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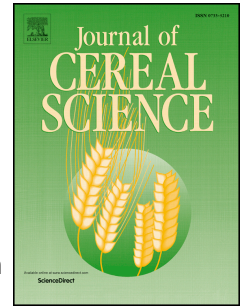
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# Accepted Manuscript

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

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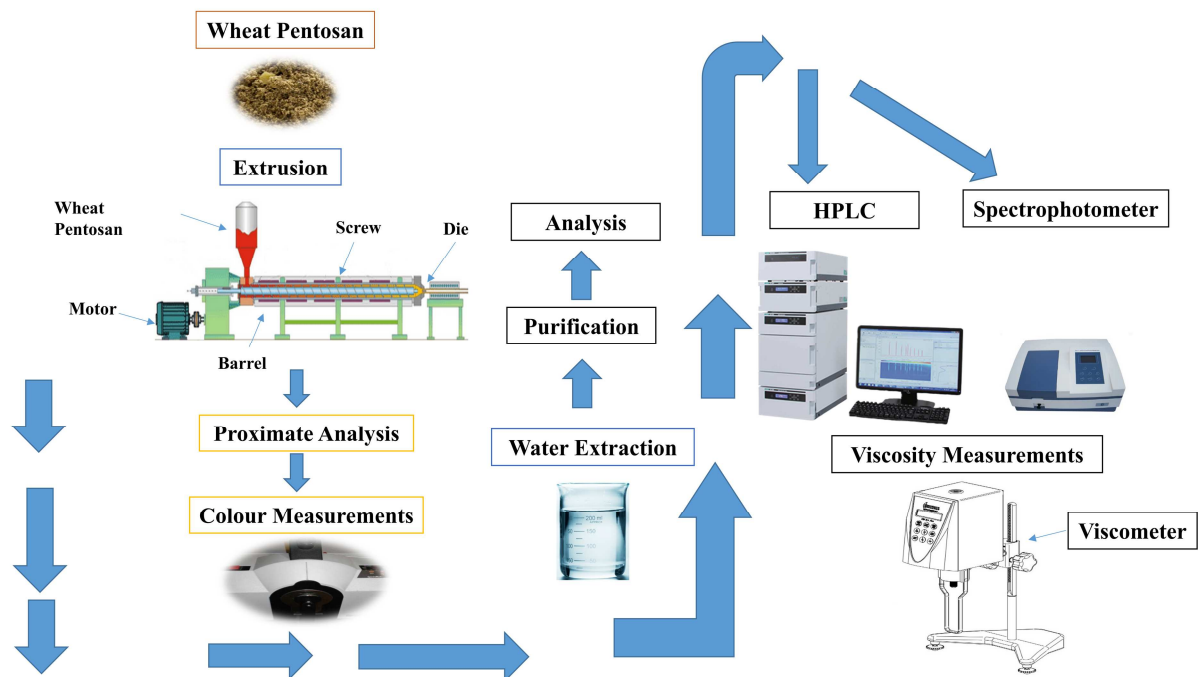
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1 **Improving the extractability of arabinoxylans and the molecular weight of**  
2 **wheat endosperm using extrusion processing**

3

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16

17

18 **Abstract**

19 Cereal derived arabinoxylans (AXs) are non-starch polysaccharides that have  
20 immunomodulatory activities. These activities are thought to be related to the low  
21 molecular weight fractions of AXs. Wheat and wheat by-products are rich in AXs,  
22 however, the water extractable fraction of AXs in wheat products is low. Water  
23 extraction of AXs can be improved by extrusion processing, which increases the  
24 extractability of the water soluble fraction. The aim of this study was to determine the  
25 extractability and molecular weight of the water soluble fraction of AXs from wheat  
26 endosperm after extrusion at screw speeds of 80 and 160 rpm. Extrusion processing  
27 significantly ( $P<0.05$ ) increased the water extractability of AXs in a screw-speed  
28 dependent manner ( $13.07\pm 0.12\%$  at 80 rpm and  $15.45\pm 0.16\%$  at 160 rpm compared  
29 to  $8.95\pm 0.10\%$  in the non-extruded control) due to a significant increase ( $P<0.05$ ) in  
30 low molecular weight fractions of AXs in extruded samples.

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

33

## 34 1. Introduction

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

45 AXs are classified into water-unextractable AXs (WUAXs) and water-extractable AXs  
46 (WEAXs) based on their solubility in water. The solubility of AXs depends on the  
47 balance between chain-chain interactions and any change in the structural features  
48 such as molecular weight, chain length, branching pattern and degree of branching  
49 (Fadel et al. 2017b). The amount of AXs is different from one plant to another; total  
50 AXs in rice comprise 5.63 - 7.15 % of the grain, with only 0.90 % of this being water-  
51 extractable (Fadel et al. 2017a). In contrast, the amount of total AXs in wheat is 6-8  
52 % (Li et al. 2013), 25 % of which is water-extractable (Fadel et al. 2017a).  
53 Differences in the amounts of AXs between plant species gives rise for the need to  
54 apply different extraction techniques to optimize the extraction of AXs. Indeed, the  
55 characteristics and extraction yield of AXs are determined by the extraction method  
56 applied (Li et al. 2015). Moreover, the bioactivity of AXs has been reported to be  
57 associated with their molecular features(Li et al. 2015).

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

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

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

## 81 **2. Experimental**

### 82 **2.1. Materials and chemicals**

83 Henan Lianhua Monosodium Glutamate Group Co. Ltd. (Xiangchen, China) kindly  
84 provided wheat endosperm pentosan (WEP). The WEP preparation was previously  
85 reported by Li et al. (2015). D-(+)-Xylose, D-(-)-Arabinose, anhydrous dextrose (D-  
86 glucose), acetic acid (glacial), hydrochloric acid, phloroglucinol and ethanol were  
87 purchased from Sigma-Aldrich (Brøndby, Denmark) for the determination of xylose in  
88 wheat pentosan. Five Pullulan (linear  $\alpha$ -(1-4) glucans with no side chain) standards  
89 of varying molecular weights (ranging from 5-708 kDa) were purchased from Shodex  
90 (Shanghai, China) to characterise the Mw of AXs by SEC-HPLC. Sodium nitrate  
91 ( $\text{NaNO}_3$ ) and sodium azide ( $\text{NaN}_3$ ) were purchased from Sigma-Aldrich (Gillingham,  
92 UK) for HPLC mobile phase. Termamyl ( $\alpha$ -amylase), type XII-A, A3403-1MU and  
93 proteinase, type XXIII, P4032 were purchased from Sigma-Aldrich (Brøndby,  
94 Denmark).

95

96

97 **2.2. Methods**98 **2.2.1. Extrusion processing**

99 The extrusion processing conditions were adapted from methods described by Jing  
100 and Chi (2013). Pentosan without extrusion (PW) was used directly. A Werner  
101 Pfleiderer Continua 37 co-rotating, self-wiping twin-screw extruder (Werner  
102 Pfleiderer, Stuttgart, Germany) was used for the extrusion processing of wheat  
103 pentosan (3 repeats). The extruder had the following characteristics: a length-to-  
104 diameter ratio (L/D) of 27:1, screw-speeds (SS) of 80 and 160 revolutions per minute  
105 (rpm) and a feed rate of 10 kg/h. The barrel temperature was controlled in two zones  
106 and was set at 80 and 140°C (feed end and die end, respectively) with a fixed  
107 moisture content of 30% (w/w wet weight basis). Extruded samples were dried at  
108 60°C for 12 hours. The only extrusion condition that was varied was the screw speed  
109 (80 or 160 rpm). The torque was recorded during each run by means of an inbuilt  
110 gauge in the instrument panel.

111 **2.2.2. Proximate analyses**112 **2.2.2.1. Fat**

113 Fat content was determined using methods adapted from Pérez-Palacios et al.  
114 (2008). A 10 g sample was weighed in an extraction thimble (n=3) (Buchi,  
115 Switzerland), placed in a hot extraction beaker and 40 mL of petroleum ether (Fisher  
116 Scientific, Loughborough, UK) was added before transferring to an E-812/E-816 HE  
117 extraction unit (Buchi, Switzerland). The percentage of fat was obtained using the  
118 following equation:

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

119 **2.2.2.2. Moisture**

120 Moisture content was measured following the method described by (n=3) Latimer  
121 (2012).



$$\text{Moisture (\%)} = \left(1 - \frac{\text{Weight}_{\text{dry sample}}}{\text{Weight}_{\text{wet sample}}}\right) \times 100$$

### 122 2.2.2.3. Protein

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

### 125 2.2.2.4. Ash

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

### 129 2.2.3. Color determination

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

136 :

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

### 137 2.2.4. Extraction and purification of water-extractable AXs (WEAXs)

138 AXs were extracted and purified using the method described by Li et al. (2013).  
139 Briefly, 1000 g of samples (PW, P80 and P160; n = 3) were extracted with 3333 mL  
140 water, by incubating in a shaking water bath (Precision SWB 15, ThermoScientific,  
141 London, UK) for 2 hours at 40 °C prior to purification. Following centrifugation at  
142 6000 x g for 40 minutes, supernatants were adjusted to pH 7 using 1M NaOH or 1M  
143 HCl before incubating with 400 ppm thermostable  $\alpha$ -amylase (500 Units/mg) in a  
144 shaking water bath at 91°C for 60 minutes. The amylase activity was stopped by  
145 boiling in a glycerin bath for 30 minutes at 120°C. Protein digestion was carried out  
146 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<sub>2</sub>SO<sub>4</sub> 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 x 4.6  
175 mm) guard column (Phenomenex, Macclesfield, UK) and SUPELCOGEL Pb (30 cm  
176 x 7.8 mm) column (ion exclusion separation mode) (Phenomenex, Macclesfield, UK)  
177 was used to determine the sugar content of samples. The column temperature,

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

### 183 **2.2.7. Molecular weight standard curve**

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

### 190 **2.2.8. Determination of the molecular weight distribution of AXs by HPLC**

191 Dry samples were prepared for analysis by dissolving 2 mg of each sample in 1 mL  
192 of the mobile phase and leaving overnight at 5°C. The mobile phase was prepared  
193 by dissolving 0.65 g NaN<sub>3</sub> and 17g NaNO<sub>3</sub> in 2000 mL HPLC-grade water.

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

### 200 **2.2.9. Viscosity alteration**

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

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

#### 211 **2.2.10. Fourier transform infra-red (FT-IR) spectroscopy**

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

#### 217 **2.2.10. Statistics**

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

### 223 **3. Results and discussion**

#### 224 **3.1. Proximate analysis**

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

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

### 252 3.2. Color changes

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

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

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

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

### 276 3.3. Extraction yield of AXs

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

### 288 3.4. Monosaccharide Composition

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

297 In wheat endosperm pentosan, WEAXs were 25 % (Fadel et al. 2017a). The low  
298 extractability of AXs could be due to their large molecular weight (Fadel et al. 2017a)  
299 and to their ferulic acid content (0.31-0.56 mg/g) (Michniewicz et al. 1990). Ferulic  
300 acid side chains are esterified to some arabinose residues (Snelders et al. 2013),  
301 which form covalent/non-covalent bonds with the cell wall materials, thus decreasing  
302 the solubility of AXs in water. Jeon et al. (2014) stated that the use of extrusion  
303 processing as a pre-treatment is an efficient, environmentally friendly and low-cost  
304 process to increase the level of WEAXs in corn fibre. The results of this study agree  
305 with the findings of Jeon et al. (2014) showing an increase in the WEAXs content in  
306 the extruded wheat pentosan with increasing screw-speed from 80 to 160 rpm. The  
307 WEAXs content in extruded samples increased by 0.23-fold and 0.4-fold in pentosan  
308 samples extruded at 80 and 160 rpm, respectively. This is supported by the recorded  
309 torque values which show a reduction (49 to 30%) with increasing screw speed (from  
310 80 to 160 rpm) respectively, suggesting greater shearing and break down of the  
311 material. There are several possible explanations for the increasing level of WEAXs  
312 in the samples post-extrusion, including the rupture of the di-ferulic linkages that  
313 allows AXs molecules to separate, exposing polar side groups which then interact  
314 with water and increase solubility, softening of the lignin and reduction of Mw by high  
315 mechanical shear forces.

316 Holguín-Acuña et al. (2008) found that the ferulic acid content increased from 0.2  
317 mg/g in non-extruded maize bran to 2.5 mg/g in extruded maize bran. Moreover, the  
318 increase in screw-speeds from 80 to 160 rpm might soften the lignin (Yoo et al.  
319 2012). Since AXs act as a glue between lignin and cellulose (Vermaas et al. 2015),  
320 exposing AXs chains to water, consequently increases their solubility.

### 321 **3.5. Molecular weight analysis of AXs using HPSEC**

#### 322 **3.5.1. Pullulan standard curve construction**

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

### 328 3.5.2. Molecular weight distribution of AXs

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

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

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

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



358 It should also be noted that the percentage levels of high Mw AXs within the Mw  
359 range 3.16 to 794.3 kDa were significantly higher ( $P<0.05$ ) in the extruded samples  
360 at 80 and 160 rpm compared to non-extruded samples. The percentage levels of  
361 high Mw range AXs increased significantly ( $P<0.05$ ) from 77.3 % in PW samples to  
362 78.1% and 78.4% in P80 and P160 respectively. This may be due to the greater  
363 shearing created inside the barrel of the extruder which facilitates the breakdown of  
364 cell walls, thus providing smaller molecular weight fractions.

### 365 **3.6. Viscosity measurements**

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

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

### 386 **3.7. FT-IR spectra of WEAXs**

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

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

396

#### 397 4. Conclusions

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

405

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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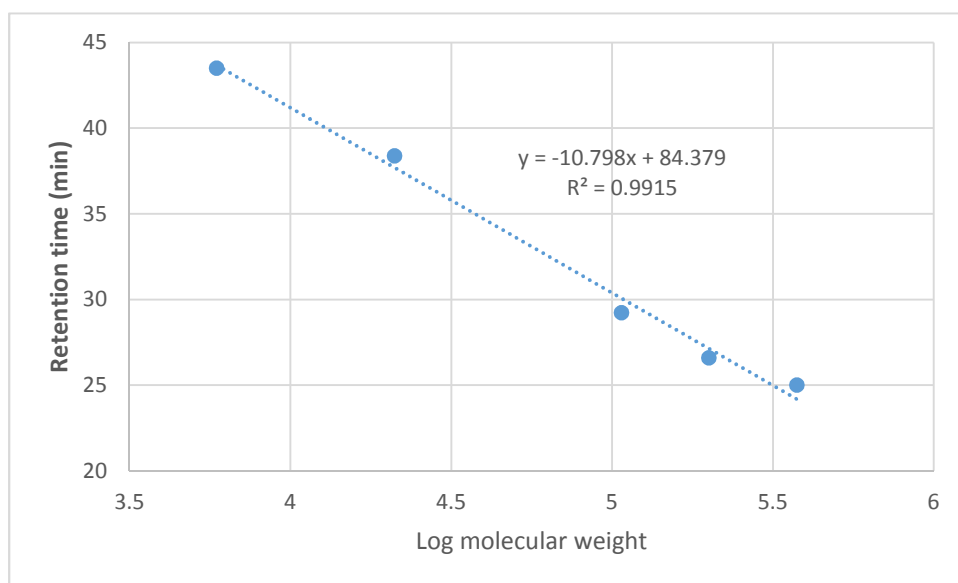
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438 **Supplementary III**

439

440 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)  $\pm$  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  $\Delta E$ . 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)  $\pm$  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.

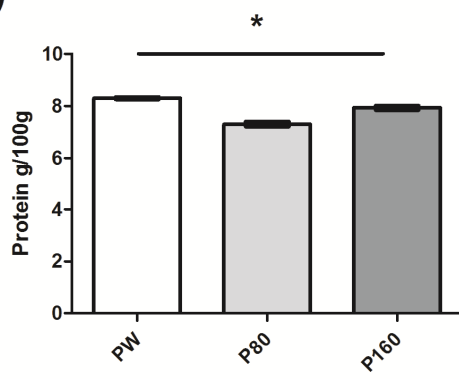
Fig. 6. FT-IR spectrum of WEAXs from extruded and non-extruded samples. PW represents wheat pentosan without extrusion, whereas P80 represents wheat pentosan extruded at 80 rpm and P160 represents wheat pentosan extruded at 160 rpm

**Table with legend**

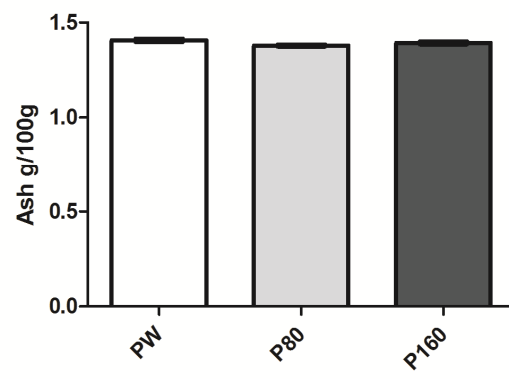
Table 1. Molecular Weight (Mw) distribution and percentage levels (%) of AXs extracted from wheat pentosan by water extraction alone (PW) or by extrusion at 80 rpm (P80) and 160 rpm (P160). Data are presented as mean  $\pm$  SEM (n = 3). \* indicates significant difference ( $P < 0.05$ ) in percentage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

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

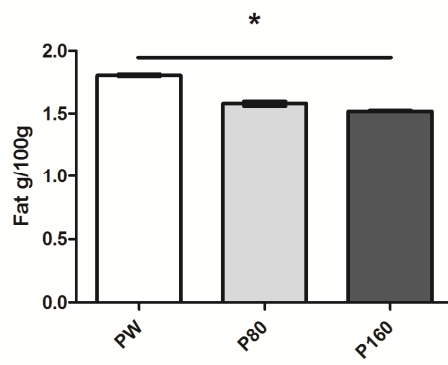
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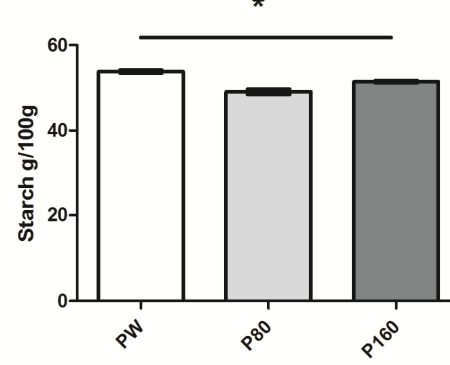
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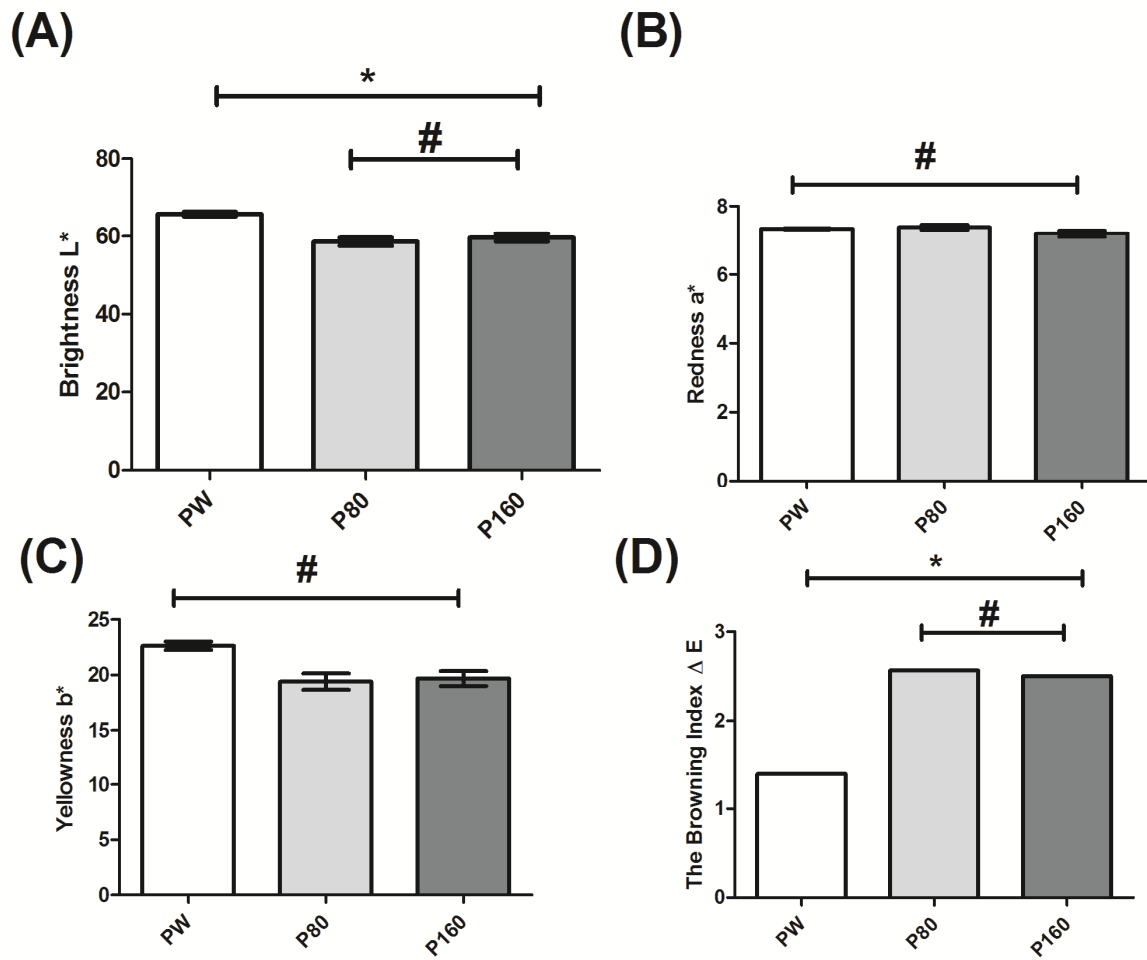


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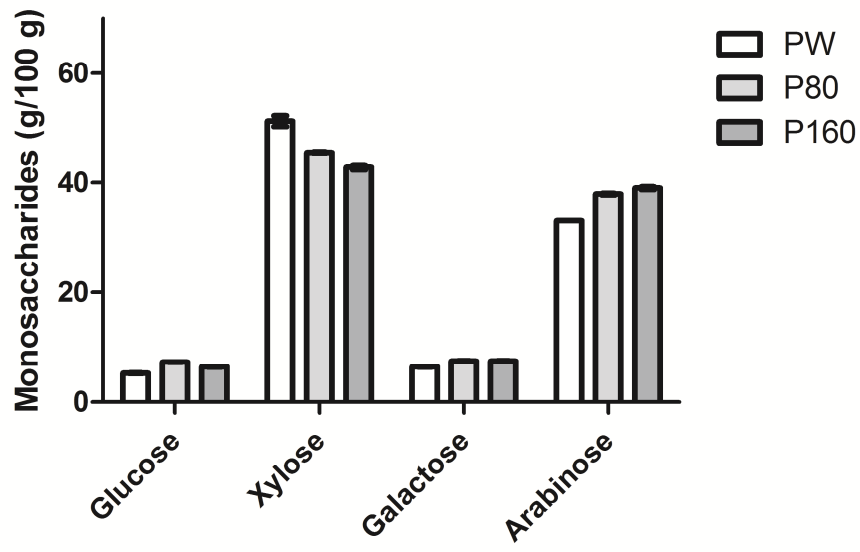


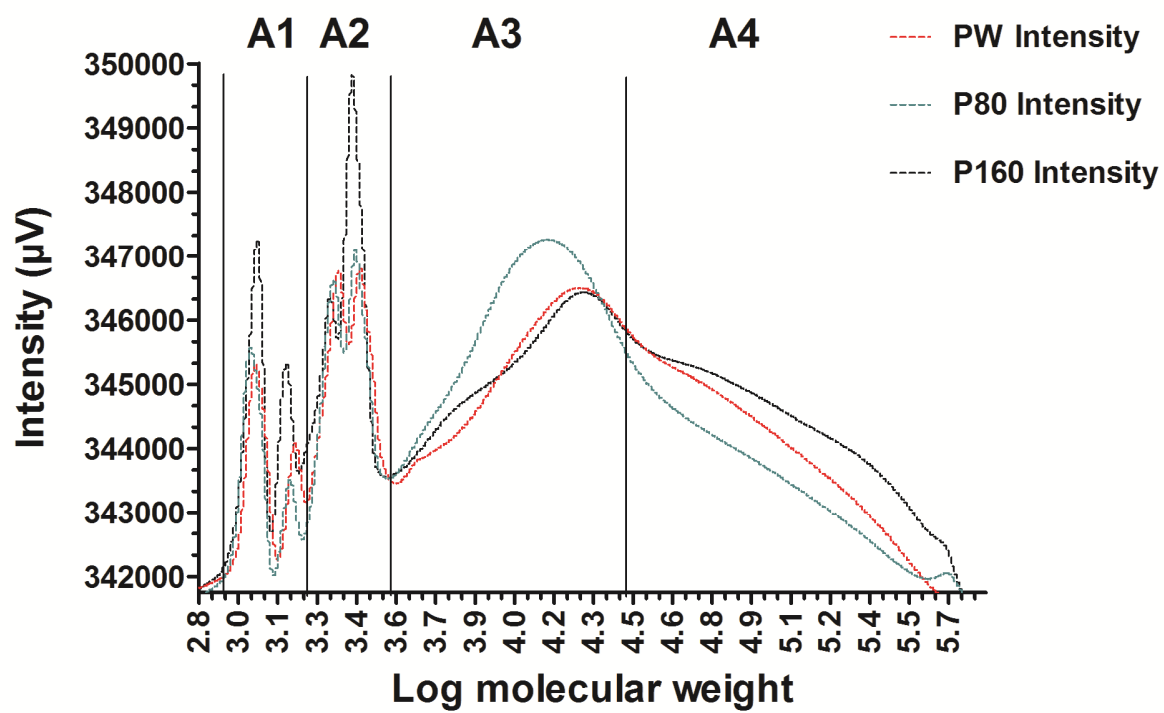
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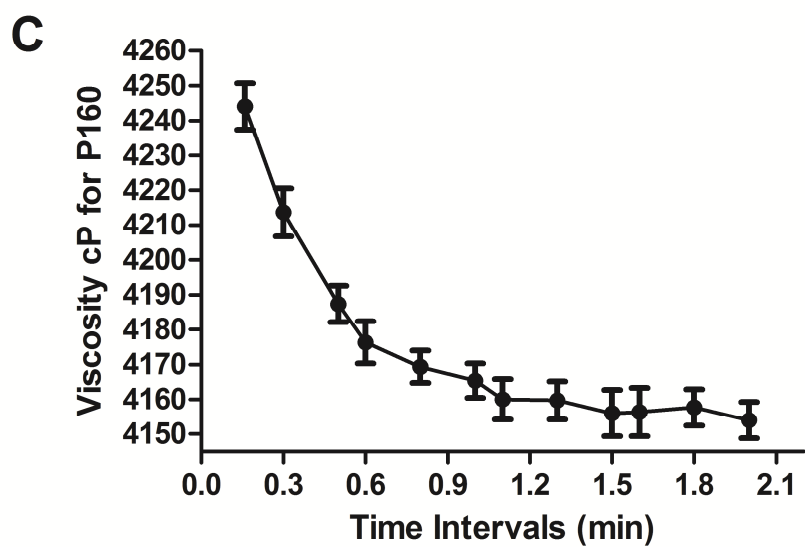
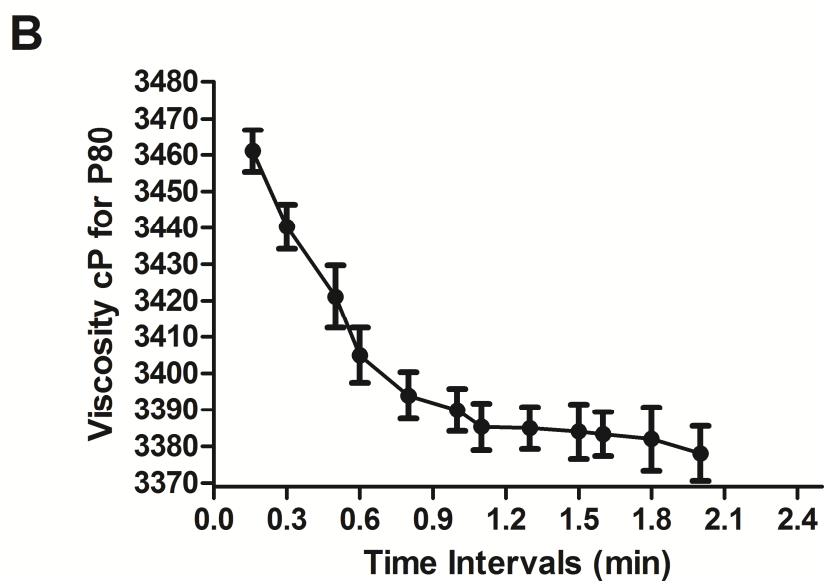
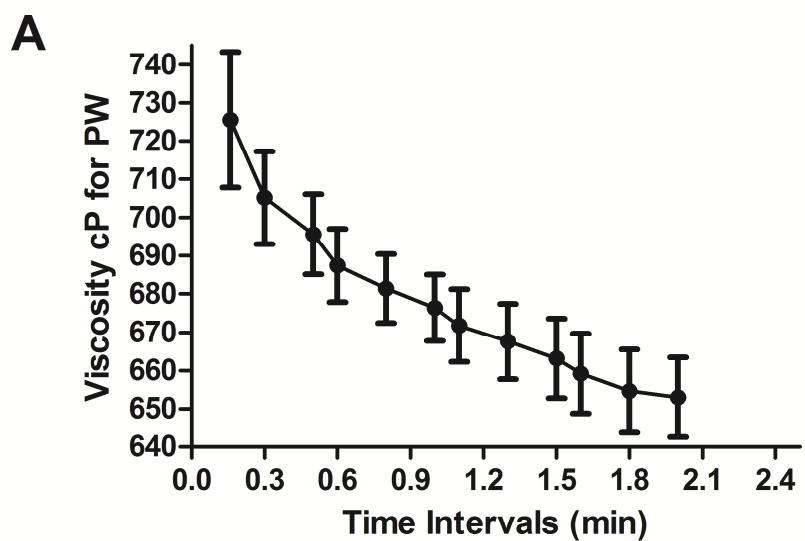




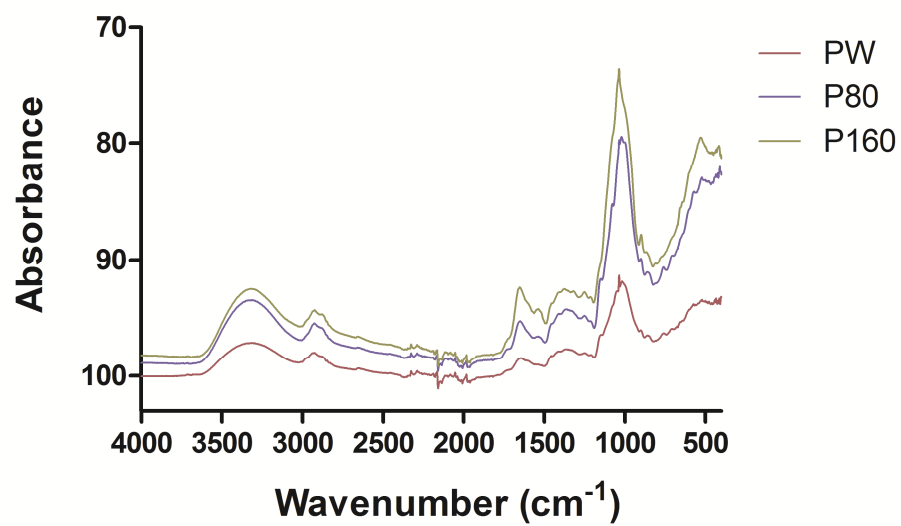
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**Highlights**

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

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