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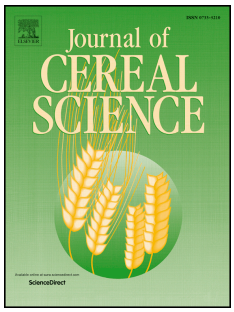

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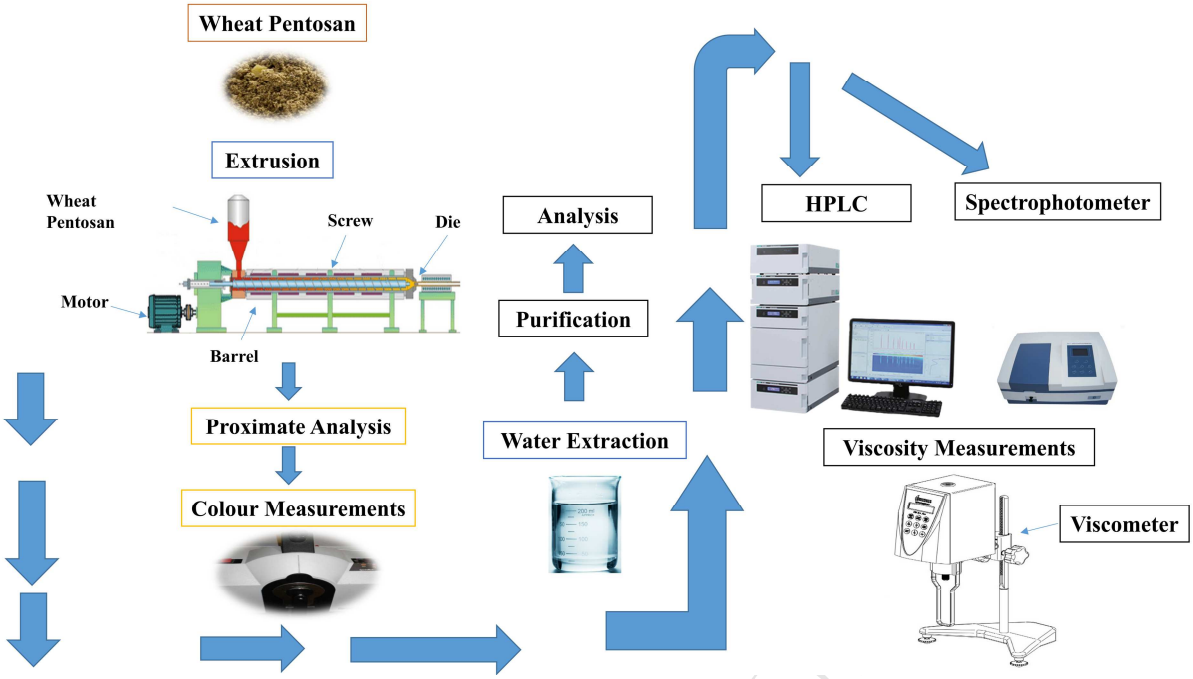

Improving the extractability of arabinoxylans and the molecular weight of wheat endosperm using extrusion processing

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Abstract

Cereal derived arabinoxylans (AXs) are non-starch polysaccharides that have immunomodulatory activities. These activities are thought to be related to the low molecular weight fractions of AXs. Wheat and wheat by-products are rich in AXs, however, the water extractable fraction of AXs in wheat products is low. Water extraction of AXs can be improved by extrusion processing, which increases the extractability of the water soluble fraction. The aim of this study was to determine the extractability and molecular weight of the water soluble fraction of AXs from wheat endosperm after extrusion at screw speeds of 80 and 160 rpm. Extrusion processing significantly ($P<0.05$) increased the water extractability of AXs in a screw-speed dependent manner (13.07±0.12% at 80 rpm and 15.45±0.16% at 160 rpm compared to8.95±0.10% in the non-extruded control) due to a significant increase ($P<0.05$) in low molecular weight fractions of AXs in extruded samples.

Keywords: non-starch polysaccharides; arabinoxylans; extrusion processing; size-exclusion chromatography

1. Introduction

Non-starch polysaccharides (NSP) are major components of dietary fiber that are present in cereal endosperm (including the aleurone layer), cell walls, husk, and bran (Fadel et al. 2017b). The main polymers of NSP are arabinoxylans (AXs). The chemical structure of AXs is based on backbone chains of β-(1-4)-linked d-xylopyranosyl residues to which α-1-arabinofuranose units are linked as side chains in the second and/or third carbon positions, often called pentosans. Recently, AXs have been reported to have biological activities, such as antioxidant properties, lowering serum cholesterol, enhancing haemoglobin A1c concentration, improving glucose tolerance and promoting immunity (Fadel et al. 2017a; Fadel et al. 2017b; Fadel et al. 2018).

AXs are classified into water-unextractable AXs (WUAXs) and water-extractable AXs (WEAXs) based on their solubility in water. The solubility of AXs depends on the balance between chain-chain interactions and any change in the structural features such as molecular weight, chain length, branching pattern and degree of branching (Fadel et al. 2017b). The amount of AXs is different from one plant to another; total AXs in rice comprise 5.63 - 7.15 % of the grain, with only 0.90 % of this being water-extractable (Fadel et al. 2017a). In contrast, the amount of total AXs in wheat is 6-8 % (Li et al. 2013), 25 % of which is water-extractable (Fadel et al. 2017a). Differences in the amounts of AXs between plant species gives rise for the need to apply different extraction techniques to optimize the extraction of AXs. Indeed, the characteristics and extraction yield of AXs are determined by the extraction method applied (Li et al. 2015). Moreover, the bioactivity of AXs has been reported to be associated with their molecular features(Li et al. 2015).

There are many possible methods that could be used to modify the solubility of AXs, including enzymatic treatment, alkaline treatment, extrusion processing and combinations of all three. Extrusion processing has been used as a pre-treatment method combined with alkaline solutions to extract AXs in the form of hemicellulose from different cereal fractions such as wheat bran (Fadel et al. 2017a). However, the use of chemicals for extraction has several disadvantages such as the production of hazardous waste, adverse effects on human health, high cost and often the need for specialist disposal or recycling treatments (Fadel et al. 2017a; Jeon et al. 2014). The

modification of rice bran dietary fibres with enzymes extracted from Shiitake mushrooms give rise to AXs with a molecular weight of 30-50 KDa and reported immune modulatory effects, both *in vivo* and *in vitro* (Fadel et al. 2017a).

Extrusion processing is a reliable and cheap physical pre-treatment applied to modify the extractability of AXs. It combines temperature and mechanical shear to disrupt the structure of the cell wall compartments(Fadel et al. 2017a). Extrusion processing is also a valuable and desirable food processing technique as it has many positive features including unique product shapes, low cost, energy savings, high speed and high productivity(Fadel et al. 2017a). Moreover, the solubility of dietary fibres can improve during extrusion (Jeon et al. 2014). However, there is little research examining the influence of extrusion on water-extractable AXs present in wheat endosperm pentosan. Therefore, the objective of this study was to determine the influence of extrusion screw speed (80 rpm and 160 rpm) on the extraction yield and molecular weight (Mw) distribution of water-soluble AXs from wheat endosperm pentosan.

2. Experimental

2.1. Materials and chemicals

Henan Lianhua Monosodium Glutamate Group Co. Ltd. (Xiangchen, China) kindly provided wheat endosperm pentosan (WEP). The WEP preparation was previously reported by Li et al. (2015). D-(+)-Xylose, D-(−)-Arabinose, anhydrous dextrose (D-glucose), acetic acid (glacial), hydrochloric acid, phloroglucinol and ethanol were purchased from Sigma-Aldrich (Brøndby, Denmark) for the determination of xylose in wheat pentosan. Five Pullulan (linear α-(1-4) glucans with no side chain) standards of varying molecular weights (ranging from 5-708 kDa) were purchased from Shodex (Shanghai, China) to characterise the Mw of AXs by SEC-HPLC. Sodium nitrate ($NaNO_3$) and sodium azide (NaN_3) were purchased from Sigma-Aldrich (Gillingham, UK) for HPLC mobile phase. Termamyl (α-amylase), type XII-A, A3403-1MU and proteinase, type XXIII, P4032 were purchased from Sigma-Aldrich (Brøndby, Denmark).

2.2. Methods

2.2.1. Extrusion processing

The extrusion processing conditions were adapted from methods described by Jing and Chi (2013). Pentosan without extrusion (PW) was used directly. A Werner Pfleiderer Continua 37 co-rotating, self-wiping twin-screw extruder (Werner Pfleiderer, Stuttgart, Germany) was used for the extrusion processing of wheat pentosan (3 repeats). The extruder had the following characteristics: a length-to-diameter ratio (L/D) of 27:1, screw-speeds (SS) of 80 and 160 revolutions per minute (rpm) and a feed rate of 10 kg/h. The barrel temperature was controlled in two zones and was set at 80 and 140°C (feed end and die end, respectively) with a fixed moisture content of 30% (w/w wet weight basis). Extruded samples were dried at 60°C for 12 hours. The only extrusion condition that was varied was the screw speed (80 or 160 rpm). The torque was recorded during each run by means of an inbuilt gauge in the instrument panel.

2.2.2. Proximate analyses

2.2.2.1. Fat

Fat content was determined using methods adapted from Pérez-Palacios et al. (2008). A 10 g sample was weighed in an extraction thimble (n=3) (Buchi, Switzerland), placed in a hot extraction beaker and 40 mL of petroleum ether (Fisher Scientific, Loughborough, UK) was added before transferring to an E-812/E-816 HE extraction unit (Buchi, Switzerland). The percentage of fat was obtained using the following equation:

$$Fat\ (\%) = \frac{Weight_{(extraction\ beaker+residue)} - Weight_{(extraciton\ beaker)}}{Weight_{sample}}$$

2.2.2.2. Moisture

Moisture content was measured following the method described by (n=3) Latimer (2012).

$$Moisture\ (\%) = (1 - \frac{Weight_{drysample}}{Weight_{wetsample}}) \times 100$$

2.2.2.3. Protein

The protein content was determined using automatic flash combustion (n=3) (LECO FP628, Stockport, UK).

2.2.2.4. Ash

The ash content of all samples was determined by placing samples in a muffle furnace (n=3) (Carbolite™ RHF14/8 Chamber Furnace, Fisher Scientific, Loughborough, UK) at 550°C. The residual material was cooled and weighed.

2.2.3. Color determination

The color of wheat pentosan samples was measured (n=6) using a reflectance spectrophotometer Datacolor sf600 plus ct (Cheshire, UK). The CIE L*a*b* color system was used, in which L* is lightness, a* is redness, and b* is yellowness. The color difference (ΔE) was calculated using the following equation provided by Ramírez-Jiménez et al. (2003), whereby *ΔE*, *ΔL, Δa* and *Δb* indicate changes in colour, intensity brightness, redness and yellowness respectively:

:

$$\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2}$$

2.2.4. Extraction and purification of water-extractable AXs (WEAXs)

AXs were extracted and purified using the method described by Li et al. (2013). Briefly, 1000 g of samples (PW, P80 and P160; n = 3) were extracted with 3333 mL water, by incubating in a shaking water bath (Precision SWB 15, ThermoScientific, London, UK) for 2 hours at 40 °C prior to purification. Following centrifugation at 6000 x g for 40 minutes, supernatants were adjusted to pH 7 using 1M NaOH or 1M HCl before incubating with 400 ppm thermostable α-amylase (500 Units/mg) in a shaking water bath at 91°C for 60 minutes. The amylase activity was stopped by boiling in a glycerin bath for 30 minutes at 120°C. Protein digestion was carried out with the addition of 400 ppm proteinase (3 Units/mg) at 50°C for 12 hours. The

147 samples were then placed in a boiling water bath for 15 minutes to deactivate the
148 proteinase and then centrifuged at 4,600 x g for 20 minutes. Ethanol (70:30 v/v in
149 distilled water) was added to the supernatants at 4°C overnight. The precipitate that
150 formed was recovered by centrifugation at 4,600 x g for 20 minutes. The supernatant
151 was discarded and the residue was weighed before washing and vortexing twice with
152 20 mL absolute ethanol (minimum 99%). Finally, 20 mL of acetone was added and
153 the samples were vortexed for one minute followed by centrifugation at 4,600 x g for
154 20 minutes. The final precipitates were dried for 48 hours at 45°C in a drying oven
155 before being transferred to vacuum-sealed, food-grade bags using a Turbovac
156 SB425 Vacuum Packer (Stockport, UK) and kept at 21°C for further analysis.

157 **2.2.5. Determination of water-extractable AXs (WEAXs)**

158 Two methods were used to measure the WEAXs in samples, a phloroglucinol assay
159 and HPLC (Li et al. 2015). The percentage of xylose in extracts was determined
160 using a phloroglucinol assay following the method described by Li et al. (2015). The
161 absorbance of each sample was measured at 552 nm and 510 nm using a
162 ThermoScientific GENESYS 10S Bio Spectrophotometer (London, UK). A xylose
163 standard curve was constructed to determine the xylose content of wheat pentosan
164 samples, which was subsequently used to calculate the amount of AXs in wheat
165 pentosan extracts (n=3).

166 **2.2.6. Determination of sugar composition of purified extracts by HPLC**

167 The sugar composition of purified extracts was determined using a method adapted
168 from Li et al. (2015). Purified samples (20 mg) of AXs from PW, P80 or P160 were
169 added to 1 mL of 1 M H_2SO_4 and vortexed for 5 minutes then incubated in a glycerin
170 bath at 100 °C for 2 h. The pH was then adjusted to 7 using 1 M NaOH and the
171 solution was diluted using HPLC-grade water to 1 mg/mL. Samples (n=3) were then
172 filtered and transferred to a 1 mL glass vial for HPLC analysis.

173 A Shimadzu LC-20 AB HPLC system, (Shimadzu Corporation, Tokyo, Japan),
174 equipped with a Refractive Index Detector (RID) 10A, SUPELGUARD Pb (5 cm × 4.6
175 mm) guard column (Phenomenex, Macclesfield, UK) and SUPELCOGEL Pb (30 cm
176 × 7.8 mm) column (ion exclusion separation mode) (Phenomenex, Macclesfield, UK)
177 was used to determine the sugar content of samples. The column temperature,

mobile phase and flow-rate were 80°C, HPLC-grade water and 0.5 mL/min respectively in an isocratic run**.** Different concentrations (0.25, 0.5, 0.75 and 1 mg/mL) of glucose, xylose, galactose and arabinose were prepared as standards to plot a series of calibration curves from which the amount of each sugar was calculated based upon the relevant peak areas.

2.2.7. Molecular weight standard curve

Five Pullulan standards ranging from 5-375 kDa were used to construct a standard curve. Standards were prepared at 0.5 mg/mL using mobile phase and left overnight at 5°C. All samples and standards were filtered through a 0.45 µm nylon membrane and transferred to 1 mL glass shell vials. To prepare the Pullulan standard curves, the Pullulan molecular weights were converted to log molecular weights before plotting against their retention times (Supplementary Data 1, 2 and 3).

2.2.8. Determination of the molecular weight distribution of AXs by HPLC

Dry samples were prepared for analysis by dissolving 2 mg of each sample in 1 mL of the mobile phase and leaving overnight at 5°C. The mobile phase was prepared by dissolving 0.65 g NaN_3 and 17g $NaNO_3$ in 2000 mL HPLC-grade water.

The molecular weight distribution of AXs was determined using size exclusion chromatography. All samples were analysed using a Shimadzu LC-10 HPLC (Shimadzu Corporation, Kyoto, Japan) equipped with a JASCO RI-2031 refractive rndex (RI) Detector (Jasco Corporation, Tokyo, Japan), and BioSep-SEC-S 4000 and BioSep-SEC-S 3000 columns (Phenomenex, Macclesfield, UK). An isocratic run was used, with a flow rate of 0.6 mL/min (Li et al. 2013).

2.2.9. Viscosity alteration

The experimental set-up for the viscosity measurements consisted of an automated viscometer, DV-11+PRO (Brookfield Engineering Laboratories, Essex, UK). Spindles were driven by the viscometer immersed in the wheat sample solution (3.3 g/mL). The rotating spindle drags the viscous fluid against itself, the effect of which is determined by the deflection on the calibrated spring. The type of spindle used was determined by the viscosity measurement. Spindle RV1 was used to calibrate the

viscometer using de-ionised water. Spindles RV2 and RV4 were required to measure the viscosity of PW, P80 and P160 respectively. The temperature of all samples was carefully maintained at 30°C throughout and the viscosity was measured at 10 second intervals for 2 minutes at 50 rpm.

2.210. Fourier transform infra-red (FT-IR) spectroscopy

FT-IR spectra of WEAX samples were obtained according to the method described by Morales-Ortega et al. (2013). Universal attenuated total reflectance (ATR) was measured on a PerkinElmer 200i spectrometer (PerkinElmer.London, United Kingdom). Spectra were recorded between 800 and 4000 cm^{-1} with 24 scans and a resolution of 4 cm^{-1}.

2.2.10. Statistics

Data were expressed as mean ± standard error of the mean (SEM) in all cases. Significant differences between samples were determined by one-way analysis of variance ANOVA with Tukey's multiple comparison tests on SPSS 23 software. A *P* value of less than 0.05 was considered statistically significant. Graphpad Prism version 5 was used to produce the figures.

3. Results and discussion

3.1. Proximate analysis

Fig. 1 presents the proximal content of extruded/non-extruded wheat pentosan samples (fat, protein, ash and starch). The percentages of ash, starch, protein and fat in the non-extruded wheat pentosan was within the range reported previously by Li et al. (2013). The ash content in all the samples was notably similar ($P>0.05$). The fat, protein and starch content of P80 and P160 were significantly lower ($P<0.05$) than PW samples. Moreover, the fat, protein and starch content of P160 was significantly lower than P80, suggesting these significant decreases were mediated through increases in extrusion screw speed. The change in screw speed is known to have a direct effect on the generation of shear stress and the residence time of extrudates (Villmow et al. 2008).

It has been reported that lower screw speeds result in a longer residence time which encourages prolonged shearing, subsequently affecting the starch content (Ziegler and Aguilar 2003). In addition, Ortolan et al. (2015) reported that extrusion processing significantly (P<0.05) reduces the protein content in the extruded wheat flour. The observed reduction in protein and starch content in the extruded samples might be related to the cross-linking of protein and starch and the gelatinization of starch (Kim et al. 2006). Furthermore, the high temperature in the barrel is responsible for producing colorful compounds (Maillard reaction), which are highly dependent on the temperature, reducing sugar content and free amino acid content. Moreover, the high shear stresses and mix in the barrel along with the high temperature have been reported to liberate starch and make it more accessible and available for enzymatic- and non-enzymatic browning. Djurle et al. (2016) reported that the extrusion of wheat bran at 400 rpm using a twin-screw extruder can reduce the starch content compared to a non-extruded samples. The fat content in the extruded samples was significantly (P<0.05) reduced in the extruded samples at 80 and 160 rpm which might be due to the formation of complexes of fat with protein or liberated amylose.

3.2. Color changes

The color changes in the extruded samples can provide us with information about the extent of browning such as the Maillard reaction and degree of cooking (Altan et al. 2008). The color analysis of PW showed a brightness (L*) of 65.8, a redness (a*) of 7.34 and a yellowness (b*) of 22.6 (Fig. 2). There was no significant increase or decrease (P>0.05) in a* or b* between the extruded and non-extruded samples. However, there was a significant reduction (P<0.05) in L* of extruded samples at 80 and 160 rpm compared to non-extruded samples. There was a non-significant increase (P>0.05) in L* level of the extruded samples at 160 rpm in comparison with samples extruded at 80 rpm.

The significant reduction of brightness in extruded samples could be explained by the high temperature developed in the barrel and the violent mixing, as well as the high shear stress. High temperature has been shown to contribute to the formation of browning material (Maillard reaction). On the other hand, the residence time of extruded material at the high screw speed (160 rpm) is less than that at 80 rpm since

the higher screw speed forces material through the barrel more quickly and results in a shorter treatment period. This may explain why the brightness level of the extruded sample at 160 rpm was modestly higher than that of the sample extruded at 80 rpm.

In concordance with the brightness data, the browning development (ΔE) was significantly increased (P<0.05) in extruded samples at 80 and 160 rpm compared to non-extruded samples. The browning index was non-significantly (P>0.05) reduced in extruded samples at 160 rpm compared to samples extruded at 80 rpm and can be explained in a similar fashion to the modest increase in brightness observed in samples extruded at 160 rpm (Mesquita et al. 2013).

3.3. Extraction yield of AXs

The extrusion processing had a positive effect on the extraction yield of AXs from wheat pentosan. An increase in extrusion screw-speed resulted in a significant increase in the extraction yield. The total AXs presented in samples were calculated using the xylose standard curve and arabinose/xylose ratio (Ar/Xy) obtained by HPLC. The extrusion process significantly (P<0.05) increased the percentage of WEAXs from 8.95±0.10 % in the control to 13.07±0.12 % and 15.45±0.16 % in the samples extruded at 80 and 160 rpm respectively. This may be due to a greater mechanical energy input and increased shear, resulting in a reduction in molecular weight. In practice, this suggests it becomes easier to extract AXs from the material with extrusion. Thus, extrusion could provide a versatile methodology to produce higher extraction yields of AXs from cereals.

3.4. Monosaccharide Composition

Glucose, arabinose, galactose and xylose monosaccharides were identified in the purified AXs from wheat pentosan (Fig. 3). The Ar/Xy ratio decreased in wheat pentosan samples as the extrusion screw speed increased. For WEAXs from un-extruded wheat pentosan Ar/Xy was 0.76±0.001. The Ar/Xy ratios for extruded wheat pentosan samples were 0.81±0.005 and 0.80±0.003 at screw speeds of 80 and 160 rpm, respectively. Hence, AXs from unextruded penotasan differ from AXs from pentosan extruded at 80 and 160 rpm in both the degree of branching and molecular weight.

In wheat endosperm pentosan, WEAXs were 25 % (Fadel et al. 2017a). The low extractability of AXs could be due to their large molecular weight (Fadel et al. 2017a) and to their ferulic acid content (0.31-0.56 mg/g) (Michniewicz et al. 1990). Ferulic acid side chains are esterified to some arabinose residues (Snelders et al. 2013), which form covalent/non-covalent bonds with the cell wall materials, thus decreasing the solubility of AXs in water. Jeon et al. (2014) stated that the use of extrusion processing as a pre-treatment is an efficient, environmentally friendly and low-cost process to increase the level of WEAXs in corn fibre. The results of this study agree with the findings of Jeon et al. (2014) showing an increase in the WEAXs content in the extruded wheat pentosan with increasing screw-speed from 80 to 160 rpm. The WEAXs content in extruded samples increased by 0.23-fold and 0.4-fold in pentosan samples extruded at 80 and 160 rpm, respectively. This is supported by the recorded torque values which show a reduction (49 to 30%) with increasing screw speed (from 80 to 160 rpm) respectively, suggesting greater shearing and break down of the material. There are several possible explanations for the increasing level of WEAXs in the samples post-extrusion, including the rupture of the di-ferulic linkages that allows AXs molecules to separate, exposing polar side groups which then interact with water and increase solubility, softening of the lignin and reduction of Mw by high mechanical shear forces.

Holguín-Acuña et al. (2008) found that the ferulic acid content increased from 0.2 mg/g in non-extruded maize bran to 2.5 mg/g in extruded maize bran. Moreover, the increase in screw-speeds from 80 to 160 rpm might soften the lignin (Yoo et al. 2012). Since AXs act as a glue between lignin and cellulose (Vermaas et al. 2015), exposing AXs chains to water, consequently increases their solubility.

3.5. Molecular weight analysis of AXs using HPSEC

3.5.1. Pullulan standard curve construction

A standard curve was constructed using five Pullulan standards (P5, P20, P100. P200 and P400) analysed by high-pressure size exclusion chromatography, HPSEC, and used to determine the Mw and retention time of AXs in samples. The Mw of the five Pullulan standards ranged between 5.9 and 375 kDa (Supplementary Data 1, 2 and 3).

3.5.2. Molecular weight distribution of AXs

The Mw distribution of AXs from wheat pentosan samples was characterized by HPLC-SEC. Table 1 and Fig. 4 illustrate the Mw range of AXs and percentage levels obtained. Most notably, extrusion with a screw speed of 80 rpm (P80) and 160 rpm (P160) resulted in significantly (P<0.05) higher levels (7.33±0.02% and 7.63±0.01% respectively) of very low Mw (0.85-1.54 kDa) AXs compared to extraction without extrusion (PW). Thus, extrusion could provide a promising methodology to produce high quality yields of low molecular weight AXs from cereals. Low molecular weight AXs have been shown to enhance immune responses and may have beneficial effects on human health (Fadel et al. 2017).

Molecular weight determinations for whole wheat AXs were reported to be within the ranges of 56-65 kDa using gel permeation chromatography and 6-600 kDa for wheat endosperm using HPSEC (Li et al. 2013), with differences most likely arising from the type of wheat material used and the methodology applied. In this study, HPSEC showed the Mw of AXs from extruded/non-extruded wheat pentosan samples was between 0.85-794.3 kDa, in concordance with the Mw range of AXs (1-700 kDa) previously reported from wheat pentosan by Li et al. (2013).

Higher percentage levels of low Mw AXs were obtained from extruded wheat pentosan samples compared to non-extruded samples. These increases in the percentage levels of low Mw AXs is probably due to the extrusion processing, such as high shear forces and high temperatures resulting in depolymerisation of the fibre (Svanberg et al. 1995). It is also possible that extrusion processing breaks down the glyosidic bonds, resulting in depolymerisation of the cell wall material and reducing the Mw of AXs (Margareta and Nyman 2003).

Levels of low Mw (1.54-3.16 kDa) AXs were significantly (P<0.05) increased in extruded samples compared to non-extruded wheat pentosan samples. This could be related to the xylan backbone, which carries more arabinose side chains (Grootaert et al. 2007) that can be esterified by ferulic acids. It has been reported that extrusion breaks up ferulic acid side chains, thus reducing the Mw of AXs (Holguín-Acuña et al. 2008).

It should also be noted that the percentage levels of high Mw AXs within the Mw range 3.16 to 794.3 kDa were significantly higher (P<0.05) in the extruded samples at 80 and 160 rpm compared to non-extruded samples. The percentage levels of high Mw range AXs increased significantly (P<0.05) from 77.3 % in PW samples to 78.1% and 78.4% in P80 and P160 respectively. This may be due to the greater shearing created inside the barrel of the extruder which facilitates the breakdown of cell walls, thus providing smaller molecular weight fractions.

3.6. Viscosity measurements

It has been reported that higher Mw AXs have higher viscosity at a given concentration (Saulnier et al. 2007). Fig. 5 shows the mean viscosity (cP) for each sample over time (minutes). The results showed that extrusion screw-speed significantly (P<0.05) increased the viscosity of samples, with higher viscosity obtained following extrusion at 160 rpm compared to 80 rpm. It has been reported that temperatures higher than 70 °C causes starch to fold extensively, leading to increased viscosity (Malumba et al. 2013). Gelatinization promotes the irreversible collapse of molecular order within granules, resulting in granular swelling and enhanced viscosity development. In a similar way, the structure of the plant cell wall material (i.e. AXs) is disrupted, allowing greater molecular interaction. However, the extrusion process in this study was carried out at the same temperature (80 °C for zone 1 and 140 °C for zone 2) for both extrusion screw speeds, suggesting the increase in viscosity was due to screw speed alone.

Another explanation for the increase in viscosity might be the formation of gels during extrusion processing which may occur due to covalent cross-links and non-covalent bonds (such as hydrogen bonds) between the chains of AXs (Niño-Medina et al. 2010). Furthermore, the significant (P<0.05) increase in viscosity in extruded samples at 80 and 160 rpm concurs with the Mw findings showing a significant increase in the percentage levels of high Mw (3.16-794.3 kDa) AXs in samples extruded at 80 and 160 rpm.

3.7. FT-IR spectra of WEAXs

The FT-IR spectrum of WEAXs shown in Fig.6 presents a broad absorbance band of polysaccharides between 800 and 1200 cm^{-1}.

The FT-IR profile correspondes to previously published polysaccharide profiles (Morales-Ortega et al. 2013; Robert et al. 2005). There was an absorbance band observed at 1720 cm^{-1} corresponding to a low degree of esterification with aromatic esters like ferulic acid (Morales-Ortega et al. 2013). Absorbance bands were observed between 800 and 1200 cm^{-1} that are indicative of functional groups present on AXs (Robert et al. 2005), thus confirming the presence of AXs in the extruded and non-extruded samples.

4. Conclusions

Extrusion increases the yield of AXs compared with non-extracted methods in a screw speed dependent manner. In particular, high screw speeds result in higher yields of low molecular weight AXs which have been shown previously to have immunomodulatory properties. These findings suggest extrusion may be a novel method to produce high yields of low molecular weight AXs from cereals. Extrusion-assisted extraction may open the possibility to the develop cereal-based products fortified with low molecular weight AXs that enhance innate immunity in humans.

406 **Supplementary I**

407 Molecular weight of pullulan standards

Sample	Molecular weight (Dalton)
P-5	5,900
P-20	21,100
P-100	107,000
P-200	200,000
P-400	375,000

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422 **Supplementary II**

423 Molecular weights of pullulan standards in relation to their retention times

Pullulan sample	Molecular weight (Da)	Retention time (Min)	Log Mw
P5	5,900	43.50	3.77
P20	21,100	38.40	4.32
P100	107,000	29.24	5.03
P200	200,000	26.61	5.30
P400	375,000	25.01	5.57

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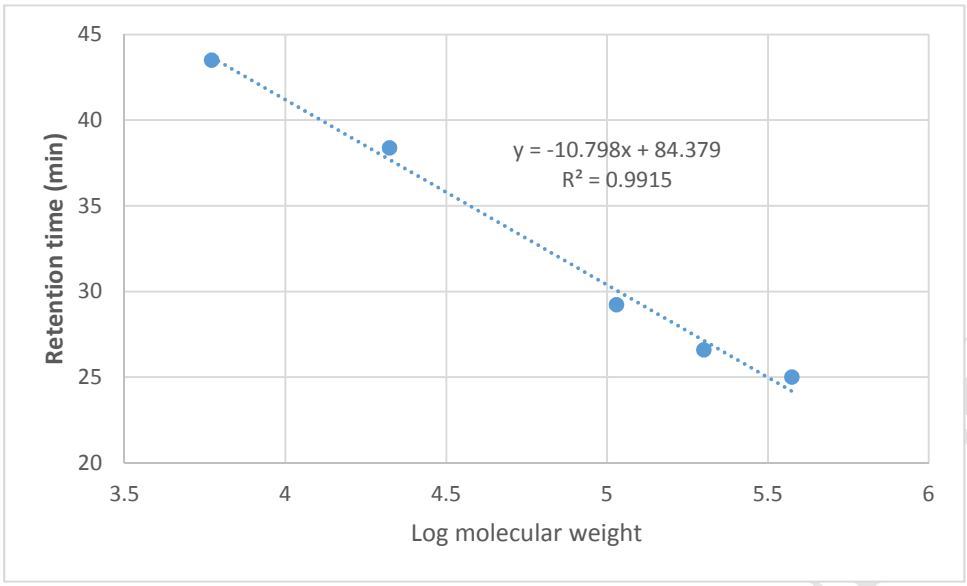
Supplementary III



The five pullulan standard curve used to characterise the Mw of PW, P80 and P160.

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Fig. 1. Proximate analysis of the samples. (A) Represents the protein content. (B) Represents the ash content. (C) Represents the fat content. (D) Represents the starch content. * represents significant differences ($P<0.05$) between PW, P80 and P160 extraction yields. Values are mean (n=3) dry weight (gram per 100 gram dry weight) ± standard error of the mean (SEM). PW represents wheat pentosan without extrusion, whereas P80 represents wheat pentosan extruded at 80 rpm and P160 represents wheat pentosan extruded at 160 rpm.

Fig. 2. Color changes in extruded and non-extruded samples. (A) Shows the difference in brightness L*, (B). Shows the difference in redness a*, (C) Shows the difference in yellowness b* and (D) Shows browning index ΔE. The # symbol above the samples indicates no significant differences were identified between PW, P80 and P160 color changes, whereas the * symbol represents significant ($P<0.05$) differences between PW, P80 and P160 color changes. Values are mean (n=3) dry weight (gram per 100 gram dry weight) ± standard error of the mean (SEM). PW represents wheat pentosan without extrusion, whereas P80 represents wheat pentosan extruded at 80 rpm and P160 represents wheat pentosan extruded at 160 rpm.

Fig. 3. Sugar composition for purified AXs from wheat pentosan obtained by water extraction alone (PW) or via extrusion at 80 rpm (P80) and 160 rpm (P160). PW represents wheat pentosan without extrusion, whereas P80 represents wheat pentosan extruded at 80 rpm and P160 represents wheat pentosan extruded at 160 rpm.

Fig. 4. Mw distribution of AXs in PW, P80 and P160 analyzed by HPSEC. The dashed lines separate the area under the curve into four areas (A1 to A4), each of which represents a distinct Mw range. PW represents wheat pentosan without

extrusion, whereas P80 represents wheat pentosan extruded at 80 rpm and P160 represents wheat pentosan extruded at 160 rpm.

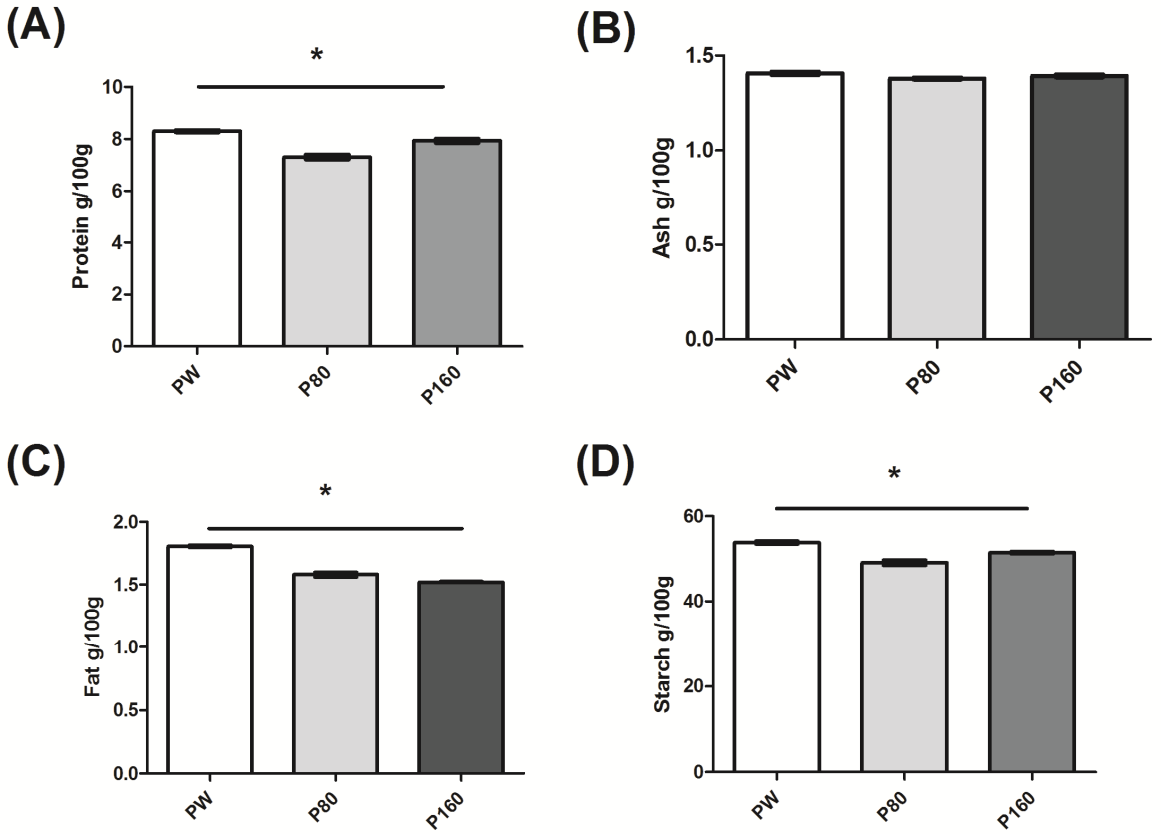
Fig. 5. Viscosity measurements for extruded and non-extruded wheat pentosan. PW represents wheat pentosan without extrusion, whereas P80 represents wheat pentosan extruded at 80 rpm and P160 represents wheat pentosan extruded at 160 rpm.

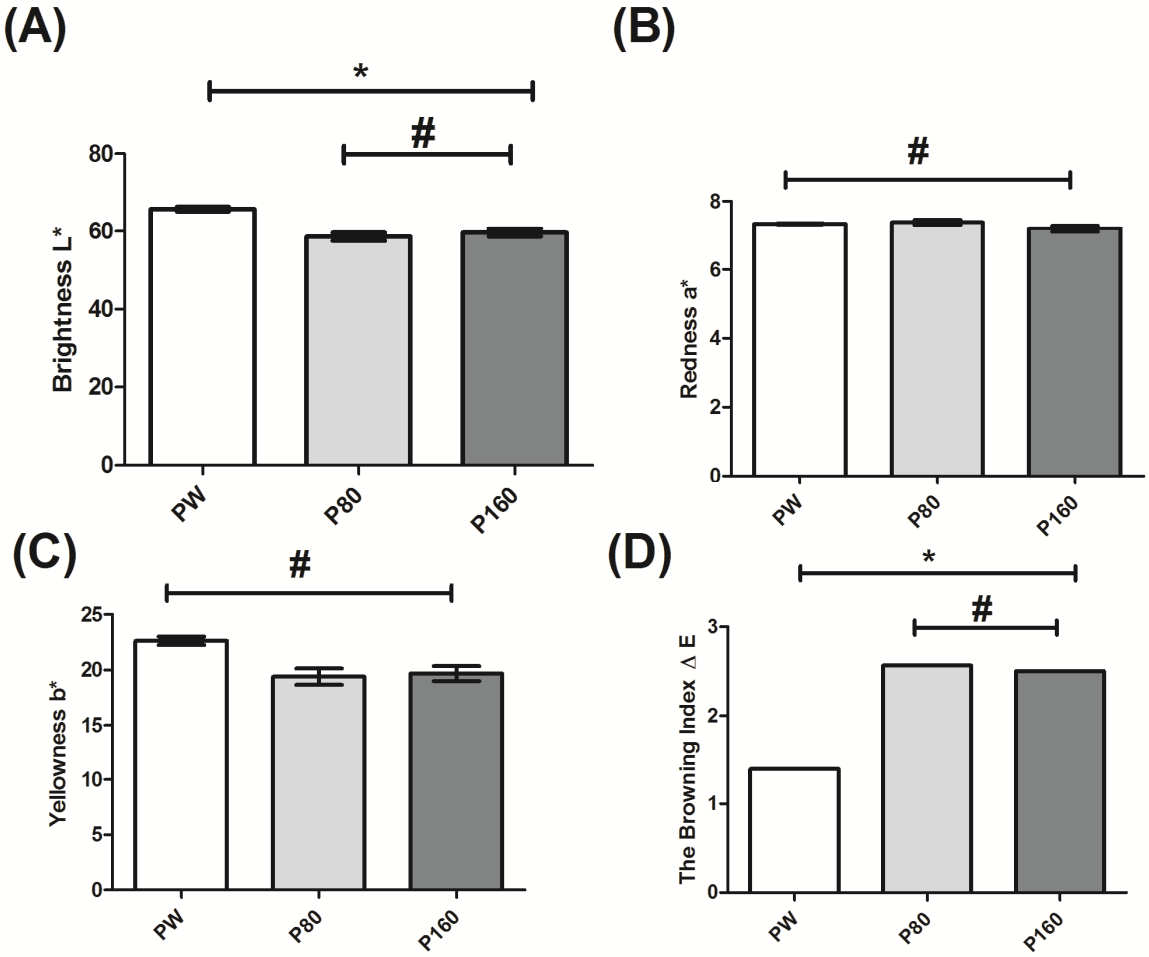
Fig. 6. FT-IR spectrum of WEAXs from extruded and non-extruded samples. PW represents wheat pentosan without extrusion, whereas P80 represents wheat pentosan extruded at 80 rpm and P160 represents wheat pentosan extruded at 160 rpm

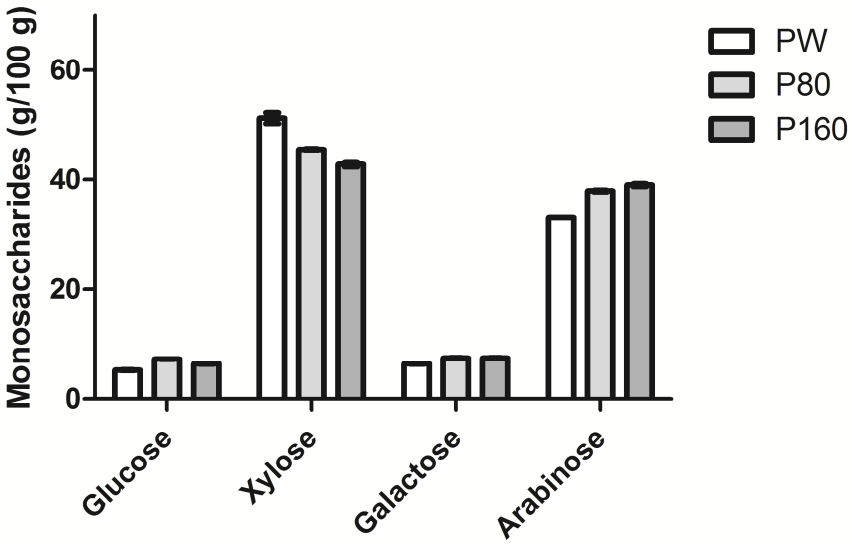
Table with legend

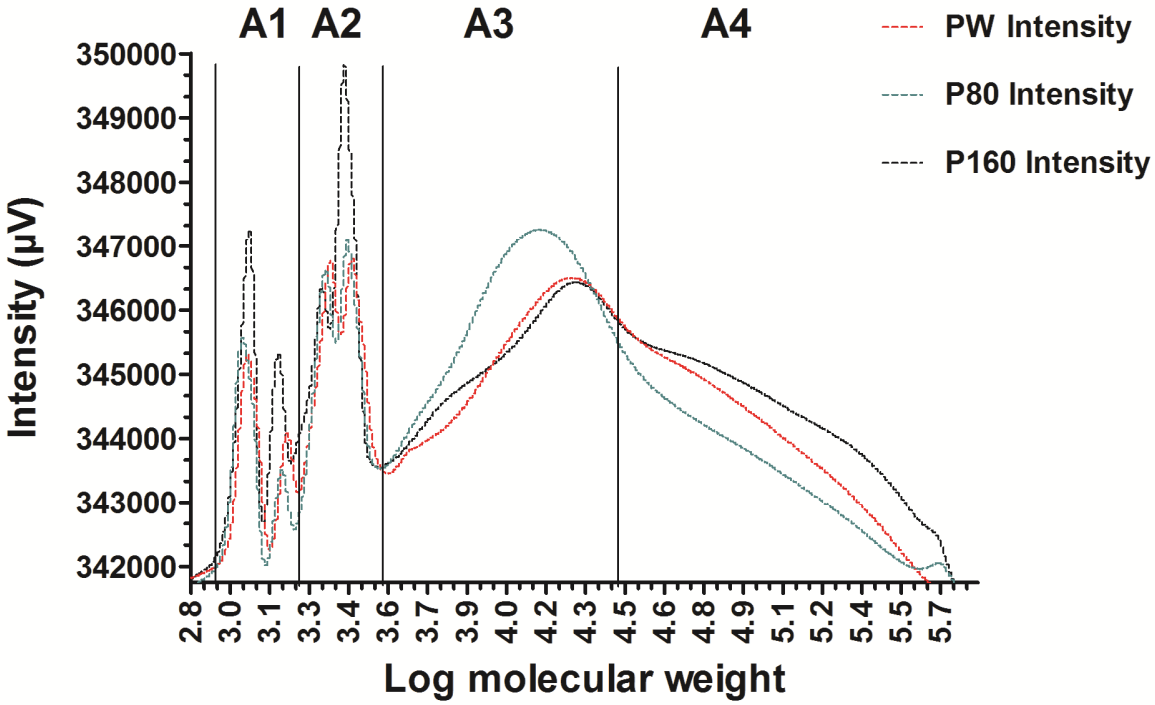
Table 1. Molecular Weight (Mw) distribution and percentage levels (%) of AXs extracted from wheat pentosan by water extraction alone (PW) or by extrusion at 80 rpm (P80) and 160 rpm (P160). Data are presented as mean ± SEM (n = 3). * indicates significant difference (P< 0.05) in percentage level of AXs compared to the PW sample. # indicate significant difference (P< 0.05) in percentage level between P80 and P160

Areas	Log Mw	Mw range (kDa)	%		
			PW	P80	P160
A1	2.93-3.19	0.85-1.54	7.13±0.01	7.33±0.02*,#	7.63±0.01*,#
A 2	3.19-3.50	1.54-3.16	14.0±0.00	14.6±0.01*,#	15.6±0.03*,#
A 3	3.50-4.50	3.16-31.62	46.9±0.01	45.1±0.01*,#	43.0±0.01*,#
A 4	4.50-5.90	31.62-794.3	30.4±0.02	33.0±0.01*,#	35.4±0.01*,#

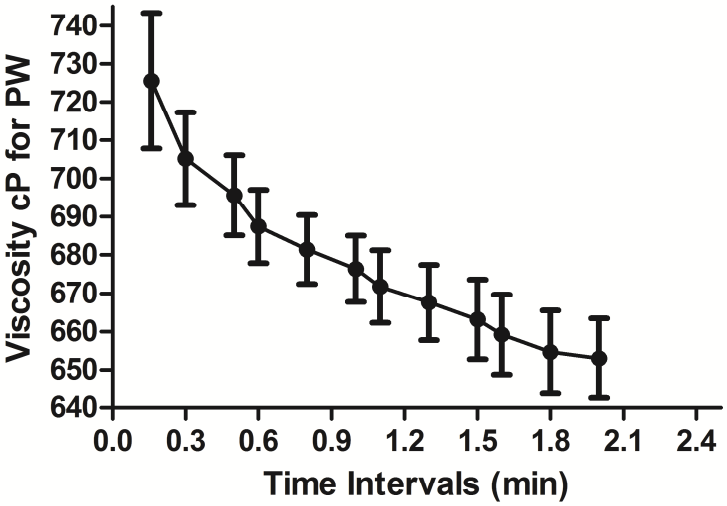



(A)
*
#
Brightness L*
80
60
40
20
0
PW
P80
P160
(B)
#
Redness a*
8
6
4
2
0
PW
P80
P160
(C)
#
Yellowness b*
25
20
15
10
5
0
PW
P80
P160
(D)
*
#
The Browning Index Δ E
3
2
1
0
PW
P80
P160

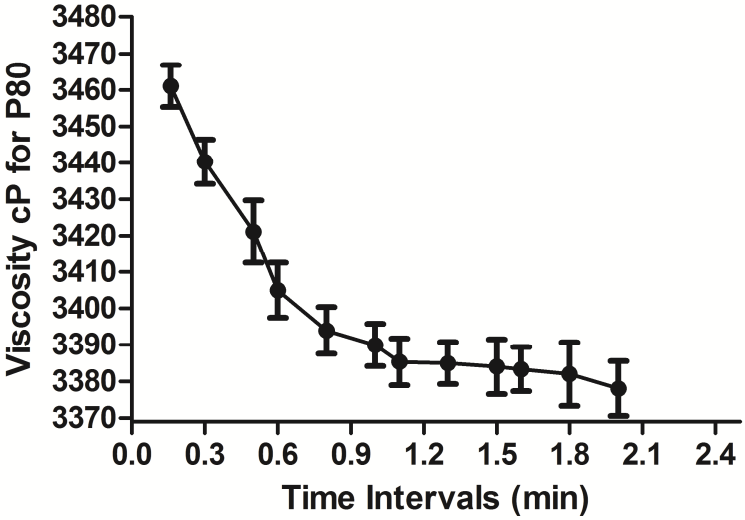




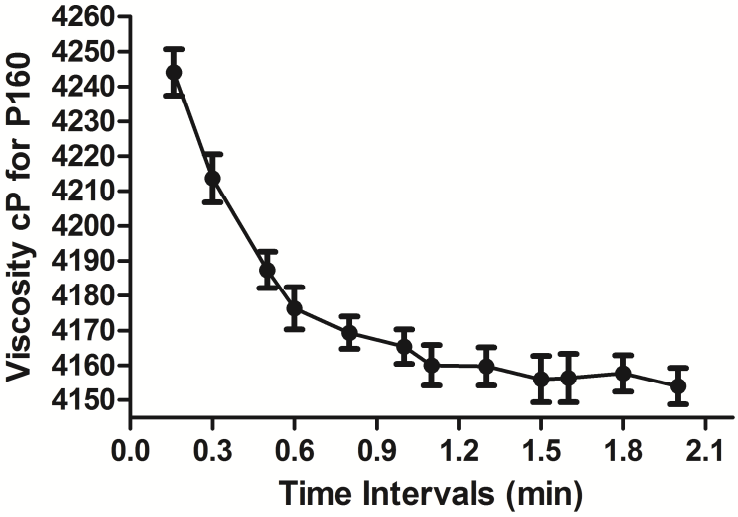
A

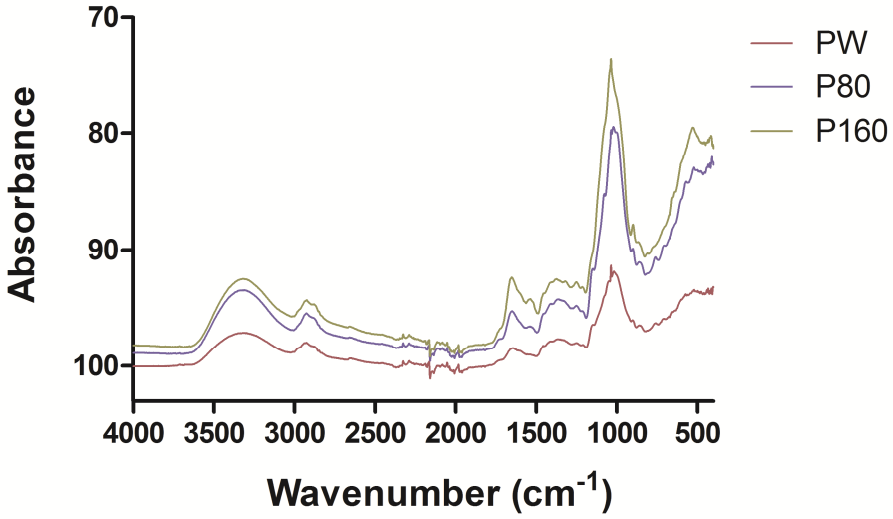

Viscosity cP for PW
740
730
720
710
700
690
680
670
660
650
640
0.0
0.3
0.6
0.9
1.2
1.5
1.8
2.1
2.4
Time Intervals (min)

B


Viscosity cP for P80
3480
3470
3460
3450
3440
3430
3420
3410
3400
3390
3380
3370
0.0
0.3
0.6
0.9
1.2
1.5
1.8
2.1
2.4
Time Intervals (min)

C


Viscosity cP for P160
4260
4250
4240
4230
4220
4210
4200
4190
4180
4170
4160
4150
0.0
0.3
0.6
0.9
1.2
1.5
1.8
2.1
Time Intervals (min)



Highlights

- Extrusion screw speeds reduces the molecular weight of arabinoxylans
- Extrusion technology increases the solubility of rice bran arabinoxylans