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RESEARCH ARTICLE

Extraction of Arabinoxylans from deoiled rice bran

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ABSTRACT

This study is focused on the extraction of arabinoxylans (AX) from cereal by-products, with a specific emphasis on deoiled rice bran. The research investigates the influence of various experimental conditions, including the concentrations of sodium hydroxide and sodium chlorite, as well as the temperature, with the goal of optimizing the extraction and delignification process. The optimal conditions were determined to be a 40% sodium hydroxide pretreatment and a 30% sodium chlorite delignification step conducted at a temperature of 60°C, resulting in a consistent 12% yield of purified arabinoxylan. The purified AX exhibited a moisture content of 9.07% and a carbohydrate content of 76.89%. FTIR analysis confirmed the presence of DORB-AX, while TLC and HPLC analyses identified the presence of arabinose and xylose, with an A:X ratio determined to be 0.58. These findings provide valuable insights into the compositional and structural characteristics of the arabinoxylan sample, which is a significant constituent of cereal grain cell walls, particularly in wheat and rice bran. The methodology presented in this study enhances our understanding of AX extraction and holds promise for potential applications in diverse industrial sectors..

Keywords: De-oiled rice bran (DORB), Alkaline extraction, Arabinoxylan

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INTRODUCTION

Arabinoxylan (AX) stands as the principal non-starch polysaccharide present in the bran of cereals and millets, emerging as a byproduct of agro-processing. Structurally, arabinoxylan is typically composed of a β 1-4-linked xylose backbone, with arabinose (α 1-2/1-3) substituting, and this structure is additionally associated with about 1-3% phenolic acid (Zannini et al., 2022). The distinctive attributes of arabinoxylans (AXs) render them advantageous in cereal based products, such as bread. Integrating AX has the potential to enhance the gluten matrix, a vital factor in shaping the aerated structure and overall quality of bread. Supplementing one's diet with prebiotics can significantly impact the growth and functionality of beneficial bacteria, notably lactobacilli and bifidobacteria, which have positive effects on human health. This area of research is a focal point in the advancement of functional foods. Arabinoxylans (AX), a primary dietary fiber component in the human diet, have the potential to function as prebiotics, contributing to these beneficial effects (Saeed et al., 2016).

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Arabinoxylans have a linear chain backbone made of ß-d-xylopyranosyl (Xylp) residues connected by glycosidic linkages (1–4). There are four structural components in the molecular structure of arabinoxylans: monosubstituted Xylp at O-2 or O-3, disubstituted Xylp at O-2,3, and unsubstituted Xylp. -l-Arabinofuranosyl (Araf) residues are connected to some of the Xylp residues at O-3, O-4, and/or at both O-2, (Izydorczyk, 2021). Arabinoxylan extracted from rice bran offers a range of applications across various industries. As a dietary fiber supplement, it aids in digestive health and weight management, while its prebiotic qualities promote a balanced gut microbiota. Notably, its ability to bind to bile acids suggests the potential for lowering cholesterol levels. In the pharmaceutical sector, arabinoxylan serves as an excipient, assisting in controlled drug release. Its incorporation in gluten-free baking enhances texture and moisture retention. The compound's antioxidants benefit cellular health, reducing oxidative stress in functional foods. Arabinoxylan, a complex polysaccharide with hydroxyl groups, can effectively bind water and create viscous solutions, making it valuable in the food industry for thickening and gelling. Arabinoxylans from maize, wheat, and rye positively impact cecal fermentation, short-chain fatty acid production, lower serum cholesterol, and enhance calcium and magnesium absorption. They are not digested in the small intestine but serve as fermentable carbon sources for large bowel bacteria (Nino-Medina et al., 2010).

MATERIALS AND METHODS

Materials and chemicals

The raw materials utilized in this study included wheat bran, rice bran, de-oiled rice bran, and corn bran. In the analytical work, chemicals were employed were α-amylase, protease, sodium chlorite (NaClO2), H_2SO_4 and NaOH. All the chemicals required for the analytical work were used of analytical reagent (AR) or guaranteed reagent (GR) grade.

Selection of bran for extraction

In the initial stage of the study, a diverse collection of wheat bran, rice bran, de-oiled rice bran, and corn bran was meticulously gathered from various sources to facilitate primary analyses crucial for subsequent processes. Wheat bran and corn bran were sourced from Ganapati Mill, Kolhapur, Maharashtra, India, while rice bran and de-oiled rice bran were obtained through a partnership with Birla Rice Mill in Sangli, Maharashtra, India, ensuring a representative sample of cereal byproducts. Among the different cereal bran, corn bran stood out for its high dietary fiber and phenolic content, primarily insoluble in nature. Despite its nutritional value, it was excluded from further study due to its low yield, typically around 3%. The initial bran treatment involved fat and starch removal, with de-oiled rice bran being exceptional for its low fat content (around 1%). For other bran samples, excluding DORB, fat removal was essential. This defatting process is crucial for suitability in various applications. The study continued with de-oiled rice bran, offering a cleaner starting material for further analysis and potential applications in fields like food science, animal feed, and industrial processes. This comprehensive approach underscores the significance of each step in preparing cereal byproducts for investigation and utilization.

Corn bran has the highest content of dietary fiber and phenolic among the different cereal bran. As a major component of corn bran and corn fiber, these food fibers are primarily insoluble. However, due to a notably low yield (approximately 3%), corn bran was excluded from further study. The initial treatment carried out on bran involves the removal of fat and starch. De-oiled rice bran (DORB) contains an exceptionally low fat content (around 1%). With the exception of DORB, the removal of fat from bran is crucial for wheat bran, rice bran samples. To avoid the need for defatting, the study continued with de-oiled rice bran.

Data analysis and optimization during extraction of arabinoxylan from De-oiled bran :

De-oiled rice bran was treated under various circumstances to get information on the best pretreatment for removing starch, lignin, and extracting arabinoxylan. This study expresses the percentage of sodium chlorite and sodium hydroxide for extraction on the initial rice bran (de-oiled) ratio (Bataillon et al.,1998).

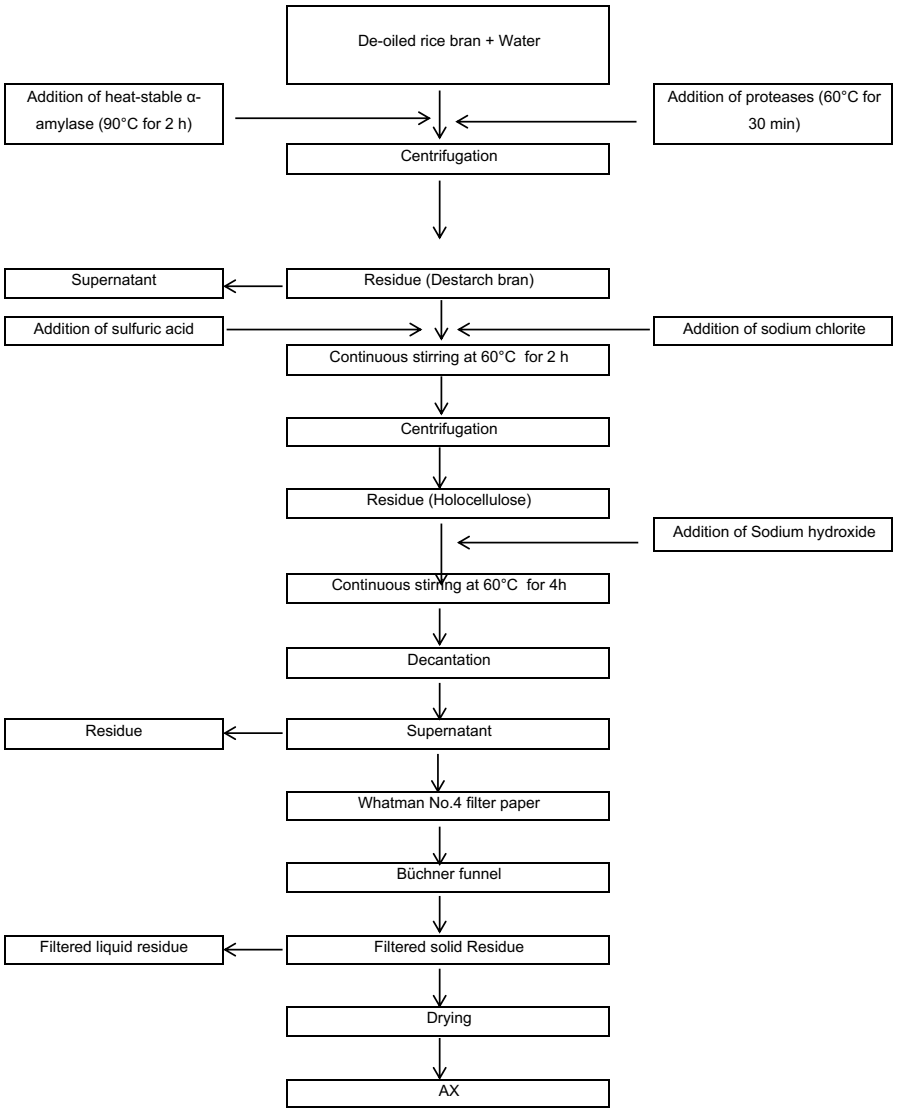

Fig. 1: Process flow sheet for extraction of arabinoxylan

The initial step in the process typically involved destarching the bran using an optimal amount of water. To determine the optimal water-to-bran ratio, three different bran solutions were prepared: A1= 1:15 A2= 1:10 A3= 1:5 (Bran: Water weight ratio). The process begins with de-oiled rice bran mixed with water, followed by the addition of (0.2%, 0.5% and 1%) heat-stable α-amylase and incubation at 90°C for 2 hours. Subsequently, proteolytic enzymes are introduced, and the mixture is incubated at 60°C for 30 minutes. Afterward, centrifugation is employed to separate components, yielding destarched bran as the final product. This method employs enzymatic and thermal treatments to reduce starch and protein content.

Optimization of sodium chlorite in delignification and water extraction

To optimize the quantity of sodium chlorite, a systematic study was carried out to determine the most effective concentration for the delignification process. Four different concentrations (10% to 40%) of sodium chlorite were tested while maintaining a constant amount of sulfuric acid. The goal was to identify the concentration that would yield the desired delignification results while minimizing excess use or undesired effects.

Optimization of sodium hydroxide in alkaline extraction of arabinoxylan

Sieved holocellulose was subjected to extraction using sodium hydroxide solutions of varying concentrations: 0.25 M, 0.5 M, 0.75 M, and 1 M. These concentrations corresponded to 10%, 20%, 30%, and 40% of sodium hydroxide relative to the starting bran. This extraction was conducted for a duration of 4 hours at a temperature of 60°C. Subsequently, the mixture was cooled to 15°C to facilitate decantation.

Filtration and drying process

The cooled supernatant was subjected to filtration using a Büchner funnel in combination with Whatman No.4 filter paper. Through this process, the clear acidic supernatant was separated and subsequently subjected to purification via filtration. The resultant retentate was then concentrated further through an evaporation process.

Analysis of extract

$$\%\, Yeild = \frac{Amount\ of\ extract}{Weight\ of\ sample} \times 100$$

$$\%\, Yeild = 12$$

Drying at 110 ^{0}C for 6 hours has found the moisture content of residues that occur during fractionation. All calculations of yield and composition have been carried out with no moisture content (Brillouet and Mercier, 1981). Ash content was determined by drying 6 hours in a muffle furnace at 550°C. High Pressure Liquid Chromatography was used to analyse monomeric sugars such xylose, arabinose, and glucose at a temperature of 40 °C with an flow rate of 0.2 ml/min. (Bataillon et al.,1998).

Water holding capacity (WHC) determination for isolated crude arabinoxylan (AX) involves measuring its water retention ability. After drying the AX to remove moisture, known water volume was added. After equilibration, the final weight was measured. WHC was calculated as (final weight - initial dry weight) / initial dry weight, expressed as g water/g AX. It assesses AX's water absorption and retention properties (Kaur et al., 2020).

FTIR spectroscopy is a valuable analytical approach that facilitates the identification of distinctive functional groups and chemical bonds within polysaccharides. The obtained FTIR spectra for DORB-AX were acquired in a solid-state manner and are presented in the chromatograph for analysis. The spectral range covered 4000–400 cm^{-1}, with a resolution of 4 cm^{-1} and 16 scans for each sample. Thin-layer chromatography (TLC) was carried out using silica gel 60F254 plates measuring 10 × 20 cm. Application of all standards and samples was accomplished using a capillary tube method.

RESULTS AND DISCUSSION

De-oiled rice bran was mixed with water to remove water-soluble starch and other components. Enzymes like α-amylase and proteases (0.5%) were added for this purpose (Table 1). The mixture was stirred in a container at a set temperature. To remove lignin, the destarched bran underwent treatment with sodium chlorite (30%) and sulfuric acid at 60°C for 2 hours. Holocellulose was obtained and separated through centrifugation, washed, and dried. Alkali solvents disrupted cell wall bonds, releasing polysaccharides. Sieved holocellulose was treated with sodium hydroxide of varying concentrations (0.25 M to 1 M) for 4 hours at 60°C. After cooling, the mixture was decanted. The cooled supernatant was filtered using a Büchner funnel and Whatman No.4 paper. The clear supernatant was purified and concentrated through evaporation.

After the evaporation process, a white powder weighing 12 grams was obtained. This powder was stored under low temperature and dry conditions to preserve it for future studies.

Table 1 : Summary of stage wise results

Initial weight	100 grams	100%
Weight after water treatment	62 grams	62%
Weight after Delignification	41 grams	41%
Weight after alkaline extraction	25 grams	25%
Weight after filtration	12 grams	12%

The extracted arabinoxylan powder exhibited a moisture content of 9.07%. The ash content of the arabinoxylan powder was measured as 11.38%. Quantification of the protein content within the arabinoxylan powder reveals a value of 1.64%. Analysis indicates a fat content of 1.02% within the arabinoxylan powder, with the predominant constituent being carbohydrates at 76.89%.

Determination of water holding capacity (WHC)

This study investigates how changes in the molecular weight of water-extractable arabinoxylans (WE-AX) impact water holding capacity during breadmaking using Differential Scanning Calorimetry (DSC). The unfreezable water content in WE-AX ranged from 0.42 to 0.45 grams per gram of dry matter across three grain varieties, consistent with prior research (Nishitsuji et al.,2022).

Similar water binding capacities for arabinoxylans from different grains. Nishitsuji et al.,2022 found varying unfrozen water content in soluble and insoluble pentosans. These results contribute to understanding WE-AX water-holding properties in different grains, with relevance in breadmaking processes. Water holding capacity (WHC) was determined as 164% (164 g per 100 g of AX) (Nishitsuji et al.,2022).

FT-IR analysis

FT-IR spectroscopy is a highly effective analytical technique used for identifying distinct functional groups and chemical bonds in polysaccharides. The FTIR spectrum for DORB-AX was obtained in solid-state and is depicted in Figure 2.

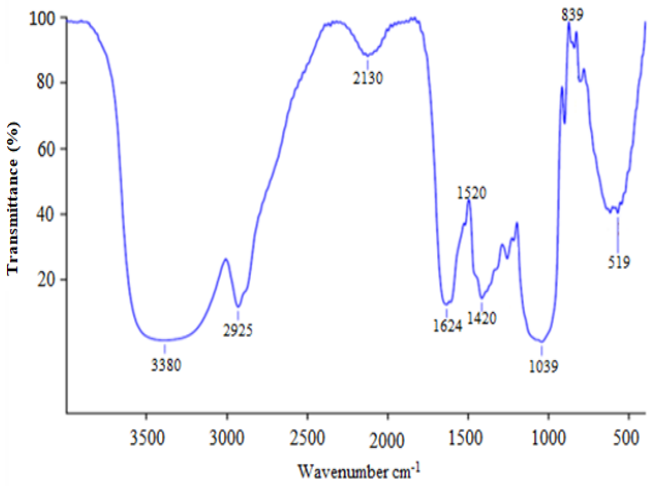

Fig 2 : FTIR spectrum of AX from DORB

In the study by Butylina et al. (2022) both xylan and arabinoxylan exhibited shared spectral characteristics associated with their xylopyranose backbone, including a peak at 1035 –1041 cm^{-1} related to C-O bond stretching, along with peaks at 2919 cm^{-1} and 3341–3343 cm−1 corresponding to C–H and O–H bond stretching, respectively, as discussed by Duan et al. (2019). The FT-IR spectroscopy results presented in this research highlight specific features of DORB-AX, such as absorbance bands at 3380 cm^{-1} (indicating free OH group stretching), 2925 cm^{-1} (C–H stretching vibrations), and 2130 cm^{-1} (associated with saccharides). An absorption signal at 1624 cm^{-1} suggested the presence of conjugated double bonds and phenyl rings, while signals at 1520 cm^{-1} and 1420 cm^{-1} implied the existence of uronic acid residues linked to carboxyl group stretching. Moreover, the absorption signal at 1043 cm−1 confirmed the nature of DORB-AX as a xylan polysaccharide (Nandiyanto et al., 2019).

High-pressure liquid chromatography (HPLC)

By using an Agilent 1200 chromatograph and high-pressure liquid chromatography(HPLC), monosaccharide qualitative and quantitative studies were performed (Fig. 3).

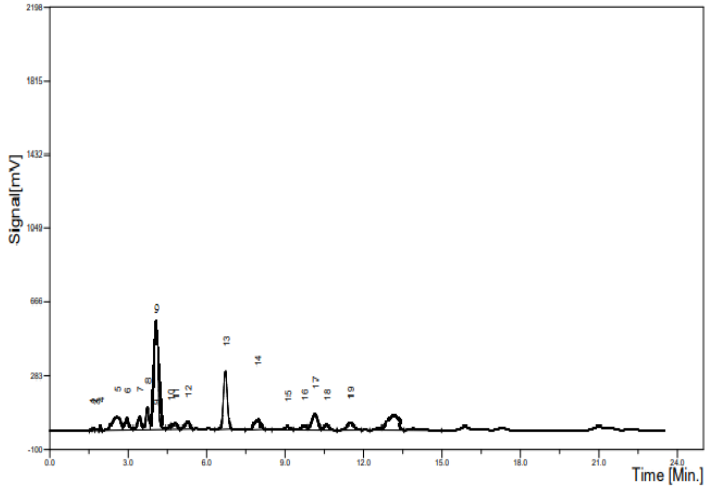

Fig. 3: Analysis of DORB-AX by HPLC

RT- 4.05 (peak no. 9) Xylose; RT- 5.26 (peak no.12) Glucose; RT-6.71 (peak no. 13) Arbinose; RT- 9.70 (peak no. 16) Mannose; RT- 11.48 (peak no. 19) Galactose.

The composition of the sample were as follows: 7.2% Uronoic Acid, 27.05% Arabinose, 46.68% Xylose, 0.035% Mannose, 0.36% Glucose, and 0.82% Galactose. A/X ratio is 0.58.

TLC analysis

After conducting TLC analysis, the developed plate showed well-defined spots representing the separated components. In the figure 4, two purified sample fractions 'a' and 'b' were chromatographed, resembling standard sugars, indicating their carbohydrate composition. TLC analysis confirmed Arabinose (Rf = 0.47) and Xylose (Rf = 0.55) presence, with slight variations from literature values. This consistency confirms the sugars' presence, aligning with known standards and validating the analytical methods (Thakur et al., 2020).

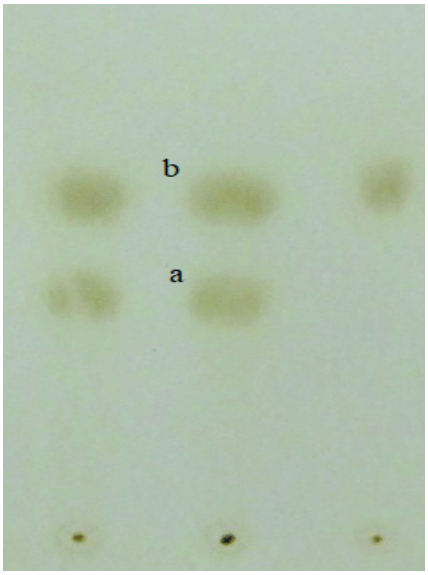

Fig. 4: Analysis of DORB-AX by TLC

CONCLUSION

This report provides a comprehensive study on arabinoxylan (AX) extraction from de-oiled rice bran, encompassing both extraction process parameters and the physico-chemical properties of the extracted AX. The isolated AX had 9.07% moisture and was predominantly composed of carbohydrates (76.89%), with minimal amounts of ash, protein, and fat. The extraction process involved holocellulose isolation, including water washing, enzymatic treatment, and lignin removal. Holocellulose was then subjected to an alkali-based extraction process at 60°C, resulting in a 12% yield of white powder. Characterization of the extracted AX confirmed the presence of DORB-AX through FTIR, displaying β-glycosidic linkages at 1039 cm^{-1} and 839 cm^{-1}, indicative of ring structures. TLC and HPLC analyses corroborated the presence of arabinose (Rf = 0.47) and xylose (Rf = 0.55) within the AX sample. The total content of arabinose and xylose in the sample amounted to 73.73 g per 100 g, with an A:X ratio of 0.58. These findings contribute significant insights into the composition and structure of arabinoxylan, important in various applications.

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