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Extraction of water soluble beta-glucan from rice bran

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Original Research Article

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Abstract: Beta- glucans are dietary fibers with many health benefits that are found in cereals. Extraction of beta- glucan from rice bran was not given much attention because of the lesser amounts of beta-glucan in them. This work explains the extraction, interaction with Congo red and FTIR spectral analysis of beta-glucan isolated from rice bran and comparison of results with that of oat beta-glucan. Rice bran contained 0.03-0.08 % of water-soluble beta- glucan. Both beta-glucans showed comparable FTIR spectra however, rice bran beta-glucan did not show bathochromic shift as shown by oat beta-glucan.
Keywords: Oats, Rice, Beta- glucan, Natural fiber, Congo red, Rice bran, Cereals

INTRODUCTION

In recent years, a great deal of interest has been developed to explore the health benefits of natural foods. Beta- glucan, a natural fiber found in microorganisms such as fungi, yeast etc., and cereals such as oats, barley, wheat and rice is conferred with many health benefits. Beta-glucan consumption inhibits the absorption of bile acid and cholesterol, thus reducing plasma cholesterol levels. It also increases HDL level and decreases LDL level in plasma [1-4]. Beta-glucan also boosts our immune responses by increasing blood cytokine level and phagocytic efficiency of natural killer cells and macrophages [5].

Beta-glucan from different sources varies in their length, degree of branching, type of glycosidic linkages, molecular weight, solubility and action. In cereals, beta-glucan is mainly found in the endosperm and the quantity of beta- glucan vary with the cereal variety and the growth conditions [6]. The largest quantities of beta- glucan are seen in barley (3-11 %), oats (3-7 %), rye (1-2 %) and wheat (<1 %).

Beta- glucan isolated from cereals are known for promoting metabolic activity especially in diabetes mellitus patients. Food formulations containing beta-glucan reduced postprandial glucose in Type 2 diabetic subjects [7]. Bread enriched with beta-glucan significantly improved the metabolic control and reduced glycated hemoglobin in Type 2 diabetes mellitus patients [8]. As dietary intake of beta- glucan is suggested to reduce the risk factor associated with diabetes and its complications and as it helps in the beneficial gut bacteria to flourish, beta-glucan is advised to diabetes patients as a prebiotic.

In countries like China, India, Indonesia, Thailand, Burma and Philippines where the production of cereals like rice is extensive, rice bran may act as an alternate source of beta-glucan. In India, rice production amounts to more than 40 % of total food grain production and the annual production of one million tons of bran is currently used for the production of rice bran oil and animal feed [9]. Though rice bran is similar to oats in crude protein, fat, fiber and energy, it was not investigated as a potential source of beta-glucan. This work was therefore attempted to extract beta- glucan from rice bran and the isolated beta- glucan was compared with that of oats.

MATERIALS AND METHODS

Isolation of beta- glucan

The isolation of rice bran beta-glucan and oats beta glucan was carried out according to the procedure of Jiang et al. (2000) [10] with slight modifications. For the extraction of water soluble beta-glucan, 20 g of rice bran/ oat flakes was mixed with 200 mL of distilled water with constant stirring at 37^{o}C for 20 h. Supernatant containing beta-glucan was collected by centrifugation at 7000 rpm for 15 min. The residue was repeatedly extracted for getting maximum yield. The pooled supernatants were treated with 250 µL of α-amylase and 175 mg of $CaCl_2$ at 40^{o}C for 16 h for removing starch. The reaction was stopped by boiling for 5 min. The resultant solution was filtered and made to 60 % using absolute ethanol before centrifuging again at 5000 rpm for 5 min. The resultant precipitate was washed, re-suspended in distilled water and lyophilized.

Absorption spectra after incorporation with Congo red

This procedure was performed according to procedures of Smiderle *et al*. (2014) [11] and Ishikawa et al. (1998) [12]. The dye concentration was optimized at 50 mM for the analysis. Dextran was used as a random coil control and the absorbance was recorded from 400 to 640 nm.

Quantification of β- glucan

Estimation of beta-glucan was carried out using Aniline blue -binding microassay that estimate 1, 3 β-glucan [13, 14]. Laminarin (0-10µg/mL) was used as reference standard. The fluorescence of the *β*-glucan-aniline blue complex was measured at an emission wavelength of 460 nm with an excitation wavelength of 400 nm.

Infrared transmission spectroscopy

FTIR analysis was conducted on Shimadzu IR 21 prestige FTIR spectrometer. The spectra were scanned in the range of 4000-640 $cm^{-1}$and the diffuse reflectance mode at a resolution of 4 cm^{-1}.

Statistics

All the experiments were conducted at least thrice and the yield is represented as mean± SEM.

RESULTS AND DISCUSSION

Consumption of soluble dietary fiber like beta-glucan is highly advocated as it possesses many immunomodulatory functions and cholesterol lowering ability. The highest amount of beta-glucan is observed in barley and in all cereals, it is concentrated mainly in the bran. Though some of the Asian countries produce major share of rice, the possibility of using rice bran as an alternative source of beta glucans have not been attempted. This study reports extraction of soluble beta-glucan from rice bran and its comparison with oats beta-glucan. By aniline blue method, the soluble beta-glucan extracted from rice bran showed a yield of comprised of 0.03-0.08 % of the rice bran. Rice beta-glucan was then compared with oats beta- glucan isolated from oats flakes.

Binding of beta-glucan with Congo red resulted in bathochromic shift in the absorption maxima from 490 nm to 500 nm for oats beta- glucan (Fig.2B). However, bathochromic shift was not observed with rice beta- glucan and therefore, estimation with Congo red was not possible in the case of rice beta-glucan [Fig.2C]. FTIR analysis showed absorption at 3593 cm^{-1}, 3510 cm^{-1}, 3383 cm^{-1}, 3122 cm^{-1} for oats and 3603 cm^{-1}, 3701 cm^{-1}, 3522 cm^{-1}, 3116 cm^{-1} and 3855 cm^{-1} for rice in the region of 4000-3000 cm^{-1}spectra pointing out the normal vibrational modes of asymmetric and symmetric stretching of OH groups of polysaccharides. The vibrational modes of asymmetric and symmetric stretches of CH groups was observed at 2941 cm^{-1} for oats and 2818 cm^{-1} and 2883 cm^{-1} for rice in the region of 3000-2840 cm^{-1}. The absorption peaks of β-glycosidic anomeric bonds was observed at 891 $cm^{-1}$for oats and 894 $cm^{-1}$for rice (Fig.1B,1D) [15]. The extracts however also contained protein as detected by the strong absorption peak at 1531 $cm^{-1}$for rice and1536 $cm^{-1}$for oats, which indicates the presence of amide linkages. Ring structure of glucose can be recognized by peaks at wave numbers of 1045 $cm^{-1}$for rice and 1093 $cm^{-1}$for oats [16] (Fig.1A,1C).

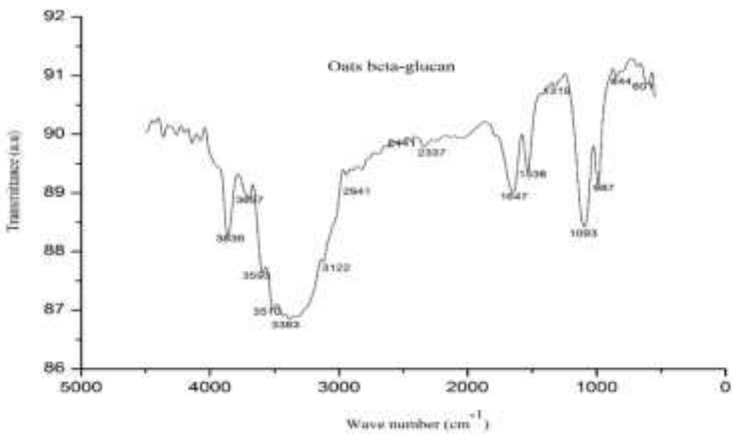

Fig-1A

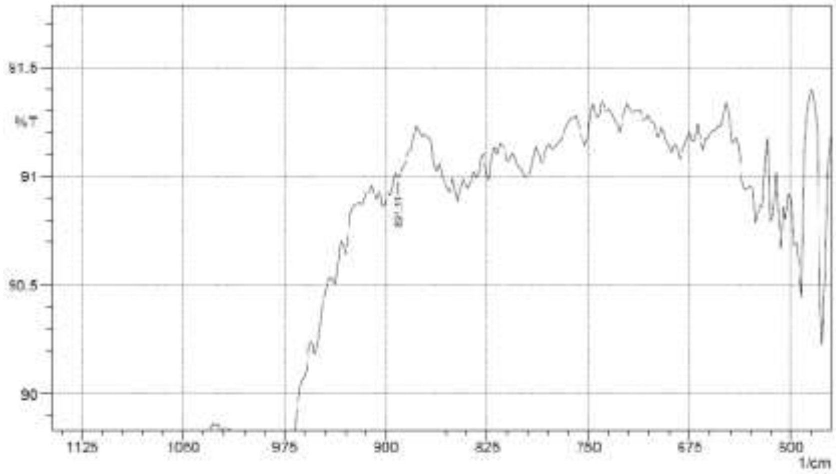

Fig-1B

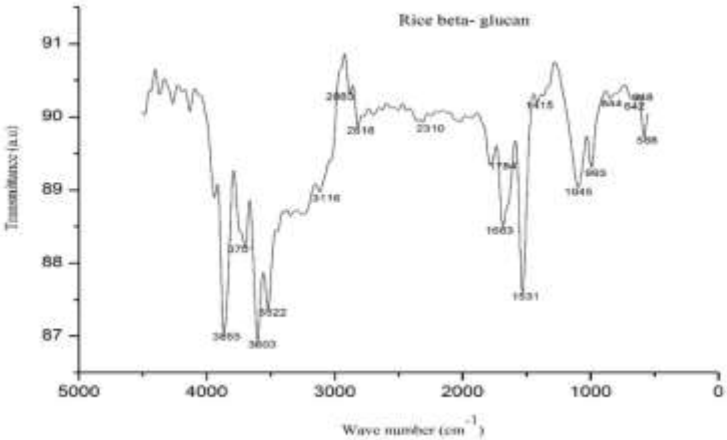

Fig-1C

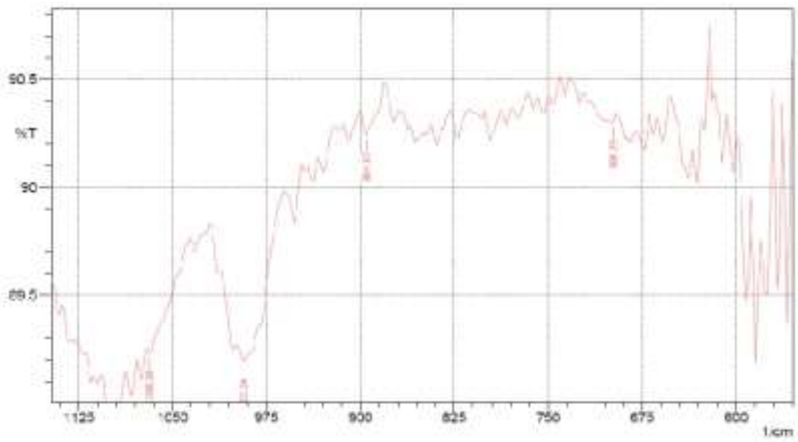

Fig-1D

Fig-1: Infrared spectra of (A) Oats beta- glucan (B) Peak denoting β-glycosidic anomeric bonds of oats beta-glucan (C) Rice beta- glucan (D) Peak denoting β-glycosidic anomeric bonds of rice beta- glucan.

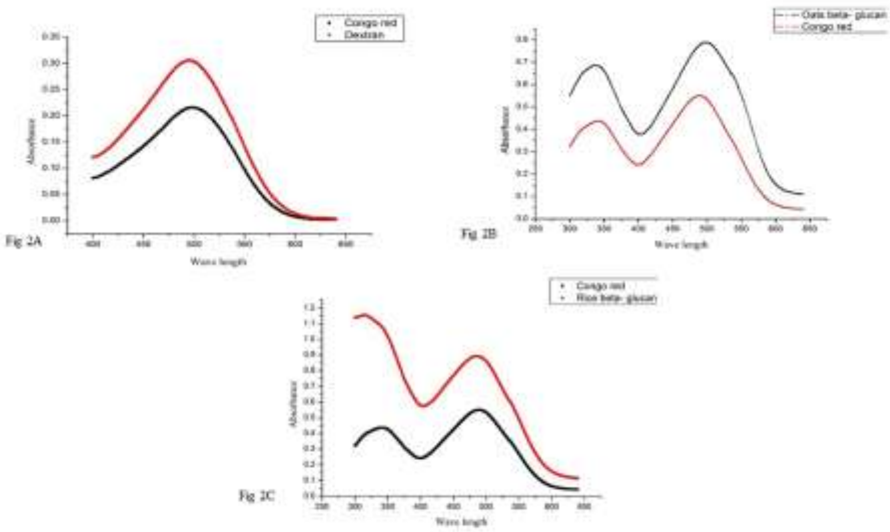

Fig-2: Absorption spectra of (A) Congo red and dextran (B) Congo red and oats beta- glucan (C) Congo red and rice beta- glucan

Several methods have been used earlier for the extraction of cereal beta- glucans from different cereal varieties. The quantity, quality and immunomodulatory effect of these dietary fibers can vary with the source, extraction procedure, structure, solubility etc. The enzymatic procedure described in this study gives considerable yield of water soluble beta-glucan from rice bran and can be used in large-scale extraction procedures. Further studies on their *in vivo* activity are necessary to assess their suitability to use them as a functional food. However, in major rice producing countries the milling by-product rice bran can be used as an alternative source of soluble beta-glucan or alternatively, rice bran can be consumed as a source of beta-glucan.

CONCLUSION

The results from above study show that beta-glucan can be effectively isolated from rice bran and in rice-producing countries; rice bran can be a potential source for beta-glucan. Beta-glucan isolated from rice bran and oat showed similar FTIR spectra however beta-glucan from rice-bran did not show bathochromic shift as shown by oat beta-glucan. The potential health benefits of rice-bran beta-glucan should be further studied and compared with other beta-glucan so as to bring it as a functional food.

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