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Characterization of xylan from rice bran and finger millet seed coat for functional food applications

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ABSTRACT

The purpose of this work is to establish alternative renewable resources by a process of chemical free water based extraction of xylan which is an important plant component with diverse industrial applications including food and pharmaceuticals ones. In this respect, we have shown an alternative source of water soluble xylan (WSX) from rice bran which is usually wasted and seed coat of underutilized finger millet. Extractions were carried out using water at ambient temperature (25 ± 2° C). Hydrolysis and derivatization of the extracted WSX revealed the predominance of xylose followed by arabinose and glucose, with Ara/Xyl molar ratios of 0.55 and 0.68 respectively. Elemental analysis showed similarities in carbon and hydrogen, while nitrogen and sulfur contents showed some variations. Structural identification of β - glycosidic linkages and acetyl groups from the FTIR spectrum; β-D-1, 4-xylopyranose backbone with α-L-arabinofuranose and 4-*O*-methylglucuronic acid substituents evident from ^{1}H and ^{13}C NMR confirmed the presence of xylan. Characteristics of the extracted WSX (SEM, particle size, XRD and TGA) showed similarities in the two sources. Thus both rice bran and finger millet seed coat are potential sources of xylan and further optimization of the process can provide economic and environmental benefits.

1. Introduction

Cereals which comprise over 73% of the total world cultivation provide dietary fiber, energy, proteins etc., required for human health (Charalampopoulos et al., 2002). The milling of cereals (rice/paddy and millet) for the production of food constituents results in a number of low-value by-products/wastes, such as bran and seed coat. Cereal and millet polysaccharides present in the plant cell wall consist of water soluble and water insoluble polysaccharides. The water soluble polysaccharides include hemicelluloses, α-cellulose and pectin. Agricultural by-products including cereal grain cell wall are rich in hemicellulose (20–35%) with xylan being the major component (Tan et al., 2008). The bioconversion of agricultural by-products into value added functional food components is a key emerging technology for addressing the need for environment friendly and sustainable resources. In this context, raw materials such as rice bran and finger millet rich in xylan, 1, 3/1, 4-β-D-glucans, polyphenols and dietary fibre represent vast renewable resources of xylan that can be enzymatically converted into bioactive compounds such as oligosaccharides, sugar alcohols and phenolic acids. Xylooligosaccharides (XOS), a class of non-digestible functional food ingredients having lower degree of polymerization is produced during the hydrolysis of xylan. The prebiotic potential of XOS is gaining recognition in the global nutraceuticals market, though it is less exploited compared to fructooligosaccharides (FOS) and galactooligosaccharides (GOS). Presently corn cob is the major commercial source for XOS production. Rice bran and finger millet as alternate sources of xylan need to be explored.

Rice bran, an important by-product of cereal industry and finger millet or *ragi* (*Eleusine coracana*) is a minor cereal in the semi-arid regions of Africa and India, which forms one of the staple cereals for a wide segment of the population and unique among the minor cereals because of its superior nutritional qualities and several health benefits (Hilu and Dewet, 1976). Rice bran has low economic value and only a small percentage is used for oil production and the remaining usually wasted creating environmental pollution.

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Appropriate technologies can make it a suitable renewable source for xylan production. Finger millet is fermented to obtain alcohol for industrial use and the non-starch polysaccharide components which are wasted can also serve as a source of xylan.

Hromadkova et al. (1999) identified two different structural types of corn cob xylan, a low-branched arabinoglucuronoxylan, which was mostly water-insoluble (wis-X), and a highly branched water soluble heteroxylan (ws-X). The structure of xylan differs greatly depending on the origin and is found in several variations, but has one common 1, 4-β-D-xylopyranose component backbone mainly substituted with α-L-arabinofuranose or its feruloylated derivatives at *O*-2 or *O*-3 positions of the xylose residues (Sun et al., 2000), by 4-*O*-methylglucuronic acid in the position of 2-*O*, and/or by acetyl groups in the positions of 2-*O* and/or 3-*O* (Aspinall, 1959). Due to the limited availability of xylan at commercial scale, and the natural structural diversity, there is an increasing need for well-characterized xylan from agro based wastes for several applications.

To the best of our knowledge, there are few reports on the extraction of hemicellulose (xylan) from rice bran and finger millet seed coat. As early as 1985, Shibuya and Iwasaki (1985) extracted hemicellulose (4.6%) from defatted rice bran using alkali (4 M NaOH) and determined monosaccharide composition, but the characterization of same still needs to be investigated. Structural characterization of water unextractable portion from finger millet (*v.* Indaf 15, red variety) bran determined after sequential extraction with alkali showed the presence of xylan (6–10%) (Savitha Prashanth and Muralikrishna, 2014). In this study, finger millet CO9 was used. Unlike other varieties, it is unique in being white with very low polyphenols (Ramachandra et al., 1977; Sudha Rani and Antony, 2014) and since fiber is bound strongly to polyphenols (Hadimani and Malleshi, 1993), xylan from the white variety may be more accessible. Considering all these factors, the present study focuses on extraction of water soluble xylan (WSX) from rice bran and finger millet CO9 seed coat and their characterization, for further food applications.

2. Materials and methods

2.1. Materials

Rice (*Oryza sativa*) bran resulting from the milling of parboiled rice was collected from a local modern rice mill in Chennai. Finger millet (*Eleusine coracana)*, white variety CO9 was procured from Tamil Nadu Agricultural University (TNAU), Coimbatore, India. Glucoamylase (EC 3.2.1.3) from *Aspergillus niger* and α-amylase (EC 3.2.1.1) from *Bacillus licheniformis,* standard beech wood xylan, arabinose and xylose were obtained from Sigma, USA, while rhamnose, glucose, galactose and all other chemicals and reagents of analytical grade were purchased from HiMedia, Mumbai, India.

2.2. Methods

While rice bran was used as such, the seed coat from finger millet was separated as described below.

2.2.1. Preparation of finger millet CO9 seed coat

The finger millet grain was sprayed with 7% water, equilibrated for 10 min, and then pulverized in a local flour mill. The resulting meal was sifted through 85 mesh (180 μm) sieve, and the tailings were re-pulverized and sifted again. The process of pulverizing and sieving was repeated twice. The fraction which passed through the sieve at 1st, 2nd and 3rd stage were pooled and called as refined millet flour. Tailings from the 3rd stage grinding (with particle size 60 mesh, 250 μm) were collected and designated as seed coat (Shobana et al., 2009).

2.2.2. Extraction of water soluble xylan (WSX)

Prior to extraction, the sample was de-fatted using petroleum ether (boiling point 60° – 80° C) in a Soxhlet apparatus for 16 h. The de-fatted material was de-starched using enzymes as described below. Fifty grams of de-fatted samples were mixed in 100 mL of sodium acetate buffer (0.1 M, pH 4.8) and incubated with 1 mL of α– amylase (1000 U) at 95° C for 1 h. After cooling to room temperature (25±2° C), 100 mg of glucoamylase (7000 U) was added to the mixture and incubated at 55° C for 48 h followed by centrifugation at 3000 rpm for 20 min at room temperature (25±2° C). The supernatant consisting of glucose emanated from starch was discarded and the residue used for extraction of water soluble xylan (WSX).

De-starched samples (100 g) were extracted with double distilled water (250 mL) four times at room temperature (25±2° C) for 2 h each. The pooled supernatant obtained after centrifugation at 3000 rpm for 20 min, 25±2° C was precipitated with three volumes of absolute alcohol to remove the free sugars present. The precipitated xylan was further purified by dialysis (against double distilled water with 8 KDa dialysis membranes) and lyophilized to obtain WSX (Chithra and Muralikrishna, 2010). These samples were subjected to further analysis to understand the characteristics of the xylan.

2.2.3. Composition of WSX

The extracted WSX from the two sources were analyzed for its constituent sugars and elements by the methods described below.

2.2.3.1. GC-MS. The extract (10 mg) was hydrolyzed with 2 mL of 2 M trifluoroacetic acid (TFA) at 100 °C for 2 h. After removing TFA with methanol, derivatization of the released monosaccharide was carried out using hydroxylamine, acetic anhydride and pyridine according to the method of Albersheim et al. (1967). The derivatives were loaded onto a TG-5MS column (30 m × 0.25 mm x 0.25 μm) and determined by Gas Chromatography Mass Spectrometry (GC-MS) analysis (Thermo Scientific, TSQ QUANTUM XLS, USA). The settings were as follows: injector temperature: 300 °C; detector temperature: 280 °C; column temperature programmed from 140 to 300 °C at 10 °C min^{-1}; carrier flow rate at 1.0 mL min^{-1}; injection

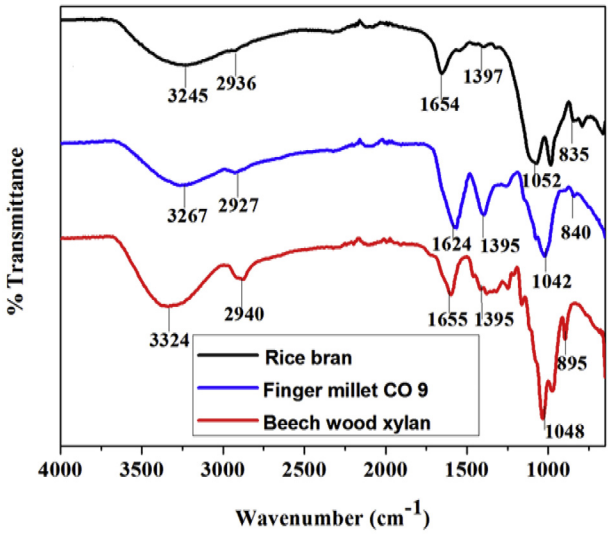

Fig. 1. ATR-FTIR characterization of water soluble xylan from rice bran, finger millet CO9 seed coat and standard beech wood xylan.