Mini Review

Prebiotic Oligosaccharides Prepared by Enzymatic Degradation of Dietary Fibers in Rice Grains

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Summary Rice are consumed mainly as polished rice grains. In the threshing and polishing processes of paddy rice, a considerable amount of husk and bran are separated as by-products. Rice bran is utilized for oil production, whereas rice husk as well as straw is not fully utilized. Defatted rice bran is rich in proteins and non-digestible polysaccharides, while husk and straw consist mainly of plant cell wall components, including cellulose, hemicellulose and pectin. Such non-digestible polysaccharides function in gastrointestinal lumen as dietary fiber, though physiological functions and their application are limited. Non-digestible oligosaccharides have recently been interested as prebiotics from a viewpoint of health benefit via utilization by intestinal microbiota. A diversity of non-digestible polysaccharides in rice bran and husk are good and ecological sources for production of both prebiotic and potentially prebiotic oligosaccharides. In this review, we summarize non-digestible polysaccharides constituting cell wall of rice grains including husk and degradation of the polysaccharides into oligo- and monosaccharides by microbial glycoside hydrolases. Prebiotic potential of such oligosaccharides derived from rice non-digestible polysaccharides are also introduced. Finally, our recent attempt for effective production of cello-oligosaccharides by regulated enzymatic degradation is briefly described.

Key Words prebiotic oligosaccharides, cellulose, xylan, dietary fiber, rice grain

Rice grains are consumed mainly as polished rice. Rice bran as a by-product from rice polishing is utilized for food oil production. The defatted rice bran as a by-product from oil extraction is not fully utilized for food, though it is used in part as feed for domestic animals and farmed fish. Moreover, rice husk, which is an abundant by-product from grain threshing, is little utilized and disposed. Basic components of defatted rice bran are non-digestible polysaccharides and proteins, while those of husk are non-digestible polysaccharides and silicic acids. The non-digestible polysaccharides in plant food are mainly from cell wall components, cellulose, hemicellulose and pectin. These non-digestible polysaccharides are insoluble or poorly soluble and therefore non-digestible or poorly digestible for intestinal microbes, even though the microbes have polysaccharide-hydrolytic enzymes. Thus, non-digestible and insoluble polysaccharides in plant food are poorly prebiotic to intestinal microbes. On the other hand, some oligosaccharides produced by enzymatic degradation from such non-digestible polysaccharides are more soluble and reach the hindgut, and can be utilized by some intestinal microbes. Such non-digestible oligosaccharides are promising prebiotics to intestinal microbiota good for health. We briefly outline non-digestible polysaccharides in rice grains and possible prebiotic oligosaccharides that could be produced by partial or restricted enzymatic hydrolysis of the non-digestible polysaccharides.

1. Non-Digestible Polysaccharides of Rice Grains: Polished Rice, Rice Bran and Rice Husk

Paddy rice grains are separated by threshing into brown rice and husk, which account for about 80% and 20% of a paddy grain by weight, respectively. Brown rice is polished to remove outer brown layer termed aleurone layer, and resultant polished rice accounts for about 70% of paddy rice by weight. Rice bran as a by-product from brown rice polishing accounts for no less than 10% of paddy rice grains. Non-digestible polysaccharides of plant food are mainly from cell wall components, cellulose, hemicellulose and pectin. Cellulose is a crystalline linear polymer and hemicellulose is amorphous and mostly branched chains. Pectin that is a minor component of rice grains unlike fruits is present with lignin that is phenolic polymer responsible for formation of woody plant tissue. Non-digestible polysaccharides in rice bran as well as polished rice are cellulose, hemicellulose and pectin, while rice husk polysaccharides are mainly cellulose and xylan that is a xylose-backbone
polymer with substituted arabinosyl, acetyl and glucuronic groups of xylose hydroxy groups. Polished rice grains contain about 77% of carbohydrate, most of which is starch accumulated in an endosperm cell organelle, amyloplast. In addition, relatively much smaller amount of non-digestible polysaccharides is present also as cell wall components of starchy endosperm cells. The endosperm cell walls contain about 40% glucuronoarabinoxylan GAX and arabinoxylan AX, 10% pectin, 15% glucomannan, and a trace of xyloglucan (1, 2). Chemical structures of typical cellulose, GAX and AX, glucomannan and xyloglucan are shown in Fig. 1A–E.

Rice bran (aleurone layer and germ) contains about 25–45% carbohydrate, depending on polishing methods and rice variety. Major components of non-digestible polysaccharides in rice bran are also cell wall components, including cellulose, hemicellulose, pectin and lignin. Mono-saccharides constituting rice bran polysaccharides including starch were determined by GC to be glucose (65%), galactose (12%), arabinose (8%), xylose (10%) and mannose (4%) (3). Non-starch polysaccharides of rice bran were reported to be glucose (32%), galactose (31%), xylose (27%), arabinose (9%) and rhamnose/fucose (1%) (4). Thus, diverse structures of matrix polysaccharides including arabinoxylan, mannan, xyloglucan are present in rice bran preparations.

Major polysaccharides constituting the cell wall of rice straw and husk are cellulose, hemicellulose and lignin (5, 6). Hemicellulose includes GAX, and mixed-linkage glucan (MLG) with β-1,3- and β-1,4-linked glucosyl residues. Cellulose forms fibrous network structure and called load-bearing wall of plant tissue cell wall, while GAX and MLG are matrix polysaccharides of the cell wall. Cellulose and GAX account for about 30% and 20% of the cell wall, respectively (5). MLG accounts for 10–20% in cell walls (7). Chemical structure of typical MLG is shown in Fig. 1F.

2. Oligosaccharides Enzymatically Produced from Rice Non-Digestible Polysaccharides

In general, oligosaccharides are relatively more soluble than polysaccharides because of their smaller degree of polymerization and molecular mass and, thereby, oligosaccharides can be attacked as substrates and degraded by glycosidases more easily than polysaccharide fibers. Moreover, some intestinal bacteria can directly absorb some non-digestible di- and tri-saccharides into the cell and utilize them metabolically as an energy source and, thus, more prebiotic than original non-digestible polysaccharides to intestinal microbiota (8). Production of prebiotic oligosaccharides from various non-digestible polysaccharides of rice grains seems to be beneficial and important from ecological and health-promotion points.
of view. Functional enhancement and effective utilization of paddy rice whole grains would add value of rice as a staple food in the world, especially Southeast Asia and West Africa.

In nature, plant cell wall including woody plants is degraded enzymatically by glycoside hydrolases (GH) and polysaccharide lyases (PL) secreted from various microbes, especially those belonging to a kingdom fungi (9). A total of more than 100 GH gene families have been identified, and only 22 among them can degrade plant cell wall polysaccharides. Interestingly, 20 of the 22 GH gene families responsible for plant cell wall degradation are from filamentous fungi (mold) (10). These GH gene families for the cell wall degradation are cellulases of both endo- and exo-types, hemicellulases, debiranching enzymes and glucosidases (10). To produce non-digestible oligosaccharides with prebiotic potential, not such final monosaccharides as glucose, appropriate order and/or balance of enzyme reactions are required, because oligosaccharides once produced are intermediates of polysaccharide degradation process and, therefore, gradually degraded to monosaccharides.

Cellulose is degraded by three types of cellulases. One is an endo-type cellulase, a variety of endoglucanases, GH5 to GH9, GH12, GH44, GH45, GH48, GH51, GH61, and GH124, which cleaves internal glycoside linkages randomly at amorphous sites of cellulose microfibrils, resulting in release of glucose as well as cellobiose internal linkages of cellulose molecules.

Hemicellulose as a major component of cell wall matrix includes a variety of linear and branched polysaccharides as described above and, therefore, more and diverse glycoside hydrolytic enzymes are required. These enzymes are xylanases, arabinofuranosidases, and mannanases.

Table 1. Enzymatic properties and specific substrates for the cellulose- and hemicellulose-degradation enzymes from fungi*.

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Glycoside hydrolase family (CAZy database)</th>
<th>Substrate</th>
<th>Cleavage site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>cellbiohydrolases</td>
<td>GH6 and GH7</td>
<td>cellulose</td>
</tr>
<tr>
<td>B</td>
<td>β-1,4-endoglucanases</td>
<td>GH5 to GH9, GH12, GH44, GH45, GH48, GH51, GH61, and GH124</td>
<td>cellulose</td>
</tr>
<tr>
<td>C</td>
<td>β-glucosidases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemicellulases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Xylanases</td>
<td>GH10 and GH11</td>
<td>xylan homopolymer</td>
</tr>
<tr>
<td>2</td>
<td>Arabinofuranosidases</td>
<td>GH3, GH51, GH54 and GH62</td>
<td>arabinoxylan, arabinan, other arabinose-containing hemicelluloses</td>
</tr>
<tr>
<td>3</td>
<td>α-Glucuronidases</td>
<td>GH67 and GH115</td>
<td>glucoronoxylan</td>
</tr>
<tr>
<td>4</td>
<td>Xyloglucanases</td>
<td>GH12 and GH74</td>
<td>xyloglucan</td>
</tr>
<tr>
<td>5</td>
<td>Arabinanases</td>
<td>GH43 and GH93</td>
<td>arabinan</td>
</tr>
<tr>
<td>6</td>
<td>Mannosidases or mannanases</td>
<td>GH2, GH5, and GH26</td>
<td>mannan</td>
</tr>
<tr>
<td>7</td>
<td>Acetyl xylan esterases</td>
<td>CE1, CE3, CH4, CE5, CE7, and CE16</td>
<td>(O-acylated) xylan</td>
</tr>
<tr>
<td>8</td>
<td>Feruloyl esterases</td>
<td>CE1</td>
<td>polysaccharides esterified with phenolic acid</td>
</tr>
<tr>
<td>9</td>
<td>Xylosidases</td>
<td>GH3 and GH43</td>
<td>xyloligosomers</td>
</tr>
<tr>
<td>10</td>
<td>β-1,3-exo-endoglucanases</td>
<td>GH55</td>
<td>β-1,3-glucans</td>
</tr>
</tbody>
</table>

*This table is prepared according to the reference (9).
α-glucuronidases, xylglucanases, arabinanases, mannosidases/mannanases, acetyl xylan esterases, furfuryl esterases and xylosidases (9). Contribution of each enzyme to polysaccharide degradation depends on cell wall composition specific for plant variety and plant tissues. Enzymatic properties and specific substrates for these hemicellulose degradation enzymes are summarized in Table 1.

3. Prebiotic Potential of Oligosaccharides Derived from Rice Non-Digestible Polysaccharides

Prebiotic potential of disaccharides as analyzed by some animal feeding models and diet intervention trials. In a intervention trial an energy-restricted diet supplemented with each of rice husk powder, rice bran and negative control was given to the overweight and obese adults (n=35 per group) for 12 wk (11). Although significant reduction in body weight, BMI and waist circumference observed in all three groups showed no statistical difference between groups, reduction in two serum inflammatory markers, serum levels of IL-6 and hs-CRP (high sensitive C-reactive protein), for tissue inflammation and injury and bacterial infection were significantly (p<0.05) higher in the rice husk powder and rice bran groups than that of control group. Non-digestible polysaccharides of rice husk and bran, and their degradation products such as short chain polysaccharides and oligosaccharides may affect the microbiota via prebiotic effect and may indirectly reduce and improve diet and/or obese-induced inflammation in colon and adipose tissue, though detailed mechanisms remain unknown.

The term, prebiotics, are used generally for carbohydrates with relatively short chain length that can escape from intestinal digestion and absorption, reach the cecum and colon and be utilized as carbon and energy sources for production of beneficial metabolites such as short chain fatty acids and organic acids (12). Utilization efficiency of nondigestible poly- and oligosaccharides for anaerobic fermentation by intestinal microbes could be evaluated by in vitro anaerobic culture systems using human fecal bacteria. Among several strains of *Bifidobacterium*, *Clostridium*, *Bacteroides*, and *Lactobacillus*, few could ferment arabininoxylan, while many strains fermented xynulo-oligosaccharide well (12). In general, linear oligosaccharides are utilized more effectively than branched ones, and *Bifidobacteria* utilized polysaccharides with low-degree of polymerization first (12). Therefore, disaccharides from rice non-digestible polysaccharides, such as cello-oligosaccharides, xylo-oligosaccharides, and manno-oligosaccharides are promising prebiotic oligosaccharides, that could be enzymatically produced from rice grain non-digestible polysaccharides. In fact, cellobiose enhanced growth of some beneficial intestinal microbes, including *Bifidobacterium* and *Lactobacillus*, and induced higher production of butyric acid (13). Moreover, feeding mice cellobiose-containing diet facilitated the production of the cecal short-chain fatty acids and the circadian clock phase adjustment, suggesting that ingestion of cellobiose modulates food-induced peripheral clock entrainment (14). On the other hand, purified xylo-oligosaccharides, which are partially O-acetylated typical oligosaccharides derived from the methylgucluroxylan-type hemicelluloses of rice husk, could be well fermented by human fecal bacteria, resulting in the production of short chain fatty acids in vitro (15). In another recent study using commercially available xylo-oligosaccharides, which are mixture of xylobiose (33%), xylotriose (29%), xylotetraose (17%), xylopentose (9%), xylohexose (10%), a few species of lactic acid bacteria, including *Weissella confusa* and *Lactobacillus johnsonii* were shown to grow well in the media containing the xylo-oligosaccharides (16).

4. Cello-Oligosaccharide Production from Cellulose Using Fungal Cellulolytic Enzymes

For effective production of cello-oligosaccharides and xylo-oligosaccharides from bran, husk and straw of rice plants, we have been studying on cellulolytic and xylanolytic enzymes from filamentous fungi. Promising fungal lines with cellulolytic activity were screened and isolated from rice straw waste. Some lines secreting enzymes with high cellulolytic and xylanolytic activities were identified by sequence analysis of 16S rRNA D1/D2 region to be *Aspergillus* and *Penicillium* species. These fungal lines secreted cellobiohydrolases predominantly among secreted proteins in a modified culture medium. Cellulolytic activity of the crude enzymes secreted from the fungal lines was evaluated using carboxymethyl cellulose (CMC), crystalline cellulose and rice straw powder as substrates. After chemical derivatization of enzymatic degradation products, both of cellobiose and cellotriose were identified in the enzyme-CMC reaction mixture using an LC-MS/MS system. By the enzyme reaction, cellotriose was produced more than cellobiose from CMC, whereas the same level of cellobiose but only a little cellotriose was produced from crystalline cellulose under the same condition. Cellobiose was produced also from rice-straw powder substrate, even though productivity was fewer than half of that of crystalline cellulose. These results suggest that under appropriate conditions cello-oligosaccharides could be produced effectively from rice straw, and husk as well, using enzymes from selected fungal lines and utilized as prebiotic food supplements.

Disclosure of State of COI

No conflicts of interest to be declared.

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