ผลของการปรับสภาพด้วยด่างและการลดความเป็นโซ่กิ่งด้วยกรดในแกลบ ฟาง และกากรำข้าวสกัดน้ำมัน ต่อการผลิตไซโลไบโอสด้วยเอนไซม์ไซแลนเนสทางการค้า

THE EFFECT OF ALKALI PRETREATMENT AND ACID DEBRANCHING ON RICE HUSK, RICE STRAW, AND DEFATTED RICE BRAN FOR XYLOBIOSE PRODUCTION BY COMMERCIAL XYLANASES

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บทคัดย่อ

งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาผลของการปรับสภาพด้วยด่างและการลดความเป็นโซ่กิ่งด้วยกรดต่ออัตราส่วนน้ำตาลอะราบิโนสต่อไซโลส (A/X) ของอะราบิโนไซแลน (AX) ในฟางข้าว แกลบ และกากรำข้าวสกัดน้ำมัน เพื่อเตรียมสำหรับการผลิตไซโลไบโอส และ/หรือ อะราบิโนไซโลโอลิโกแซคคาไรด์ด้วยเอนไซม์ไซแลนเนสทางการค้า โดยเริ่มจากนำวัสดุชีวมวลแต่ละชนิดทำการปรับสภาพด้วยโซเดียมไฮดรอกไซด์ความเข้มข้น 2% และนำไปลดความเป็นโซ่กิ่งด้วยกรดฟอร์มิก พบว่าน้ำหนักมวลของแกลบ ฟางข้าว และกากรำข้าวสกัดน้ำมันหลังการปรับสภาพด้วยด่างเท่ากับ 68.7, 44.8 และ 24.3% ในขณะที่ปริมาณน้ำตาลทั้งหมดเพิ่มขึ้นจาก 19.9 เป็น 28.0%, 21.7 เป็น 26.4% และ 11.3 เป็น 20.0% เมื่อเปรียบเทียบกับวัสดุชีวมวลที่ไม่ผ่านการปรับสภาพ ตามลำดับ และเมื่อทำการลดความเป็นโซ่กิ่งด้วยกรดฟอร์มิกพบว่าปริมาณน้ำตาลทั้งหมดในวัสดุชีวมวลเพิ่มขึ้นเป็น 4% นอกจากนี้การปรับสภาพด้วยด่างสามารถลดอัตราส่วน A/X ในกากรำข้าวสกัดน้ำมัน แกลบ และฟางข้าว จาก 1.08 เป็น 0.82, 0.14 ถึง 0.13 และ 0.22 เป็น 0.22 ในขณะที่ปริมาณ AX เพิ่มขึ้นจาก 5.8 เป็น 14.3%, 11.5 เป็น 18.6% และ 11.0 เป็น 18.3% ตามลำดับ ซึ่งการปรับสภาพด้วยด่างและการลดความเป็นโซ่กิ่งด้วยกรดส่งผลทำให้อัตราส่วน A/X ในกากรำข้าวสกัดน้ำมันลดลงจาก 1.08 เป็น 0.63 หลังจากกระบวนการดังกล่าว วัสดุชีวมวลถูกนำมาใช้เป็นสารตั้งต้นในการผลิตไซโลไบโอสด้วยเอนไซม์เอนโดไซแลนเนสทางการค้าชนิด Pentopan Mono BG และ Pentopan 500 BG ที่ระดับความเข้มข้น 132.5 U และ 26.5 U ต่อหนึ่งกรัมของสารตั้งต้น ตามลำดับ ในโซเดียมฟอสเฟตบัฟเฟอร์ (พีเอช 6.0) ที่อุณหภูมิ 50 องศาเซลเซียส เป็นระยะเวลา 4 ชั่วโมง โดยเขย่าอย่างต่อเนื่องที่ความเร็ว

150 รอบต่อนาที จากผลการศึกษาพบว่าสารชีวมวลที่มีอัตราส่วน A/X สูงจะถูกย่อยได้น้อยกว่าสารชีวมวลที่มีอัตราส่วน A/X ต่ำ นอกจากนี้ยังพบอีกว่าเอนไซม์ Pentopan 500 BG แสดงประสิทธิภาพการย่อยได้ดีกว่า Pentopan Mono BG ถึงแม้ว่าผลของโครมาโตรกราฟฟีแสดงให้เห็นว่าผลิตภัณฑ์ที่ได้จากการย่อยด้วยเอนไซม์ไซแลนเนสทั้งสองชนิดมีชนิดของน้ำตาลที่เหมือนกัน ได้แก่ น้ำตาลไซโลส น้ำตาลอะราบิโนส น้ำตาลไซโลไบโอส และน้ำตาลที่เป็นพอลิเมอร์ขนาดใหญ่ อย่างไรก็ตาม Pentopan 500 BG ได้แสดงการผลิตน้ำตาลไซโลสและอะราบิโนสที่โดดเด่นกว่า Pentopan Mono BG จากการศึกษาครั้งนี้จึงสามารถกล่าวได้ว่า ฟางข้าวและแกลบสามารถใช้การปรับสภาพด้วยด่างเพียงอย่างเดียวก็เพียงพอต่อการเตรียมสำหรับการผลิตไซโลไบโอส ในขณะที่กากรำข้าวสกัดน้ำมันจำเป็นต้องใช้การปรับสภาพด้วยด่างร่วมกับการลดความเป็นโซ่กิ่งด้วยกรด

คำสำคัญ: วัสดุชีวมวล การปรับสภาพด้วยด่าง การลดความเป็นโซ่กิ่งด้วยกรด เอนโดไซแลนเนส ไซโลไบโอส

Abstract

The use of alkali pretreatment and acid debranching to optimize the arabinose/xylose ratio of arabinoxylan from rice straw (RS), rice husk (RH), and defatted rice bran (DRB) as preparation for production of xylobiose and/or arabino-xylooligosacharides using commercial xylanases were investigated. Firstly, each biomass was treated with alkali pretreatment with 2% NaOH and acid debranching with formic acid. The yields of RH, RS, and DRB after alkali pretreatment appeared to be 68.7, 44.8, and 24.3% while the total sugar contents were increased from 19.9 to 28.0%, 21.7 to 26.4%, and 11.3 to 20.0%, compared to untreated biomass, respectively. The total sugar content of acid debranched biomass was also increased up to 4%. In addition, the alkali pretreatment slightly decreased arabinose/xylose ratio (A/X) of DRB, RH, and RS from 1.08 to 0.82, 0.14 to 0.13 and 0.22 to 0.22. At the same time, arabinoxylan contents were increased from 5.8 to 14.3%, 11.5 to 18.6% and 11.0 to 18.3%, respectively. Superior to alkali pretreatment, the following acid debranching dramatically decreased A/X ratio of DRB from 1.08 to 0.63. After mentioned processes, xylobiose was separately produced by using Pentopan Mono BG and Pentopan 500 BG at 132.5 U and 26.5 U/g of substrate, respectively. The hydrolysis was performed in sodium phosphate buffer (pH 6.0) at 50°C for 4 h with continuously shaking at 150 rpm. The results showed that the biomass with higher A/X ratio was lesser hydrolysed. Pentopan 500 BG exhibited greater hydrolysis effect than that of Pentopan Mono BG. However, the Thin layer chromatography and Ion chromatography results confirmed that the hydrolysates from both xylanases had the similar sugar patterns showing xylose, arabinose, xylobiose, and the components with a higher degree of polymerization. However, Pentopan 500 BG remarkably produced monosaccharides mainly xylose and arabinose. The alkali pretreatment singly was enough while from DRB, the acid debranching should be combined following the pretreatment process.

Keywords: Biomass, Alkali Pretreatment, Acid Debranching, Endoxylanase, Xylobiose

Introduction

In recent years, the huge amount of residual plant biomass from rice farming, rice milling and by-products from rice bran oil industry such as rice straw (RS), rice husk (RH), and defatted rice bran (DRB) were considered as wastes. Most of them are potentially used to produce value-added products because they are rich in lignocellulose. The previous study found that RS, RH, and DRB consisted of 32, 22, and 35% of cellulose content and 24, 23, and 25% of hemicellulose content, respectively [1]. Arabino-xylooligosaccharides and xylooligosaccharides ((A)XOS) are sugar oligomers made up of 2-10 xylose units and with or without the branch of arabinose units and considered as non-digestible food ingredients. They are commonly reported as the prebiotic when consumed as a part of a diet. Moreover, xylobiose and (A)XOS have acceptable that they do not exhibit toxicity or negative effects on human health. These properties make them suitable for many food products such as beverage, dairy products, bakery and confectionaries and also show a remarkable potential for pharmaceuticals, feed formulations and agricultural applications as well [2]. Xylobiose and (A)XOS can be produced from biomass such as wheat straw, wheat bran, rice bran, corncobs, etc. Various methods have been studied for (A) XOS production such as chemical process, autohydrolysis, direct enzymatic hydrolysis or a combination of these methods. Generally, hemicellulose exists as the complex structure in the lignocellulosic materials which is resistant to hydrolysis. Therefore, many researchers are usually carried out in two stages for (A)XOS production: first is alkali extraction of hemicellulose. Second is enzymatic hydrolysis [3]. However, alkali extraction of hemicellulose requires the high amount of acid to neutralize alkali-rich hemicellulose solution. As a result, the undesirable salt will be produced. Then the purification process will be required before performing enzymatic hydrolysis. Millett, et al [4] reported that alkali pretreatment by soaking with diluted NaOH has been widely used to improve the digestibility of lignocellulosics in ruminants. Fan, et al. [5] revealed that the increasing external surface area of the pretreated biomass could enhance the rate of enzymatic hydrolysis.

The high amount of (A)XOS production is usually accomplished by xylanases with negligible as the presence amount of β-xylosidase and exoxylanase activity to prevent high amount production of xylose which causes inhibitory effects on the production of XOS [6] and purity of oligosaccharides. However, most of the endoxylanases from glycoside hydrolase families 10 or 11 are highly active on unsubstituted parts of the xylose backbone chain and also have limited tolerance for arabinose substituents. Thus, it can be considered that removal of arabinose substituents can be useful for the complete degradation of the biomass into (A)XOS.

Objectives

Here, the use of residues from rice milling and rice bran oil industry which are

rich in arabinoxylan by alkali pretreatment and acid debranching to optimize the arabinose/xylose ratio of arabinoxylan from RS, RH, and DRB for production of xylobiose and/or arabino-xylooligosacharides using commercial xylanases were investigated.

Methods

Materials

DRB used in this research was purchased from King Rice Oil group, Ltd., Thailand. RS and RH were purchased from Phitsanulok and Pichit, Thailand, respectively. DRB was sieved into 60 sieving mesh to ensure their uniformity. Rice straw was sized reduction to 2 cm long. To remove the undesirable contamination, rice husk was passed through screen filter. All of them were dried overnight in the hot air oven at 60°C. Commercial xylanases were Pentopan Mono BG powder recombinant, expressed in Aspergillus oryzae (xylanase activity ≥ 2500 units/g) and Pentopan 500 BG (xylanase activity 500 units/g) gifted from Novozymes, Bagsvaerd, Denmark. All chemicals and solvents used in this research were analytical grades.

Alkali Pretreatment of Biomass

In brief, one hundred grams of biomass were soaked in 2%NaOH and covered with a lid to prevent evaporation for 6 days of pretreatment time. After complete pretreatment, the alkali solution was decanted and then the residues were washed until reaching pH 6.0-7.0. The pretreated biomass was dried at 45°C for 24 h in hot air oven, then ground and filtered through 60 mesh sieving size to obtain the pretreated rice husk (P-RH), pretreated rice straw (P-RS), and pretreated defatted rice bran (P-DRB).

Preparation of Acid Debranched Biomass

Acid debranching method was modified from McCleary, et al. [7]. In brief, 10 g of pretreated biomass was added to 500 ml of distilled water and heating to 80°C in water bath. Concentrated formic acid (20 ml) was added, adjusted pH to 1.45 by the addition of 5 M HCl. This solution was incubated for 1 h at 80°C in water bath. The solution was quickly decanted and transferred into 500 ml of 95% ethanol and mixed for 2 h. The residues were recovered by filtration. The debranched biomass was re-washed with 95% of ethanol squeezed free of liquid and then dried at 60°C for 24 h in hot air oven to obtain the debranched rice husk (D-RH), debranched rice straw (D-RS), and debranched defatted rice bran (D-DRB).

Preparation of Biomass for Monosaccharides Compositional Analysis

The determination of structural carbohydrates in a solid biomass sample was carried out by modified method of NREL: Determination of Structural Carbohydrates and Lignin in Biomass. 400 mg of processed biomass was mixed with 4.5 ml of 72% sulfuric acid, stirred the sample for 30 min. The slurry was diluted by acid to a 4% concentration. After completion of the autoclave cycle for 1 h at 121°C, the hydrolysates were allowed to cool down and filled up to 100 ml. The total sugar as pentosan was measured by the method of orcinol-HCl with xylose as standard. Monosaccharides analysis was done using barium carbonate

to neutralize each sample to pH 5.0–6.0, then performed Ion chromatography (IC) analysis with Dionex CarboPac PA-1 column (250 mm x 4 mm) and a guard column (50 mm x 4 mm) at flow rate 1.0 ml/min. The post-column pump has controlled flow rate at 0.5 ml/min of 300 mM NaOH. A stepwise linear gradient was applied over 20 min by 100% distilled water (Solution A) and was applied over 16 min by mixing solutions of 200 mM NaOH (Solution B) and 200 mM NaOAc in 170mM NaAc (Solution C). Eluted oligosaccharides were monitored by PAD detection using an Au electrode. Peaks of monosaccharides were assigned by using xylose (Merck), arabinose (Sigma), mannose (Merck), galactose (Sigma), and glucose (Sigma) standards.

Commercial Xylanase Treatments

Hydrolysis of no treatment, pretreated, and acid debranched biomass was performed with Pentopan Mono BG or Pentopan 500 BG for (A)XOS production. One gram of biomass was suspended in 20 ml of 100 mM sodium phosphate buffer (pH 6.0). Then Pentopan mono BG and Pentopan 500 BG were separately added at 132.5 and 26.5 U/g substrate, respectively. The samples were incubated at 50°C in water bath shaker at 170 rpm for 4 h of incubation time. The reaction was stopped by boiling for 30 min and determined the production of oligosaccharides by DNS method [8] using xylose as the standard. Sugar hydrolysate was checked qualitatively by thin layer chromatography (TLC) which was performed on Merck TLC (aluminum sheets 20 x 20 cm) of silica gel 60. The plates were developed once with 1-butanol: acetic acid: water in the ratio of 2:1:1. Spots were detected by spraying with 10% sulfuric acid in ethanol with 0.2% of orcinol and heating in hot air oven at 110°C compared with mixed XOS (X_1-X_5) standard. For oligosaccharides analysis by IC, Dionex CarboPac PA-200 column (250 mm x 4 mm) and a guard column (50 mm x 4 mm) was used at constant flow rate 0.4 ml/min. The sample pumps gradient elution of the neutral carbohydrate was performed followed by McCleary, et al. [7]. Peaks of oligosaccharides were assigned by using arabinose (Sigma, USA), xylose (Merck, Germany), xylobiose (Wako, Japan), xylotriose (Wako, Japan), 1,3-arabinosyl-xylobiose and 1,2-arabinosyl-xylotriose standards were purchased from Megazyme, Ireland.

One Unit of xylanase activity is defined as the amount of enzyme required to release one ตmole of xylose reducing sugar equivalents per minute from wheat arabinoxylan (5 mg/ml) in sodium phosphate buffer (100 mM), pH 6.0.

Statistical Analysis

Data were analyzed by one way Analysis of variance (ANOVA). Significant differences ($p \leq 0.05$) between samples were evaluated using Duncan's new multiple range test. Two replications were performed in the experiment.

Results

Sugar Composition of Biomass

The carbohydrate portion of the biomass is made up of holocellulose. Results showed

that DRB contained arabinose, xylose, glucose, galactose, and mannose as the main structural carbohydrates in the polymeric form based on dry weight while RH and RS did not contain mannose. Glucose is the sugar mainly found in most plants structure. High amounts of glucose were determined in DRB, RS, and RH showing the highest content about 76.8, 70.4, and 68.1%, respectively. Interestingly, RH and RS also contained the higher amount of xylose than that of DRB up to 15% (Table 1). Whereas, the arabinose content was found at 3.6% and 4.9% in RH and RS, respectively which are lower than in DRB (9.4%). The content of arabinoxylan (AX) was estimated from the amounts of xylose and arabinose. It was 11.5% and 11.0% in RH and RS, respectively indicating higher amount compared to DRB (5.8%). The A/X ratio was 1.08, 0.14, and 0.22 in DRB, RH, and RS respectively.

Table 1 Monosaccharides analysis results of biomass obtained by alkali pretreatment and acid debranching from DRB, RH, and RS expressed as % w/w.

Samples	% Monosaccharides (w/w)					A/X	AX
	Ara	Xyl	Glu	Gal	Man	ratio	(%)
DRB	9.45	8.79	76.84	3.57	1.35	1.08	5.8
P-DRB	11.97	14.54	67.89	3.92	1.69	0.82	14.3
D-DRB	9.26	14.62	69.39	4.29	2.44	0.63	9.9
RH	3.61	26.38	68.18	1.83	ND	0.14	11.5
P-RH	3.87	29.03	65.56	1.54	ND	0.13	18.6
D-RH	3.95	21.50	71.50	2.05	ND	0.18	9.4
RS	4.88	22.29	70.44	2.39	ND	0.22	11.0
P-RS	3.94	18.10	76.60	1.36	ND	0.22	18.3
D-RS	3.43	23.12	72.29	1.17	ND	0.15	19.7

Glu = Glucose, Gal = Galactose, Man = Mannose, A/X = Arabinose/Xylose Ratio, AX = Arabinoxylan [Calculate from 0.88 x (%arabinose+%xylose)]

Sugar Composition and Yield of Biomass from Different Processes

After pretreatment, the yields of RH, RS, and DRB were decreased to 68.7, 44.8, and 24.3% while the increment of total sugar contents from 9.4 to 12.0% and 8.8 to 14.5%, respectively, in DRB. However, the xylose content of RH increased from 26.4 to 29.0% while the arabinose content was not changed. Interestingly, the alkali pretreatment decreased A/X ratio of DRB and RH from 1.08 to 0.82 and 0.14 to 0.13, respectively. While the increment of AX content of DRB, RH and RS from 5.8 to 14.3%, 11.5 to 18.6% and 11.0 to 18.3% were reported, respectively (Table 1). Diluted acid catalysis enables pre-hydrolysis and thereby reduces sugar decomposition [12]. After acid debranching, the yield of biomass was reduced around 83% in DRB, 60% in RS, and 41% in RH (Table 2). Interestingly, results from Table 1 exhibited that acid debranching reduced arabinose content in DRB from 12.0 to 9.3%. On the other hands, xylose content was not changed in DRB but was slightly reduced in RH from 29.0 to 21.5%. The A/X ratio was dramatically decreased from 1.08 to 0.63 after acid debranching.

Table 2 The percentage yield and total sugar as pentosan content after alkali pretreatment and debranching step of rice husk, rice straw, and defatted rice bran

Samples	Yield (% w/w)	Total Sugar (%w/w)
DRB	100±0.00	11.33±0.13
P-DRB	24.32±0.13	19.94±0.00
D-DRB	17.51±0.49	24.58±0.32
RH	100±0.00	19.89±0.57
P-RH	68.74±0.68	28.00±0.89
D-RH	59.09±0.23	32.01±0.70
RS	100±0.00	21.74±0.32
P-RS	44.80±0.52	26.42±0.13
D-RS	40.32±0.38	29.98±0.25

Oligosaccharides Produced by Commercial Xylanases

Incubations using Pentopan Mono BG and Pentopan 500 BG xylanases were performed in the untreated, pretreated and debranched biomass. Figure 1 demonstrated the total reducing sugar at 4 h of hydrolysis time at 50°C. Pentopan 500 BG gave higher total reducing sugar while sugar patterns of both enzymes similarly exhibited (Figure 2 and 3). The untreated biomass seemed to be an inappropriate

substrate for both enzymes. The total reducing sugar contents from Pentopan Mono BG hydrolysis of untreated, alkali pretreated and acid debranched biomass were 0.2, 1.5, and 1.6% in RH, 2.0, 4.0, and 4.1% in RS, and 0.2, 0.3, and 0.4% in DRB, while those of Pentopan 500 BG hydrolysis were 0.9, 7.9 and 7.9% in RH, 5.3, 8.4 and 20.6% in RS and 5.3, 19.9 and 13.7% in DRB, respectively. However, the determination of sugar pattern by TLC confirmed that both enzymes gave the similar sugar patterns (Figure 2) but Pentopan 500 BG produced predominantly monosaccharides mainly xylose and arabinose. Considering RH and RS, the A/X ratios and total reducing sugar contents in alkali pretreated as well as acid debranched biomass were not significantly different. While A/X ratio of D-DRB was significantly different to the P-DRB ($p \leq 0.05$). From these results, the hydrolysates of P-RH, P-RS, and D-DRB from Pentopan Mono BG were chosen as the representatives for sugar patterns analysis by IC.

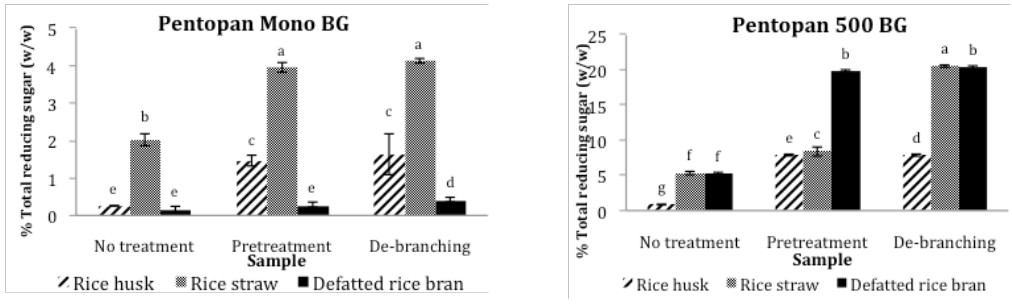

Figure 1 Enzymatic production of oligosaccharides from untreated, pretreated, and debranched RH, RS and DRB at using Pentopan Mono BG and Pentopan 500 BG endoxylanases (133 U, 50°C, 4 h)

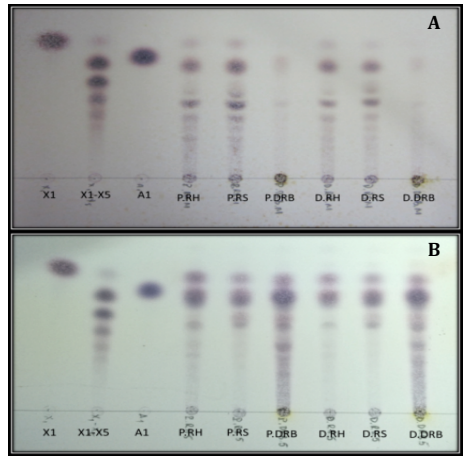

Figure 2 TLC chromatogram of enzymatic hydrolysates of alkali pretreated and acid debranched RH, RS and DRB by (A) Pentopan Mono BG and (B) Pentopan 500 BG (133 U, 50°C, 4 h). Xylose (x1), mixture of xylobiose (x2), xylotriose (x3), xylotetraose (x4) and xylopentaose (x5) and arabinose (A1) were used as standard.

The IC results exhibited that xylobiose was the main oligosaccharide found in P-RH and P-RS hydrolysate while xylose followed by xylobiose were the sugars mainly found in D-DRB hydrolysate (Figure 3). Interestingly, all of hydrolysates found 1,3-arabinosyl-xylobiose and 1,2-arabinosyl-xylotriose in a small amount (data not shown) because of the specific activity of enzymes on the substrate was limited.

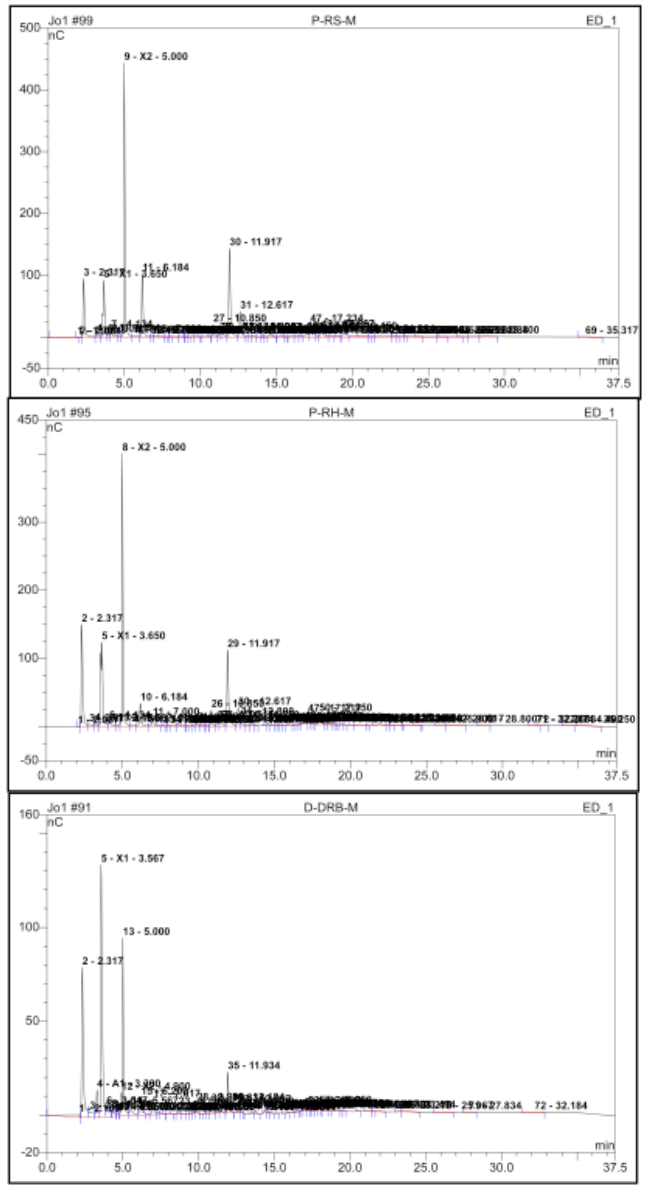

Figure 3 IC Chromatograms of enzymatic hydrolysates of (A) P-RS, (B) P-RH, and (C) D-DRB by Pentopan Mono BG at 133 U, 50°C, 4 h. Xylose, xylobiose, xylotriose, 1,3-arabinosyl-xylobiose, 1,2-arabinosyl-xylotriose, and arabinose were used as standard.

Conclusions and Discussion

The major source of sugars is cellulose and hemicellulose, which can include xylose, mannose, galactose, rhamnose, and arabinose monomers. The neutral sugar composition in RH was similar to the previous report which was analyzed the neutral sugar; glucose, xylose, and arabinose, from RH in ratio 66.68±0.97, 27.61±1.06 and 5.71±0.17%, respectively [9]. The carbohydrate of RS was similar as reported by Roberto, et al. [10] which in range of glucose 41-43.4%, xylose 14.8-20.2%, arabinose 2.7-4.5%, mannose 1.8%, and galactose 0.4%. Their occurrence could depend on the plant source. However, the yields of all biomass were decreased while the total sugar contents were increased after pretreatment. Furthermore, alkali pretreatment increased the proportion of sugar content, especially arabinose and xylose in DRB (Table 1). Tarkow and Feist [11] indicated that alkali treatment increased intra-particle porosity and channel size. Changing in structural carbohydrates proportion and yield in biomass during alkali pretreatment could result from the dissolution of extractives such as terpenes, resins, phenol, and lignin or non-extractives such as starches, pectins, and proteins [4]. Acid debranching was used for modifying the hemicellulose structure which contained high branched of sugars. For example, commercially oat-spelt xylan characteristic substrate contains a single O-3-connected α-L-arabinofuranosyl residue on every eight to ten xylosyl residues of the linear xylan backbone [18]. The effect of acid debranching on RH, RS, and DRB in this study revealed that the A/X ratio of RH and RS was not reduced much but in DRB was dramatically decreased (Table 1). These results could be considered that the hemicellulose structure of DRB would be short chain polymer with high branched while RH and RS would be a long-chain polymer with low branched. However, cleavage of the side groups of sugars is a prerequisite to achieving fast and complete hydrolysis of branched hemicellulosic substrates by endoxylanase action [19].

Endoxylanases of GH 10 and 11 are able to saccharify holocellulose to digestible polymeric sugar at β-1,4 glycosidic linkage, primarily producing (A)XOS [13]. The untreated biomass seemed to be an inappropriate substrate for both enzymes. This could result from the presence of lignin, poorly accessible surface area, high crystallinity and so on [14], which may physically restrict or otherwise block enzymatic binding to the lignocellulosic surface. As expected, the lesser branched biomass (lower A/X ratio) was able to be hydrolysed prior to the highly branched biomass (high A/X ratio). Pentopan 500 BG was more effective than Pentopan Mono BG in hydrolysing all biomass showing higher total reducing suger contents (Figure 1). Pentopan mono BG was classified in glycoside hydrolase (GH) 11 which preferentially cleave the unsubstituted regions of the arabinoxylan backbone [15], whereas Pentopan 500 BG cannot classified because it gave several bands on SDS-PAGE which

made it difficult to determine their molecular weight and GH family [16]. In this study, use of Pentopan 500 BG on pretreated biomass releases relatively higher amount of xylose compared to Pentopan Mono BG (data not show). This can be explained as Pentopan 500 BG has more cleavage sites compared to Pentopan mono BG and are efficient in making xylose and arabinose product which may consist of β-xylosidase, α-L-arabinofuranisidase, and/or exoxylanases. Stone, et al. [17] introduced the hypothesis that the initial rate of hydrolysis is a function of the accessible surface area, then the hydrolysis rate increases with increasing coverage of mixture enzymes. The TLC and IC chromatogram (Figure 2 and 3) revealed that both xylanases are quite effective on hydrolyzing arabinoxylan into short-oligosaccharides which xylobiose was the main oligosaccharide found in P-RH, P-RS, and D-DRB hydrolysate. However, hydrolyzing with Pentopan 500 BG could be required purification step for monosaccharides removal because they are not show prebiotic properties.

This study showed that decreasing the A/X ratio and increasing the content of AX by alkali pretreatment and acid debranching followed by commercial xylanase treatment is a promising method for producing mixtures of short oligosaccharides mainly xylobiose and some kind of (A)XOS from RH, RS, and DRB. The type of xylanase influences the amount and pattern of monosaccharides of the hydrolysate. Acid debranching could potentially be integrated on DRB preparation to modify its structure for increasing accessible surface area binding attack with the enzyme. This research has valuable impacts especially in finding alternative uses of the RH, RS, and DRB is expected to increase the benefits for the value-added aspect in food industry.

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