Chemical Properties of Corn Pericarp as a Renewable Resource

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Contents

Chapter 1

General Introduction

1.1. Introduction

Corn, referred as Maize, is an annual plant which belongs to family Poaceae and Genus *Zea*. Corn is one of the most important cereals for a food and feed since it contains starch in the endosperm abundantly, as well as proteins, oils, vitamin in the embryo or aleurone layer. There are many races of corn over the world. They are commonly classified as 6 general types related to the quality of accumulated starch and its appearance (Baker, 1970); Dent corn (*Zea mays* var. *indentata*), Waxy corn (*Zea mays* var. *certain*), Popcorn (*Zea mays* var. *everta*), Flint corn (*Zea mays* var. *indurate*), Sweet corn (*Zea mays* var. *saccharata* and *Zea mays* var. *rugosa*), Flour corn (*Zea mays* var. *amylacea*) (Figure 1-1).

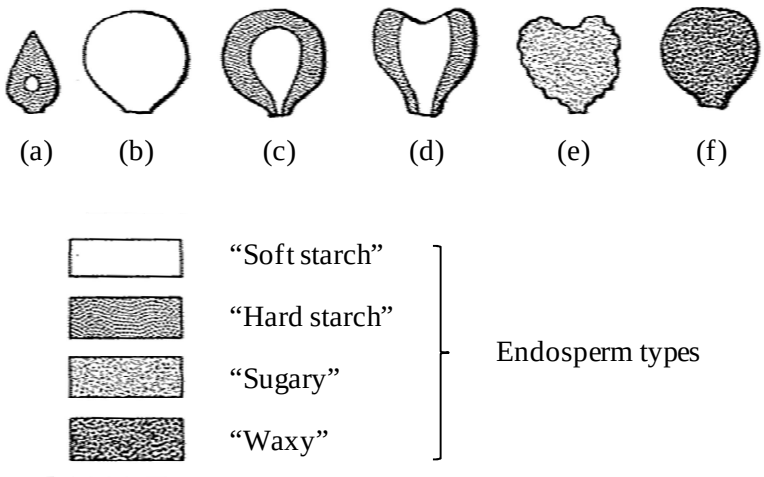

Figure 1-1. Diagrammatic sections of corn kernels: (a) popcorn, (b) flour corn, (c) flint corn, (d) dent corn, (e) sweet corn, (f) waxy corn (Baker, 1970).

Most of present hybrid corn varieties and cultivars are derived from dent corn (Hadi, 2004; Troyer, 2009), known as yellow dent corn or field corn. It contains a “hard” starch confined to the sides of the kernel with “soft” starch in the core and cap. It has a small indentation at the crown of each kernel. It is generally used as a source of corn starch in food manufactures and as a feed for livestock. The kernels used for a food are mainly a type of sweet corn and popcorn. Sweet corn, sometimes called sugar corn, contains a rather high concentration of sugar instead of starch and is put on table use. Popcorn is a type of corn that possesses exceptional popping qualities. It contains “hard” starch with minor portion of “soft” starch in the center of the endosperm. The other types of corn, waxy corn, flint corn and flour corn, are occasionally used as a food or an industrial raw material.

1.2. Production and consumption of corn

The great adaptability and high yield of corn leads to the way for a large-scale production. The annual production of corn in the world is increasing more and more every year, and attained over 820 million tons in 2009 (FAOSTAT, 2012) (Figure 1-2a) ［831 million tons in 2010 (USDA, 2012)］. The consumption of corn was also increased and mainly consumed as a feed for cattles and food. Recently, its use as a source of starch for bio-ethanol or bio-based-plastic has caused a remarkable increment of consumption in starch manufacture (USDA, 2013; FAOSTAT, 2012) (Figure 1-2b). Both production and consumption of corn continue to increase since it expands and diversifies as a crop for feed and food and as an industrial resource (Bennetzen, 2009).

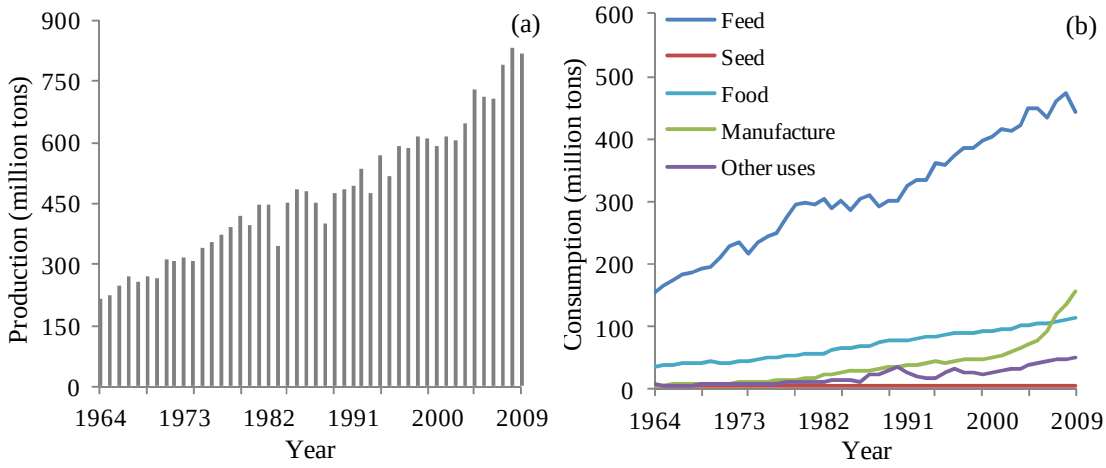

Figure 1-2. World production (a) and consumption (b) of corn kernels (FAOSTAT).

1.3. Corn pericarp as a by-product

Corn pericarp (CP) is produced from corn starch manufactures as a by-product. In corn industry, starch is extracted from corn kernels by dry-milling process or wet-milling process. These processes have been developed to fractionate the kernels into starch, oil, protein and fiber. Pericarp fraction is concentrated in fiber fraction, and called corn bran for the dry-milling process and corn fiber for the wet-milling process. The difference between these processes appeared in a change of the protein content in the pericarp fraction (Yadav *et al*., 2007a). Its global production the marketing year October 2010-September 2011 were estimated to be over 4 million tons, calculated from the percentage of the world corn production (831 million tons in 2010) (USDA, 2012) used for food manufacture (8.6%) (Kim and Dale, 2004) multiplied by the percentage pericarp content (6.2%) (Wolf *et al*., 1969), and are predicted to increase continuously because of widening of its utilization for production of bio-ethanol or bio-based-plastic as described above. Although it is generally used as an animal feed with the addition of corn protein (Shukla and Cheryan, 2001), development of more valuable products is

expected.

1.4. Effective use of corn pericarp as a renewable resource

CP is mainly composed of carbohydrates including cellulose, hemicellulosic polysaccharides and resistant starch, which are important polysaccharides as renewable biomass resources present in nature. Therefore, polysaccharides are to be targeted for effective use of CP as a renewable resource. In this thesis, the author focused on extraction, isolation and characterization of polysaccharides from CP in a direction to use them as a kind of biomaterial.

An effective extraction procedure of these polysaccharides is necessary for reuse of CP as a biomass resouce for the first step. To date, polysaccharides present in CP have been extracted by various methods, including hydrolysis with alkali (Doner and Hicks, 1997) or acid (Vidal *et al*., 2009), steam fractionation (Allen *et al*., 2001), hot compressed water (Dien *et al*., 2006) and microwave-assisted extraction (Benkö *et al*., 2007). In this thesis, the author intended to examine two different approaches with microwave-assisted extraction and NaOH-urea solvent system for achievement of their effective extraction.

1.5. Arabinoxylan

Arabinoxylan is a principal hemicellulose in CP (67%) (McKee and Latner, 2000) and also found in almost all annual plants and in soft wood. It has a backbone of β-(1,4)-linked D-xylopyranose substituted with side chains consisting largely of α-L-arabinosefranose on *O*-3 and/or *O*-2 of the xylose residue (Izydorczyk and Biliaderis, 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 arabinoxylan attractive as an ingredient in functional foods (Crittenden and Playne, 1996). Moreover, recent studies indicate that arabinoxylan can form a film without any plasticizer having moisture and oxygen barrier properties (Zhang *et al*., 2004; Gröndahl *et al*., 2004). Nowadays, arabinoxylan become attracted as a biomass resource expected for providing innovative products in material science.

1.6. β-(1,3;1,4)-Glucan

β-(1,3;1,4)-Glucan (β-glucan) is commonly present in cereal grains and included in many kinds of commercially available cereal based foods as a nutritional important ingredient, because it improves food qualities such as mouthfeel and texture (Lazaridou and Biliaderis, 2007), and also provides some specific health benefits, such as attenuating blood postprandial glycemic and insulinemic responses, lowering blood total cholesterol and low-density lipoprotein (LDL) cholesterol, and improving high-density lipoprotein (HDL) cholesterol and blood lipid profiles (Daou and Zhang, 2012; Brennan and Cleary, 2005). It is a linear homopolysaccharide comprising two types of D-glucopyranosyl residues linked by a mixture of β-(1-3) and β-(1-4) linkages, with blocks of (1-4)-linked residues (oligomeric cellulose-like segments) separated by (1-3)-linkages. Its structural features, such as linkage ratio, units of cellulose-like segments and distribution of the cellulose-like segments, are known to be important determinants for its physical properties and functionalities, including its use as food

additives (Lazaridou *et al*., 2004).

1.7. Microwave assisted extraction

Microwave-assisted extraction is a process of using microwave energy for solubilization of important components from samples. It can reduce times and solvent volumes required for the extraction by the particular effects of microwave irradiation (Eskilsson and Björklund, 2000). Microwave irradiation allows heating a sample uniformly and rapidly due to the direct and internal heating by the frictions of molecules occurring with dipole rotation (Eskilsson and Björklund, 2000). Extraction of polysaccharides from biomass by using this technique has been proposed for a rapid extraction method as an attractive alternative for conventional techniques (Okushi *et al*., 2006; Azuma *et al*., 1984). Solubilization of polysaccharides with microwave-assisted extraction is accompanied with their depolymerization by heating above 180ºC (Yu *et al.* 2008), and further heating leads to secondary degradation (Shafizadeh, 1968). Thus, the microwave heating conditions for the extraction of CP is significant for its effectiveness, however, it has not been fully explored by using statistical method.

1.8. NaOH-urea solvent system

Recently, NaOH-urea solvent system has developed for dissolution of cellulose (Cai and Zhang, 2005). This cheap and environmentally friendly solvent can rapidly dissolve native cellulose by treatment with an aqueous solution of 7% NaOH-12% urea pre-cooled to -12ºC (Cai and Zhang, 2005; 2006; Cai *et al*., 2008). It seems that the formation of a urea and cellulose-NaOH complex at low temperatures contributes for splitting of the intermolecular and intramolecular hydrogen bonds of cellulose (Cai *et*

al., 2008). Additionally, this solvent system allows preparation of transparent regenerated cellulose films with excellent optical transmittance and tensile strength via a green process (Qi *et al.*, 2009). In plant cell wall, it is well known that cellulose is interacted with hemicelluloses. For this aspect, this solvent system is considered to affect the extractability of hemicelluloses. However NaOH-urea solvent system has not been applied for extraction of hemicelluloses.

1.9. Objective of this thesis

The author intended to develop a new systematic method for effective use of CP as a renewable resource of carbohydrates. The method was focused on extraction, isolation and characterization of its polysaccharides. This research work is expected to open more effective usability of corn kernels for its total utilization.

For achievement of this objective, at the beginning of this thesis, the effectiveness of microwave-heating and NaOH-urea solvent system were investigated for extraction of polysaccharides from CP in chapters 2 and 3, respectively.

In chapter 2, the conditions for extraction of carbohydrates from CP as a by-product of corn starch manufactures were discussed by using a statistical method of combinations of response surface methodology and experimental design method in order to achieve an optimum yield. Detailed effects of microwave irradiation on chemical components in CP were also noted for total recycle of this material.

In chapter 3, a novel method was proposed for effective extraction of hemicellulosic polysaccharides from CP by using NaOH-urea solvent system to produce transparent biofilms. The mechanical properties of biofilms prepared from the isolated hemicellulosic polysaccharides were described in this chapter.

In chapter 4, in order to characterize water sorption behavior of arabinoxylan isolated from CP, a convenient preparation method of partially dearabinofuranosylated arabinoxylan was described by hydrolysis with weak acid. Effects of the treatment conditions on chemical properties of arabinoxylan were noted in detail with characterization of its solubility and crystallinity. The role of arabinose substituents for water adsorption behavior of arabinoxylan was comparatively investigated on basis of the independent dual sorption model and the Hailwood-Horrobin model.

In chapter 5, the conditions of NaOH-urea solvent system were optimized to develop a technique for extraction of β-glucan from CP. The effects of crystalline property of cellulose present in CP on its extractability were noted. Applicability of the present technique to the other cereal materials was also discussed in this chapter.

In chapter 6, for practical utilization of β-glucan from CP, a simple and useful purification technique was proposed by using a combination of anion-exchange chromatography and affinity chromatography on cellulose column. Chemical structure of the isolated β-glucan from CP confirmed by ^{13}C-NMR spectroscopic, methylation and lichenase treatment analyses was described by comparing with the previous results of other cereal β-glucans.

Chapter 2

Microwave-assisted extraction of carbohydrates from corn pericarp

2.1. Introduction

Corn fiber containing pericarp of corn kernels is rich in hemicellulosic polysaccharides (Doner and Hicks, 1997). Within the hemicellulosic polysaccharides arabinoxylan is the major polysaccharide. In general, arabinoxylan and xylo-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 (Benkö *et al.*, 2007; Swennen *et al.*, 2006). These bioactivities make arabinoxylan-type hemicelluloses attractive as ingredients in functional foods (Crittenden and Playne, 1996). Arabinoxylan also has emulsifying and gelling property (Yadav *et al.*, 2006; 2008; Lapierre *et al.*, 2001; Carvajal-Millan *et al.*, 2006). Corn fiber is also an attracting source of carbohydrates to produce bioethanol (Mészáros *et al.*, 2009).

Arabinoxylan has been extracted from corn fiber or bran by various methods, including hydrolysis with alkali (Doner and Hicks, 1997) or acid (Bernardo *et al.*, 2009), steam fractionation (Allen *et al.*, 2001), and hot compressed water (Dien *et al.*, 2006). Microwave-assisted extraction (MAE) is known to allow great reduction in time required for extraction of polysaccharides from fruiting body of mushrooms (Ookushi *et al.*, 2006) and tea residues (Tsubaki *et al.*, 2008). Recently, Benkö *et al.* (2007) and Rose and Inglett (2010) have applied MAE to extract hemicellulosic polysaccharides from corn fiber. However, the extraction conditions have not been fully explored using

statistical method.

In this chapter, after characterization of gross chemical properties of CP produced from corn starch industry, detailed effects of microwave irradiation on chemical components in CP were investigated for total recycle of this material. Subsequently, the author applied 3-step of experimental design including fractional factorial design, the path of steepest ascent and central composite design for accurate prediction of the optimum condition of MAE of carbohydrates in CP. Finally the relationship between carbohydrates yield and severity parameter was calculated for better interpretation of heating condition necessary for MAE of carbohydrates in CP from corn starch industry.

2.2. Materials and Methods

2.2.1. Materials

CP as a by-product fiber obtained after the wet-milling process of dent corn was supplied from a corn starch manufacture (Sanwa Cornstarch Co., Ltd.) in Nara Prefecture, Japan. The solvent and all other regents used were analytical grades. A mixture of xylo-oligosaccharides having degree of polymerization of 2-10 was prepared by partial hydrolysis of beech 4-*O*-methyl-glucuronoxylan with 0.1 N H_2SO_4 for 1 h at 92°C followed by neutralization with barium carbohydrate and passage through a joint column of Dowex 50 × 8 (H^+) and Dowex 1 × 8 (AcO^-).

2.2.2. Compositional analysis

CP was powdered by electric mixer (≦1.0 mm) before compositional analysis. Moisture content was determined according to the AOAC official method 32.1.02. Carbohydrate content was determined by the phenol-sulfuric acid method using a mixed

standard solution of glucose, xylose, arabinose and galactose according to the composition determined after Saeman hydrolysis (1945) and HPAEC as described in section 2.2.4. Protein content was determined from elemental analysis and multiplied by 6.25 as a protein conversion factor (AOAC official method 32.2.02.). Ash content was determined according to the AOAC official method 32.1.05. Lipid content was determined by using soxhlet extraction with 75% aqueous 1-propanol as an extracting solvent according to the method given in the *Handbook of Food Analytical Chemistry* (Wrolstad *et al*., 2004). Acid insoluble lignin content was determined by the TAPPI test method T222 om-88.

2.2.3. Microwave-assisted extraction

Microwave irradiation was performed by using MicroSYNTH Labstation (maximum output 1 kW, 2.45 GHz) microwave oven (Milestone Inc., Shelton, CT, USA) using a Monobloc High-pressure Digestion Rotor (HPR-100). CP (0.5 to 1 g) was suspended in 20-30 mL distilled water in the HPR-100 and mixed with a stirrer bar for 15 min to give sufficient penetration of water. Then reactant was heated to desired temperatures (14-220°C) in 2-4 min and held at the temperature for 1-26 min with microwave irradiation. After irradiation, the product was immediately cooled in an ice bath to room temperature and filtrated to separate solubilized fraction and residues. Solubilized fraction was preserved at -30°C until experiments, and residues were freeze-dried.

2.2.4. Chemical and morphological analysis

To examine the effects of heating temperature on solubilization of carbohydrates

and polyphenol and solubilization rate, microwave irradiations were performed at 140-220°C, solid to liquid ratio 1/30 (g/mL), come-up time 2 min and heating time 5 min. Carbohydrates content was determined by using the phenol-sulfuric acid method by using the same standard as compositional analysis. Polyphenol content was determined by the Folin-Ciocalteau method (Wrolstad *et al*., 2004) by using gallic acid as a standard and expressed as gallic acid equivalent. The solubilization rate was determined by the following equation;

Solubilization rate (%) = (*Weight of raw material – Weight of residue*) × 100 / (*Weight of raw material*)

Raw material and each fraction after microwave irradiation were hydrolyzed according to the method of Saeman (1945), and the monosaccharide composition was investigated by high-performance anion exchange chromatography (HPAEC) on a Dionex DX-500 system (Sunnyvale, CA, USA) with pulsed amperometric detector (ED-40) using 1.0 mM NaOH as a mobile phase.

Molecular weight distribution of neutral fractions of solubilized materials were analyzed by SEC on a column of MC IGEL CK04SS (7.5 × 200 mm, Mitsubishi Chemical Industry Co., Ltd.) at 80°C with refractive index detector. Eluent was deionized water and flow rate was 0.3 mL/min. To eliminate charged contaminants, the solubilized materials were purified by passage through a joint column of anion and cation exchange resins as described above with pure water.

Solid-state CP/MAS ^{13}C-NMR spectra were obtained on a Chemagnetics CMX-300 spectrometer (JEOL Ltd., Tokyo, Japan), operating at 74.7 MHz. The condition of the pulse repetition was 5 s, the sweep width was 30 kHz, the cross-polarization contact

time was 2 ms, and acquisition time was 34 ms. Magic angle spinning was performed at 4.5 kHz in a Zirconia rotor with Teflon caps. Chemical shifts were referenced to 17.3 ppm of the methyl-carbon signal of hexamethylbenzene.

The effects of heating temperature on morphological properties of residues after MAE were analyzed by low voltage scanning electron microscope (LV-SEM, VE-8800, Keyence Co., Osaka, Japan) at 1.7 kV on an amorphous carbon stage.

2.2.5. Experimental design and data analysis

To maximize the yield of carbohydrates, 3-step experimental design was used in this work. The predictor variables were coded by the following equation:

$$x_i = (X_i - X_{i,0}) / \Delta X_i$$

Where, x_i is the coded value of independent variables, X_i is the real value of the independent variable, $X_{i,0}$ is the real value of the independent variable at the center point, and ΔX_i is the step change value. The yields of carbohydrates extraction were determined by the same method as mentioned in the section 2.4. The software, JMP 6 (SAS institute Inc.) was used for data analysis. The qualities of fitted models obtained by experimental data were examined by the coefficient of determination R^2. The experimental results were analyzed by using the least-square method (LSM). The regression model was analyzed by using analysis of variance (ANOVA).

2.2.5.1. Fractional factorial designs

At first, 2^{4-1} fractional factorial design (FFD) was designed to identify factors which have significant effects on the carbohydrates yield (Montgomery, 2005). FFD was

designed at conditions far away from the optimum condition with 8 experimental runs and 3 replications at the center point of the design. The following regression equation was obtained by the LSM:

$$\hat{Y} = \hat{\beta}_0 + \sum_{i=1}^{k} \hat{\beta}_i x_i + x_1 \sum_{i=2}^{k} \hat{\beta}_{1i} x_i$$

Where, $\hat{Y}$ is the estimated carbohydrates yield, x_i is the coded value of independent variables, $\hat{\beta}$'s are the regression coefficients. After screening, adequacy of the factors in this model was confirmed by ANOVA, and nonsignificant terms were eliminated in the refined model.

2.2.5.2. The path of steepest ascent

Next, region in the vicinity of the optimum condition was determined by using the method of steepest ascent. In this work, experiments were carried out along the steep path of refined model obtained from FFD until the response value showed no more increase.

2.2.5.3. Central composite design

Finally, the central composite design (CCD) was performed to determine the optimum condition. CCD was constructed with 5 replications at the center points, with 4 axial points and 4 star points (± α). In this work, 1.68 was used as α for rotatable design (Montgomery, 2005). The following regression equation was fitted to the response resulted from CCD by the LSM:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i<j} \sum \beta_{ij} x_i x_j$$

Where Y is the estimated carbohydrates yield, x_i is the coded value of an independent variables, β's are the regression coefficients. Adequacy of this model was confirmed by ANOVA. Finally, the model was reconfirmed by 3 replications at the estimated optimum condition.

2.2.6. Calculation of severity parameter

Severity parameter of hydrothermal treatment without addition of acid catalyst was calculated as following equation (Overend and Chornet, 1987):

$$\log R_0 = \log\left[\int_0^t \exp\left(\frac{T(t) - T_{ref}}{14.75}\right) dt\right]$$

Where, t is the reaction time (min), $T(t)$ is the temperature-time function for gradually heated processes (°C) and T_{ref} is the reference temperature which was set to 100°C in this paper.

2.3. Results and Discussion

2.3.1. Chemical composition of corn pericarp

The chemical composition (%, dry weight basis) and relative monosaccharide composition of the raw CP were summarized in Table 1-1. The results showed that the native sample is mainly composed of carbohydrates. The composition showed slightly higher content in protein and lipid than that of corn fiber reported by Shukla and Cheryan (2001), indicating the native sample included tip caps of the corn kernels.

Relative monosaccharide composition of the native sample is also shown in Table 1-1. Total content of xylose, glucose and arabinose accounted for 90.2% showing abundance of arabinoxylan and cellulose as main polysaccharides.

Table 1-1. Chemical composition of the raw dent corn pericarp (%, dry weight basis).

Component	
Chemical composition (%)	
Moisture[a]	8.0 ± 0.1
Carbohydrate[a]	83.2 ± 3.2
Holocellulose[a]	78.6 ± 0.5
α-clellulose[a]	25.1 ± 0.8
Acid-insoluble lignin[a]	4.0 ± 0.3
Uronic acid[a]	0.6 ± 0.1
Protein[b]	9.5 ± 0
Lipid[a]	6.6 ± 2.0
Ash[a]	0.6 ± 0
Relative monosaccharide composition (%, w/w)[b]	
Mannose	4.1 ± 1.3
Xylose	33.4 ± 0.5
Glucose	36.8 ± 0.4
Galactose	5.9 ± 0.6
Arabinose	20 ± 0.4

[a] Values are expressed as mean ± SD (n = 3).
[b] Values are expressed as mean ± SD (n = 2).

2.3.2. Effects of microwave heating on corn pericarp

The effects of heating temperature on the solubilized fraction from CP were investigated by microwave irradiation at 140°C to 220°C under fixed solid to liquid ratio of 1/30 (g/mL), 2 min of come-up time and 5 min of heating time at the desired temperatures. Solubilization rate, carbohydrates yield, polyphenol yield and uronic acid

yield in soluble fraction were shown in Figure 1-1. The solubilization rate increased with increase in heating temperature, and reached 75.2% at 220°C (Figure 1-1a). Carbohydrates yield also increased with increase in heating temperature and attained 489 mg/g at 200°C, however, decreased by heating at 220°C (Figure 1-1b), due to secondary degradation of carbohydrates into furfural and other derivatives.

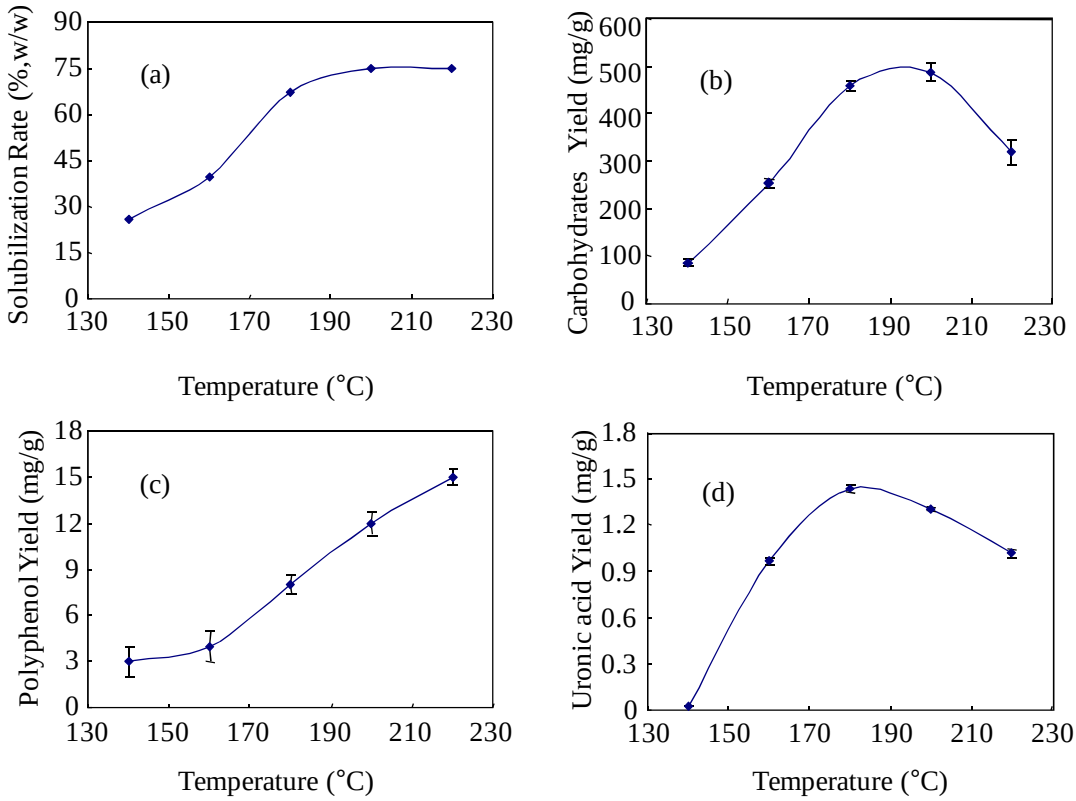

Figure 1-1. Effects of MAE on (a) solubilization rate (%), (b) carbohydrates yield (mg/g), (c) polyphenol yield (mg/g) and (d) uronic acid yield (mg/g). Error bars represent standard deviation (n = 3).

Table 1-2 shows relative monosaccharide composition of the solubilized fraction

and residues. In the solubilized fraction, the content of xylose increased with increase in temperature and attained 42.6% of total neutral monosaccharide at 200°C. The abundance of xylose and arabinose in the solubilized fraction suggested predominant extraction of arabinoxylan from CP. The decrease in xylose and arabinose content at 220°C in the solubilized fraction was ascribed to their secondary degradation. The behavior of solubilization of xylose and arabinose strongly corresponded with that of carbohydrates yield. Carbohydrates yield and total content of xylose and arabinose showed good correlation (R^2 = 0.9506, *p*-value <0.0001). In the residue, glucose content in the solubilized fraction increased with increase in heating temperature and attained 95.7% at 220°C, suggesting survival of cellulose in the residue after MAE.

Production of polyphenol accelerated above 160°C by MAE (Figure 1-1c), however, the maximum yield was only <15 mg/g. Lapierre *et al*. (2001) reported that little phenolic acids such as ferulate and diferulate are bounded to arabinoxylan and contribute to cross-linkage between xylan chains and lignin chains. Uronic acid yields also attained maximum at 1.5 mg/g at 180°C (Figure 1-1d). The uronic acids are probably derived from 4-*O*-methylglucuronic acid binding with xylan chains. These results indicated that the solubilization rate was largely affected by the solubilization of carbohydrates.

Table 1-2. Relative monosaccharide compositions of residues and solubilized fractions obtained after microwave irradiation (%, dry weight basis).

Temperature (°C)	Ara	Gal	Glc	Xyl	Man
		Residues			
Native[a]	20.0 ± 0.4	5.9 ± 0.6	36.8 ± 0.4	33.4 ± 0.5	4.1 ± 1.3
Room temperture[a,d]	21.2 ± 0.4	5.7 ± 0.1	40.0 ± 0.4	30.5 ± 0.1	2.8 ± 0.0
140[a]	18.0 ± 1.0	6.8 ± 0.6	35.7 ± 0.6	37.2 ± 0.6	2.4 ± 0.4
160[a]	13.2 ± 0.3	5.8 ± 0.1	38.0 ± 0.3	38.7 ± 0.1	4.3 ± 0.8
180[a]	3.2 ± 0.1	2.3 ± 0.4	72.4 ± 0.9	19.4 ± 0.1	2.8 ± 0.2
200[a]	0.7 ± 0.0	0.9 ± 0.4	89.4 ± 0.9	7.3 ± 0.1	1.7 ± 0.2
220[a]	0.5 ± 0.1	0.6 ± 0.4	95.7 ± 1.1	2.3 ± 0.4	1.0 ± 0.5
Optimum point[b,e]	1.8 ± 0.6	2.0 ± 1.1	83.3 ± 3.4	10.5 ± 0.7	2.6 ± 1.0
		Solubilized fraction			
Room temperture[a,d]	33.4 ± 1.4	7.0 ± 0.5	46.8 ± 0.5	12.9 ± 1.3	tr[c]
140[a]	16.5 ± 0.1	3.0 ± 0.4	71.5 ± 1.0	8.8 ± 0.4	0.3 ± 0.4
160[a]	25.8 ± 0.4	5.4 ± 0.1	42.7 ± 0.4	24.9 ± 0.1	1.4 ± 0.1
180[a]	21.8 ± 0.3	6.7 ± 0.9	31.4 ± 0.6	39 ± 0.8	1.3 ± 0.1
200[a]	20.8 ± 0.1	7.7 ± 0.5	26.9 ± 0.1	42.6 ± 0.4	2.3 ± 0.1
220[a]	16.8 ± 0.4	7.9 ± 0.1	34.9 ± 0.1	38.6 ± 0.1	1.9 ± 0.0
Optimum point[b,e]	22.5 ± 0.4	7.2 ± 0.3	26.5 ± 0.1	41.6 ± 0.2	2.2 ± 0.6

[a] Values are expressed as mean ± SD (n = 2).

[b] Values are expressed as mean ± SD (n = 3).

[c] tr represents trace.

[d] Room temperature represents stirring for 15 min in water without microwave irradiation.

[e] Optimum point represents treatment under the optimum microwave irradiation condition (175ºC, 18 min).

Solid-state CP/MAS ^{13}C-NMR was further applied to characterize the chemical components in the residues after microwave heating and native CP nondestructively (Figure 1-2). Signal assignments for ^{13}C spectra were referred to the report by Love *et al*. (1998). The spectra showed increase in heating temperature induced weakening of xylan

signals (C-4: 82 ppm, C-1: 101 ppm) and enhancement of cellulose signals (C-1: 105 ppm, C-2, 3, 5: 72 and 75 ppm, C-4 of amorphous and crystalline cellulose: 84 and 89 ppm, C-6: 65 ppm) supporting the chemical analysis which showed solubilization of xylan and survival of cellulose in the residues. The signals due to acetyl methyl (21 ppm) and carbonyl carbons (173 ppm) decreased with increase in temperature, which indicated deacetylation of xylan as well as deesterification of ferulic acid and *p*-coumaric acid binding with xylan chains (Lapierre *et al*., 2001) with generation of acetic acid and polyphenol in the solubilized material. Solubilization of polyphenol was also confirmed by decrease in the signal of C-3 of esterfied phenol units (149 ppm) above 180°C. The presence of acetic acid might have promoted the hydrolysis of hemicellulose (Shafizadeh, 1968).

The molecular weight distributions of the neutral fractions of solubilized materials were monitored by using SEC (Figure 1-3) after purification with ion exchange resins. Peaks equivalent to xylo-oligosaccharides started to appear above 180°C, and production of xylose (peak X_1) largely increased at 220°C (Figure 1-3a-e), showing generation of xylo-oligosaccharides by degradation of higher molecular weight components.

Typical LV-SEM images of the residues after microwave heating were shown in Figure 1-4. Morphology of native corn pericarp was thin fibered film. The residue after MAE at 160°C had several cracks on the surface. The residue after MAE at 180°C was appreciably tore indicating solubilization of cell wall components.

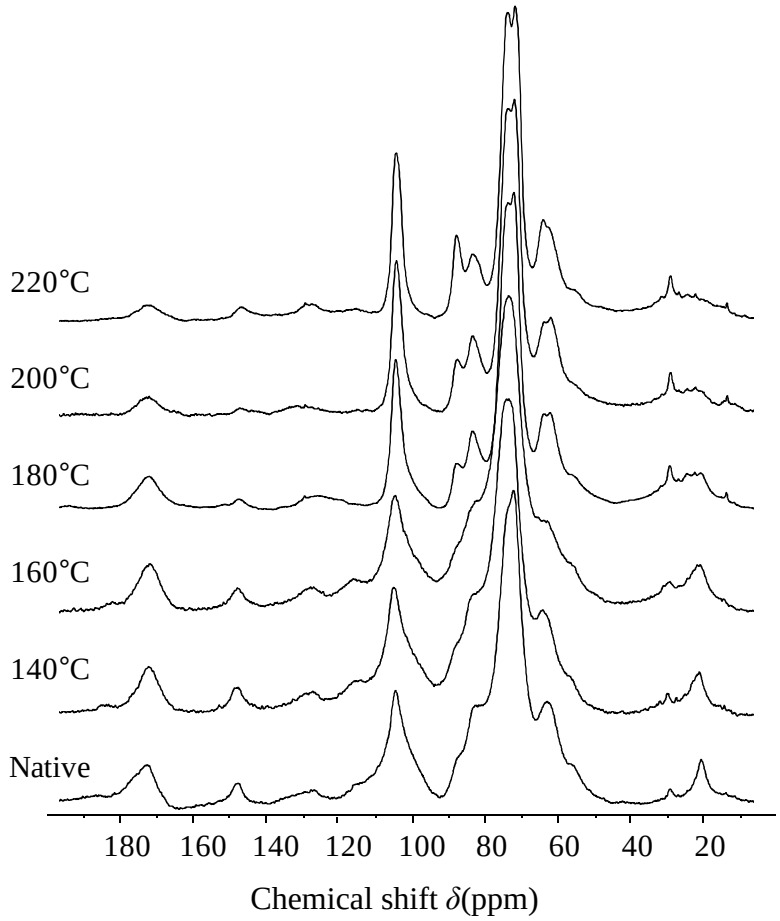

Figure 1-2. Solid-state CP/MAS ^{13}C-NMR spectra of native corn pericarp and residues obtained after microwave irradiation.

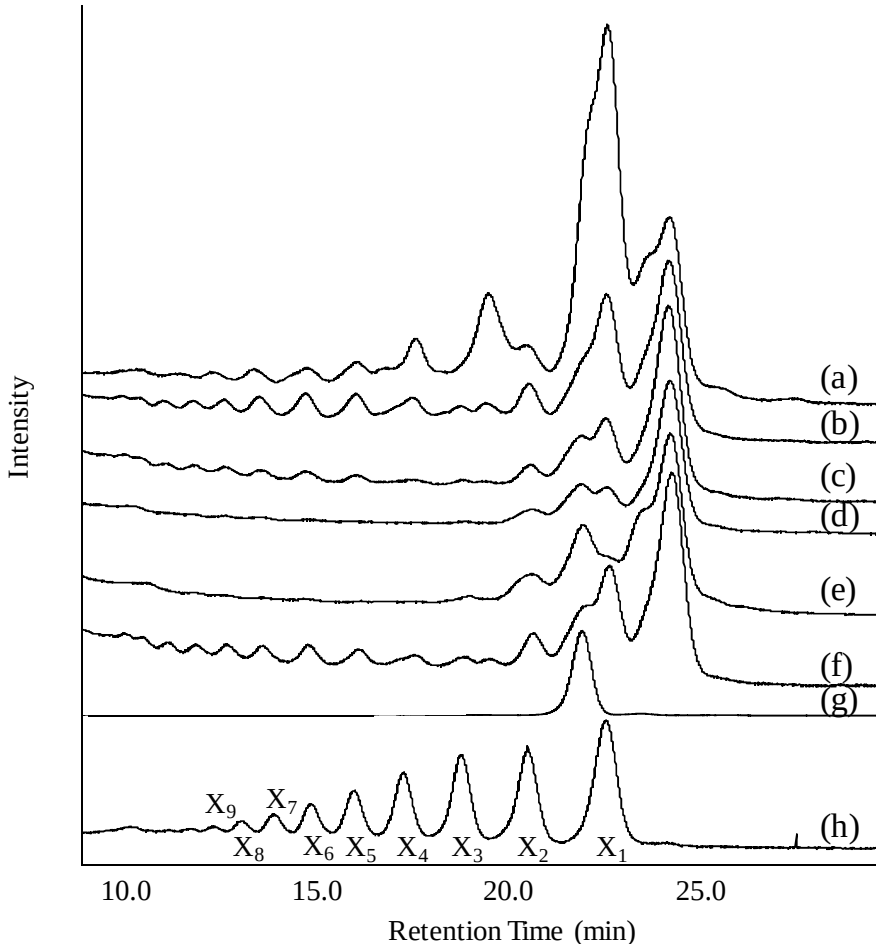

Figure 1-3. Size exclusion chromatograms of glucose, xylo-oligosaccharides and solubilized material obtained by microwave irradiation. Chromatograms (a), (b) , (c), (d), (e) and (f) show molecular weight distribution of extracted components by MAE at 220°C, 200°C, 180°C, 160°C, 140°C and optimum point, respectively. Chromatograms (g) and (h) show standard glucose and xylo-oligosaccharides, respectively. Peaks X_1, X_2, X_3, X_4 and X_5 show components having molecular weights equivalent to xylose, di-, tri-, tetra- and penta-xylo-oligosaccharides, respectively.

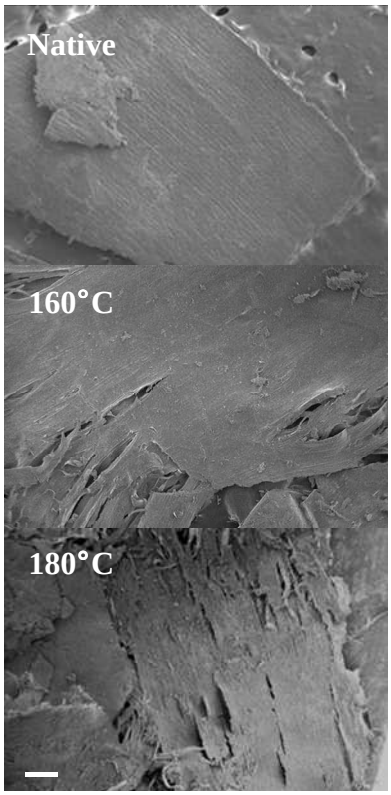

Figure 1-4. Typical LV-SEM images of native sample and residues remaining after microwave irradiation. White bar indicates 200 μm.

2.3.3. Optimization of microwave irradiation conditions for extraction of carbohydrates from corn pericarp

Heating temperature, come-up time, heating time and solid to liquid ratio were examined as factors that might affect carbohydrate yield by using FFD. The conditions and the results of FFD were shown in Table 1-3. ANOVA of the regression model was summarized in Table 1-4. The regression model was shown in equation (1-1):

$$Y = 96.0909 + 41.0000x_1 + 23.0000x_2 + 5.5000x_3 + 1.0000x_4 + 10.2500x_1x_2 + 6.7500x_1x_3 - 1.2500x_1x_4 \quad (1\text{-}1)$$

Table 1-3. Independent variables of FFD and carbohydrates yields.

Run	Coded variables				Real variables				Yield (mg/g)
	x_1	x_2	x_3	x_4	X_1	X_2	X_3	X_4	
1	0	0	0	0	150	2	0.75	3	92
2	0	0	0	0	150	2	0.75	3	87
3	0	0	0	0	150	2	0.75	3	84
4	-	-	+	-	140	1	1	2	42
5	+	-	+	+	160	1	1	4	119
6	-	-	-	+	140	1	0.5	4	49
7	+	-	-	-	160	1	0.5	2	95
8	-	+	+	+	140	3	1	4	72
9	-	+	-	-	140	3	0.5	2	70
10	+	+	-	+	160	3	0.5	4	161
11	+	+	+	-	160	3	1	2	186

Table 1-4. Analysis of variance for regression models of FFD and CCD.

	Degree of freedom	Sum of square	Mean square	F -value	p -value
Regression model of FFD					
Model	7	19147.500	2735.357	25.218	0.00114*
Residual	3	325.409	108.470		
Lack of fit	1	292.742	292.742	17.923	0.05152041
Pure error	2	32.667	16.333		
total	10	19472.909			
R^2	0.983				
Screening regression model of FFD					
Model	2	17680.000	8840.000	39.444	<0.0001*
Residual	8	1792.909	224.114		
Lack of fit	6	1133.242	566.621	5.154	0.17140047
Pure error	2	659.667	109.944		
total	10	19472.909			
R^2	0.908				
Regression model of CCD					
Model	5	13892.805	2778.560	24.188	0.0003*
Residual	7	804.118	114.870		
Lack of fit	3	368.118	122.706	1.126	0.4383
Pure error	4	436.000	109.000		
total	12	14696.923			
R^2	0.945				

* represents significant at the level of 5%.

The *p*-value of the model (0.0011) was significant enough at the probability level of 5%. Significant factors (intercept; <0.0001, x_1; 0.0016 and x_2; 0.0083) were selected for the refined model:

$$Y = 96.0909 + 41.0000\ x_1 + 23.0000\ x_2 \quad (1\text{-}2)$$

The ANOVA for the refined model was also summarized in Table 1-4. The *p*-value of the model was significant enough (<0.0001) with no significance in lack of fit (0.0515) (Table 1-4). The value of R^2 (0.9079) also showed good fitting of this model.

The path of steepest ascent was determined by the equation (1-2) obtained by FFD and the results were shown in Table 1-3a. The nonsignificant factors screened out by FFD (come-up time and solid to liquid ratio) were fixed at 2 min and 1/20 (g/mL), respectively, to minimize the reaction time and solid to liquid ratio. Further reduction in come-up time and solid to liquid ratio was difficult for precise temperature control and homogeneous stirring of the material in the reactor. The increment was 5.6 unit of x_2 for each unit of x_1. The results clearly showed that the carbohydrates yield would be maximized around the conditions of run 13 and 14 (Figure 1-5a).

The conditions of CCD, the responses and the predicted yields estimated by the regression model were summarized in Table 1-5. Center point was set at 180°C (heating temperature) and 19 min (heating time). Come-up time and solid to liquid ratio were fixed at the same condition as previously employed in the path of steepest ascent. The regression model was follows:

$$Y = 530.8191 - 25.1177\ x_1 - 11.5657\ x_2 - 29.2227\ x_1^2 - 8.2500\ x_1x_2 - 7.8606\ x_2^2 \quad (1\text{-}3)$$

The result of ANOVA of the model was also summarized in Table 1-4. ANOVA showed significant *p*-value (0.0003), no significant lack of fit and 0.945 of determination coefficient (R^2), which showed good fitting of the model. The response surface and contour plot of the model (1-3) were shown in Figure 1-5b, c. Carbohydrates yield was estimated to be 538 mg/g at the optimum point [heating temperature; 176.5°C, heating time; 16 min, come-up time; 2 min and solid to liquid ratio; 1/20 (g/mL)]. To reconfirm the adequacy of the model, 3 replications were carried out at the estimated optimum point. Carbohydrates yield attained 542 ± 3 mg/g clearly showing this model is sufficiently adequate.

At the optimum point, total rate of arabinose and xylose in the solubilized fraction showed the highest value of 64.1%, which obviously indicated that the extraction of arabinoxylan was improved by optimization process (Table 1-2). Previously Benkö *et al*., (2007) have reported that 30% of polysaccharides can be recovered from corn fiber by microwave-assisted heat treatment at 210°C 2 min. Rose and Inglett (2010) have investigated microwave-assisted autohydrolysis and reported that 51.6% of the initial arabinoxylan content in maize bran was recovered. Although our stating material contains residual starch, carbohydrate yield greatly improved to 70.8% of total carbohydrate content in CP by optimization process.

In the size exclusion chromatogram, the peaks of small molecular weight components equivalent to xylo-oligosaccharides were clearly observed at the optimum condition (Figure 1-3f). Xylo-oligosaccharides were apparently more extracted at the optimal condition than that at 180°C, indicating the optimal condition was also desirable for production of xylo-oligosaccharides.

Table 1-5. Independent variables, experimental and predicted carbohydrate yields of the CCD.

	Coded variables		Real variables		Yield (mg/g)	
Run	x_1	x_2	X_1	X_2	Eperimenta	Predicited
17	-1.68	0	165	19	507	503
18	-	-	170	14	522	522
19	-	+	170	24	506	516
20	0	-1.68	180	12	530	532
21	0	0	180	19	542	532
22	0	0	180	19	530	532
23	0	0	180	19	514	532
24	0	0	180	19	535	532
25	0	0	180	19	534	532
26	0	1.68	180	26	511	499
27	+	-	190	14	488	488
28	+	+	190	24	439	449
29	1.68	0	195	19	432	427

X_1 and X_2 represent heating temperature (°C) and heating time (min), respectively.

2.3.4. Relationship among carbohydrates yield, solubilization rate and severity parameter

The relationships among carbohydrates yield, solubilization rate and severity parameter were shown in Figure 1-6. The maximal carbohydrates yield increased proportionally with increase in the value of log R_0 until the optimum condition (3.42), however, higher severity resulted in decrease (Figure 1-6a). The log R_0 for the optimum condition will be crucial for industrial scale-up of this system by using continuous microwave apparatus (Magara *et al*., 1988).

The solubilization rate increased linearly with increase in log R_0 attaining 75% at around 3.42, which also correspond to optimum condition. Since the native material

contains 25.1% of α-cellulose, the optimal condition is also sufficient for solubilization of CP and preparation of cellulose for bioethanol production with minimum loss of carbohydrates (Figure 1-6b). Previously, Dien *et al*. (2006) has reported hot water treatment of dissolved 58% of corn fiber which is a similar corn starch by-product as CP (160°C, 20 min). By employing MAE and RSM optimization, the solubilization was greatly improved by 17%.

Previously, Ookushi *et al*., (2006) have reported that MAE (140°C, 5 min, log R_0 = 1.89) of polysaccharides from fruiting body of mushrooms is comparable to 100°C, 6 h of traditional extraction (log R_0 = 2.57) showing great reduction in extraction time and severity by MAE. Fischer and Bipp (2005) have reported that reaction time and reagent demand is reduced by microwave application for generation of organic acid and monosaccharides from food processing residues. Addition of salts will be an interesting method to improve microwave absorption for autohydrolysis of polysaccharides (Tsubaki *et al*., 2010).

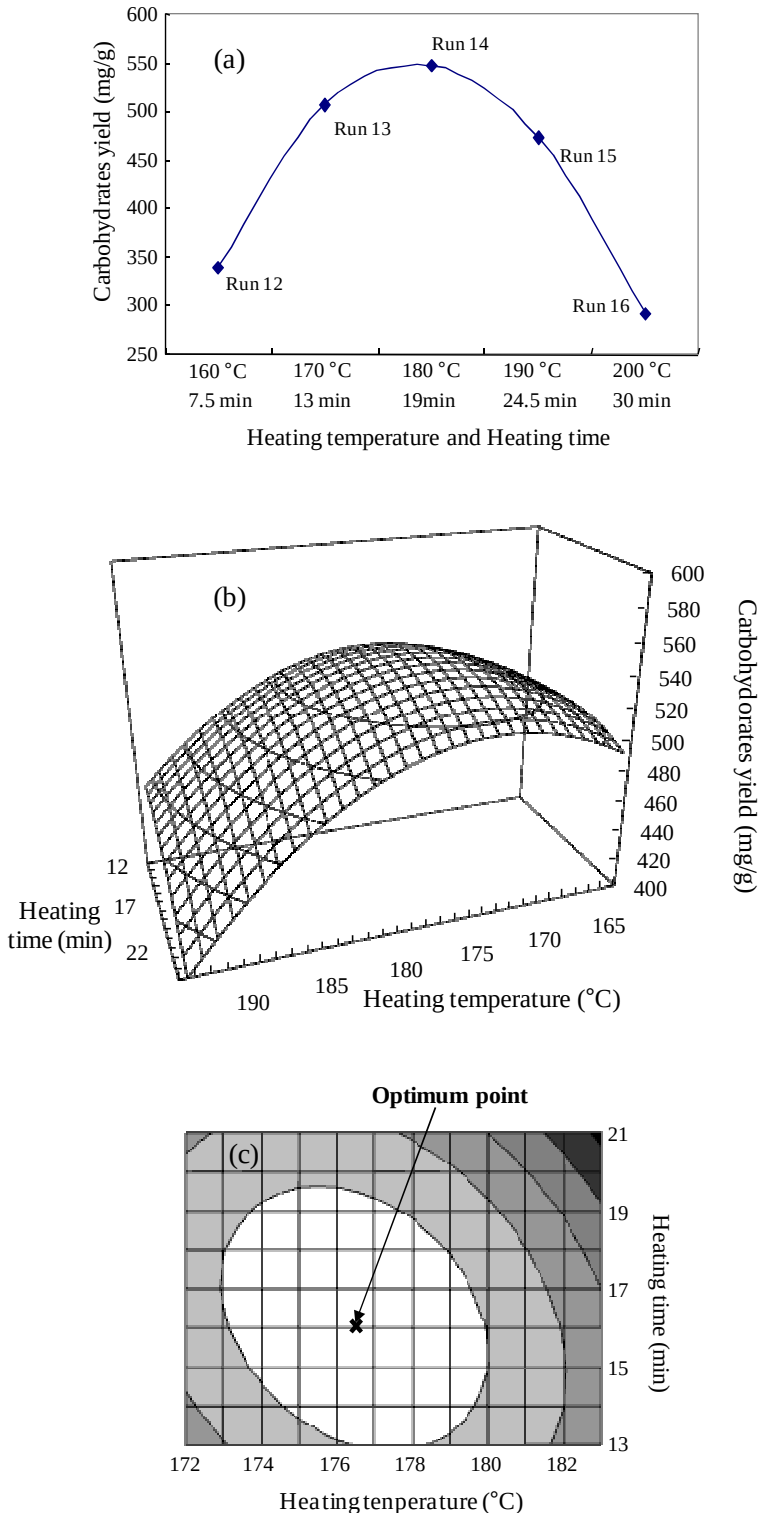

Figure 1-5. (a) Responses of carbohydrates yield along the path of steepest ascent. (b) Response surface and (c) contour plot of estimated amount of carbohydrates yield calculated from model (1-3) in the text.

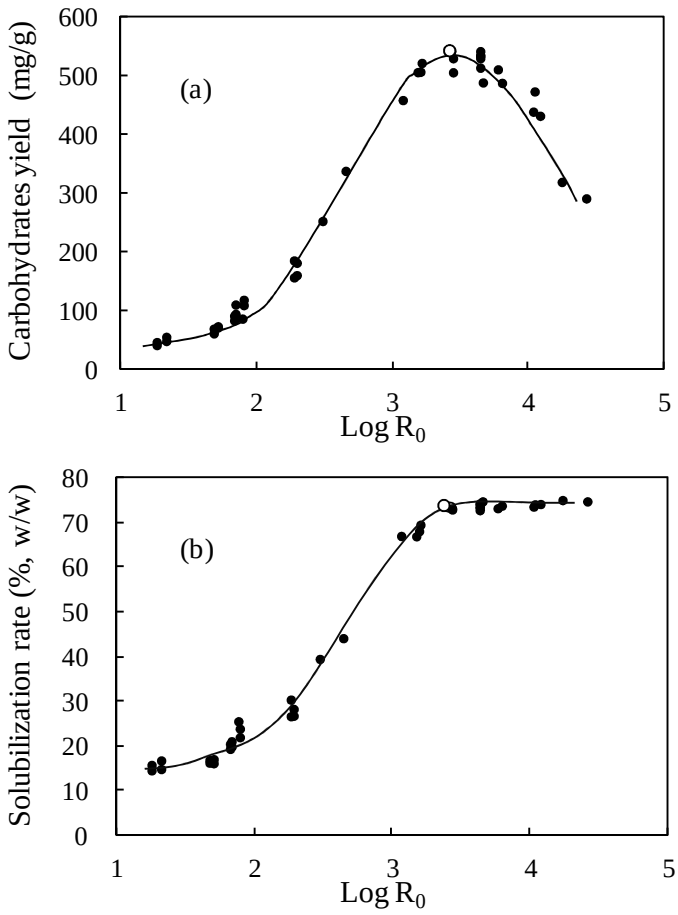

Figure 1-6. Relationships between (a) severity parameter and carbohydrates yield, (b) severity parameter and solubilization rate. White and black circles represent optimum condition and other conditions, respectively.

2.4. Summary

Carbohydrates were effectively extracted from CP by MAE. The optimum condition for MAE of carbohydrates was determined by using response surface methodology with experiment design including fractional factorial design, the path of steepest ascent and central composite design. Carbohydrate yield attained 70.8% of total carbohydrate content in CP at the optimum condition [heating temperature 176.5°C, come-up time 2 min, heating time 16 min and solid to liquid ratio 1/20 (g/mL)].

Additionally, the optimum condition was desirable for predominant production of xylo-oligosaccharides. In this chapter, the author clearly demonstrated applicability of MAE as an effective tool for reuse of CP as a carbohydrates resource.

Chapter 3

Development of a new mild method for extraction of hemicellulosic polysaccharides including arabinoxylan from corn pericarp

3.1. Introduction

CP as a wasted corn fiber obtained from industrial corn starch production is rich in arabinoxylan. The chemical structure of the CP arabinoxylan was clarified to have a backbone of β-(1,4)-linked D-xylose with side chains of α-L-arabinose (Saulnier *et al*., 1995). Extraction of this arabinoxylan from CP is attractive because of its importance as a food additive for expectation of its benefit for human health and good emulsion property (Yoshida *et al*., 2013). In chapter 2, MAE was attempted to extract hemicellulosic polysaccharides including arabinoxylan from the CP obtained from industrial corn starch production. The results, however, indicate that MAE was too severe for extraction of arabinoxylan in the higher molecular state and nonspecific degradation of arabinoxylan occurred at the optimum solubilization condition, although carbohydrate yield attained 70.8% of total carbohydrate content in CP. Therefore development of more mild extraction method seems necessary to obtain arabinoxylan from CP. In addition, the content of hemicelluloses in CP produced from industrial corn starch industry was not extremely high and heavily contaminated with lignin, protein and lipid, which hampered selective extraction of hemicellulosic polysaccharides including arabinoxylan.

In this chapter, pure CP was obtained by manual peeling of sweet corn kernels and a new technique mild enough for extraction of hemicellulosic polysaccharides from this CP sample in a higher molecular state was developed by using NaOH-urea solution as

an extraction solvent.

The NaOH-urea solvent system was originally used for rapid dissolution of native cellulose (Cai and Zhang, 2006) and preparation of transparent regenerated cellulose films with excellent properties (Qi *et al*., 2009). However, this solvent system has not been applied for extraction of hemicelluloses yet. Then, in this chapter, the author applied this solvent system for extraction of hemicellulosic polysaccharides present in CP. Feasibility of the isolated hemicelluloses to produce films was also undertaken with characterization of their mechanical properties by tensile test.

3.2. Materials and Methods

3.2.1. Materials

Six types of matured kernels, dent corn, waxy corn, popcorn, flint corn, sweet corn and flour corn, were purchased local shops in Japan. β-(1,3;1,4)-Glucan enzymatic assay kit were purchased from Sigma (St Louis, Missouri, U.S.A.) and Megazyme (Megazyme International Ireland Ltd., Wicklow, Ireland), respectively.

3.2.2. Preparation of corn pericarp and arabinoxylan

Corn kernels were vertically cut into two parts and pericarp of each upper half portion of the excided kernels was manually taken out as CP. CP was treated with hot water (121°C, 1h), and dried over night at 45°C. Standard arabinoxylan was prepared from CP with the modified method of Dervilly *et al*. (2000). 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 from CP.

3.2.3. Compositional analysis

Cellulose and hemicellulose contents were determined according to the method of López-Casado *et al*. (2007). Lipid content was determined from chloroform-methanol extraction. β-Glucan content was determined according to the AOAC official methods of 995.16. Protein, starch, ash and uronic acid contents, and monosaccharide composition were determined as described in section 2.2.2., respectively.

3.2.4. Extraction of corn pericarp with NaOH-urea solution

CP from sweet corn was soaked in 0-8% 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 as described in section 2.2.4.

3.2.5. Preparation and mechanical analysis of films

The hemicelluloses extracted by the NaOH-urea solution, arabinoxylan, (1:1, w/w) 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 maxmum 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.

3.3. Results and Discussion

3.3.1. Chemical composition of corn pericarp

The chemical compositions including relative monosaccharide composition of CP from 6 types of corn kernels were listed in Table 3-1. The results clearly indicate over-all similarity in the gross values. The only notable difference was in the higher β-glucan content in the sweet corn. Hemicellulose and cellulose contents in CP from sweet corn were 69.1-73.6% and 19.7-20.5%, respectively, indicating the abundance of hemicellulose, with less lignin content and lower content in protein and lipid than the corresponding values given in CP obtained from corn starch industry (chapter 2). On the basis of these suitable aspects, CP from sweet kernels was used for clarification of the effects of NaOH-urea solution on extraction of hemiclluloses in this chapter. The present distribution of hemicellulose and cellulose was similar to the values (67% and 18%) reported previously (McKee and Latner, 2000). β-Glucan content in CP (3.2%) was higher than that of corn grain (0.5-1.3%) (Demirbas, 2005), but was comparable to that of oat and barely (3-5%) (Ebringerová *et al.*, 2005). β-Glucan is a hemicellulose characteristic for monocots and is located mainly in the endospermic cell walls. β-Glucan contained in cereal grains is worth mentioning because of its bioactivities

(Ebringerová *et al.*, 2005), such as lowering blood cholesterol (Brennan and Cleary, 2005). Monosaccharide composition of CP (Table 3-1) was similar to that of the maize bran which contained pericarp, tip cap and hull (Saulnier, *et al.*, 1995). Total content of arabinose and xylose in CP amounted over 60%, showing abundance of arabinoxylan as the main hemicellulose in CP.

Table 3-1. Chemical compositions of corn pericarps from 6 types of corn kernels.

Component	Dent corn[a]	Waxy corn[b]	Popcorn[a]	Flint corn	Sweet corn[a]	Flour corn[a]
Chemical compostion (%)						
Cellulose	22.9-23.6	14.0-20.9	21.4-22.5	19.0	19.7-20.5	17.8-25.4
Hemicellulose	66.7-71.1	72.8-74.8	67.6-67.8	62.8	69.1-73.6	62.9-63.9
β-Glucan	0.1-0.3	0-0.2	0.1-0.2	0.1	0.1-3.2	0.2
Starch	0.7-0.9	0.7	0.9	0.7	0.8-3.5	0.4-1.3
Uronic acid	3.9-6.1	3.6-6.2	4.9-5.5	5.4	6.7-8.6	6.5-6.9
Protein	2.9-3.1	3.2-3.9	4.3-5.5	4.5	4.3-5.1	2.5-3.4
Lipid	5.9-6.3	6.4-9.7	8.3-10.0	12.1	4.9-7.2	4.5
Ash	0.4-0.7	0.6-0.7	0.9-1.0	0.7	0.5-1.1	0.7-0.9
Relative monosaccharide composition (%, w/w)						
Xylose	41.6-44.1	44.2-46.6	39.8-43.8	43.6	40.0-44.1	39.4-43.5
Glucose	23.8-25.3	18.8-25.1	23.2-24.1	20.2	28.6-29.7	28.3-31.3
Galactose	5.8-9.0	3.4-7.1	7.0-13.0	8.2	4.7-5.4	5.3-6.2
Arabinose	24.7-25.6	25.9-31.2	23.9-25.1	28.0	21.5-26.1	22.0-24.0

[a] Values are expressed as 2 cultivars.

[b] Values are expressed as 3 cultivars.

3.3.2. Extraction of corn pericarp with NaOH-urea solution

Solubility of CP in 0-8% NaOH solutions containing 0-8 M urea and relative monosaccharide composition of the solubilized materials are listed in Figure 3-1. The solubility of CP in 2% NaOH containing 0-8 M urea was firstly analyzed (Figure 3-1a). The value increased with increase in urea concentration and reached a maximum

(72.2%) at 6 M urea. Subsequently, the solubility of CP and monosaccharide composition of the solubilized materials in 0-8% NaOH solutions containing 6 M urea were analyzed (Figure 3-1a, c). Solubility increased with increase in NaOH concentration and reached a maximum at 2% NaOH with 6 M urea (Figure 3-1b). Addition of urea clearly enhanced the solubility of CP below 4% NaOH. No significant effect, however, was detected above 6% 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% NaOH (Figure 3-1c). The residue was, however, rich in cellulose. Previously arabinose to xylose ratio (A/X) has been shown to affect the solubility of arabinoxylan (Doner *et al*., 1998). With increasing the values of A/X, the solubility increased because of decreasing in intermolecular and intramolecular hydrogen bonds (Höije *et al*., 2008). Our results showed that the value of A/X changed from 0.84 to 0.72 by addition of 6 M urea in 2% 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% NaOH as evident from the higher glucose content of solubilized materials with urea (25.2%) than that without urea (14.9%) (Figure 3-1c, d). Comparable data was previously reported by Bhatty (1993) who showed that 81% of β-glucan was recovered from barley by extraction with 4% NaOH.

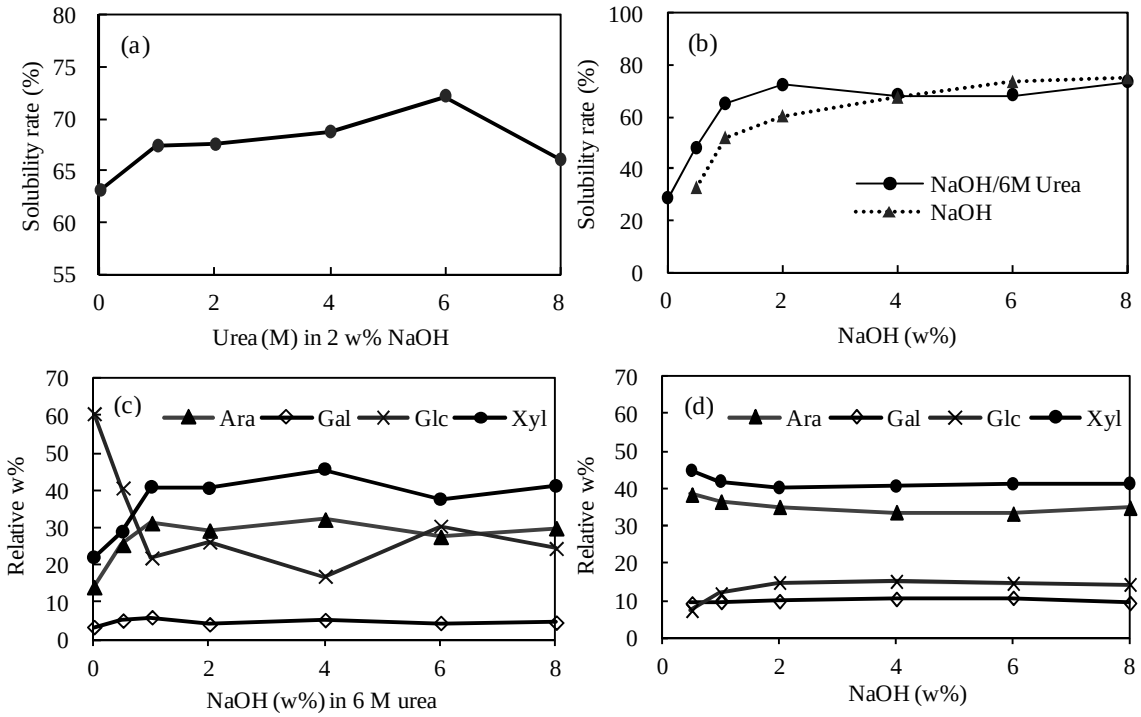

Figure 3-1. Effects of urea (a) and NaOH (b) on the solubility of CP and the carbohydrate compositions of the materials solubilized by extraction with 0-8% NaOH-6 M urea (c) and 0.5-8% NaOH (d).

Typical LV-SEM images of the native CP and the residue after treatment with 2% NaOH-6 M urea are shown in Figure 3-2. 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.

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 (Cai and Zhang, 2006). Cai *et al.* (2008) proposed a solubilization mechanism that the formation of a urea and cellulose-NaOH complex at low temperatures allowed the rapid dissolution of cellulose. The present study also shows that the optimal condition for solubilization of

hemicelluloses in CP is 2% of NaOH in 6 M urea which differed from that of cellulose, 7% NaOH-12% urea-81% water (Cai and Zhang, 2006).

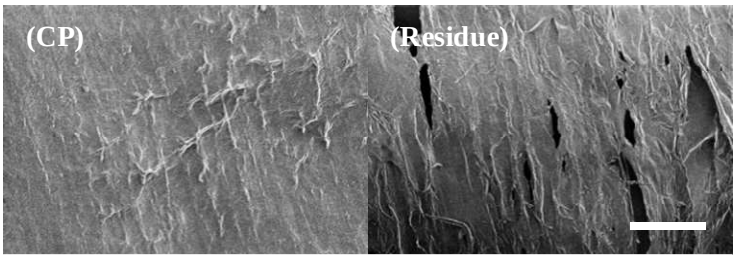

Figure 3-2. Typical LV-SEM images of the native CP and the residue after treatment with 2% NaOH-6 M urea. Scale bar represents 50 μm.

3.3.3. Mechanical properties of films prepared with extracted hemicelluloses

Since the extracted hemicelluloses form transparent films after simple drying of their aqueous solutions, mechanical properties of their films was subsequently analyzed by the tensile test. The representative stress-strain curves are shown in Figure 3-3. The values of breaking stress (σ_{max}), maximum strain (ε_{max}) and elastic modules (E) are summarized in Table 3-2. 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. β-Glucan, with about 70% of (1,4)-linked and

30% of (1,3)-linked D-Glc*p* residues, has been shown to have strong intermolecular hydrogen bonds along the (1,4)-linked units (Ebringerová *et al.*, 2005). On the other hand, arabinoxylan has been shown to possess limited intermolecular hydrogen bonds because of steric hindrance due to highly substituted arabinose residues (Ebringerová *et al.*, 2005). Randomly dispersed β-(1,3)-Glc*p* units among the (1,4)-linked regions have been demonstrated to increase flexibility of the molecule (Wood *et al*., 2003). 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 (2000) 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 (Höije, *et al*., 2008; Zhang and Whistler, 2004; Stevanic, *et al*., 2011). 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 (Zhang and Whistler, 2004), 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 (Höije, *et al*., 2008). In addition, Stevanic *et al.* (2011) reported an interesting result that addiction 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.

Table 3-2. 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 ± SD (*n* = 5).

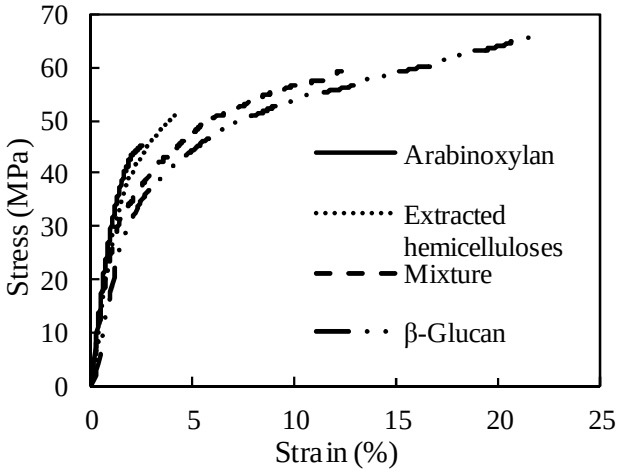

Figure 3-3. Representative Stress-Strain curves of biofilms prepared from arabinoxylan, the extracted hemicellulose containing 4.3% β-glucan content, (1:1) mixture of arabinoxylan and β-glucan, and β-glucan.

3.4. Summary

Extractability of hemicellulosic polysaccharides including arabinoxylan and β-glucan by using NaOH-urea solvent system from CP was firstly found in this chapter. Solubilization rate of CP by the present system attained 72.2% at the condition of 2% NaOH-6M urea. The extracted hemicelluloses 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 the developed system is an attractive technique desirable not only for convenient extraction of hemicelluloses from CP but also for the utilization of the extracted materials as a source of flexible biofilms. Further, similarity of the over-all chemical compositions observed among the CPs isolated from 6 types of the matured corn kernels. The exception was only obsereved in the β-glucan content (3.2%) of CP from sweet corn.

Chapter 4

Water sorption property of corn pericarp arabinoxylan

4.1. Introduction

Quality control of the corn grain during cultivation and harvest is highly important for the corn industry. One of the factors which determines the quality and preservability of foods including corn flour and bran is moisture content regulated mainly by their water vapor sorption properties (Kumar, 1974; Dural and Hines, 1993; Al-Muhtaseb *et al*., 2002). Since CP is the outermost layer which protects the seed from the external environment, the water vapor sorption characteristics of this layer is of principal importance. For example, CP contributes the popping quality of popcorn as the moisture barrier. Pericarp withstands the pressure built during the heating due to the expansion of moisture inside the kernel or its turn to steam (Baker, 1970). Chemically, the hemicellulose portion of CP is mainly composed of arabinoxylan as described in chapters 2 and 3. Because water adsorbability of crystalline cellulose is low (Cristensen and Kelsey, 1959; Ishikura and Nakano, 2005), arabinoxylan seems to act as a determinant role for water vapor sorption property of CP. Therefore, the water vapor sorption characteristic of CP arabinoxylan has to be clarified.

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; 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 CP 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 arabinofuranosidase treatment, 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.

On account of the water vapor sorption property, this behavior is considered as a process of penetrant solution and diffusion in microheterogeneous media and usually described as a concept of the dual sorption theory (Vieth *et al*., 1976). In the field of wood science this process, usually shown by the sigmoid shaped sorption isotherm, has been successfully characterized by the Haliwood and Horrobin (H-H) model (Hailwood and Horrobin, 1946). Detailed descriptions of the H-H model can be found in Skaar (1988). Briefly, in this model the water sorption of woody materials is considered to be composed of the dual sorption processes of the formation of a water monolayer hydrated by hydrogen bonds (hydration process) and the formation of a water polylayer consisting of a solid solution of water in the polymer (dissolution process) through an equilibrium of three chemical species, unhydrated polymer, polymer hydrates and dissolved water. So far, many applications of the H-H model to woody materials have been published (Spalt, 1958; Skaar, 1972; 1988; Okoh and Skaar, 1980; Simpson, 1980; Hill *et al*., 2009; Yasuda *et al*., 1994; Yamamoto *et al*., 2005; Zaihan *et al*., 2009). For the constituents of wood, Cristensen and Kelsey (1958; 1959) analyzed the detailed water sorption behavior of cellulose, hemicellulose and lignin and concluded that approximately 80% of water sorption is attributed to hemicellulose and cellulose. The

hydration sites of cellulose are only present in the non-crystalline region because strong hydrogen bonding within the crystalline region prevents access by the water molecules (Cristensen and Kelsey, 1959; Ishikura and Nakano, 2005). Acetylation of glucuronoxylan decreased its water sorption capacity (Gröndahl *et al*., 2003) in comparison to other polymers (Krevelen and Hoftyzer, 1976).

However, the physical presence of water in wood cannot be explained by the H-H model alone (Hartley et al., 1992) because the model does not deal with the exposure of new hydration sites as a polymer swells due to water sorption, or the resistance of the structure to swelling, all of which lead to difficulties in describing the dissolution process. Nakano (2006) has, therefore, improved a dual gas sorption model (Koros and Paul, 1978) to describe the water vapor sorption behavior of woody materials strengthened at the dissolution process by a linear combination of the Langmuir and Henry's equations. His model differs from the H-H model in that the hydration process and dissolution process are linearly dealt with independently, so that we designated this new model as the independent dual sorption model. By using this sorption model, it is possible to investigate the detailed water sorption characteristics of arabinoxylan associated with its chemical and physicochemical properties.

By taking these problems into consideration, in this chapter, therefore, preparation of aranbinoxylans different in degree of arabinose substitution was firstly carried out by partial acid hydrolysis of the arabinoxylan extracted from CP. Because of necessity of production of 10 g order purified arabinoxylan in order to characterize properties of arabinoxylan, CP obtained from corn starch industry was used as a raw material. Regression analysis with the method of least squares has been applied to describe the removal of arabinose from the arabinoxylan. Next, their chemical and physicochemical

properties of CP arabinoxylan were investigated in detail. Finally, the role of arabinose substituents for water adsorption behavior of arabinoxylan was comparatively investigated on basis of the independent dual sorption model and the H-H model.

4.2. Materials and Methods

4.2.1. Materials

CP 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.

4.2.2. Preparation of arabinoxylan

Arabinoxylan was prepared from CP as described in section 3.2.2. with slight modification. CP was treated three times with dimethyl sulfoxide at room temperature to remove starch, and the residue was extracted three times with 4% KOH for 24 h at room temperature. The extracted solution was treated by graduated ethanol precipitation. The materials precipitated between 30-50% EtOH were obtained as arabinoxylan.

4.2.3. Preparation of partially degraded arabinoxylans

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 CP arabinoxylan samples.

4.2.4. Chemical analysis

The monosaccharide composition of the arabinoxylan samples was analyzed as described in section 2.2.2. The molecular weight distribution of the arabinoxylan samples was analyzed with size exclusion chromatography on a column (8 × 50 cm) 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 θ (5-40°) was conducted with speed of 2°/min. JMP 9 (SAS institute Inc.) was used for data analysis.

4.2.5. Characterization of water adsorption behavior

Water adsorption isotherms of all arabinoxylan samples were determined by the gravimetric method. Arabinoxylan samples were vacuumed-dried over night at 70°C, and weighted. The water vapor sorption experiments were conducted for over 3 weeks at 20°C under 0-8% relative humidity (RH); conditioned with 14 kinds of saturated salt solutions consisting of LiCl (11% RH), CH_3COOK (25% RH), $MgCl_2$ (33% RH), $CaCl_2$ (40% RH), K_2CO_3 (43% RH), $Mg(NO_3)_2$ (53% RH), NaBr (63% RH), NaCl (75% RH), $SrCl_2$ (77% RH), $(NH_4)_2SO_4$ (82% RH), KCl (87% RH), $BaCl_2$ (92% RH), KNO_3 (96% RH), and K_2SO_4 (98% RH). Then the water vapor sorption behavior was characterized by the independent dual sorption model (equation 4-1) and the H-H model (equation 4-2), respectively:

$$M = M_H + M_D = \frac{M_0 b p_0 H}{100 + b p_0 H} + \frac{1800}{W}\frac{K_D H}{100 - K_D H} \quad \cdots \quad (4\text{-}1)$$

$$M = M_H + M_S = \frac{1800}{W}\frac{K_1 K_2 H}{100 + K_1 K_2 H} + \frac{1800}{W}\frac{K_2 H}{100 - K_2 H} \quad \cdots \quad (4\text{-}2)$$

where M is the equilibrium moisture content (w%) at a given RH, M_H is the moisture content (w%) associated with water of hydration, M_D and M_S are the moisture contents (w%) associated with dissolved water for the independent dual sorption model and the H-H model, respectively. M_0 is the saturation concentration (w%) in Langmuir sorption process, b is hole affinity constant (KPa^{-1}) and p_0 is the saturated water vapor pressure (KPa), H is the relative humidity (w%). K_D is Henrry's parameter. W is molecular weight of polymer per mole of water sorption sites. K_1 is the equilibrium constant between water of hydration and dissolved water, and K_2 is the equilibrium constant between water vapor and dissolved water. The detailed independent dual sorption model used in this study can be found in Nakano (2006). In this study, the moisture contents under 22-43% (RH) and 88-95% (RH) were used as sorption data at low humidity and high humidity, respectively, for calculation of the independent dual sorption model. The values M_0, b and K_D are determined by plotting H/M against H to give a linearity of the form shown in equations 4-3 and 4-4.

$$\frac{H}{M_H} = \frac{1}{M_0 b} + \frac{1}{M_0} H \quad \cdots \quad (4\text{-}3)$$

$$\frac{H}{M_D} = \frac{W}{1800}\frac{1}{K_D} - \frac{W}{1800} H \quad \cdots \quad (4\text{-}4)$$

4.3. Results and Discussion

4.3.1. Removal of arabinose substituents from corn pericarp

The relative monosaccharide compositions of the untreated arabinoxylan and the partially acid-hydrolyzed arabinoxylans are listed in Table 4-1. The raw CP 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 CP arabinoxylan 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.*, 2007b; 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 4-1), indicating effective removal of arabinose residues in accord with the observation reported by Whistler and Corbett (1955) and Whistler and BeMiller (1956).

Table 4-1. Relative monosaccharide compositions of the untreated and partially hydrolyzed arabinoxylans.

Hydrolysis condtions		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
		Acidic 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

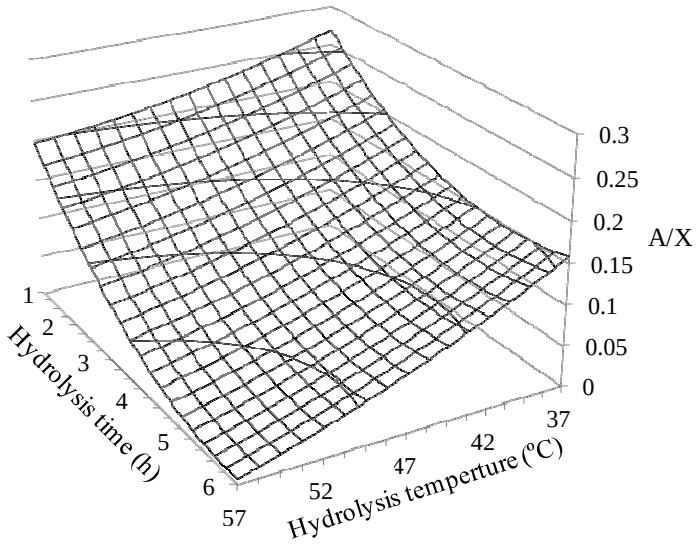

Figure 4-1. Effects of hydrolysis temperature and time on removal of arabinose from corn pericarp arabinoxylan.

The effects of the hydrolysis temperature and time on the extent of removal of arabinose from the CP arabinoxylan were further evaluated by regression analysis with the method of least squares (Figure 4-1). When the value of A/X was used as the degree of removal of arabinose, the regression model was expressed as in the following equation:

$$Y = 7.087\times10^{-1} - 5.357\times10^{-2}\ X_1 - 1.421\times10^{-2}\ X_2 - 7.533\times10^{-4}\ X_1X_2 + 8.373\times10^{-3}\ X_1\ X_1 + 1.189\times10^{-4}\ X_2X_2$$

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 4-2). 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 CP arabinoxylan. Thus, the partial acid hydrolysis conditions for the preparation of CP 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 4-1).

Table 4-2. 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	0.0075
Residual	3	0.0010	0.0003		
total	8				
R^2	0.98				

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 CP arabinoxylans in water was analyzed (Table 4-3). The results showed fairly good solubility was still maintained ~~in~~ for the arabinoxylans with low A/X ratios, but a clear trend towards decreasing solubility with lowering the A/X ratios is evident.

The molecular weight distributions of the native 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 4-2). 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 CP arabinoxylan (Table 4-3). 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 CP arabinoxylan indicates that hydrolysis at lower temperature for longer time resulted in removal of arabinose substituents with less degradation of the main xylan chain.

Next the crystalline properties of the CP arabinoxylans were analyzed by X-ray diffraction (Figure 4-3). All X-ray diffraction patterns showed a broad peak centered at around 18°, indicating the presence in amorphous state. Dea *et al.* (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 CP arabionoxylans, only the untreated one could form a film after simple drying of its aqueous solution. All the samples of desubstituted CP arabinoxylans had lost the ability to form films probably due to their low molecular weights.

Table 4-3. Effects of the acid hydrolysis of arabinoxylan on solubility in water.

Hydrolysis condtions		Solubility (%)	A/X
Temperature (°C)	Time (h)		
Untreated arabinoxylan		99.1	0.35
		Acid hydrolysis	
37	1	93.1	0.26
47	3	88.0	0.12
57	3	86.2	0.05

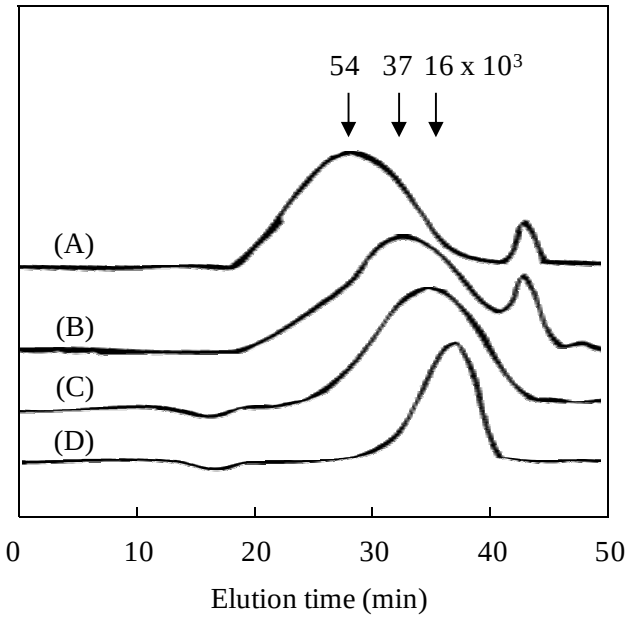

Figure 4-2. Molecular weight distributions of corn pericarp arabinoxylan samples; untreated arabinoxylan (A) and arabinoxylan hydrolyzed at pH 1.0 and 37°C for 1 h (B), at 47°C for 3 h (C) and at 57°C for 3 h (D). The A/X ratios of arabinoxylan samples are 0.35 (A), 0.25 (B), 0.12 (C) and 0.03 (D).

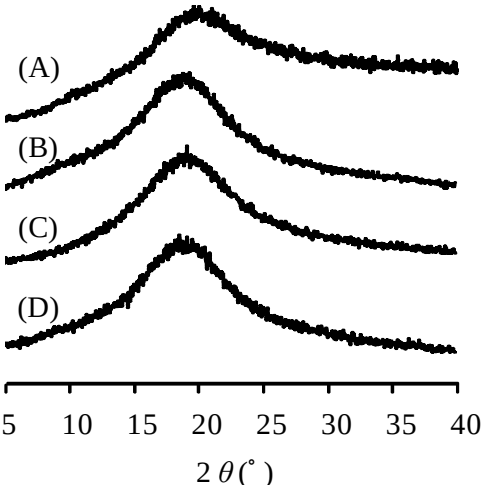

Figure 4-3. X-ray diffraction patterns of arabinoxylan samples; untreated arabinoxylan (A) and arabinoxylan hydrolyzed at 37°C for 1 h (B), at 47°C for 3 h (C) and at 57°C for 3 h (D). The A/X ratios of arabinoxylan samples are 0.35 (A), 0.25 (B), 0.12 (C) and 0.03 (D).

4.3.2. Water adsorption behavior of corn pericarp arabinoxylan

All CP arabinoxylans having different arabinose content showed sigmoidal shaped adsorption isotherms grouped in IUPAC Type II similar to those of hemicelluloses isolated from softwood and hardwood (Sadoh 196; 1962). The profiles except that of AX-03 which had the lowest A/X value of 0.03 are shown in Figure 4-4. Previous results on corn flour and bran also showed similar sigmoidal shaped sorption isotherms (Kumar, 1974; Dural and Hines, 1993). The author did not, however, further mentioned about characterization of the isotherm of AX-03 because of necessity of taking contribution of terminal residues into consideration due to its lowness in molecular weight of the order of 10^3. When the isotherms given at low and high RHs were analyzed individually, the behavior of arabinoxylans observed at low RH was similar to hemicellulose, while the behavior at high RH was shifted to somewhat higher region than the level of hemicellulose (Sadoh, 1961; 1962). In addition, Sadoh (1962) has also reported the presence of a slight difference in water sorption behaviors between hemicelluloses from softwood and hardwood at high humidity. Degree of steepness of the increase in sigmoidal isoforms constantly evident at higher RH in all CP arabinoxylans was similar to that of wood hemicellulose but substantially higher than those of cellulose and lignin (Cristensen and Kelsey, 1959). These results suggest that the type of hemicellulose affects its water sorption behavior especially at high relative humidity. Indeed, moisture content of CP arabinoxylans increased with increase in the A/X ratio at high humidity conditions (Table 4-4) in good agreement with the water absorption of rye arabinoxylan films (Höije *et al*., 2008). On the other hand, inverse relationship was slightly observed at low humidity. These results suggest that the effects of degree of substitution in arabinoxylan on their water sorption behavior are

specifically detectable at high relative humidity.

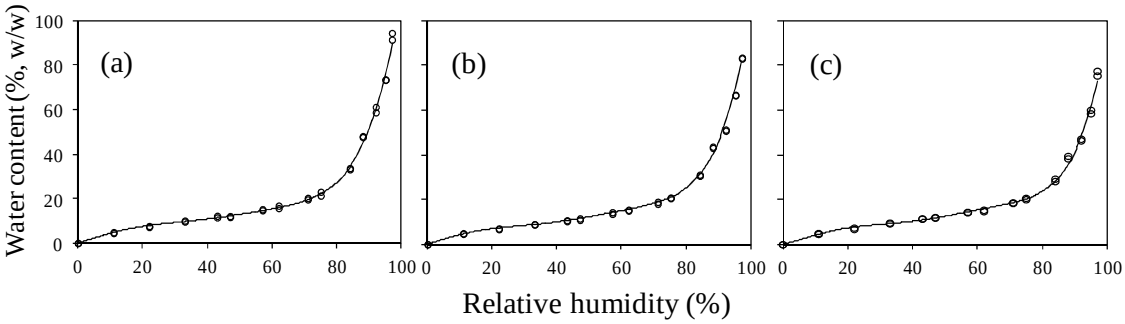

Figure 4-4. Effects of degree of arabinose substitution on water vapor sorption isotherm of corn pericarp arabinoxylans; the values of A/X ratio are (a) 0.35, (b) 0.26 and (c) 0.12, respectively.

Table 4-4. Effects of the degree of arabinose substitution on the moisture content of arabinoxylan samples at low (11%), middle (62%) and high relative humidity (97%).

Material	Moisture content (w%)		
	Relative humidity		
	11 %	62 %	97 %
AX-12	4.84	15.11	76.58
AX-26	4.80	15.25	83.35
AX-35	4.69	16.26	93.05

For further characterization of the water vapor sorption behavior of CP arabinoxylans, the experimental results were fitted by using two models, the independent dual sorption model (Nakano, 2006) and the H-H model (Hailwood and Horrobin, 1946). The data in the two different moisture content regions of low humidity (22-43%, RH) and high humidity (88-95%, RH) were used for interpretation according to the independent dual sorption model, while whole data throughout 0-98% RH were

included in the H-H model. The parameters and coefficient correlation value of fitting the independent dual sorption model and H-H model were listed in Table 4-5. The results indicate that both regression models showed good fit to all experimental results with high correlation values (R^2) of above 0.93.

Firstly, the data in the former regions were described by the independent dual sorption model. The value of modified affinity constant (b) for the native CP arabinoxylan (89.46 KPa^{-1}) decreased with decrease in the degree of arabinose substitution (lowering in the A/X ratio). Because b represents the affinity of the water for hydration sites in arabinoxylan (Koros and Paul, 1978), the present results implies that an increase in the degree of arabinose substitution in arabinoxylans increased its affinity to water molecule. Similarly the composite parameter, K (= $M_0 b/100K_D$), which implies the magnitude of Langmuir sorption relative to Henry's law sorption (Koros and Paul, 1978), decreased with lowering in the A/X ratio. These parallel alleles implies that removal of arabinose pendants from the main (1,4)-linked xylan chain gradually reduces the Langmuir capacity of the CP arabinoxylan.

Inversely, the value of M_0 which is interpreted as a site capacity constant or a value of saturated concentration for Langmuir sorption process originally given as 24.24% for arabinoxylan with A/X ratio of 0.35 increased with decrease in the A/X ratio (Table 4-5). The present estimated values of saturated concentration were substantially higher than the previous results such as 10.4 for Japanese cypress, 10.8 for Japanese cedar, 9.7 for Japanese red pine, 10.7 for Japanese beech, 10.4 for Shinanoki, 9.9 for Makanba (Sadoh 1961; 1962), 5.10-5.60% for sitka spruce (Yasuda *et al*., 1994; Hill *et al*., 2009), and 2.65-4.53% for natural fibers (Hill *et al*., 2009). Indeed the values of M_0 as saturated concentration for Langmuir sorption in the present cases are desirably

obtained around 10% based on the isotherms shown in Figure 4-4. This discrepancy may be originated from mismatching of the estimation according to Nakano (2006). Further research is necessary for correction.

By using the H-H model, the values of K_1 showed no apparent relationship with the degree of arabinose substitution in arabinoxylan. The previously reported values for K_1 varied in a range within 8.21-28.80 for woods, 4.38-5.87 for oil palm trunk fibers, 3.30-5.24 for bamboo and 3.29-14.07 for acetylated wood (Spalt, 1958; Yamamoto *et al.*, 2005). The present values for the CP arabinoxylans (11.18-12.26) were closer to the woods than monocotyledonous materials.

Secondly, the data in the high humidity region were described by the individual dual sorption model. The Henrry's parameter (K_D) in the independent dual sorption model (equation 4-1) is corresponding to the equilibrium constant of K_2 in the H-H model (equation 4-2). Both Henrry's parameter (K_D) and equilibrium constant of K_2 were estimated at 0.90-0.89 and 0.95-0.92, respectively, for the CP arabinoxylans. These values were in the similar order of the K_2 values previously reported ranging within 0.750-0.83 for woods, 0.89-0.95 for oil palm trunk fibers, 0.81-0.84 for bamboo, and 0.73-0.86 for acetylated wood (Spalt, 1958; Yamamoto *et al.*, 2005; Zaihan *et al.*, 2009; 2011). The number of hydration sites in arabinoxylans was represented as $1/W$ in the independent dual sorption and H-H models (Table 4-5). By using the independent dual sorption model, the calculated number of hydration sites decreased from 0.00707 to 0.00587 with a decrease of the degree of arabinose substitution, while no trend could be shown in the H-H model. The value of number of hydration sites has been reported ranging within 0.0027-0.0037 for wood, 0.0015 for acethylated wood and 0.0023-0.0029 for bamboo (Spalt, 1958; Yamamoto *et al.*, 2005; Zaihan *et al.*, 2009,

2011).

The water sorption behavior of polymers at high humidity is depended on their physicochemical properties, such as intermolecular association of polymers and the area (free volume) available for water molecule to adsorb in the polymer matrix (Hartley and Peemoeller, 1992). Nakano (2003) analyzed the effects of size of woody samples on the dissolution process and reported that the moisture content of powder samples was higher than block samples at high humidity because of restrictions of swelling by tight cell-wall structure in the latter cases. The present results suggest that the higher branched arabinoxylans are more susceptible to swelling and, thus, have much more free volume in the matrix than the lower branched ones. This interpretation was in good agreement with the previous findings that the substituted arabinose in arabinoxylan prevents intermolecular association of xylan chains (Höije *et al*., 2008) and induces to take more extended conformation (Andrewartha *et al.,* 1979).

Based on the results presented above, it will suffice to conclude that the independent dual sorption model is desirable for characterization of the water sorption behavior of arabinoxylans in comparison with the H-H model, although improve in a direction to attain more precise estimation of the saturated concentration (M_0) for Langmuir sorption in the former model is desirable. The results also opened applicability of the independent dual sorption model to characterize physicochemical properties of a wide variety of the related branched natural polymers.

Table 4-5. The parameters and coefficient correlation values of the individual dual sorption model and the H-H model.

Analysis model		Parameter	Material		
			AX-35	AX-26	AX-12
Independent dual sorption model	Low humidity	b, KPa^{-1}	89.46	60.08	40.84
		M_0, %	24.24	28.68	39.56
		R^2	0.99	1.00	0.99
	High humidity	K_D	0.90	0.89	0.89
		$1/W$, g^{-1}	0.007070	0.006691	0.005870
		R^2	1.00	0.93	0.99
	$M_0 b/100K_D$	K	24.21	19.42	18.16
Hailwood & Horrobin model		K_1	12.26	11.18	11.21
		K_2	0.95	0.95	0.92
		$K_1 * K_2$	11.66	10.56	10.35
		$1/W$, g^{-1}	0.003852	0.003697	0.003864
		R^2	0.97	0.98	0.96

4.4. Summary

In this chapter, the role of arabinose substituents of arabinoxylan for its water sorption properties was discussed. Preparation of the CP arabinoxylan with removal of arabinose substituents was firstly 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 from native arabinoxylan (A/X ratio 0.35 and mean molecular weight of 53.6×10^3). All CP arabinoxylans were in the amorphous state and the film forming ability of the native state was lost after partial acid hydrolysis. The decrement in the degree of arabinose substitution decreased its solubility.

All arabinoxylan samples gave similar sigmoid shaped adsorption isotherms grouped in IUPAC type II. When isotherms were analyzed on the basis of the independent dual sorption model and the H-H model, the former model was found to be usable for demonstrating that removal of arabinose pendants from the main (1,4)-linked xylan chain gradually reduces the Langmuir capacity of the CP arabinoxylan. Results may provide crucial information for industrial utilization of arabinoxylan and indicate the future applicability of the independent dual sorption model to characterize hydration behavior of the other kind of branched polymers.

Chapter 5

Extraction of β-(1,3;1,4)-glucan from corn pericarp

5.1. Introduction

Commercially important cereal grains, such as oat, barley, rye, wheat and corn grains, are commonly known to contain β-glucan whose content varied in ranges of 1.8-8.5% for oat (Wood, 2010; Wood and Beer, 1998), 3-11% for barley (Wood and Beer, 1998), 1-3.4% for rye (Roubroeks and Åman, 2000), 0.18-1.8% for wheat (Pritchard *et al*., 2011) and 0.5-1.3% for corn (Demirbas, 2005). Presence of β-glucan in the grain is rather localized in the cell-walls of storage tissues, such as aleurone layer and endosperm (Wood *et al*., 1983; Philippe *et al*., 2006; Ebringerová *et al*., 2005).

The functionalities of β-glucan have increased the popularity and consumption of cereal-based foods and stimulated the research of its extraction and concentration. Previously, fractions rich in β-glucan have been obtained from cereal grains by dry milling processes (Kiryluk *et al*., 2000) or solvent-extraction procedures (Ahmad *et al*., 2009; Beer *et al*., 1996; Bhatty, 1993; Wood *et al*., 1989; 2003). The key extraction method of β-glucan has been developed by Wood *et al.* (1989), that is a treatment with sodium carbonate solution at pH 10 to give a preparation of 78% β-glucan from oat flours. Although these approaches have resulted in concentrates or isolates containing high purity β-glucan (up to 95%) (Lazaridou and Biliaderis, 2007), the extraction rate of β-glucan has been reported in range from 16.6 to 85% (Benito-Román *et al*., 2011).

The NaOH-urea solvent system allowed increasing extractability of hemicelluloses including β-glucan from CP at 2% NaOH-6 M urea under low temperature as described in chapter 3. In this chapter, the condition of this solvent system was optimized for

extraction of β-glucan present in CP. Additionally, effects of cellulose crystallinity on extrability of β-glucan were investigated.

5.2. Materials and Methods

5.2.1. Materials

CP was prepared from sweet corn kernel as described in section 3.2.2. Barley flour and oat meal were purchased in a local supermarket in Japan. Water soluble materials were extracted with hot water (121°C) for removal of starch. Water insoluble materials were recovered by centrifugation (3000 rpm, 20°C, 10 min), and dried over night at 55°C.

5.2.2. Extraction of β-(1,3;1,4)-glucan

β-Glucan was extracted from CP, water-soluble free barley flour and water-soluble free oat meal with 2% NaOH-6 M urea solution as described in section 3.2.4. with slight modification. Samples were soaked in 2% NaOH solution containing 6 M urea at the fixed liquid to solid ratio of 125 mL/g. Solutions were freezed at -20°C over night and thawed at room temperature. After repetition of freezing and thawing treatments (0-7 times), the solutions were filtrated to remove insoluble residues. The solubilized fraction was neutralized with acetic acid, dialyzed against water and subjected to ethanol precipitation. The precipitated materials were recovered by filtration, and dried over night at 45°C after thorough washing with ethanol and acetone.

CP was extracted with 3 different solvents which have previously applied for extraction of β-glucan from oat under the fixed liquid to solid ratio of 125 mL/g; a solution whose pH was adjusted to 10 with 20% Na_2CO_3 (45°C for 30 min) according to

Wood *et al*. (1989), 1 M NaOH (at room temperature for 5 h) according to Ahmad *et al*. (2009), and 1 M HCl containing 0.09 g/mL of KCl (at room temperature for 1 h) according to Batty *et al*. (1991).

CP was powdered by ball milling for 1-10 min (Model JFC-300 Cryogenic Sample Crusher, Japan Analytical Industry Co., Ltd.) with use of a set of a titanium pot-type cell (capacity 12 mL) and a tungsten carbide steel ball under liquid nitrogen. Powdered CP samples were extracted with hot water (90°C), dilute alkaline solution adjusted to pH 10 with 20% Na_2CO_3 at 50°C, 2% NaOH, and 2% NaOH in 6 M urea each 24 h at room temperature. Solubilized materials and residues were recovered as described above.

Content of β-glucan was determined as described in section 3.2.3. and β-glucan extraction rate was determined by an equation:

β-Glucan extraction rate (%) = 100 × (1- (*amount of β-glucan in residue*/ *amount of β-glucan in raw material*))

5.2.3. Chemical analysis

The monosaccharide composition and molecular weight distribution of solubilized materials were investigated as described in section 2.2.2. and section 4.2.3., respectively. X-Ray diffraction profiles of residues were measured as described in section 4.2.3. The crystalinity of cellulose was determined by following equation according previous report (Goto *et al*., 1985):

Cellulose crystallinity (%) = 100 × (*An intensity of a peak originated cellulose crystalline plane* (002) - *An intensity of a peak at* 18°)/(*An intensity of a peak originated cellulose crystalline plane* (002))

5.3. Results and Discussion

5.3.1. Optimum condition of extraction of β-(1,3;1,4)-glucan

CP was soaked in 2% NaOH solution containing 6 M urea, and the mixtures were freeze (-20°C)-thawed for 0-7 times. Solubility initially depressed from the value without the freeze-thaw treatment (78.2%), but gradually increased by repeating the freeze-thaw treatment, and attained 79.7% after seven treatments (Figure 5-1a). The results of sugar compositional analysis indicate that the extracted materials were mainly composed of hemicellulosic polysaccharides consisting mainly of arabinoxylan with 4.3-6.1% of β-glucan (Table 5-1). The amount of extracted β-glucan did not show depression by the freeze-thaw treatmet, increased evidently from the value without the freeze-thaw treatment (75.7%) until three treatments (97.8%), and showed a plateau between three to seven treatments, attaining the maximum value at seventh treatment (98.2%) (Figure 5-1b). The inclement of the extraction rate of β-glucan induced by the freeze-thaw treatments in the presence of 6M urea was estimated as 22.1% and 22.5% after three and seven repetitions, respectively.

In the cases of NaOH alone, both solubility and extraction rate of β-glucan gradually increased from the values of 67.1% and 67.0% to 75.0% and 85.4%, respectively, after three repetitions of the freeze-thaw treatment (Figure 5-1a, b). By comparing the results obtained by addition of urea, inclement evoked by inclusion of 6 M urea was observed 11.1% in solubility and 8.7% for extraction rate of β-glucan

without freeze-thaw treatment. However, additional promoting effects induced by the freeze-thaw treatment were clearly observed only in the extractability of β-glucan, the inclement being calculated as 10.7% after seven repetitions. Namely, the inclement of the extraction rate of β-glucan induced by the freeze-thaw treatments of the simple 2% NaOH solutions was estimated as 18.4% and 20.5% after three and seven repetitions, respectively, cloth to the corresponding values obtained by the inclusion of 6M urea as above. Enhancement of the extraction rate of β-glucan by addition of urea may be originated from splitting hydrogen bonds induced by urea at peripheral region of β-glucan molecules.

5.3.2. Effects of NaOH-urea solvent system on cellulose crystallinity

The X-ray diffraction and carbohydrate analyses indicate that the residues remained after these treatments were mainly cellulose. Immersion of CP in both 2% NaOH and 2% NaOH-6 M urea solutions at room temperature did not clearly disrupt the crystalline property of cellulose in CP (Figure 5-2). One freeze-thaw treatment was, however, enough to disrupt the natural cellulose I crystalline allomorph of CP (Figure 5-2). These results suggest that the increase in extractability of β-glucan for approximately 20% induced by the freeze-thaw treatment is ascribable to disruption of crystalline property of the cellulose in CP.

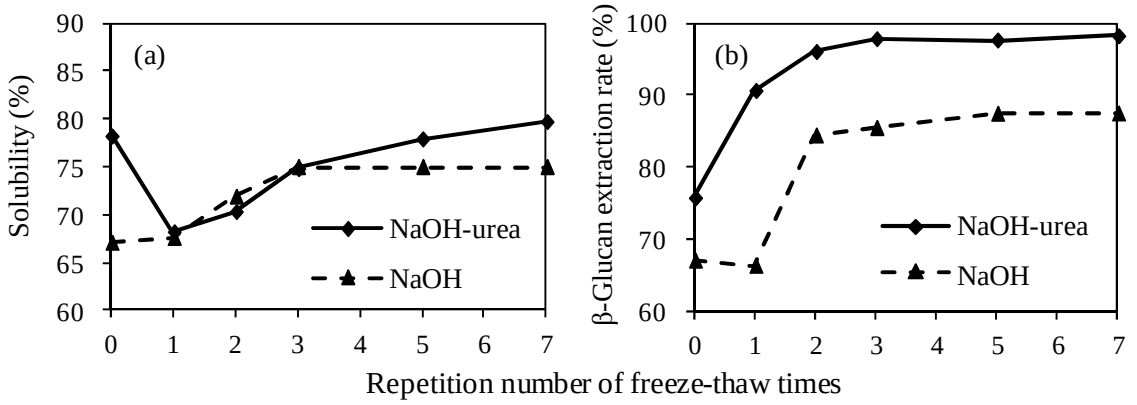

Figure 5-1. Effects of repetition of freeze-thaw treatments of NaOH-urea solvent system on solubility (a) and β-glucan extraction (b).

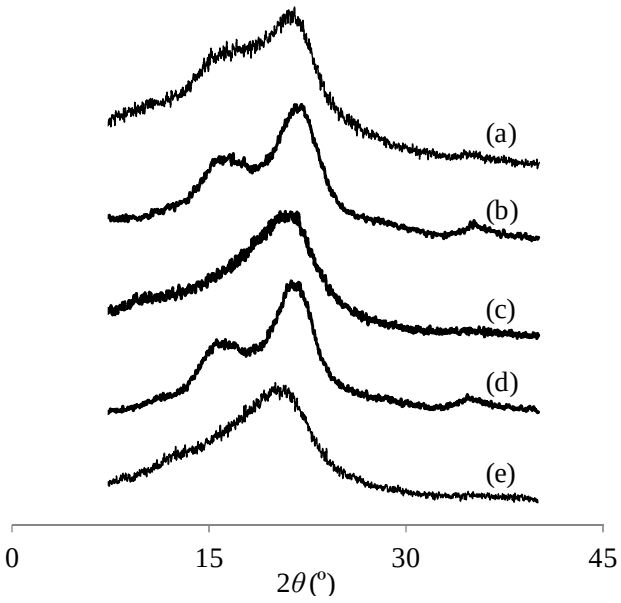

Figure 5-2. X-ray diffraction patterns of (a) native corn pericarp, residues (b) without and (c) with one freeze-thaw treatment with 2% NaOH-6M urea, (d) without and (e) with one freeze-thaw treatment with 2% NaOH.

Table 5-1. Effects of NaOH-urea solvent system on molecular weight and sugar composition of solubilized materials.

	Freeze-thaw times	Molecular weight ($\times 10^4$ Da)	Relative monosaccharide composition (%, w/w)				β-Glucan content (%)	β-Glucan extraction rate (%)
			Ara	Gal	Glc	Xyl		
Corn pericarp	-	-	26.1	5.4	28.6	40.0	3.2	–
NaOH-urea	0	50.2	33.8	6.2	7.8	52.2	4.3	75.7
	3	55.4	31.0	6.3	16.6	46.2	5.7	97.8
NaOH	0	49.0	35.5	7.0	6.8	50.7	4.8	67.0
	3	50.0	32.1	7.0	14.4	46.5	6.1	85.4

For further investigation of the effects of crystalline property of cellulose on extractability of β-glucan, CP was pre-treated with ball-milling to disrupt crystallinity of the cellulose in CP. The rate of β-glucan extraction with 4 kinds of solvents (hot water, Na_2CO_3, NaOH, NaOH-urea) increased with decrease in crystallinity (Table 5-2). Again the NaOH-urea solvent gave the highest extractability of β-glucan from CP. These results clearly indicate the existence of firm interactions between β-glucan and cellulose in CP and thus disruption of cellulose resulted in enhancement of the solubility of β-glucan. Lazaridou *et al.* (2004) showed that cereal β-glucan has cellulose-like blocks of (1,4)-linkage sequences longer than the cellotetraose (up to 9-14 glucopyranosyl residues were observed). These oligomeric cellulose-like regions in β-glucan might allow the strong interaction with cellulose.

Similar disruption of the crystalline property of cellulose was known to occur by the freeze-thaw treatment with 8-10% NaOH solution (Isogai and Atalla, 1998). The NaOH-urea solvent could dissolve cellulose completely and rapidly at optimum concentration of 7-8% NaOH / 11-12% urea at low temperature (Cai and Zhang, 2006). The formation of a urea and cellulose-NaOH complex at low temperatures seems to

allow splitting of not only the intermolecular and intramolecular hydrogen bonds of cellulose (Cai *et al*., 2008) but also inter molecular hydrogen bonds operated between cellulose and β-glucan leading to dissolution of β-glucan.

Table 5-2. Effects of cellulose crystallinity on extrability of β-glucan.

Ball milling time (min)	Crystallinity (%)	β-Glucan extraction rate (%)			
		Hot water	Na_2CO_3	NaOH	NaOH-urea
0	19.8	-	-	67.0	75.7
1	15.7	34.6	31.1	71.4	88.4
2.5	14.4	43.3	35.6	80.3	88.8
5	13.1	41.0	48.5	81.7	89.7
10	11.0	44.6	55.6	85.6	93.1

5.3.3. Comparison of NaOH-urea solvent system with other solvents

The content of β-glucan in the solubilized materials with the 2% NaOH-6 M urea solution was comparatively low (5.7%) because of contamination win arabinoxylan (Table 5-1). It has been reported that the materials extracted with NaOH and Na_2CO_3 (pH 10) contain β-glucan at 59.7-57.1% and 54.7-72.9% from oat or barley, respectively (Bhatty, 1993; Beer *et al*., 1996; Wood *et al*., 1989; Burkus and Temelli, 1998).

The molecular weight of the solubilized materials (49.0-55.4 × 10^4 kDa) was slightly affected by addition of urea to the NaOH solution and repetition of the freeze-thaw treatment (Table 5-2). Comparative data have been reported for arabinoxylans from corn fiber (0.93-30.0 × 10^4 kDa) (Yadav *et al*., 2008) and for β-glucans from oat (3-6.5 × 10^4 kDa) and barley (15-250 × 10^4 kDa) (Ebringerová *et al*., 2005).

For extraction of β-glucan from CP, the NaOH-urea solvent system was superior to

the other solvents previously applied for oat and barley (Table 5-3). Moreover, this solvent system also allowed the effective extraction of β-glucan from oat meal (82.7%) and barley flour (92.5%), giving materials which are rich in β-glucan (8.0% for oat and 11.2% for barley). Much lower extraction rates were reported previously; extraction of 49.5-70% β-glcuan with Na_2CO_3 solution from oat and barley (Bhatty, 1993; Burkus and Temelli, 1998), 78.1-84% extraction for barley β-glcuan with NaOH solution (Ahmad *et al*., 2009; Bhatty, 1993), and 44.7% extraction with HCl solution from barley (Bhatty *et al*., 1991).

Table 5-3. Comparable data of NaOH-urea solvent system and previous β-glucan extraction solvents.

Sample	Solvent	Solubility (%)	β-Glucan extraction rate (%)
Corn pericarp			
	NaOH-urea	74.3	97.8
	1 M NaOH	66.0	65.0
	Na_2CO_3, pH 10	5.9	20.4
	1 M HCl	5.5	16.2
Oat meal			
	NaOH-urea	82.8	82.7
Barley flour			
	NaOH-urea	78.1	92.5

5.4. Summary

Three repetitions of the freeze (-20°C)-thaw treatment allowed extraction of almost all β-glucan (97.8%) present in CP. Disruption of cellulose crystalline property was prerequisite for extraction of β-glucan fraction amounting approximately 20% which was firmly interacted with cellulose. Availability of this technique to extract β-glucan

from oat meal and barley flour in high rates could also be shown, 82.8% for the former and 92.5% for the latter. The present NaOH-urea solvent system with repetition of the freeze-thaw treatments may be a new versatile extraction technique for extraction of β-glucan from cereal materials.

Chapter 6

Isolation and properties of corn pericarp β-(1,3;1,4)-glucan

6.1. Introduction

For development of a new innovating utilization of corn starch residues a technique for refinary of β-glucan from CP is desirable. In the previous chapters 3 and 5, the author demonstrated the effectiveness of a NaOH-urea solvent system for extraction of hemicellulosic polysaccharides including arabinoxylan and β-glucan from CP. Especially the latter polysaccharide could be almost quantitatively (97.8%) solubilized in 2% NaOH-6 M urea solution by three repetitions of freeze-thaw treatment. Then the final step is isolation of β-glucan by separation of large amount of coexisting arabinoxylan. Previously, rather tedious steps were usually required for isolation of β-glucan from monocotyledonous crops (Ahmad *et al*., 2010; 2009; Beer *et al*., 1996; Bhatty, 1993; Burkus and Temelli, 1998; Wood *et al*., 1989; Izydorczyk et al., 1998; Morgan and Ofman, 1998). Therefore, in this chapter the author intended to develop a convenient purification method specific for CP β-glucan by using affinity chromatography on a cellulose column and present chemical properties of the isolated β-glucan.

6.2. Materials and Methods

6.2.1. Materials

CP was prepared from kernels of sweet corn as described in section 3.2.2. and treated with hot water (121°C) for 1h. A mixture of CP hemicelluloses (CPHs) consisting of 40.5% of xylose, 29.2% of arabinose, 26.2% of glucose and 4.1% of

galactose was prepared by extraction of CP with 2% NaOH-6 M urea in a yield of 74.8% as described in section 5.2.2. β-Glucan from barley (>95%) was purchased from Sigma (St Louis, Missouri, USA). Amounts of β-glucan and starch were determined as described in section 3.2.3 and 2.2.2, respectively. Whatman CF-11 cellulose powder (Whatman^{TM}, a part of GE Healthcare Life Science, Ltd., Buckinghamshire, UK) was used for affinity chromatography after pre-washing with 5% NaOH and neutralization with acetic acid.

6.2.2. Isolation of β-(1,3;1,4)-glucan

Anion exchange chromatography was firstly applied for partial purification of β-glucan based on its neutral property in contrast to acidic property of arabinoxylan. Hot water-soluble fraction of CP hemicelluloses amounted at 94.2 ± 0.5% (n = 4) was obtained by extraction of CP with 100 fold excess amount of water for 2-3 h at 80^{o}C and applied to a column (15 × 150 mm) of TOYOPEARL DEAE-650M (Tosoh Co., Tokyo, Japan) equilibrated with 5 mM sodium phosphate buffer (pH 6.8) and eluted with the same solution to recover neutral β-glucan. Acidic arabinoxylan was further recovered by elution with the same buffer containing 1.2 M NaCl. Elution was monitored by the phenol-sulfuric method. Both polysaccharide fractions were separately pooled, dialyzed against water and freezed-dried. The β-glucan-rich fraction (8.7 ± 2.4% on the basis of CPHs, n = 3, Table 6-1) was dissolved in 5 mM sodium acetate buffer, pH 5.0, and applied on a cellulose column (15 × 150 mm). After allowing for 30 min at room temperature, the column was washed with the same buffer to remove unadsorbed material, eluted with distilled water, and finally adsorbed β-glucan was recovered by elution with 2% NaOH (yield 3.3 ± 1.3% on the basis of the raw

hemicellulose mixture, *n* = 3, Table 6-1). All carbohydrate containing fractions were pooled, neutralized, dialyzed against water and freeze-dried.

6.2.3. Chemical analysis

Chemical analysis of the separated fractions including β-glucan was carried out as described in chapters 2-5. Liquid state ^{13}C-NMR spectrum of the isolated BG was recorded in D_2O on a Bruker DPX-400 instrument (Billerica, Bruker, MA, USA) operating at 400 MHz for 2-3 h and the chemical shifts in ppm were normalized as downfield values from that of TSP (sodium 2,2,3,3-tetradueterio-3-(trimethylsilyl) propionate as internal standard.

Permethylation of polysaccharides was carried out according to the Hakomori method (Hakamori, 1964). The permethylated polysaccharides were subjected to two-step hydrolyses with 90% formic acid for 2 h at 100°C and 0.5 N sulfuric acid for 12 h at 100°C. After neutralization with barium carbonate, the hydrolyzate was reduced with sodium borohydride and acetylated with a mixture of acetic anhydride and pyridine (1:1, v/v) for 1 h at 100°C. The resulting mixture of partially methylated alditol acetates was analyzed by GC/MS with a Shimadzu Parvum 2 (70 eV) using a column of CBP-1 (0.25 μm, 0.25 mm × 25 m) and a linear temperature gradient from 140°C to 220°C at 2°C/min.

Distribution of (1,4)-linked-glucosyl segments in β-glucan was determined by lichenase treatment and HPLC. β-Glucan samples were dissolved in sodium phosphate buffer (20 mM, pH6.5) and incubated with lichenase [(1,3;1,4)-beta-glucan-4-glucanohgydrolase, 1000 U/mL, included in the Megazyme kit for measurement of β-glucan content] for 2 h at 50°C. After centrifugation, supernatant was purified by

passage through a joint column of anion (Dowex 50 × 8, H^+) and cation (Dowex 1 × 8, AcO^-) exchange resins with pure water. The distribution of segments was analyzed by HPLC on a column of MCI GEL CK04SS as described in section 2.2.2

6.3. Results and Discussion

6.3.1. Isolation of β-(1,3;1,4)-glucan

The raw hemicellulosic mixture was shown to be composed of neutral and acidic fractions by anion exchange chromatography on a DEAE-column. Elution profile is shown in Figure 6-1a. Neutral fraction was further separated into three fractions by affinity chromatography on a cellulose column. Elution profile is shown in Figure 6-1b. Yields of the separated fractions are listed in Table 6-1. The relative monosaccharide composition of the raw hemicellulose mixture and the fractions separated by anion exchange and affinity chromatographic techniques were listed in Table 6-2. The results indicate that the neutral fraction contained β-glucan (28.4%) with contamination of a small amount of xylan (10.1%). On the other hand, the acidic fraction was arabinoxylan consisting of xylose (50.9%), arabinose (35.6%) and glucose as a minor constituent (3.5%). These results indicate that the anion exchange chromatography was effective for partial purification of β-glucan in CP. Previously, Gruppen *et al.* (1992) have reported that anion exchange chromatography is an efficient tool for fractionation of arabinoxylans present in wheat flour. Present results further indicate its convenience for removal of acidic arabinoxylan abundantly present in the raw hemicellulose mixture.

In the present study, affinity chromatography on cellulose column was found to be applicable for selective purification of β-glucan. After affinity chromatography glucose contents of the fractions eluted with SAB, distilled water and 2% NaOH were 29.5, 50.6

and 91.5%, respectively (Table 6-1). β-Glucan was recovered from the column by elution with 2% NaOH at 84.7% purity (Table 6-1). Starch contents in SAB, water and 2% NaOH fractions were as low as 4.3, 5.7 and 3.5%, respectively.

When water-soluble portion of the raw hemicellulose mixture was directly applied to the cellulose column, a higher amount of xylose (23.2%) was contaminated in the adsorbed fraction. This result indicates the importance of the order of combination of two chromatographic techniques; the necessity of operating anion-exchange chromatography prior to cellulose affinity chromatography.

Table 6-1. Yields of the separated fractions and sugar composition of corn pericarp hemicelluloses.

Sample	Yield[a] (%)	Relative monosaccharide composition (%, w/w)					β-Glucan	Starch
		Ara	Gal	Glc	Xyl	Man	content[b] (%)	content[b] (%)
CPHs	-	29.2	4.1	26.2	40.5	tr[c]	6.6	1.4
Anion exchange chromatography								
Neutral fraction	8.7 ± 2.4	1.0	2.0	80.3	10.1	6.7	28.4	5.0
Acidic fraction	42.8 ± 4.8	35.6	8.2	3.5	50.9	1.9	0.6	0.6
Affinity chromatography on cellulose column								
SAB fraction	8.6 ± 4.1	23.4	7.6	29.5	35.8	3.7	1.3	4.3
Water fraction	1.0 ± 0.7	14.3	7.1	50.6	23.4	4.6	1.4	5.7
2 % NaOH fraction	3.3 ± 1.3	1.3	0.9	91.5	6.3	-	84.7	3.5

[a] Values are expressed as a percentage on the basis of the native corn pericarp hemicelluloses. Values are expressed as mean ± SD (n = 3).

[b] Values represent the average of duplicate data.

[c] tr represents trace.

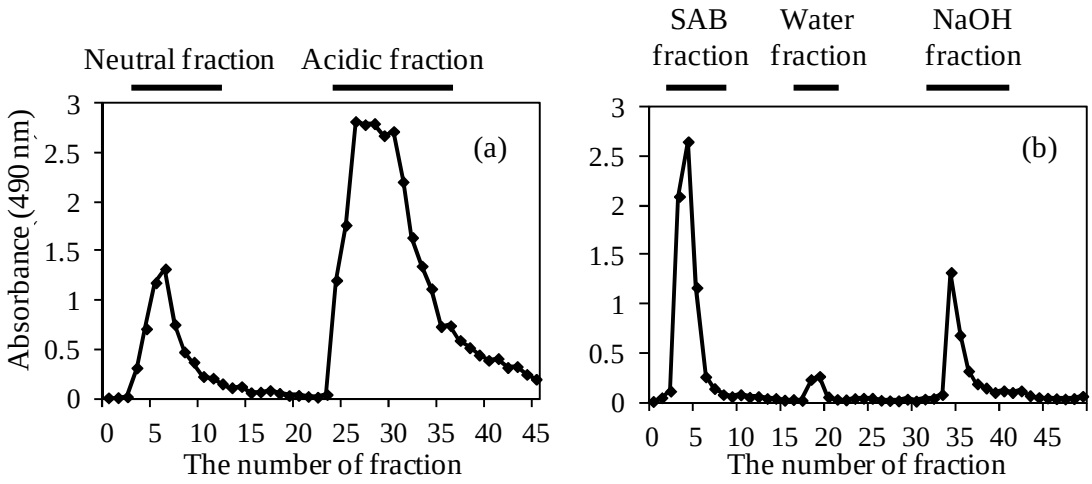

Figure 6-1. Anion exchange chromatographic (a) and affinity chromatographic (b) profiles. (a) The neutral and acidic fractions shown as separate bars at the top of the figure were pooled. (b) Neutral fraction was applied on cellulose column. Three fractions eluted with SAB, distilled water and 2% NaOH were recovered. Fractions of 5-15 and 25-36 separated by anion exchange chromatography were obtained as neutral and acidic fraction, respectively. Fractions of 4-11, 19-21 and 35-44 separated by affinity chromatography on cellulose column were obtained as SAB, water and NaOH fraction, respectively. Elution for anion exchange chromatography was changed at fraction number 21 from 5 mM sodium phosphate buffer (pH 6.8) to the same buffer containing 1.2 M NaCl. Eluted solution for cellulose column was changed by fraction of 17 from SAB to water, and by fraction of 33 to 2% NaOH.

6.3.2. Properties of β-(1,3;1,4)-glucan from corn pericarp

Figure 6-2 shows the ^{13}C-NMR spectra of the materials separated into NaOH fraction. Each signal was assigned according to the previous report (Roubroeks *et al*., 2000; Cui *et al*., 2000) and the results are listed in Table 6-2. The spectrum was identical to that of pure β-glucan reported by Wood (1991); characteristic intense signals at 81.3 and 86.6 ppm, assignable to the signal of C-3 of 1,4-linked- and 1,3-linked-D-glucopyranose residues of β-glucan, respectively. Although weak signals not assigned were also detected at 71.38 and 72.45, no other intense signals other than β-glucan could be detected. Detection of similar weak signals in bareley β-glucan fractionated with $(NH)_4SO_4$ was reported by Izydorczyk and MacGregor (2000). These results also indicate that highly purified β-glucan could be isolated following the procedure described above.

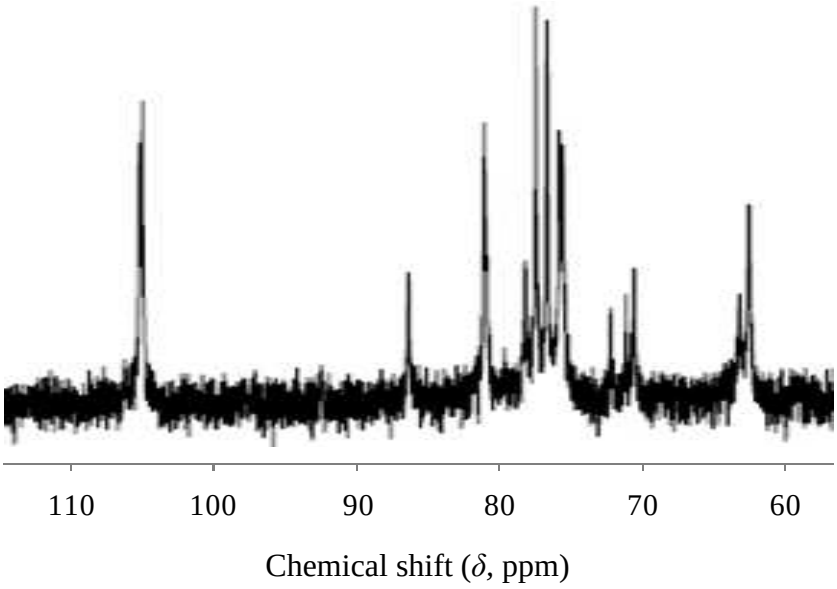

Figure 6-2. ^{13}C-NMR spectrum of the material isolated in NaOH fraction by using cellulose column.

Table 6-2. Assignment of ^{13}C-chemical shifts (ppm) of β-glucan in D_2O.

Linkage type	Chemical shift (ppm)					
	C1	C2	C3	C4	C5	C6
→4)-β-Glc*p* (1→3)	105.41	76.08	81.29	76.92	77.68	62.81
→3)-β-Glc*p* (1→4)	105.22	75.84	86.60	70.81	78.45	63.41
→4)-β-Glc*p* (1→4)	105.22	76.08	81.29	76.92	77.68	62.81

The glucosidic linkage analysis of the purified β-glucan was investigated by methylation analysis. The results shown in Table 6-3 indicate that the CP β-glucan was consisted of 2,3,6-Me-Glc*p*, 2,4,6-Me-Glc*p* and 2,3,4,6-Me-Glc*p* in a molar ratio of 58.1 (1,4-linked Glc*p*), 22.3 (1,3-linked Glc*p*) and 0.5% (terminal Glc*p*), respectively. The (1,4) to (1,3) glucosidic linkage ratio was calculated as 2.60, which was slightly higher than that from barley (2.37) but included in the ranges previously reported; 2.3-2.8 for oat, 1.9-2.8 for barley and 2.3 for rye (Lazaridou and Biliaderis, 2007). In addition, high affinity of the CP β-glucan to cellulose suggests the existence of strong interaction between these polysaccharides in CP.

The distribution of (1,4)-linked glucose segments in CP β-glucan was examined by fragmentation analyses with lichenase which splits (1,3)-β-D-glycosidic linkages in β-glucan (Table 6-4). After lichenase treatment, oligomers below cello-octamer were detected in CP β-glucan. The value of cellotriosyl/cellotetraosyl distribution ratio of CP β-glucan was 2.5, which was slightly higher than that from barley (2.2). Previously, the ratios of cellotriosyl/cellotetraosyl segments in the native cereal β-glucan structures were reported to be within the range of 1.5-2.3 for oat, 1.8-3.5 for barley, 1.9-3.8 for rye and 3.0-4.5 for wheat (Lazaridou and Biliaderis, 2007). β-Glucan with higher ratios of cellotriosyl/cellotetraosyl units and larger amount of contiguous (1,4)-linked segments

have been reported to show lower solubility, because of operation of intermolecular interactions by strong hydrogen bondings along the cellulose-like regions (Ebringerová *et al*., 2005). The present chromatographic system might be desirable for isolation and purification of β-glucan with higher cellotriosyl/cellotetraosyl ratios, because affinity of β-glucan to cellulose was the basis for its isolation.

Table 6-3. Methylation analysis of the material isolated in NaOH fraction by using cellulose column and barley β-glucan.

Methylation position	Linkage type	Molar ratio (%)	
		NaOH fraction	Barely β-glucan
2,3,6-Me_3-Glc*p*	1,4-	71.8	69.4
2,4,6-Me_3-Glc*p*	1,3-	27.6	29.3
2,3,4,6-Me_4-Glc*p*	Terminal	0.6	1.0
3,6-Me_2-Glc*p*	1,2,4-	-	0.3

Values are expressed as a relative percentage of the total partially methylated glucose residues.

Table 6-4. Fragmentation analysis of the materials isolated in NaOH fraction after affinity chromatography on cellulose column and barley β-glucan with lichenase.

Oligmer	DP	Molar ratio (%)	
		NaOH fraction	Barley
Cellobiose	2	0.3	1.3
Cellotrimer	3	67.5	63.8
Cellotetramer	4	26.7	28.7
Cellopentamer	5	3.4	4.4
Cellohexamer	6	1.7	1.1
Celloheptamer	7	0.2	0.4
Cellooctamer	8	0.1	0.3
Cellotrimer + Cellotetramer	3 + 4	94.2	92.5
Cellotrimer/Cellotetramer	3/4	2.5	2.2

DP represents degree of polymerization.

6.4. Summary

Effectiveness of the combination of anion-exchange and cellulose affinity chromatographic techniques for isolation of β-glucan from CP was established for the first time. By using the present system, purity of the β-glucan was increased from 6.6% to 84.7%. The methylation and fragmentation analyses showed that the isolated β-glucan has the ratios of (1,4)/(1,3) linkage and cellotriosyl/cellotetraosyl segments of 2.60 and 2.5, respectively. The chemical structure of the β-glucan was also confirmed by ^{13}C-NMR spectroscopic analysis.

Conclusions

The objective of this thesis is directed to develop new innovative methods for effective reuse of corn pericarp (CP) as a renewable source of polysaccharides. For achievement of this objective, microwave-assisted extraction (MAE) was firstly applied to extract polysaccahrides present in CP, and a kind of cellulose solvent comprising NaOH and urea was successfully applied to extract hemicellulosic polysaccharides in CP. Further the author clarified water sorption behavior and film forming ability of arabinoxylan, which was the major hemicellulose of CP. Finally, the author developed a new technique of affinity chromatography on cellulose whose use enabled one-step purification of β-glucan.

For the first step, the author combined MAE with a statistical method of combinations of response surface methodology and experimental design for solubilization of CP and succeeded to give the optimum conditions as heating temperature 176.5°C, come-up time 2 min, heating time 16 min and solid to liquid ratio 1/20 (g/mL) with 70.8% carbohydrate recovery. Induction of autodegration of arabinoxylan to produce useful xylo-oligosaccharides was also noted.

In the second step, the author found for the first time that the cellulose solvent comprising 2% NaOH-6 M urea was quite useful to solubilize the hemicellulosic polysaccharides selectively from CP. Productionability of transparent biofilms of the solubilized polysaccharide mixture, presence of β-glucan in CP (≤3.2%) from sweet corn and plasticizing function of β-glucan were also three major discoveries of the present thesis. Moreover, the results strongly indicate that composite formation of highly branched arabinoxylan and β-glucan is important for investment of flexibility to the CP membrane.

Next, the author analyzed the role of afrabinofuranosyl substituents of the arabinoxylan during water vapor sorption behavior by the Haliwood and Horrobin model and the independent dual sorption model. Best fitting of the isotherms by the latter model was found to be applicable for demonstrating that removal of arabinose pendants from the main (1,4)-linked xylan chain gradually reduces the Langmuir capacity of the CP arabinoxylan.

In addition, the author could optimize the extraction condition of β-glucan as three repetitions of freeze (-20°C)-thaw treatment in 2% NaOH-6 M urea solution at the fixed liquid to solid ratio of 125 mL/g. By following this process 97.8% extractability of β-glucan from CP was attained. Necessity of destruction of crystalline portion of cellulose in CP for promotion of solubilization of β-glucan indicates presence of strong interaction of β-glucan with cellulose in the native CP.

Finally, a simple and useful method for isolation of β-glucan was presented by the author by combination of anion-exchange chromatography to remove acidic arabinoxylan and affinity chromatography on cellulose to purify β-glucan. The isolated β-glucan had the ratios of (1,4)/(1,3) linkage and cellotriosyl/cellotetraosyl segments of 2.60 and 2.5, respectively. This rather higher ratios indicated presence of lager amount of contiguous (1,4)-linked segments which is desirable to increase in molecular association between β-glucan and cellulose.

In conclusion, the present thesis showed important characteristics of polysaccharides in CP and provided the new applications and techniques for reuse of CP as a biomass resource, allowing more effective utilization of corn kernels not only for food and feed, but also for preparation of valuable polysaccharide-based innovative materials in the future.

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Original papers

1. **T. Yoshida**, Y. Honda, H. Uyama and J. Azuma: Removal of arabinose substituents from corn pericarp arabinoxylan. *Indonesian Wood Research Journal* 4: 46-50. 2013.
2. **T. Yoshida**, Y. Honda, H. Uyama and J. Azuma: Water vapor sorption behavior of arabinoxylan from corn pericarp. *Indonesian Wood Research Journal* 4: 51-56. 2013.
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4. **T. Yoshida**, S. Tsubaki, Y. Teramoto and J. Azuma: Optimization of microwave-assisted extraction of carbohydrates from industrial waste of corn starch production using response surface methodology. *Bioresource Technology* 101: 7820-7826. 2010.
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6. **T. Yoshida**, Y. Honda, T. Tujimoto, H. Uyama and J. Azuma: Extraction of β-(1,3;1,4)-glucan from corn pericarp with urea-NaOH solvent system. *Journal of Polymer-Plastics Technology and Engineering*: submitted.
7. **T. Yoshida**, Y. Honda, T. Tujimoto, H. Uyama and J. Azuma: Selective isolation of β-glucan from corn pericarp hemicelluloses by affinity chromatography on cellulose column. *Carbohydrate Polymer*: in preparation.

International symposium

1. **T. Yoshida**, Y. Honda, T. Tujimoto, H. Uyama and J. Azuma: Optimization of NaOH-urea solvent system for extraction of hemicelluloses from corn pericarp. HPI (Indonesia Polymer Association)-FAPS (Federation Asian Polymer Societies) International Conference on Innovation in Polymer Science and Technology. November, 2013. Yogyakarta, Indonesia. Poster session.
2. **T. Yoshida**, Y. Honda, H. Uyama and J. Azuma: Release of arabinose from corn pericarp arabinoxylan. The 4th International Symposium of Indonesian Wood Research Society. November, 2012. Makkasar, Indonesia. Oral session.
3. **T. Yoshida**, Y. Honda, T. Tujimoto, H. Uyama and J. Azuma: Selective isolation of β-glucan from corn pericarp hemicelluloses by affinity chromatography on cellulose column. 19th Regional Symposium on Chemical Engineering. November, 2012. Bali, Indonesia. Oral session.
4. **T. Yoshida**, M. Sakamoto and J. Azuma: Extraction of hemicelluloses from corn pericarp by the NaOH-urea solvent system. HPI (Indonesia Polymer Association)-APA (Asian Polymer Association) International Conference on Innovation in Polymer Science and Technology. November, 2011. Bali, Indonesia. Poster session. Received two poster prizes; “Polymer Chemistry Poster Prize” and “The Best Student Poster Presenter”.
5. **T. Yoshida**, S. Tsubaki, Y. Teramoto and J. Azuma: Microwave-assisted extraction of carbohydrates from corn pericarp (a byproduct from corn starch production). International Microwave Power Institute's 44th Annual Symposium July, 2010. Denver, Colorado, USA. Oral and Poster session

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