

EXTRACTION AND CHARACTERIZATION OF ARABINOXYLANS FROM BRANS OF BG352, BW367 AND BG300 RICE VARIETIES AND EVALUATE ITS PROPRIETY FOR GLUTEN-FREE BREAD

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Abstract

Arabinoxylans (AXs) were isolated from brans of three varieties of rice. AX1, AX2, and AX3, respectively extracted from Bg 352, Bw 367 and Bg 300 rice varieties. The different AXs fractions were characterized and evaluated by incorporating into a gluten-free rice bread. The extractions consisted of $27.43 \pm 5.72\%$ amounts of AXs and the highest amount was resulted by AX1. The water-holding capacities of the extracted AXs were at a range of $1-2\text{g g}^{-1}$ (DM basis) which showed a significant difference among the varieties ($p < 0.05$). The highest oil holding capacity was observed in AX1 which was $3.74 \pm 0.09\text{g g}^{-1}$ (DM basis) and the range was $1-4\text{g g}^{-1}$ (DM basis). There was a positive linear relationship between the relative viscosities and concentrations of the AXs solutions. The relative viscosity decreased with the temperature of the AXs solutions. The AXs incorporated gluten-free rice bread showed a significant increment in moisture content ($p < 0.05$). Every treated bread with AXs showed a high increment in the water activity yet the rate of decrement showed no significant difference with the control ($p > 0.05$). The addition of AXs increased the moisture retention of the gluten-free rice bread but did not affect the rate of moisture loss during storage. The loaf volume showed significant increment at the 2% supplementation level of AXs in treated bread compared to 1% supplementation level and the control which indicates 2% supplementation is ideal to incorporate to gluten-free rice bread.

Keywords: Arabinoxylans, extraction, gluten-free bread, extraction, rice bran

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1. INTRODUCTION

In the early nineteenth century, a polysaccharide with high viscosity was extracted from wheat flour. Those compounds were named pentosans and found to be composed of mainly xylose and arabinose (Biliaderis *et al.*, 1995). The same polysaccharide was extracted from other cereals like durum wheat, rye, and barley. After that, these so-called pentosans became a research interest over the past few decades due to its functional and physicochemical properties. By being major non-starch polysaccharides in cereal by-products, the arabinoxylans have inspired considerable interest due to their viscosity enhancement in solutions, gelation ability, water absorption, solubility properties, and their effect on the rheological behavior of doughs and the properties in bakery products. Comparing to other polysaccharides like dextran, gum

Arabic, the AXs has reported a higher range of 2.5- 3.1 dl/g intrinsic viscosities (Pavlovich-Abril *et al.*, 2016). Due to the high ferulic acid, AXs has high oxidative gelation capacity (Izydorczyk and Biliaderis, 1992). Addition of Wheat AXs has shown to have a positive impact on the dough rheology, enhancing the water absorption and dough development time and bread quality parameters such as loaf volume, crumb texture and staling characteristics (Biliaderis, Izydorczyk and Rattan, 1995). As mentioned in Izydorczyk and Biliaderis, (1995) when the AXs was added to the wheat flour it competed with the other compounds for the added water. This increased the farinograph water absorption and dough development time. According to Koegelenberg and Chimphango, (2016) the addition of wheat bran AXs to the dough, the water absorption increased by 2%. The water absorption decrement due to the removal of sugar and gluten when the flour is removed can be

replaced using the AXs due to its inherent high water absorption capacity. The AXs has a positive effect on the loaf volume of bread also. As experimented by Izydorczyk and Biliaderis, (1995), the addition of purified AXs has been shown to enhance the loaf volume of bread.

Various procedures have been followed to isolate this compound from the cereal brans due to the water un-extractability such as Alkaline treatment (Gruppen *et al.*, 1991; Aguedo *et al.*, 2014; Pavlovich-Abril *et al.*, 2016), using enzymes (Beaugrand *et al.*, 2004; Collins *et al.*, 2005), and hydrothermal methods (Aguedo *et al.*, 2014).

Recently efforts were made to extract AXs from other cereals such as wheat (Gruppen *et al.*, 1991; Bergmans *et al.*, 1996; Courtin and Delcour, 2001; Aguedo *et al.*, 2014; Koegelenberg and Chimphango, 2017), rye (Nilsson *et al.*, 1996; Buksa *et al.*, 2016), Corn (Doner *et al.*, 1998) and were studied on their physicochemical and functional properties, a great deal of uncertainty, however, remains about those aspects in rice bran AXs.

Rice as the staple food for the Sri Lankan population, is produced around 1.47 million metric tons annually (Satharasinghe, 2017). The rice bran removed in Sri Lanka is around 4 % (Saunders, 1985) thus the annual rice bran production is around 58.8 thousand metric tons which are highly under-utilized except in some cases as animal feed. Hence the rice bran may be a source of choice in extracting AXs to utilize in food product development.

In this work, AX fractions were obtained from brans of three varieties of rice (Bg352, Bw367, and Bg300 respectively). Then the samples will be characterized and evaluated by incorporating into gluten-free rice bread.

2. MATERIALS AND METHODS

2.1 Sample Collection

Bg352, Bw367, and Bg300 rice varieties were obtained from Rice Research center, Bathalegoda, Sri Lanka and the respected brans were obtained from dry milling in a commercial two-stage mill.

2.2 Sample preparation

2.2.1 Lipid removal

A defatted rice bran was obtained by washing 300 g of rice bran from petroleum ether (0.2 L/100 g) twice. Vacuum filtration using Whatman 110 mm filter paper, was done to separate the fat dissolved petroleum ether from the bran residue. The bran residue was air-dried and passed through 0.25 mm sieve. The larger particles were collected, grounded and re-sieved (Annison, Moughan and Thomas, 1995).

2.2.2 De-starching

The procedure used to de-starch the de-fatted rice bran was based on a method described by Aguedo *et al.*, (2014) with some modifications. De-fatted rice bran was de-starched by mixing for 10 minutes with water at 95°C in a ratio of 1/10 (w/w). The suspension was filtered using muslin cloths and the retentate was dried at 100°C for one hour.

2.2.3 Protein removal

The dried retentate (200 g) resulted in the de-starching process was mixed with water (50-55°C) and incubated with the pancreatic enzyme (0.48 g, 1hour, pH 6.5-7.0) and cooled up to 40°C (Aguedo *et al.*, 2014).

2.3 Isolation of AXs from rice bran

The procedure used to extract rice bran AXs was based on the method described in Annison, Moughan and Thomas, (1995) with some modifications. The prepared rice bran solution was added sodium hydroxide (16 g) and sodium borohydride (0.768 g) and the mixture was allowed to stand for 1 hour. Then the mixture was neutralized using glacial acetic acid and allowed to stand for 16 hours. The mixture was centrifuged (10000 g, 30 minutes) and the supernatant was collected. Two volumes of ethanol were added and the precipitate allowed to settle. The precipitate was collected, freeze-dried and ground immediately. This material was rice bran AXs. This isolate was subjected to further analysis.

2.4 Characterization of AXs

2.4.1 Determination of total pentosan

Isolate (10 mg) was weighed into a test tube, 2 ml of 2N HCl was added, and the mixture was hydrolyzed at 100 °C for 2.5 hours. After

cooling, the neutralization was effected by the addition of 2 mL of 2 N Sodium carbonate. Fermentable sugars were removed by fermentation; 2 ml of 25mg/ml of 0.2M Na phosphate buffer (pH 7.0) was added in a suspension of fresh yeast (*Saccharomyces cerevisiae*), incubated, and mixed on a vortex mixer every 20 minutes for 1.5 hours at 30°C. The mixture was centrifuged (1000 g, 10 minutes). The supernatant (2 ml) was obtained and 1 ml of distilled water, 3 ml of FeCl₃ Solution (0.1% FeCl₃ in CONC HCl) and 0.3 ml of Orcinol (1.0% Orcinol in Ethanol) was added. The mixture was vortexed and heated in boiling water for 30 minutes. The absorbance was obtained at 670 nm after been cooled (Shogren, Hashimoto and Pomeranz, 1987).

2.4.2 Water holding capacity (WHC) and oil holding capacity (OHC)

WHC and OHC were determined according to Muhammad, (2012). Distilled water or commercial coconut oil (10 ml) was added to 20 mg of isolates stirred and left at room temperature for 1 hour. After centrifugation, the residue was weighed. The WHC was expressed as grams of water per grams of sample, while the OHC was expressed as grams of oil held per grams of sample.

2.4.3 Relative viscosity

The relative viscosity of the solutions was determined (concerning water) as a function of concentration (0.1-1%) and temperature (20-80°C) in an Oswald viscometer (Muralikrishna, Bhat and Tharanathan, 1987).

2.4.4 Solubility

Solutions (0.1% (w/w) dry basis) was prepared at the room temperature and stirred under magnetic stirring for 0.5,1,2, and 3 hours. Then those solutions were centrifuged (6000 g, 30 minutes) and recovered the supernatant. The supernatants were dried at 105°C for 2 hours (García *et al.*, 2004).

Equation

$$\text{Solubility} = \frac{\text{Supernatant concentration (mg/ml)}}{\text{Initial preparation concentration (mg/ml)}}$$

2.5 Evaluation of AXs

2.5.1 Gluten-free rice bread formulation

A straight dough process was employed in

preparing the gluten-free bread. The following ingredients were (as g/100g on flour basis) used: Sunflower oil (6 g/100 g), sugar (5 g/100 g), salt (2 g/100 g), instant yeast (3 g/100 g), and the extracted AXs (in 1%, and 2% on flour basis) (de la Hera *et al.*, 2013). The amount of water added was 130 g/100 g (Lazaridou *et al.*, 2007) which was adequate to give a consistent dough in the presence of other ingredients.

2.5.2 Bread making process

The baking was carried out in an electric oven. The yeast was dissolved in warm water (35 °C) and was added to the dry ingredients and the sunflower oil and then the mixture was blended for 2 minutes. The dough proofed at 25-30 °C for 20 minutes and subsequently baked at 215°C for 20-25 minutes following steaming for 10 minutes (Lazaridou *et al.*, 2007)

2.5.3 Bread quality parameters

After baking the loaves were kept until cooled to room temperature and weighed.

The loaf volume was determined using the volume displacement method using mustard seeds. Loaves were placed in a container of known volume into which mustard seeds were run until the container was full. The volume of seeds displaced by the loaf was considered as the loaf volume.

Loaf specific volume was calculated according to the following formula:

$$\text{Loaf Specific volume} = \frac{\text{Loaf volume (cc)}}{\text{Loaf weight (g)}}$$
 (Ranasalva and Visvanathan, 2014)

The moisture content of the crumb was determined by drying for 1 hour at 130°C (Buksa, Nowotna and Ziobro, 2016).

The water activity of the crumb was determined using the water activity meter in the intervals of 1 and 3 days after the baking of the bread which incorporated 1% and 2% AXs and the control.

3. RESULTS AND DISCUSSION

3.1 The total pentosan of the extracts

AX is a polysaccharide with predominantly two monomeric sugars arabinose and xylose mainly which are pentosans. Thus by the determination of the total pentosans in the

extracted samples, the amount of arabinoxylans present could be estimated.

In this study, the orcinol- hydrochloric method was followed where the interference from hexoses was minimized using yeast fermentation (Shogren, Hashimoto and Pomeranz, 1987). The results showed a significant difference between the total pentosan amount of the extracts from different varieties ($p < 0.05$), (**Table 1**).

Table 1. Total Pentosans (TP) of the AX fractions obtained from brans of Bg352, Bw367 and Bg300 rice varieties (AX1, AX2 and AX3 respectively). Value \pm SD with $n=3$ independent samples.

Extract AXs	Total pentosans %
AX1	33.18 ± 4.00^a
AX2	28.09 ± 0.78^b
AX3	21.02 ± 1.68^c

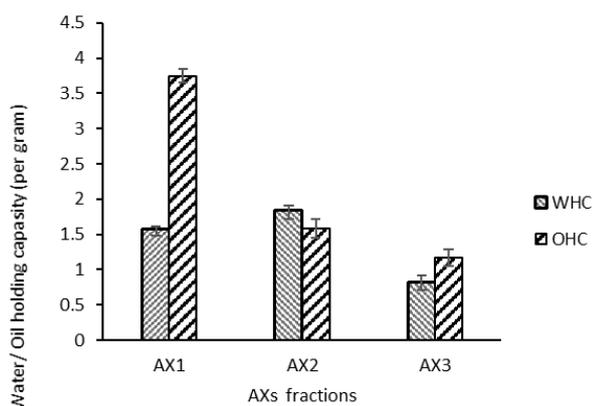


Fig. 1. The water holding capacity (WHC) and the Oil holding capacity (OHC) of the AX fractions obtained from brans of Bg352, Bw367 and Bg300 rice varieties (AX1, AX2 and AX3 respectively). Values were the average of three replicates. BG, Bathalegoda; BW, Bombuwala.

This result might indicate that the AXs amount in rice bran vary or the extraction procedure might affect differently on different varieties. Some studies have reported that the AXs are physically and chemically interlinked to lignin, cellulose or loosely bound to the cell materials which vary with the variety (Iiyama, Lam and Stone, 1994; Delcour, Van Win and Grobet, 1999) The hemicellulose fractions could

present different compositions according to the extraction method used (Höjje *et al.*, 2005). Hence the AXs present in the selected varieties would differ quantitatively and qualitatively which might cause the alkali treatment to act differently on different varieties.

The highest pentosan content was with AX1 sample obtained from Bg352 rice variety. A considerable $27.43 \pm 5.72\%$ of an average could be obtained from the followed procedure although compared to other processes, the amount and the purity were less. Wheat bran had resulted in 37.6% of AXs from alkali extraction (Aguedo *et al.*, 2014) while Koegelenberg and Chimphango, (2017) extracted $49.3 \pm 0.9\%$. The higher purity resulted in those processes would be due to the ultrafiltration and the dialysis they had followed for the purification. Further extraction using advanced purifying steps would result in similar observations from rice bran.

According to previous studies, the AXs extracted from rice bran using this method contained several impurities. The isolates were predominantly non-starch polysaccharides {63.5% (w/w, DM basis)} with less amount of protein {16.4% (w/w, DM basis)} and starch {14.1% (w/w, DM basis)} (Annison, Moughan and Thomas, 1995). Annison *et al.*, (1995) recorded that there was a high concentration of galactose with the isolates due to unbreakable covalent bondages present between galactose and AX molecules.

3.2 Water holding capacity (WHC) and oil holding capacity (OHC)

Concerning the water holding capacity (WHC) of the extracts, it could be observed that the AX2 demonstrated the higher value where the WHC range of the obtained isolates was between 1 to 2 g g^{-1} (DM basis). (Fig. 3) However relating the WHC values, the previous studies had shown otherwise. According to the review of Wang *et al.*, (2002), the AX has average 10g g^{-1} (DM basis) of WHC. The fewer results in this research might cause by impurities present or comparatively less amount of pentosan present. The oil holding capacity (OHC) values of the isolated

AXs fractions were in the same range {1-4g g⁻¹ (DM basis)}, as other hydrocolloids such as xanthan gum {1.28 ± 0.03 g g⁻¹ (DM basis)}, apple pectin {2.11 ± 0.17 g g⁻¹ (DM basis)}, and agar-agar {2.25 ± 0.07 g g⁻¹ (DM basis)}.

3.3 Relative viscosity

Stone, (1979) stated that the increment of arabinose substitution stiffens the xylan backbone by forming a rigid rod-like conformation which increases the viscosity of the prepared solutions. According to Annison, Moughan and Thomas, (1995), the A/X ratio of AXs from rice bran was 1.23 which indicates high arabinose substitution. That would explain the relative viscosity increment of the prepared AXs solutions. There was a significant strong linear correlation between the relative viscosity and the concentration of the extracted AXs solutions (Fig. 2).

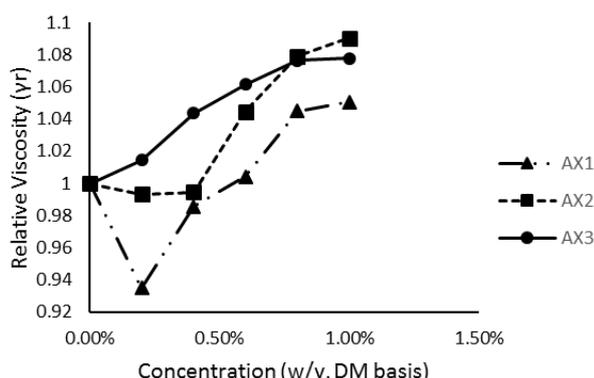


Fig. 2. Relative viscosity variation according to the concentration {(w/v), DM basis} of AXs solutions. AX1, AX2, and AX3 were the isolated AXs from brans of Bg352, Bw367 and Bg300 rice varieties respectively. BG, Bathalegoda; BW, Bombuwala.

The correlations for solutions of extracted AXs from Bg352, Bw367 and Bg300 were 0.960, 0.958 and 0.863 respectively.

The relative viscosity of the AX solutions decreased with temperature (20 °C 80 °C) (Fig. 3). A possible explanation of this result would be the high amount of chain interactions and increased thermal mobility of the molecules (Whistler, 1973).

3.4 Solubility

The solubility was measured in the extracts using the same amount dissolving in the same amount of distilled water (0.1% (w/w) dry

basis) stirring for different time durations (0.5,1,2, and 3 hours). No any significant variation was detected between the varieties or time durations. The results indicated that the isolates were highly soluble in water thus less effort should be provided to dissolve them.

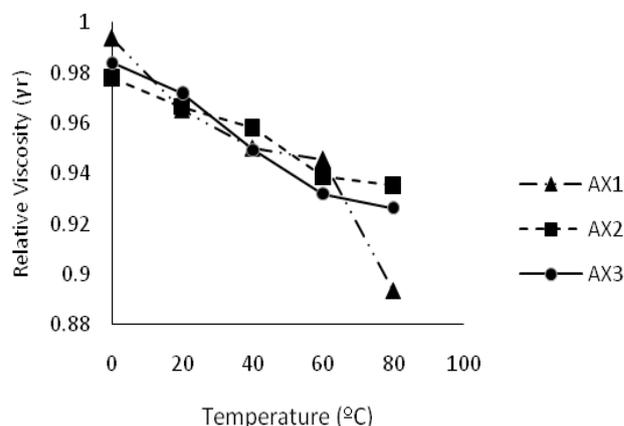


Fig. 3. Relative Viscosity variation according to the temperature (°C) of the AXs solutions {0.70% (w/v), DM basis}. AX1, AX2, and AX3 were the isolated AXs from brans of Bg352, Bw367 and Bg300 rice varieties respectively. BG, Bathalegoda; BW, Bombuwala

3.5 Bread quality parameters

The effect of the incorporation of isolated AXs from different rice brans into some quality parameters of fresh gluten-free bread is summarized in Table. 2, whereas the images of the slices of bread are illustrated in Fig. 4. Comparing the mean values obtained, there was a significant increase in the bread weight and moisture content which might be due to the increased water-absorbing ability due to AXs. However the variety or level of incorporation did not appear to affect the above parameters (p>0.05).

The specific volume of bread increased with the addition of isolated AXs whereas the higher specific volume was observed at 2% (w/w, flour basis) supplementation level compared to 1% (w/w, flour basis) supplementation and control. A possible explanation for the increment of loaf volume as given in many researches might be the increment of the dough viscosity. Hydrocolloids have water retention properties because of their hydrophilic nature. In contrast, the hydrophobic groups form gel networks which upon heating strengthen the boundaries of the expanding cells in the dough.

Table 2. Comparison of the properties of bread samples containing extracted AXs from brans of Bg352, Bw367 and Bg300 rice varieties (AX1, AX2 and AX3 respectively). Values \pm SD with n=2 independent samples.

Level of AXs incorporated (w/w, Flour basis)	Bread weight (g)	Specific Volume (cm ³ /g flour)	Final Moisture (%)
Control	116.00 \pm 0.71 ^a	1.04 \pm 0.02 ^a	1.56 \pm 0.00 ^a
AX1			
1%	128.00 \pm 1.41 ^b	1.04 \pm 0.02 ^a	0.87 \pm 0.00 ^b
2%	130.75 \pm 2.47 ^b	1.12 \pm 0.01 ^b	1.85 \pm 0.00 ^d
AX2			
1%	137.50 \pm 10.60 ^b	1.04 \pm 0.00 ^a	1.21 \pm 0.24 ^b
2%	131.50 \pm 2.82 ^b	1.16 \pm 0.00 ^b	2.36 \pm 0.55 ^d
AX3			
1%	138.25 \pm 2.47 ^b	0.98 \pm 0.02 ^c	1.63 \pm 0.03 ^c
2%	123.75 \pm 6.68 ^b	1.21 \pm 0.06 ^b	1.49 \pm 0.16 ^d

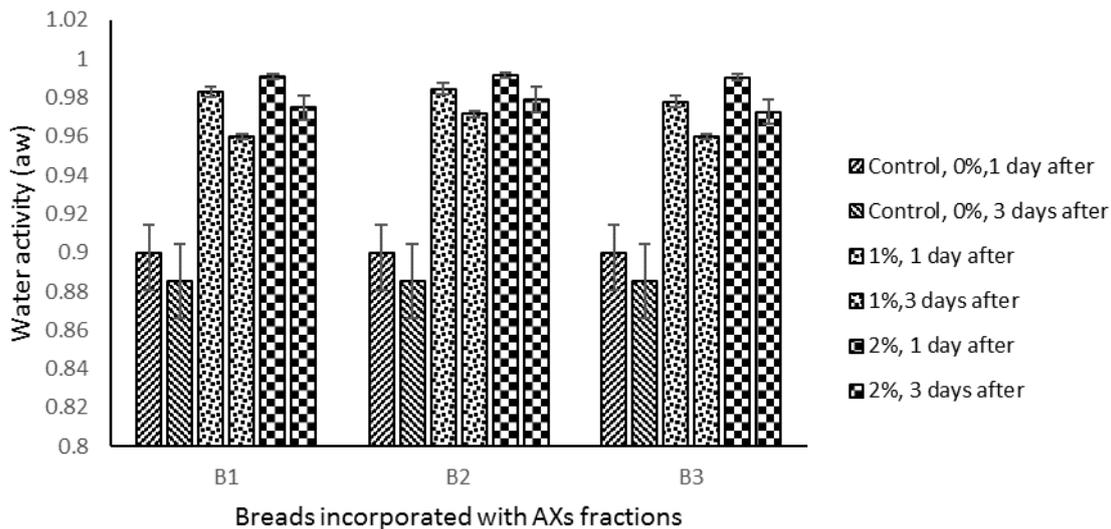


Fig. 4 Effect of the addition of different levels {0%, 1% and 2% (w/w, Flour basis)} of AXs isolated from Bg352, Bw367 and Bg300 on water activity (aw) of crumb of gluten free breads (B1, B2 and B3 respectively) after storage for 1 and 3 days at 5 °C. Values are the average of two replicates where error bars represent the standard deviations. ; BG, Bathalegoda; BW, Bombuwala

This increases the gas retention throughout the baking process which leads to better loaf volume (Rosell, Rojas and Barber, 2001).

In Fig. 4 the water activity (aw) values for the bread crumb of all gluten-free bread formulations after 1 and 3 days of storage are given. Water activity is one of the important factors which can be changed in bread during the storage period. Due to the moisture loss the water activity. decreased in both controlled and AXs added to bread. In controlled bread, the aw varied from 0.88 to 0.9 at the 1st and 3rd day of storage. However, when AXs were added

the bread showed higher aw compared to control and the level of supplementation did not significantly affect initial moisture retention ($p > 0.05$). The decrement of aw between 1st day and 3rd day was similar in both controlled and AXs added to bread. The controlled bread retained the least amount of water probably due to its evaporation in the initial stages of baking before starch gelatinization. The addition of AXs inhibited this process thus allowing retention of a higher amount of moisture in bread crumb resulting in a soft crumb. However, the factors affecting the

moisture loss during the storage period were not harmed by the supplementation thus the aw decrement was as same as the controlled sample. Nevertheless, the AXs incorporated bread might remain softer for a longer duration than the control.

4. CONCLUSION

Extractions from tested varieties consisted considerable $27.43 \pm 5.72\%$ amount of AXs. Isolated rice bran AXs had the water holding capacity at a range of $1-2\text{g g}^{-1}$ (DM basis), and oil holding capacity at a range of $1-4\text{g g}^{-1}$ (DM basis) The relative viscosity increased with the concentration of AXs in the solutions and decreased with the temperature of the AXs solutions. The AXs incorporated bread showed higher moisture content and water activity compared to the control. Addition of AXs allows retention of a higher amount of moisture in bread thus the factors affecting the moisture loss during the storage period were not impacted by the supplementation The 2% supplementation is ideal in terms of increased loaf volume of gluten-free rice bread. The AXs extracted from the rice brans have the potential to utilize as a food ingredient. Further studies should address the methods of extraction in terms of purity and yield and the composition of the extracts.

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