Pertanika J. Trop. Agric. Sci. 47 (1): 25 - 40 (2024)

PERTANIKA JOURNALS

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

Ultrasound and Steam Explosion Treatments on the Quantity and Molecular Size of Soluble Fibre Obtained from Un-purified and Purified Rice Bran

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ABSTRACT

Rice bran (RB) is a major by-product of the rice industry, and the high proportion (~90%) of insoluble fibre (IF) is the main reason limiting its applications in foods. Thus, the objective of this research is to enhance the solubility of rice bran fibre and decrease the molecular weight (MW) of the soluble fibre (SF) fraction through ultrasound (US) and steam explosion (SE) treatments. The main sugars in the RB fibre were xylose and arabinose, with glucose, galactose, and mannose present in the side chains. The ratio of Ara/Xyl was 0.92 for the un-purified and 1.02 for the purified RB, reflecting the high degree of substitution of the xylan backbone. The highest amount of SF was obtained from RB treated at 60% US amplitude, 20 min treatment, where 7.8% (un-purified) and 35.2% (purified), respectively. For SE treatments, the amount of SF in un-purified RB increased as the pressure increased from 0.3 and 0.6 MPa, which were 6.10±0.34 and 8.83±0.56%, respectively. Meanwhile, the highest SF fraction (35.2%) of purified RB was obtained from the SE treatment at 0.6 MPa. The SF produced from both treatments mainly contained oligosaccharides with MW <1 kDa, with those produced by the SE treatment generally smaller than those by the US treatment. Purification of RB significantly enhanced the efficiency of the US and SE treatments in breaking down the IF into the SF.

Keywords: Fibre, purified, rice bran, solubility, steam explosion, ultrasound

ARTICLE INFO

Article history:
Received: 08 June 2023
Accepted: 04 October 2023
Published: 03 January 2024

DOI: https://doi.org/10.47836/pjtas.47.1.03

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ISSN: 1511-3701
e-ISSN: 2231-8542

INTRODUCTION

Physical treatments have been well used to modify the native structure of cereal fibre as they offer many advantages over chemical treatments, such as being economical, environmentally safe, and industrially practical (Brodeur et al., 2011). Among the physical treatments, ultrasound and steam explosion are well known for their effectiveness in cutting off the polymeric structure of cereal bran fibre into shorter chains, which led to an increase in fibre solubility by generating soluble oligosaccharides and improving the technological and health properties of the fibre (Daou & Zhang, 2012a; Jiang & Guo, 2016). With regard to the modification of dietary fibre, ultrasound treatment has been applied to a number of cereal bran and fibre materials including corn bran and cob, wheat bran and straw, rice bran, sugarcane bagasse, buckwheat hulls, hazelnut skin, mulberry leaves, cellulose, and citrus pectin (Daou & Zhang, 2012b; Ebringerová & Hromádková, 2002, 2010; Hromádková & Ebringerová, 2003; L. Zhang et al., 2013; Sfalcin et al., 2015; Sun & Tomkinson, 2002; Wang et al., 2014; Yilmaz & Tavman, 2016; Ying et al., 2011; You et al., 2014).

Meanwhile, steam explosion treatment has been applied in many non-food areas, including producing bioenergy, biomass and chemicals, and environmental protection. More recently, it has attracted the attention of food researchers for its capacity to break down lignin (Han et al., 2010; L.-H. Zhang et al., 2008) and fibrous networks such as cellulose and hemicellulose (Jiang & Guo, 2016), which leads to modification of their physicochemical properties. However, the treatment conditions explored in these studies are limited, and, to date, there is no report on the use of this technique to treat rice bran fibre to improve its chemical properties, specifically on the sugar composition and solubility. Therefore, this study aims to determine the effect of ultrasound and steam explosion treatments on the solubility of un-purified and purified rice bran fibre and the molecular weight distribution of the SF fractions, making it more applicable in a variety of food products and potentially be used as a prebiotic.

MATERIALS AND METHODS

Samples, Chemicals, and Materials Used

RB used in this study was donated by SunRice (Australia). Dextran standards with molecular weight (MW) of 2000, 670, 410, 80, 50, 25, 12, 5, and 1 kDa, raffinose pentahydrate ≥99% (high-performance liquid chromatography [HPLC], 594.51 g/mol), dinitro-salicylic acid, 1-methyl-imidazole, allose, rhamnose, fucose, calcium chloride dihydrate, sodium tetra-hydroborate, potassium sodium tartrate, pancreatin, and pullulanase were purchased from Sigma-Aldrich (Australia). Termamyl α-amylase was donated by Novozymes Australia Pty. Ltd. (Australia). Ethanol, acetone, glacial acetic acid, benzoic acids, hydrochloric acid, D-glucose anhydrous, arabinose, xylose, mannose, galactose, ammonium hydroxide, potassium hydrogen phosphate, and potassium hydroxide

were purchased from Chem Supply Pty. Ltd. (Australia). Sulphuric acids, acetic anhydrate, octan-2-ol, bromophenol blue, monobasic sodium phosphate, and dibasic sodium phosphate were purchased from Ajax Chemical Pty. Ltd. (Australia). Phenol and dimethyl sulfoxide (DMSO) were purchased from Ajax Finechem Pty. Ltd. (Australia).

Rice Bran Purification

RB was defatting following the method described by Uraipong and Zhao (2016). The bran samples were dried at 60ºC overnight in an oven and finally stored in sealed polyethene bags at 4°C before purification. RB fibre was purified by removing starch and protein following the procedure described by Hu et al. (2015) with minor modifications. The defatted rice bran 74 was mixed with MiliQ water at 1:10 (w/v) and placed in a shaking water bath with continuous shaking at 250 rpm at 55°C for 2 hr. After that, the pH of the mixture was adjusted to 9.5 by dropwise addition of 5% sodium hydroxide (NaOH) solution. The mixture was then centrifuged at 9,600 ×*g* for 30 min, the supernatant discarded, and the residue washed with MiliQ water several times (each with centrifugation) to remove protein.

Biuret reagent was used to confirm the absence of protein in the supernatant. The protein-free residue was added with MiliQ water at a ratio of 1:5 (w/v), and the pH of the mixture was adjusted to 6.5 with 10% (v/v) acetic acid before adding 1 ml of Termamyl α-amylase. The mixture was digested in a water bath at 100°C with continuous shaking at 250 rpm for 1 hr. After cooling to room temperature, the mixture was centrifuged again at 9,600 ×*g* for 20 min, the supernatant was removed, and the residue was washed with MiliQ water twice (by centrifugation) to remove starch. The absence of starch in the digested rice bran was confirmed using an iodine staining solution.

Ultrasound Treatment

Ultrasound (US) cavitation treatment was applied to defat and purify RB using a sonication immersion probe (20 kHz and 450 W, Branson Sonifier 450, USA). Defatted RB was dispersed in water at a ratio of 1:30 (w/v) in a 250 ml beaker, and the treatment was performed at three different power amplitudes, 60, 80, and 95% and time for 5, 10, 15, and 20 min for each power setting. The beaker containing the sample was partially immersed in an ice water bath to ensure that the samples did not become overheated. The temperature of the slurry during the treatment was maintained at 25±5°C. The sonicated samples were lyophilised (Leybold Lyovac GT2, Germany), sieved to pass 450 µm mesh size, and stored at -18°C until further analysis (Ismail & Zhao, 2022).

Steam Explosion (SE) Treatment

SE treatment (SE) of the bran samples (defatted and purified RB) was performed using a QBS-80 batch SE apparatus (Hebi Steam Explosion Research Center, China). Samples (50 g) were treated at 0.3 MPa

(144°C) and 0.6 MPa (165°C) for 120 and 180 s, respectively. All treated samples were lyophilised (Christ Alpha 1-2LD, Germany), sieved to pass 450 µm mesh size and kept at -18°C for further analysis (Ismail & Zhao, 2022).

Determination of Sugar Composition and Solubility of Rice Bran Fibre

The solubility of RB dietary fibre before and after US and SE treatments was determined by analysing the total amount of neutral sugars (rhamnose, fucose, arabinose, xylose, mannose, galactose, and glucose) in the total fibre and IF fractions of the samples by gas chromatography with flame ionised detector (GC-FID, Agilent 7890A, USA) according to the method introduced by Englyst et al. (1992) and optimised by Ma et al. (2017) with a minor modification. The soluble fraction was derived by subtracting the total and insoluble fractions. The soluble fraction was derived by subtracting the total and insoluble fractions. The samples were subjected to enzymatic digestions to release the simple sugars, which were then derivatised to make them volatile before the gas chromatography (GC) analysis.

Reducing Sugar

Reducing sugars released after US and SE treatments were measured using dinitrosalicylic acid (DNSA) reagent with a spectrophotometric method. The treated RB fibre (1 g) was dispersed in 50 ml deionised water, vortex mixed and centrifuged at 1,000 ×*g* for 20 min. The supernatant was collected and used to analyse the reducing sugar. The supernatant (2 ml) was diluted with 1 ml deionised water, added with 3 ml DNSA reagent, and heated in a boiling water bath for 15 min. After that, the sample was taken out, and 1 ml of 40% (w/v) potassium sodium tartrate solution (Roschell salt) was added immediately and vortex mixed. The mixture was let to cool to room temperature for 30 min, and the intensity of colour produced was read using a spectrophotometer (Spectramax M5e microplate reader, Molecular Device LLC, USA) at 540 nm. A standard curve was plotted by using xylose as a standard at the concentration range of 0.01-0.07 mg/ml in deionised water to determine the concentration of reduced sugar in the supernatant after US and SE treatments.

Determination of Molecular Weight Distribution of Rice Bran Soluble Fibre

The molecular weight distribution of soluble fractions after treated with SE and US was determined using size exclusion high-performance liquid chromatography (SE-HPLC) (LC-20AD, Shimadzu DGU-20A5 Degasser and Shimadzu SIL-20A HT Autosampler, USA) equipped with a refractive index (RI) detector (Shimadzu RID-10A, USA) and a size exclusion column PolySep-GFC-P-Linear column (L:300 mm × i.d: 7.5 mm; Phenomenex, Inc., USA). The elution conditions were as follows: isocratic elution with deionised water as mobile phase, column temperature 35°C, flow rate 0.8 ml/min for 30 min and injection volume 15 µl. Nine dextran standards with molecular weight (MW)

2000, 670, 410, 80, 50, 25, 12, 5, 1 kDa, and raffinose (0.6 kDa), maltose (0.3 kDa), and xylose (0.15 kDa) were used to construct a standard curve. Each standard was dissolved in deionised water at concentrations 0.25, 0.5, 2.0, 3.0, 4.0, and 5.0 mg/ml and left overnight at 4°C to allow it to swell.

For sample preparation, 1 g of RB was dispersed in 30 ml of deionised water, mixed well for 30 min, and centrifuged at 1,400 ×*g* for 20 min. The supernatant was collected and freeze-dried for 72 hr to yield a dry mass of the soluble fraction. After that, the soluble fraction was dissolved in deionised water at a concentration of 1 mg/ml and left overnight at 4°C to allow it to swell. The standards and samples were then filtered through a 0.45 µm membrane filter before being transferred into 1 ml glass vials for injection. A standard curve was built individually (0.25, 0.5, 2.0, 3.0, 4.0, and 5.0 mg/ml), and it was confirmed that the standard concentration is directly proportional to the peak area of the chromatogram ($R^2 = 0.98$). A mixed standard curve (log MW vs retention time) consisting of all dextran standards was also prepared to determine each standard's retention times. The equation from the curve was used to estimate the molecular weight of the SF components in the samples.

Statistical Analysis

The experiments were repeated twice, and all data was collected in triplicate. Data were analysed by one-way analysis of variance (ANOVA) to determine the significant differences, and Tukey pairwise comparisons were used to compare the significant differences between the treatments. The statistical analysis was performed using Minitab version 17 (USA).

RESULTS AND DISCUSSION

Sugar Composition of Rice Bran Fibre

The sugar compositions of un-purified and purified RB are shown in Table 1. Purification led to changes in sugar compositions, particularly the loss of glucose. The loss of glucose could be due to the high heat treatment (100°C) applied during the removal of starch in the purification process, which might have caused the breakdown of some glucose in the side chains of the rice bran fibre. The main sugars found in the rice bran fibre were xylose and arabinose. Rice bran fibre is expected to have a xylan backbone (β-1, 4-D-xylan) with a highly branched structure made mainly of arabinose and xylose (Shibuya & Iwasaki, 1985). Glucose, galactose, and mannose were also present in these samples and suspected to be located at the side chains. The degree of substitution or branching of

Table 1
Sugar composition in the total fibre fraction of un-purified and purified rice bran fibre

Neutral sugars	Amount (%)	
	Un-purified	Purified
Xylose	49.40±3.21	48.07±3.51
Arabinose	45.32±3.42	48.80±2.74
Glucose	3.78±0.05	0.99±0.03
Galactose	1.21±0.01	1.14±0.02
Mannose	0.13±0.02	0.14±0.01
Rhamnose	0.06±0.00	0.06±0.00
Fucose	0.04±0.00	0.05±0.00
Total sugars	99.94±6.71	99.77±6.30

the xylan backbone is reflected by the ratio of arabinose to xylose (Ara/Xyl). A higher ratio indicates a higher degree of substitution on the xylan backbone (Izydorczyk & Biliaderis, 1995). As reported in previous studies (Shibuya & Iwasaki, 1985; Shiiba et al., 1993), the ratio of Ara/Xyl is particularly high in cereal bran (0.98–1.76), which agrees with the finding in this study, where it was 0.92 for the un-purified and 1.02 for the purified RB. The higher degree of branching also suggested higher heterogeneity of rice bran fibre structure, which could influence the hydrolysis of the polysaccharide chain.

Effect of Ultrasound and Steam Explosion Treatment on Chemical Composition of Rice Bran Fibre

The effect of US and SE treatments on the chemical properties of RB fibre was investigated by analysing changes in solubility, molecular weight, and reducing sugar released after the treatments.

Solubility of Rice Bran Fibre After Ultrasound and Steam Explosion Treatments

The effect of US and SE treatments on the solubility of RB fibre was analysed by comparing the amount of IF retained after the treatments for both un-purified and purified RB with the total fibre content before the treatments. The total fibre content of untreated RB (un-purified and purified) was used as a control to obtain the amount of soluble compounds (A) after the treatments. Then, the reducing sugar content (B) in the soluble fraction was determined to obtain the amount of SF by subtracting B from A (A-B), and the data are shown in Tables 2 and 3 for US-treated samples and Table 4 for SE-treated samples, respectively. For the untreated RB (both un-purified and purified), the SF is directly calculated by the difference between the amounts of total fibre and IF in the samples. However, for the US and SE-treated RB, the amount of SF could not be directly calculated by the difference as the treatments had caused changes in the native structure of the fibre, leading to changes or reductions in the total fibre content as some of the SF, which had shorter polysaccharide chains could be broken down into simple sugars (i.e., disaccharides and monosaccharides), and lost during the extraction process.

In un-purified RB (Table 2), the total fibre, IF, and SF were 20.67±0.37, 17.47±0.24, and 3.20±0.6%, respectively. A fluctuating trend of decreases in IF content was observed after US treatment at 60% amplitude for various lengths of time. It could be due to the presence of starch and protein, which hindered the effect of US treatment on the fibre, as discussed in the previous study (Ismail & Zhao, 2022).

However, after treatment at 80 and 95% US amplitudes, the IF content decreased significantly ($p<0.05$) as a function of both US amplitude and time, with corresponding increases in the amount of SF. Reducing sugar content increased after the US treatments compared to the untreated sample. The most soluble fraction was obtained at 60% US amplitude and 20 min treatment. At 95% amplitude of

US treatment, an inconsistent trend in the SF amount produced was observed as the treatment time increased from 5 to 20 min. One probable reason for this could be that the high amplitude of US further broke down the SF into reducing sugars as the amount of reduced sugar increased when the treatment time increased.

The untreated purified RB contained 65.90±4.4% total fibre, 49.27±2.7% IF, and 16.64±0.9% SF (Table 3). It was assumed that the other 34.1% of the material (other than total fibre) was comprised of lignin, which was not made of sugar components and, thus, could not be detected by the GC analysis. After treatment with US or SE, a significant decrease in IF fraction was observed under all conditions with respect to time, amplitude, and pressure. The SF fraction of purified RB increased after US

Table 2
Effects of ultrasound treatment on un-purified rice bran fibre fractions

Ultrasound amplitude (%)	Ultrasound time (min)	Total fibre (%)	Insoluble fibre (%)	Soluble compound (%) A	Reducing sugars (%) B	Soluble fibre (%) A-B
0 (Untreated)	0	20.7±0.4	17.5±0.2$^{A,\ a,\ x}$	5.7±0.1$^{C,d,\ y}$	2.5±0.1$^{C,\ b,\ z}$	*3.2±0.6$^{B,\ c,\ x}$
60	5	n/a	13.7±0.2^{B}	6.9±0.2^{B}	3.1±0.1^{B}	3.8±0.6^{B}
	10	n/a	15.3±2.4^{A}	5.5±0.3^{C}	3.2±0.1^{B}	2.3±0.2^{C}
	15	n/a	14.3±2.2^{B}	6.5±1.2^{B}	3.0±0.1^{B}	3.5±1.1^{B}
	20	n/a	9.1±0.7^{C}	11.7±1.3^{A}	4.0±0.1^{A}	7.8±1.4^{A}
80	5	n/a	13.3±0.5^{b}	7.5±0.5^{c}	4.8±0.1^{a}	2.8±0.3^{d}
	10	n/a	12.0±3.9^{b}	8.9±0.5^{b}	4.8±0.1^{a}	4.1±0.8^{b}
	15	n/a	9.7±1.7^{c}	11.2±0.2^{a}	4.7±0.1^{a}	6.5±0.3^{a}
	20	n/a	9.6±0.8^{c}	11.2±1.6^{a}	4.7±0.1^{a}	6.5±0.8^{a}
95	5	n/a	17.4±0.2^{x}	3.3±0.1^{z}	3.1±0.1^{y}	0.1±0.7^{z}
	10	n/a	12.8±2.5^{y}	7.9±2.3^{x}	3.2±0.1^{y}	4.7±0.4^{w}
	15	n/a	13.3±0.5^{y}	7.4±1.5^{x}	5.5±0.1^{w}	1.9±0.6^{y}
	20	n/a	10.0±0.4^{z}	10.7±1.3^{w}	5.1±0.1^{x}	5.6±0.6^{v}

Note.
For untreated, the soluble fibre* is directly calculated by deducting total fibre from insoluble fibre.
For ultrasound-treated, the soluble compounds (A) are calculated by deducting the total fibre of untreated samples from the insoluble fibre of ultrasound-treated soluble compound (A), which is the sum of reducing sugar and soluble fibre.
For ultrasound treatment, the soluble fibre is calculated by deducting soluble compounds (A) to reduce sugar (B), A-B.
$^{A-C}$ = Means with different superscript letters within the same column of similar US amplitude differ significantly (p<0.05).
$^{a-c:}$ = Means with different superscript letters within the same column of similar US amplitude differ significantly (p<0.05).
$^{w-z}$ = Means with different superscript letters within the same column similar US amplitude differ significantly (p<0.05).
Values are means±S.D.; n = 3; n/a = Not available.

Table 3
Effects of ultrasound treatment on purified rice bran fibre fractions

Ultrasound amplitude (%)	Ultrasound time (min)	Total fibre (%)	Insoluble fibre (%)	Soluble compound (%) A	Reducing sugar (%) B	Soluble fibre (%) A-B
0 (Untreated)	0	65.9±4.4	49.3±2.7$^{A, a, x}$	22.0±1.7$^{C, b, y}$	5.4±0.0$^{C, b, z}$	*16.6±0.9$^{C, c, z}$
60	5	n/a	44.1±4.2^{A}	21.8±3.0^{C}	7.1±0.0^{A}	14.7±2.1^{C}
	10	n/a	26.2±4.1^{B}	39.7±2.1^{B}	6.8±0.1^{B}	32.9±1.0^{B}
	15	n/a	26.9±0.9^{B}	39.0±6.9^{B}	7.0±0.0^{A}	32.0±0.3^{B}
	20	n/a	23.6±1.2^{B}	42.3±2.2^{A}	7.2±0.1^{A}	35.2±0.6^{A}
80	5	n/a	32.8±6.4^{b}	33.1±7.1^{a}	6.8±0.0^{a}	26.3±0.4^{b}
	10	n/a	32.3±3.5^{b}	33.6±3.6^{a}	5.3±0.0^{b}	28.3±1.2^{b}
	15	n/a	29.3±3.2^{b}	36.6±1.3^{a}	5.4±0.0^{b}	31.2±1.3^{a}
	20	n/a	27.1±3.3^{b}	38.3±3.3^{a}	5.6±0.2^{b}	33.2±1.0^{a}
95	5	n/a	33.9±0.8^{y}	32.1±8.5^{x}	7.5±0.1^{w}	24.6±0.3^{y}
	10	n/a	27.9±2.2^{z}	38.0±7.2^{x}	6.8±0.0^{y}	31.3±0.7^{w}
	15	n/a	31.2±1.0^{z}	34.8±8.3^{x}	7.0±0.0^{x}	27.8±0.5^{x}
	20	n/a	34.3±1.5^{y}	31.6±7.8^{x}	6.8±0.2^{y}	24.7±0.8^{y}

Note.
For untreated, the soluble fibre* is directly calculated by deducting total fibre from Insoluble fibre.
For ultrasound treated, the soluble compounds (A) are calculated by deducting the total fibre of untreated samples from the insoluble fibre of ultrasound treated.
For ultrasound treatment, the soluble fibre is calculated by deducting soluble compounds (A) to reduce sugar (B), A-B.
$^{A-C}$ = Means with different supersript letters within the same column of similar US amplitude differ significantly (p<0.05).
$^{a-c}$ = Means with different supersript letters within the same column of similar US amplitude differ significantly (p<0.05).
$^{w-z}$ = Means with different supersript letters within the same column of similar US amplitude differ significantly (p<0.05).
Values are means±S.D.; n = 3; n/a = Not available.

treatment at all amplitudes. The highest amount of SF was achieved at 60% US amplitude and 20 min. However, at 95% US amplitude, a further increment of US treatment time from 10 to 20 min led to an increase in IF content and a corresponding decrease in the SF fraction and reduced sugars compared to 5 min treatment. Furthermore, it was also observed that high levels of reducing sugars were released after 5 minutes of treatment at all US amplitudes, with the highest amount recorded at 95% US amplitude. It demonstrated that, for a shorter treatment time, the US broke down the side chains of the RB fibre, releasing reducing sugars.

For un-purified RB treated with the SE, the amount of reduced sugars decreased compared to untreated samples, as shown in Table 4. It was likely due to the high temperature and pressure (0.3 MPa, 144°C and 0.6 MPa, 165°C) used that had caused

Table 4
Effect of steam explosion treatment on purified and un-purified rice bran fibre fractions

Rice bran	Time (min)	Steam explosion pressure (MPa)	Total fibre (%)	Insoluble fibre (%)	Soluble compounds (%) A	Reducing sugar (%) B	Soluble fibre (%) A-B
Un-purified bran	0	0.0	20.7±0.4	17.5±0.2^{A}	5.8±0.2^{C}	2.5±0.1^{A}	*3.2±0.6^{C}
	2	0.3	n/a	13.9±0.2^{B}	6.9±0.1^{B}	0.8±0.0^{C}	6.1±0.3^{B}
	2	0.6	n/a	10.9±0.3^{C}	9.9±0.4^{A}	1.1±0.0^{B}	8.8±0.6^{A}
Purified bran	0	0.0	65.9±4.4	49.3±2.7^{a}	22.0±1.7^{c}	5.4±0.0^{b}	*16.6±0.9^{c}
	2	0.3	n/a	27.1±2.9^{b}	38.9±0.7^{b}	6.6±0.1^{a}	32.3±0.4^{b}
	2	0.6	n/a	24.3±1.0^{c}	41.6±1.0^{a}	6.4±0.0^{a}	35.2±0.7^{a}

Note.
For untreated, the soluble fibre* is directly calculated by deducting total fibre from insoluble fibre.
For steam explosion treated, the soluble compounds (A) are calculated by deducting the total fibre of untreated samples from the insoluble fibre of steam explosion treated.
For steam explosion treated, the soluble fibre is calculated by deducting soluble compounds (A) to reducing sugar (B), A-B.
$^{A-C}$ = Means with different superscript letters within the same column of un-purified rice bran differ significantly (p<0.05).
$^{a-c}$ = Means with different superscript letters within the same column of purified rice bran differ significantly (p<0.05).
Values are means±S.D.; n = 3.

a further breakdown of the SF and reducing sugars into molecules such as furfural and hydroxymethylfurfural (HMF) (Álvarez et al., 2017), thus making it unable to be detected in the sugar analysis. Furfural and HMF can be formed from the dehydration of pentoses or hexoses due to intense SE treatment (Ramos, 2003).

Similar effects were also observed by Gong et al. (2012), who found that a reduction in soluble carbohydrates occurred after severe SE treatment (temperature range: 210-250°C; time range: 10-120 s) was applied to barley bran. Although the temperatures used in this study were lower, the treatment time was longer, thus making it possible for the formation of furfural and HMF that contribute to the browning effect on the samples, and this is confirmed by the colour analysis as shown in the previous work (Ismail & Zhao, 2022). Nevertheless, the amount of SF in the stream explosion treated samples increased from 6.10±0.34 to 8.83±0.56% as the pressure increased from 0.3 and 0.6 MPa, respectively.

The insoluble fraction of SE-treated purified RB showed the same decrease trend as the un-purified RB (Table 4), where treatment at the higher pressure caused a greater reduction in the amount of IF. In contrast, the level of SF showed a corresponding increase. However, the amount of reducing sugars showed an opposite trend to the un-purified rice bran, i.e., a significant (p<0.05) increase was observed after treatment at both 0.3 and 0.6 MPa. It could be due to the breakdown of side chains of the rice bran fibre, which

mainly consisted of arabinose, as in Table 1 and reported by Truong and Rumpagaporn (2019).

Changes in Molecular Weight Distribution of Rice Bran Soluble Fibre after Ultrasound and Steam Explosion Treatment

The molecular weights (MW) of the saccharides in the soluble fractions of purified RB fibre were measured by size-exclusion high-performance liquid chromatography (SE-HPLC).

Four peaks appeared in the chromatogram of the soluble fraction of untreated purified RB (Table 5), of which peak 4 was dominant while the other three peaks were much smaller in comparison. Peak 4 was eluted at 12.0 min, and the last peak eluded, indicating that it was the smallest oligosaccharide present (0.5 kDa). The second-largest peak was peak 1 (7.7 min), which had a molecular weight between 670 and 410 kDa.

After US treatment, both the number of peaks and their retention times changed from those of the untreated samples, demonstrating that the composition of the oligosaccharides in the soluble fractions had changed as a result of the treatment (Table 6). The soluble fraction produced after US treatment at 60% amplitude for 5–20 min gave an extra peak, eluted at 7.4 min, earlier than the first peak in the untreated sample, indicating a bigger molecular mass than the others. It demonstrated that the US treatment broke down some long-chain polymers (IF) into water-soluble shorter-chain polymers.

However, the area of peak 1' decreased as the treatment time increased. The same trend was also observed for peak 2'. Meanwhile, the area of peak 4' increased with increases in treatment time, demonstrating that some of the high molecular weight polymers were further broken down into lower molecular weight molecules.

After US treatment at 80% amplitude, four peaks appeared at 5.0, 8.3, 12, and 14.5 min, different from those in the untreated sample and those treated with US at 60% amplitudes. A polymer with a large molecular weight (>2,000 kDa) was released after the treatment (peak 1*), and the area of the peak increased as the treatment time increased from 5 to 20 min. Furthermore,

Table 5
Retention time and peak areas of the soluble fraction of untreated purified rice bran

Treatment	Peak	Retention time (min)	Calculated MW (kDa)	Area count of soluble polysaccharides or oligosaccharides ($\times 10^4$)
				Treatment time (min)
				0
Untreated	1	7.7±0.1	~724	1.2±0.1
	2	8.0±0.1	~617	0.2±0.0
	3	8.6±0.1	~537	0.4±0.1
	4	12.0±0.0	~0.5	4.7±0.0
Total area count ($\times 10^4$)				6.5±0.2

Table 6
Retention time and peak areas of soluble fraction of purified rice bran after ultrasound treatment

Ultrasound amplitude (%)	Peak	Retention time (min)	Calculated MW (kDa)	Area count of soluble polysaccharides or oligosaccharides (× 10^4)			
				Treatment time (min)			
				5	10	15	20
60	1'	7.4±0.1	~794	0.3±0.0^{a}	0.2±0.0^{b}	0.2±0.0^{c}	0.1±0.0^{c}
	2'	7.6±0.1	~724	0.2±0.0$^{a'}$	0.2±0.0$^{a'}$	0.1±0.0$^{b'}$	0.1±0.0$^{b'}$
	3'	8.5±0.0	~537	0.2±0.0^{A}	0.2±0.5^{A}	-	-
	4'	11.0±0.1	~6.8	1.0±0.1$^{B'}$	1.4±0.4$^{B'}$	2.9±0.2$^{A'}$	2.9±0.3$^{A'}$
	5'	12.0±0.0	~0.5	11.1±0.5^{A*}	12.7±0.4^{A*}	12.8±2.2^{A*}	13.7±1.3^{A*}
Total area count (× 10^4)				12.8±0.4	14.7±0.6	16.0±1.7	16.8±1.2
80	1*	5.0±0.0	>2,000	0.1±0.0^{c}	0.2±0.0^{b}	0.2±0.0^{b}	0.3±0.0^{a}
	2*	8.3±0.2	~537	0.1±0.0$^{b'}$	0.1±0.0$^{b'}$	0.1±0.0$^{b'}$	0.3±0.0$^{a'}$
	3*	12.0±0.0	~0.5	5.4±0.1^{C}	7.4±0.2^{A}	6.6±0.1^{B}	7.6±0.6^{A}
	4*	14.5±0.1	nd	-	-	0.3±0.0$^{B'}$	0.8±0.1$^{A'}$
Total area count (× 10^4)				5.6±0.1	7.7±0.2	7.2±0.2	9.0±0.7
95	1#	6.0±0.1	>2,000	-	-	-	0.3±0.0
	2#	7.5±0.1	~794	1.8±0.2$^{a'}$	0.9±0.1$^{c'}$	0.5±0.0$^{d'}$	1.5±0.0$^{b'}$
	3#	7.8±0.1	~724	-	0.4±0.0^{A}	0.2±0.0^{B}	-
	4#	8.4±0.2	~537	0.2±0.0$^{A'}$	0.2±0.0$^{A'}$	0.1±0.0$^{B'}$	0.2±0.0$^{A'}$
	5#	10.8±0.2	~10.7	0.2±0.1^{A*}	-	-	0.2±0.^{1A*}
	6#	12.0±0.0	~0.5	3.2±0.2$^{C\#}$	10.4±0.7$^{A\#}$	10.9±0.8$^{B\#}$	1.8±0.1$^{D\#}$
	7#	12.3±0.0	~0.3	-	-	-	0.1±0.0
	8#	13.4±0.0	nd	-	-	-	0.8±0.0
	9#	16.0±0.1	nd	-	0.3±0.1^{x}	0.2±0.0^{x}	-
Total area count (× 10^4)				5.4±0.6	12.2±0.9	11.9±0.8	4.9±0.1

Note.
$^{a\text{-}c}$ = Means with different letters within the same row differ significantly (p<0.05).
$^{A\text{-}C}$ = Means with different letters within the same row differ significantly (p<0.05).
$^{a'\text{-}d'}$ = Means with different letters within the same row differ significantly (p<0.05).
$^{A*\text{-}D*}$ = Means with different letters within the same row differ significantly (p<0.05).
$^{A'\text{-}C'}$ = Means with different letters within the same row differ significantly (p<0.05).
$^{A\#\text{-}D\#}$ = Means with different letters within the same row differ significantly (p<0.05).
$^{x\text{-}y}$ = Means with different letters within the same row differ significantly (p<0.05).
nd = Not determined.
Values are means±S.D.; n=3.

a smaller molecular weight (< 25 kDa) polymer was observed after the treatment for 15 and 20 min (Peak 4*). However, the 80% US amplitude is less effective in producing SF with smaller molecular weight compared to the 60% US amplitude, as the total area of peaks is much lower than the total area of peaks in 60% amplitude samples.

Ultrasound treatment at 95% amplitude produced nine peaks in the chromatogram.

Peak 1#, which was only observed in the sample treated for 20 min, was eluted at 6 min, which means that the polymer had a molecular weight bigger than 2,000 kDa. Peak 2# decreased after 10- and 15-min treatment but increased after 20 min-treatment. This peak was estimated to have a molecular weight of 794 kDa. The area count was increased for peak 6# (<25 kDa) from 5 to 10 min-treatment. However, a further increment in treatment time (15 to 20 min) caused a decrease in area count of peak 6#, indicating that re-polymerisation had occurred, and this result agreed with and supported the GC results in 4.3.1.2, where a decrease in the SF fraction was observed after the same treatment time. Peaks 4# and 6# had similar retention times with peaks 3' and 5' (in 60% amplitude samples) and peaks 2* and 3* (in 80% amplitude samples), eluted at 8.4 and 12.0 min, respectively. The area counts of peak 4# (8.4 min) did not show significant differences among the US amplitudes and treatment times. However, the area count of peak 6# (12.0 min) increased over the treatment time for each US amplitude, except for 95% amplitude, which decreased after 20 min of treatment.

In summary, US treatments caused a breakdown of some of the large polymers of IF into soluble polymers and some of the high molecular weight polymers of SF into lower molecular weight ones. The total area count ($\times 10^4$) increased by 60 and 80% US amplitude as treatment time increased. A contrasting trend was observed for the sample treated with 95% US amplitude, where the total area count ($\times 10^4$) increased after 5- and 10-min treatment and decreased when the treatment time was prolonged to 15- and 20-min. The highest total area count ($\times 10^4$), representing the highest amount of SF, was observed in samples treated at 60% US amplitude at 20 min. It eventually supports the results

Table 7
Retention time and peak areas of soluble fraction of purified rice bran after steam explosion treatment

Peak	Retention time (min)	Calculated MW (kDa)	Area count of soluble polysaccharides or oligosaccharides ($\times 10^4$)	
			Steam explosion pressure (MPa)	
			0.3	0.6
1*	7.3±0.1	~794	-	0.9±0.1
2*	8.2±0.1	~537	1.1±0.1	-
3*	8.7±0.3	~275	1.1±0.1^{b}	1.4±0.1^{a}
4*	13.7±0.1	<1	5.8±0.1$^{b'}$	6.2±0.0$^{a'}$
5*	14.6±0.0	<1	2.7±0.0^{A}	2.8±0.3^{A}
		Total area count ($\times 10^4$)	10.7±0.3	11.3±0.1

Note.
$^{a\text{-}b}$ = Means with different letters within the same row differ significantly (p<0.05);
$^{A\text{-}C}$ = Means with different letters within the same row differ significantly (p<0.05);
$^{a'\text{-}b'}$ = Means with different letters within the same row differ significantly (p<0.05);
Values are means±S.D.; n = 3; - = No peak detected.

in Table 3. The most abundant soluble polymers produced after the US treatment had molecular weights less than 25 kDa for all US amplitudes applied. However, the effect was not always cumulative as the US amplitude and treatment time increased.

Steam explosion-treated purified RB also showed changes in the molecular weight distribution of soluble fractions, as summarised in Table 7. Four peaks appeared in the chromatogram of the soluble fractions of purified RB fibre after the treatments at both 0.3 and 0.6 MPa; however, the retention times of the peaks differed for the samples treated at the two different pressures. The largest peaks were peak 4* for both samples, with the sample treated at 0.6 MPa, giving the larger area. The largest overall peak area of all the peaks was also obtained from the sample treated at 0.6 MPa.

The longer retention time of peak eluted is evidence of steam explosion treatment, which caused a breakdown of some of the polymers of the IF into soluble, smaller MW polymers. The steam explosion treatment also caused a loss of some SF due to the degradation of soluble polymers, which were seen as smaller peak areas compared to ultrasound-treated samples. The smaller MW oligosaccharides and/or by-products (i.e., hydroxymethylfurfural) produced by further breakdown of the SFs were unable to be detected by the SE-HPLC system as the limit of detection of the column used was 1 kDa. This finding agreed with the decrease in reduced sugar content, as discussed previously.

CONCLUSION

Results presented in this study showed that the ultrasound and steam explosion treatments can break down the IF of rice bran into SF with various molecular weights. The treatment intensity affected the SF yield; however, the relationship between ultrasound intensity and SF yield was still unclear. Generally, for US treatment at lower US amplitudes (60 and 80%), the SF yield increased as the treatment time increased. At high ultrasound amplitude (95%), the yield of SF increased initially with time but began to decline with prolonged treatment time (after 10 min). The amount of SF produced for SE treatment was directly proportional to the pressure applied (treatment intensity). The highest SF yield (35.2%) was obtained from US treatment at 60% amplitude for 20 min. The highest yield (35.2%) for SE treatment was obtained at 0.6 MPa SE pressure and 2 min treatment. The SF produced from both treatments mainly contained oligosaccharides with MW smaller than 25 kDa, with those produced by steam explosion treatment generally smaller than those by ultrasound treatment. Purifying rice bran by removing starch and proteins enhanced the efficiency of the ultrasound and steam explosion treatments in breaking down the IF into the SF.

DATA AVAILABILITY

The data used to support the findings of this study are available from the corresponding author upon request.

ETHICAL APPROVAL

No ethical approval is needed.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ACKNOWLEDGMENTS

The authors acknowledge The Ministry of Higher Education Malaysia, Universiti Malaysia Terengganu (UMT) and the University of New South Wales, Sydney, for the financial support of this study. The first author thanks the Government of Malaysia and UMT for providing a PhD Scholarship under SLAM.

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