ISSN: 2347-467X, Vol. 12, No. (3) 2024, Pg. 1433-1444

Current Research in Nutrition and Food Science

www.foodandnutritionjournal.org

Isolation and Purifying of Xylooligosaccharides from various Mongolian Food Processing by-Products

ODGEREL CHINBAT[1], PUREVDULAM ERDENETSOG[1], BATTOGTOKH CHIMEDDORJ[2], MUNKHJARGAL BURENJARGAL[3]* and MUNKHTSETSEG JANLAV[1]

[1]Department of Biochemistry, School of Biomedicine, Mongolian National University of Medical Sciences, Ulaanbaatar, Mongolia.
[2]Department of Microbiology, Infection Prevention and Control, School of Biomedicine, Mongolian National University of Medical Sciences, Ulaanbaatar, Mongolia.
[3]Department of Chemistry, School of Arts and Sciences, National University of Mongolia, Ulaanbaatar, Mongolia.

Abstract

Xylooligosaccharides (XOS) are hemicellulose constituents that naturally occur in wheat bran peels and brewery-spent grain. XOS comprises two to six xylose residues connected by β-(1,4) linkages. These compounds are vulnerable to enzymatic breakdown by gut microorganisms. In this study, some industrial by-products, specifically wheat bran peels (WBP) and brewers' spent grain (BSG), underwent treatment using microwave-facilitated enzymatic hydrolysis. The current study produced 8.1 g of purified dried XOS from 100 g of WBP material and 7.2 g of pure dried XOS from 100 g of BSG material. Singular microwave irradiation of WBP and BSG before enzymatic hydrolysis, along with a precisely determined enzyme-to-raw-material ratio of 0.25 g/100 g, resulted in optimal conditions. This approach significantly enhanced the efficacy of xylanases on both WBP and BSG. Results of our study proved that microwave assisted pretreatment improves the xylanase enzymatic hydrolysis which increase yield of XOS.

Article History
Received: 19 September 2024
Accepted: 05 November 2024

Keywords
By-Product;
Enzymatic Hydrolysis;
Prebiotic, Xylanase;
Xylooligosaccharides;
Brewer's Spent Grain;
Wheat Bran Peels.

Introduction

Wheat bran peel (WBP) and brewers' spent grain (BSG) represent the primary by-products arising from the wheat milling and beer manufacturing industries, respectively.[1, 2] These residues find common use as livestock feed. Globally, the annual production of WBP and BSG is estimated at approximately 90 million tons and 39 million tons,

CONTACT Munkhjargal Burenjargal munkhtsetseg.j@mnums.edu.mn Department of Chemistry, School of Arts and Sciences, National University of Mongolia, Ulaanbaatar, Mongolia.

Doi: https://dx.doi.org/10.12944/CRNFSJ.12.3.35

respectively. Wheat bran peel (WBP) is a residual by-product arising from the wheat processing industry during flour production.[1, 3, 4] Brewers' spent grain (BSG) stands out as a prolific waste by-product resulting from the beer fermentation process essentially the remnants left behind after brewing. Due to the relatively low economic value of these by-products, recent research has concentrated on converting them into innovative food products with higher commercial value and improved health benefits.[5, 6] Recent advancements in the use of biopolymers for tissue engineering and drug delivery highlight their ecological benefits and biomedical efficacy.[7, 8]

WBP contains 5.9-6.8% fats, 15-20% proteins, 11-23% starch, and 43-53% dietary fiber, while BSG contains 5.8-6.0% of lipid, 15.3-16.2% protein, 6.4-7.0% of moist and 46% dietary fiber.[5, 9] The major insoluble fiber WBP contains lignin (5-20%), cellulose (16-30%), arabinoxylan (38-55%), and other nonstarch polysaccharides. Researchers have shown particular interest in the production of xylooligosaccharides (XOS) from xylan, which can specifically promote the proliferation of probiotic bifidogenic and lactic acid bacteria inhabiting the human gut.[3, 10] XOS are defined as oligomers composed of 2-10 xylose residues and selectively fermented.

They promote the growth of a restricted quantity of gut microbiota species, providing health benefits to the host, and are produced through the hydrolysis of arabinoxylan. There are three primary methods for extracting XOS: (1) enzymatic hydrolysis of xylan-containing sources; (2) Chemical fractionation to extract xylan from appropriate sources, succeeded by enzymatic hydrolysis to yield XOS; and (3) hydrolytic destruction of xylan utilizing steam or diluted mineral acid and alkali solutions.[10, 11]

Before enzymatic hydrolysis, plant materials undergo essential pretreatment steps, including grinding, soaking in an appropriate solution, and microwave irradiation. Microwave-facilitated separation serves as a possible pretreatment method, particularly for material extraction. Microwaves react with unbound molecules of water in the system, ultimately releasing active compounds from the plant material. In this WBP and BSG are subjected to microwave irradiation to release xylooligosaccharides (XOS).[11, 12] This research is significant because it introduces a cost-effective method for processing and purifying biologically active XOS from by-product materials, facilitating substantial waste recycling in Mongolia. The authors argue that microwave-facilitated hydrolysis for XOS extraction is an economical process. However, it is important to note that large-scale implementation would require a much larger system. This study focused on extracting XOS from industrial by-products, specifically WBP and BSG, using microwave-assisted hydrolysis by enzymes.

Materials and Methods

Methodology

This investigation followed strict protocols within a controlled laboratory setting. All experiments were rigorously conducted three times, and the resultant results were presented as mean values with associated standard deviations.

Materials

During the experimental phase, two distinct sample types were collected. The first sample consisted of wheat bran peels (unprocessed waste), which constitute the primary residue discarded during wheat bran processing at flour mills. The second sample originated from brewery's spent grain (chemically treated debris), typically disposed of after the brewing process. Our laboratory experiments aimed to effectively separate xylooligosaccharides (XOS) from arabinoxylan-rich hemicellulose-rich organic waste.

WBP were procured from the local mill company, Altantaria Co., Ltd., while dried BSG was obtained from the local beverage brewing company, APU Co., Ltd. These raw materials underwent thorough washing use distilled water and subsequent drying at 45°C in an oven operates for a duration of two days. The dried WBP and BSG were further processed using an electromechanical mill and filtered using a 45-mesh sieve, resulting in fine powder. This powder was maintained at room temperature for subsequent experiments.

For enzymatic hydrolysis, commercialized xylanase (≥2500 units/g, recombinant, taken in *Aspergillus oryzae*) was sourced from Sigma-Aldrich Co. (USA). Additionally, Xylobiose (X2), xylotriose (X3), and xylotetrose (X4), used as standards, were provided

by MedChemExpress Co., Ltd. (USA). Chemical reagents, including 1-phenyl-3-methyl-5-pyrazolone (PMP), HPLC-grade sodium hydroxide, and HPLC-grade acetonitrile, were procured from Sigma-Aldrich (USA) and Merck KGaA (Germany), respectively.[13, 14]

Chemical Analysis

The WBP and BSG protein amount was as certained using the Kjeldahl method, and lipid content was quantified by weight, obtained utilizing the Soxhlet method. Moisture content was at 105 °C in the drying oven for 2h, and mineral content was determined after the samples were incinerated at 650 °C for four hours. The total carbohydrate in the oligosaccharides was estimated by the phenol-sulfuric acid method. Briefly, a 50 ml sample of WBP and BSG was placed in a test tube; phenol (5%,0.3 ml) and concentrated sulfuric acid (1.8 ml) was included and blended comprehensively to increase the adjust the sample volume to 500 ml. The mixture was allowed to cool to room temperature for 15 minutes. The solution's absorbance was subsequently measured at 540 nm. The sugar content was determined by referencing a graph, using D-xylose (5 µg/50 ml) as a standard.

Microwave-Assisted Enzymatic Hydrolysis

The synthesis of xylooligosaccharides was achieved through repeated microwave-assisted enzymatic hydrolysis following the established technique.[10] WBP and BSG powders (50 g) were soaked in distilled water (100 mL) and then mixed in a 0.6 L heat-resistant and microwave-safe Pyrex flask (Guandong, ROC). The powders were subsequently subjected to microwave radiation in a microwave oven (1400W power, 0.9 cubic feet, Galanz RMW 1480, China) at 200 ºC for 5 minutes and then allowed to cool to ambient temperature, after which distilled water was incorporated to create a 10% slurry. Subsequent to microwave irradiation, the slurry was allowed to cool to ambient temperature, and distilled water was incorporated to achieve an H_2O to slurry solution ratio of 10:1 (v/w). Xylanase (≥2500 units/g, recombinant, expressed in *Aspergillus oryzae*; Sigma-Aldrich) was added at rates of 0.062 g /100 g substrate, 0.125 g/ 100 g substrate, 0.25 g/ 100 g substrate, 0.5 g/ 100 g substrate, 0.75 g /100 g substrate and 1 g / 100 g substrate, and mixed for 30 min at 50°C with continuous mild stirring. The enzymatic reactions were carried out in a 55ºC water bath with an orbital shaker, set to 200 rpm, for 24 hours.[15] To evaluate the hydrolysis, reducing sugars in the supernatant were determined with dinitrosalicylic acid (DNS).[16] After immersing the mixture in heated water for 5 min, the enzymatic reaction was stopped and the mixture was centrifuged at 9,000 rpm for 10 minutes, and reducing sugars were quantified by DNS with D-xylose as the standard.[16]

Isolation of xylooligosaccharides

The supernatant of the enzymatic reaction solution was isolated via centrifuged at 9000 rpm for 10 min. XOS was purified using the adsorption of activated carbon method. Briefly, activated carbon powder was included into the supernatant at a final concentration of 10% (w/w) and incubated at ambient temperature on a shaker at 200 rpm for 30 minutes. Final mixtures were filtered twice through a 0.45µm mixed cellulose ester filter (SciLab, Korea) and rinsed with deionized water. Oligosaccharides absorbed onto activated carbons were eluted with 50% ethanol and freeze-dried.

Scanning Electron Microscopy

Electron microscopy was employed to confirm the damage to the hydrolyzed sample's cell wall. The samples were dried, ground into a powder, fixed onto an aluminium stub, and sputter-coated with gold to enhance conductivity. They were then scanned using an electron microscope (Hitachi TM-1000, Tokyo, Japan).

Fourier Transform Infrared Spectroscopy (FT-IR)

Using infrared spectroscopy, structural units and functional groups can be determined directly, regardless of the state or size of the substance. The Vjorkman technique was utilized. FT-IR spectra of oligosaccharide samples were acquired with an FT-IR spectrophotometer (Bruker Alpha) utilizing a potassium bromide disc containing 1% of finely pulverized specimens. A total of 32 scans were conducted for each sample, recorded from 4,000 to 400 cm^{-1} at a resolution of 2 cm^{-1} in transmission mode. Then, the FT-IR spectra of the samples were compared with the xylotetrose standard.[17]

High-Performance Liquid Chromatography (HPLC)

The PMP derivatization was conducted as previously outlined by Pu in 2016.[14] In summary, 100 µL of a 5 mg/mL solution of the XOS sample or a standard mixture of XOS (X2 to X4, each at 5 mg/mL)

was combined with 100 µL of 0.3 mol/L sodium hydroxide. To a tiny sample tube, 120 µL of a 0.5 mol/L methanolic solution of PMP was introduced and vigorously mixed using a vortex mixer. The entire mixture was heated to 70˚C and incubated for 1 hour, then neutralized with 100 µL of 0.3 mol/L hydrochloric acid after cooling to room temperature. The resulting solution was extracted thrice with 500 µL of chloroform each time. The chloroform layer was eliminated, and the aqueous layer was subjected to filtration using a 0.22 µm membrane filter prior to HPLC analysis. The HPLC system (Agilent 1260, USA) features a quaternary pump, an autosampler, a column oven, and a diode-array detector (Agilent, USA). The data were obtained using an Open LAB CDS Chemstation Edition (version C.01.05) supplied by Agilent Technologies (USA). A Zorbax NH2 column (4.6 × 150 mm, 5 µm), acquired from Agilent Technologies (USA), was employed at 30˚C for HPLC analysis. The PMP derivatives were eluted with a 20:80 (v/v) mixture of acetonitrile (A) and 10 mmol/L ammonium acetate buffer (B, pH 5.5) at a flow rate of 1.0 mL/min. The effluent's UV absorbance was measured at 245 nm.

Results

Compositions of Wheat Bran Peel (WBP) and Brewers' Spent Grain (BSG)

As illustrated in Table 1, the compositions of WBP and BSG were analyzed and compared. The results indicate that WBP has a higher dietary fiber content, whereas BSG contains more protein and lipids.

Table 1. Chemical composition in 100 g of WBP and BSG samples

Chemical composition	WBP sample (%)	BSG sample (%)
Moisture	5.98±0.12	7.64±0.09
Protein	7.38±0.43	15.75±0.37
Lipid	0.4±0.05	4.81±0.02
Ash	18.62±0.64	4.25±0.57

The findings indicated that wheat possessed a moisture percentage of 5.98%, protein content of 7.38%, oil content of 0.41%, and mineral content of 18.62%. For BSG, the moisture content was 7.64%, protein content was 15.75%, oil content was 4.81%, and mineral content was 4.25%. The total carbohydrate content was estimated to be 67.61% in WBPs and 67.55% in BSGs, with the samples containing between 67.55% and 67.61% carbohydrates.

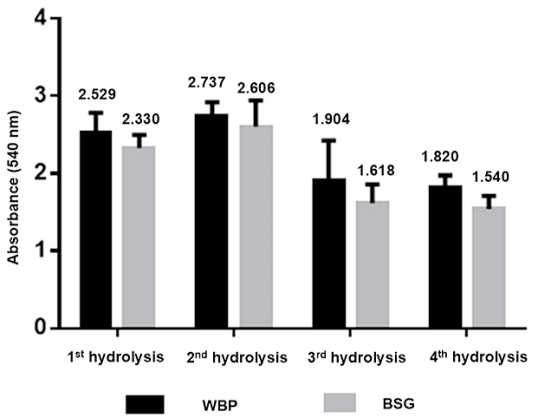

Fig. 1. The efficacy of recurrent microwave-assisted enzymatic hydrolysis. Black column indicates WBP, gray column BSG. The absorbance was measured against the reagent blank at 540 nm

Microwave Pretreatment of WBP and BSG Increases Reducing Sugar Yield with Enzymatic Hydrolysis

For the successful production of xylooligosaccharides from WBP and BSG, hydrolyzing enzyme (xylanase) must effectively reach the xylan through a cellulose and lignin barriers. An effect of microwave pretreatment on enzymatic hydrolysis was demonstrated, as it increased the effectiveness of enzymatic hydrolysis.[12, 18] In this study, we attempted to apply microwave pretreatment to the xylanase action of WBP and BSG. XOS production from microwave-pretreated WBP and BSG with xylanase was characterized by an increased reduction in sugars. Figure 1 shows the effect of repeated microwave pretreatment on reducing sugar production. The greatest amount of sugar production reduction in both samples occurred with

the second hydrolysis, and the lowest was with the fourth hydrolysis.

We established that microwave pretreatment alone enhanced enzymatic hydrolysis; yet, it seemed that a singular pretreatment did not effectively increase the hydrolysis yield. The decreased sugar assay, in conjunction with scanning electron microscopy observations (Figure 2), validated the efficacy of a singular pretreatment compared to many pretreatments. Figure 2 illustrates the impact of one and four microwave treatments on WBP and BSG.

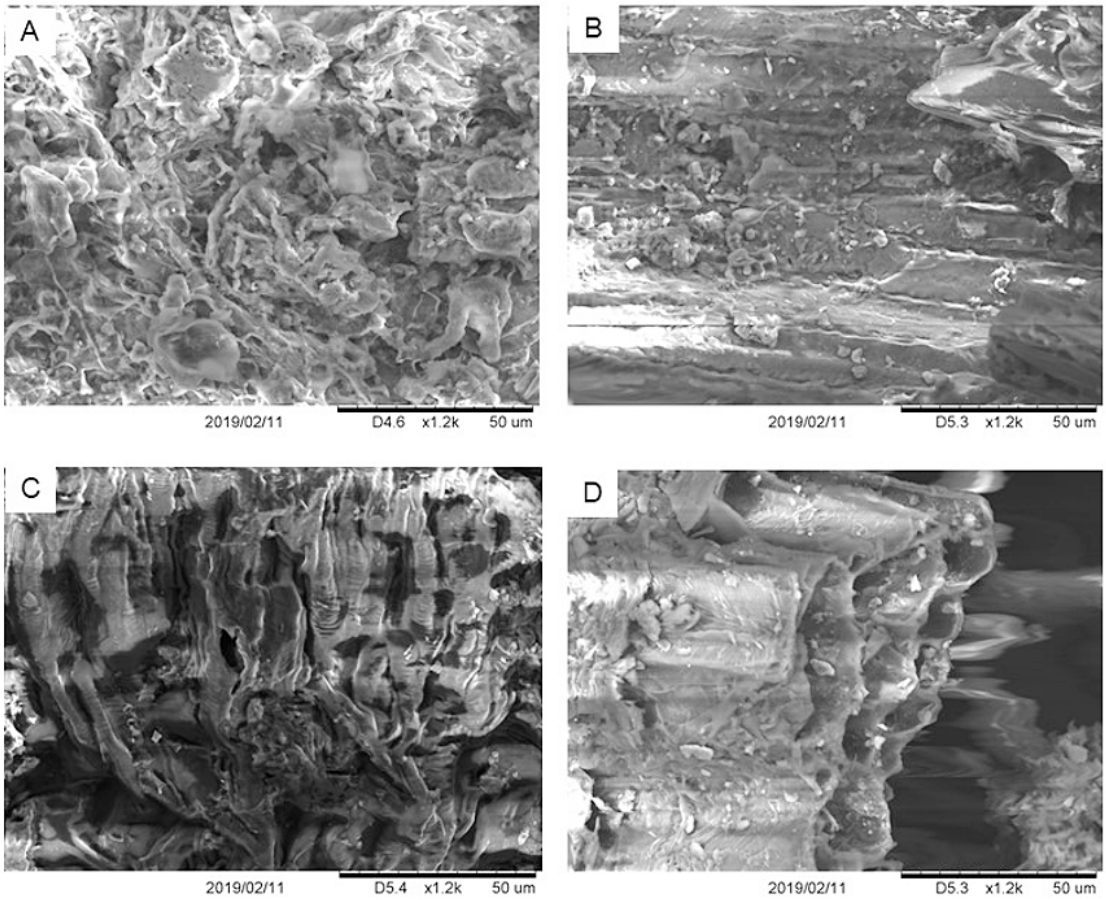

Fig. 2. Scanning electron micrographs of the original WBP (A) at 1200× and after the fourth (B) microwave-assisted enzymatic hydrolysis; the original BSG (C) at 1200× and after the fourth (D) microwave- assisted enzymatic hydrolysis

Upon microwave treatment, the highest XOS release during the second hydrolysis was observed: 2.529 g from WBPs, 2.330 g from BSGs, 2.737 g from WBPs, and 2.606 g from BSGs. This yield exceeded that of the third and fourth hydrolysis steps. Overall, enzymatic hydrolysis using four microwave cycles resulted in the extraction of 8.092 g of XOS from 100 g samples of WBPs and 8.991g of XOS from 100 g samples of BSGs.

Isolation of Xylooligosaccharides

Following enzymatic hydrolysis, the water-soluble fraction was isolated using centrifugation. Wang showed that the water-soluble fraction comprised starch, pectin, protein, and tannin, along with hydrolysis products. Activated carbon adsorption and membrane filtration were employed to eliminate these contaminants. The general amount of sugar was quantified using the phenol-sulfuric acid method

with a D-xylose reference total carbohydrate was 87.6% in the WBP hydrolysate and 89% in the BSG hydrolysate. Since BSG had high protein content, membrane dialysis was used to purify XOS from BSG samples.

The supernatant obtained after four rounds of microwave-pretreated enzymatic hydrolysis underwent a three-step purification process: activated carbon absorption, ethanol precipitation, and 0.22 µm membrane filtration. Subsequently, the solution was freeze-dried, and the resulting dry weight was measured.

Scanning Electron Microscopy

The reduced sugar assay in conjunction with scanning electron microscopy analysis (Figure 2) confirmed the effectiveness of a single pretreatment over multiple ones. Figure 2 shows the effects of one and four microwave treatments on BSG. Following repeated enzymatic hydrolysis with microwave pretreatment, samples were prepared from the sediment after each hydrolysis step. Then the electron microscope was adjusted to a magnification of 150x to capture the images.

Figure 2A depicts the BSGs powder before microwave pretreatment of enzymatic hydrolysis. In this image, the intact structure of the cell wall is clearly visible, with intact cellulose and hemicellulose connections. Figures 2 B, C, and D provide a comparative view of BSGs subjected to repeated enzymatic hydrolysis. As the samples undergo multiple cycles of pretreated enzyme hydrolysis with microwaves, the orderly structure of the cell walls is disrupted. Cellulose and hemicellulose bonds break down, leading to cell structure disruption and xylan dissolution.

Fourier Transform Infrared Spectroscopy (FT-IR) Analysis

The FT-IR analysis is a quick and sensitive method extensively employed to examine the physicochemical and structural characteristics of carbohydrate materials.

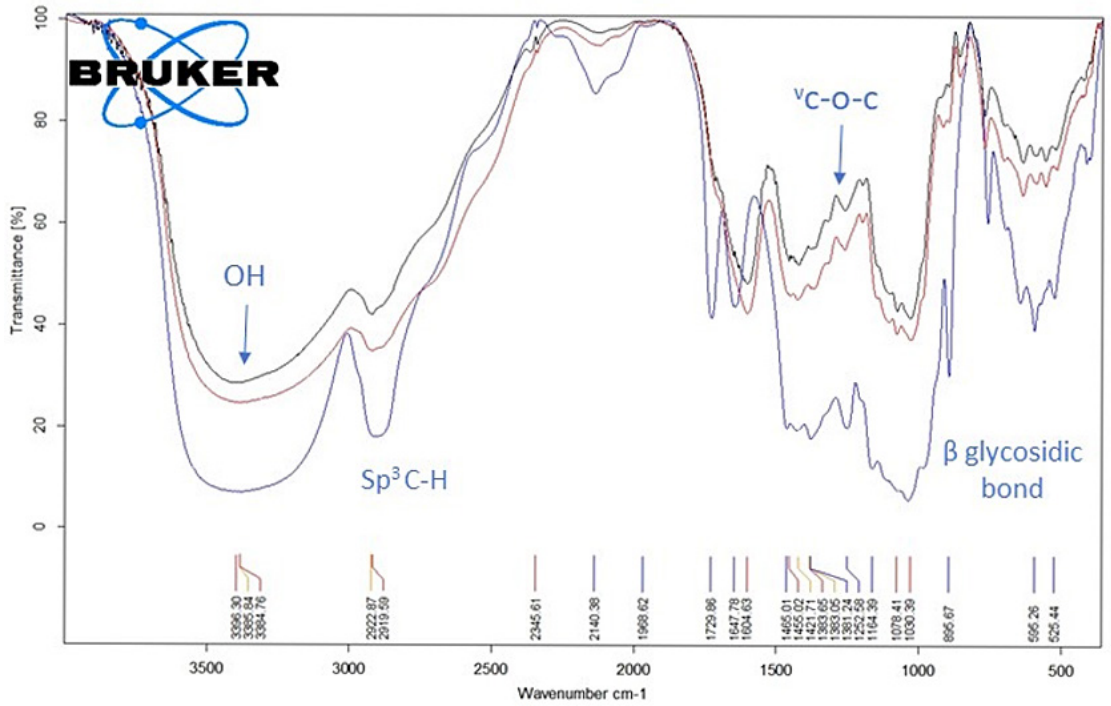

Fig. 3. FT-IR spectra of XOS extracted from microwave- assisted enzymatic hydrolysis of WBP and BSG compared with xylotetrose standard (Blue line xylotetrose, red line XOS from WBP and black line XOS from BSG)

The FT-IR spectra for the WBP sample, the BSG sample, xylotetrose standard (Biosynth Co., UK) are shown in Figure 3. The FT-IR spectra of standard (xylotetrose) showed major bonds at 3406.80 cm^{-1} is ascribed to the elongation of the hydroxyl (-OH) groups present, 2979.06 cm^{-1} and 2918.84 cm^{-1} pertaining to C-H bending. The absorbance bands1465.70 cm^{-1}, 1043.40 cm^{-1}, and 896.73 cm^{-1} were associated with xylan.[19] The bands ranging from 1166 cm^{-1} to 1000 cm^{-1} were likewise characteristic of the xylan molecules. As expected, the three spectral profiles of the majority of bands were largely analogous, suggesting that the structure of the sugars were similar.

The absorption value at 3406.80 cm^{-1} indicates the stretching of the O–H bond. The absorbances at 2918.84, 2979.06, and 1638.14 cm^{-1} signify the C–H stretching of carbohydrates. The highest point at 1465.70 denotes the stretching of C–O, C–C–C, and C–OH in hemicelluloses. The peak of 1043.40 cm^{-1} is represented by furanose. The peak of 896.73 cm^{-1} represents the β glycoside bond.

In our study, the purification of xylooligosaccharides (XOS) is attributed to variations in the deformation of the C-H_2 bond at 1040 cm^{-1} and the vc-o-s vibration of furanose at 1100 cm^{-1}.

Furthermore, a weak peak observed at 870 cm^{-1} indicates the presence of a β-glycoside bond and matches the spectrogram of standard xylotetrose FT-IR spectra, thereby confirming the presence of XOS in the analyzed sample.[20]

Xylooligasaccharide Determination using High-Performance Liquid Chromatography HPLC

The efficacy of this detector set a limit on the concentration values of xylotetrose, xylotriose, and xylobiose included in the sample. In 3.2±0.5 min, xylobiose is identified, followed by xylotriose in 7.5±0.5 and xylotetrose in 12.04±0.5 min. The findings of a 5-time transmissibility analysis are shown in Figure 4.

The peak's form varies, depending on the sample's purity and the detector's capabilities.

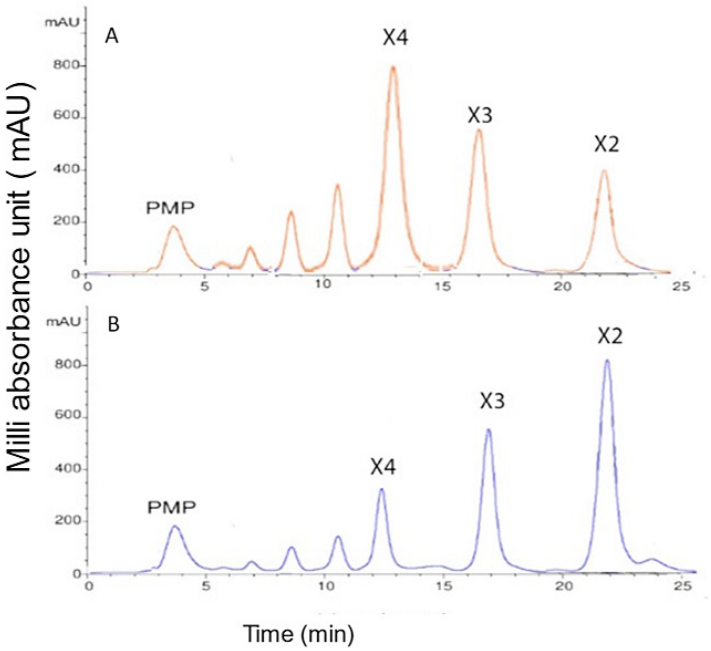

Fig. 4. The HPLC chromatogram of the purified sample shows the presence of xylobiose, xylotriose, and xylotetrose. (a) XOS extracted from microwave- assisted enzymatic hydrolysis of WBP. (b) XOS extracted from microwave-assisted enzymatic hydrolysis of BSG. PMP- stands for 1-Phenyl-3-methyl-5-pyrazolone, X1-D-xylose, X2-xylobiose, X3-xylotriose, X4- xylotetrose

The results indicate that the purified samples contain xylotetrose, xylotriose, and xylobiose.

Table 2. The monosaccharide composition in the purified xylooligosaccharides (XOS)

	Refractor time (min)	Oligosaccharides composition in XOS purified from WBPs (%)	Oligosaccharides composition in XOS purified from BSGs (%)
Xylotetrose	13.279±0.5	47.84±0.5	21.54±0.5
Xylotriose	17.556±0.5	33.27±0.5	30.98±0.5
Xylobiose	22.040±0.5	18.89±0.5	47.48±0.5

In the WBP derived xylooligosaccharides (XOS), xylobiose constituted 47.84%, xylotriose 33.27%, and xylotetrose 18.89%. Meanwhile, the XOS purified from brewers' spent grain (BSG) comprised 21.54% xylotetrose, 30.98% xylotriose, and 47.48% xylobiose.

Upon completion of this process, the purity of the extracted substance was determined xylooligosaccharides (XOS) was 87.6% in the dry WBP powder, with a yield of 8.1 grams. In the dry BSG powder sample, the XOS purity was 89%, accompanied by a yield of 7.2 grams.

Discussion

Producing XOS from wheat bran has garnered significant interest due to its high xylan content, a type of hemicellulosic polysaccharide. Wheat bran, which is a by-product of the milling process, constitutes about 20% of total wheat output and contains approximately 40% xylan. This xylan can be hydrolyzed into XOS using specific enzymes. The enzymatic hydrolysis of wheat bran not only enhances the extraction of valuable oligosaccharides but also promotes the valorization of agricultural waste, aligning with sustainable food production practices.[9]

BSG is produced during the processing of malt (cereal grain) and the separation of wort. It constitutes approximately 85% of the solid by-products generated in the brewing industry. According to the State Statistics Committee of Mongolia, beer production increased from 82,415.2 liters in 2017 to 91,246 liters in 2018, and then to 91,975 liters in 2019. This trend highlights an annual growth in beer production21. The extraction of Xylo-oligosaccharides (XOS) from BSG has become a significant topic due to the rising emphasis on sustainable food production and effective waste management practices. BSG, being a by-product of the brewing industry, accounts for about 85% of the total by-products produced during beer manufacturing.[22]

During the WB processing at Altan Taria Co., Ltd., seven types of waste are generated. In this study, the outer peels and dust, referred to as "black dust," were previously not used for livestock feed. As a result, the ash content in the WBP was found to be relatively high. Additionally, the protein and lipid compositions in BSG were significantly higher than those in WBP. Mongolia is pioneering the combination of established methods and the testing of a technique for purifying prebiotic oligosaccharides from waste materials produced by two major food industries in the country. The practical significance of this research lies in its demonstration of how to process biologically effective oligosaccharides using a cost-effective isolation method from by-products generated by these two large food industries.

According to our findings, the chemical composition of BSG includes 16.2% protein, 5.8% fat, and 3.8% minerals, based on a study conducted by E. Solongo (2018) in Mongolia. The amounts of these constituents in BSG can vary by country, influenced by the raw materials used, weather conditions, and geographical features. In Mongolia, BSG contains lower levels of protein and fat compared to other countries, but has a higher mineral content. However, no studies have been conducted to determine the chemical composition of WBP.[21]

Purifying xylooligosaccharides (XOS) using microwave-assisted enzymatic hydrolysis represents a significant advancement in bioprocessing, particularly in the valorization of lignocellulosic biomass. The production of XOS not only enhances the health benefits of food products but also encourages the sustainable use of agricultural by-products, aligning with the principles of a circular economy. Microwave-assisted enzymatic

hydrolysis has emerged as an innovative technique that improves the efficiency of XOS production. By harnessing microwave energy, this method facilitates the breakdown of complex lignocellulosic structures, making xylan more accessible to enzymatic action.[1]

In Coelho's study, the preparation of xylo-oligosaccharides (XOS) involved using a water-based extract. The process began with collecting the supernatant, which was then precipitated with 70% ethanol, centrifuged, and freeze-dried. This formed a substance that was heated at 180°C for 2 minutes. The supernatant was collected again, producing a centrifuged and freeze-dried product.

Next, the precipitate was treated with a 0.1M KOH solution, and the supernatant was again heated at 180°C for 2 minutes before being centrifuged with acetic acid and freeze-dried. The samples underwent centrifugation with 70% ethanol before freeze-drying, resulting in XOS with 62% purity. Moreover, increasing the microwave temperature from 140 to 210°C led to higher XOS production. The microwave-assisted enzymatic hydrolysis technique achieved a purity of 89%. The study aimed to increase the purity level of produced XOS. This was accomplished by adding the xylanase enzyme and employing activated carbon absorption through enzymatic hydrolysis, combined with physical treatment at 200°C for 3 minutes. This process resulted in a product with an elevated degree of purity.[23]

Aachary, XOS were derived by hydrolyzing corn with the enzyme xylanase (*Aspergillus oryzae* MTCC 5154), and enzyme hydrolysis was conducted at 50°C for 24–36 h, after which it was boiled for 15 min to terminate the hydrolysis. The amount of XOS produced decreased when enzyme hydrolysis was conducted for a maximum of 24 h, and the xylanase (*Aspergillus oryzae* MTCC 5154) enzyme increased the production of XOS more so than the xylanase enzyme. Xylanase units/g (Sigma-Aldrich Germany, X2753) experienced enzymatic hydrolysis at 55°C for 24 h. In addition, it was placed in a water involvement at 96°C for 5 min to stop enzymatic hydrolysis. The current research used a different sample, but the same enzyme and time for enzymatic hydrolysis were employed as described in our study.[24]

A microenvironment of an enzyme-catalyzed process was subjected to microwave radiation. The microstructure of the first bran samples posts the fourth microwave-assisted enzymatic hydrolysis was examined via scanning electron microscopy. In the purification phase, the high molecular weight components were isolated, and several XOS, specifically xylopentose and xylohexose exceeded the size of X4. Techniques like thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) are frequently used to analyze the composition and purity of XOS. These methods offer valuable insights into the degree of polymerization and the presence of specific oligosaccharide fractions, which are crucial for evaluating the prebiotic potential of the final product. Without a standard, these oligosaccharides are denoted as >xylotetrose. The HPLC chromatograms of XOS compositions derived from wheat bran predominantly exhibited xylobiose, xylotriose, and xylotetrose, with negligible quantities of >X4.[14]

Both XOS samples purified from WBP and BSG samples showed structures similar to the standard one, which represent the stretching of O-H, C-H stretching of carbohydrate, C-O, C-C-C, and C-OH bonds in hemicellulose, and the β-glycoside bond, suggesting the presence of the xylan group in the XOS mix, reported earlier by Liang.[17] As it has been revealed earlier that the band at 896.73 cm^{-1} represents the presence of glycosidic bonds between the sugar molecules,[19] the graph shown in Figure 4 could be related to the previous study's results. The band structure resembled that presented by Liang in their FTIR analysis of the XOS mixture derived from Bian's FTIR examination of XOS from sugarcane bagasse.[17, 20, 25]

The XOS generated by the charcoal adsorption process largely comprised xylobiose and xylotriose, with a negligible quantity of xylotetrose. The findings demonstrate that the adsorption approach utilizing activated carbon outperformed the precipitation technique involving ethanol. Subsequent tests employed activated carbon adsorption according to the nature of the product.

Wang XOS derived from wheat bran using microwave-assisted enzymatic hydrolysis. Thus, 3.2 grams of XOS were extracted from 50 grams of the sample the microwave alone does not positively affect production, but it increases the amount produced when combined with the enzyme hydrolysis method,

where four instances of enzyme hydrolysis and 24 h per instance were found effective.[15]

Over 24-hour period of enzymatic hydrolysis, 5.4 grams of XOS were extracted from 50 grams of the WBP sample, while 3.6 grams of purified dry XOS were obtained from 50 grams of dry BSG powder.

Conclusion

We conducted an extensive examination of the effectiveness of microwave-assisted enzymatic hydrolysis for wheat bran peel (WBP) and brewers' spent grain (BSG). This method not only enhanced the yield of XOS but also facilitated the removal of contaminants from the final product. By integrating this process with xylanase, we outlined essential steps for the industrial-scale production of food-grade XOS. Moving forward, the study will focus on evaluating the biological effects of purified XOS, including its role as a prebiotic, its antioxidant properties, and its impact on metabolism. Additionally, we will conduct sub-acute toxicity studies on the purified product as part of this research.

Acknowledgement

The research team would like to thank the Joint Field Research Center with Nagoya University, Japan of the Mongolian University of Sciences and Technology for Scanning electron microscopy with energy dispersive spectroscopy TM-1000. We appreciate Mr. Curt Chatelain for English editing of the manuscript.

Funding Sources

This study was implemented by "Technology development study to isolate prebiotic oligosaccharides from wheat coarse bran by-product" grant (No: 2016/06-2022/13) financially supported by the Science and Technology Promoting Fund of Mongolian National University of Medical Sciences, 2022.

Conflict of Interest

The authors declare that they have no competing interests.

Authors' Contribution

Odgerel Chinbat - Data Collection, Analysis, Methodology, Writing – Original Draft.
Purevdulam Erdenetsog - Data Collection, Analysis
Battogtokh Chimeddorj - Supervision, Writing – Review & Editing
Munkhjargal Burenjargal - Conceptualization, Methodology, Writing – Review & Editing, Supervision
Munkhtsetseg Janlav – Conceptualization, Funding Acquisition, Resources, Writing – Review & Editing, Supervision.

Data Availability Statement

The manuscript incorporates all datasets produced or examined throughout this research study.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

References

1. Wang L, Pang T, Kong F, Chen H. Steam Explosion Pretreatment for Improving Wheat Bran Extrusion Capacity. *Foods*. 2022;11(18):2850. doi:10.3390/foods11182850
2. Serraj R, Pingali P. Agriculture & Food Systems To 2050: Global Trends, Challenges And Opportunities. *World Scientific Publishing Company*; 2018.
3. Manicardi T, Silva GBe, Longati AA, Paiva JF, Souza TB, Pádua TP, Purlan FF, Giordano LC, Giordano RC, Milessi TS. Xylooligosaccharides: A Bibliometric Analysis and Current Advances of This Bioactive Food Chemical as a Potential Product in Biorefineries' Portfolios. *Foods*. 2023;12(16):3007. doi:10.3390/foods12163007
4. Chen M, Liu S, Imam KMSU, Sun L, Wang Y, Gu T, Wen B, Xin F.. The Effect

of Xylooligosaccharide, Xylan, and Whole Wheat Bran on the Human Gut Bacteria. *Frontiers in Microbiology.* 2020;11doi:10.3389/fmicb.2020.568457

5. Lebiocka M, Montusiewicz A, Szaja A, Rembisz S, Nowakowska E. Thermophilic Co-Digestion of Sewage Sludge and Brewery Spent Grain. *Journal of Ecological Engineering*. 2019;20(10):118-124. doi:10.12911/22998993/113139
6. Chinbat O, Erdenetsog P, Tuvshintur B, Burenjargal M,Chimeddorj B, Janlav M. In Vitro and In vivo Investigation of the Biological Action of Xylooligosaccharides Derived From Industrial Waste. *Food Science & Nutrition.* 2024;doi:10.1002/fsn3.4391
7. Mahmud MZA, Islam MD, Mobarak MH. The Development of Eco-Friendly Biopolymers for Use in Tissue Engineering and Drug Delivery. *Journal of Nanomaterials.* 2023/01/01 2023;2023(1):9270064. doi:https://doi.org/10.1155/2023/9270064
8. Mahmud MZA, Mobarak MH, Hossain N. Emerging trends in biomaterials for sustainable food packaging: A comprehensive review. *Heliyon*. 2024;10(1)doi:10.1016/j.heliyon.2024.e24122
9. Si D, Shang T, Liu X, Zheng Z, Hu Q, Hu C, Zhang R.. Production and characterization of functional wheat bran hydrolysate rich in reducing sugars, xylooligosaccharides and phenolic acids. *Biotechnol Rep (Amst). Sep* 2020;27:e00511. doi:10.1016/j.btre.2020.e00511
10. Kumar V, Satyanarayana T. Secretion of Recombinant Thermo-Alkali-Stable Endoxylanase of Polyextremophilic Bacillus Halodurans TSEV1 and Its Utility in Generating Xylooligosaccharides From Renewable Agro-Residues. *Process Biochemistry.* 2014;49(11):1875-1883. doi:10.1016/j.procbio.2014.08.012
11. Rashid R, Sohail M. Xylanolytic Bacillus Species for Xylooligosaccharides Production: A Critical Review. *Bioresources and Bioprocessing.* 2021;8(1)doi:10.1186/s40643-021-00369-3
12. Ha SH, Mai NL, An G, Koo Y-M. Microwave-assisted pretreatment of cellulose in ionic liquid for accelerated enzymatic hydrolysis. *Bioresource Technology.* 2011/01/01/ 2011;102(2):1214-1219. doi:https://doi.org/10.1016/j.biortech.2010.07.108
13. Ratnadewi AAI, Fauziyah KR, Indarti D. Purification of Xylooligosaccharide From Casavva Pulp by Ultrafiltration Method. *Berkala Sainstek.* 2021;9(2):51. doi:10.19184/bst.v9i2.23388
14. Pu J, Zhao X, Wang Q, Wang Y, Zhou H. Development and validation of a HPLC method for determination of degree of polymerization of xylo-oligosaccharides. *Food Chem.* Dec 15 2016;213:654-659. doi:10.1016/j.foodchem.2016.07.014
15. Wang T-H, Lu S. Production of xylooligosaccharide from wheat bran by microwave assisted enzymatic hydrolysis. *Food Chemistry.* 2013/06/01/ 2013;138(2):1531-1535. doi:https://doi.org/10.1016/j.foodchem.2012.09.124
16. Bailey MJ. A note on the use of dinitrosalicylic acid for determining the products of enzymatic reactions. *Applied Microbiology and Biotechnology.* 1988/11/01 1988;29(5):494-496. doi:10.1007/BF00269074
17. Liang N, Lu X, Hu Y, Kitts DD. Application of Attenuated Total Reflectance-Fourier Transformed Infrared (ATR-FTIR) Spectroscopy To Determine the Chlorogenic Acid Isomer Profile and Antioxidant Capacity of Coffee Beans. *J Agric Food Chem.* Jan 27 2016;64(3):681-9. doi:10.1021/acs.jafc.5b05682
18. Ooi BG, Rambo AL, Hurtado MA. Overcoming the recalcitrance for the conversion of kenaf pulp to glucose via microwave-assisted pre-treatment processes. *Int J Mol Sci.* 2011;12(3):1451-63. doi:10.3390/ijms12031451
19. Ren J-L, Peng F, Sun R-C, Kennedy JF. Influence of hemicellulosic derivatives on the sulfate kraft pulp strength. *Carbohydrate Polymers.* 2009/01/22/ 2009;75(2):338-342. doi:https://doi.org/10.1016/j.carbpol.2008.08.011
20. Mathias TRdS, Alexandre VMF, Cammarota MC, Mello PPMd, Sérvulo EFC. Characterization and Determination of Brewer's Solid Wastes Composition. *Journal of the Institute of Brewing*. 2015;121(3):400-404. doi:10.1002/jib.229
21. E Solongo. The potential to extract bioactive

compounds from brewery spent grains. Mongolian National University; 2018.

22. Mussatto SI. Brewer's Spent Grain: A Valuable Feedstock for Industrial Applications. *Journal of the Science of Food and Agriculture*. 2014;94(7):1264-1275. doi:10.1002/jsfa.6486
23. Coelho E, Rocha MA, Saraiva JA, Coimbra MA. Microwave superheated water and dilute alkali extraction of brewers' spent grain arabinoxylans and arabinoxylo-oligosaccharides. *Carbohydr Polym.* Jan 2014;99:415-22. doi:10.1016/j.carbpol.2013.09.003
24. Aachary AA, Prapulla SG. Value addition to corncob: production and characterization of xylooligosaccharides from alkali pretreated lignin-saccharide complex using *Aspergillus oryzae* MTCC 5154. *Bioresour Technol.* Jan 2009;100(2):991-5. doi:10.1016/j.biortech.2008.06.050
25. Feng Z-g, Bian H-j, Li X-j, Yu J-y. FTIR analysis of UV aging on bitumen and its fractions. *Materials and Structures*. 2016/04/01 2016;49(4):1381-1389. doi:10.1617/s11527-015-0583-9