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Oligosaccharides from rice straw and rice husks produced by glycoside hydrolase family 10 and 11 xylanases

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Abstract

Rice straw (RS) and rice husks (RH) are the by-products obtained from rice farming, which are the remaining non-starch polysaccharides, called cellulose and hemicellulose. The objectives of this study were to investigate the abilities of the glycoside hydrolase family 10 (GH10) and 11 (GH11) commercial xylanases on the production of oligosaccharides from RS and RH by hydrothermal assisted enzymatic hydrolysis. Firstly, RS and RH were pretreated with acetone/ethanol. Then the pretreated biomass was heated by autoclave at 180 ˚C for 10-30 min. The oligosaccharides content in the RS and RH hydrolysates (HRS and HRH) were analyzed by High Performance Anion Exchange Chromatography (HPAEC-PAD). The results indicated that RS and RH treated for 10 min had shown the highest total oligosaccharides content. After that, the HRS and HRH were hydrolyzed with Ultraflo Max (UM10) and Ultraflo L (UL11), belonged to GH10 and GH11 respectively, under condition at 50 ˚C pH of 6.0 for 0-4 h. The highest sugar-reducing content was found while incubating HRS and HRH for 1 h with the aforementioned xylanases. The sugar-reducing contents of HRS and HRH treated with UM10 increased up to 0.24% and 0.17%, respectively, whereas those treated with UL11 increased up to 0.18% and 0.14%, respectively. The results revealed that HRS and HRH treated with UM10 had mainly consisted of xylobiose, while those treated with UL11 had mainly consisted of xylotriose. This study has suggested the potential of GH10 and GH11 xylanases on Xylooligosaccharide (XOS) production using RS and RH as alternative sources.

Keywords: Rice husks, Rice straw, Oligosaccharide, Glycoside hydrolase 10, Glycoside hydrolase 11

1. Introduction

Rice is an important economic crop in Thailand for domestic consumption and for exportation. In 2017, Thailand exported 11 million t of rice, which increased from 5 million t in 2012 [1]. Increasing rice production causes huge amounts of by-products, such as rice straw (RS) and rice husks (RH). Previous studies have reported that RS and RH are abundant in lignocellulosic materials, which can be a precursor to the production of oligosaccharides. A previous report found that RS consists of 32% of cellulose and 29% of hemicellulose content, whereas RH consists of 36% and 29%, respectively [2]. Hemicellulose is a group of non-starch polysaccharides, which are found abundantly in plants. The hemicellulose structure of RS and RH consist of long chain xylose units as the backbone, called xylan. Xylan is a group of hemicelluloses, which are made up of β-1,4-linked xylose residues with or without side branches of α-arabinofuranose and α-glucuronic acids. Arabinoxylans (AX) is one of the four main families of xylans [3]. AX can be converted into XOS, which are considered to be a functional food due to their potential prebiotic properties.

In recent years, oligosaccharide production by enzymatic hydrolysis has gained much interest due to the mild conditions used and the specific products obtained. Therefore, for oligosaccharide production, it is necessary that it be carried out in more than one stage using a hydrothermal treatment assisted with enzymatic hydrolysis. Hydrothermal treatment is used to extract hemicellulosic materials. In this process, polysaccharides undergo hydrolysis at high temperatures and pressure in the presence of hydronium ions, which are generated by water autoionization and act as catalysts, that can break down the hydrogen bonded structure [4]. Temperatures below 210-220 ˚C are able to partially degrade cellulose molecules and temperatures higher than 120 ˚C could be successfully used to extract hemicelluloses. Moreover, some hemicelluloses may be further hydrolyzed and converted into oligomers and monomers [5].

Among all the xylanases, the endo-xylanases are the most important due to their direct involvement in cleaving the glycosidic bonds and in liberating the short XOS [6]. Xylanases as glycoside hydrolase (GH) families can catalyze the hydrolysis of the glycosidic bond in 1,4-β-D-xylosidic linkages of xylan. Diverse forms of these enzymes exist and display varying folds, mechanisms of action, substrate specificities, hydrolytic activities, and physicochemical characteristics. This research has mainly focused on only two of the xylanases containing glycoside hydrolase families, namely Families 10 and 11 [7]. The GH10 xylanase family compose the activities of endo-1,4-β-xylanases and endo-1,3-β-xylanases (EC 3.2.1.32) [8]. The members of this family are also capable of hydrolyzing the aryl β-glycosides of xylobiose and xylotriose at the aglyconic bond. Furthermore, these enzymes are highly active on the short-chain XOS, thereby indicating small substrate-binding sites. Meanwhile, the GH11 (EC3.2.1.8) family displays several interesting properties, such as a high substrate selectivity, a high catalytic efficiency, a small size, and a variety of optimum pH and temperature values [9]. GH11 is exclusively active on D-xylose-containing substrates [10]. Therefore, GH11 xylanases preferentially cleave the unsubstituted regions of the arabinoxylan backbone, whereas the GH10 enzymes, being less hampered by the presence of substituents along the xylan backbone, cleave the decorated regions [10]. Therefore, it can be mentioned here that the difference in substrate specificity has important implications in the deconstruction of xylan in a biomass [11]. Based on the evidence mentioned above, this research aimed to study the effects of GH10 and GH11 xylanases on the oligosaccharides production from hydrothermal-treated RS and RH.

2. Materials and methods

2.1 Materials

The RS and RH, used in this research, were purchased from a rice mill in Phichit Province in Thailand. Both biomasses were ground and sieved through a 40 sieving mesh to ensure their uniformity. All of them were dried in a hot air oven at 50 ˚C until moisture content was determined to be less than 10%. The commercial xylanases, Ultraflo max (700 U/ml from *Aspergillus oryzae* and *Trichoderma reesei*), and Ultraflo L (3550 U/ml from *Humicola insolens*), were provided by Novozyme Co., Ltd., Denmark. All chemicals and solvents used in this research were of analytical grade.

2.2 Pretreatment of biomass

In brief, 100 g of biomass powder was separately soaked in Acetone/Ethanol at ratio 2:1 with continuous stirring, which was performed by an overhead stirrer for 24 h. After pretreatment, the solvent was decanted and then the residues were boiled for 30 min and washed with distilled water until a pH of 6.0-7.0 had been reached. The biomasses were then incubated at 50 ˚C overnight to obtain the pretreated RS and RH powders [12].

2.3 Hydrothermal treatment

The pretreated biomass powder was suspended with distilled water at a ratio of 1:30. In brief, ten grams (dry matter, dm) of biomass was weighed into a 1 L stainless vessel, and distilled water was added to make a final volume of 300 ml. The vessels were then sealed with lids. Temperature probes were inserted down into the slurry. While undergoing constant stirring at 200 rpm, the mixture was then heated in an autoclave reactor (Amar, India) to raise the temperature from room temperature to final temperature of 180 ˚C for 10-30 min. This temperature program was controlled and monitored in the autoclave reactor. After completing the treatment, the slurries were immediately cooled to <45 ˚C according to the manufacturer's instructions. Following the treatments, the slurries were then separated by vacuum filtration to obtain the HRS and HRH. The optimum hydrolyzing time for oligosaccharides production was selected as the treatment time. This maximized the release of oligomers (the sum of polymeric sugar of the arabinose, galactose, glucose, and xylose), which are present in a polysaccharide. The sugar oligomer was calculated from the following equation:

$$\text{Sugar oligomer} = (C_{acid} - C_{hydrolysate}) \times \textit{Anhydro correction} \quad (1)$$

in which.

C_{acid} = sugar concentration in mg/ml of a sugar in the hydrolyzed sample after 4% H_2SO_4 hydrolysis.
$C_{hydrolysate}$ = sugar concentration in mg/ml of a sugar in the hydrolysate.
Anhydro correction = the corresponding monomeric sugars, using anhydro correction of 0.88 for C-5 sugars (xylose and arabinose) and 0.90 for C-6 sugars (glucose and galactose).
Total oligosaccharide = Sum of C-5 and C-6 oligomers (2)

2.4 Determination of oligomers in HRS and HRH

The determination of oligomers in HRS and HRH was carried out by the method of NREL [13]. In brief, 20 ml of HRS and HRH was mixed with 697 µl of 72% sulfuric acid. After completing the autoclave cycle for 1 h at 121 ˚C, the hydrolysates were allowed to cool down. Monosaccharide analysis was done using barium carbonate to neutralize each sample to a pH of 5.0–6.0 and was then passed through 0.45 µm of CA membrane. Next, the HPAEC-PAD analysis was performed [14]. In brief, the Dionex CarboPac PA-1 column (250 mm×4 mm) with a guard column (50 mm×4 mm) was performed at flow rate of 1.0 ml/min. The post-column pump had a controlled flow rate of 0.5 ml/min. A stepwise linear gradient was applied over 20 min. Peaks of monosaccharides were assigned by using xylose (Merck), arabinose (Sigma), mannose (Merck), galactose (Sigma), and glucose (Sigma) standards at 1-5 mg/L.

2.5 Commercial xylanase treatments

The hydrolysis of HRS and HRH was performed with a representative of GH10 and GH11 xylanases: UM10 and UL11. Twenty milliliters of HRS and HRH were diluted to 1% of reducing sugar content by DNS assay [15]. Sodium phosphate buffer (pH 6.0) was prepared by adding sodium dihydrogen phosphate directly into the HRS and HRH and then adjusting the pH until it had reached the point. Each commercial xylanase was then added to a concentration of 150 U/g of substrate, and then the samples were incubated at 50 ˚C with stirring at 170 rpm for 1-4 h of incubation time [16]. The reaction was stopped by boiling for 5 min. The products were then analyzed to determine the total reducing sugar and oligosaccharides profile by DNS assay and TLC, respectively. TLC was performed on aluminum sheets silica gel 60 (Merck). The running mobile phase was 1-butanol: acetic acid: water at the ratio of 2:1:1. The color was developed by spraying with 10% sulfuric acid in ethanol containing 0.2% of orcinol and then by heating at 110 ˚C in hot air oven for 15 min. The quantitative analysis of the oligosaccharide contents was further performed by HPAEC-PAD. A Dionex CarboPac PA-200 column (250 mm×4 mm) and a guard column (50 mm×4 mm) were used at a constant flow rate of 0.4 ml/min. The gradient elution of the neutral carbohydrate was then performed [17]. Peaks of oligosaccharides were assigned by using arabinose (Sigma, USA), xylose; X_1 (Merck, Germany), xylobiose; X_2 (Wako, Japan), xylotriose; X_3 (Wako, Japan), xylotetraose; X_4, 1,3-arabinosyl-xylobiose; and 1,2-arabinosyl-xylotriose; (Megazyme, Ireland) at concentrations of 1-5 mg/L of sugar standards.

The data was then statistically analyzed by a 2x3 asymmetric factorial experiment in a completely randomized design (CRD). Then, the interaction was determined and the treatment means or treatment combination means were compared statistically using Duncan's New Multiple Range Test.

3. Results and discussion

3.1 The effect of hydrothermal treatment on oligosaccharides production

RS and RH were pretreated in an autoclave reactor at 180 ˚C for 10, 20, and 30 min to optimize the oligosaccharide content. At 180 ˚C, the cellulose molecules were partially degraded and then the hemicelluloses were extracted [5,17]. Table 1 demonstrates that the hydrothermal treatment had provided various kinds of oligosaccharides, among which XOS was the most dominant. The total oligosaccharide contents in HRS and HRH was 4.92 g/100 g and 5.50 g/100 g, respectively. At high temperatures and pressure, the presence of hydronium ions was generated by water auto-ionization, which act as catalysts, resulting in the breakdown of the hydrogen bonds and the partial degradation of the polymers of the plant cell walls.

Table 1 Oligosaccharide Contents in HRS and HRH after Autoclaving at 180 ˚C for 10-30 min.

Sample	Oligosaccharides (g/100 g)	Time (min)		
		10	20	30
HRS	AOS	0.10±0.02[a]	0.14±0.02[a]	0.02±0.01[b]
	Gal-OS	0.30±0.03[a]	0.28±0.01[a]	0.23±0.03[b]
	Glc-OS	0.67±0.13[b]	0.75±0.11[a]	0.76±0.06[a]
	XOS	3.84±0.05[a]	2.68±0.08[b]	2.70±0.17[b]
	Total	4.92±0.23[a]	3.81±0.21[b]	3.72±0.28[c]
HRH	AOS	0.15±0.02[a]	ND[b]	ND[b]
	Gal-OS	0.29±0.02[a]	0.13±0.02[b]	ND[c]
	Glc-OS	0.54±0.03[a]	0.41±0.03[b]	0.20±0.03[c]
	XOS	4.52±0.08[a]	2.16±0.02[b]	0.80±0.06[c]
	Total	5.50±0.15[a]	2.70±0.07[b]	0.99±0.09[c]

[a-c]Means in a row without a common superscript letter differ ($P < 0.05$) as a factorial experiment in a completely randomized design and the Duncan's new multiple range test. ND = not detected, AOS = Arabinooligosaccharide, Gal-OS = Galactooligasaccharide, Glc-OS = Glucooligosaccharide, XOS = Xylooligosaccharide.

In addition, arabino-oligosaccharide (AOS) and galacto-oligosaccharide (Gal-OS) were found to be minor products in both hydrolysates due to having lesser amounts of arabinose and galactose content in their carbohydrate structure. According to previous studies, the structural carbohydrate of RS and RH are composed of 4 kinds of monosaccharides: glucose, xylose, arabinose, and galactose. The arabinoxylan content was reported at 11.0 and 11.5% with the Arabinose to xylose ratio (A/X) being 0.20 and 0.14, respectively [12]. Although, glucose was the major sugar found in plant polysaccharides, the content of Gluco-oligosaccharide (Glc-OS) after treatment was low because the temperature selected was not high enough to complete the hydrolyzation of the cellulose. It has been previously reported that the maximal yield of cellulose conversion was 87.9% at a temperature of 250 ˚C [18]. In this study, increasing the hydrolyzing time had affected production by significantly decreasing XOS content at 3.84, 2.68, and 2.70% of HRS and at 4.52, 2.16, and 0.80% of HRH at 10, 20, and 30 min, respectively. This evidence can be explained by the fact that a longer hydrolyzing time had completely degraded the RH and RS arabinoxylan and/or xylose oligomers into the xylose (Figure 1). The different xylose contents after the hydrothermal treatment could have resulted from the different structures of biomass.

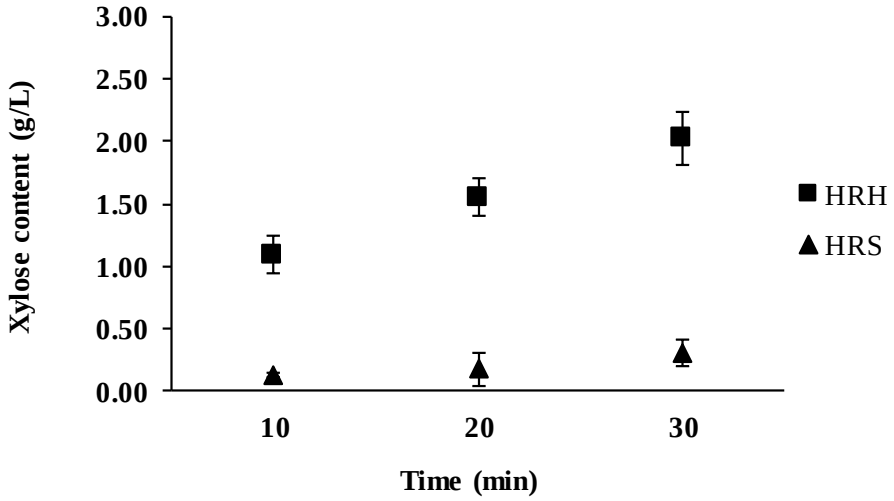
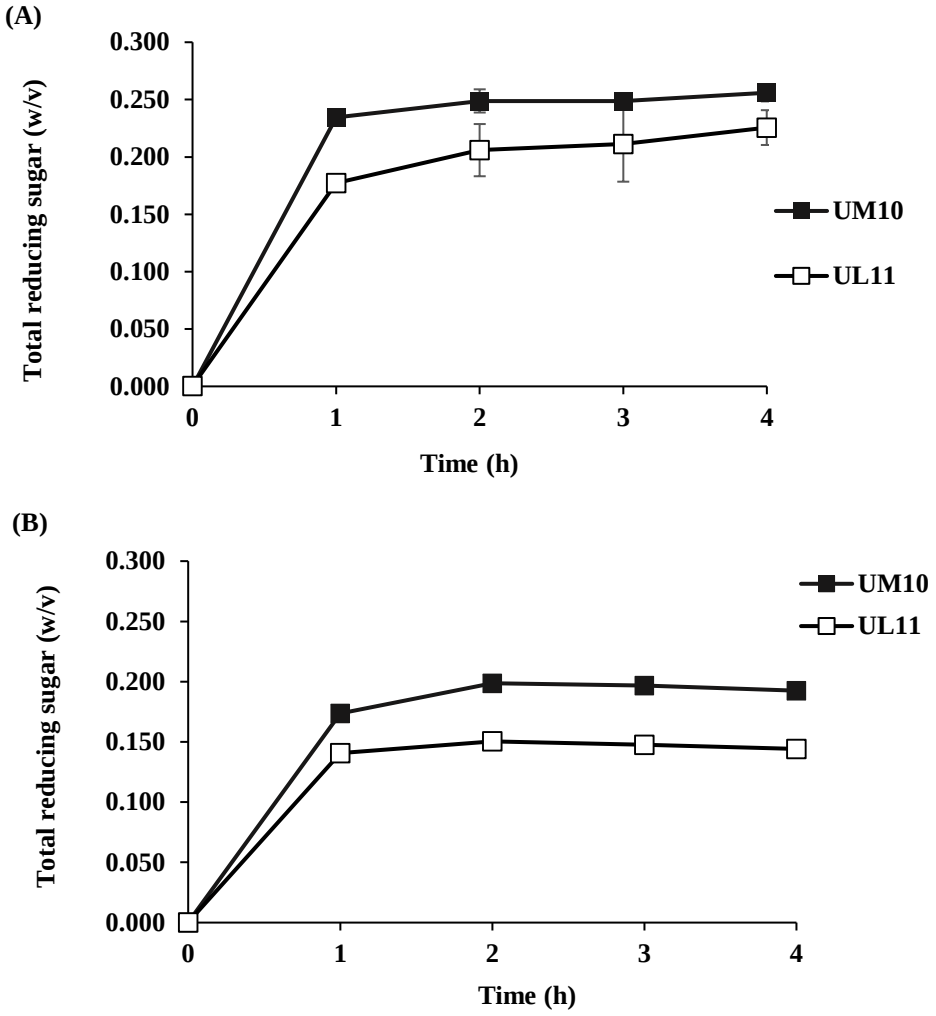


Figure 1 The xylose content at different times in HRS and HRH after autoclaving at 180 ˚C.

3.2 Modification of oligosaccharides from HRS and HRH with GH10 and GH11 commercial xylanases

Commercial xylanases, UM10 and UL11, at a concentration of 150 U/g of reducing sugar in 100 mM of Sodium phosphate buffer (pH 6) were used to hydrolyze HRS and HRH at 1% reducing sugar. Figure 2 demonstrates the total reducing sugar content at 50 ˚C during 0-4 h of incubation time. The reducing sugar content was dramatically increased at 1h. After that, increases in the incubation time were not found to affect the reducing sugar content. At 1 h of hydrolyzing time with UM10 and UL11, reducing sugar content of HRS increased up to 0.24 and 0.18%, respectively, while the HRH increased up to 0.17 and 0.14% (w/v), respectively.



[a-b]Means in each incubation time without a common superscript letter differ ($P < 0.05$) as factorial experiment in a completely randomized design and the Duncan's new multiple range test.

Figure 2 Total reducing sugar contents of (A) HRS and (B) HRH hydrolyzed by commercial xylanases (150 U/g, 50 ˚C, pH 6, 0-4 h).

An incubation time of longer than 1h was not found to be significantly different in reducing sugar content in both UM10 and UL11. This result could be due to the limited substrates in hydrolysates. However, the HRS and HRH profiles showed a degree of polymerization (DP) 1 to 7 for the xylose oligomers, for some branched xylose oligomers, and for arabino-xylooligosaccharides (AXOS) (data not shown). It might be concluded that the HRS and HRH substrate had been completely hydrolyzed to the products within 1 h of incubation time (Figure 2 and 3).

The oligosaccharides profile was primarily observed by TLC during 0-4 h of incubation, as compared to mixtures of xylose (X_1), xylobiose (X_2), xylotriose (X_3), xylotetraose (X_4) and xylopentaose (X_5) as standards (Figure 3). Before the incubation of HRS and HRH with xylanases, the profiles of oligosaccharides demonstrated a DP range from 1 to 5 and greater than 5. UM10 and UM11 had shown some differences in sugar profiles. UM10 seemed to greater hydrolyze a small DP (X_1, X_2, and X_3) more than UL11 in both HRS and HRH because the characteristics of this family are composed of the activity of the endo-1,4-β-xylanases and the endo-1,3-β-xylanases [8]. Members of this family are also capable of hydrolyzing the aryl β-glycosides and are highly active on short-chain XOS, thereby indicating small substrate-binding sites. In contrast, the GH11 family has high substrate selectivity, and high catalytic efficiency. Plus the family preferentially cleaves the unsubstituted regions of the arabinoxylan backbone. Therefore, a hydrolyzing time of 1h was chosen as the representative for confirming the sugar profile and the quantitative analysis by HPAEC-PAD.

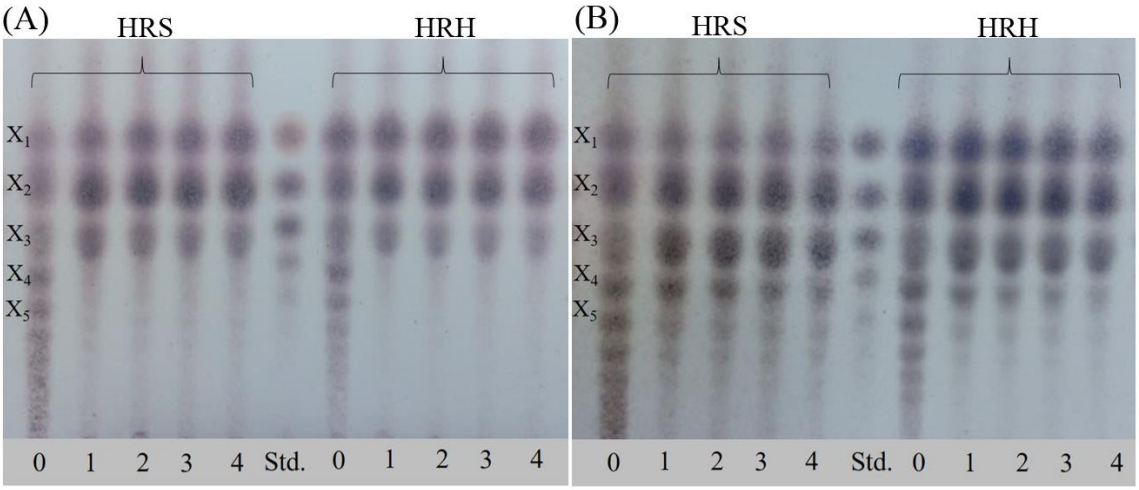


Figure 3 TLC chromatograms of HRS and HRH after being hydrolyzed with different commercial xylanases at 150 U/g of reducing sugar, at 50 ˚C, and at a pH of 6.0 for 0-4 h. (A) UM10 and (B) UL11. A mixture of xylose (X_1), xylobiose (X_2), xylotriose (X_3), xylotetraose (X_4), and xylopentaose (X_5) were used as the standard.

Table 2 shows the modified oligosaccharides of 1% HRS and HRH after hydrolyzed with UM10 and UL11 at 50 ˚C and at a pH of 6.0 for 1 h. The oligosaccharides of HRS and HRH were the same sugar profiles. Those consisted of X_2, X_3, X_4, and X_5 including some AXOS; 2^3-α-L-arabinofuranosyl-xylotriose (A_2XX); 3^2-α-L-arabinofuranosyl-xylobiose (A_3X); and 3^3-α-L-arabinofuranosyl-xylotetraose (XA_3XX).

Table 2 Modification of oligosaccharides from HRH and HRS with GH10 and GH11 commercial xylanases at 150 U/g of reducing sugar, at 50 ˚C and a pH of 6.0 for 1 h of incubation time.

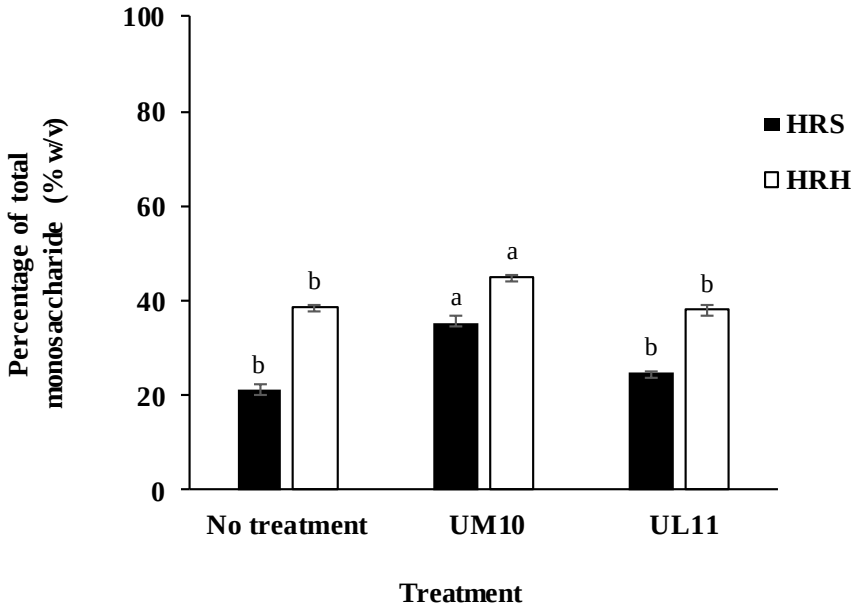
Sample	Oligosaccharides (g/100 g)	Treatments		
		Control	UM10	UL11
HRS	X_2	1.80±0.01^c	6.90±0.00^a	3.33±0.16^b
	X_3	2.16±0.01^c	2.95±0.03^b	5.05±0.06^a
	X_4	1.82±0.00^b	0.14±0.00^c	2.44±0.00^a
	X_5	1.64±0.01^a	ND	0.49±0.05^b
	X_6	1.28±0.01^a	0.11±0.00^b	0.09±0.00^b
	A_2XX	0.98±0.01^a	ND	0.03±0.00^b
	A_3X	0.54±0.00^a	0.14±0.00^b	0.12±0.00^b
	XA_3XX	0.69±0.02^a	0.18±0.01^c	0.27±0.05^b
HRH	X_2	2.55±0.08^c	5.71±0.03^a	3.81±0.10^b
	X_3	2.11±0.03^c	2.22±0.04^b	4.21±0.01^a
	X_4	1.71±0.03^a	ND	1.59±0.01^b
	X_5	1.20±0.02^a	ND	0.21±0.01^b
	X_6	0.93±0.03^a	ND	ND
	A_2XX	0.52±0.01^a	ND	ND
	A_3X	0.29±0.01^a	ND	0.12±0.04^b
	XA_3XX	0.28±0.05^a	ND	ND

$^{a-c}$Means in a row without a common superscript letter differ ($P < 0.05$) as a factorial experiment in a completely randomized design and the Duncan's new multiple range test. ND = not detected, X_2 = xylobiose, X_3 = xylotriose, X_4 = xylotetraose, X_5 = xylopentaose, X_6 = xylohexaose, A_2XX = 2^3-α-L-arabinofuranosyl-xylotriose, A_3X = 3^2-α-Larabinofuranosyl-xylobiose, XA_3XX = 3^3-α-Larabinofuranosyl-xylotetraose.

Notably, both of xylanases had effectively hydrolyzed the oligosaccharides (with DP values greater than 4) into small oligomers, such as X_2 and X_3. During hydrolysis, the X_2 content in the HRS had increased significantly from 1.80% to 6.90% and 3.33% while, the HRH had increased from 2.55% to 5.71% and 3.81% with UM10 and UL11, respectively. In addition, when compared to the control, the X_3 content had also increased in HRS and HRH at up to 2-3% after being hydrolyzed with UL11. Interestingly, the X_4 content in the HRS had significantly increased from 1.82% to 2.44% with UL11. Former studies conducted on wheat bran have reported that xylobiose had also been remarkably produced by GH10 endoxylanase, while GH11 had shown xylotriose as a major component [19]. However, the DP≥X_5 in both of hydrolysates had significantly decreased. This study showed the effectiveness of the XOS modification with the UM10 and UL11 commercial xylanases into X_2 and X_3. Many

researchers have reported that XOS with different DP had exhibited different prebiotic properties according to the independent sugar utilization of the target microorganisms. It also has been previously reported that most *Bifidobacterium lactis* strains HN019 had been able to utilize XOS with DP 2 greater than long chain XOS. However, *B. lactis* strains (Bb-12 and Bl-04) had preferred the long-chain XOS and had supported the growth at the same level as pure glucose [20].

Nonetheless, due to the side activities of the xylanases, the monosaccharide content is also of concern. Most of commercial xylanases contain many side activities of xylanase, such as α-arabinofuranosidase and β-xylosidase, which might produce arabinose and xylose during enzymatic hydrolysis.



[a-c]Means in each enzyme without a common superscript letter differ ($P < 0.05$) as factorial experiment in a completely randomized design and in the Duncan's new multiple range test.

Figure 4 The percentage of total monosaccharides in the HRS and HRH hydrolysate after being hydrolyzed by the commercial xylanases.

The results demonstrated that UM10 had significantly increased the content of the monosaccharides in HRS and HRH. Furthermore, UM10 had tended to produce xylose more than other sugars (data not shown). The product description of UM10 claims that it contains β-xylosidase, β-glucanase and α-L-arabinofuranosidase, which is able to cleave a branch chain and long-chain xylan from the reducing end and as a result, produce short-chain xylose oligomers, as well as xylose, arabinose, and glucose [21]. However, in order to eliminate the monosaccharides in hydrolysate, a purification process, such as using activated charcoal, nanofiltration, or yeast fermentation, would be appropriate for pharmaceutical products. Otherwise, undesirable monosaccharides would be absorbed in the upper gastrointestinal tract and would enhance the growth of pathogens in human microbiota [22].

4. Conclusion

The results from this study showed that RS and RH, which are both by-products of rice farming, can be used as alternative sources for oligosaccharide production. The enzymes GH10 and GH11 were able to produce xylooligosaccharide from these by-products showing different sugar profiles. However, the purification process should be performed to eliminate the monosaccharides in order to enhance the quality of the final oligosaccharide product from enzymatic hydrolysis. Currently, there are many pretreatment methods, including physical, chemical, physicochemical and biological methods. Pretreatment methods are aimed at increasing the enzyme accessibility to biomass and the yield of the product. Yet, a few more challenges still remain, such as understanding the structure of biomass cell walls, finding a suitable pretreatment method, and determining the extent of cell wall deconstruction in order to generate value-added products.

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