Comprehensive evaluation of combining hydrothermal pretreatment (autohydrolysis) with enzymatic hydrolysis for efficient release of monosaccharides and ferulic acid from corn bran

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A B S T R A C T

The combination of hydrothermal pretreatment (autohydrolysis) and enzymatic hydrolysis was comprehensively evaluated for the efficient release of monosaccharides and ferulic acid from corn bran. Arabinan was depolymerized and solubilized more easily during autohydrolysis compared to xylan, esterified ferulic acid, and the acetyl group. Also, the enzymatic xylose yield showed strong linear correlation with arabinan, ferulic acid, and acetic acid content in autohydrolysis residues while correlations between enzymatic glucose yield and hemicellulose contents were separated into two stages with different slopes. The addition of a few debranching enzymes to commercial cellulase and xylanase only slightly enhanced enzymatic hydrolysis of autohydrolysis residues, whereas an enzyme blend from Aspergillus oryzae and Eupenicillium parvum showed a significant synergistic effect. Desirable combined hydrolysis yields of glucose (72.26%), xylose (75.87%), arabinose (76.95%), and ferulic acid (74.13%) were obtained after autohydrolysis at 165 °C for 40 min and subsequent hydrolysis by an equal mixture blend produced by A. oryzae and E. parvum at an enzyme loading dosage of 14.1 mg protein/g dry destarched corn bran.

1. Introduction

Corn is one of the three most important food and industrial crops in the world. China is one of the world’s two largest producers of corn with approximately 218 × 10\textsuperscript{6} t of corn grown in 2016, of which more than 10% is allocated for the manufacture of food products (Liu and Guo, 2013). With a cautious estimation, 5% (by weight) of the corn is separated as corn bran during the process of obtaining corn starch. The recovered corn bran has low value and is often used for animal feed alone or in combination with corn germ cake or meal (Rose et al., 2010). Corn bran is mainly composed of arabinoxylan, comprising up to 56% of the biomass dry matter. Furthermore, it contains approximately 20% cellulose and has a high content of ferulic acid (approximately 3%) (Rose et al., 2010). Therefore, corn bran can serve as a low-cost organic source of sugars and natural ferulic acid production.

One of the primary requirements in the utilization of corn bran is to achieve an efficient decomposition of corn bran into monosaccharides and ferulic acid. However, the complete enzymatic hydrolysis of corn bran is still a challenge because of its native recalcitrant properties (Appeldoorn et al., 2010; Faulds and Williamson, 1995). The recalcitrance could be attributed to a combination of several factors. First, corn bran has an exceptional rigid and tight exterior that leaves it virtually impenetrable to enzymes. Second, up to 70% of the xylpyranosyl moieties in arabinoxylan are heavily substituted with various components, such as α-L-arabinofuranosyl, α-galactopyranosyl, α-glucuronyl, acetyl residues, and ferulic acid (Nghiem et al., 2011; Saha 2003). This heterogeneous arabinoxylan is crosslinked through covalent linkages between arabinofuranosyl residues and ferulic acid, which makes it more difficult for even the correct enzymes to catalyze complete hydrolysis (Dodd and Cann, 2010). In addition, corn bran contains lignin (10–14%) and structural proteins (5%). It has been suggested that the lignin and structural proteins also participate in intermolecular interactions with arabinoxylan through diferulate cross-linking, giving rise to a highly complex network of heterogeneous molecules.

Therefore, in order to enhance the susceptibility and substantially release the monosaccharides and ferulic acid from corn bran, pretreatment before enzymatic hydrolysis seems indispensable at present. Furthermore, a full complement of cellulolytic and hemicellulolytic enzymes would be required to cooperatively act in order to completely release the monosaccharides and ferulic acid from pretreated corn bran.
To date, the reported corn bran pretreatment methods include hydrothermal pretreatment, acidic or alkaline pretreatment, and steam explosion technology (Agger et al., 2011; Bonnin et al., 2002; Dien et al., 2006; Grohmann and Bothast, 1997; Saulnier et al., 2001; Zhao et al., 2014). Among all of the pretreatment methods, hydrothermal pretreatment (autohydrolysis) is relatively simple, environmentally friendly, and cost effective (Batalha et al., 2015; Cara et al., 2012). However, this pretreatment method also causes side effects, such as the production of degraded compounds, including furfural and acetic acid, especially at extreme processing conditions (Baêta et al., 2015; Zhang et al., 2013a,b). A few experiments with autohydrolysis pretreatment and enzymatic hydrolysis of corn bran have been reported (Bonnin et al., 2002; Dien et al., 2006), but details on the solubilization and degradation of hemicellulose and the chemical composition changes in solid residues with various autohydrolysis pretreatment severity have not been well understood. The effect of autohydrolysis pretreatment severity on subsequent enzymatic hydrolysis of autohydrolysis products (the solid and liquid fractions) has not been comprehensively elucidated. Additionally, the commercial cellulase/hemicellulases were ineffective for enzymatic saccharification of corn bran because of the lack or insufficiency of some key enzyme constituents (Agger et al., 2010). The proper pretreatment conditions and a more efficient enzyme cocktail are still needed to be designed in the pursuit of full component valorization of corn bran, particularly with respect to glucose, xylose, arabinose, and furfural acid.

In this paper, the combination of hydrothermal pretreatment and enzymatic hydrolysis was thoroughly investigated with the aim of maximizing monosaccharide and furfural acid release from corn bran. To this end, the first step of this work was to hydrothermally treat corn bran to formulate a solubilization model of hemicelluloses of corn bran based on the combined severity (CS) factor. Second, crude enzyme preparations from *Eupenicillium parvum* 4–14 and *Aspergillus oryzae* were blended for the synergetic hydrolysis of autohydrolyzed corn bran for the efficient release of monosaccharides and furfural acid. The objective of this work was to complete the information about the influence of the autohydrolysis and enzymatic hydrolysis on the efficient decomposition of corn bran into monosaccharides and furfural acid.

2. Materials and methods

2.1. Materials

Corn bran was collected from Nanyang, Henan Province, China. Destarched corn bran was prepared by treating raw material with amylase and papain according to the method used by Rose and Inglett (2010) with modifications. Amylase and papain were purchased from Imperial Jade Bio-Technology Co., Ltd. (Ningxia, China). The commercial enzymes of cellulase, β-glucosidase, and xylanase were purchased from Sigma Chemicals (St. Louis, MO, USA). Arabinofuranosidase and acetyl xylan esterase were purchased from Megazyme (Bray, Ireland). Ferulic acid esterase from *Myceliophthora thermophila* ATCC 42464 was recombinantly expressed in *Pichia pastoris* strain X33, which was kindly provided by Prof. Christakopoulos at Luleå University of Technology in Sweden (Antonopoulou et al., 2017). Crude enzymes were produced by fungal strains using modified Mandels’ medium with corn bran as the carbon source under solid state fermentation following the method used by Long et al. (2016). All of the other chemicals used in the study were analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). *Eupenicillium parvum* 4–14 was isolated from soil (Nanjing, China) and deposited in the China Center for Type Culture Collection (CCTCC) (Long et al., 2016). *Aspergillus oryzae* was obtained from the China Center of Industrial Culture Collection (CICC). *Thielavia heterothallica* D-76003, *Trichoderma reesei* D-86271 (Rut C-30), and *Thermomyces lanuginosus* D-96488 were obtained from the VTT Culture Collection (VTTCC, Finland). *Myceliophthora thermophila* ATCC 42464 was supplied by the American Type Culture Collection (ATCC, USA).

2.2. Autohydrolysis processing

Autohydrolysis was conducted in duplicate in a stainless steel batch reactor (model YRG2-10 × 1.25 L, ZhengJie Technology and Development Co., Ltd., Nanjing, China). The combined severity factor (CS) was used to characterize the pretreatment intensity by coupling the reaction conditions of time and temperature into one single variable. The CS factor was calculated by the following equation (Overend and Chornet, 1987):

\[
CS = \log\left(1 - e^{-(TR - TS)/14.75}\right)
\]

where \(t\) is reaction time (min); \(TS\) is the target temperature (°C); \(TR\) is a reference temperature (most often 100 °C); and the value of 14.75 is present as an empirical parameter related to the activation energy (pseudo first-order kinetics).

Destarched corn bran (30 g) and ultrapure water (300 mL) were mixed in reactors and then immersed in an oil bath. The autohydrolysis was conducted at different temperatures (155 °C, 165 °C, and 175 °C) for different times (10, 20, 30, and 40 min, excluding heating and cooling periods) based on a preliminary experiment. The rotation speed of the reactors during autohydrolysis was approximately 10 rpm. The

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### Table 1

The content of carbohydrates, esterified furfural acid, and acetyl group in destarched corn bran and autohydrolysis residues.

<table>
<thead>
<tr>
<th>Destarched corn bran or autohydrolysis residue</th>
<th>CS(^a)</th>
<th>Cellulose (%)</th>
<th>Xylan (%)</th>
<th>Arabinan (%)</th>
<th>Acetic acid (%)</th>
<th>Furfural acid (%)</th>
<th>Acid insoluble lignin (%)</th>
<th>Ara.(^b)/Xyl.(^c)</th>
<th>Ace.(^d)/Xyl.(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Destarched corn bran</td>
<td>–</td>
<td>22.50</td>
<td>30.50</td>
<td>16.62</td>
<td>4.20</td>
<td>2.06</td>
<td>9.06</td>
<td>0.54</td>
<td>0.14</td>
</tr>
<tr>
<td>155 °C, 10 min</td>
<td>6.03</td>
<td>24.38</td>
<td>29.43</td>
<td>15.95</td>
<td>3.25</td>
<td>1.90</td>
<td>9.80</td>
<td>0.54</td>
<td>0.11</td>
</tr>
<tr>
<td>155 °C, 20 min</td>
<td>6.72</td>
<td>25.85</td>
<td>29.74</td>
<td>14.41</td>
<td>3.17</td>
<td>1.85</td>
<td>9.83</td>
<td>0.49</td>
<td>0.11</td>
</tr>
<tr>
<td>175 °C, 30 min</td>
<td>7.13</td>
<td>27.42</td>
<td>28.76</td>
<td>12.87</td>
<td>3.16</td>
<td>1.82</td>
<td>11.17</td>
<td>0.45</td>
<td>0.11</td>
</tr>
<tr>
<td>155 °C, 40 min</td>
<td>7.42</td>
<td>29.66</td>
<td>27.93</td>
<td>11.00</td>
<td>3.11</td>
<td>1.51</td>
<td>12.73</td>
<td>0.39</td>
<td>0.11</td>
</tr>
<tr>
<td>165 °C, 10 min</td>
<td>6.71</td>
<td>25.64</td>
<td>29.30</td>
<td>14.33</td>
<td>3.33</td>
<td>1.77</td>
<td>10.27</td>
<td>0.49</td>
<td>0.11</td>
</tr>
<tr>
<td>165 °C, 20 min</td>
<td>7.40</td>
<td>29.58</td>
<td>27.74</td>
<td>11.78</td>
<td>3.06</td>
<td>1.59</td>
<td>12.70</td>
<td>0.43</td>
<td>0.11</td>
</tr>
<tr>
<td>165 °C, 30 min</td>
<td>7.81</td>
<td>36.63</td>
<td>24.08</td>
<td>8.00</td>
<td>2.60</td>
<td>1.06</td>
<td>15.87</td>
<td>0.33</td>
<td>0.11</td>
</tr>
<tr>
<td>165 °C, 40 min</td>
<td>8.10</td>
<td>43.61</td>
<td>19.52</td>
<td>6.13</td>
<td>2.14</td>
<td>0.71</td>
<td>17.97</td>
<td>0.31</td>
<td>0.11</td>
</tr>
<tr>
<td>175 °C, 10 min</td>
<td>7.39</td>
<td>37.51</td>
<td>22.22</td>
<td>7.26</td>
<td>2.59</td>
<td>0.97</td>
<td>15.03</td>
<td>0.33</td>
<td>0.12</td>
</tr>
<tr>
<td>175 °C, 20 min</td>
<td>8.08</td>
<td>51.18</td>
<td>12.08</td>
<td>2.56</td>
<td>1.48</td>
<td>0.28</td>
<td>21.37</td>
<td>0.21</td>
<td>0.12</td>
</tr>
<tr>
<td>175 °C, 30 min</td>
<td>8.49</td>
<td>54.31</td>
<td>9.24</td>
<td>1.44</td>
<td>1.12</td>
<td>0.16</td>
<td>20.87</td>
<td>0.16</td>
<td>0.12</td>
</tr>
<tr>
<td>175 °C, 40 min</td>
<td>8.77</td>
<td>53.95</td>
<td>8.97</td>
<td>1.27</td>
<td>1.01</td>
<td>0.15</td>
<td>25.33</td>
<td>0.14</td>
<td>0.11</td>
</tr>
</tbody>
</table>

\(^a\) CS, combined severity.
\(^b\) Ara., arabinan.
\(^c\) Xyl., xylose.
\(^d\) Ace., acetic acid.
corresponding CS under different pretreatment conditions was calculated and is presented in Table 1. After autohydrolysis pretreatment, the reaction system was cooled to room temperature by immersing the reactors in cool water. The solid (residue) and liquid fraction was separated by vacuum filtration. The solid was repeatedly washed with water three times (200 mL) and then freeze-dried. The water from the washings and the liquid fraction were pooled and centrifuged to obtain the supernatant, followed by dilution to 1 L. The residues and autohydrolysis liquid were kept for either compositional analysis or the enzymatic hydrolysis experiments. The ratio of the content of components in the autohydrolysis liquid to that in destarched corn bran was calculated as the percent of component solubilized.

2.3. Enzymatic hydrolysis of autohydrolysis residues using commercial enzymes

The enzymatic hydrolysis of autohydrolysis residues was conducted at a substrate loading of 5% (w/v), a total working volume of 5 mL at 50 °C with 50 mM sodium citrate buffer (pH 5.0) in 50-mL conical flasks. The flasks were shaken at 150 rpm for 72 h. The enzyme dosages added were 15 FPU cellulase (Sigma C2730, 117 FPU/g), 15 CBU β-glucosidase (Novozyme 188, 269 CBU/g), and 200 U xylanase (Sigma X2629, 7700 U/g) per gram of dry biomass. Sodium azide (10 mM) was added to prevent microbial contamination. The reactions were stopped by placing the flasks in a boiling water bath for 10 min and hydrolysates were clarified by centrifugation. Glucose and xylose in the supernatants in the supernatant were analyzed by HPLC. All the experiments were carried out in duplicate, and the results were presented by considering the average value. The yields of glucose and xylose were calculated according to Eqs. (1) and (2) as follows:

Glucose yield (%) = 100 × (0.9 × amount of released glucose after enzymatic hydrolysis)/amount of glucan in residue. (1)

Xylose yield (%) = 100 × (0.88 × amount of released xylose after enzymatic hydrolysis)/amount of xylan in residue. (2)

The conversion factor for dehydration on polymerization to glucose was 162/180 (0.9) for glucose; to xylan and arabinan, it was 132/150 (0.88) for xylose and arabinoise.

As for the addition of debranching enzymes, all of the operations were similar to the above, except that the additional 1 U of arabinofuranosidase (Megazeme, A-ABFAN), acetyl xylan esterase (Megazeme, A-AXEAO), and ferulic acid esterase complemented 15 FPU of cellulase, 15 CBU of β-glucosidase, and 200 U of xylanase. Also, sodium hydrogen sulfite (100 mg/L) was added to prevent ferulic acid oxidation. The yields of glucose and xylose were calculated as Eqs. (1) and (2), respectively, while the yields of arabinoise, ferulic acid, and acetic acid according to Eqs. (3)–(5), respectively, were calculated as follows:

Arabinose yield (%) = 100 × (0.88 × amount of released arabinose after enzymatic hydrolysis)/amount of arabinan in residue. (3)

Ferulic acid yield (%) = 100 × (amount of released ferulic acid after enzymatic hydrolysis)/amount of ferulic acid in residue. (4)

Acetic acid yield (%) = 100 × (amount of released acetic acid after enzymatic hydrolysis)/amount of acetic acid in residue. (5)

2.4. Screening for a crude fungal enzyme blend

First, crude enzymes from E. parvum 4–14, A. oryzae, and T. reesei D-86271 (Rut C-30) were screened by comparing the hydrolysis yield of autohydrolysis residue with six individual crude enzymes. Second, one enzyme blend was selected from three enzyme blends consisting of two crude enzymes from these three strains by comparing the hydrolysis yield of the autohydrolysis residue. In all of the above experiments, the autohydrolysis residue was obtained by autohydrolysis at 165 °C for 40 min, and the enzymatic hydrolysis was carried out at the same conditions as described for commercial enzymes.

2.5. Separate enzymatic hydrolysis of autohydrolysis residue and liquid using a crude enzyme blend

The enzymatic hydrolysis of residue and liquid was conducted separately at 50 °C with 50 mM sodium citrate buffer (pH 5.0) at 150 rpm in 50-mL conical flasks and 10-mL centrifuge tubes, respectively, for 72 h using the enzyme blend produced by Eupenicillium parvum 4–14 and Aspergillus oryzae. The total working volume for enzymatic hydrolysis of the residue and liquid was 5 mL and 2 mL, respectively. Sodium azide (10 mM) and sodium hydrogen sulfite (100 mg/L) were added to prevent microbial contamination and ferulic acid oxidation, respectively. For hydrolysis of the residue, the total enzyme dosage was 1.0% (w/v) (0.5% each for individual enzymes) of dried autohydrolysis residue, and substrate loading was 5% (w/v, 0.25 g dry mass in a total working volume of 5 mL). For the hydrolysis of liquids, the total enzyme dosage was 300 μg of enzyme protein (150 μg each for individual enzymes) per mL of liquid. After the hydrolysis reactions were stopped by placing the flasks in a boiling water bath for 10 min, the mixtures were then diluted to 50 mL and clarified by centrifugation at 10,000 rpm for 5 min. The hydrolysis yields of monosaccharide and ferulic acid were calculated on the basis of the content of corresponding components in residue or liquid, respectively. All of the experiments were carried out in duplicate, and the average value was presented.

2.6. Analysis of carbohydrates, ferulic acid, and inhibitors

The structural carbohydrates, including glucon, xylan, and arabinan in destarched corn bran and autohydrolysis residues, were determined using a two-step sulfuric acid hydrolysis technique (Shuter et al., 2005). Glucose, xylene, and arabinoise in the acid hydrolysate were analyzed by HPLC (Agilent 1100, USA) equipped with a Bio-Rad Aminex HPX-87H column (300 mm × 7.8 mm; USA) and a refractive index detector. The analytical column was operated at 55 °C using 5 mM H2SO4 as the mobile phase with a flow rate of 0.6 mL/min. The conversion factor for dehydration on polymerization to glucon was 162/180 (0.9) for glucose; to xylan and arabinan, it was 132/150 (0.88) for xylose and arabinoise. The experiment was performed in triplicate, and the average of the data was reported.

The monosaccharides in the autohydrolysis liquid and enzymatic hydrolysate, as well as the inhibitors (formic acid, acetic acid, levulinic acid, 5-hydroxymethylfurfural, and furfural) were determined by HPLC as described above. To determine the total content of monosaccharides and oligosaccharides, autohydrolysis liquid was subjected to hydrolysis in 4% (w/v) sulfuric acid at 121 °C for 1 h prior to HPLC detection. All of the experiments were performed in triplicate, and the average of the data was reported.

The content of esterified ferulic acid in destarched corn bran and autohydrolysis residues was detected after NaOH saponification as previously reported (Jiang et al., 2016). Free ferulic acid was analyzed by HPLC (Agilent Technologies 1260 Infinity) on a ZORBAX Eclipse Plus C18 (4.6 × 100 mm, Agilent, Santa Clara, CA, USA) column with the mobile phase of acetic acid (0.1%)-methanol (65:35) at a flow rate of 0.8 mL/min at 30 °C. The quantitative analysis was performed by the external standard method at 320 nm. Free ferulic acid in the acidified liquid was directly analyzed by HPLC, as mentioned above. Total ferulic acid in autohydrolysis liquid was detected after NaOH saponification followed by HPLC analysis according to Rose and Ingleit (2010). Esterified ferulic acid in the autohydrolysis liquid was determined by the difference between the free ferulic acid content of the saponified and unsaponified samples. The experiment was performed in triplicate, and the average of the data was reported.
2.7. Activity assay of crude enzymes

Activities of crude enzymes were determined at 50 °C and pH 5.0 (50 mM sodium phosphate buffer) as follows. Cellulase was measured as the release of reducing sugars (Somogyi-Nelson method) from carboxymethylcellulose or filter paper (FP, Whatman no. 1). Xylanase was determined as the release of reducing sugars from soluble beechwood xylan with one unit defined as the release of 1 μmol sugar/min. β-xylosidase, β-glucosidase, arabinofuranosidase, and glucuronidase activities were determined by the release of p-nitrophenol from p-nitrophenyl β-D-xyloside, p-nitrophenyl β-D-glucoside, p-nitrophenyl α-L-arabinoside, and p-nitrophenyl β-D-glucuronide, respectively. One unit was defined as 1 μmol p-nitrophenol released/min. Ferulic acid esterase activity was determined by measuring the conversion of methyl-ferulate to ferulic acid. Acetyl xylan esterase activity was determined by measuring the release of acetic acid from 4-methylumbelliferyl acetate. Glucuronoyl esterase was determined by measuring the release of benzyl alcohol from benzyl-D-glucuronate by HPLC. Proteolytic activity was determined against bovine serum albumin. One unit of activity was defined as the amount of enzyme that was required to increase the corresponding absorbance value at 280 nm by 0.01 units per min. All of the assays were performed in triplicate, and the average of the data was reported.

3. Results and discussion

3.1. Composition of destarched corn bran

As shown in Table 1, the weight percent of major components of destarched corn bran are glucan, xylan, arabinan, and acid insoluble lignin, attesting to the richness of this biomass in carbohydrates. The content of ferulic acid and acetic acid in destarched corn bran is 2.06% and 4.2%, respectively. It was reported that corn bran contained approximately 2.8–3.1% ferulic acid (Chanliaud et al., 1995; Mathew and Abraham, 2004). The difference between the contents may be partially attributed to differences in variety, climate, location of cultivation, or other factors. The high amount of polysaccharides and ferulic acid indicate that corn bran is a promising source for both natural ferulic acid and multiple fermentable monosaccharides.

3.2. Effect of combined severity on soluble components released from destarched corn bran

The autohydrolysis of destarched corn bran was carried out at different CS. The autohydrolysis products in the liquid fraction were mainly oligosaccharides, monosaccharides, and byproducts. The profiles of oligosaccharides and monosaccharides with the increase of CS during the autohydrolysis are presented in Fig. 1A. As shown in Fig. 1A, the dissolution of the total amount of xylose and xyloligomers increased sharply as the CS increased from 7.13 to 8.10; the dissolution reached the maximum of 75.9% when CS was equal to 8.49. Subsequently, the total amount of xylose and xyloligomers decreased. On the other hand, the maximum xylose production accounted for only 17.4% of the total amount of xylose and xyloligomers when the CS was 8.77, indicating that most of the solubilized xylan existed as oligomers rather than monomers.

The total amount of arabinose and arabinooligomers increased with elevated CS and reached a peak of 74.3% when CS was 8.10 then gradually decreased. Furthermore, the maximum arabinose production accounted for up to 69.3% of the total amount of arabinose and arabinooligomers when the CS was 8.49, implying that most of the arabinan was depolymerized as monomers. Also, it was observed that the total dissolution of arabinose and arabinooligomers was significantly higher than that of the total xylose and xyloligomers within the low CS, ranging from 6.0 to 7.8, reflecting a higher sensitivity of arabinan to autohydrolysis pretreatment than xylan in corn bran. These results are
in agreement with previous reports (Silva-Fernandes et al., 2015; Xiao et al., 2013). Free ferulic acid was not detected in the autohydrolysis liquid, indicating that the ester linkage survived the temperature and reaction time and ferulic acid remained as an esterified form linked to carbohydrates. In addition, it was noteworthy that the color of the autohydrolysis liquid was related to the CS; the liquid appeared very dark after treatment at 175 °C for over 30 min (CS 8.49) (data not shown).

In contrast to hemicelluloses, a small amount of glucan was hydrolyzed. The maximum dissolution of the total amount of glucose and glucooligomers reached only 17.4% with the CS of 8.49. This vulnerable portion of glucan most likely originated from hemicelluloses or the amorphous region of cellulose (Li et al., 2005; Carpita and McCann, 2000). As shown in Table 1, increases in pretreatment severities led to the decrease of hemicelluloses, but increase of cellulose content in the autohydrolysis residues implied that the severe autohydrolysis condition promoted the dissolution of hemicelluloses but not of cellulose (Branco et al., 2015; Sidiras et al., 2011). Moreover, the arabinan/xylan ratio of the residues declined with autohydrolysis time at a fixed temperature while the acetic acid/xylan ratio remained almost constant, implying that the dissolution rate of arabinan from corn bran was faster than that of xylan during autohydrolysis, while the dissolution rate of acetyl was similar with that of xylan.

In addition to the oligosaccharides and monosaccharides, various organic acids (formic acid, acetic acid, and levulinic acid) and other byproducts (furfural and hydroxymethylfurfural) were generated during the autohydrolysis process. As seen in Fig. 1B, the amount of acetic acid and furfural were low when the CS was below 7.4 but dramatically increased when the CS reached 8.77. Acetic acid, which is formed by the removal of the acetyl group from the hemicellulose moiety, was the main organic acid found in the autohydrolysis liquid. The final concentration of acetic acid was 0.78 g/L with the CS equal to 8.77, corresponding to 55% of acetyl groups in destarched corn bran, which indicated that 45% of acetyl groups was linked to xylooligomers or still located in the residue. Furfural is formed by the degradation of pentose, and its final concentration was 0.68 g/L with the CS equal to 8.77. Hydroxymethylfurfural (HMF), which is formed from hexose degradation, was at a low level (a final concentration of 0.03 g/L), whereas formic acid (from both furfural and HMF degradation) and levulinic acid (from HMF degradation) respectively reached 0.27 and 0.12 g/L with the CS at 8.77 (Palmqvist and Hahn-Hägerdal, 2000).

3.3. Relationships between the content of hemicelluloses components and saccharification yields of autohydrolysis residues

The autohydrolysis residues were mainly composed of cellulose, and a lesser portion of lignin and xylan with different degrees of substituent groups such as acetyl, arabinose, and ferulic acid depending on the severity of the pretreatment. The autohydrolysis residues were still difficult to be enzymatically hydrolyzed due to the complexity of the structure and substitutes, but if and how there are any correlations between the hemicelluloses components and the enzymatic digestibility is not fully understood. Therefore, in this section, the autohydrolysis residues obtained under the various levels of autohydrolysis pretreatment were enzymatically hydrolyzed by commercial cellulase and xylanase. Subsequently, the correlation between the content of hemicelluloses components and enzymatic glucose yield as well as the correlation between the content of side chain groups and enzymatic xylose yield are discussed.

Fig. 2 shows the enzymatic glucose and xylose yield from the autohydrolysis residues pretreated at different conditions. When the autohydrolysis temperature was set at 155 °C, the enzymatic glucose yield...
increased sharply as the autohydrolysis time increased from 10 min to 30 min, whereas it did not significantly rise as the time further increased to 40 min. The enzymatic glucose yield reached approximately 85% for three autohydrolysis residues pretreated at 165 °C for 20, 30, and 40 min. Once again, the enzymatic glucose yields were nearly all at the same level (approximately 90%) for four residues from autohydrolysis at 175 °C for 10, 20, 30, and 40 min. These results indicated that glucose could be abundantly released by commercial cellulase from autohydrolysis residue pretreated at relatively low severity (e.g., 165 °C for 20 min) and excessively increased autohydrolysis severity was not favorable to enhanced enzymatic hydrolysis of cellulose in corn bran.

In contrast, the enzymatic xylose yield of the residues autohydrolyzed at 155 °C or 165 °C constantly increased with autohydrolysis time. However, further increasing the time from 30 min to 40 min at 175 °C did not significantly promote xylose yield. In general, as shown in Fig. 2, the enzymatic xylose yields of autohydrolysis residues were less than 50%, unless the residue was obtained at the severe autohydrolysis condition of 175 °C for 20 min because of recalcitrance of residual xylan.

The correlation between the contents of xylan, arabinan, and ferulic acid as well as acetic acid and enzymatic glucose yield of the residues is shown in Fig. 3. The crossover point is marked in each figure. As shown in Fig. 3, the decreases of xylan, arabinan, ferulic acid, and acetic acid caused the increase of enzymatic glucose yield. However, the increasing process could be separated into two stages with different slopes. In the first stage, when the xylan in the residue was decreased to 27.88%, arabinan to 12.76%, ferulic acid to 1.71%, and acetic acid to 3.06%, the enzymatic glucose yield increased rapidly to approximately 83%. In the second stage, the enzymatic glucose yield increased slowly with further decrease of hemicelluloses components (from approximately 83% to 92.32%), which implied that the excessive removal of hemicelluloses was less significant for the enhanced enzymatic glucose yield of corn bran. Zhang et al. (2013a, b) reported that the xylan content and enzymatic glucose yield showed a strong linear correlation (R² = 0.8) during the pretreatment of switchgrass with sulfite and subsequent enzymatic hydrolysis of pretreated material. Ewanick and Bura (2011) also pointed out that after sugarcane bagasse was impregnated with SO₂ and pretreated with steam, the linear correlation of the enzymatic glucose yield and xylan content was strong (R² = 0.78). The difference of our study from those previously reported might be due to the specific composition and structure of hemicelluloses in corn bran as well as the different pretreatment methods applied.

The relationship between the content of arabinan and ferulic acid as well as acetic acid and enzymatic xylose yield is shown in Fig. 4. As seen in Fig. 4, the linear correlations between the arabinan and ferulic acid content as well as acetic acid and enzymatic xylose yield were very strong (R² = 0.9863, 0.9717, and 0.9632, respectively). These side chain groups prevented xylanase from accessing the xylan backbone, leading to a limited degradation of xylan (Agger et al., 2010; Biely et al., 2016; Li et al., 2016; Liu and Ding, 2016). When three slopes in Fig. 4 were compared, it was clear that the slope for the linear correlation between the content of acetic acid and enzymatic xylose yield were steeper than the other two, indicating that the degree of acetyl substitution affects the enzymatic hydrolysis of xylan in autohydrolysis residues more significantly than arabinose and ferulic acid. This phenomenon is consistent with the previous work by Agger et al. (2010).

### 3.4. Effect of debranching enzymes on monosaccharide and ferulic acid release from autohydrolysis residues

As the enzymatic xylose yields of the autohydrolysis residues were low, especially for the residues autohydrolyzed at 155 °C and 165 °C (Fig. 2), the effects of debranching enzymes (namely arabinofuranosidase, ferulic acid esterase, and acetyl xylan esterase) on monosaccharide and ferulic acid release from the residues autohydrolyzed at 165 °C for 10, 20, and 40 min (CS = 6.71, 7.40, and 8.10, respectively) were respectively investigated. As shown in Fig. 5A1–A3, the addition of FAE to cellulase/xylanase could improve ferulic acid yields of the three residues by 55.39%, 34.3%, and 27.76%, respectively. However, supplementation of arabinofuranosidase did not remarkably promote arabinose release from the three residues. The arabinose yields of the three residues were generally no more than 10%, even if all three of the debranching enzymes were added. Besides, although acetic acid yields of the three residues increased with autohydrolysis time, the addition of AXE hardly facilitated the release of acetic acid from individual residue. Furthermore, it was observed that both xylose and glucose yields of the three residues were not remarkably improved in spite of a combined addition of all the three debranching enzymes to cellulase/xylanase. The enzymatic xylose yields of the three residues autohydrolyzed at 165 °C for 10, 20, and 40 min remained low after the addition of all of the three debranching enzymes (15.79%, 24.92%, and 51.27%, respectively). Therefore, it seems that the heterogeneous nature of arabinoxylan in corn bran makes it difficult for a combination of a few individual debranching enzymes to cooperatively catalyze the hydrolysis of hemicelluloses. The hydrolysis was still restricted, probably due to the resistance of arabinoxylan itself.
to steric hindrance or lack of additional enzymes, such as β-xylosidase, glucuronyl esterase, and glucuronidase, to catalyze the hydrolysis of certain unusual bonds (glycosidic bond, glucuronyl ester, and glucuronide linkage, etc.) (Biely et al., 2016). A full complement of enzymes, including different activities, is of crucial importance to enhance the release of monosaccharides and ferulic acid from corn bran.
Table 2
Activity profiles of the crude enzymes from solid state fermentation by Eupenicillium parvum 4–14 and Aspergillus oryzae.

<table>
<thead>
<tr>
<th>Enzyme produced by</th>
<th>Enzyme produced by</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eupenicillium parvum 4–14 (U/mL)</td>
<td>Aspergillus oryzae (U/mL)</td>
</tr>
<tr>
<td>Filter paper activity</td>
<td>0.05 ± 0.01</td>
</tr>
<tr>
<td>CMC-Na’s activity</td>
<td>19.74 ± 0.79</td>
</tr>
<tr>
<td>β-glucosidase</td>
<td>0.62 ± 0.01</td>
</tr>
<tr>
<td>Xylanase</td>
<td>64.42 ± 2.29</td>
</tr>
<tr>
<td>β-xylosidase</td>
<td>4.85 ± 0.84</td>
</tr>
<tr>
<td>Acetyl xylan esterase</td>
<td>2.46 ± 0.02</td>
</tr>
<tr>
<td>Arabinofuranosidase</td>
<td>80.81 ± 3.20</td>
</tr>
<tr>
<td>Furfuryl esterase</td>
<td>0.11 ± 0.01</td>
</tr>
<tr>
<td>Glucuronoyl esterase</td>
<td>0.39 ± 0.03</td>
</tr>
<tr>
<td>Glucuronidase activity</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>Proteolytic activity</td>
<td>4.00 ± 0.12</td>
</tr>
</tbody>
</table>

* CMC-Na, sodium carbosymethyl cellulose.

3.5. Screening for a crude fungal enzyme blend with high enzymatic hydrolysis ability

As shown in Fig. 5B1, the crude enzymes from A. oryzae, E. parvum 4–14, and T. reesei exhibited relatively better hydrolysis performance in the hydrolysis of the residue than the other strains in terms of enzymatic hydrolysis yields of glucose and xylose (Fig. 5B1). Furthermore, the enzyme blend from A. oryzae and E. parvum 4–14 hydrolyzed the residue better than the individual enzymes at the same dose of enzyme (Fig. 5B2). The 72 h enzymatic hydrolysis yields of glucose, xylose, arabinose, and ferulic acid reached 84.02%, 90.39%, 76.72%, and 74.14% based on their corresponding content in the residue, respectively, which was also higher than that of the other two blends. In comparison with crude enzymes from E. parvum 4–14 alone on the basis of equivalent dosage of protein, the enzyme blend enhanced the enzymatic hydrolysis yields of glucose, xylose, arabinose, and ferulic acid by 45.43%, 16.77%, 19.95%, and 1.5% respectively. Furthermore, the enzymatic hydrolysis yields of glucose, xylose, arabinose, and ferulic acid were increased by 6.96%, 31.04%, 32.23%, and 14.15%, respectively, compared with crude enzymes from A. oryzae alone on the basis of equal protein. The cellulolytic and hemicellulolytic activity profiles of the crude enzymes from A. oryzae and E. parvum 4–14 are shown in Table 2. The E. parvum 4–14 enzyme contained higher hemicellulase activities (e.g., xylanase and arabinofuranosidase), while the A. oryzae enzyme had higher cellulase activity than the E. parvum 4–14 enzyme. The enhanced enzymatic hydrolysis seems to be attributed to the synergism in the activities of the enzymes produced by E. parvum 4–14 and A. oryzae. Therefore, we selected the crude enzymes from A. oryzae and E. parvum 4–14 for subsequent study.

Additionally, the effect of a blend of enzymes (produced by A. oryzae and E. parvum 4–14) with different protein dosages (0.25%–1%, w/w) was also studied on the enzymatic hydrolysis of corn bran residue (165 °C/40 min). As shown in Fig. 5B3, the enzymatic hydrolysis yields of glucose, xylose, and arabinose increased with the enzyme dosage, whereas the ferulic acid yield did not. On the other hand, the enzymatic hydrolysis yields of glucose, xylose, and arabinose when using the enzyme blend of 0.5% improved by 26.31%, 22.85%, and 32.23%, respectively, compared with that when using the enzyme blend of 0.25%. However, the enzymatic yields of glucose, xylose, and arabinose when using the enzyme blend of 1% only improved by 5.93%, 3.87%, and 5.7%, respectively, compared with that when using the enzyme blend of 0.5%, suggesting that the dosage of 0.5% may have reached the saturation point. Therefore, the dosage of 0.5% was selected for the subsequent enzymatic hydrolysis.

3.6. Separate enzymatic hydrolysis of autohydrolysis residue and liquid with different severity of autohydrolysis using a crude enzyme blend

The hydrolysis yields of the residues and liquids with different severity of autohydrolysis are presented in Fig. 6A1–A3 and B1–B3, respectively. As shown in Fig. 6A1, the enzymatic hydrolysis yields of glucose, xylose, arabinose, and ferulic acid in residues increased with autohydrolysis time at 155 °C. This was due to the more accessible substrates in residues with longer autohydrolysis time at 155 °C. The enzymatic hydrolysis yields of the four products in residues also increased as autohydrolysis time increased from 10 min to 30 min at 165 °C (Fig. 6A2). However, they just slightly increased as the autohydrolysis time further increased to 40 min, suggesting that the severity of autohydrolysis had an insignificant effect on the enzymatic digestibility of the residues. As shown in Fig. 6A3, the enzymatic hydrolysis yields of monosaccharides for the three residues autohydrolyzed at 175 °C for 20–40 min were almost at the same level, which further confirms this point of view.

Since the autohydrolysis liquid fraction is rich in complex xylan-based oligosaccharides, enzymatic hydrolysis of the autohydrolysis liquid by the enzyme blend was carried out to convert oligosaccharides into monosaccharides and ferulic acid (Fig. 6B1–B3). In general, the glucose yield of the liquid fractions after enzymatic hydrolysis was higher than the yield of the other three products (Fig. 6B1–B3). The overall yields of glucose, xylose, arabinose, and ferulic acid reached more than 80% for most of the samples pretreated under various severities. Although the complex xylan-based oligosaccharides are in soluble form, they are resistant to be converted into monosaccharides such as xylose and arabinose by enzymatic hydrolysis. For example, Bonnin et al. (2002) pointed out that the xylose and arabinose yields respectively reached 29.5% and 62.3% after enzymatic hydrolysis of autohydrolysis liquor fraction of corn bran (160 °C/60 min) using a crude enzyme from Aspergillus niger. These data suggested that the enzyme blend used in this work was very efficient in digesting oligosaccharides in the soluble fraction into the monosaccharides and ferulic acid.

Because a difference exists between the effects of the autohydrolysis condition on the separate enzymatic hydrolysis of liquids and residues, combined hydrolysis yields of liquids and residues obtained at 155–175 °C were calculated and compared in Fig. 6C1–C3, respectively. The combined hydrolysis yields of glucose, xylose, arabinose, and ferulic acid were based on the content of glucon, xylan, arabinan, and ferulic acid in destarched corn bran (160 °C/60 min) using a crude enzyme from Aspergillus niger. These data suggested that the enzyme blend used in this work was very efficient in digesting oligosaccharides in the soluble fraction into the monosaccharides and ferulic acid.

In conclusion, this work was very efficient in digesting oligosaccharides in the soluble fraction into the monosaccharides and ferulic acid.
saccharification of corn bran with various pretreatments such as, acidic, autohydrolysis, and flash-explosion pretreatments (Saulnier et al., 2001; Bonnin et al., 2002; Dien et al., 2006; Agger et al., 2011). However, some of these studies concentrated on enzymatic release of a single component, such as ferulic acid (Saulnier et al., 2001), xylose (Agger et al., 2010), or arabinose (Fehér et al., 2015), from the liquid fraction and/or the residue. Although other studies focused on the overall hydrolysis of all of the components in the liquid and/or residue, the hydrolysis yields were far from satisfactory. Saulnier et al. (2001) reported that the maximum enzymatic yields of glucose (35%), xylose (60%), and arabinose (60%) were obtained after flash-explosion (190 °C/1 min) of corn bran and subsequent hydrolysis of insoluble residue using 1% (w/w) of Novozym 342. Agger et al. (2011) found that the maximum combined hydrolysis yields of xylose and arabinose respectively reached 50% and 60% of their original amounts present in corn bran after acidic pretreatment (150 °C/65 min, pH 2) followed by enzymatic hydrolysis using mixed commercial enzymes, including xylanase, arabinofuranosidase, acetyl xylan esterase, and feruloyl esterase. Dien et al. (2006) reported that when enzymatically hydrolyzing the autohydrolysis products of corn bran (165 °C/20 min) using the crude enzyme blend from solid state fermentation by T. reesei and A. niger, the hydrolysis yields of xylose and arabinose were 74% and 54%, respectively. A complete enzymatic release of ferulic acid together with a significant release (76–100%) of pentoses, such as xylose and

Fig. 6. Monosaccharide and ferulic acid yields from autohydrolysis residues (A), liquid (B), and the combined yield (C) after enzymatic hydrolysis of autohydrolysis residues and liquid by the enzyme blend. 1–3: autohydrolysis at 155 °C, 165 °C, and 175 °C, respectively. Glu., glucose; Xyl., xylose; Ara., arabinose; Fer., ferulic acid. The hydrolysis yields from autohydrolysis residues and liquid were respectively calculated on base of the content of corresponding components in residues and liquids, while the combined yield was based on the content of corresponding components in destarched corn bran.
Biohydrolysis of complex plant polysaccharides and other constituents. The desirable mono-
saccharides and other components could be obtained by enzymatic hydrolysis.

4. Conclusions

The autohydrolysis conditions had a great influence on depoly-
merization and solubilization of carbohydrates, ferulic acid, and acetyl
methyl group as well as on the enzymatic hydrolysis of autohydrolysis residues.
Arabinan was depolymerized and solubilized more easily compared to
xylan and other constituents. Different linear correlations existed be-
tween enzymatic glucose and xylose yields with other constituent
contents. The enzyme blend from A. oryzae and E. parvum 4–14 showed a
significant synergistic effect, making it superior to individual crude
enzymes or a commercial enzyme mixture. The desirable mono-
saccharide and ferulic acid yields could be obtained by autohydrolysis
at 165 °C for 40 min and enzymatic hydrolysis using the enzyme blend.

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