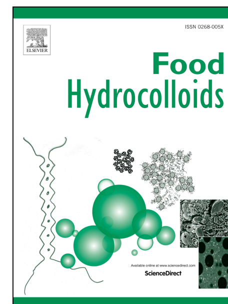
Accepted Manuscript



Characterisation of soluble and insoluble cell wall fractions from rye, wheat and hull-less barley endosperm flours

Penny Comino, Helen Collins, Jelle Lahnstein, Cherie Beahan, Michael J. Gidley

PII: S0268-005X(14)00135-0

DOI: 10.1016/j.foodhyd.2014.04.005

Reference: FOOHYD 2566

To appear in: *Food Hydrocolloids*

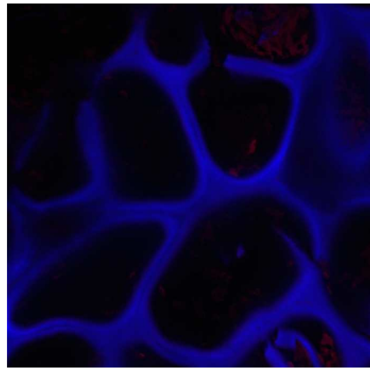
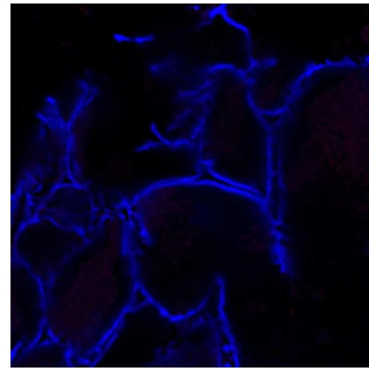
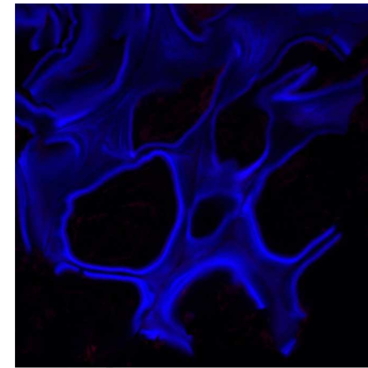
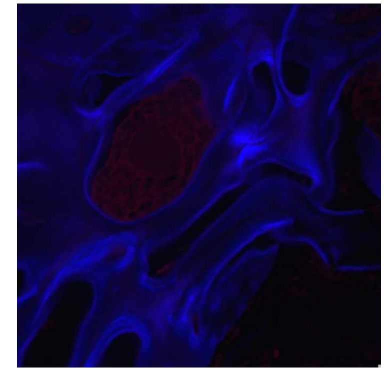
Received Date: 27 November 2013

Revised Date: 30 March 2014

Accepted Date: 2 April 2014

Please cite this article as: Comino, P., Collins, H., Lahnstein, J., Beahan, C., Gidley, M.J, Characterisation of soluble and insoluble cell wall fractions from rye, wheat and hull-less barley endosperm flours, *Food Hydrocolloids* (2014), doi: 10.1016/j.foodhyd.2014.04.005.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Degrees of Solubilisation of Wheat Endosperm Cell Wall

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

1 **Characterisation of soluble and insoluble cell wall fractions from rye, wheat**

2 **and hull-less barley endosperm flours**

3

4 ***Penny Comino*[1], *Helen Collins*[2], *Jelle Lahnstein*[2], *Cherie Beahan*[3], *Michael J***
5 ***Gidley*[1*]**

6

7 [1] The University of Queensland, Centre for Nutrition and Food Sciences, ARC Centre of Excellence in
8 Plant Cell Walls, Queensland Alliance for Agriculture and Food Innovation, St. Lucia, 4072, Australia

9 [2] The University of Adelaide, ARC Centre of Excellence in Plant Cell Walls, School of Agriculture, Food
10 and Wine, Waite Campus, Urrbrae, 5064, Australia

11 [3] The University of Melbourne, ARC Centre of Excellence in Plant Cell Walls, Plant Cell Biology
12 Research Centre, School of Botany, VIC 3010, Australia

13

14

15

16

17

18

19

20

21

22

23 Corresponding Author Mike Gidley *Email: m.gidley@uq.edu.au Phone: +61 7 3365 2145

24

ACCEPTED MANUSCRIPT

25 **Abstract:**

26 Within cereal endosperm flours, arabinoxylan and β-glucan molecules exist in either a
27 soluble or an insoluble form. From a nutritional functionality viewpoint, soluble and
28 insoluble forms offer different potential health advantages, so it is important to define both
29 the features controlling solubilisation and the properties of each of the soluble and
30 insoluble fractions. Factors known to affect the stability of arabinoxylan (AX) and β-glucan
31 (BG) solutions include AX branching extent and type, and the ratio of cellotriose to
32 cellotetraose units (DP3/DP4) in BG. Through studying the solubilisation of AX and BG from
33 wheat, rye, and hull less barley endosperm under conditions that avoid the use of alkali or
34 ethanol during the solubilisation process, we report (a) similar A/X ratios and fine structures
35 for extracted soluble arabinoxylan and the corresponding insoluble AX within the cell walls
36 for rye and wheat endosperm flours, (b) comparable DP3/DP4 ratios for soluble β-glucan,
37 flour and insoluble β-glucan within the endosperm cell wall of hull less barley, and (c)
38 evidence for enrichment of β-glucan at the exterior of residual insoluble cell walls.
39 Therefore, the factors determining solubilisation of AX and BG from endosperm cell walls
40 are different to those that determine the stability of aqueous solutions of the same
41 polymers, and β-glucan may show limited solubilisation by being trapped within restraining
42 cross-linked arabinoxylans in the cell wall.

43

44 Keywords: arabinoxylan; β-glucan; phenolic acid; cross-linking; soluble dietary fibre;
45 endosperm cell wall

46 ## 1 Introduction:

47 Polysaccharide hydrocolloids (dietary fibre) are important for the maintenance of human
48 colonic health. During transport through the digestive system, they exist mainly in either a
49 viscous soluble/swollen form or within an encapsulating matrix (Gidley, 2013). Soluble forms
50 of dietary fibre are associated with the potential to reduce plasma cholesterol and can also
51 attenuate the glycemic and insulinemic response to foods (Collins, Burton, Topping, Liao,
52 Bacic & Fincher, 2010). A more recently appreciated nutritional functional role of
53 polysaccharide hydrocolloids is that they can act as carriers of nutrients to the large
54 intestine, not just through encapsulation, but also from direct binding of e.g. phytonutrients
55 such as anthocyanins, phenolic acids, and other plant derived phenolic compounds
56 (Padayachee, Netzel, Netzel, Day, Mikkelsen & Gidley, 2013; Saura-Calixto, 2010).

57

58 Cereal endosperm (white) flours contain approximately 70-80% starch, 5-15% protein and
59 0.5-4% non starch polysaccharides (NSP). The NSP's contribute to dietary fibre and mainly
60 consist of cell wall associated arabinoxylan (AX) and β-glucan (BG), and to a lesser extent
61 arabinogalactan (AG). Broadly speaking, AX and β-glucan are generally categorised in terms
62 of potential nutritional functionality based on their aqueous solubility into either soluble or
63 insoluble fractions (Topping, 2007).

64

65 β-glucan is mainly composed of two major building blocks: cellotriose (DP3) and
66 cellotetraose (DP4) units linked β 1-3 (Cui & Wang, 2009; Wood, 2010; Woodward, Fincher
67 & Stone, 1983; Woodward, Phillips & Fincher, 1988). The ratio of DP3/DP4 is used as a

ACCEPTED MANUSCRIPT

68 fingerprint for identifying various types of cereal β-glucans, and is considered to be related
69 to polymer solubility. The further this ratio deviates from 1.0, the higher the relative
70 amounts of either trisaccharide or tetrasaccharide units, which favours more intermolecular
71 associations of regular repeat regions within the β-glucan chains, thus decreasing stability of
72 aqueous solutions (Izydorczyk, Biliaderis, Macri & MacGregor, 1998; Izydorczyk, Macri &
73 MacGregor, 1998).

74

75 The chemical structure of AX, is based on a chain of linear (1,4)-β-D-xylopyranose units,
76 which can be substituted with α-L-arabinofuranose in the O-2 or the O-3 position, or both.
77 Highly substituted AXs are generally soluble in aqueous media and do not tend to form
78 aggregates (Saulnier, Guillon, Sado & Rouau, 2007). On the other hand, low-substituted
79 isolated AXs have a strong tendency to form aggregates after dissolution, (Saulnier et al.,
80 2007). However, current evidence suggests that the arabinose and xylose features which
81 seem to control stability in solution do not appear to be the determinants of extractability
82 from endosperm cell walls (Dervilly-Pinel et al, 2001; Saulnier et al, 2012).

83

84 The AX structure also has occasional arabinofuranosyl residues esterified at O-5 with ferulic
85 acid (Collins et al., 2010; Muralikrishna, Rao & Subba, 2007; Nino-Medina, Carvajal-Millan,
86 Rascon-Chu, Marquez-Escalante, Guerrero & Salas-Munoz, 2010; Saulnier et al., 2007). The
87 amount of ferulic acid linked to AX is low and represents 0.2-0.4% of water-extractable AX
88 (WEAX) (w/w) and 0.6-0.9% of water-unextractable AX (WUAX) in wheat (Saulnier et al.,
89 2007). The insoluble forms of arabinoxylans within the cell walls thus contain higher levels

ACCEPTED MANUSCRIPT

90 of bound phenolic acids which may form oxidative cross-links (Muralikrishna et al., 2007)
91 and restrict extractability (Saulnier et al, 2012).

92

93 Soluble fibre typically has the ability to lower plasma cholesterol, reduce glycemia and other
94 health benefits (Lewis & Heaton, 1999; Moore, Park & Tsuda, 1998; Ou & Kwok, 2004;
95 Srinivasan, Sudheer & Menon, 2007) which are not shared by insoluble fibre. Insoluble fibre
96 may have different benefits in providing faecal bulk and delivering fermentable
97 carbohydrates and associated phenolic antioxidants throughout the colon (Fardet, 2010;
98 Topping, 2007; Lazaridou & Biliaderis, 2007); these benefits may result in a reduction in
99 colo-rectal cancer risk (Shewry, 2009; Vitaglione, Napolitano & Fogliano, 2008).

100

101 Water soluble β-glucans have been particularly well studied, and have been shown to
102 improve blood glucose regulation (Fardet, 2010; Topping, 2007) and reduce serum
103 cholesterol levels (Cui et al., 2009; Wolever et al., 2010) in diabetic and
104 hypercholesterolemic patients, respectively. Reducing blood serum cholesterol and
105 regulating blood glucose levels, are also correlated with the amount and molecular weight
106 of the solubilised β-glucans in the gastro-intestinal tract (Lazaridou et al., 2007; Wolever et
107 al., 2010; Wood, 2010). Such beneficial health effects have been attributed to the solubility
108 of β-glucans in water and their capacity to form highly viscous solutions (Kahlon, Chow,
109 Knuckles & Chiu, 1993; Tosh et al., 2010; Wood, Braaten, Scott, Riedel, Wolynetz & Collins,
110 1994b).

111

ACCEPTED MANUSCRIPT

112 Characterisation of rye, wheat and hull less barley AX, β-glucan and phenolic acid levels
113 within the soluble and insoluble fractions of endosperm cell walls, gives the opportunity to
114 tailor hydrocolloid fibre functionality through selection of cereal varieties and food
115 processing conditions. This paper uses a recently-reported method (Comino et al, 2013) for
116 the separation of soluble and insoluble endosperm cell wall fractions and the purification of
117 each as well as the fractionation of soluble AX and BG, and reports the structure and
118 properties of both soluble and insoluble fibre fractions from rye, wheat, and hull-less barley
119 endosperm flours to identify (a) factors affecting solubilisation and (b) the architectural
120 features of insoluble CW fractions.

121

122 2 Materials and Methods:

123 Wheat endosperm flour was supplied from the Macro Food Company (Sydney; NSW), rye
124 endosperm flour (*Bevy*) from Laucke Mills (Strathalbyn; SA), barley hull-less endosperm flour
125 (*Finniss*) was from the University of Adelaide, Waite Campus; Urrbrae, SA.

126

127 ^{1}H-NMR Materials: DMSO-*d*6 (methyl sulfoxide-D_6, 99.9 atom % D 151874-100g CAS 2206-
128 27-1), TFA (Trifluoroacetic acid T6508 – 5ml CAS 76-05-1), TSP (Trimethylsilyl propanoic acid
129 269913 – 1G CAS 24493-21-8), D_2O (deuterium oxide 151882 – 10G CAS 7789-20-0), were
130 purchased from Sigma–Aldrich, St Louis, MO, USA.

131

132 Phenolic assay Materials: 2 M sodium hydroxide, 12 M hydrochloric acid, ethyl acetate, and
133 internal standard - 3,4-dimethoxy-cinnamic acid, Tri-Sil (1-Trimethylsilyl imidazole - Pyridine

134 mixture) were purchased from Sigma–Aldrich, St Louis, MO, USA. Standards – o-coumaric
135 acid, syringic acid, ferulic acid, p-coumaric acid, trans-cinnamic acid, were purchased from
136 Sigma–Aldrich, St Louis, MO, USA.
137
138 Histological, Immuno-labelling Materials: PST plastic moulds were from ProSciTech Pty Ltd
139 Thuringowa, 4817 Australia, OCT (Jung tissue freezing medium from Leica Microsystems
140 Systems, Nussloch Germany Order Number 0201 08926), 1% bovine Serum Albumin (BSA)
141 Sigma–Aldrich, St Louis, MO, USA, Phosphate Buffer Solution (PBS) Sigma–Aldrich, St Louis,
142 MO, USA, primary antibody (LM11) Monoclonal antibody to (1-4)-β-D-Xylan / Arabinoxylan
143 (Rat IgM, LM11) Cat. No. LM11 from Plantprobes Leeds, UK, primary antibody (BG1)
144 Monoclonal Antibody to (1-3,1-4)-β-Glucan (Mouse IgG, Kappa Light) Cat. No. 400-3 from
145 BioSupplies Pty Ltd Parkville Victoria 3052, secondary antibody (fluorescent anti-rat Dy 405),
146 catalogue number 112-475-003 Jackson ImmunoResearch Laboratories, Inc. 872 West
147 Baltimore Pike West Grove, PA 19390 USA, secondary antibody (fluorescent anti-mouse
148 Alexafluor 647) Code number 115-605-003 Jackson ImmunoResearch Laboratories Inc, West
149 Grove, PA 19390 USA, Vectashield (mounting medium for fluorescence H-1000) from Vector
150 Laboratories Inc, Burlingame, CA, 94010 USA.
151

152 *2.1 Separation of water extractable and Insoluble (cell wall) Fractions from*
153 *Endosperm Flours*

154 The rye, wheat and hull less barley endosperm flours were fractionated in duplicate into
155 soluble (water extractable) and insoluble cell wall fractions (including separation of soluble
156 arabinoxylan and β-glucan from hull less barley) and the fractions treated to remove starch
157 and protein using the methods detailed in Comino et al, 2013.

ACCEPTED MANUSCRIPT

158

159 All water extractable fractions were characterised using ^{1}H-NMR, and monosaccharide
160 contents and DP3/DP4 ratios were determined using HPLC in duplicate. The insoluble cell
161 wall fractions were characterised by monosaccharide analysis and DP3/DP4 ratios (HPLC),
162 phenolic acid profiling (GC) in duplicate, and by both confocal and scanning electron
163 microscopy. The monosaccharide analysis, DP3/DP4 ratio determinations, ^{1}H-NMR, and β-
164 glucan assays were performed as described in Comino et al, 2013. Results reported are the
165 average of duplicate measurements on each of duplicate fractionations.

166

167 *2.2 Total Phenolic Acid Profile by GC-MS*

168 300µL of 2M NaOH was added to 5mg of dry extracted cell wall sample (in duplicate) in a
169 1.5ml tube. The tubes were flushed with N_2 and left overnight at room temperature in the
170 dark. Samples were then acidified with 55 µL 12 M hydrochloric acid (to pH 3.0) and internal
171 standard (3,4-dimethoxy-cinnamic acid - 5 µg) was added. Samples were then extracted
172 three times with 1 mL ethyl acetate. Extracts were then combined and dried under a
173 constant stream of nitrogen and then silylated with the addition of 50 µL N-O-bis
174 (trimethylsilyl acetamide). Samples were left at 100˚C for 5 min, and then resuspended in
175 dichloromethane and injected onto a GC-MS fitted with a CP SIL 5 column (Agilent
176 Technologies Australia Pty Ltd; 679 Springvale Road, Mulgrave Victoria 3170 Australia).
177 Results reported are the average of duplicate measurements.

178

179 *2.3 Histological Sample Preparation*

180 Samples were placed into PST (ProSciTech) plastic moulds, and covered with Jung tissue
181 freezing medium (Leica Systems, Wetzlar,Germany). Embedded samples were left at room

ACCEPTED MANUSCRIPT

182 temperature for 10min, then transferred into new plastic moulds and embedded again with
183 Jung tissue freezing medium at room temperature for 10min. This process was repeated
184 twice, and the samples stored at -20˚C. The additional use of Jung tissue freezing medium
185 ensures that it has been adequately absorbed into the cell wall sample to prevent crumbling
186 once the sample is sliced.

187

188 The frozen sample was mounted onto a circular flat disc located inside a Leica cryostat unit
189 (Leica CM1860 clinical cyrostat; Nth Ryde, Australia) by applying freezing medium, and
190 pressed gently into place. The adhered frozen sample was sliced to a thickness of
191 approximately 6 microns. The slice thickness was determined based on even and
192 representative staining with Haematoxylin and Eosin (H&E) staining in preliminary trials.

193

194 *2.4 Immunolabelling of AX and β-glucan*
195 Slides were covered and incubated in blocking buffer (1% bovine serum albumin (BSA) in
196 phosphate buffer solution (PBS)) for 30 min at room temperature. The AX primary antibody
197 (LM11 at a 1:20 dilution in blocking buffer eg: 250uL:5ml) was applied for 15mins at room
198 temperature, 25˚C. The β-glucan primary antibody (BG1 at a 1:500 dilution in blocking
199 buffer eg; 10uL:5ml) was applied over the primary AX antibody (LM11) and left for 1hr at
200 room temperature 25˚C, and then left overnight at 4˚C. Slides were washed twice with PBS
201 and three times with blocking buffer.

202

203 The secondary antibody for AX localisation (fluorescent anti-rat Dy 405) was applied for
204 15mins at room temperature (ca 25˚C), and then the secondary antibody for β-glucan

205 localisation (fluorescent anti-mouse Alexafluor 647) was applied and left for 1hr at room
206 temperature, and then overnight at 4˚C. Secondary antibody concentrations used were 10µL
207 Alexafluor 647 added to 500µL of blocking buffer for a final dilution 1:100 or 250µL blocking
208 buffer 10µL of Dy 405 for a final concentration of 1:50.

209

210 Slides were then washed four times with deionised water and dried for a few seconds in an
211 oven at 40˚C. The Vectashield was applied onto a cover slip and then gently pressed onto
212 the slide removing air bubbles in the process. Nail polish was applied around the edges of
213 the slide to secure the cover slip and dried in a fume cabinet at room temperature.

214 *2.5 Confocal Imaging*

215 The confocal microscope (Leica LSM 510META or LSM510) computer software (AIM 4.2) was
216 used. Samples were viewed using a 20x objective under fluorescent filters, and slides were
217 placed onto the stage. Images were viewed on an adjacent screen. Optimal image intensity
218 settings were selected prior to acquisition of final images. A typical example of the settings
219 used whilst scanning a final image were frame size 1024, scan speed 6, data depth 12 bit,
220 average 4.

221

222 *2.6 Scanning Electron Microscopy*

223 A 0.5mg sample of dried extracted cell wall powder was placed onto a steel pin (circular flat
224 head of approximately 10mm in diameter). A carbon coating was then applied followed by a
225 10 nm coating of platinum (Pt) using an Eiko IB-5 sputter coater and examined using a field
226 emission Scanning Electron Microscope (JEOL JSM 6300F) at 6 kV and 3–5 mm working
227 distance.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

228 # 3 Results and Discussion

229 ## 3.1 *Molecular composition of soluble and insoluble fractions*

230 Molecular compositions have been widely reported for wheat and, to a lesser extent, rye
231 endosperm cell wall polysaccharides. However, hull less barley, which is also regarded as an
232 important food cereal, has not been so widely studied. These three cereal types were
233 chosen for this study, not only for their consumer relevance and extensive utilisation within
234 the food manufacturing industry, but also for their diverse cereal fibre characteristics and
235 polymer fine structure.

236

237 Water extractable arabinoxylan (WEAX) and β-glucan (WEBG) and cell wall solubility
238 characteristics were compared between the three endosperm flour types using an
239 extraction method (Comino et al., 2013) designed to avoid treatments which could affect
240 the solubilisation process (such as alkali or alcohol) prior to separation of soluble and
241 insoluble fractions. This ensured, as far as is practical, that the dietary fibre was not
242 subjected to solubility and possible nutritional functionality changes caused by the
243 extraction and purification method, and extraction solvents. The WEAX yields obtained
244 (Comino et al., 2013), were 0.42g/100g for hull less barley (40˚C), 0.56g/100g for wheat and
245 1.64g/100g for rye endosperm flours. The WEBG yield obtained from endosperm hull less
246 barley was 0.47g/100g.

247

248

ACCEPTED MANUSCRIPT

249 *3.1.1 Aqueous solubilisation is not related to arabinoxylan A/X or β-glucan DP3/DP4*
250 *ratios*

251 A/X ratios for the endosperm wheat, rye and hull less barley WEAX extracts (Table 1) were
252 all in the range 0.52-0.65, consistent with previous reports (Collins et al., 2010; Ordaz-Ortiz
253 & Saulnier, 2005). The A/X ratios found for all samples studied here indicate extensive
254 substitution of the xylan backbone with arabinose which causes the polymer to be stable in
255 aqueous solution once dissolved. If A/X ratios are low, < 0.3 (Vinkx, Stevens, Gruppen,
256 Grobet & Delcour, 1995), or <0.43 (Andrewartha, Phillips & Stone, 1979) then the AX
257 polymer will tend to aggregate from solution through association of unsubstituted regions
258 (Andrewartha et al., 1979) of the xylan backbone. Thus based on solution stability criteria,
259 the AX from the insoluble cell walls, with A/X ratios of 0.54, 0.59 and 0.79 for rye, wheat,
260 and hull less barley respectively (Table 1), should have solubilised. The fact that only a small
261 percentage does, illustrates that stability in solution is a very different property from the
262 ability to solubilise AX from endosperm cell walls.
263
264 In addition to the A/X ratios, the DP3/DP4 ratio for hull less barley (Table 1) were similar in
265 the major water-extractable fraction to the water-unextractable fraction (2.5) both at room
266 temperature (ca 25 ˚C ; 2.6) and 40˚C (2.5; 2.6). The DP3/DP4 ratios determined for the
267 endosperm flours of wheat, hull less barley and rye were 2.2, 2.6 and 2.3 respectively (data
268 not shown). No values for DP3/DP4 ratio for wheat or rye endosperm flour have been
269 reported, but DP3/DP4 ratios for whole wheat flours are higher than those reported here
270 for endosperm flour eg; (hard red spring) DP3/DP4 ratio 3.04 – whole wheat flour cv.
271 Frederick DP3/DP4 ratio 3.84 (Wood, Weisz & Blackwell, 1991). Likewise reported DP3/DP4
272 ratios for rye wholegrain/wholemeal flour of 3.3 – 3.4 (Brummer, Jones, Tosh & Wood,
273 2008; Ragaee, Wood, Wang, Tosh & Brummer, 2008) and 3.45 (Tosh, Brummer, Wood,

ACCEPTED MANUSCRIPT

274 Wang & Weisz, 2004) are larger than the values found here for endosperm flour, suggesting
275 that endosperm β-glucan may have a different fine structure to β-glucans from other parts
276 of the grain. Izydorczyk and Dexter, 2008 reported DP3/DP4 ratios for different cell wall
277 tissues in the kernel of hull less barley (cv. *McGwire*), namely 4.24 (pericarp), 3.98
278 (aleurone), and 2.68 (endosperm). Higher DP3/DP4 ratios were found for the aleurone and
279 pericarp tissues compared to their counterparts in the endosperm CW. The higher DP3/DP4
280 ratios from the barley pericarp and aleurone layers (Izydorczyk and Dexter 2008) were
281 similar to ratios reported for wheat bran (Cui, Wood, Blackwell & Nikiforuk, 2000; Tosh et
282 al., 2004). Thus our findings of lower DP3/DP4 ratios for wheat and rye endosperm than
283 reported values for the corresponding wholegrain suggests a similar variation in ratio across
284 wheat and rye tissues as that found in barley.
285
286 This study has shown that with an initial dry heating step to inactivate the endogenous
287 enzymes, and the avoidance of either alcohol or alkali solvents prior to removal of AX
288 and/or β-glucan from cell walls, then the A/X, and DP3/DP4 ratios for the major soluble and
289 insoluble cell wall polysaccharides (AX in wheat and rye and β-glucan in barley) are virtually
290 the same.
291
292 Interestingly, the minor extractable components (β-glucan in wheat and rye, and AX in
293 barley) show different structural characteristics from the corresponding insoluble fraction.
294 The β-glucan co-extracted with AX from wheat and rye showed lower and higher DP3/DP4
295 ratios than the residual cell wall material respectively (Table 1), and the AX co-extracted
296 with β-glucan from hull less barley had a higher (WEBG fraction) and lower (WEAX fraction)
297 A/X ratio than the unextracted residue (Table 1). This difference between WEBG and WEAX

ACCEPTED MANUSCRIPT

298 composition may reflect the solvent conditions used in purifying these fractions (Comino et
299 al., 2013). However, these variations in composition do not show a consistent pattern and
300 represent only a minor fraction of the extractable polymers.
301

302 *3.1.2 Molecular Branching Characteristics of WEAX Using ^{1}H-NMR*

303 Table 2 shows the O-3 and O-2 mono substitution and di-substitution levels determined for
304 extractable fractions from the three endosperm cereal flours. The current results are similar
305 to literature reports of 19.2% (mono O-3 substitution) and 17% (O-3,O-2 di-substitution) in
306 wheat white flour WEAX (Cleemput et al., 1995), and 5.7-10.6% (O-3) and 17.6-23.1% (O-
307 3,O-2) in barley hull-less flour (Trogh, Courtin & Delcour, 2004). Cleemput et al (Cleemput et
308 al., 1995) started their extractions by heating the original white wheat flour at 130ºC for
309 90min and precipitating WEAX at 65% ethanol concentration after use of *B.lichenformis* α-
310 amylase, similar to the process used here. Trogh et al, 2004 used 80% ethanol whilst boiling
311 to inactivate endogenous enzymes in European hull less barley flour, then precipitated
312 WEAX using 65% ethanol. It should be noted that the values obtained by ^{1}H-NMR are
313 averages across a presumed range of arabinoxylan fine structures as has been previously
314 shown by selective precipitation of WEAX fractions (Cleemput et al., 1995; Trogh et al.,
315 2004; Verwimp, Van Craeyveld, Courtin & Delcour, 2007). In summary, AX substitution
316 patterns are characteristically different for the three cereals, but there is no apparent
317 relationship between substitution patterns and extractability.
318

319 *3.1.3 Molecular Composition of Insoluble Cell Wall Fractions- Phenolic acids*

320 As analysis of soluble fractions suggested that polysaccharide structural characteristics were
321 not the determinants of extractability form endosperm flours, we determined the phenolic

322 acid contents of the insoluble fractions. Saulnier et al (Saulnier, Guillon et al. 2007; Saulnier,
323 Guillon et al. 2012) have suggested that covalent cross-linking of AX chains through ferulic
324 acid dehydrodimers and trimers may be the major mechanism contributing to wall assembly
325 in cereal grains, promoting tissue cohesion and restricting cell expansion (Saulnier, Guillon
326 et al. 2012). Phenolic acid contents of the insoluble or water-unextractable cell wall
327 fractions from hull less barley, rye and wheat endosperm flours are shown in Table 3. The
328 wheat cell wall fraction had approximately twice the total amount of phenolic acids than the
329 rye and hull less barley flours ie; 2119μg/g for wheat, 884μg/g for rye and 1010μg/g for hull
330 less barley. The dominant phenolic acid was trans-ferulic acid with contents of 2109 µg/g
331 (wheat), 794 µg/g (rye) and 891 µg/g (hull less barley); *p*-coumaric, and syringic acids were
332 detected in minor amounts as found previously (Nyström et al., 2008; Rybka, Sitarski &
333 Raczynska-Bojanowska, 1993).

334

335 A wide range (491-1082μg/g of dm) of total phenolic acid contents has been reported for
336 different wholemeal rye flours, with the mean value being 685μg/g (Nyström et al., 2008)
337 but there are no reports for isolated endosperm flours. Lempereur et al, 1997 (Lempereur,
338 Rouau & Joel, 1997) also reported very high genetic variability with respect to the FA
339 contents of various durum wheat varieties studied, but there are apparently no previous
340 reports for wheat endosperm flour. Quinde et al, 2006 reported ferulic acid contents for hull
341 less regular barley of whole flours ranging from 355 to 493 μg/g, and total phenolic acid
342 values of 377-514 µg/g, somewhat lower than the value found in the present study. The *ρ*-
343 coumaric acid content was significantly lower in hull less genotypes (4-21 μg/g) (Quinde-
344 Axtell & Baik, 2006).

345

346 Compared with AX contents within the endosperm cell walls of approximately 20% (hull less
347 barley), 70% (wheat), and 60% (rye) (Table 1), there is no apparent correlation between
348 total AX and total phenolic acid contents shown in Table 3. Thus assuming that all phenolic
349 acid substitution is on AX, substitution levels vary for the three endosperm flours, having
350 values of 3.03mg/g, 1.47 mg/g, and 5.05 mg/g for wheat, rye, and hull less barley
351 respectively.

352

353 *3.2 Microstructure of insoluble cell wall fractions*

354 The microstructures of the extracted endosperm cell walls were examined by SEM and
355 confocal microscopy. The SEM images demonstrated the effective removal of starch and
356 protein during the extraction process (Figure 1). Cereal cell wall thicknesses were observed
357 to vary. The hull less barley appears to have the thickest cell walls, whilst the wheat cell
358 walls appear to be the thinnest (Figure 1). Autio et al, 2001 used fluorescence microscopy
359 to examine the endosperm cell walls of wheat and rye grains, and reported that the rye
360 grain had clearly thicker primary cell walls than the wheat grain, and that the thickness of
361 the walls was uniform in the different parts of the starchy endosperm (Autio Karin & and
362 Salmenkallio-Marttila Marjatta, 2001). It has been demonstrated (Bhatty, 1997; Oscarsson,
363 1996) through Calcofluor staining that the cell walls in the endosperm of barley grains with
364 high levels of β-glucans are thicker than in barley grains with low levels of β-glucans
365 (Izydorczyk & Dexter, 2008). The thinner wheat endosperm cell walls shown in Figure 1 are
366 consistent with less swelling during the solubilisation process, which may be related with
367 the relatively high level of phenolic acids (Table 3).

368

ACCEPTED MANUSCRIPT

369 To further probe the microstructure of unextractable cell wall fractions, confocal
370 microscopy has been used with individual selective labelling of β-glucan and arabinoxylan
371 using monoclonal antibodies. Images viewed without immunolabelling showed that
372 autofluorescence of phenolic acids was much less than that observed for antibody labels for
373 all endosperm tissues.
374

375 The confocal images of rye, wheat and hull less barley water unextractable cell walls from
376 endosperm flours (Figure 2) show varying amounts of β-glucan (red) and arabinoxylans
377 (blue). Where the arabinoxylan and β-glucans are co-localised, intermediate magenta
378 colours are observed (see Figure 2c). The wheat and rye cell walls clearly show higher levels
379 of arabinoxylan than β-glucan whilst the rye (Figure 2c) and hull less barley (Figure 2b)
380 showed increased levels of β-glucans, when compared to the wheat, (Figure 2a) consistent
381 with the monosaccharide results in Table 1. The β-glucans can also be located in the interior
382 of cells, loosely associated with cell walls, as shown in Figure 2a, Figure 2b and Figure 2c.
383 Guillon et al, 2004 reported that in the central starchy endosperm from wheat, β-glucans
384 were restricted to the inner border of the walls, and are therefore unlikely to contribute to
385 cell adhesion (Guillon, Tranquet, Quillien, Utille, Ordaz Ortiz & Saulnier, 2004). Saulnier et al,
386 2007 (Saulnier et al., 2007) showed a fluorescent micrograph of an aleurone cell wall from
387 wheat grain showing that arabinoxylans were more abundant at the interface between cells
388 and cell corners, whereas mixed-linked β-glucans were concentrated close to the plasma
389 membrane.

390

ACCEPTED MANUSCRIPT

391 Another interesting point is the apparent solubilisation of some wheat cell walls as shown in
392 Figure 3, although this was not found throughout the sample. According to Knudsen &
393 Laerke, 2010, the insoluble AX present in the non dispersed cell wall matrix has the ability to
394 swell and hold water in the cell wall matrix, thereby enhancing the water binding capacity of
395 digesta (Knudsen & Lærke, 2010). The swollen wheat cell walls (Figure 3b and c) appear to
396 show higher arabinoxylan concentrations around the inside edges of the walls, with the
397 same effect seen for rye (Figure 2c).

398

399 Partial swelling of arabinoxylan is found in the hull less barley cell walls (Figure 2b), and in
400 the rye cell walls (Figure 2c). The amount of swelling was found to vary for individual cell
401 wall residues within the insoluble wheat endosperm cell walls as illustrated in Figure 3.
402 However, a tight band of blue (arabinoxylan) fluorescence was seen as a common feature at
403 the inside edges of the cell wall even during cell wall swelling. This is shown most
404 prominently in the wheat endosperm flour cell wall in Figure 3c, and suggests that the
405 limited extractability of wheat endosperm cell walls is due to the anchoring of
406 polysaccharides to a dense inner layer of the cell wall, possibly through phenolic acid
407 dimers. The extensive apparent swelling observed in Figure 3c and d is consistent with
408 hydration-driven swelling being constrained by effective physical or chemical cross-links.

409

410 A common feature seen for all three cell wall fractions is the apparently much looser
411 attachment of β-glucan to the cell wall compared with arabinoxylan. The red labelling of β-
412 glucan is typically seen at the outer edge of cell walls or apparently dispersed away from cell
413 walls. This is particularly marked for hull less barley where the apparent low intensity of β-

414 glucan labelling (Fig 2B) compared with the high β-glucan content (Table 1) seems to be due
415 to a more widespread dispersion of loosely-associated β-glucan compared with rye and
416 wheat residues (Fig 2 A, C; Fig 3) in which the β-glucan appears to be more localised and
417 thus intense in the confocal images. However, it is important to note that this loosely
418 attached β-glucan is not fully soluble, as the fractions examined were all recovered as an
419 insoluble residue. These observations suggest that arabinoxylan acts as an anchor polymer
420 for the cell wall with β-glucan retained within the cell wall but able to swell to some extent.
421 This behaviour is consistent with phenolic acid cross-linking of arabinoxylan, but the
422 mechanism responsible for allowing β-glucan to swell away from the wall but not dissolve is
423 not clear and deserves further investigation.

424 *3.3 Implications for nutritional functionality*

425 The low levels of solubilisation of AX and BG clearly have great potential to be increased
426 through (a) greater understanding of the factors restraining solubility from walls and (b)
427 examining the effects of food processing on the extent of solubilisation. Confocal imaging
428 shows that at least some of the insoluble cell wall fraction exhibits significant swelling of
429 polymers which may provide some properties intermediate between those of solubilised
430 polymers (expected to influence glycemic response and plasma cholesterol levels) and those
431 of insoluble cell walls (e.g. bran) as bulking agents for promotion of regular digestive
432 transport. The data obtained supports the view that AX within the cell walls (particularly rye
433 and wheat) is anchored by means of phenolic acid cross linkages which prevent the active
434 solubilisation of AX from the cell walls. However, β-glucan acts differently.

435

436 Even though the solubility levels of β-glucan were relatively low, confocal microscopy
437 suggests that this polymer has an enhanced ability to swell away from cell walls with the

ACCEPTED MANUSCRIPT

438 potential to impart viscous properties to cell wall residues and thereby potentially generate
439 some of the nutritional properties normally associated with the viscosity enhancing
440 properties of soluble fibre. The mechanism responsible for allowing β-glucan to swell away
441 from the wall but not dissolve is unclear and deserves further investigation.
442
443 Cell wall swelling and the consequent enhanced water binding ability of the insoluble
444 fraction may have an impact on both small intestinal digestion (e.g. potential modulation of
445 enzymic digestion of starch, protein and/or triglycerides) and large intestinal fermentation
446 (e.g. changing the water holding capacity and the fermentation rate and extent).

447 ## 4 Conclusion:
448

449 The results in this study suggest that (1) the extractability of the major polymers from the
450 endosperm cell walls of wheat (AX), rye (AX) and hull less barley (β-glucan) are not related
451 to the intrinsic solution stability properties of the component polysaccharides but are more
452 likely to be controlled by physical and/or chemical cross-linking within the cell wall, (2) the
453 low levels of extractable polymers are consistent with efficient phenolic cross linkages that
454 retain the polysaccharides within the cell walls, (3)phenolic acid contents are inversely
455 correlated with the swelling of unextractable cell walls as observed in SEM images, (4) there
456 is no apparent difference in the molecular fine structures for either AX in wheat and rye or
457 β-glucan in barley between extractable and unextractable fractions, and (5) β-glucan is
458 more loosely associated with cell walls whereas arabinoxylans appear to be more tightly
459 held within the cell walls, presumably through occasional phenolic acid cross-linkages.
460

ACCEPTED MANUSCRIPT

461 These results thus suggest relationships between cell wall constituents and polysaccharide
462 solubility / swelling in cereal endosperms, and provide hypotheses for testing effects of cell
463 wall structure and processing on nutritional functionality for these important components
464 of many human diets.
465

466 Acknowledgements
467 Funding was made available from the CSIRO Flagship Collaborative Research Programme,
468 provided to the High Fibre Grains Cluster via the Food Futures Flagship.
469

470 Dr Deirdre Mikkelsen is thanked for her expertise in taking the scanning electron
471 micrographs. The authors acknowledge the facilities, and the scientific and technical
472 assistance of the Australian Microscopy & Microanalysis Research Facility at the Centre for
473 Microscopy and Microanalysis, The University of Queensland.
474
475

476 5 References:

477

478 Andrewartha, K. A., Phillips, D. R., & Stone, B. A. (1979). Solution properties of wheat-flour
479 arabinoxylans and enzymically modified arabinoxylans. *Carbohydrate Research*, *77*(1), 191-204.
480 Autio K., & Salmenkallio-Marttila, M. (2001). Light microscopic investigations of cereal grains,
481 doughs and breads. *Lebensmittel-Wissenschaft & Technologie*, *34*, 18-22.
482 Bhatty, R. S. (1997). Milling of regular and waxy starch hull-less barleys for the production of bran
483 and flour. *Cereal Chemistry*, *74*(6), 693-699.

ACCEPTED MANUSCRIPT

484 Brummer, Y., Jones, S., Tosh, S. M., & Wood, P. J. (2008). Extraction and physicochemical
485 characterization of rye β-glucan and effects of barium on polysaccharide molecular weight. *Cereal*
486 *Chemistry*, *85*(2), 174-181.
487 Cleemput, G., van Oort, M., Hessing, M., Bergmans, M. E. F., Gruppen, H., Grobe, P. J., & Delcour, J.
488 A. (1995). Variation in the degree of D-xylose substitution in arabinoxylans extracted from a
489 European wheat flour. *Journal of Cereal Science*, *22*(1), 73-84.
490 Collins, H. M., Burton, R. A., Topping, D. L., Liao, M.-L., Bacic, A., & Fincher, G. B. (2010). Variability in
491 fine structures of noncellulosic cell wall polysaccharides from cereal grains: potential importance in
492 human health and nutrition. *Cereal Chemistry*, *87*(4), 272-282.
493 Comino, P., Collins, H., Lahnstein, J., & Gidley, M., J. (2013). Separation and purification of soluble
494 polymers and cell wall fractions from wheat, rye and hull less barley endosperm flours for structure-
495 nutrition studies *Journal of Agricultural and Food Chemistry*, *61(49),* 12111−12122. Cui, S., & Wang,
496 Q. (2009). Cell wall polysaccharides in cereals: chemical structures and functional properties.
497 *Structural Chemistry*, *20*(2), 291-297.
498 Cui, W., Wood, P., Blackwell, B., & Nikiforuk, J. (2000). Physicochemical properties and structural
499 characterization by two-dimensional NMR spectroscopy of wheat β-D-glucan—comparison with
500 other cereal β-D-glucans. *Carbohydr. Polym*, *41*(3), 249-258.
501 Fardet, A. (2010). New hypotheses for the health-protective mechanisms of whole-grain cereals:
502 what is beyond fibre? *Nutrition Research Reviews*, *23*, 65–134.
503 Gidley, M. J. (2013). Hydrocolloids in the digestive tract and related health implications. *Current*
504 *Opinion in Colloid & Interface Science*, *18*(4), 371-378.
505 Guillon, F., Tranquet, O., Quillien, L., Utille, J.-P., Ordaz Ortiz, J. J., & Saulnier, L. (2004). Generation of
506 polyclonal and monoclonal antibodies against arabinoxylans and their use for immunocytochemical
507 location of arabinoxylans in cell walls of endosperm of wheat. *Journal of Cereal Science*, *40*(2), 167-
508 182.

ACCEPTED MANUSCRIPT

509 Izydorczyk, M., Biliaderis, C., Macri, L., & MacGregor, A. (1998). Fractionation of oat (1-3), (1-4)-β-d-
510 glucans and characterisation of the fractions. *Journal of Cereal Science*, *27*(3), 321-325.
511 Izydorczyk, M., Macri, L., & MacGregor, A. (1998). Structure and physicochemical properties of
512 barley non-starch polysaccharides—I. Water extractable β-glucans and arabinoxylans. *Carbohydrate*
513 *Polymers*, *35(3-4)*, 249-258.
514 Izydorczyk, M. S., & Dexter, J. E. (2008). Barley beta-glucans and arabinoxylans: molecular structure,
515 physicochemical properties, and uses in food products-a review. *Food Research International*, *41*(9),
516 850-868.
517 Kahlon, T. S., Chow, F. I., Knuckles, B. E., & Chiu, M. (1993). Cholesterol lowering effects in hamsters
518 of beta-glucan enriched barley fraction, dehulled whole barley, rice bran, and oat bran and their
519 combinations. *Cereal Chemistry*, *70*, 435–440.
520 Knudsen, K. E. B., & Lærke, H. N. (2010). Rye arabinoxylans: molecular structure, physicochemical
521 properties and physiological effects in the gastrointestinal tract. *Cereal Chemistry*, *87*(4), 353-362.
522 Lazaridou, A., & Biliaderis, C. G. (2007). Molecular aspects of cereal b-glucan functionality: Physical
523 properties, technological applications and physiological effects. *Journal of Cereal Science*, *46*, 101–
524 118.
525 Lempereur I., Rouau X., & Joel, A. (1997). Genetic and agronomic variation in arabinoxylan and
526 ferulic acid contents of durum wheat (Triticum durum l.) grain and its milling fractions. *Journal of*
527 *Cereal Science*, *25*, 103-110.
528 Lewis, S. J., & Heaton, K. W. (1999). The metabolic consequences of slow colonic transit. *American*
529 *Journal of Gastroenterology*, *94*(8), 2010-2016.
530 Moore, M. A., Park, C. B., & Tsuda, H. (1998). Soluble and insoluble fiber influences on cancer
531 development. *Critical Reviews in Oncology/Hematology*, *27*(3), 229-242.
532 Muralikrishna, G., Rao, M., & Subba, V. (2007). Cereal non-cellulosic polysaccharides: structure and
533 function relationship—an overview. *Critical Reviews in Food Science and Nutrition,*, *47*, 599–610.

ACCEPTED MANUSCRIPT

534 Nino-Medina, G., Carvajal-Millan, E., Rascon-Chu, A., Marquez-Escalante, J. A., Guerrero, V., & Salas-
535 Munoz, E. (2010). Feruloylated arabinoxylans and arabinoxylan gels: structure, sources and
536 applications. *Phytochemistry Reviews*, *9*(1), 111-120.
537 Nyström, L., Lampi, A.-M., Andersson, A. A. M., Kamal-Eldin, A., Gebruers, K., Courtin, C. M., Delcour,
538 J. A., Li, L., Ward, J. L., Fraś, A., Boros, D., Rakszegi, M., Bedő, Z., Shewry, P. R., & Piironen, V. (2008).
539 Phytochemicals and dietary fibre components in rye varieties in the heathgrain diversity screen.
540 *Journal of Agricultural and Food Chemistry*, *56*(21), 9758-9766.
541 Ordaz-Ortiz, J., & Saulnier, L. (2005). Structural variability of arabinoxylans from wheat flour.
542 Comparison of water-extractable and xylanase-extractable arabinoxylans. *Journal of Cereal Science*,
543 *42*(1), 119-125.
544 Oscarsson, M. (1996). Chemical composition of barley samples focusing on dietary fibre
545 components. *Journal of Cereal Science*, *24*(2), 161-170.
546 Ou, S., & Kwok, K.-C. (2004). Ferulic acid: pharmaceutical functions, preparation and applications in
547 foods. *Journal of the Science of Food and Agriculture*, *84*(11), 1261-1269.
548 Padayachee, A., Netzel, G., Netzel, M., Day, L., Mikkelsen, D., & Gidley, M. J. (2013). Lack of release
549 of bound anthocyanins and phenolic acids from carrot plant cell walls and model composites during
550 simulated gastric and small intestinal digestion. *Food & Function*, 4, 906-916.
551 Quinde-Axtell, Z., & Baik, B.-K. (2006). Phenolic compounds of barley grain and their implication in
552 food product discoloration. *Journal of Agricultural and Food Chemistry*, *54*(26), 9978-9984.
553 Ragaee, S., Wood, P., Wang, Q., Tosh, S., & Brummer, Y. (2008). Extractability, structure and
554 molecular weight of β-glucan from canadian rye (Secale cereale l.) whole meal. *Cereal Chemistry*,
555 *85*(3), 283-288.
556 Rybka K, Sitarski J, a., & Raczynska-Bojanowska (1993). Ferulic acid in rye and wheat grain and grain
557 dietary fibre. *Cereal Chemistry*, *70*(1), 55-59.
558 Saulnier, L., Guillon, F., Sado, P., & Rouau, X., eds (2007). *Plant Cell Wall Polysaccharides in Storage*
559 *Organs: Xylans (Food Applications)*. Elsevier Ltd.

560 Saura-Calixto, F. (2010). Dietary fiber as a carrier of dietary antioxidants: an essential physiological
561 function. *Journal of Agricultural and Food Chemistry*, *59*(1), 43-49.
562 Shewry, P. R. (2009). Wheat. *Journal of Experimental Botany*, *60*(6,), 1537–1553.
563 Srinivasan, M., Sudheer, A. R., & Menon, V. P. (2007). Ferulic acid: therapeutic potential through its
564 antioxidant property. *Journal of Clinical Biochemistry and Nutrition*, *40*(2), 92-100.
565 Topping, D. (2007). Cereal complex carbohydrates and their contribution to human health. *Journal of*
566 *Cereal Science*, *46*(3), 220-229.
567 Tosh, S., Brummer, Y., Wood, P., Wang, Q., & Weisz, J. (2004). Evaluation of structure in the
568 formation of gels by structurally diverse (1→3),(1→4)-β-d-glucans from four cereal and one lichen
569 species. *Carbohydrate Polymers*, *57(3)*, 249-259.
570 Tosh, S. M., Brummer, Y., Miller, S. S., Regand, A., Defelice, C., Duss, R., Wolever, T. M. S., & Wood, P.
571 J. (2010). Processing affects the physicochemical properties of β-glucan in oat bran cereal. *Journal of*
572 *Agricultural and Food Chemistry*, *58*(13), 7723-7730.
573 Trogh, I., Courtin, C. M., & Delcour, J. A. (2004). Isolation and characterization of water-extractable
574 arabinoxylan from hull-less barley flours. *Cereal Chemistry*, *81*(5), 576-581.
575 Verwimp, T., Van Craeyveld, V., Courtin, C. M., & Delcour, J. A. (2007). Variability in the structure of
576 rye flour alkali-extractable arabinoxylans. *Journal of Agricultural and Food Chemistry*, *55*(5), 1985-
577 1992.
578 Vinkx, C., Stevens, I., Gruppen, H., Grobet, P., & Delcour, J. (1995). Physiochemical and functional-
579 properties of rye nonstarch polysaccharides 6. variability in the structure of water-unextractable
580 arabinoxylans. *Cereal Chemistry*, *72*(4), 411-418.
581 Vitaglione, P., Napolitano, A., & Fogliano, V. (2008). Cereal dietary fibre: a natural functional
582 ingredient to deliver phenolic compounds into the gut. *Trends in Food Science & Technology*, *19*(9),
583 451-463.
584 Wolever, T. M., Tosh, S. M., Gibbs, A. L., Brand-Miller, J., Duncan, A. M., Hart, V., Lamarche, B.,
585 Thomson, B. A., Duss, R., & Wood, P. J. (2010). Physicochemical properties of oat β-glucan influence

ACCEPTED MANUSCRIPT

586 its ability to reduce serum LDL cholesterol in humans: a randomized clinical trial. *American Journal of*
587 *Clinical Nutrition*, *92*(4), 723-732.

588 Wood, P., Braaten, J., Scott, F., Riedel, K., Wolynetz, M., & Collins, M. (1994b). Effect of dose and
589 modification of viscous properties of oat gum on plasma glucose and insulin following an oral
590 glucose load. *British Journal of Nutrition*, *72*, 731–743.

591 Wood, P., Weisz, J., & Blackwell, B. (1991). Molecular characterization of cereal β-d-glucans.
592 structural analysis of oat β-d-glucan and rapid structural evaluation of β-d-glucans from different
593 sources by hplc of oligosaccharides released by lichenase. *Cereal Chem.*, *68*, 31-39.

594 Wood, P. J. (2010). Oat and rye β-glucan: properties and function. *Cereal Chemistry*, *87*(4), 315-330.

595 Woodward, J., Fincher, G., & Stone, B. (1983). Water-soluble (1→3), (1→4)-β-D-glucans from barley
596 (Hordeum vulgare) endosperm. II. Fine structure. *Carbohydrate Polymers*, *3*(3), 207-225.

597 Woodward, J., Phillips, D., & Fincher, G. (1988). Water-soluble (1 → 3,1 → 4)-β-d-glucans from barley
598 (Hordeum vulgare) endosperm. IV. Comparison of 40°C and 65°C soluble fractions. *Carbohydrate*
599 *Polymers*, *8*(2), 85-97.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Table 1: HPLC monosaccharide sugar composition, A/X and DP3/DP4 ratio, and β-glucan % analysis for endosperm flours and soluble and insoluble extracts

Sample	Mannose	Glucose	Galactose	Xylose	Arabinose	Total AX *	A/X Ratio	% β-glucan Megazyme (AOAC 995.16)	DP3/DP4 Ratio
	HPLC Monosaccharide Release (%w/w)								
Barley *Finnis*									
Barley WEAX RT	0.1	9.6	3.6	51.5	33.0	74.4	0.64		2.0
Barley WEAX 40˚C	0.2	9.8	4.2	51.0	32.7	73.6	0.64		1.9
Barley WEBG RT	0.4	80.4	0.4	0.6	0.8	1.3	1.02	74.8	2.6
Barley WEBG 40˚C	0.4	89.3	0.5	0.8	0.8	1.4	1.03	90.9	2.6
Barley Cell Wall	2.7	72.9	0.0	12.8	10.1	20.2	0.79	68.2	2.5
Wheat *Macro*									
Wheat WEAX	0.0	3.7	0.5	60.0	34.6	83.3	0.58		2.1
Wheat Cell Wall	3.6	19.5	0.8	47.7	28.3	66.8	0.59	19.2	2.3
Rye *Bevy*									
Rye WEAX	0.0	2.3	1.3	61.2	32.2	82.1	0.53		2.9
Rye Cell Wall	2.4	10.9	0.5	42.9	23.1	58.0	0.54	10.6	2.2

All samples analysed in duplicate with very small standard deviations - average values shown. *Total arabinoxylan contents calculated by adding arabinose and xylose sugar amounts and multiplying by 0.88 to account for the water molecule added during hydrolysis.

ACCEPTED MANUSCRIPT

Table 2: Summary of AX Branching Patterns for WEAX fractions from Wheat, Hull less Barley and Rye Endosperm flours

	Arabinose Branched Linkages ^{1}H-NMR				
Endosperm Flour	Mono O-3 %	Mono O-2 %	Total Mono	di O-2, O-3 %	Unsub %
Wheat ***AX***	17.8	2.2	20.0	13.6	66.3
Barley *Finnis* ***AX RT***	5.9	2.2	8.0	13.2	78.8
Barley *Finnis* ***AX 40˚C***	5.6	1.2	6.9	15.0	78.2
Rye *Bevy* ***AX***	31.3	0.8	32.1	4.8	63.1

ACCEPTED MANUSCRIPT

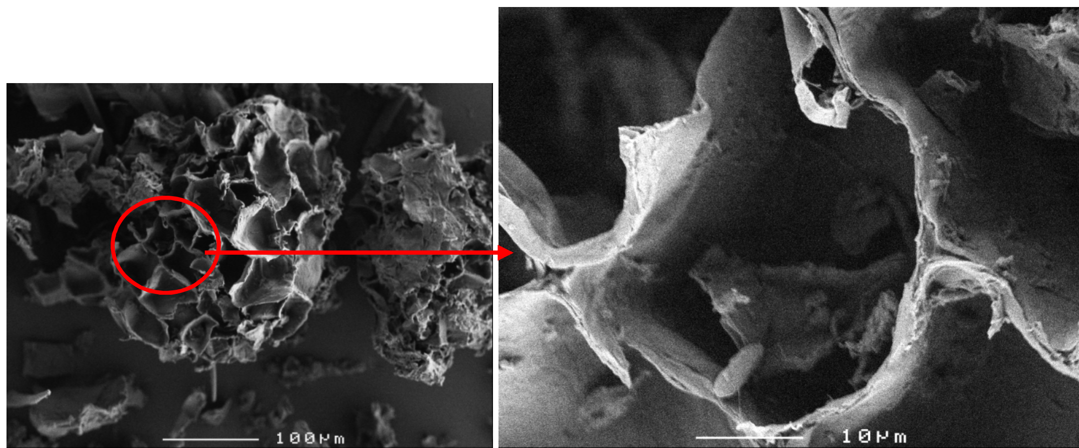
ACCEPTED MANUSCRIPT

Table 3: Phenolic acid profiles for wheat, rye and hull less barley insoluble cell wall fractions (CW) from endosperm flour. Results shown are the average of duplicate measurements.

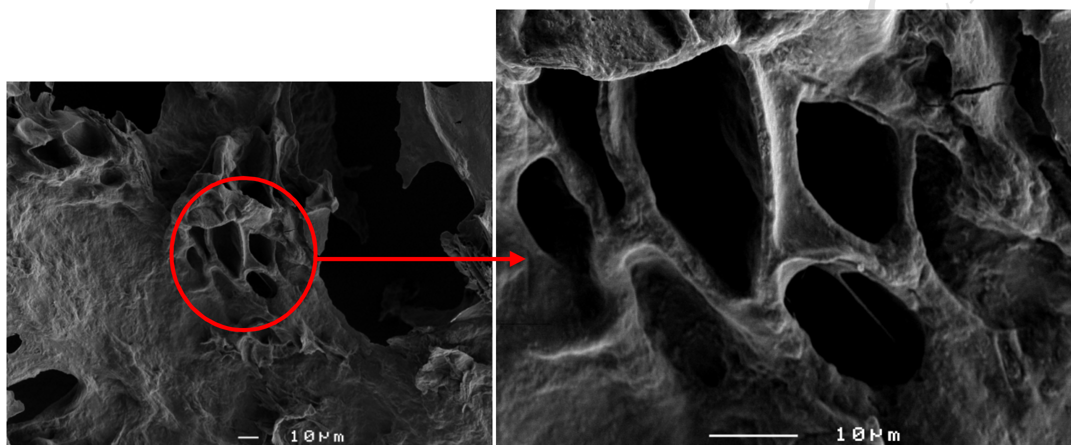
	Wheat CW		Rye CW		Hull less Barley CW	
	% Phenolics	µg/g	% Phenolics	µg/g	% Phenolics	µg/g
t-cinnamic	0	0	0	0	0	0
o-coumaric	0	0	0	0	0	0
syringic	0.4	7	1.5	14	7.3	96
p-coumaric	2.1	3	8.5	76	2.4	23
trans-ferulic	97.4	2109	90	794	90.4	891
TOTAL	100	2119	100	884	100	1010

ACCEPTED MANUSCRIPT

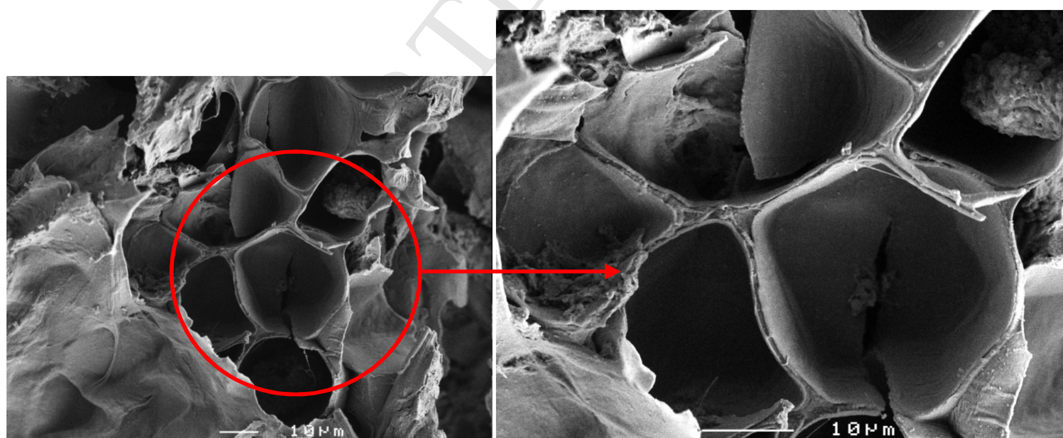
ACCEPTED MANUSCRIPT



A



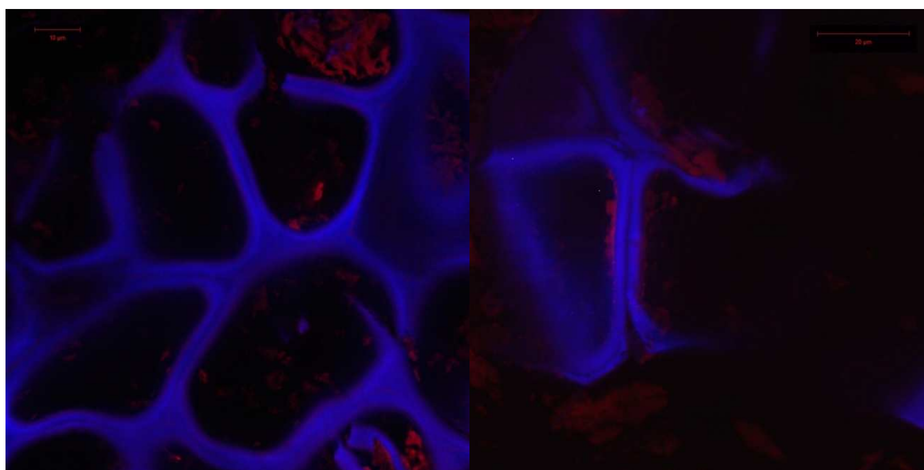
B



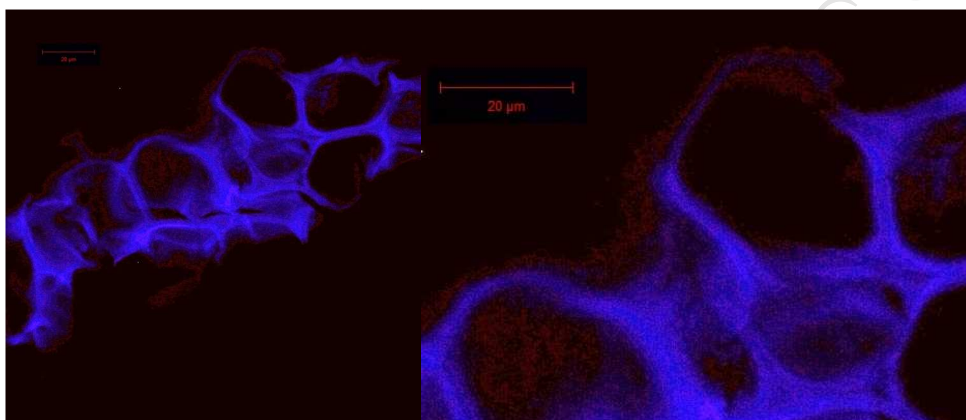
C

Figure 1: SEM images of extracted wheat (A), hull less barley (B) and rye (C) endosperm cell walls

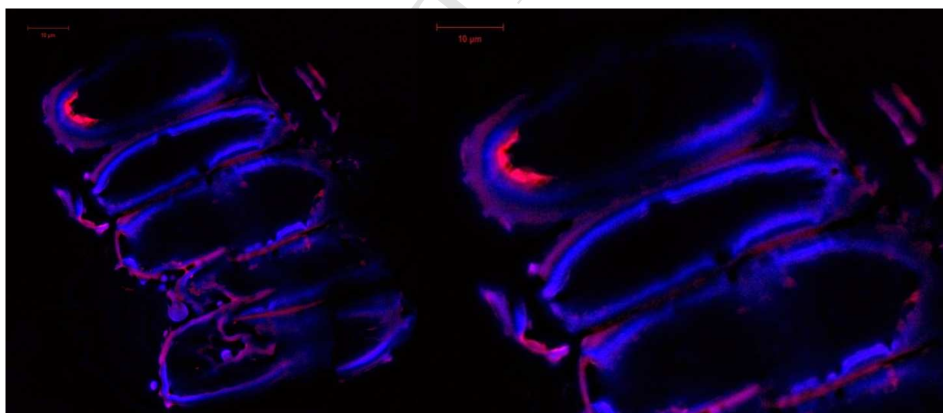
ACCEPTED MANUSCRIPT



A Wheat Endosperm Cell Wall Fraction (scale bar 10 µm left; 20 µm right)



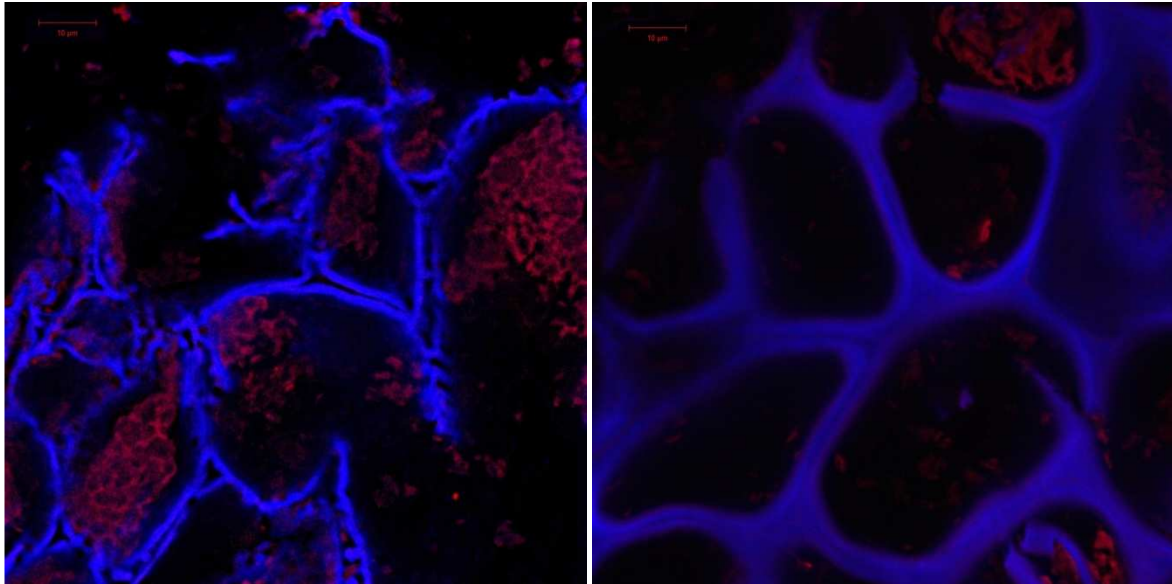
B Hull less Barley Endosperm Cell Wall Fraction (scale bars 20 µm)



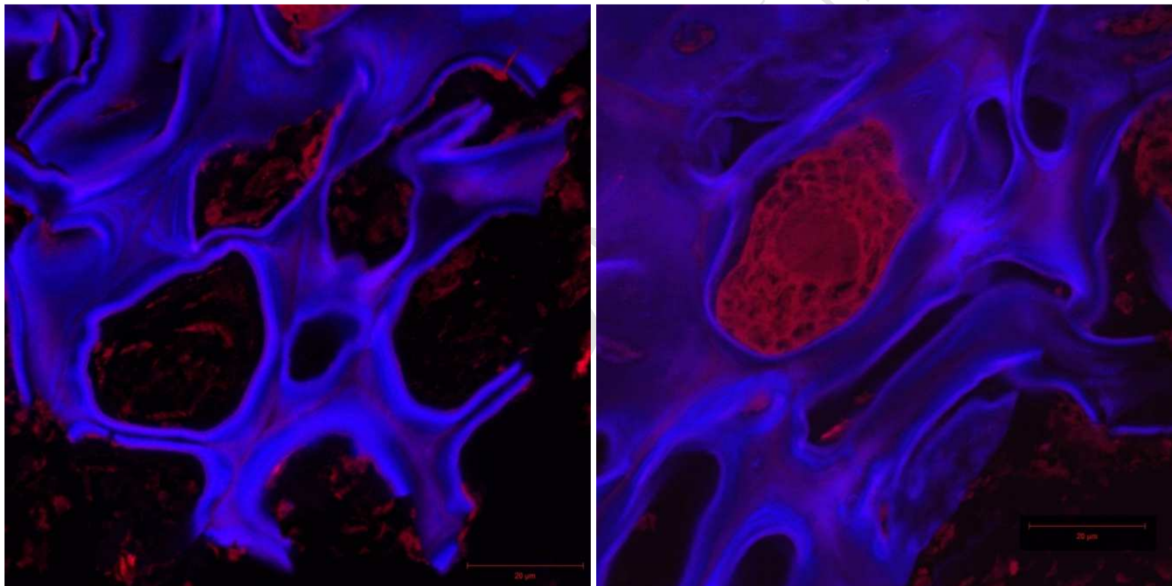
C Rye Endosperm Cell Wall Fraction (scale bars 10 µm)

Figure 2: Confocal Images of wheat (A), hull less barley (B) and rye (C) endosperm cell walls showing β-glucan (red) arabinoxylan (blue) and co-localisation of arabinoxylan and β-glucan (magenta)

ACCEPTED MANUSCRIPT



A **B**



C **D**

Figure 3: Confocal images showing varying degrees of swelling of the wheat endosperm flour cell wall CW (A, B scale bar 10µm; C, D scale bar 20µm)

ACCEPTED MANUSCRIPT

- Factors controlling extractability of polymers from cereal endosperm cell walls investigated.
- Extractability not correlated with molecular structure for either arabinoxylan or β – glucan.
- β-glucan more loosely held by unextractable cell walls compared to more tightly held arabinoxylans.

ACCEPTED MANUSCRIPT