

Structural Changes of Polysaccharides Isolated from Corn Bran by Hydrothermal Treatment after Twin-Screw Extrusion

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The objective of this investigation was to elucidate the structural changes of the polysaccharides isolated by hydrothermal treatment of corn bran after twin-screw extrusion. The structures and antioxidant activities of the purified polysaccharides were investigated and compared by monosaccharide analysis, Fourier transform infrared (FT-IR), gel permeation chromatography (GPC), and nuclear magnetic resonance (NMR). The results showed that the structures of the linkages and monosaccharide components of the purified polysaccharides were not affected strongly by the twin-screw extrusion pretreatment. However, the purified polysaccharides isolated from pretreated samples displayed significant differences in monosaccharide ratios, degree of branching/linearity, and molecular weight. These physical changes may be related to the decrease of antioxidant activities of the polysaccharides. The present investigation contributes to the knowledge of how pretreatment by twin-screw extrusion affects the chemistry of corn bran polysaccharides. Results can be applied to improve the efficiency of hydrothermal extraction of polysaccharides from corn bran.

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

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INTRODUCTION

Corn bran is the most abundant byproduct of the industrial corn milling process used to produce corn starch. The recovered material is often used as animal feed alone or in combination with the corn germ meal and cake (Rose *et al.* 2010; Jiang *et al.* 2018). Corn bran is composed of approximately 56% hemicelluloses (mainly arabinoxylan), 20% cellulose, and 3% ferulic acid. All of these are potential low-cost organic sources of polysaccharides (Rose *et al.* 2010; Jiang *et al.* 2018).

Natural polysaccharides have specific biological activities that play primary roles in the growth and development of organisms (Liu *et al.* 2016). Efficient degradation of corn bran into polysaccharides such as arabinoxylan is one of the important requirements in corn bran utilization (Jiang *et al.* 2018). Reflux extraction technology commonly used to extract polysaccharides from plant materials requires high temperatures and long extraction times (Chen *et al.* 2015). However, long extraction time and high temperatures can lead to a decrease of the activities and decomposition of polysaccharides (Hromádková *et al.* 2002). The ideal extraction technology should be non-destructive, should be complete in a short period of time, and should give a high yield of polysaccharides (Chen *et al.* 2015). In the authors' previous investigation, it was found that twin-screw extrusion pretreatment did indeed improve extraction conditions (including decreases in both temperature and

time) and enhance the yield of intact polysaccharides from subsequent hydrothermal treatment of corn bran (Liu *et al.* 2017). In other words, the twin-screw extrusion pretreatment changed the thermochemical behavior and physical structure of the corn bran, with the result that the process took 20% less time, reduced the optimum treatment temperature, and increased the yield of polysaccharides. However, at that time it was not known what effect pretreatment has on the structure and by association the activities, of the polysaccharides. To the best of our knowledge, there are no published studies on the structural changes of polysaccharides isolated by hydrothermal treatment of corn bran after twin-screw extrusion.

In the present investigation, the main objective was to elucidate the structural changes of the polysaccharides isolated from corn bran by hydrothermal treatment after twin-screw extrusion. The extracted polysaccharides were purified and their structural properties were characterized by monosaccharide analysis, Fourier transform infrared (FT-IR), gel permeation chromatography (GPC), and nuclear magnetic resonance (NMR). Additionally, the antioxidant activities of the polysaccharides were evaluated by DPPH free radical scavenging.

EXPERIMENTAL

Materials

Corn bran was kindly provided by Henan Yonglong Medicine & Food Technology Co., Ltd. (Zhengzhou, China). The raw material was pretreated according to the authors' previous report (Liu *et al.* 2017). Briefly, the corn bran was ground and then passed through a 40-60 mesh screen. The obtained powder was dried at 80 °C for 24 h before use. All chemicals and solvents used in the present investigation were analytical grade.

Methods

Pretreatment

A continuous twin-screw extruder (Saixin Co., Ltd., Jinan, China) was used to treat the corn bran before isolation of the polysaccharides by hydrothermal treatment. The L/D ratio of the extruder was 36/1 with the screw diameter of 28 mm. Details of the pretreatment process have been described previously (Liu *et al.* 2017). In the present investigation, the process temperatures of the extruder were set at 110 °C and 180 °C. The raw corn bran samples after pretreatment at 110 °C and 180 °C were named as R110 and R180, respectively.

Polysaccharides isolation and purification

The crude polysaccharides from the raw and pretreated corn bran materials were isolated based on the previously reported process (Liu *et al.* 2016). For a typical run, 10 g of raw material and 100 mL of water were mixed in a 250 mL pressure glass reactor in a ratio of 1:10 (g/mL) and heated to the set temperature for the desired holding time using an IKA (Guangzhou, China) heating stirrer. After the isolation, the water-soluble fraction was filtered through gauze, and the filtrate was collected for precipitation with 95% ethanol. The precipitates were washed three times with 75% ethanol and freeze-dried to obtain crude polysaccharides. The crude polysaccharides isolated from raw corn bran, R110, and R180 were defined as RMP, 110P, and 180P, respectively.

The crude polysaccharides were redissolved in ultrapure water and deproteinized using Sevage solution (Staub 1965). Then the deproteinized polysaccharides were decolorized by AB-8 resin. Next, the supernatants were further purified on a cellulose DEAE-52 column (2.6 cm × 40 cm). The column was eluted stepwise with a linear gradient from 0 to 0.2 mol/L NaCl (0, 0.1, and 0.2 M) at 1.17 mL/min. The eluent fractions were collected in test tubes (10 mL per tube), and the phenol-sulfuric acid method was used to determine the total sugar content of the fractions. The elution curves were drawn using the tube number, and the absorbance at UV 490 nm as X and Y axes, respectively. The major peaks in the DEAE-52 chromatography were collected, concentrated, dialyzed against water, and lyophilized to obtain pure fractions. Finally, the purified polysaccharides thus obtained were used for structural characterization.

Structural characterization

Monosaccharide component and molecular weights of the purified polysaccharides were analyzed by HPAEC and GPC, respectively, as described previously (Wang *et al.* 2017). The IR spectra of the purified polysaccharides were recorded using a FT-IR spectrophotometer (Nicolet iN10, USA) with a liquid nitrogen-cooled MCT detector. The spectra were obtained at a resolution of 4 cm⁻¹ in the region of 4000 to 650 cm⁻¹. The UV spectra were obtained on a T6 UV-Vis spectrophotometer (Puxi, Beijing City, China) in the range of 200 to 600 nm at room temperature.

For NMR analysis, a Bruker ARX500 spectrometer (Rheinstetten, Germany) was used to obtain the ¹H and ¹³C NMR spectra at 500 and 125 MHz, respectively. The spectra were recorded after 60000 scans at 25 °C and the Stand Bruker Topspin-NMR software (Bruker, Germany) was used to analyze the NMR data.

Thermogravimetric analyses of the purified polysaccharides were performed with a thermogravimetric analyzer (Shimadzu, Kyoto City, Japan). Before thermal analysis, the freeze-dried polysaccharide samples were further dried at 80 °C for 24 h. Then 3 to 5 mg of samples were heated under nitrogen protection from room temperature to 750 °C at a heating rate of 10 °C/min.

Antioxidant activity

The antioxidant activities of the polysaccharides were evaluated by DPPH free radical scavenging capability, as described previously (Braca *et al.* 2001). Briefly, 1 mL of various concentrations of polysaccharide solutions (0 to 3.0 mg/mL) were mixed with 3 mL DPPH solution (0.1 mM DPPH in 95% ethanol) and then reacted at 37 °C in darkness for 30 min. The antioxidant activities in scavenging the DPPH radical were determined by the following equation,

$$\text{DPPH radical scavenging activity (\%)} = [1 - (A_1 - A_2) / A_0] \times 100 \quad (1)$$

where A_0 , A_1 , and A_2 refer to the absorbance values of the mixture with sample replaced by deionized water, the DPPH solution with polysaccharide samples, and the DPPH solution without samples, respectively.

RESULTS AND DISCUSSION

Extraction and Purification of Crude Polysaccharides

The crude polysaccharides isolated by hydrothermal treatment of raw corn bran, R110, and R180 samples were defined as RMP, 110P, and 180P, respectively. All the crude polysaccharides were further purified by using the same DEAE-cellulose column. Figures 1A, B, and C exhibit the various peaks of polysaccharides separated clearly by eluting stepwise with a linear gradient from 0 to 0.2 mol/L NaCl, indicating that the method was ideal for separation of the crude polysaccharides. Three various polysaccharides were obtained from each stepwise elution of crude polysaccharides. For each polysaccharide fraction, stepwise elution yielded three different polysaccharides, which were named 1, 2, and 3. Thus, for RMP the three polysaccharides obtained were named RMP-1, RMP-2, and RMP-3; for 110P the fractions were 110P-1, -2, and -3; and for 180P the fractions were 180P-1, -2, and -3. All the polysaccharides of RMP, 110P, and 180P eluted by water and 0.1 mol/L NaCl constituted the bulk (more than 90%) of total polysaccharides. Then the fractions were further investigated for elucidation of any structural changes caused by twin-screw extrusion. Unfortunately, the fractions eluted by 0.2 mol/L NaCl such as RMP-3, 110P-3, and 180P-3 could not be further investigated because of their extremely low yields. UV spectra (Fig. 1D) show that all the polysaccharides had no obvious absorption at 280 nm, indicating the absence of protein.

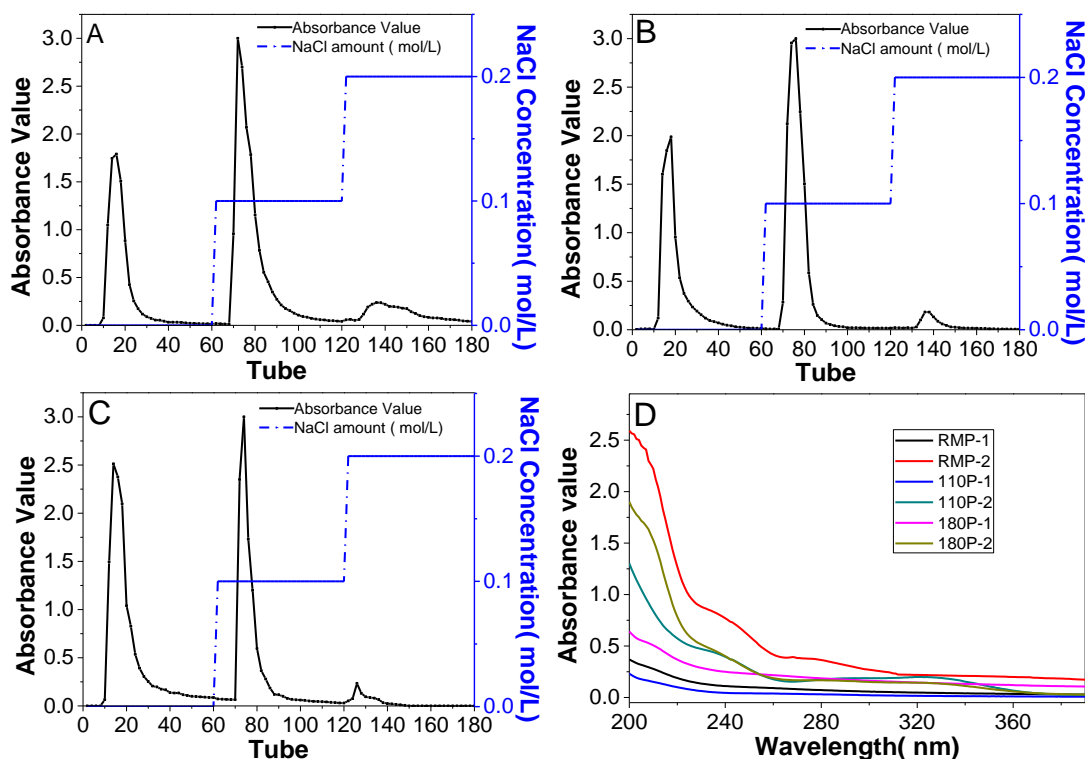


Fig. 1. Elution profiles: Crude polysaccharides from raw corn bran (A), R110 (B), and R180 (C) on DEAE-cellulose column; UV spectra of all the purified polysaccharides (D)

Sugar Analysis of Purified Fractions

The amounts of monosaccharides in the purified fractions were identified, and the results are presented in Table 1. Neutral polysaccharides of RMP-1, 110P-1, and 180P-1 were mostly composed of glucose, which comprised more than 95% of the total sugars. A small amount of galactose (around 2.5%) and arabinose (around 1.3%) were also identified in the neutral polysaccharides. The sugar analysis results indicated that the neutral polysaccharides were mainly composed of glucan. Additionally, all the neutral polysaccharides had similar monosaccharide compositions, and the monosaccharide ratios of the fractions were almost equal, which indicated that twin-screw extrusion did not obviously change the sugar components and ratios of the neutral polysaccharides.

Table 1. Monosaccharide Components of the Purified Polysaccharides

Sample ^a	Molar composition					
	Ara ^b	Gal ^b	Glu ^b	Xyl ^b	UA ^b	Ara/Xyl
RMP-1	1.23±0.08	2.41±0.04	96.36±0.09	ND ^b	ND	ND
110P-1	1.47±0.04	2.83±0.05	95.70±0.11	ND	ND	ND
180P-1	1.15±0.05	2.77±0.07	96.08±0.14	ND	ND	ND
RMP-2	11.48±0.07	19.56±0.14	18.02±0.08	47.15±0.11	3.41±0.04	0.25
110P-2	12.08±0.09	15.74±0.03	12.37±0.02	54.03±0.15	5.10±0.06	0.23
180P-2	9.58±0.04	18.21±0.06	14.56±0.07	49.54±0.19	7.20±0.03	0.20

^a RMP-1, 110P-1, 180P-1, RMP-2, 110P-2, and 180P-2 represent the polysaccharides extracted by hydrothermal treatment corn bran samples before and after twin-screw extrusion.

^b Abbreviation: Ara, arabinose; Gal, galactose; Glu, glucose; Xyl, xylose; UA, uronic acid; ND, not detectable.

Total uronic acid (included glucuronic acid and galacturonic acid) in RMP-2, 110P-2, and 180P-2 fractions occurred in the relatively small amounts of $3.41 \pm 0.04\%$, $5.10 \pm 0.06\%$, and $7.20 \pm 0.03\%$, respectively, indicating that the fractions were acidic. As shown from Table 1, xylose was the dominant monosaccharide component ($47.15 \pm 0.11\%$ to $54.03 \pm 0.15\%$). The high amounts of xylose in the fractions were taken to indicate correspondingly more xylans. The polysaccharides isolated by hydrothermal treatment of agricultural residues have been widely investigated, and the results indicate that hydrothermal treatment favors the isolation of xylose with high percentages (Lawther *et al.* 1995; Ma *et al.* 2012; Liu *et al.* 2016); these previous results are in agreement with the present investigation. In comparing the acidic polysaccharides, the monosaccharide components were the same, but the amounts were clearly different. The amounts of xylose and uronic acid in 110P-2 and 180P-2 were relatively higher compared with those in RMP-2, indicating that the xylan with acidic monosaccharide components were preferentially isolated during hydrothermal treatment after twin-screw extrusion pretreatment. Generally, the ratio of Ara/Xyl could be considered to determine the degree of branching or linearity of hemicellulosic polysaccharides. A low ratio of Ara/Xyl indicated a linear hemicellulosic polysaccharide with little monosaccharide branching, while a high ratio of Ara/Xyl suggested a short-chain polymer with much bonding (Peng *et al.* 2017). The clearly decreasing Ara/Xyl ratios, ranging from 0.25 to 0.23 and 0.20, after twin-screw extrusion pretreatment at 110 °C and 180 °C, respectively, indicated a change of the xylan backbone in the acidic polysaccharides after pretreatment. The results also indicated that the acidic polysaccharides isolated after twin-screw extrusion pretreatment appeared to be more linear. The explanation for the results could be that twin-screw extrusion pretreatment

changed the major physical and chemical structures of corn bran (Liu *et al.* 2017). Thus, these changes affected the polysaccharides isolation by hydrothermal treatment.

Average Molecular Weight of Purified Polysaccharides

The molecular weights of the purified polysaccharides are presented in Table 2. As shown from Table 2, the molecular weight of the acidic and neutral polysaccharides ranged from 13.3 kDa to 39.4 kDa and 6.2 kDa to 6.7 kDa, respectively. The results also confirmed that the neutral polysaccharides had higher molecular weights than their acidic polysaccharides. All the polysaccharides isolated from untreated corn bran exhibited a relatively higher degree of polymerization compared with their fractions isolated from pretreated corn bran samples. For the neutral polysaccharides, the molecular weight of RMP-1 was nearly 1.5 to 2 times larger than those of 110P-1 and 180P-1 fractions. This indicated that pretreatment with the twin-screw extrusion easily released polysaccharide polymers with low molecular weights. The decrease of molecular weights may be attributable to the cleavage of some sensitive linkages or to the increase in accessible surface area after twin-screw extrusion pretreatment (Mosier *et al.* 2005; Liu *et al.* 2011). These linkages were easily subsequently cleaved by the hydrothermal treatment, thus facilitating the solubilization and depolymerization of polysaccharides.

Table 2. Molecular Weight of the Purified Polysaccharides

Fractions ^a	Time (min)	M_w (Da)
RMP-1	19.5	39400
110P -1	19.8	27100
180P -1	20.7	13300
RMP-2	21.6	6700
110P -2	21.8	6200
180P- 2	21.7	6300

^a RMP-1, 110P-1, 180P-1, RMP-2, 110P-2, and 180P-2 represent the polysaccharide fractions extracted by hydrothermal treatment corn bran samples before and after twin-screw extrusion.

FT-IR Analysis of Purified Fractions

The spectra of purified polysaccharides are shown in Figs. 2A and B. All UV spectra (Fig. 1D) of the purified polysaccharides exhibited no peak at 280 nm, indicating the absence of nucleic acids or proteins in the fractions. Thus, a variety of typical absorption bands of polysaccharides could be determined in the FT-IR spectra of the purified fractions. As shown in Fig. 2A, the three neutral polysaccharides exhibited very similar spectra. The absorption peak at 3408 cm^{-1} is attributed to the stretch vibration of hydroxyl groups, and the signal in the region of around 2920 cm^{-1} is assigned to the stretch vibration of C-H bonds (Chen *et al.* 2012a). The absorption bands at around 1643 cm^{-1} and 1369 cm^{-1} are attributed to acetyl groups (Chen *et al.* 2012b; Suvakanta *et al.* 2014; Yang *et al.* 2016; Gao *et al.* 2017). The absorption peaks at around 1154 and 930 cm^{-1} in the FT-IR spectra of the neutral polysaccharides were possibly due to the stretching vibrations of the pyranose ring (Zhang *et al.* 2014). In Fig. 2, comparison with A and B, it shows that most of the absorption peaks were similar; however, the three acidic fractions showed more intense absorbance peaks at 1732 cm^{-1} , 1420 cm^{-1} , and 1248 cm^{-1} , which are attributed to the characteristic absorption of C=O stretching vibration, C-O stretching vibration, and O-H bending, respectively, confirming the structure of -COOH (Chen *et al.* 2012a). The

strong absorption peak at around 1046 cm^{-1} is attributed to glycosidic (C-O-C), C-C and C-O stretching contributions, confirming a dominant xylan structure in the acidic polysaccharides (Bian *et al.* 2012). Additionally, the sharp absorption peak at around 896 cm^{-1} is assigned to the β -configuration of the $1\rightarrow4$ glycosidic bond among the units of xylopyranose (Xylp) in the main xylan structures (Bian *et al.* 2012). The results were in agreement with the monosaccharide analysis, in which the amounts of xylose were above 40% (relative to total sugars). A comparison of the fingerprint region of the neutral or acidic polysaccharides indicated that the major absorption peaks were similar. Although the intensities differed, the similarity of the major bands indicated that the linkages of the polysaccharides were not affected strongly by the twin-screw extrusion pretreatment.

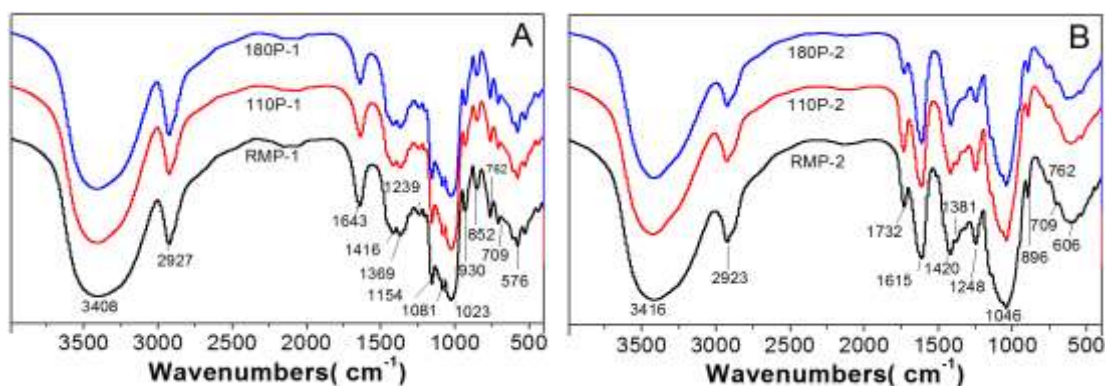


Fig. 2. FT-IR spectra of purified polysaccharides

NMR Spectra of Polysaccharides

FTIR analysis of the acidic polysaccharides indicated that the types of linkages in the fractions had no obvious differences. Thus, to further elucidate the structural features of the acidic polysaccharides, only the 110P-2 fraction was investigated by ^1H , ^{13}C NMR, and 2D HSQC NMR spectra. In the ^1H NMR spectrum (Fig. 3A), the strong signal peak at 4.66 ppm is due to the solvent chemical shift of HDO. The signal peaks in the spectral region of 5.20 to 5.40 ppm correspond to the α -anomeric protons, and the region between 4.20 and 4.90 ppm is attributed to the β -configuration (Peng *et al.* 2014; Jing *et al.* 2015). The major signal peaks at δ 4.32 (H-1), 3.96 (H-5eq), 3.63 (H-4), 3.39 (H-3), 3.29 (H-5ax), and 3.14 (H-2) ppm are attributed to units of β -D-xylopyranosyl residues (Peng *et al.* 2011). This also confirmed that the xylose residues present in the polysaccharide fraction is β -glycosidic linked, which agrees with the small bond peak observed at 896 cm^{-1} in the IR spectrum. As shown in Fig. 3B, the ^{13}C NMR spectrum exhibits five major signal peaks at δ 102.90 (C-1), 76.12 (C-4), 74.68 (C-3), 73.43 (C-2), and 63.08 (C-1) ppm, contributing to $(1\rightarrow4)$ β -D-xylopyranosyl residues (Bendahou *et al.* 2007). Other less intense signal peaks at δ 109.50 (C-1), 86.12 (C-4), 77.33 (C-3), 80.82 (C-2), and 61.44 (C-1) ppm, are assigned to Araf units (Roubroeks *et al.* 2000). Small signal peaks at 177 (COOH), 97.92 (C-2), 72.78 (C-3), 73.77 (C-2), and 60.08 (C-1) ppm, are also characteristic, probably due to the few $\text{OCH}_{3,4}$ of 4-O-methy- α -D-glucuronic acid units (Vignon and Gey 1998). Additionally, a high-field resonance at 21.06 (CH_3) ppm and a strong signal peak at the low-field of 173.7 (C=O) ppm indicated the presence of O-acetyl groups in the polysaccharide fraction (Peng *et al.* 2012).

2D HSQC NMR analysis was carried out for more specific information about the polysaccharide fraction (Fig. 3C). As shown, the marked $^1\text{H}/^{13}\text{C}$ cross-peaks could be

expressly identified at δ 101.7/4.41, 76.31/3.73, 75.68/3.38, and 62.93/4.05–3.32, which are attributed to C₁-H₁, C₄-H₄, C₃-H₃, C₂-H₂, and C₅-H₅ of the (1→4) β -D-xylopyranosyl residues, respectively (Lisboa *et al.* 2005). In addition, ¹H/¹³C cross-peaks in the HSQC NMR spectrum at δ 74.89/3.65, 84.66/4.20, and 97.98/5.24 were also observed, which confirmed the structural units of (1→4)- α -D-Glcp, Araf at O-3, and 4-O-Me- α -D-GlcpA-(1→2) units at position O-2, respectively (Van-Hazendonk *et al.* 1996). Therefore, according to the results of sugar analysis and the NMR analysis, it could be concluded that the polysaccharide fraction isolated from corn bran was mainly composed of (1→4) β -D-xylopyranosyl residues and Araf units attached with some amounts of substituted sugars and glucuronic acids.

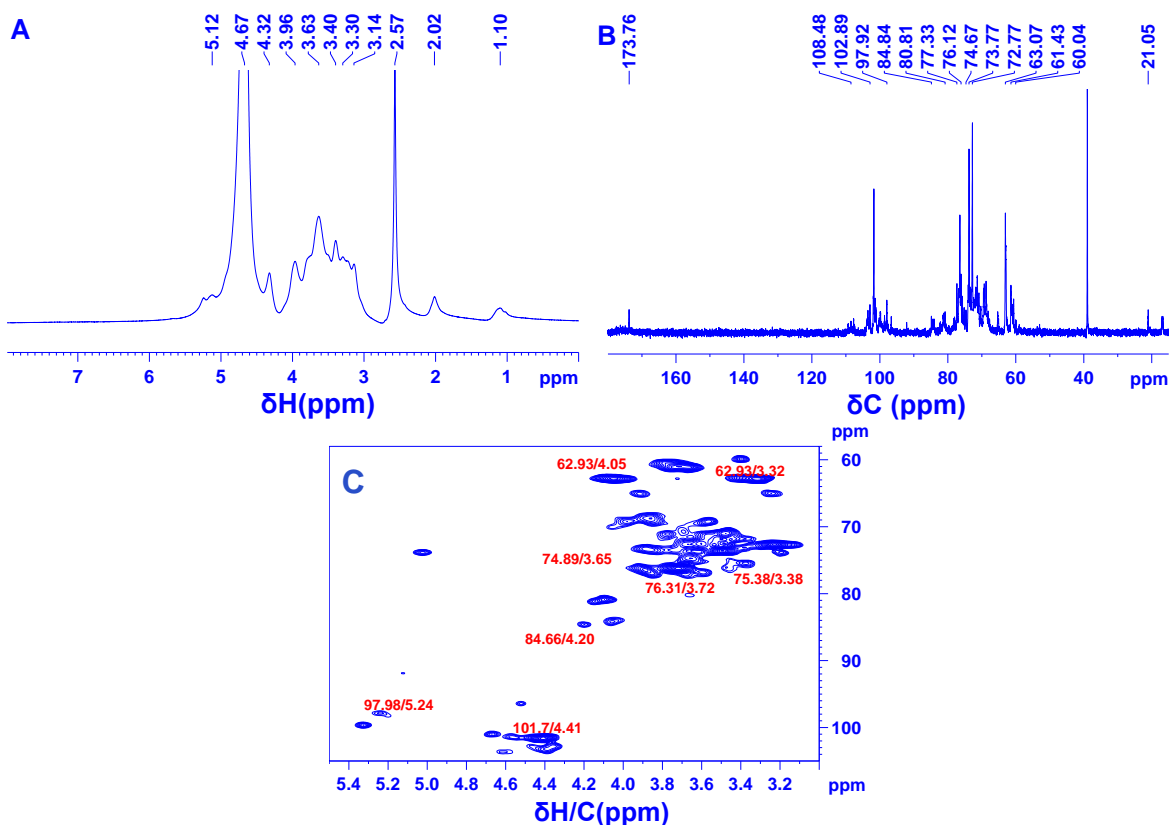


Fig. 3. NMR spectra of 110P-2 (A: ¹H NMR spectrum, B: ¹³C NMR spectrum, and C: 2D HSQC NMR spectrum)

Thermal Analysis of the Polysaccharides

Figure 4 exhibits the thermogram curves of the purified polysaccharides. In Fig. 4, it can be noticed that the TG/DTG curves of all purified polysaccharides exhibited three major steps in the temperature range from 30 to 750 °C. The first step of weight loss, appearing before 150 °C, was associated with water. At this stage, all the purified fractions had slight weight loss rates of about 9%, which was probably because the polysaccharides absorbed a small amount of moisture from the atmosphere. At the second step, although the thermal curves exhibited similar behavior with one major stage of weight loss for all the purified polysaccharides, the TG/DTG plots indicated that there were differences in the kinetics of the pyrolysis process among the purified polysaccharides. These differences were attributed to the structural and compositional variation among the polysaccharides.

From the DTG curves of the neutral polysaccharides, it was clear the decomposition temperature peaks for RMP-1, 110P-1, and 180P-1 occurred at 302.8 °C, 286 °C, and 292.4 °C, respectively. Additionally, the DTG curve for RMP-2 showed the peak maximum at 269 °C, and for 110P-2 and 180P-2 at 232.4 °C and 232.7 °C, respectively. The results confirmed that the polysaccharides isolated from untreated corn bran samples had higher thermal stability compared with those of pretreated corn bran samples. The main reason was the lower molecular weights of the purified polysaccharides isolated from the corn bran after twin-screw extrusion.

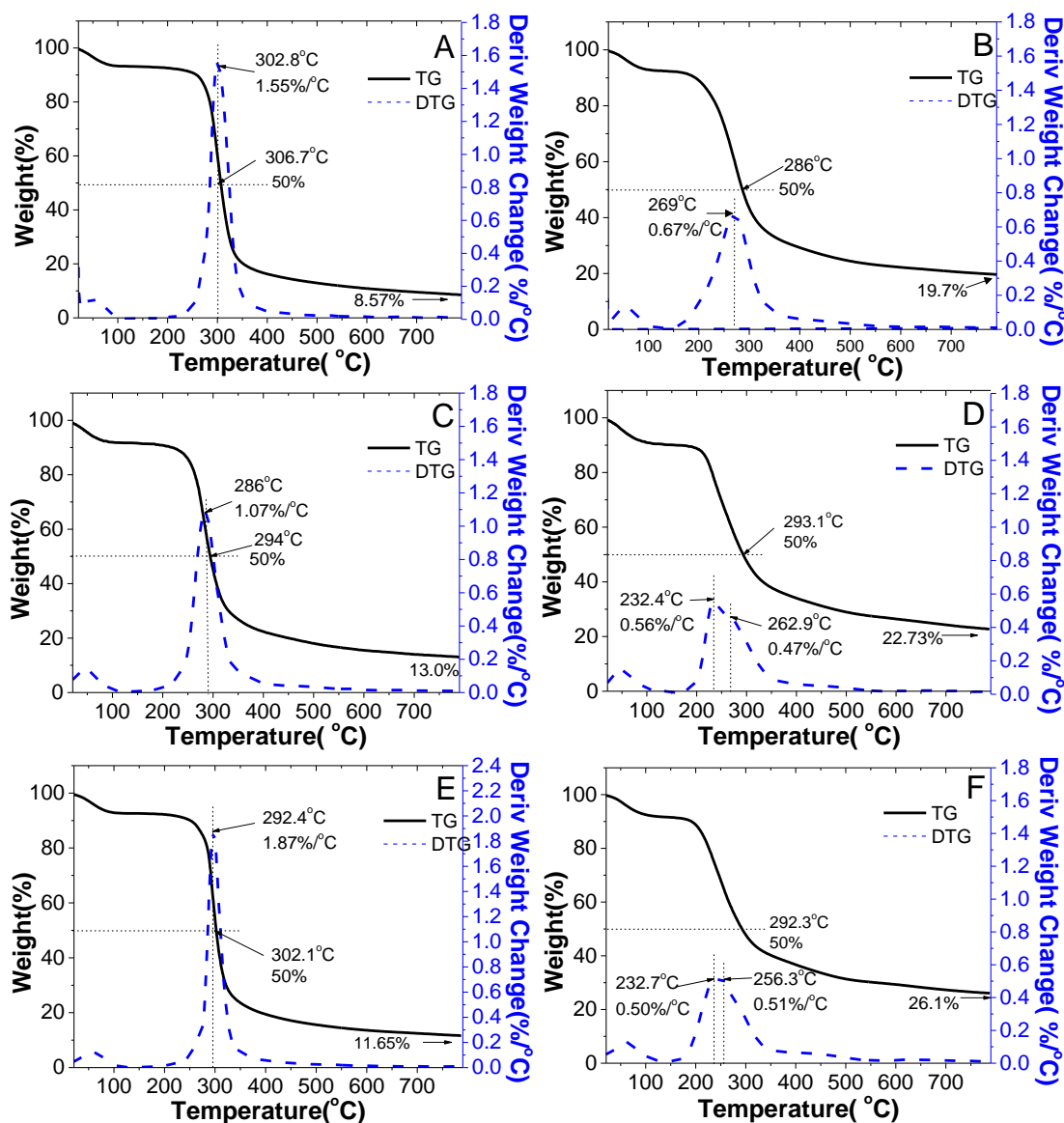


Fig. 4. TG and DTG curves of the RMP-1 (A), RMP-2 (B), 110P-1 (C), 110P-2 (D), 180P-1 (E), and 180P-2 (F)

Antioxidant Activity of the Polysaccharides

DPPH is a stable free radical that has been commonly used as a tool for evaluating the antioxidant activities of various materials including polysaccharides. The DPPH radical scavenging abilities of the purified polysaccharides isolated from corn bran before and after

pretreatment are exhibited in Fig. 5. As shown, the DPPH radical scavenging activities of all the purified polysaccharides are different and appear to be concentration-related. As shown in Fig. 5A, the free radical scavenging activities of DPPH in the samples increased with the amount of Vc, RMP-2, 110P-2, and 180P-2 tested, from 0.5 mg/mL to 3.0 mg/mL, reaching maximum values of 97.2%, 91.9%, 75.3%, and 87.9%, respectively. Thus, the abilities to scavenge the DPPH radical among all the tested samples were observed in the following order: Vc>RMP-2>180P-2>110P-2. The strongest scavenging activity of RMP-2 fraction was 91.9%, almost the same as 97.2% of Vc, which was much higher than 110P-1 and 180P-1 fractions at the concentration of 3.0 mg/mL. As shown in Fig. 5B, the order of the scavenging activities of DPPH for the neutral polysaccharides was different as compared with that of the acidic polysaccharides isolated from various corn bran samples before and after pretreatment by twin-screw extrusion. At 3 mg/mL, the scavenging abilities of RMP-1, 110P-1, and 180P-1 were 93.5%, 64.1%, and 47.3%, respectively. Thus, the scavenging activities of the neutral polysaccharides were higher, in the following order: RMP-1>110P-1>180P-1. At the same concentration, the acidic polysaccharides (110P-2 and 180P-2) isolated from pretreated corn bran had stronger scavenging activities as compared with those of neutral fractions (110P-1 and 180P-1). This difference could be due to the differences in the amount of glucuronic acid present in the acidic fractions, as glucuronic acid can chelate the metal ion and then scavenge the DPPH radical (Wang *et al.* 2010). However, according to the results of antioxidant activity tests, twin-screw extrusion pretreatment led to lower antioxidant activity of the polysaccharide fraction isolated by hydrothermal treatment, even though the pretreatment shortened the process duration, reduced the optimum treatment temperature, and increased the yield of polysaccharides.

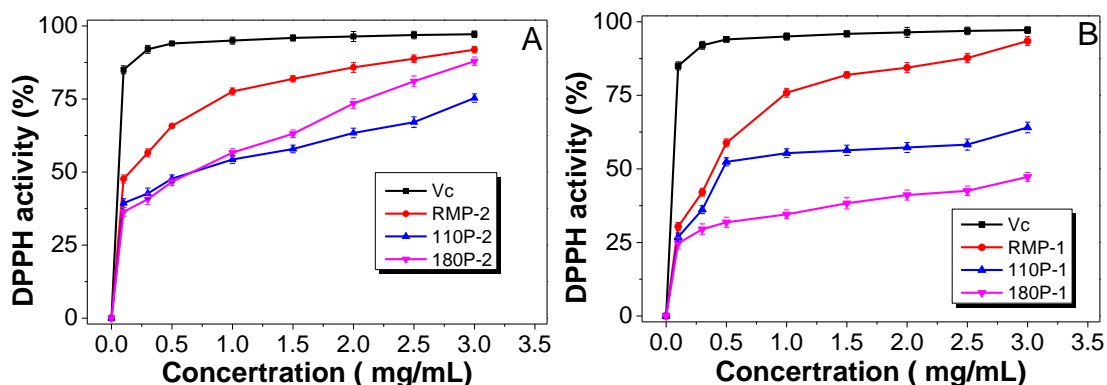


Fig. 5. Antioxidant activities against DPPH of the polysaccharides (A: Acidic fractions; B: Neutral fractions)

The antioxidant activity of polysaccharides is mainly related to their configuration, structure, molecular size, and monosaccharide components (Al-Sheraji *et al.* 2012). In the present investigation, RMP-1 and RMP-2 showed the highest antioxidant activities among all the polysaccharides. This might be due to the molecular structure and sugar components of the two fractions, which had higher hydrogen-donating abilities (Wu *et al.* 2015). Many researchers have reported that the polysaccharides with low molecular weight have higher antioxidant activities, because more free hydroxyl groups could increase the solubility of components (Chen *et al.* 2015; Wang *et al.* 2015). However, some reporters also indicated that the polysaccharides with a relatively high molecular weight showed stronger biological activity (Chen *et al.* 2009; Yang *et al.* 2012). RMP-1 and RMP-2 had larger molecular

weights than the fractions isolated from pretreated corn bran samples; thus, the present results also indicate that larger molecular weight polysaccharides have higher antioxidant activities. It should be noted that the acidic polysaccharides isolated after twin-screw extrusion pretreatment appeared to have lower degrees of branching, which could be one factor to influence the antioxidant activities of polysaccharides in the present investigation. However, deep and further investigation is needed to elucidate the relationship between functionality and structure of polysaccharides isolated from corn hulls by hydrothermal treatment after the twin-screw extrusion pretreatment and antioxidant mechanisms of the polysaccharides.

Isolation Mechanism of Hydrothermal Treatment after

Extrusion is a promising and novel physical method for pretreating lignocellulosic materials. During extrusion pretreatment, the material is subjected to shearing, heating, and mixing, leading to chemical modifications and physical disruption of lignocellulosic material during the passage through the extruder (Zhang *et al.* 2012; Negro *et al.* 2015). According to the authors' previous investigation, screw barrel temperature has an important influence in that it disrupts the corn bran structure, resulting in changes of thermochemical behavior and changes of the physical structure of the corn bran. In the end, it leads to more efficient polysaccharide isolation from corn bran by subsequent hydrothermal treatment (Liu *et al.* 2017). Based on the experimental results, the effect of twin-screw extrusion on the structure changes of polysaccharides isolated from hydrothermal treatment has been determined and described. The results show that the linkages and monosaccharide components of the purified polysaccharides were not affected strongly by the twin-screw extrusion pretreatment. However, the purified polysaccharides isolated from the corn bran after twin-screw extrusion pretreatment displayed significant variability in the monosaccharide ratios, degrees of branching/linearity, and molecular weight. This variability may cause the changes in functional properties of the polysaccharides. In the present investigation, the polysaccharides were isolated by hydrothermal treatment of corn bran at temperatures ranging from 110 to 180 °C. Thus, the lignocellulosic material was degraded during the thermal treatment process, so the large-chain compositions such as hemicelluloses were broken down into simpler and smaller molecules, some of which were dissolved into water-soluble fractions (Yuliansyah *et al.* 2010). Twin-screw extrusion pretreatment increased the surface area of the raw material available to the solvent water. Thus, the depolymerization and solubilization processes were facilitated by the pretreatment, which resulted in differences in the molecular structures of the polysaccharides compared to corn bran that had not been subjected to extrusion pretreatment.

CONCLUSIONS

1. Two kinds of polysaccharides, neutral and acidic, were successfully obtained from the crude polysaccharides of corn bran samples that had been pretreated with twin-screw extrusion before hydrothermal extraction, and of samples that had not been pretreated before hydrothermal extraction.

2. Twin-screw extrusion pretreatment did not strongly affect the structure of linkages nor the monosaccharide components of the purified polysaccharides subsequently extracted by hydrothermal treatment.
3. The purified polysaccharides isolated from corn bran samples after twin-screw extrusion pretreatment displayed significant differences in the monosaccharide ratios, degree of branching/linearity, and molecular weight. These changes may cause the decrease of antioxidant activities of the polysaccharides.

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