JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Effects of Molecular Weight and Concentration of Arabinoxylans on the Membrane Plugging

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In this paper, arabinoxylans from wheat were isolated, purified, and degraded into four fractions with different molecular weight. The distribution of particle size was employed to evaluate the membrane plugging by beer. These arabinoxylans fractions were added into arabinoxylan-free beer to investigate the effects of molecular weight and concentration on the distribution of particle size in beer. Results showed that the fraction of arabinoxylans retained by a 0.22-μm pore size membrane was more easily affected by shearing and arabinoxylan concentration than the fraction of arabinoxylans retained by a 0.45-μm membrane. The effects of shearing, pH, and ethanol concentration of beer on the particle size distribution of the highest molecular weight arabinoxylan fraction in beer were also examined. General linear models (GLM) equations indicated that there was a positive correlation between the particle size distributions of arabinoxylans and shearing and ethanol content, while high molecular weight arabinoxylans particle size retained by the 0.45-μm membrane was not significantly ($p > 0.05$) influenced by pH value. Scanning electron microscope (SEM) photos showed that membrane plugging was significantly affected by the molecular weight of the arabinoxylans.

KEYWORDS: Arabinoxylans; beer; membrane plugging; shear

INTRODUCTION

In cereal grains, arabinoxylans are nonstarch polysaccharides from cell walls. Arabinoxylans consist of a linear backbone of (1→4)-β-D-xylopyranosyl units, to which α-L-arabinofuranosyl substituents are attached through O-2, O-3, or O-2,3 (*1*). Arabinoxylans from endosperm are partially water-extractable because they contain polymers with a high molecular weight, which influence viscosity and membrane filterability of solutions which contain them. Ferulic acid has also been detected in arabinoxylans indicating that they might be partially cross-linked; their cross-links may also contribute to filtration problems (*2*, *3*).

Arabinoxylans can be extracted from walls with hot water and form solutions of high viscosity. This property can be attributed to the fact that arabinoxylans behave as a stiff rodlike structure (*1*). So, arabinoxylans from barley grains may be responsible for various problems in the beer brewing industry such as low extract yields, high wort viscosity, decreased rate of filtration, or haze formation (*4*, *5*) in beer.

Historically, reduced beer filtration efficiency has been mainly attributed to β-glucans (*6*−*9*), another important nonstarch polysaccharide in barley grain. Studies on beer solution made from barley β-glucans of various molecular weights indicated that β-glucans with high molecular weight were more likely to increase wort viscosity (*10*−*13*) and reduce membrane filtration performance (*14*).

In fact, the content of water-soluble arabinoxylans in wheat is higher than the content of water-soluble β-glucans (*15*). It has been reported that the total arabinoxylan amount in commercial beer is approximately 10 times greater than that of the high molecular weight β-glucans (*16*). Paul et al. (*17*) investigated the effects of arabinoxylans, β-glucans, and dextrins on the viscosity and membrane filterability of beer. They found that filterability of beer was decreased by arabinoxylans and that the effects of arabinoxylans on viscosity and filterability were at least as important as the effects of β-glucans. Stewart et al. (*18*) found that pilot-brewed beer viscosity and membrane filterability correlated with arabinoxylan content, whereas β-glucan was correlated only with viscosity. Their results indicated that arabinoxylans might have a substantial role in filter plugging and suggested that further investigations into the effects of arabinoxylans on membrane plugging should be carefully considered. Egi et al. (*19*) investigated the effects of arabinoxylan concentration, molecular weight, and membrane pore size on filtration of a beer model. An analysis of variance indicated that all the three parameters had a significant effect on the V_{max} values ($p < 0.05$). Other researchers (*20*) reported poor correlations between filterability and total arabinoxylan concentration in beer, implying that the potential importance of the molecular weight distribution of these polymers in beer

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10.1021/jf0480694 CCC: $30.25
Published on Web 05/21/2005

should be considered. Membrane pores used in filtration tests were expected to be more easily plugged by these polymers, especially when polymers were affected by other environmental factors during brewing.

In our previous work, the effects of mashing parameters on the solubilization and hydrolysis of arabinoxylans have been extensively investigated (*21*). A mathematical model predicting arabinoxylan concentration during the mashing process was also developed (*22*). However, the effects of arabinoxylan molecular weight were not considered in these studies; if native arabinoxylans isolated from the raw material were added to beer, this would provide further information about the behavior of these polysaccharides which are potentially cross-linked with proteins or β-glucans and are also affected by other environmental factors. Knowledge about such interactions would provide brewers with a better understanding of the behavior of these biopolymers in brewing.

In this paper, we aimed at investigating the effects of molecular weight and concentration on the distribution of the particle sizes of arabinoxylans in beer. The effects of shearing, pH, and ethanol concentration of beer on the particle size distribution of the highest molecular weight arabinoxylans were also examined. This work was expected to provide a better understanding of the effects of arabinoxylans on membrane plugging during brewing, so as to supply brewers with useful informative indexes, with regard to whether and what further operational actions should be taken for the purpose of process optimization.

MATERIALS AND METHODS

Isolation, Fractionation, and Purification of Water-Extractable Arabinoxylans. Arabinoxylans was isolated from Chinese Hard Red Spring Wheat that was milled with a Buhler Miag disk mill (Braunschweig, Germany). The isolation, fractionation, and purification of water-extractable arabinoxylans were based on the methodology of Cleemput et al. (*23*) and Izydorczyk et al. (*24*). Samples of wheat flour were transferred into stainless steel bowls and heated in a drying oven (130 °C, 90 min) to inactivate the enzyme before extraction with water (5:1, v/w, 30 °C, 15 min). Subsequent isolation procedures were accomplished at room temperature. After centrifugation (3000*g*, 15 min), the supernatant solution was heated to 90 °C to precipitate the soluble protein. Residual starch was then hydrolyzed by adding α-amylase (Genencor Ltd. Co.). The solution was kept at 90 °C for 30 min, cooled, and centrifuged as above. The resulting supernatant solution was isolated from the extract by stepwise addition of aliquots of ethanol (96%, v/v) to a final concentration of 65% (v/v). The mixture was stirred for 30 min, kept at 4 °C overnight, and centrifuged (10 000*g*, 30 min).

The precipitate obtained was dissolved in 150 mL of water and stirred for 30 min. Ethanol was added to a final concentration of 60% (v/v) as above. After stirring for 30 min, the mixture was stored at 4 °C for 2 h and centrifuged at 10 000*g*. The precipitate was redissolved in distilled water. This solution was subjected to ultrafiltration at room temperature with a 10 000 molecular weight cut off (Vivascience, Sartorius Ltd. Co., Germany).

The precipitate was suspended in aliquots of ethanol (96%, v/v) and acetone with intermediate stirring and then centrifuged (20 000*g*, 30 min). The final precipitate was lyophilized to give AX1.

Preparation of Different Molecular Weight Arabinoxylans. To obtain four fractions of different molecular weight arabinoxylans, the isolated arabinoxylans (AX1) were enzymatically degraded with endo-xylanase. The commercial preparation, endo-xylanase (Laminex XL), was supplied by Genencor International Ltd. Co. (Wuxi, China). According to the manufacturer, this product contained 2500 U/mL of endo-xylanase activity, assayed by the dinitrosalicylic acid (DNS) method (*25*). Arabinoxylans (1:100, w/v) were dissolved into double deionized water by constant agitation. The solution was then heated to 50 °C and enzyme preparation (1 U/mL) was added. Enzymatic degradation of arabinoxylans in wort and beer has been extensively investigated in our previous work. Enzymatic degradation was performed for 10 min (AX2), 30 min (AX3), and 120 min (AX4), respectively, to obtain the three low molecular weight fractions. The reaction was stopped by heating the solution to boiling for 15 min to inactivate the endo-xylanase activity. The polysaccharide solutions were lyophilized, respectively.

Preparation of Arabinoxylan-Free Beer. A commercial beer "Suntory" was supplied by Suntory Co. Ltd. (Kunshan, China) and was used as the beer base in which exogenous arabinoxylans were hydrolyzd. The beer was first degassed by filtration. The degassed beer was then boiled for 2 h to remove ethanol and other volatile components. The concentrated beer was cooled to 50 °C, endo-xylanase (16 U/mL) was added, and it was incubated for 4 h. The arabinoxylan concentration of retentates was negligible after dialysis against deionized water (24 h, 5 °C).

Preparation of Beer Samples at Different Arabinoxylans Levels. Prepared arabinoxylans (AX1, AX2, AX3, AX4) were dissolved in double deionized water. The beers containing different arabinoxylans levels were prepared by mixing adequate amounts of the arabinoxylan stock, arabinoxylan-free beer, anhydrous ethanol, and double deionized water. This was done to investigate the effects of molecular weight and concentration of arabinoxylans on beer membrane filtration. Arabinoxylan (AX1, AX2, AX3, AX4) concentrations in beers were 50, 100, 250, 500, and 1000 mg/L. Beer samples were also subjected to shearing in a Lourdes Blender (Lourdes instrument Ltd. Co., NY) with the speed control set to 50 for 10 min (approximate shear rate $\geq 5000\ s^{-1}$) to investigate the effect of shearing on membrane filtration according to the method described by Egi et al. (*19*).

Beers containing the highest concentration (1000 mg/L) and highest molecular weight (963 kDa) of arabinoxylans were prepared to investigate the effects of pH (3.8, 4.2, and 5.4) and ethanol (0, 5, and 10%, v/v) on membrane plugging. These samples were also subjected to shearing at 5 °C and were analyzed immediately.

Determination of Arabinoxylans. Arabinoxylan contents were determined by measuring the total monosaccharide composition. Monosaccharide composition of biological samples was determined by acid hydrolysis of the polysaccharides followed by conversion of the monosaccharides to alditol acetates (*26*) and analysis by gas chromatography (*27*). Separation of the alditol acetates was carried out with a Finnigan (GC-FID) chromatograph using an SP-2330 column (30 m × 0.25 mm). The temperatures of injection and detection (flame ionization detector) were 260 °C and 280 °C, respectively. Arabinoxylans contents were calculated as 0.88 × (% arabinose + % xylose) (*28*).

Determination of Average Molecular Weight of Arabinoxylans. The average molecular weight of arabinoxylans was determined by high-performance size-exclusion chromatography (HPSEC). The HPSEC system comprised a Rheodyne model 7725 sample injector, a Waters 510 pump, a Waters 2410 refractive index detector, a Waters 740 data module, and a guard column (7.8 × 300 mm; Ultrahydrogel and Ultrahydrogel 500, Waters) connected in series. The mobile phase was a sodium acetate buffer (0.05 M, pH 3.65) with a flow rate of 0.8 mL/min. The standards used to calibrate the column system included maltotriose (Sigma) and maltoheptaose (Sigma) and dextrans (M_w 41 000, 70 000, 188 000, 482 000, 580 000, and 2 000 000, Sigma). Data analysis was performed using Millennium 2010 software (Waters).

Determination of Protein. Protein content was determined by using the method of Lowry (*29*) with the Bio-Rad *DC* protein assay kit. Bovine serum albumin was used as standard.

Evaluation of Membrane Plugging. Membrane plugging tests using 25-mm diameter membranes (modified polyethersulfone membrane, American Membrane Corporation, U.S.) with nominal pore sizes of 0.45 μm or 0.22 μm were controlled at 5 °C. The membrane filtration method was based upon the work of Sudarmana et al. (*30*). The membrane filterability apparatus used in this study consisted of a liquid

Table 1. The Composition, Number, Weight Molecular Masses, and Dispersion Degree of Arabinoxylans Fractions

	AX1	AX2	AX3	AX4
AX content (%)	88.2	84.2	85.1	86.7
protein content (%)	9.5	11.2	9.4	10.2
M_n (kDa)[a]	266	85	72	16
M_w (kDa)[b]	963	389	282	102
d (M_w/M_n)	3.6	4.5	3.9	6.3

[a] Number-average molecular weight. [b] Weight-average molecular weight.

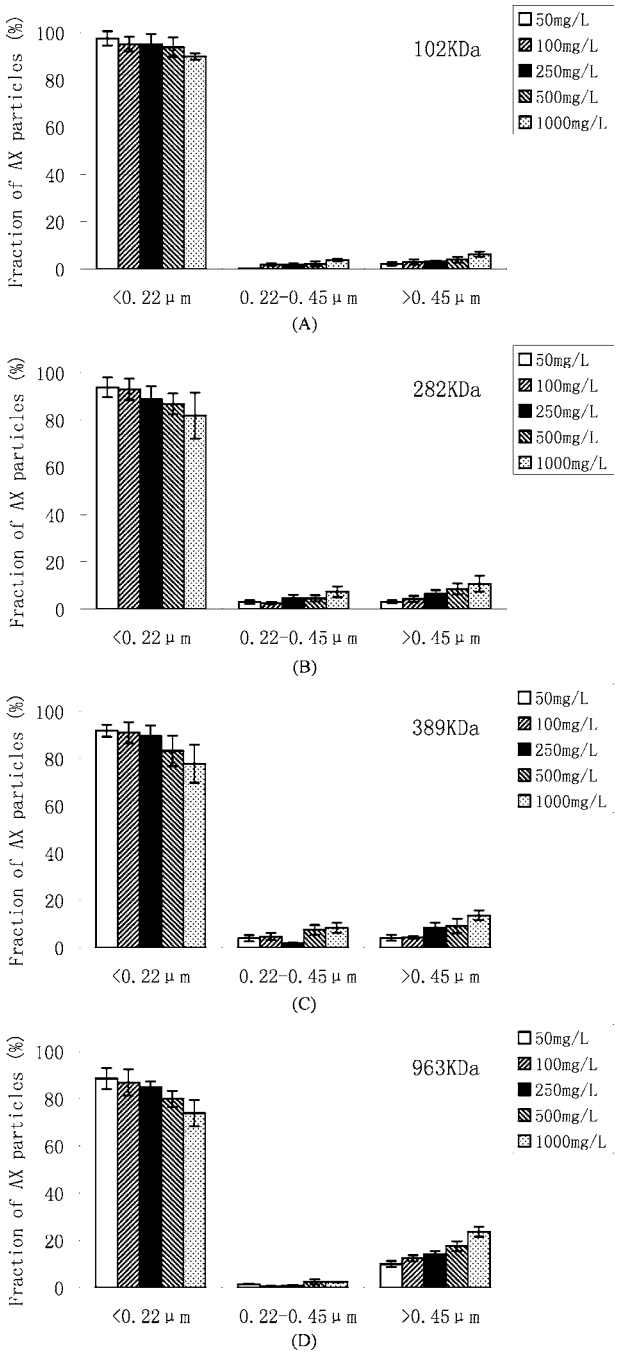

Figure 1. Effect of concentration of arabinoxylan fraction with M_w of **(A)** 102 kDa, **(B)** 282 kDa, **(C)** 389 kDa, and **(D)** 963 kDa in unsheared beer on distribution of arabinoxylans following filtration.

holding chamber, with a capacity of 20 mL. The system was held at a constant temperature of 5 °C and was pressurized to 200 kPa with nitrogen. The distribution of arabinoxylans particle sizes between

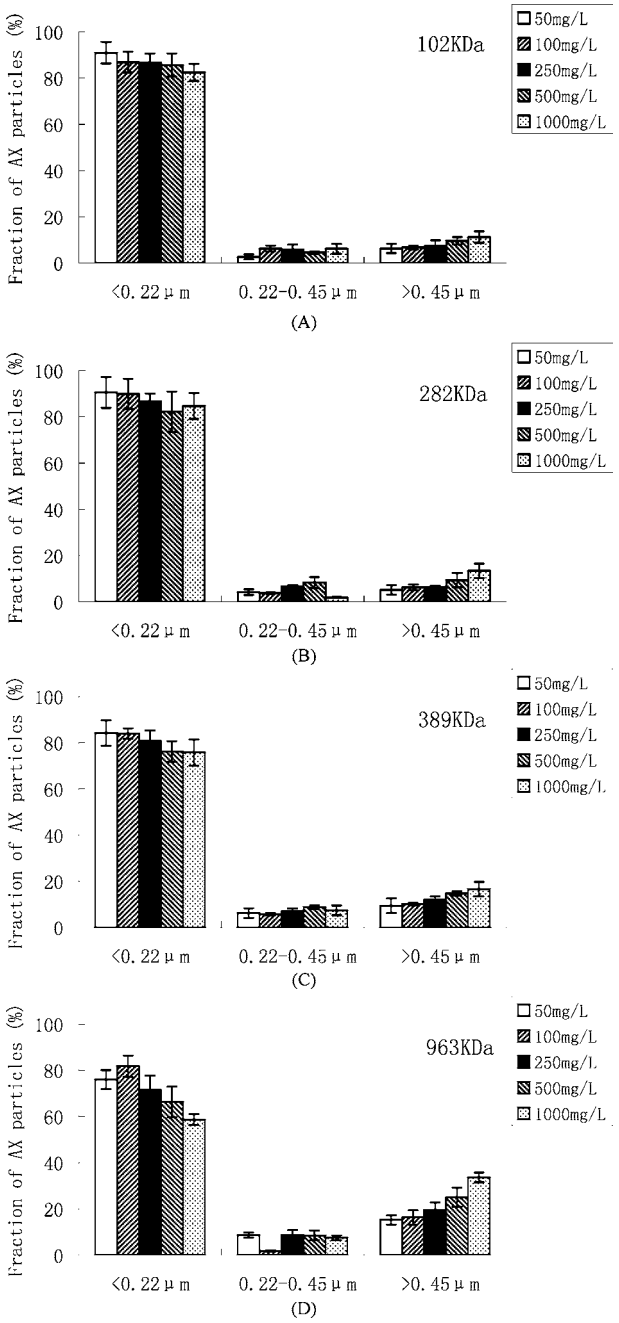

Figure 2. Effect of concentration of arabinoxylan fraction with M_w of **(A)** 102 kDa, **(B)** 282 kDa, **(C)** 389 kDa, and **(D)** 963 kDa in sheared beer on distribution of arabinoxylans following filtration.

nominal pore size of 0.45 μm and that of 0.22 μm was calculated as follows:

$$F_{>0.45} = \{[\text{initial AX in solution} - \text{AX filtrate thorough 0.45-}\mu\text{m membrane}]/\text{initial AX in solution}\} \times 100 \quad (1)$$

$$F_{0.22-0.45} = \{[\text{AX filtrate thorough 0.45-}\mu\text{m membrane} - \text{AX filtrate thorough 0.22-}\mu\text{m membrane}]/\text{initial AX in solution}\} \times 100 \quad (2)$$

$$F_{<0.22} = \text{AX filtrate thorough 0.22-}\mu\text{m membrane/initial AX in solution} \times 100 \quad (3)$$

$$F_{>0.22} = 100 - F_{<0.22} \quad (4)$$

where $F_{>0.45}$ is the arabinoxylans retained by the 0.45-μm membrane,

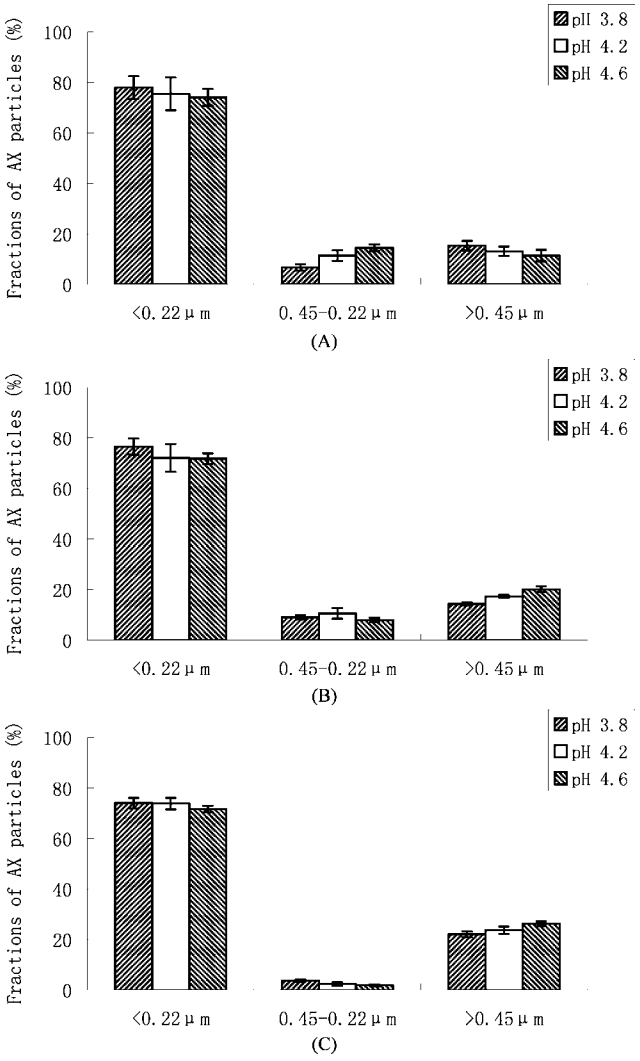

Figure 3. Effects of ethanol content and pH on the distribution of arabinoxylans in unsheared beer after addition of 963 kDa fraction (1000 mg/L) **(A)** ethanol content = 0, **(B)** ethanol content = 5%, and **(C)** ethanol content = 10%.

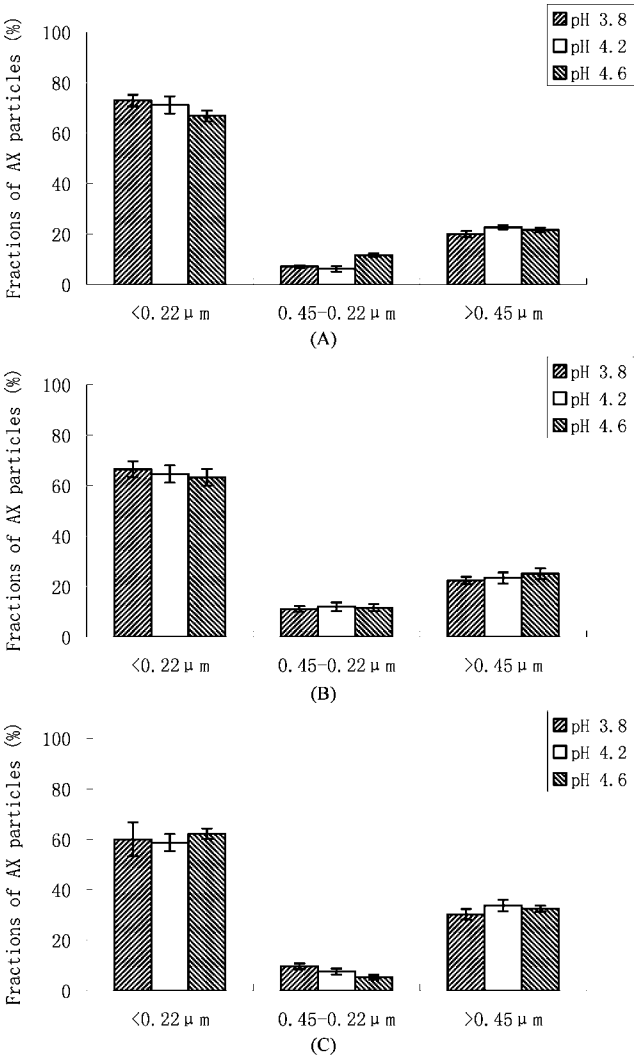

Figure 4. Effects of ethanol content and pH on the distribution of arabinoxylans in sheared beer after addition of 963 kDa fraction (1000 mg/L) **(A)** ethanol content = 0, **(B)** ethanol content = 5%, and **(C)** ethanol content = 10%.

$F_{0.22-0.45}$ is the arabinoxylans that passed through the 0.45-μm membrane but were retained by the 0.22-μm membrane, $F_{<0.22}$ is the arabinoxylans that passed through the 0.22-μm membrane, and $F_{>0.22}$ is the arabinoxylans retained by the 0.22-μm membrane.

Scanning Electron Microscopy (SEM). Membrane filters (pore diameter size of 0.45 μm) were used to filter beer with arabinoxylan concentration of 1000 mg/L and were examined by scanning electron microscopy (SEM) after 5 min of filtration. The filters were plunge-frozen in liquid propane cooled with liquid nitrogen. They were freeze-dried in an FTS EZ585Q freeze-dryer (FTS systems Inc., NY) and held at −70 °C overnight. The microscopic images of membrane plugging were observed by scanning electron microscopy (Quanta 200, FEI Co. Ltd.) at 5 kV.

Statistical Analysis. Statistical analyses were preformed by using the procedures outlined in the Statistical Analysis System (version 8.1, SAS Institute, Cary, NC). The main effects and interactions were evaluated by using the general linear models (GLM) procedure. The GLM was used to perform a forward stepwise regression with α-to-enter = 0.15 and α-to-remove = 0.15. Stepwise regression was used to evaluate how much variability could be explained by each independent variable (such as arabinoxylan concentration and arabinoxylan molecular weight) for the dependent variable (the distribution of arabinoxylans particle sizes).

RESULTS AND DISCUSSION

The Composition and Average Molecular Weight of Arabinoxylans. On the basis of the determination method of arabinoxylans by neutral sugar analysis, arabinoxylan contents of all the four arabinoxylans fractions were in excess of 84% (**Table 1**), similar to the report of Paul et al. (*17*) and Fessas et al. (*31*). The protein contents ranged from 9.4% to 11.2%.

Weight-average molecular weights (M_w), polydispersity indices (M_w/M_n), and elution patterns of water-extractable fractions were measured using an HPSEC system to gain further insight into the molecular characteristics of arabinoxylan fractions (**Table 1**). The average molecular weights of four arabinoxylan fractions were measured as 102 kDa, 282 kDa, 389 kDa, and 963 kDa, respectively. The lowest molecular weight fraction (AX4) showed a rather broad distribution, while a much narrower dispersion was found for the other three fractions. The high ratio of weight-average molecular weight to number-average molecular weight (M_w/M_n) reported for water-extractable arabinxylans wheat (4.1) strongly pointed to their inherent polydispersity (*32*).

Effects of Molecular Weight and Concentration of Arabinoxylans on the Particle Size Distribution in Unsheared Beer. Beers containing different arabinoxylans levels (50−1000

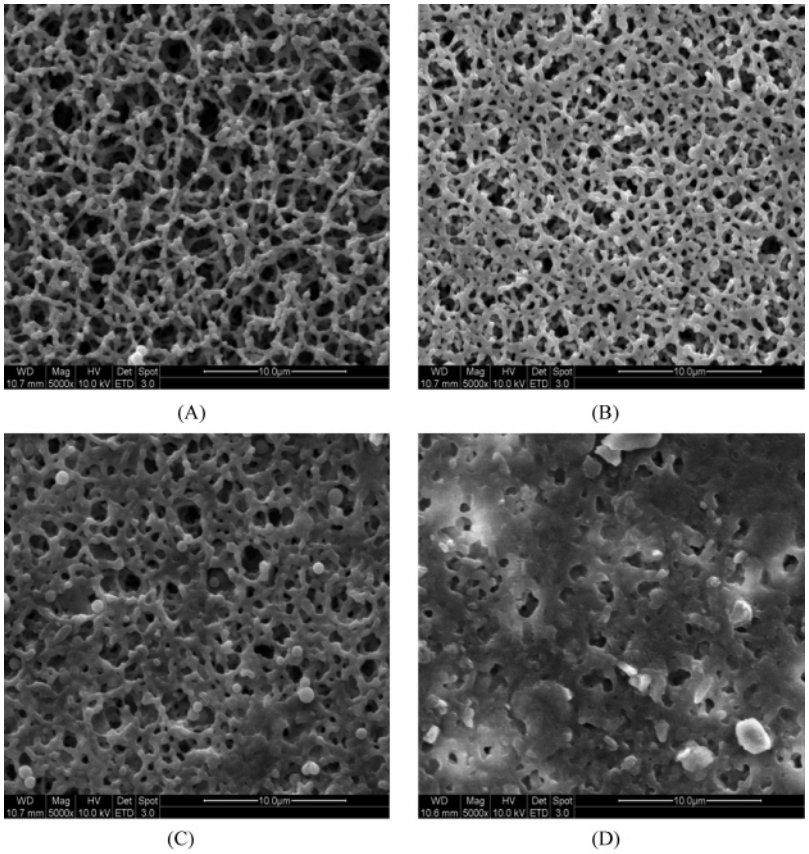

Figure 5. SEM photo of 0.45-μm membrane surface **(A)** and SEM photos of membranes after filtration of beer containing arabinoxylan fraction with M_w of **(B)** 102 kDa, **(C)** 389 kDa, and **(D)** 963 kDa.

mg/L) were prepared to investigate the effects of molecular weight and concentration of added arabinoxylans on distribution of arabinoxylans following filtration. **Figure 1A**−**D** showed the distribution of arabinoxylans between nominal pore sizes of 0.45 μm and 0.22 μm in unsheared beer. The majority (more than 90%) of the low molecular weight arabinoxylans (102 kDa) was smaller than a pore size of 0.22 μm at concentrations between 50 and 1000 mg/L (**Figure 1A**). However, only minor fractions (2.2−6.3%) of the low molecular weight arabinoxylans were retained by 0.45-μm membranes. With middle molecular weight arabinoxylans (282 kDa and 389 kDa), the percentage of arabinoxylan particle sizes below 0.22 μm was decreased with the increase of molecular weight and arabinoxylan concentration (**Figure 1B**, **C**). Those fractions retained by 0.45-μm membranes were increased with the increase of molecular weight and arabinoxylan concentration. When the highest molecular weight arabinoxylans (963 kDa) were added to beer, the lowest proportion (73.9%) of arabinoxylans passed through a 0.22-μm membrane when 1000 mg/L was added (**Figure 1D**). However, the fraction retained by 0.45-μm membranes was increased to 23.7% under the above conditions.

The percentage of arabinoxylans retained by 0.22-μm membrane (>0.22 μm) can be estimated from their molecular weight and concentration:

$$F_{>0.22} = 3.21 + 0.0089M_w + 0.0091C \ (n = 40, R^2 = 0.87, p < 0.01) \quad (5)$$

where M_w is molecular weight in kDa, and C is the arabinoxylan concentration (mg/L).

The percentage of arabinoxylans retained by 0.45-μm membrane (>0.45 μm) can be estimated by the following equation:

$$F_{>0.45} = 0.94 + 0.0096M_w + 0.0043C + 0.00001M_w \times C \ (n = 40, R^2 = 0.97, p < 0.01) \quad (6)$$

Comparing eq 5 and eq 6, the higher value of the multiplier of arabinoxylan concentration in eq 5 suggested that $F_{>0.22}$ was more easily affected by arabinoxylan concentration than $F_{>0.45}$, while the multipliers of molecular weight gave similar values. The interaction between molecular weight of arabinoxylans and arabinoxylan concentration in beer was only observed in eq 6.

Effects of Molecular Weight and Concentration of Added Arabinoxylan Fraction on the Particle Size Distribution in Sheared Beer. These beer samples were also sheared at 5 °C to investigate the effects of molecular weight, arabinoxylan concentration, and shearing on the particle size distribution. The results of the particle size distribution of arabinoxylans in sheared beer are shown in **Figure 2 A**−**D**. The proportion of arabinoxylans in the <0.22-μm fraction of the sheared beer containing the low molecular weight arabinoxylans (102 kDa) was similar to that of the unsheared beer. The proportion of arabinoxylans in the >0.45-μm fraction ranged from 6.3% to 11.3%, higher than that of unsheared beer (2.2−6.3%). When the highest molecular weight arabinoxylans (963 kDa) at a concentration of 1000 mg/L was added, the lowest proportion (58.7%) of arabinoxylans was retained by 0.22-μm membrane, much lower than that value (73.9%) observed in unsheared beer. However, the proportion retained by 0.45-μm membrane was increased to 33.7% in sheared beer. These data indicate that arabinoxylan particle size was significantly ($p < 0.01$) increased by shearing. The proportion of arabinoxylan with a particle size

>0.22 μm in sheared beer can be described by the following equation:

$$F_{>0.22} = 9.28 + 0.012M_w + 0.0031C + 0.000018M_w \times C \ (n = 40, R^2 = 0.86, p < 0.01) \quad (7)$$

The proportion of arabinoxylans with particle size >0.45 μm in sheared beer can be described by the following equation:

$$F_{>0.45} = 4.24 + 0.011M_w + 0.0030C + 0.000016M_w \times C \ (n = 40, R^2 = 0.95, p < 0.01) \quad (8)$$

Similar multipliers of molecular weight and arabinoxylan concentration were observed in both equations (eq 7 and eq 8).

The particle size distribution of arabinoxylans between nominal pore sizes of 0.45 μm and 0.22 μm can be described by the following two equations:

$$F_{>0.22} = 2.71 + 7.08S + 0.011M_w + 0.0061C + 0.000013M_w \times C \ (n = 80, R^2 = 0.86, p < 0.01) \quad (9)$$

$$F_{>0.45} = 0.43 + 4.31S + 0.010M_w + 0.0037C + 0.000013M_w \times C \ (n = 80, R^2 = 0.95, p < 0.01) \quad (10)$$

Where $S = 0$ for unsheared beer and $S = 1$ for sheared beer. The higher multiplier of shearing and arabinoxylan concentration in eq 9 compared with that in eq 10 suggested that the fraction of arabinoxylans retained by 0.22-μm membrane (>0.22 μm) was more easily affected by shearing and arabinoxylan concentration than the fraction of arabinoxylans retained by 0.45-μm membrane (>0.45 μm).

Effects of pH and Ethanol Concentration on the Distribution of the Highest Molecular Weight Arabinoxylans in Unsheared and Sheared Beer after Filtration. The highest molecular weight arabinoxylans (963 kDa) at the highest concentration of 1000 mg/L was used to investigate the effects of pH, ethanol concentration, and shearing on the distribution of particle size of arabinoxylans after filtration. The effects are shown in **Figure 3A−C** and **Figure 4A−C**.

The proportion of arabinoxylans with particle size >0.22 μm and >0.45 μm in unsheared and sheared beer can be described by the following equations:

$$F_{>0.22} = 6.81 + 9.09S + 0.63E + 3.77\text{pH} \ (n = 36, R^2 = 0.85, p < 0.01) \quad (11)$$

$$F_{>0.45} = 1.80 + 7.54S + 1.08E \ (n = 36, R^2 = 0.89, p < 0.01) \quad (12)$$

where E is the ethanol concentration (%, v/v), pH is the pH value of the beer samples, $S = 0$ for unsheared beer, and $S = 1$ for sheared beer. Eq 11 and eq 12 suggested that the distributions of particle sizes of arabinoxylan had a positive correlation to shearing and ethanol content. However, the proportion of high molecular weight arabinoxylans particle size retained by the 0.45-μm membrane was not significantly ($p > 0.05$) influenced by pH value.

SEM Photos of Membrane Plugging by Beers Containing Different Molecular Weight Arabinoxylan Fractions. Cross-flow microfiltration (CFMF) is attracting increased technical and commercial interest as an alternative method for beer clarification in the brewing industries (*33*). However, membrane filtration is more susceptible to clogging by colloidal beer particles. When nominal pore diameter of 0.45 μm was chosen, a membrane with this pore size can readily be blocked depending on the amount and size of particulates present in beer (*34*). The interception of a host of large particles of biological origin and the required transmission of macromolecular species through the membrane pores often lead to a severe and unavoidable fouling, which are very complex in terms of fouling constituents, formation, and structure. Proteins and some of carbohydrates (such as β-glucans and dextrins) are known membrane foulants (*35*, *36*), but they are also essential beer quality elements (*18*). Only few studies on the effect of arabinoxylans on membrane plugging have been reported (*17*, *18*).

Figure 5**A** showed the SEM photo of the membrane surface without any foulants. Beers containing arabinoxylans of three different molecular weights (102 kDa, 389 kDa, 963 kDa) at concentrations of 1000 mg/L were prepared and filtered through the 0.45-μm membrane to investigate membrane plugging; SEM photos were shown in **Figure 5B**, **C**, **D** respectively. When low molecular weight arabinoxylans (102 kDa) were added to beer, the pores were blocked by discrete particles and the pore pathways were slightly plugged by smaller colloids entrapped in the structure (**Figure 5B**). When arabinoxylans with higher molecular weight (389 kDa) were added to beer, this problem was more pronounced (**Figure 5C**). When arabinoxylans with the highest molecular weight (963 kDa) were added (**Figure 5D**), the fouling problem was more severe and almost all of the membrane pores were plugged by arabinoxylans. These SEM photos showed that membrane plugging was significantly affected by the arabinoxylans molecular weight.

ACKNOWLEDGMENT

We greatly appreciated the help of Mr. Terry Pickett of The Department of Post Graduate Studies, Southern Yangtze University, China, for his kindness in editing our manuscript.

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Received for review November 18, 2004. Revised manuscript received April 18, 2005. Accepted April 26, 2005.

JF0480694