Effects of Steam Explosion Pretreatment on the Extraction of Xylooligosaccharide from Rice Husk

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Rice husk, which contains hemicellulose, can be used as a renewable resource to produce xylooligosaccharide (XOS). However, it is difficult to destroy the lignin structure of rice husk. Steam explosion (SE) is an effective method in destroying the lignin structure to enhance the release of hemicellulose and cellulose. In this study, SE pretreatment was used at different high pressures. The results showed that the lignin structure of rice husk could be collapsed by SE pretreatment, and the chemical structures of rice husk were evaluated by Fourier transform infrared spectroscopy (FTIR). The SE pretreatment resulted in the significant increase of XOS content and antioxidant activities. In summary, SE pretreatment under 2.5 MPa was chosen as a good option for the production of XOS from rice husk.

Keywords: Rice husk; Steam explosion (SE); Xylooligosaccharide (XOS)

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INTRODUCTION

Rice is one of the most widely grown grain crops in the world. In the processing of rice, a huge amount of rice husk is generated, and rice husk accounts for about 23% of the total weight of rice, resulting in 800 million tons of rice husk produced every year, while 50% of rice husk comes from China (Shen *et al.* 2021). The composition structure of rice husk is approximately 26 to 31% lignin, 25 to 35% cellulose, 18 to 21% hemicelluloses, and 15 to 17% silica (Alam *et al.* 2020). The application of rice husk is mainly to use its rice husk ash to make concrete (Pachla *et al.* 2021). However, its higher value utilization deserves more attention.

In addition to biorefineries, rice husk can also be used to produce xylooligosaccharides (XOS). The XOS are functional oligosaccharides composed of 2 to 7 xylose molecules combined with β-1,4 glycosidic bonds (Pinales-Márquez *et al.* 2021). XOS is considered as an indigestible oligosaccharide and can be used as a dietary sweetener in low energy food (Zhang *et al.* 2016). It is also semi-prebiotic. Both *in vitro* and *in vivo* studies show that XOS can produce prebiotic effects when fermented by specific beneficial bacteria (such as *Lactobacillus* and *Bifidobacterium*). XOS can enhance the balance of microorganisms in the intestinal tract (Palaniappan *et al.* 2021). Furthermore, XOS also have antioxidant activity (Zhou *et al.* 2018). Rice husk contains a lot of hemicellulose, which is the source of xylan (Remedios *et al.* 2006). XOS is produced by xylanase hydrolysis of hemicellulose (Mathew *et al.* 2018). The maximum XOS production of rice husk is 17.35 ± 0.31 mg/mL xylan (Banerjee *et al.* 2009; Nuntawat *et al.* 2018). The specific enzyme hydrolysis of rice husk generates the highest proportion of XOS with degrees of polymerization in the range 2 to 3 (Vegas *et al.* 2008).

XOS can be obtained by physical, chemical, or enzymatic methods (Samanta *et al.* 2012; Jayapal *et al.* 2013). In addition, agricultural plant biomass rich in xylan can also be directly autohydrolysed under high temperature and high pressure to produce XOS. Due to the specificity of the hydrolase, there is less unnecessary pollution during enzymatic hydrolysis (Samanta *et al.* 2015). Pretreatment of rice husk contributes to the release of sugar; for example, alkaline and acid soaking pretreatment can greatly improve the subsequent enzymatic hydrolysis (Kawet *et al.* 2016). Another pretreatment method is steam explosion (SE). The high shear force of SE is released during the high temperature explosion, and it can destroy and depolymerize cellulose and hemicellulose (Sui and Chen 2014). For example, SE pretreatment has proven to be an effective method to enhance the water solubility of raw materials (Chao *et al.* 2010). Previous studies have shown that, compared to conventional laboratory-scale methods, SE can produce XOS-rich hydrolysates with properties similar to or even better than prebiotics (Carvalho *et al.* 2017).

Rice husk is composed of a heterogeneous complex of carbohydrate polymers. Cellulosic sugars (such as cellulose and hemicellulose) are tightly packed by the lignin layer, thereby protecting them from enzymatic hydrolysis. Therefore, it is important to include a pretreatment process to destroy the lignocellulose structure to expose cellulose and hemicellulose to promote enzymatic action (Abbas and Ansumali 2010). The SE is an effective pretreatment method that can mechanically decompose lignocellulosic structure to make lignocellulosic biomass more vulnerable to enzyme attack (Shimizu *et al.* 2017). In this study, rice husk was pretreated with SE under different pressures and then hydrolyzed with xylanase. The lignin content and microstructure of SE rice husk was measured. The antioxidant activity and XOS content of the product through the combination of SE and enzymatic reaction were analyzed. This study aims to explore the impact of SE preprocessing on XOS extraction. Furthermore, this investigation will assist in the production of XOS from rice husk.

EXPERIMENTAL

Materials and Chemicals

Rice husk was purchased from Jinyuan Grain Trade Company (Jilin, China). The xylanase (the enzyme activity was more than 100000 u/mg) was purchased from Biotopped Company (Beijing, China). All the standard samples were obtained from Aladdin (Beijing, China). The other analytical reagents were purchased from Kemiou Chemical Reagent Company (Tianjin, China).

SE Pretreatments for Rice Husk

400 mL of rice husk was put into the SE reactor (QB-300, Tsing-Gentle Eco-Technology, Suzhou, China). The high-pressure steam was gradient risen and kept at varying pressures of 1.2 MPa (188 ℃), 1.4 MPa (195 ℃), 1.6 MPa (201 ℃), 1.8 MPa (207 ℃), 2.0 MPa (212 ℃), or 2.5 MPa (224 ℃) for 5 min. Samples were stored in an oven at 50 ℃ for 12 h until dried. Untreated sample was labeled as US, and the pretreated samples were labeled as SE-1.2, SE-1.4, SE-1.6, SE-1.8, SE-2.0, and SE-2.5, respectively.

Lignin Content Analysis

Samples were ground in a vertical cyclone mill (Type3001 Perten Instruments Co., Ltd., Sweden) and then passed through a 35-mesh sieve. Lignin was determined using the

NREL method (Sluiter *et al.* 2010). The lignin content was determined by two-step acid hydrolysis. After 72% and 4% sulfuric acid hydrolysis, the material was processed with a crucibled filter, baked in oven to constant weight, and burned in a Muffle furnace. The lignin content was calculated according to Eq. 1. Moisture and ash content were tested using the method of American Association of Cereal Chemists. The moisture content was tested by baking rice husk in a oven to constant weight. Ash content was tested by burning rice husk in a muffle furnace to constant weight (AACC 2000),

$$\text{Lignin content}(\%) = \frac{[(\text{Weight}_{\text{G4 plus acid insoluble residue}} - \text{Weight}_{\text{G4}}) - (\text{Weight}_{\text{G4 plus Ash}} - \text{Weight}_{\text{G4}})]}{\text{ODW}_{\text{sample}}} \times 100 \quad (1)$$

where $\text{Weight}_{\text{G4}}$ means the weight of G4 (a kind of glass sand core crucible) that has been incinerated to a constant weight in a Muffle furnace. $\text{Weight}_{\text{G4 plus Acid insoluble}}$ means the sum of the weight of G4 and sample that has been filtered and dried after two-step acid hydrolysis. $\text{Weight}_{\text{G4 plus Ash}}$ means the sum of the weight of G4 and acid insoluble residue that has been incinerated in a Muffle furnace. $\text{ODW}_{\text{sample}}$ means the oven dry weight of sample.

Scanning Electronic Microscopy (SEM) Analysis

Each sample was coated on the copper sheet with conductive adhesive and then stored in the vapor deposition chamber for gold plating (D07-19BM; Ketan Instrument Equipment Co., Ltd., Zhengzhou, China). The sample was observed and imaged using a scanning electron microscope (SU3800; Hitachi Scientific Instruments Co., Ltd., Beijing, China).

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

The different rice husk samples were sifted through 100-mesh sieve. The SE pretreated sample (1 mg) was ground and pressed with 100 mg KBr. All spectra were captured using a FTIR spectrophotometer (Nicolet FT-IR6700; Thermo Fisher Scientific, Madison, WI, USA).

XOS Content Analysis after Enzymolysis

The content of XOS was expressed by the reducing sugar content of the enzymatic hydrolysate of rice husk, which was detected by DNS method (in xylose, mg/g) (Yang *et al.* 2007). The pretreated rice husks were enzymolyzed by the xylanase. The solid-liquid ratio was 1:15. The addition amount of xylanase was 3%, at pH 4.80, 50 °C for 2 h. The enzyme was inactivated at 100 °C for 10 min, then centrifuged at 8000 rpm for 10 min (Martin-Sampedro *et al.* 2012). The collected supernatant was diluted to detect reducing sugar and total sugar content. The XOS content was determined by the dinitrosalicylic acid (DNS) method with xylose as the standard (Zhao *et al.* 2020). Then 3 mL of diluted supernatant was mixed with 2 mL DNS, then reacted in a boiling water bath for 5 min, cooled, diluted with water to a constant volume of 25 mL, and the absorbance at 540 nm was compared with xylose standard curve, making it possible to calculate the content of XOS.

Antioxidant Activities Analysis

The antioxidant activities were expressed by DPPH and ·OH radical scavenging rates (Kallel *et al.* 2015). The collected supernatant of rice husk after xylanase hydrolysised and centrifuge was diluted to detect the DPPH and ·OH radical scavenging rates. Samples were vortexed with DPPH and incubated for 30 min in the dark. The optical density was obtained at 517 nm using an ultraviolet-visible spectrophotometer (752; Jinghua Instruments Co., Ltd., Shanghai, China). Water and ethanol were used as the control and blank, respectively (Chao *et al.* 2014). The DPPH radical scavenging property of the sample was calculated as shown below in Eq. 2:

$$\text{DPPH radical scavenging activity (\%)} = \left(1 - \frac{\text{Absorbance of samples}}{\text{Absorbance of control}}\right) \times 100 \tag{2}$$

The formula for ·OH radical scavenging activity is the same as above.

Statistical Analysis

The data were expressed as mean ± standard deviation (SD) values. The statistical methods were a one-way analysis of variance and Duncan's multiple comparison method. The results were statistically significant ($p < 0.05$). The software used for significance analysis was SPSS 20.0 (IBM Corp., Armonk, NY, USA).

RESULTS AND DISCUSSION

Lignin Content of SE Pretreated Rice Husk

The lignin contents of rice husk at different pressures used in SE pretreatment are shown in Table 1. When the pressure of SE pretreatment increased, the lignin proportions decreased. As shown in Fig. 1. Lignin content decreased from 24.48% to 15.65% after the SE pretreatment, due to the cleavage of lignin bonds (Li *et al.* 2007).

Table 1. Lignin Content of SE-pretreated Rice Husk

Samples	Moisture Content (%)	Lignin (%)	Ash (%)
US	8.79 ± 0.01^{a}	24.48 ± 0.75^{a}	17.03 ± 0.03^{e}
SE-1.2	7.29 ± 0.05^{b}	19.18 ± 0.71^{b}	16.70 ± 0.03^{f}
SE-1.4	7.16 ± 0.01^{b}	17.58 ± 0.51^{bc}	17.25 ± 0.02^{d}
SE-1.6	6.73 ± 0.02^{cd}	17.43 ± 0.62^{bc}	17.62 ± 0.05^{c}
SE-1.8	6.67 ± 0.02^{d}	16.86 ± 0.51^{c}	18.26 ± 0.04^{b}
SE-2.0	6.87 ± 0.09^{c}	16.02 ± 0.56^{c}	19.02 ± 0.03^{a}
SE-2.5	5.91 ± 0.05^{e}	15.65 ± 0.12^{c}	19.01 ± 0.01^{a}

Each value is expressed as mean ± standard deviation, the a, b c etc. indicates significant difference of results when two rows do not share any letter in common.

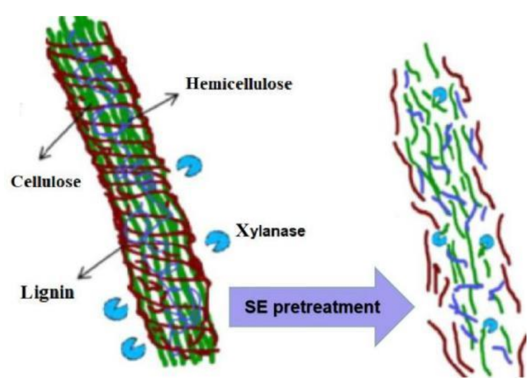


Fig. 1. The schematic of SE-pretreated cell wall of rice husk

SEM Analysis of SE-pretreated Rice Husk

Scanning electron microscopy was used to observe the samples to evaluate the effect of SE pretreatment methods on rice husks. Natural rice husk exhibited typical integrity, with no visible fractures (Fig. 2a). The rice husk pretreated with SE at 2.5 MPa showed significant concave-convex structure (Fig. 2b). The barrier structure on the surface may be damaged. This change may be caused by the high defibrillating power of the SE pretreatment with sudden decompression (Rocha *et al.* 2015). Previous studies have shown that SE pretreatment affects the solubilization and recovery of glucan, xylan, arabinan in Brewer's spent grain (Kemppainen *et al.* 2016). The results showed that the SE-2.5 might help to extract XOS from rice husk.

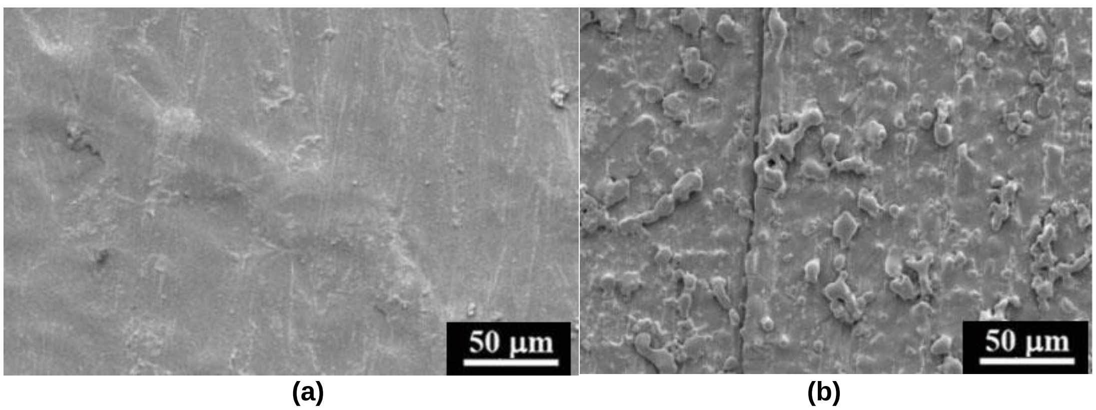


Fig. 2. The microstructure of different rice husk detected by SEM: a: US and b: SE-2.5

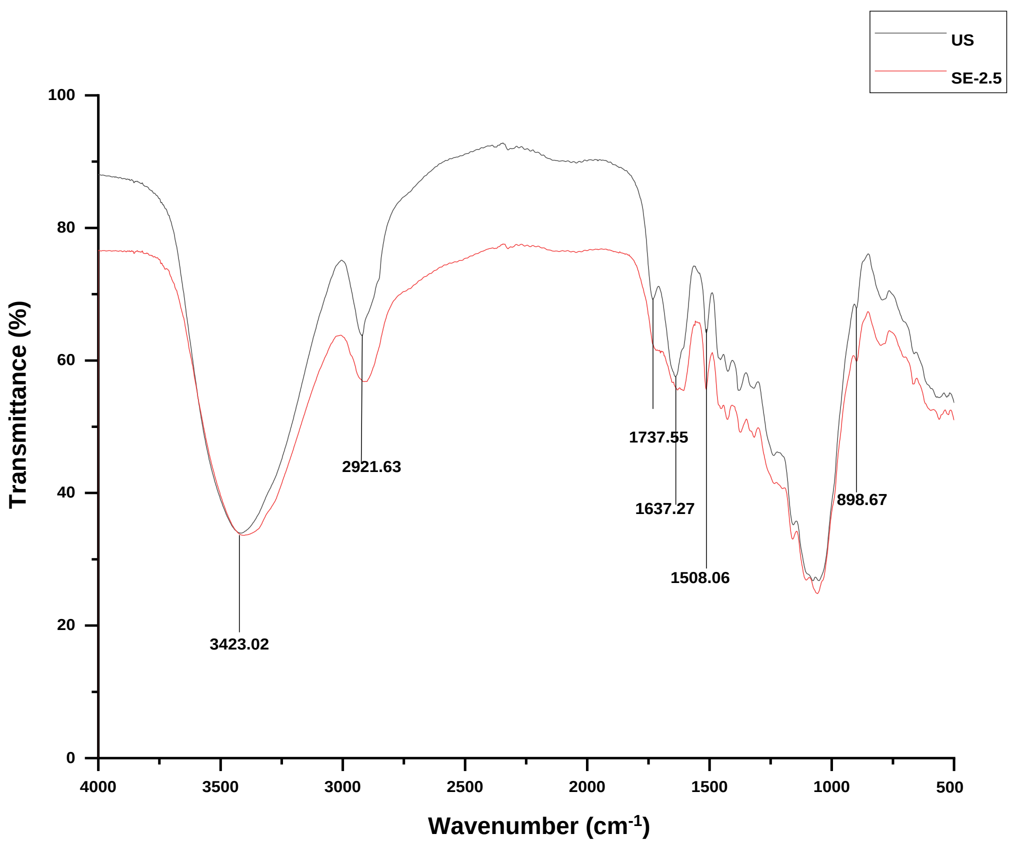


Fig. 3. FTIR spectroscopy of SE-pretreated rice husk

FTIR Analysis of SE-pretreated Rice Husk

The FTIR spectroscopy was performed to evaluate the effect of SE-2.5 on rice husk. As shown in Fig. 3, the vibration at 3423 cm^{-1} represents the -OH group of cellulose. This absorbance was weakened in SE-2.5 (Saelee *et al.* 2014). Steam explosion destroyed the structure of cellulose (Čater *et al.* 2014). This result is consistent with Sun *et al.* (2005), who studied the properties of cellulose degraded from steam-fried wheat straw and found that high-temperature steam pretreatment can lead to severe cellulose degradation or structural modification of cellulose during steam explosion.

In addition, in SE-2.5, the intensity of the band at 898 cm^{-1} related to the β-1,4-glycosidic bond of glucose in cellulose decreased (Karim *et al.* 2014). The band at 1737 cm^{-1} represents hemicellulose disappeared by comparison with the US, indicating that a considerable amount of hemicellulose was deacetylated into organic acids under high pressure. This finding confirmed that steam explosion had a great effect on hemicellulose, because hemicellulose is easily broken due to its amorphous state and low degree of polymerization (Trache *et al.* 2014). Hemicellulose is hydrolyzed in oligosaccharides or monosaccharin and can be degraded into furfural and hydroxymethyl furfural under severe conditions (Jacquet *et al.* 2015). The stretching vibration of aromatic rings at 1508 and 1637 cm^{-1} that are attributed to the C=C in lignin structures was weakened, confirming that the molecular structure of lignin was destroyed by steam explosion (Nieves *et al.* 2011). The SE pretreatment could lead to most of the hydrolysis of hemicellulose and a small part of lignin becoming soluble in the water phase (Zhang *et al.* 2008). This is consistent with the previous lignin content analysis. These spectral changes indicate that the structure of cellulose, hemicellulose, and lignin may be damaged by SE pretreatment, which indicates that rice husk can be used more effectively.

XOS Content in the SE-prepared Rice Husk

Both xylobiose and xylotriose have reductive properties. The reducing sugar content can be used to represent the XOS content (Yang *et al*. 2007). The different pressure levels of SE significantly affected the XOS content of rice husk (Fig. 4).

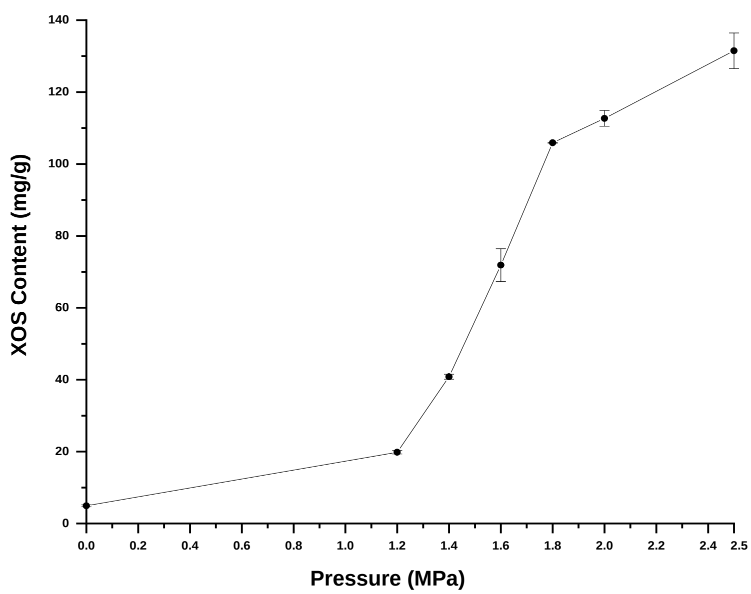
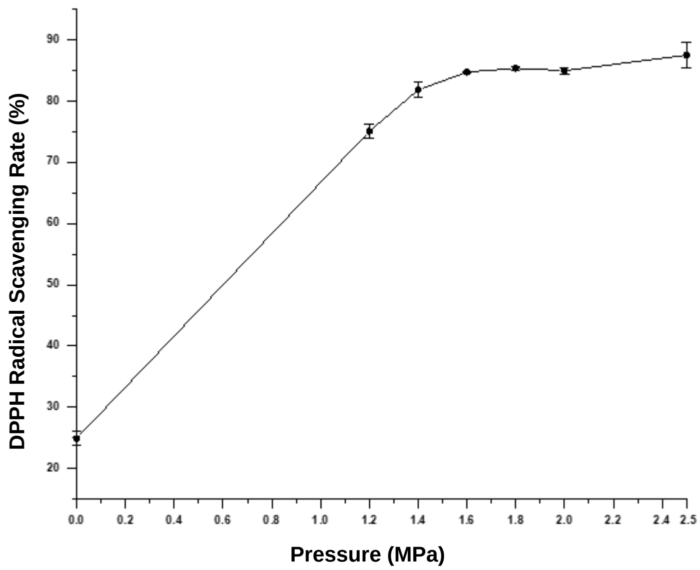
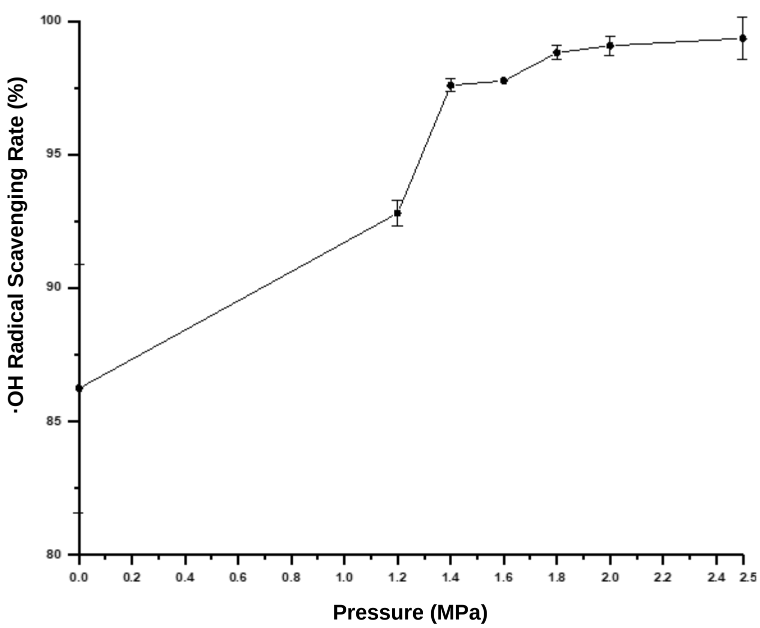


Fig. 4. XOS content of enzymatic SE-pretreated rice husk

The content of XOS significantly increased from 4.91 to 131.49 mg/g. The XOS content of pretreated rice husk was higher than US. The results showed that SE could significantly improve the enzymatic digestibility of insoluble carbohydrates (Kemppainen *et al.* 2016). The increase in XOS content and yield might be attributed to the substantial destruction of the lignocellulose structure (Singh *et al.* 2015). The results of SEM and FTIR also confirmed the dissolution of sugar and destruction of hemicellulose structure. This indicated that the enzymatic hydrolysis after steam explosion may be helpful for the extraction of XOS from rice husk.



(a)



(b)

Fig. 5. The DPPH and ·OH clearance rate of different enzymatic SE-pretreated rice husk: (a) DPPH clearance rate and (b) ·OH clearance rate

Antioxidant Activities of SE-prepared Rice Husk

The different pressure levels of SE were able to significantly affected the DPPH and ·OH radical scavenging rate of rice husk (Fig. 5). The DPPH radical scavenging rate significantly increased from 24.94% to 87.47%, and the ·OH radical scavenging rate also increased. The increase of DPPH and ·OH radical scavenging rate may be attributed to the increase of the XOS content (Zhou *et al.* 2018). After enzymatic hydrolysis, the active compound might also be more easily dissolved in the reaction buffer. Therefore, the scavenging ability of the xylo-oligosaccharide mixture was due to the effective release of phenolic compounds and the transfer of hydrogen or hydroxyl molecules from phenolic compounds (Huang *et al.* 2005).

CONCLUSIONS

1. The synergistic action of steam explosion (SE) pretreatment and xylanase enzymolysis reaction was chosen as an option to extract xylo-oligosaccharides (XOS) from rice husk.
2. Because of the SE pretreatment, the barrier function of lignin in rice husk was destroyed, and its content decreased, especially at 2.5 MPa.
3. SE pretreatment of 2.5 MPa was helpful to increase XOS yield and antioxidant activities. Steam explosion technology can obviously increase the yield of XOS, which is a feasible auxiliary extraction method. Flash steaming, high pressure are also easy to achieve. It can assist the extraction of XOS, further improving the extraction rate and reducing production costs. Therefore, SE pretreatment was suitable for effective pretreatment method of rice husk in the food processing industry.

ACKNOWLEDGEMENTS

This research was supported by the National Key R&D Program (no. 2018YFD0401000) and funded by NSFC (no. 31701636). The authors would like to acknowledge for Young Backbone Teachers in Henan province and Henan University of Technology.

Conflict of Interest

The authors declare that there is no conflict of interest

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Article submitted: May 24, 2021; Peer review completed: August 2, 2021; Revised version received: August 6, 2021; Accepted: August 22, 2021; Published: August 27, 2021.
DOI: 10.15376/biores.16.4.6910-6920