Enhanced Polysaccharides Yield Obtained from Hydrothermal Treatment of Corn Bran *via* Twin-screw Extrusion

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Lignocellulosic biomass, such as corn bran, has limited accessibility to solvents during thermal-chemical processes. However, a pretreatment can help to change the characteristics of the raw material and improve the yield of the polysaccharides. A twin-screw extrusion pretreatment was developed to enhance the polysaccharide yield and decrease the optimum temperature and time during the hydrothermal treatment of corn bran. The effects of temperature and time on the polysaccharide yield were investigated during the hydrothermal treatment with pretreated and unpretreated corn bran. All samples were comparatively analyzed by X-ray diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy, scanning electron microscopy (SEM), and thermogravimetric analysis to investigate the changes in the chemical and physical characteristics. The results showed that the twin-screw extrusion pretreatment changed the main physical structure and thermochemical behavior of corn bran, which confirmed that it could enhance the polysaccharide yield and decrease the optimum treatment temperature and reduce the process duration. The pretreatment and the hydrothermal treatment temperature also had a synergetic effect on extraction yield and the composition of polysaccharide fractions of corn bran compared to the unpretreated sample. This study contributes to the knowledge improvement of corn bran pretreatments, which can be used for the efficient production of polysaccharides.

Keywords: Twin-screw extrusion; Corn bran; Polysaccharide; Hydrothermal treatment

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INTRODUCTION

Corn bran is an agro-industrial byproduct that results from the wet milling step in corn starch production. It contains complex carbohydrate polymers, such as cellulose, hemicelluloses, and 1% to 3% lignin (Yadav *et al.* 2016). Corn bran is usually confined within the fields of animal feeding, anaerobic fermentation, and sometimes treated as a waste (Zhang *et al*. 2015). Recently, there have been industrial and scientific interests in searching for an alternative way to increase the economic value of corn bran. Although corn bran is still underutilized, it has received attention as a potential commercial source of polysaccharides.

Polysaccharides are natural polymers and constructed from several monosaccharide units and their derivatives, which are widely distributed in microorganisms, plant, and animals (Shi *et al.* 2016). Recently, much attention has been paid to polysaccharides because they may possess a variety of biological activities, such as inmunostimulating, antioxidant, antitumor, antihyperlipidemic, and antidiabetic activities (Liu *et al*. 2015; Yu *et al*. 2015). In addition, polysaccharides are usually biodegradable, nontoxic, and with few adverse effects (Jia *et al.* 2013). Therefore, they may potentially be developed as natural functional products. Water is an effective and "green" solvent for the direct extraction of polysaccharides from plant materials. The hot-compressed water process, also called subcritical water or a hydrothermal treatment, is known as an eco-friendly and economical technology (Liu *et al.* 2016). However, a hot-water extraction method of polysaccharides is commonly associated with high temperature and long extraction duration. Moreover, high temperature and long treatment time might cause the degradation of polysaccharides and decrease the pharmacological activity (Hromádková *et al.* 2002).

Because of the structural features of a lignocellulosic biomass, such as corn bran, it has limited accessibility to solvents and enzymes during the thermal-chemical and bio-chemical processes, respectively. However, a pretreatment would help to break down the lignin-carbohydrate complexes and improve the accessible surface area of lignocelluloses (Mosier *et al*. 2005; Liu *et al.* 2011). Various methods have been used for the pretreatment of lignocellulosic materials, of which include the dilute acid, hydrothermal treatment, soaking in aqueous ammonia, ammonia fiber expansion, and steam explosion methods (Díaz *et al*. 2010; Kang *et al*. 2013). However, those methods have some disadvantages, such as potential environmental pollution, low pretreatment effectiveness, and high energy consumption, which limit their large-scale utilizations (Zhang *et al.* 2014). Thus, a new method is required to homogenize lignocellulosic biomass to address those disadvantages. The twin-screw extrusion pretreatment is a relatively novel physicochemical method, through which biomass is processed by means of compression, heat, and shear forces, leading to chemical modifications and physical disruption of biomass during the passage through the extruder (Alvira *et al*. 2010; Karunanithy and Muthukumarappan 2010; Zhang *et al*. 2012). To the best of the authors' knowledge, although extractions of polysaccharides from various lignocellulosic materials by hot-compressed water method have been performed; however, there is little literature on the extraction of polysaccharides from corn bran after twin-screw extrusion pretreatment by hot-compressed water.

In the present study, the main objective is to establish the feasibility of corn bran for designing a cost-effective, less energy consuming, and environmentally friendly polysaccharides extraction system. Thus, corn bran was pretreated by the twin-screw extraction and then extracted by the hot-compressed water in a confined environment under various conditions (extraction temperature and time). In addition, the characteristics of the untreated and pretreated corn bran samples were analyzed *via* XRD, SEM, FT-IR, sugar analysis, and thermogravimetric analysis. The main polysaccharides products were also analyzed by FT-IR and a sugar analysis.

EXPERIMENTAL

Materials

The corn bran was collected from Henan Yonglong Medicine & Food Technology Co., Ltd. (Zhengzhou, China). The feedstock was ground *via* a high-speed rotary cutting mill and then sieved through a 40-mesh screen. The powder was dried at 80 °C for 24 h before use. The moisture, ash, protein, crude fiber, and extractives contents of the corn bran were determined according to the American Oil Chemists' Society (AOCS) methods (Firestong 1998). The results showed that the compositions of the corn bran were 8.0±0.6%

moisture, 1.2±0.05% ash, 9.7±0.4% protein, 16.2±0.5% crude fiber, and 2.6±0.3% extractives. All chemicals used were analytical grade.

Methods

Pretreatment

The pretreatment of corn bran was performed on a continuous twin-screw extruder (Saixin Extrusion Machinery Co., Ltd., Jinan, China) with the screw diameter of 28 mm (L/D ratio was 36/1). The screw arrangement could be varied to suit for the specific characteristics of corn bran. The extruder was designated for a maximum capacity of 200 kg of oven-dried feedstock per hour at with a twin-screw speed of 150 rpm. The temperature was controlled by an oil heater and the difference of the temperature during the pretreatment process was ±5 °C. Before the pretreatment, the corn bran was mixed with water at the solid/liquid ratio of 4:1 (g/g). The mixture of water and corn bran entered the extruder *via* a feed inlet continuously and was crushed by the twin-screw extruder at the temperatures of 110 and 180 °C, respectively.

Isolation of polysaccharides

The polysaccharides from native and pretreated corn bran materials were extracted according to the previously reported method by Liu *et al.* (2016). Briefly, a 250 mL pressure glass reactor was loaded with 10 g of corn bran and 100 mL of water with a solid to liquid ratio of 1:10 and sealed. Agitation was set at 500 rpm and kept constant for all experiments. The reactor was heated up to the setting temperature by a magnetic heating stirrer (IKA, Guangzhou, China) at a heating rate of approximately 3 °C/min, and the temperature was maintained at the setting temperature for the desired holding time. After a hot-compressed water extraction, the water-soluble fraction was retrieved by filtration, and the filtrate was collected for precipitation by anhydrous ethanol (final ethanol ratio was approximately 75%). The precipitates formed were recovered by filtration, washed with acidified 75% ethanol, freeze-dried, and labeled as polysaccharide A (P_A). The non-precipitated filtrate was concentrated under reduced pressure at 45 °C, freeze-dried, and labeled as polysaccharide B (P_B). The water-insoluble fraction was dried at 105 °C, weighed, and called the solid residue (SR). The P_A, P_B, and SR yields were calculated on a dry-ash-free basis, and each experiment was performed in duplicate. The highest standard deviations of the product yields were 3.0%.

Thermogravimetric analysis

A thermal analysis was performed by using a simultaneous thermogravimetric analysis (TGA) (Pekin-Elmer of China, Beijing, China), and differential thermal analysis (DTA) was performed on a simultaneous thermal analyzer (SDT-60, Shimadzu, Nakamura-ku, Koyoto, Japan). The apparatus was continually flushed with a nitrogen flow of 25 mL/min. The sample was weighed between 8 mg and 12 mg and heated from room temperature to 640 °C at a rate of 10 °C/min.

SEM observations

The changes in corn bran morphology before and after pretreatment were observed by scanning electron microscopy (SEM). For magnification, each sample was viewed at an accelerating voltage of 30 kV.

XRD analysis

Diffractograms were recorded in reflection mode in the angular range of 5° to 40° (2θ) by steps of 0.02° (2θ). The measurements were obtained with a diffractometer (Bruker, model D8 advance). The Cu Kα radiation generated at 40 kV and 40 mA was monochromatized using a 20 μm Ni filter.

FT-IR analysis

The FT-IR analysis of samples (P_A and corn bran before and after pretreatment) was operated on a Nicolet iN10 FT-IR spectrophotometer (Adhoc International Technologies Co., Ltd, Beijing, China).

Sugar analysis

The sugar analysis in the samples (P_A, P_B, and SR) was determined by high-performance anion-exchange chromatography (HPAEC, Agilent, Ratingen,Germany) using a Dionex ICS3000 gradient pump, amperometric detector, AS50 autosampler, and a Carbopac^{TM} PA-20 column (4 × 250 mm, Dionex). The neutral sugars and uronic acids in the samples were obtained by hydrolysis with 1.0 M H_2SO_4 at 105 °C for 2.5 h. Neutral sugars and uronic acids were separated in 5 mM NaOH isocratic eluent (carbonate free and purged with nitrogen) for 20 min, followed by a 0-75 mM NaAc gradient in 5 mM NaOH for 15 min. Then the columns were washed with 200 mM NaOH to remove carbonate for 10 min, and followed a 5 min elution with 5 mM NaOH to re-equilibrate the column before the next injection. The total analysis time was 50 min, and the flow rate was 0.4 mL/min. Calibration was performed with standard solutions of L-arabinose, D-glucose, D-xylose, D-glucose, D-mannose, D-galactose, glucuronic acid, and galacturonic acids.

RESULTS AND DISCUSSION

Thermogravimetric Analysis of Corn Bran Samples

The effect of the twin-screw extrusion pretreatment on the pyrolytic behavior of the corn bran was investigated *via* TGA, and the weight loss traces and their differential curves were recorded in the temperature range of 30 °C to 640 °C and are shown in Fig. 1. The thermograms of the untreated and pretreated at 110 and 180 °C corn bran showed one similar pyrolysis process in the TGA curves.

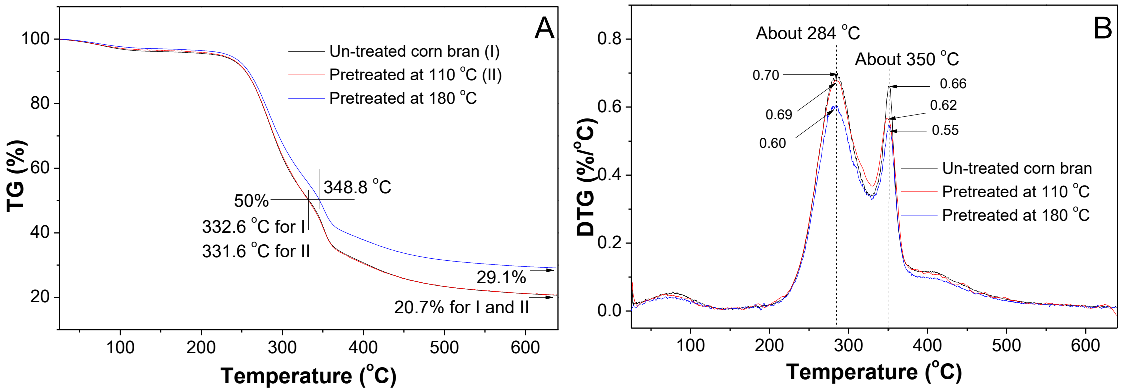

Fig. 1. TG (A) and DTG (B) curves of the untreated and pretreated corn bran samples

At the first stage, from 30 °C to 105 °C, weight loss was due to the evaporation of free and bonded water. This water represented 4.0% of the initial weight of the samples, and the main weight loss of the samples occurred from 200 °C to 400 °C. Above 400 °C, an abrupt change in the slope of the curve occurred, which indicated a slower weight loss. The weight loss was 50.0% at 332.6 °C, 331.6 °C, and 348.8 °C for the raw corn bran, the pretreated samples at 110 °C, and the pretreated samples at 180 °C, respectively. Finally, it could be clearly noted that the pretreated corn bran sample at 180 °C had a higher content of residue (29.1%) than that of the raw corn bran and the pretreated sample at 110 °C (20.7%). The TG analysis indicated that the pretreatment at 180 °C had improved the thermal stability, and assumed that the increasing amount of TG mass residue was correlated with the insoluble content.

In the DTG curves, the pretreated and untreated corn bran samples both showed two degradation peaks. The first peak occurred between 200 °C and 340 °C in three samples, mainly due to the cellulose and hemicelluloses in the corn bran samples. The stability of cellulose can be attributed to the intra-molecular and intermolecular hydrogen bonds regularly repeating along each cellulose chain (Wang *et al.* 2009). Under an inert atmosphere, the end-products of the cellulose decomposition are carbonaceous residues, which consist mainly of polycyclic aromatic compounds (Mamleev *et al.* 2007). During the thermal degradation process, the hemicelluloses were decomposed to monomeric sugars, furfural, furan, aldehyde, and acetic acid. The second degradation peak was also observed in all samples between 330 °C and 380 °C, which corresponded to the degradation of protein in the samples. During the degradation, non-covalent bonds were decomposed, including intramolecular and intermolecular hydrogen bonds, electrostatic bonds, and hydrophobic interaction, then covalent bonds between C (O)-NH, C-H, $-NH_2$, and C (O)-NH_2 of amino acid residues were broken as the temperature increased (Liu *et al.* 2015). The protein backbone was completely decomposed and released various gases, such as NH_3, CO, and CO_2 (Schmidt *et al.* 2005; Das *et al.* 2008). The high temperature pretreatment *via* twin-screw extrusion could unfold the protein structure and expose more reactive side groups, such as hydroxyl groups, carboxyl, and amino that were responsible for cross-linking reactions (Mo and Sun 2000). Therefore, this might be the possible reason for the high thermal stability of the corn bran sample with the pretreatment at 180 °C.

SEM Observations of the Corn Bran Samples

Scanning electron microscopy was used to investigate the morphological changes in the untreated and pretreated corn bran samples. The SEM images of the various corn bran samples (Fig. 2) revealed that the raw corn bran sample surface was smooth, tight, and contiguous.

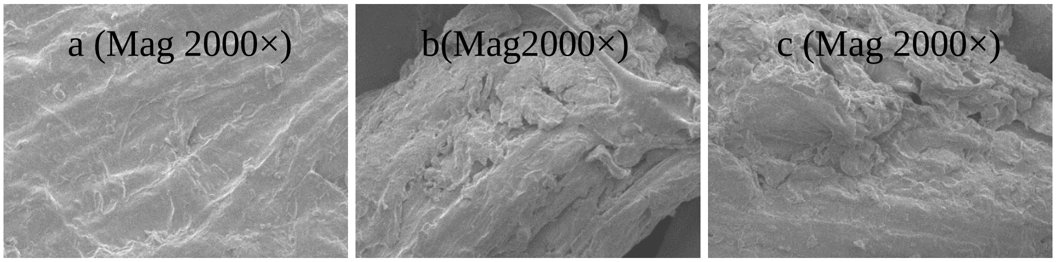

Fig. 2. SEM images of the untreated corn bran sample (a), pretreated sample at 110 °C (b), and pretreated sample at 180 °C (c)

In comparison, the twin-screw extrusion pretreatment undoubtedly changed the surface structures of the corn bran samples. Clearly, the surface of the pretreated corn bran samples showed extensive irregular pore structures and the fibers were relatively loose compared to that of the untreated corn bran. Due to the improvement of the accessibility to solvents, it would be very useful for the further degradation of corn bran to produce a high yield of polysaccharides (Liu *et al*. 2011; Wang *et al*. 2016).

XRD and FTIR Analysis of the Corn Bran Samples

Unlike the hemicelluloses, which are amorphous in nature, cellulose includes both the amorphous and crystalline domains in its structure. The crystalline structure of cellulose in corn bran samples before and after pretreatment was investigated by XRD. The XRD diffractograms are listed in Fig. 3A. All of the diffractograms showed a sharp peak and a small peak at 21.5° and 34.9°, which represented the diffraction planes of (002) and (040), respectively, are characteristic of cellulose I (Cave 1997; Guo *et al.* 2016). A weak peak in the diffraction pattern of typical cellulose I was not clearly observed in Fig. 3A, which could have been overlapped with the big peak at 21.5°. The results indicated that the low crystalline structure in the cellulose of these corn bran samples could have been easily degraded during the hydrothermal treatment process. Comparatively, after pretreatment, there was a decrease in the intensity of the (002) reflection, especially in the sample pretreated at 110 °C.

The higher intensity of the (002) reflection in the sample pretreated at 180 °C as compared with that of 110 °C might be due to a more significant breakdown of the amorphous cellulose under the pretreatment condition. Interestingly, the (040) reflection experienced no obvious change in the three samples, which indicated that the cellulose molecular chains in the corn bran samples remained largely unaffected by the twin-screw extrusion.

The FT-IR (Fig. 3B) spectra showed that most bands of the three samples exhibited similar absorption and relative intensities that indicated the pretreatment could not change the chemical structures of the three samples. According to the compositions analysis of the raw corn bran, the sample was mainly composed of cellulose, hemicelluloses, protein, and a small amount of lignin. The bands at 3350 cm^{-1} were attributed to the -OH in these samples and water. Cellulose had its characteristic absorption bands at 3350 cm^{-1}, 2922 cm^{-1}, 1369 cm^{-1}, and 1151 cm^{-1}. The absorption peaks at 1741 cm^{-1}, 1645 cm^{-1}, and 1369 cm^{-1} indicated the presence of the hemicelluloses polysaccharides in these samples (Zhang *et al*. 2016). The small absorption peaks at 1315 cm^{-1} and 830 cm^{-1} to 750 cm^{-1} represented the low content of the lignin in these samples. Therefore, corn bran can be regarded as a good resource for the polysaccharides production due to its low lignin content. According to Nelson and O'Connor (1964), the absorptions at 1420 cm^{-1} and 1430 cm^{-1} were attributed to the amorphous cellulose or crystallized cellulose II and crystallized cellulose I, respectively.

The spectra obtained from the unpretreated and pretreated corn bran samples both showed a band at 1427 cm^{-1}, which indicated that all of the samples contained a mixture of amorphous cellulose and crystallized cellulose I. The same absorption peak among these unpretreated and pretreated samples also indicated that the cellulose molecular chains in the corn bran sample were not affected by the pretreatment (Wang *et al.* 2009), which was in agreement with the XRD results.

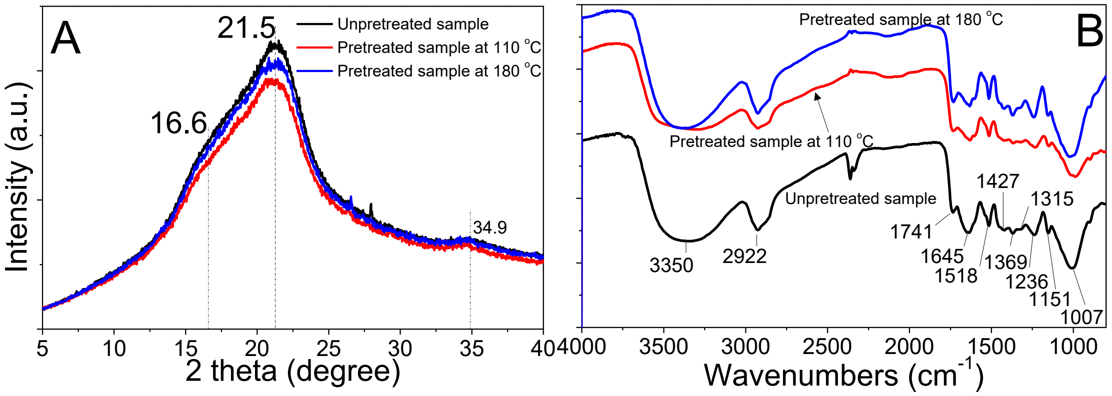

Fig. 3. XRD (A) and FT-IR spectra (B) patterns of the various corn bran samples

Polysaccharides Yield at Various Conditions

The product fractions obtained from each of the hydrothermal treatment experiments contained SR, P_A, and P_B. In the present investigation, P_A was the primary product fraction of interest.

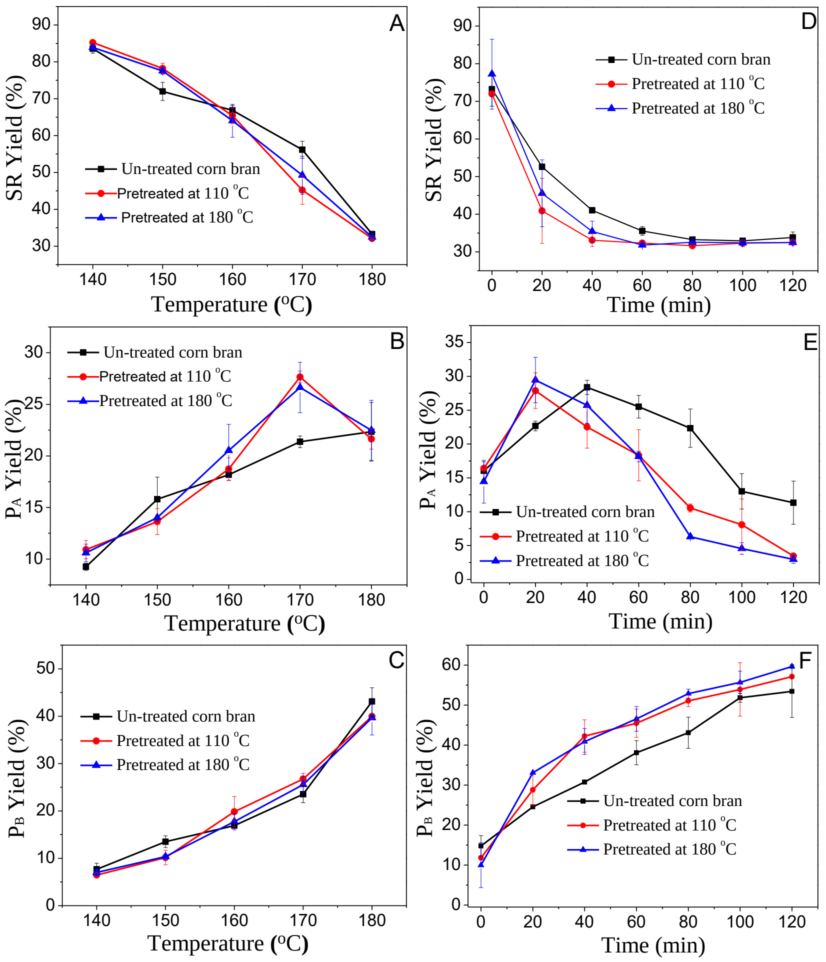

Fig. 4. Effects of temperature (A-SR yield; B-P_A yield; C-P_B yield) and time (D-SR yield; E-P_A yield; F-P_B yield) on the product yields

To increase the main product fraction yield and decrease the treatment temperature and time, the twin-screw extrusion pretreatment was performed to modify the physicochemical structural barriers and remove the compositional impediments of corn bran. These corn bran samples, untreated and pretreated by the twin-screw extrusion, were treated hydrothermally under the same conditions to investigate the pretreatment effect on the polysaccharide yields. The polysaccharide fractions and the SR of the three samples with the hydrothermal treatment at various conditions are shown in Fig. 4. As shown in Fig. 4A, regardless of the pretreatment effect on corn bran, the SR yield decreased continually when the treatment temperature was increased from 140 °C to 180 °C. Compared to the unpretreated corn bran samples during the hydrothermal treatment, one decrease from 140 °C to 160 °C, following by a more intense decrease higher than 160°C whatever the pretreatment used, which indicated that the pretreatment increased the total conversion rates at these temperatures.

As shown in Fig. 4B, when the treatment temperature increased from 140 °C to 180 °C, the P_A yield of the unpretreated corn bran sample increased from 9.3% to 22.3%. Comparatively, the pretreated corn bran samples had higher P_A yields and lower optimum hydrothermal treatment temperatures compared to that of the unpretreated sample. The highest P_A yields of the pretreated samples by the twin-screw extrusion at 110 °C and 180 °C were 27.7% and 26.6%, respectively, with the hydrothermal treatment at 170 °C. A decrease of the P_A yields of the pretreated samples was also observed as the temperature increased from 170 °C to 180 °C. At the temperature range of 140 °C to 170 °C, the P_B yields of the pretreated samples were approximately 6% to 26% (Fig. 4C). However, as the extraction temperature increased to 180 °C, the yields of P_B increased considerably to approximately 46.0% (almost two times than that at 170 °C). The reason for this behavior was that the secondary decomposition of the P_A fraction at the high temperature led to the high P_B fraction yield (Liu *et al.* 2016). Compared to the higher pretreatment temperature *via* the twin-screw extrusion, the lower pretreatment temperature at 110 °C was sufficient for the corn bran to form a higher P_A fraction yield. Therefore, the operation of the extraction system at the lower temperature with mild pretreatment was preferred due to the higher polysaccharide yield and lower heat loss per unit mass of the obtained polysaccharide.

Figures 4D through F show the effect of treatment time at the temperature of 180 °C on the product yields with the hydrothermal treatment temperature at 180 °C. Clearly, the SR yields obtained from these samples decreased continuously with the increased treatment time from 0 min to 80 min. However, when the treatment time increased to 120 min, the total conversion rates could not markedly increase. The P_A yield of the unpretreated sample reached a maximum of 28.4% at 40 min and remained approximately constant at 11.3% as the treatment time was increased from 60 min to 120 min. The yield of P_B increased continuously at the ranges of the treatment time tested for all samples (Fig. 4F). Therefore, the yield of P_A fraction decreased as the treatment time was further prolonged due to the degradation of P_A to P_B. Comparatively, the pretreatment by the twin-screw extrusion could curtail the optimization treatment time during the hydrothermal treatment of corn bran. The maximum P_A yields of the hydrothermal treatment of the pretreated samples at 110 °C and 180 °C were 27.9% and 29.5%, respectively, with the treatment time at 20 min. Therefore, the operation of the extraction system at 170 °C for shorter treatment time with mild pretreatment (110 °C) was preferred due to the higher polysaccharide yield and lower heat loss per unit mass of the obtained polysaccharide.

Sugar Analysis of Solid Residues

The main sugars of the corn bran sample were arabinose and xylose (mainly from hemicelluloses), which accounted for 34.4% and 22.1% of the sample, respectively, followed by glucose (from cellulose), which accounted for 15.7%. Small amounts of galactose, mannose, and glucuronic acid were also observed. To help understand the effect of the pretreatment on the degradation process of corn bran, the monosaccharide sugar compositions of SRs of various samples obtained from the hydrothermal treatment at 140 °C, 160 °C, and 180 °C for 0 treatment time were determined (Table 1).

Table 1. Sugar Compositions (wt%) of the SRs from the Hydrothermal Treatment of the Unpretreated and Pretreated Corn Bran Samples

Samples[a]	Ara[b]	Gal[b]	Glu[b]	Xyl[b]	Man[b]
Raw Corn Bran	34.4	3.8	15.7	22.1	1.0
UP-SR-140	45.6	5.2	11.6	35.4	2.4
UP-SR-160	29.4	6.4	15.3	43.8	3.7
UP-SR-180	5.9	4.3	51.3	31.6	6.9
P110-SR-140	45.4	5.2	11.6	35.4	2.4
P110-SR-160	33.1	5.7	18.5	39.0	2.7
P110-SR-180	21.8	5.8	33.9	35.1	3.5
P180-SR-140	43.2	6.6	10.0	38.2	2.0
P180-SR-160	32.6	6.1	16.5	42.4	2.4
P180-SR-180	8.8	4.1	42.9	41.1	3.1

[a] UP-SR-140, UP-SR-160, UP-SR-180, P110-SR-140, P110-SR-160, P110-SR-180, P180-SR-140, P180-SR-140, and P180-SR-180 represent the SRs obtained from the hydrothermal treatment of the unpretreated (UP) and pretreated (P) corn bran samples at 110 °C (P110) and 180 °C (P180) and the hydrothermal treatment at various temperatures (140 °C, 160 °C, and 180 °C), respectively; [b] Abbreviation: Ara, arabinose; Gal, galactose; Glu, glucose; Xyl, xylose; and Man, mannose

Clearly, for all of the SRs of the unpretreated and pretreated corn bran samples, the contents of arabinose decreased continuously with increased extraction temperature. During the hydrothermal treatment process, the hemicelluloses would be the first to degrade at a temperature of 180 °C, while the cellulose degraded at a relatively higher temperature (> 240 °C) (Ando *et al.* 2000; Akhtar *et al.* 2010). The contents of glucose in the SRs increased with the increasing treatment temperature. The increase in the glucose contents from 140 °C to 180 °C was mainly caused by the degradation of arabinose and galactose. For the unpretreated and pretreated corn bran samples, the xylose contents in the SRs first increased as the treatment temperature increased from 140 °C to 160 °C, and then decreased slightly as the temperature increased to 180 °C. In addition, the SRs obtained from all samples at 180 °C have higher xylose contents and lower arabinose contents as compared with raw corn bran. The results indicated that the structure of xylan in the corn bran was not drastically broken down under the treatment conditions, which was consistent with the authors' previous investigation on the hydrothermal treatment of soy hulls (Liu *et al.* 2016). Comparatively, the pretreatment had an important effect on the degradation process of corn bran during the hydrothermal treatment. For example, in comparison with the other solid resides from the hydrothermal treatment at 180 °C, a noticeable difference was observed in the P110-SR-180 sample. A higher content of xylose (35.1%) was obtained from the hydrothermal treatment of pretreated sample at the pretreatment temperature of 110 °C, but a relatively lower quantity of glucose (33.9%) was found under the same condition.

Characterization of Polysaccharide Fractions

Sugar and protein compositions

Table 2. Sugar Compositions (wt%) of the Polysaccharides Obtained from the Hydrothermal Treatment of the Unpretreated and Pretreated Corn Bran Samples

Temperature	Samples[a]	Ara[b]	Gal[b]	Glu[b]	Xyl[b]	Man[b]	UA[b]	Pro[b]
140 °C	UPS-P_A	25. 4	2.4	53.7	13.6	1.2	0.9	2.9
	PS-110-P_A	23.4	2.3	57.7	10.8	0.9	1.8	3.1
	PS-180-P_A	26.1	2.5	51.1	14.7	0.9	1.6	3.1
160 °C	UPS-P_A	23.9	3.8	39.4	27.1	1.2	1.2	3.3
	PS-110-P_A	25.5	3.3	40.4	24.5	1.5	1.7	3.2
	PS-180-P_A	26.5	3.2	40.3	24.2	1.0	1.8	3.0
180 °C	UPS-P_A	12.5	3.8	48.1	28.2	1.4	1.0	5.0
	PS-110-P_A	14.0	4.0	43.2	28.8	3.2	1.9	4.8
	PS-180-P_A	14.2	4.2	42.4	31.5	1.5	1.5	4.8

[a] UPS-P_A, PS-110-P_A, PS-180-P_A, UPS-P_B, PS-110-P_B, and PS-180-P_B represent the P_A and P_B fractions obtained from the hydrothermal treatment of the unpretreated (UPS) and pretreated (PS) corn bran samples at 110 °C and 180 °C, respectively; [b] Abbreviation: Ara, arabinose; Gal, galactose; Glu, glucose; Xyl, xylose; man, mannose; UA, uronic acid; and Pro, protein

To investigate the effect of the twin-screw extrusion pretreatment on the polysaccharide composition obtained from the hydrothermal treatment of corn bran samples at various temperatures, nine P_A fractions were prepared for the determination of their constituent sugars and proteins, and the results are listed in Table 2. Clearly, the glucose was a predominant sugar constituent in the nine polysaccharide fractions (39.4% to 57.7%). The dominant glucose residues originated from the amorphous cellulose. However, the result was not consistent with the authors' previous investigation on polysaccharide fractions obtained from the hydrothermal treatment of soy hulls (Liu *et al.* 2016). A possible explanation for the results was that the high content of the amorphous cellulose in corn bran favored the degradation of glucans during the hydrothermal treatment process. As shown in Table 2, the compositional differences were relatively large amongst the P_A fractions that originated from various samples. The differences of the components among these P_A fractions, obtained from the hydrothermal treatment of various samples, could have contributed to the distinctive characteristics of corn bran samples after the twin-screw extrusion. The content of arabinose decreased from 23.4-26.5% in the P_A fraction obtained from the hydrothermal treatment at 140-160 °C, to 12.5-14.2% in the P_A from the experiments at 180 °C. This phenomenon was probably due to the arabinose, as a side chain in corn bran hemicelluloses, which was easily solubilized during the lower temperature of the hydrothermal treatment process. When the hydrothermal treatment temperature reached 180 °C, xylose appeared as the second major sugar in these polysaccharides, which comprised of 28.2% to 31.5% of the total sample compositions. As compared with the P_A fraction obtained from the unpretreated sample extraction at 180 °C, the P_A fractions obtained from the pretreated samples appeared to have higher contents of xylose and lower contents of glucose units. In addition, the protein content in the P_A fraction increased with the increase of the hydrothermal treatment temperature, which was due to the re-polymerization of protein that occurred at higher temperatures. It could be concluded that the pretreatment and the hydrothermal treatment temperature had a synergistic effect on the compositions of the polysaccharide fractions.

FT-IR analysis

Figure 5 shows the FTIR spectra of various P_A fractions. Clearly, no considerable difference in the main absorption intensity was found among these fractions, which indicated that the twin-screw extrusion pretreatment had no significant effect on the structure of the polysaccharide fractions during the hydrothermal treatment process. The signals found at approximately 3360 cm^{-1} were assigned to the -OH stretching vibrations of all the polysaccharide fractions and one peak at approximately 2927 cm^{-1} was related to the C-H stretching. In the carbonyl stretching region of the P_A fractions, the intensity of the peaks at around 1730 cm^{-1}, indicated that the acetyl group of these polysaccharides increased with the hydrothermal treatment temperature (Jing *et al.* 2010). The weak peaks at around 998 cm^{-1} were characterized by the presence of the lower substituted arabinoxylans in these polysaccharide fractions, which corresponded to the relatively lower xylose contents in these polysaccharide fractions obtained by the sugar analysis.

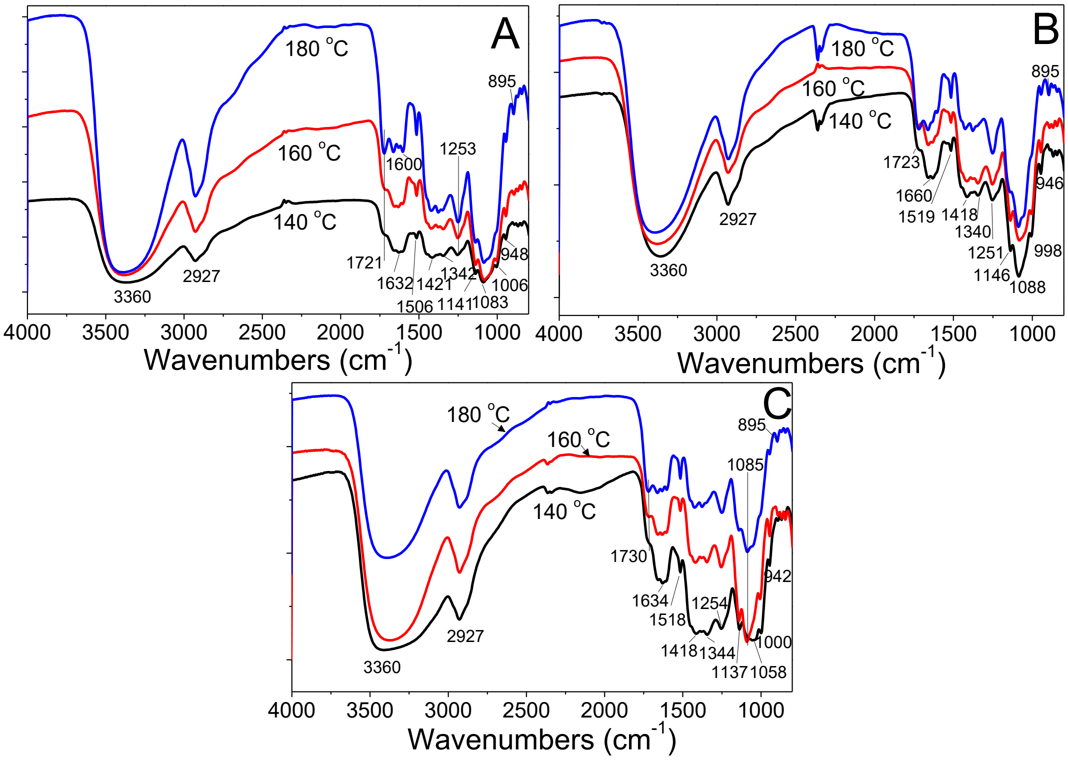

Fig. 5. FT-IR spectra of PA fractions obtained from the hydrothermal treatment of the unpretreated (A) and pretreated corn bran via Twin-screw Extrusion at 110 °C (B) and 180 °C (C)

The absorption peak at approximately 1250 cm^{-1} was assigned to the C-O linkage in the acetyl group in xylan and the OH in-plane bending of glucose units. Furthermore, the absorption peak at 1082 cm^{-1} was originated from the ring vibration (Kacurakova *et al.* 2000). The occurrence of a very small peak at approximately 1515 cm^{-1} in all the spectra was visible due to the presence of the small amount of associated lignin in these polysaccharide fractions, and because the absorption intensities in the polysaccharide fractions increased with hydrothermal treatment temperatures. This indicated that more lignin of corn bran was degraded from corn bran at higher temperatures. The broad peak at around 1635 cm^{-1} was mainly attributed to the H-O-H angle vibration, because these polysaccharide fractions have a strong affinity for water (Kačuráková *et al.* 1998). In the anometric region (700 cm^{-1} to 950 cm^{-1}), a small sharp band at 895 cm^{-1} was clearly visible in the spectra of P_A fractions obtained from the hydrothermal treatment at 180 °C, which indicated the presence of the dominant β-glycosidic linkages between these sugar units in the polysaccharide fractions obtained at the higher hydrothermal treatment temperature (Blakeney *et al.* 1983). However, the intensity of such signal became weak or could not be detected in these polysaccharide fractions obtained at the lower hydrothermal treatment temperatures (140 °C and 160 °C), which indicated that the hydrothermal treatment temperature had an important effect on these polysaccharides structures.

CONCLUSIONS

1. The twin-screw extrusion pretreatment enhanced the polysaccharide yield and decreased the optimum treatment temperature and time.
2. The pretreatment and the hydrothermal treatment temperatures had a synergetic effect on the extraction yield and the compositions of the polysaccharide fractions of corn bran.
3. The twin-screw extrusion pretreatment changed the main physical structure and the thermochemical character of corn bran.
4. The pretreatment had no substantial effect on the structure of the polysaccharide fractions.

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