Oligosaccharides Preparation from Rice Bran Arabinoxylan by Two Different Commercial Endoxylanase Enzymes

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Summary

Xylooligosaccharides (XOS) and arabinoxylooligosaccharides (AXOS), potential prebiotics, can be produced from rice bran arabinoxylan (RBAX) using commercial endoxylanase enzymes. However, differences in rice bran cultivars and endoxylanase enzyme types may affect extracted oligosaccharides (OS) yields. This study investigated extracted OS structures derived from three different RBAX using two commercial endoxylanase enzymes. RBAX extracted from commercially defatted rice bran (CDRB) yielded the greatest OS amount (456.69 mg/g RBAX), followed by that of the San-Pah-Tawng1 (SPT1) cultivar (231.7 mg/g RBAX), and lastly, the Chai-Nat1 (CN1) cultivar (172.57 mg/g RBAX), as evidenced via Ultraflo L enzyme hydrolysis. Ultraflo Max enzyme hydrolysis produced a similar trend, however, OS amounts from all RBAX sources were remarkably lower (83.39 mg/g RBAX extracted from CDRB, 27.05 mg/g RBAX from SPT1 cultivar, and 21.53 mg/g RBAX from CN1 cultivar). Interestingly, 3'-α-L-Araf-(1-4)-β-D-xylobiose (A3X) was the primary AXOS product in all RBAX hydrolysates prepared by Ultraflo Max. RBAX extracted from CDRB solubilized by Ultraflo L (45.67% weight of RBAX) had higher OS yields than that obtained via Ultraflo Max (8.3% weight of RBAX). Ultraflo L was therefore a suitable commercial enzyme for short-chain OS conversion from RBAX.

Key Words rice bran, arabinoxylooligosaccharides, xylooligosaccharides, endoxylanase, arabinoxylan

Arabinoxylan oligosaccharides (AX-OS), consisting of xylooligosaccharides (XOS) and arabinoxylooligosaccharides (AXOS), are partial hydrolysis products of arabinoxylan (AX) with beneficial prebiotic properties (1). Enzymatic hydrolysis is potentially superior for AX-OS preparation, being more environmentally-friendly with fewer undesirable by-products compared to other chemical and physical methods (2). Glycoside hydrolase 10 (GH10) and glycoside hydrolase 11 (GH11) xylanases were preferable due to their endo-action on xylan backbone (3). Previous studies reveal influence and efficiency differences between GH10 and GH11 on wheat bran (4) and its alkali-extractable AX (3). However, xylanase impact research on rice bran arabinoxylan (RBAX) is limited. This study, therefore, focused on the influence and efficiency of two commercial GH10 and GH11 xylanases on alkali-extractable RBAX for AX-OS preparation. The composition of AX-OS obtained from RBAX was investigated by high-performance anion-exchange chromatography coupled with pulsed amperomeric detector (HPAEC-PAD) and yields of AX-OS were calculated. This information will be useful for rice bran AX-OS production.

Materials and Methods

Materials. Two cultivars of Thai rice (Oryza sativa L.) paddy, San-Pah-Tawng1 (SPT1) and Chai-Nat1 (CN1), were bought from the Rice Research Center, while commercially defatted rice bran (CDRB) was kindly provided by Thai Edible Oil Co., Ltd. All enzymes were kindly given by Novozymes ( Bagsvaerd, Denmark).

Preparation of alkaline soluble arabinoxylans from different rice bran cultivars. Alkaline-soluble AX preparation from rice bran followed that of the previous publication (5): n-hexane removed fat, both Termamyl 120 L and amyloglucosidase (AMG 300 L) hydrolyzed the starch, and Alcalase® 2.4 L eliminated protein from rice bran. The defatted, destarched and deproteinized rice bran was then delignified by 72% H2SO4 with NaClO2 and 0.5 m NaOH (at 40˚C for 6 h) was used for AX extraction (6). Lastly, a dialysis bag (Spectra Por, cut-off 3.5 kDa) purified the crude isolated AX, following the method described in (7). The collected supernatant was dried in a hot-air oven prior to enzyme hydrolysis.

Xylanase treatment for alkali-soluble AX. Isolated AX (3% w/v) was re-suspended in a sodium acetate buffer (25 mM) with a fixed volume of 10 mL. The designated pH values were obtained with 0.1 N HCl and/or 0.05 M NaOH. Under continuous stirring at 50˚C for 24 h, suspensions with Ultraflo L (4 wt%) and Ultraflo Max (3.3 wt%) were incubated at pH 6 and 4.5, respectively. Later, the hydrolysate was boiled for 15 min for enzyme hydrolysis.

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inactivation and centrifuged at 4,000 \times g for 30 min to separate supernatant from residue.

**Determination of oligosaccharides.** Supernatants were characterized by HPAEC-PAD following Rivière’s method (8). CarboPac PA-200 with guard column identified and quantified extracted AX-OS and Chromelon 6.7 (Thermo Scientific) provided system control and data analysis.

Gradients of 120 mM NaOAc in 100 mM NaOH, and 100 mM NaOH were used for AX-OS analysis with a total analysis time of 37.5 min. The external standard for oligosaccharides analysis was a mixture of arabino-nose (Ara), xylose (Xyl), and AXOS (Araβ-Araf-(1-4)β-d-xylotriose (A2XX), Aα-Ara-(1-4)-β-d-xylotetraose (A3XX), 23,33-di-α-L-Araf-(1-4)-β-d-xylohexaose (A3XX), and an XOS (Degree of polymerization (DP) 2-6) mixture.

**Statistical analysis.** All statistical analysis was performed using SPSS software (Version 21, IBM Corp., USA). Analysis of variance with Turkey HSD was used to compare means. A level of 0.05 was set to determine statistical significance of differences.

**Results**

From our previous work, the monosaccharide compositions of all isolated AX were analysed and arabinose/xylose (A/X) ratios were calculated to approximate their degree of branching. The A/X ratio of RBAX extracted from CN1 cultivar (1.09) was the highest, followed by that of CDRB (1.04), and SPT1 cultivar (0.76).

OS structures derived from all RBAX by Ultraflo L and Ultraflo Max were characterized by HPAEC-PAD.
after 24 h of incubation (Figs. 1 and 3). Peaks corre-
sponding to Ara, Xyl, Xylobose (X2), Xylotriose (X3),
Xylohexose (X6), 3,4-di-α-L-Ara-(1-4)-β-D-xylotriose (A2XX),
3,4-di-α-L-Ara-(1-4)-β-D-xylotetraose (A2XXX), 2,3,4-tri-α-L-
Ara-(1-4)-β-D-xylotriose (A2XXX), and 2,3,4-tri-α-L-
Ara-(1-4)-β-D-xylotriose (A2XXX) were clearly observed in all RBAX
hydrolysates. Ultraflo L primarily produced Ara, Xyl,
X2, and X3 and a relatively small amount of XOS DP 4-6 and AXOS. Moreover, there were some unidentified
peaks found in these chromatograms (Fig. 1).

AX from CDRB yielded the most OS content
(456.69 mg/g RBAX), followed by SPT1 (231.7 mg/g
RBAX) and CN1 (172.57 mg/g RBAX) (Fig. 2). Regard-
ing Ultraflo Max, CDRB hydrolysate also yielded the
most OS content (83.39 mg/g RBAX), followed by SPT1
(21.53 mg/g RBAX) and CN1 (21.53 mg/g RBAX)
(Fig. 4). Interestingly, the main OS product found in
Ultraflo Max hydrolysates was A2X (61.22 mg/g RBAX),
followed by SPT1 (19.54 mg/g RBAX), and CN1
(14.92 mg/g RBAX). OS yields obtained from RBAX
extracted from CDRB solubilized by Ultraflo L (45.67%
weight of RBAX) were higher than that obtained via
Ultraflo Max (8.3% weight of RBAX).

Discussion
The high A/X ratio indicates a greater degree of
branching and therefore a relatively higher solubility
(9). The A/X ratio of AX from SPT1 was lower than
those from CDRB and CN1, however, the CDRB hydro-
lysate contained the highest content of OS. The side
chains of AX may hinder active xylanase sites, thereby
affecting OS production. The different structures of the
arabinose-substituted/-unsubstituted xylan backbone
also affected OS yield. The Ultraflo L commercial enzyme
contains endoxylanase belonging to glycoside hydrolase
family (GHF) 11 and other accessory enzymes, including
cellulases, endoglucanases, feruloyl esterases, acetyl-
esterases and α-L-arabinofuranosidases. The Ultraflo L
mechanism on AX substrate may cause the predomi-
nance of Ara, Xyl, X2, and X3 in hydrolysate where α-L-
arabinofuranosidase (EC 3.2.1.55) and endoxylanase
(EC 3.2.1.8) activities catalyze the release of Ara, and
XOS (primarily X2), respectively (10). Several unknown
peaks were observed in all the HPAEC-PAD chromatograms
of RBAX in the detected AXOS regions. Other studies
(11, 12) indicate that the unknown peaks might represent
longer linear XOS chains, namely Xyloheptaose (X7), Xylooctaose (X8), Xylononaose (X9), and
Xylodecasose (X10), or other AXOS structures, such as
3,4-di-α-L-Araf-(1-4)-β-D-xylotriose (X1A3X), 3,4-di-α-L-
Araf-(1-4)-β-D-xylotriose (X12XX), 3,4-di-α-L-Araf-(1-4)-β-D-
xylotetraose (X12XXX) and 3,4-di-α-L-Araf-(1-4)-β-D-
xylotetraose (X12XX). The late eluted peaks after 2,3,4-
tri-α-L-Ara-(1-4)-β-D-xylotetraose (X2A2XXX) might be due
to more complicated AXOS structures, such as 2,3,4-tri-
α-L-Ara-(1-4)-β-D-xylotriose (X2A2XX) and
3,4,5-tri-α-L-Ara-(1-4)-β-D-xylotriose (X2A3XX).
Moreover, additional AXOS structures, for example
2,3,4-tri-α-L-Ara-(1-4)-β-D-xylotriose (X2A2XX) and
2,3,4,5,6-penta-α-L-Ara-(1-4)-β-D-xylotriose (X2A5XX),
have been identified after treating barley malt AX with
Ultraflo L (13).

The Ultraflo Max commercial enzyme is a mixture of
endoxylanase (GHF10) and β-glucanases. The differ-
ent accessory enzymes containing in Ultraflo Max and
hydrolysis mechanism of GHF 10 endoxylanase may
cause the lower amount of produced OS, compared to
Ultraflo L. The presence of A'X in all rice bran hydroly-
sates clearly revealed the GHF10 endoxylanase mecha-
nism. Similar AXOS structures were found in a study by
Mathew (3) including A'X, A'XX and A'2XX when wheat bran AX was hydrolyzed with GHF10 endoxyla-
nase. This also indicated that differences in substrate
structure might also affect the final products. Fur-
thermore, the high amount of arabinose and xylose
produced from RBAX hydrolysis might be due to the
side activities of both commercial enzymes. This could
release the branched chain of AX and enhance the con-
version efficiency of RBAX into OS by lowering the A/X
ratio to favor the enzyme breakdown on the xylan main
chain per the previous study (14).

In conclusion, OS amounts from RBAX solubilized by
Ultraflo L were higher than that of Ultraflo Max. Ultraflo
L enzyme is therefore considered a suitable commercial
enzyme for hydrolyzing RBAX into short-chain OS. The
amount and type of OS produced from RBAX depends
on the cultivar. RBAX extracted from commercially
defatted rice bran is the best source for OS production,
compared to those from SPT1 and CN1.

Disclosure of State of COI
No conflicts of interest to be declared.

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