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Effects of Diverse Food Processing Conditions on the Structure and Solubility of Wheat, Barley and Rye Endosperm Dietary Fibre

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Abstract:

The effects of archetypal food processing conditions (dough formation, baking, extrusion, and cooking/boiling) on dietary fibre structure and extractability from the endosperm flours of rye, hull less barley and wheat are reported. For all flours and processes, the distributions of soluble / insoluble cell wall dietary fibre as well as the chemical composition (arabinoxylan (AX) branching patterns, β-glucan DP3/DP4 (DP = degree of polymerisation) ratios) of solubilised fractions were characterised. The results show that overall the total amounts of AX and β-glucan (BG) were not significantly affected by processing but that there were similar increases in the soluble fibre fraction (20- 29%) for baked, extruded, and boiled/cooked processes for each flour, with lower (10-15%) increases for all flours processed into dough. In all cases, solubilised fractions of AX and BG had very similar chemical structures to the starting flour, suggesting that increased solubilisation was not due to specific chemical fractions. Confocal images illustrate loosely-held associations of β-glucan with the cell walls of processed foods in contrast to some of the arabinoxylans which appear more tightly held within the residual cell walls. The similarities in behaviour across the three grains are consistent with mechanical treatments during food preparation resulting in similar extents of disentanglement of physically-constrained AX and BG leading to their partial solubilisation.

Keywords: arabinoxylan; food processing; extrusion; cell wall; β-glucan

1 Introduction

Non-starch polysaccharides (NSP) are found in cereal endosperm cell walls, as well as the aleurone layer, the bran and the husk (Nino-Medina et al., 2010) and form a major portion of dietary fibre in many diets. The nutritional benefits of dietary fibre include positive effects on important biomarkers including increased microbial fermentation, promotion of beneficial microflora, lowered plasma cholesterol (Lewis and Heaton, 1999; Moore et al., 1998; Ou and Kwok, 2004; Srinivasan et al., 2007), and controlled glycemia (Bird and Topping, 2008; Jenkins et al., 1986; Muralikrishna et al., 2007; Plaami, 1997; Shelat et al., 2010). Other benefits include reduction in colo-rectal cancer (Shewry, 2009; Vitaglione et al., 2008), and increased faecal bulk and therefore relief from constipation (Lazaridou and Biliaderis, 2007).

The two main components of all common cereal endosperm flour cell walls are arabinoxylan (AX) and (1,3;1,4)-β-glucan (BG), whose chemical structures are based on a β-(1,4)-linked xylan backbone decorated with α-(1,2) and/or α-(1,3)-linked arabinose (AX) or unbranched chains of a block co-polymer of mostly cellotriose (DP3) and cellotetraose (DP4) connected by β-(1,3) linkages (BG).

As cereals are primarily consumed in the form of processed food, it is important to ascertain the effects of processing on dietary fibre levels, solubility, and functionality within cereal endosperm flours. Major generic processing conditions include dough formation and bread baking, noodle manufacture, and extrusion. Extrusion is widely used in the cereal food processing industries, involves the application of high heat, high pressure, and shear forces to an uncooked mass (Zhang et al., 2011), and is commonly used to produce breakfast cereals and snack foods.

70 The aims of this study were therefore to characterise the effect of archetypal food
71 processing operations on dietary fibre extractability, structure and properties for each
72 of three cereal endosperm flours (wheat, rye, hull-less barley) in order to
73 systematically study the effect of raw materials and food processes on potential
74 health-benefiting properties.

2 Materials and Methods

In this study, a series of cereal food processing conditions were applied to each of wheat, hull-less barley and rye. These processing conditions were selected to be typical of the wide range of treatments experienced in cereal-based foods, although some were unusual for one or more of the cereal starting materials. The treatments assessed were dough formation and baking (relevant to bread formation), extrusion (relevant to breakfast cereal and snack product production) and yellow alkaline noodle (YAN) manufacture. YAN was used as an example of not only boiling and cooking in water, but also of chemical (alkali) process treatment effects. The introduction of the alkali serves to toughen the noodle and impart the characteristic yellow colour, aroma and firm texture (Hatcher et al., 2005).

2.1 General Characterisation

2.1.1 Materials

Wheat endosperm flour (2010 Australian: unknown variety) was purchased from the Macro Food Company, Queensland. Rye endosperm flour (2010: *Bevy*) was purchased from Laucke Mills, South Australia; Barley hull-less endosperm flour (2010: *Finnis*) was obtained from the University of Adelaide, Waite Campus; South Australia. All flours were milled using commercial break rollers, were devoid of bran and husks (i.e. contained predominantly endosperm), and had particle sizes <150 µm. Before use, flours were sifted through a 75 µm mesh and particles that came through the sieve were discarded. Full analytical characterisation of these materials, and all other materials used for analytical methods have been detailed in (Comino et al., 2013; Comino et al., 2014).

2.1.2 Methods

Endosperm flours from rye, wheat, and hull-less barley were processed and fractionated into soluble (water extractable) and insoluble cell walls after removal of starch and protein using the methods detailed in (Comino et al., 2013). Extractions

were performed in duplicate unless otherwise stated. The processed water extractable fractions were characterised using HPLC (high performance liquid chromatography) for monosaccharide contents. The processed insoluble cell wall fractions were characterised by monosaccharide analysis, β-glucan analysis and DP3/DP4 ratios, and confocal microscopy. The unfractionated processed foods were also analysed for monosaccharide contents, β-glucan content and DP3/DP4 ratios, and microstructure (confocal microscopy).

The monosaccharide analysis, and total β-glucan assays were performed as per (Comino et al., 2013). Histological sample preparation, immuno-labelling of AX and BG, and confocal imaging microscopy were performed as described (Comino et al., 2014).

2.1.2.1 Analysis of DP3/DP4 ratios

The β-glucan DP3/DP4 ratios were determined after lichenase digestion as per the mixed-linkage β-glucan assay kit (AOAC Method 995.16) (Association of Official Methods of Analysis, 2006) from Megazyme (Wicklow, Ireland), using high performance anion exchange chromatography (Dionex ICS-5000; Column: Dionex CarboPac PA200 3x250mm + guard), at a temperature of 35^{o}C. The injection volume was 25 µL and the eluents used were A) 0.1M sodium hydroxide and B) 0.1M sodium hydroxide, 1M sodium acetate. Flow rate: 0.5mL/min with a gradient as follows

Gradient:	Time (min)	0	9	10	11	12	20
	%B	1	9	100	100	1	1

All gradient segments were linear, and detection used a pulsed amperometric detection (PAD), 20^{o}C, Gold Standard PAD waveform. The area under the peak was

126 quantified by comparison to a BG-OS (β-glucan oligosaccharides) DP3 and DP4
127 standard curve.

128 *2.1.2.2 Statistical Analyses*

129 Statistical analyses were performed using Minitab software version 16 (Minitab Inc.,
130 State College, PA, USA) to calculate means ± SD (standard deviation) of values
131 measured for each sample. All significant differences are reported at a significance
132 level of 0.05.
133

134 *2.1.3 General Food Processing Materials*

135 Dough and bread ingredients and recipes (dry basis %w/w): 96% w/w flour (either
136 wheat, hull-less barley or rye), 2% w/w yeast (Tandaco Dry Yeast from Cerebos
137 Foods; Seven Hills, NSW, Australia), 1% w/w sugar (CSR, Yarraville, Victoria,
138 Australia), and 1% w/w NaCl (Sigma–Aldrich, St Louis, MO, USA). Water was added
139 at 36% w/w (of the total dry w/w% formulation). An additional 20% w/w of water (or
140 56% w/w of the total dry w/w% formulation) was added to the hull-less barley flour to
141 enable a dough to be formed due to the different hydration absorption properties of
142 the flour.

143 Extruded product ingredients and recipes (dry basis %w/w): 98.7% w/w flour, 0.78%
144 w/w NaCl, 0.48% w/w emulsifier (Dimodan ® Danisco Australia Pty Ltd; Botany, New
145 South Wales (NSW), Australia) and 0.04% w/w α-tocopherol (Danisco Australia Pty
146 Ltd; Botany, NSW, Australia) (King et al., 2008). Water was added at 20-25% w/w
147 (of the total dry w/w% formulation) for the wheat and rye flours, whilst for the hull-
148 less barley, water was added at 48% w/w (of the total dry w/w% formulation). The
149 screw speed was 200 rpm and the configuration used is detailed in Figure 1.

Alkaline yellow noodle ingredient and recipe was adapted from (Morris et al., 2000): 99.5%w/w flour, and 0.5%w/w NaCl (dry basis %w/w) were added and mixed. The Kansui alkaline salt formulation made up of 0.6 %w/w sodium carbonate (Na_2CO_3) and 0.4% w/w potassium carbonate in distilled water. The Kansui formulated mix was added to the dry noodle formulation mix at 36% w/w (of the total dry w/w% formulation) rye and wheat noodle preparations, and 56% (of the total dry w/w% formulation) for the barley formulation. The alkaline noodle formulation and production was performed at the Leslie Research Centre, Queensland Department of Agriculture, Fisheries and Forestry (203 Tor Street, Toowoomba, Queensland 4350, Australia).

2.1.4 Processed Food Cell Wall and Solubilised Yield (WEAX and WEBG) Amounts and Percentage Calculations

The processed food yield percentages were calculated by subtracting the original flour yield weight (g), from the processed food yield weight (g), then dividing by the original flour weight and converting to a percentage. The WEAX (water extractable AX) yield was calculated from monosaccharide analysis of the solubilised fraction. The solubilised BG yield amounts (WEBG) were calculated by subtracting the WEAX yield (g) differences with the original flour, from the cell wall yield (g) and original flour difference.

2.1.5 Food Processing Operations

In order to determine the full effects of various food processing operations on the characterisation and functionality of the dietary fibre, the endosperm flours were designed to be the major ingredient (>96% w/w) in the various recipes. Other ingredients were only added so that the food could be produced, for example, the

yeast, sugar and salt incorporated into the dough and bread recipes so that that fermentation could take place.

2.1.5.1 Dough Fermentation Process

The dough and bread procedures were performed using a Panasonic Bread Bakery SD 251 (Macquarie Park; NSW; Australia) bread maker.

The dough procedure involved mixing all the ingredients for 10min to provide a dough-like consistency, then resting for 40min, kneading for 20min and allowing to rise for 70min. The dough proofing temperature was 30-35˚C.

The dough was then carefully scraped out of the bread maker and spread out onto a baking tray (50x30cm) using a plastic spatula. The tray was then placed into an air forced oven without heating, and air dried for approximately 12hrs, or until the moisture content was below 5%, using a vacuum drying oven.

The dried dough was then crushed into a powder using a mortar and pestle, and stored at -20^{o}C prior to analysis.

2.1.5.2 Bread Baking Process

The basic dough and bake options of the bread maker were used. This involved mixing of the ingredients (including water) for 10min, then resting for 45min, kneading for 20min, allowing to rise for 120min and then baking for 50min at 180-200˚C.

Complete bread loaves (including crusts) were broken into pieces by hand and then blended into bread crumbs (approximately <1mm pieces) using a Kenwood Triblade Hand Blender HB724 (de'Longhi via Lodovico Seitz, 47, 31100 Treviso TV, Italy) before being spread out onto a baking tray. The tray was placed into an unheated air

197 forced oven for 12hrs or until the moisture content was below 5% by using a vacuum
198 drying oven. The dried bread crumbs were then crushed into a powder using a
199 mortar and pestle, and stored at -20^{o}C prior to analysis.

200 *2.1.5.3 Yellow Alkaline Yellow Noodle Preparation*

201 Ingredients were weighed into a stainless steel mixing bowl fitted onto a Hobart N50
202 mixer (Hobart Corporation, 701 S. Ridge Ave, Troy, Ohio, USA). The flat paddle was
203 attached and then used to mix the dry ingredients for approximately 30 sec on
204 setting 1 (low speed). The prepared Kansui alkaline salt solution was added slowly
205 into the bowl and mixed with the dry ingredients. After 1 minute, the dough mixture
206 from the paddle and sides of the mixing bowl was scraped into the centre of the
207 bowl. The mixer was then started on setting 2 (high speed) and mixed for an
208 additional 4 minutes. After mixing was completed, the dough was then compressed
209 into a stainless steel press tray or box (Morris et al., 2000) measuring 18 × 9× 2 cm
210 (D × W × H) fitted with a scaled screw clamp, to form a dough block of approximately
211 5mm thickness, before being evenly pressed between the rollers of the noodle
212 machine (Ohtake Noodle Machine Manufacturing Co., Ltd., Tokyo, Japan).

213 The dough block was passed through the noodle machine with the roller gap set to
214 3mm (Izydorczyk et al., 2005; Lagassé et al., 2006). Once through, the dough sheet
215 was folded in half and passed through the rolls again. This step was repeated twice
216 (Morris et al., 2000). The dough sheet was then stored in a plastic bag and proofed
217 in an oven set at 28˚C for 30 minutes, after which it was subjected to decreasing roll
218 gap thicknesses of 2.0mm, 1.4mm and 1.0mm (Izydorczyk et al., 2005; Lagassé et
219 al., 2006; Morris et al., 2000). The dough sheet was passed through each roll gap
220 setting three times. Once rolling was completed, the noodle sheet thickness was

221 measured using Vernier callipers, and confirmed to be approximately 1mm. The
222 noodle dough sheet was cut into noodle strands approximately 45cm long, and
223 stored in plastic bags at -20˚C.

224 The hull-less barley flour formulation required 56% w/w moisture compared with 36%
225 w/w for wheat or rye, due to the increased water holding properties of the flour
226 (Hatcher et al., 2005). The dough formed was very crumbly and not as compressed
227 as the wheat and rye noodle dough. It was difficult to feed the barley dough through
228 the rollers of the noodle machine. The dough had to be put through the rollers at
229 least five times before a sufficiently cohesive dough was formed that could be cut
230 into noodle strands. Once the noodle was cooked at 100˚C, it broke into 5/6cm
231 lengths, whereas the wheat and rye noodles remained intact after boiling.

232 *2.1.5.4 Alkaline Noodle Cooking Water Preparation*

233 Approximately 120g of noodle was cooked in 1.2L of boiling water for 5min. The
234 noodle cooking water (broth) was passed through a sieve (screen size <0.1mm) and
235 the broth and noodles fractions collected. Any noodle strands or solid pieces left on
236 the sieve were added to the noodle solids total.

237 The drained cooked noodles were spread onto a baking tray measuring 50x30cm,
238 and placed into an unheated fan forced oven. The noodles were dried to a moisture
239 content of less than approximately 5% measured using a vacuum drying oven. The
240 dried noodles were then crushed into a powder using a Kenwood Triblade Hand
241 Blender HB724 (de'Longhi via Lodovico Seitz, 47, 31100 Treviso TV, Italy), mortar
242 and pestle, and was then stored at -20^{o}C. The noodle broth was freeze dried
243 (Ingelbrecht et al., 2001) and stored at -20^{o}C.

244 *2.1.5.5 Extruded Products Preparation*

245 The dry ingredients were thoroughly mixed using a Kenwood KM330 mixer
246 (Kenwood Australia, Sydney, NSW, Australia) fitted with a flat paddle for
247 approximately 15min. Blended dry mixes were extruded in triplicate trials using a
248 laboratory scale Prism Eurolab KX16 co-rotating twin screw extruder (Thermo
249 Electron (Karlsruhe) GmbH Dieselstr. 4 76227 Karlsruhe, Germany). The extruder
250 barrel length was 640mm, with screw diameter of 16mm, giving a total length to
251 diameter ratio (L:D) of 40:1. The screw configuration (Figure **1**) was fixed for the
252 duration of the extrusion trials. The SME (specific mechanical energy) was
253 calculated for all samples containing wheat, barley and rye and ranged between 120
254 and 386 kJ/kg.

255 The extruder barrel contained 10 temperature zones. The dry feed is conveyed into
256 the first zone of the barrel using a single screw volumetric feeder (KX16 powder
257 feeder, Brabender Technologie, Duisberg, Germany) at 1.2kg/hr. Following
258 preliminary trials, the barrel temperature and twin screw speed were set to the
259 conditions shown in Figure 1, which are typical for the extrusion of food starch
260 products (Gaosong and Vasanthan, 2000; Köksel et al., 2004; Ng et al., 1999; Singh
261 et al., 2007). The die plate was fitted with two circular die inserts measuring
262 approximately 2mm in diameter. Attached to the die plate was a pressure
263 transducer, (Terwin, Nottinghamshire, UK) which was connected to a pressure
264 reader (Tracker 220 series), so that feed and water rates could be altered if needed
265 to avoid excessive pressure build up.

266 Distilled water was injected into the second zone via a peristaltic pump (Cole-Palmer
267 Masterflex L/S (laboratory standard) 7523-50 Digital Console Drive Peristaltic Pump,

Thermo Fisher Scientific, Massachusetts, USA) at 85-120mL per min. Water was added at approximately 20-25% w/w for the wheat and rye flours, and 48% w/w for the hull-less barley to prevent the twin screws within the extrusion barrel becoming jammed.

The extruded foods were dried to a moisture content of less than 5% using a vacuum oven, then crushed into a powder and stored at -20^{o}C.

3 Results and Discussion

Food processing recipes incorporated at least 96% endosperm flour in the total mix to enable the clearest assessment of the effects of the food processing measures on endosperm AX and BG, and to minimise additional effects of other ingredient components although these cannot be ruled out.

3.1 Soluble and Insoluble Material as a Function of Food Processing

Soluble dietary fibre (WEAX and calculated WEBG) yields and insoluble cell wall fibre yield values shown in Table 1 demonstrate a similar re-distribution between insoluble (cell wall) and soluble fractions for each of the various food processing operations. There is a consistent reduction in insoluble cell wall content of around 20-25% for all three cereals and final food analogues (noodle, bread, extrudate) with an increase in the percentage of WEAX of 12-15% for both wheat and rye noodles, bread and extrudates. The calculated solubilised WEBG results for hull-less barley show increased levels ranging from 13% for dough to 22% for extrudate, whereas the wheat and rye WEBG increases ranged from 2%-14% for all food processes. This difference may be accounted for by the fact that barley not only contains higher amounts of β-glucan than wheat and rye, but also that the β-glucan has been shown in confocal images to be more loosely attached to the endosperm cell walls than AX (Comino et al., 2014). The dough preparations for all cereal types show relatively smaller increases in the amounts of solubilised WEAX and WEBG (Table 1), in line with the milder process compared to the final foods.

Others have described a transition of insoluble to soluble dietary fibre after processing of various cereal flours, fruits and vegetables, (Colin-Henrion et al., 2009;

Siljeström et al., 1986; Stojceska et al., 2010; Theander and Westerlund, 1987; Vasanthan et al., 2002). Colin-Henrion et al. reported an increase of 39% soluble dietary fibre after the production of apple sauce from apples (Colin-Henrion et al., 2009). Østergård et al (1989) reported increases of 13-18% soluble dietary fibre from extruded barley products. There have been some previous studies on the effects of bread making on solubilisation of wheat endosperm NSP's. The current data (Table 1) shows that the wheat cell wall (unextractable NSP) yields decreased 7% during fermentation (dough) and 19% during baking. Cleemput et al. (1997) found that for each of three wheat flour varieties, only very low levels (0-5%) of water unextractable NSP became water extractable NSP during fermentation. This contrasts with the maximum 25% solubilisation observed by Rouau et al. (1994). The current study (Table 1) shows an increase of approximately 18.5% for the total solubilised NSP (12.5% WEAX and 6% WEBG) yield during the baking of the wheat endosperm flour. Cleemput et al. found after baking that the degree of solubilisation of NSP increased by 22-30% depending on cultivar (Cleemput et al., 1997). A decrease in insoluble AX of 35% from wholemeal flour to the baked bread product has also been reported by Hansen et al (2002).

3.1.1 Arabinoxylan and β-glucan contents and structural features in processed foods

The total arabinoxylan and β-glucan levels as well as characteristic structural features (arabinose/xylose ratio for AX and DP3/DP4 ratio for BG) before and after food processing are shown in Table 2, and show no major losses of β-glucan or arabinoxylan across the various forms of processed foods. The only marked apparent loss was β-glucan found in the YAN noodle cooking water for hull-less barley (approximately 22%). However, once the hull-less barley cooked noodle and

323 cooking water β-glucan amounts are added together, then the totals of β-glucan are
324 approximately the same as found in the original flour. Izydorczyk et al (Izydorczyk et
325 al., 2005) also reported a reduction of β-glucan in fibre-enriched noodles after
326 cooking of about 30 g/kg during cooking of fresh YAN, but β-glucan levels within the
327 noodle cooking waters or broth were not tested and might have explained where the
328 "losses" occurred. Marconi et al (Marconi et al., 2000) studied pastas enriched with
329 β-glucan, but found no significant differences between the β-glucan content in
330 cooked and raw pasta.

331 If unprocessed barley flour is boiled in water, β-glucan losses of up to 70% can occur
332 (Fincher, 1975; Fleming and Kawakami, 1977). However, if barley flour is processed
333 into a fused food matrix arrangement, then the β-glucan losses appear to be minimal
334 (Table 2). Gaosong (Gaosong and Vasanthan, 2000) reported that β-glucan from
335 barley Phoenix flour was relatively resistant to extrusion-induced fragmentation,
336 resulting in improved structural regularity enhanced by inter chain associations
337 thereby suppressing solubility and therefore losses. They also reported that
338 extrusion cooking at higher temperatures and higher moisture levels influenced the
339 water solubility of β-glucan (Gaosong and Vasanthan, 2000).

340 Data in Table 2 show that the A/X (arabinoxylan/xylose) and DP3/DP4 ratios for all
341 processed foods tested are not altered from the original endosperm flours, consistent
342 with previous reports. Andersson (Andersson et al., 2004) investigated β-glucan
343 characteristics in dough and bread made from hull-less barley milling fractions, and
344 found that baking did not affect molecular weight, nor did any of the processing steps
345 affect β-glucan DP3/DP4 ratio (Andersson et al., 2004; Brennan and Cleary, 2005).
346 Hansen (Hansen et al., 2002) have reported that the A/X ratios of rye wholemeal

(flour, dough and breads) had no significant changes during the bread-making process. Similarly Cleemput (Cleemput et al., 1997) found no changes in the level of arabinose substitution of wheat AX during bread making. Siljeström (Siljeström et al., 1986) found that non-extruded and extruded wheat products contained 0.7% w/w arabinose and 1.0% xylose (Siljeström et al., 1986), in a similar A/X ratio as found in this study (Table 2).

3.1.2 Monosaccharide Analysis of the Soluble Fraction

From the galactose found by monosaccharide analysis (Table 3), it is apparent that arabinogalactans were co-extracted to a minor extent in the rye WEAX extractions, and more so in the wheat and barley. Typically highly branched arabinogalactans (AG), are co-extracted with AX for wheat and rye under aqueous conditions (Ganguli and Turner, 2008; Robert et al., 2005; Saulnier et al., 2007).

As shown previously (Comino et al., 2013), the amounts of arabinoxylan within the original hull-less barley endosperm flour are very low at around 1.25%. The pure WEAX amounts as shown in Table 3 are therefore lower for the hull-less barley processed foods when compared to wheat and rye, although the A/X ratios are similar for both processed and unprocessed endosperm flours.

3.2 Chemical and Microscopic Structure of the Insoluble Processed Fraction

Monosaccharide analysis of the endosperm flour and processed food insoluble cell wall extracts are shown in Table 4, and indicate similar total AX and BG levels for the processed food cell walls and the starting endosperm flour cell walls. Slightly increased AX levels were observed in the hull-less barley extracts but generally no marked differences were present between processed cell wall extracts and the

original endosperm flour. For all processing conditions, the A/X and DP3/DP4 ratios for the cell wall (Table 4) and WEAX (Table 3) fractions of each cereal are very similar to each other, suggesting that neither of these structural factors is responsible for the increased polymer solubility after processing (Table 1), consistent with data for non-processed flour (Comino et al., 2014). Processed soluble WEAX (Table 3) and insoluble cell wall (Table 4) fractions show apparent overall lower A/X ratios compared to the intact processed food (Table 2), although they have similar DP3/DP4 ratios. However, this may be due to inaccuracies in the monosaccharide analysis of the processed whole foods, for which absolute values for arabinose and xylose are low (Table 2) and dwarfed by the glucose contents, primarily from starch.

The confocal microscopy images shown in Figure 2 are examples of extracted cell walls from processed foods, and illustrate the ability to observe both arabinoxylan (blue) and β-glucan (red) in processed food samples. All images seem to show loosely held (more-dispersed) β-glucan not only on the outer edges of the cell wall, but also in the background of the image as seen for the same fraction from the starting flour (Comino et al., 2014). Tosh et al noted during extrusion that the β-glucan was dispersed throughout oat bran pieces and was therefore more bio-accessible, with progressive disruption of the cell walls and dispersal of the β-glucan with more severe processing conditions (Tosh et al., 2010). In contrast, much of the arabinoxylan (blue) appears to be more tightly held within the cell walls.

Taken together the data suggest that the fractions of flour AX and BG which are solubilised by a range of food processes represent polymers which are only held in the endosperm cell wall by physical entanglements. This is analogous to the physical

entanglements of starch polymers when they are cooked under low shear conditions, but which solubilise on high shear treatment (Zhang et al., 2014).

4 Conclusions

Dietary fibre (arabinoxylan and β-glucan) amounts were not significantly affected by (a) the alkaline and/ or boiling (100˚C) conditions in YAN production, (b) extrusion pressures and/ or temperatures, or (c) fermentation of dough, and/or baking temperatures of 200ºC.

Soluble dietary fibre was enhanced for each of the three cereals to a similar extent after diverse processing conditions, with no apparent change in either A/X or DP3/DP4 ratios. Thus there was no evidence that the increase in soluble fraction after food processing is selective for particular structural features of either arabinoxylan or β-glucan.

Confocal microscopic examination of the cereals revealed progressive disruption of the cell walls and the dispersal of loosely held β-glucan, whilst at least some of the arabinoxylan appears more anchored within the cell wall.

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561 **Figure Legends**

562 Figure 1: Prism EuroLabKX16 Co-Rotating Screw Configuration. Temperatures
563 achieved in zones 1 -10 were measured to be 36^{o}C, 49^{o}C, 66ºC, 101ºC, 120ºC,
564 119ºC, 119ºC, 120ºC, 130ºC, and 93ºC

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566 Figure 2: Confocal microscope images of processed foods with fluorescent
567 antibodies against β-glucan (red) and arabinoxylan (blue), (A) rye extruded food cell
568 wall (B) cooked wheat noodle cell wall (C) wheat dough cell wall (D) hull less barley
569 extruded food cell wall.

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Table 1: Yields (g) of insoluble cell wall and solubilised WEAX (water extractable arabinoxylan), and calculated solubilised WEBG (water extractable β-glucan) for wheat, rye and hull-less barley endosperm flours and corresponding processed foods. % values show differences between processed and flour forms; all values are averages of three independent extractions.

Cell Wall % Yield Results

Cereal Type	Original Flour	Cooked Alkali Noodle	% CW Noodle - flour	Dough	% CW Dough - flour	Bread	% CW Bread - flour	Extruded	% CW Extruded - flour
Wheat	2.30	1.76	**-23.48**	2.13	**-7.39**	1.87	**-18.70**	1.79	**-22.17**
Barley	3.30	2.63	**-20.30**	2.80	**-15.15**	2.55	**-22.73**	2.51	**-23.94**
Rye	6.20 [a]	4.67 [b]	**-24.68**	5.64 [a,b]	**-9.03**	4.76 [b]	**-23.23**	4.39 [b]	**-29.19**

wheat μ= 1.97 σ= 0.24 SEM= 0.11 p value= 0.1202 Barley μ= 2.76 σ= 0.32 SEM= 0.14 p value= 0.0509

Rye μ= 5.13 σ= 0.76 SEM= 0.34 p value= 0.0068

Solubilised WEAX % Yield Results

Cereal Type	Original Flour	Cooked Alkali Noodle	% WEAX Noodle - flour	Dough	% WEAX Dough - flour	Bread	% WEAX Bread - flour	Extruded	% WEAX Extruded - flour
Wheat	0.56	0.64	**14.29**	0.59	**5.36**	0.63	**12.50**	0.64	**14.29**
Barley	0.45	0.47	**4.44**	0.46	**2.22**	0.47	**4.44**	0.46	**2.22**
Rye	1.64	1.83	**11.59**	1.71	**4.27**	1.84	**12.20**	1.89	**15.24**

wheat μ= 0.61 σ= 0.04 SEM= 0.02 p value= 0.5736 Barley μ= 0.46 σ= 0.01 SEM= 0.004 p value= 0.6681

Rye μ= 1.78 σ= 0.10 SEM= 0.05 p value= 0.1493

Calculated Solubilised WEBG % Yield Results

Cereal Type	Original Flour	Cooked Alkali Noodle	% WEBG Noodle - flour	Dough	% WEBG Dough - flour	Bread	% WEBG Bread - flour	Extruded	% WEBG Extruded - flour
Wheat	0.42	0.46	**9.19**	0.43	**2.03**	0.45	**6.20**	0.45	**7.89**
Barley	0.48	0.56	**15.86**	0.54	**12.93**	0.57	**18.28**	0.59	**21.71**
Rye	1.54	1.74	**13.09**	1.61	**4.76**	1.71	**11.03**	1.75	**13.95**

wheat μ= 0.44 σ= 0.02 SEM= 0.01 p value= 0.6813 Barley μ= 0.55 σ= 0.04 SEM= 0.02 p value= 0.6128

Rye μ= 1.67 σ= 0.09 SEM= 0.04 p value= 0.1702

[a,b] values within a single row, within the same cereal type (not including % values), with different superscripts differ significantly ($p<0.05$)

n= 5 per cereal type; μ= mean; σ= standard deviation; SEM = Standard Error of the Mean

Table 2: Arabinoxylan (AX) and β-glucan analysis of processed food from rye, wheat and hull-less barley endosperm flours (samples performed in duplicate). Total AX and A/X ratios are apparent because of the low absolute values of the monosaccharide levels in the whole flour.

Sample	Monosaccharide HPLC				% β-glucan Megazyme (AOAC 995.16)	β-glucan DP3/DP4 ratio
	Xylose	Arabinose	Apparent Total AX	Apparent A/X Ratio		
Endosperm Barley Finnis Flour	0.79	0.60	1.22	0.76	1.57	2.63 [a]
Barley Dough	0.80	0.62	1.25	0.77	1.47	2.83 [b]
Barley Bread	0.80	0.61	1.24	0.76	1.46	2.71 [a,b]
Barley Extruded	0.78	0.59	1.21	0.76	1.40	2.67 [a]
Barley Noodle Cooking Water	0.07	0.05	0.10	0.76	0.34	2.74 [a,b]
Barley Noodle	0.66	0.51	1.03	0.77	1.21	2.80 [b]
Total Barley Noodle + Cooking Water	0.73	0.56	1.13	0.77	1.55	2.74 [a,b]
μ	0.78	0.60	1.21	0.77	1.49	2.72
σ	0.03	0.03	0.05	0.01	0.11	0.07
SEM	0.01	0.01	0.02	0.00	0.04	0.02
P (<0.05 values differ significantly)	0.4554	0.5633	0.2571	0.4610	0.1704	0.0232
Endosperm Rye Bevy Flour	3.05 [a,b]	2.32 [a,b]	4.73 [a,b]	0.76	1.20	2.33 [a,b]
Rye Dough	2.94 [a]	2.26 [a]	4.58 [a]	0.77	1.24	2.30 [a]
Rye Bread	3.17 [b]	2.42 [b]	4.92 [b]	0.76	1.23	2.41 [b]
Rye Extruded	2.97 [a]	2.28 [a]	4.62 [a]	0.77	1.26	2.34 [a,b]
Rye Noodle Cooking Water	0.15	0.12	0.24	0.79	0.05	2.46 [b]
Rye Noodle	2.94 [a]	2.21 [a]	4.53 [a]	0.75	1.08	2.35 [a,b]
Total Rye Noodle + Cooking Water	3.09 [a,b]	2.33 [a,b]	4.77 [a,b]	0.77	1.13	2.41 [b]
μ	3.04	2.32	4.72	0.76	1.21	2.36
σ	0.11	0.07	0.16	0.01	0.05	0.05
SEM	0.03	0.02	0.05	0.00	0.02	0.01
P (<0.05 values differ significantly)	0.0140	0.0455	0.0011	0.4667	0.2571	0.0426
Endosperm Wheat Macro Flour	1.32	0.88	1.94	0.67	0.20	2.23
Wheat Dough	1.29	0.87	1.90	0.67	0.20	2.20
Wheat Bread	1.42	0.94	2.08	0.66	0.20	2.19
Wheat Extruded	1.30	0.88	1.92	0.68	0.20	2.08
Wheat Noodle Cooking Water	0.05	0.03	0.07	0.66	0.02	2.01
Wheat Noodle	1.38	0.92	2.03	0.66	0.13	2.16
Wheat Noodle + Cooking Water	1.43	0.95	2.09	0.66	0.15	2.09
μ	1.35	0.90	1.98	0.67	0.19	2.17
σ	0.07	0.05	0.11	0.01	0.01	0.06
SEM	0.02	0.02	0.03	0.00	0.00	0.02
P (<0.05 values differ significantly)	0.2100	0.3916	0.0791	0.5197	0.8535	0.0581

Note: Noodle cooking water and noodle values were added and statistics then calculated

n=10 per cereal type

[a,b] values within a single column, within the same cereal type, with different superscripts differ significantly ($p<0.05$)

μ= mean; σ= standard deviation; SEM = Standard Error of the Mean

Table 3: Monosaccharide analysis of water extractable NSP fractions from processed foods. Values presented are the means of duplicate samples.

Sample	Monosaccharide HPLC % w/w Man	Gluc	Gal	Xyl	Ara	Total AX	A/X Ratio
Barley Hull less Flour WEAX	0.16	9.84	4.23 [a]	51.00	32.70	73.66	0.64
Barley Hull less Dough WEAX	0.05	10.35	1.10 [b]	51.35	33.45	74.62	0.65
Barley Hull less Bread WEAX	0.13	11.74	3.11 [a,b]	51.95	32.45	74.27	0.62
Barley Hull less Noodle WEAX	0.00	12.00	3.25 [a,b]	52.00	33.00	74.80	0.63
Barley Hull less Extruded WEAX	0.12	11.44	3.80 [a]	52.00	32.75	74.58	0.63
μ	0.09	11.07	3.10	51.66	32.87	74.39	0.64
σ	0.07	0.96	1.19	0.46	0.49	0.48	0.01
SEM	0.02	0.30	0.38	0.15	0.16	0.15	0.004
P (<0.05 values differ significantly)	0.9303	0.3125	0.0127	0.6313	0.6943	0.7979	0.5381
Rye Flour WEAX	0.00	2.31	1.32	61.17	32.18	82.15	0.53
Rye Dough WEAX	0.15	2.35	1.75	60.70	33.20	82.63	0.55
Rye Bread WEAX	0.28	2.24	2.01	68.59	34.85	91.03	0.51
Rye Noodle WEAX	0.15	1.55	1.70	68.45	34.60	90.68	0.51
Rye Extruded WEAX	0.42	1.50	2.37	69.58	34.59	91.67	0.50
μ	0.20	1.99	1.83	65.70	33.88	87.63	0.52
σ	0.16	0.45	0.39	4.30	1.25	4.79	0.02
SEM	0.05	0.14	0.12	1.36	0.40	1.52	0.01
P (<0.05 values differ significantly)	0.8459	0.0778	0.1005	0.1233	0.0913	0.1375	0.6156
Wheat Flour WEAX	0.00	3.67 [b]	0.54 [a]	60.04	34.56	83.25	0.58
Wheat Dough WEAX	0.15	2.35 [a]	0.35 [a]	60.95	34.00	83.56	0.56
Wheat Bread WEAX	0.19	3.26 [a,b]	3.90 [b]	59.21	32.34	80.56	0.55
Wheat Noodle WEAX	0.05	3.50 [b]	3.40 [b]	58.75	33.00	80.74	0.56
Wheat Extruded WEAX	0.11	3.46 [b]	3.98 [b]	58.82	33.21	80.99	0.56
μ	0.10	3.25	2.43	59.55	33.42	81.82	0.56
σ	0.08	0.60	1.75	1.15	0.96	1.74	0.01
SEM	0.03	0.19	0.55	0.37	0.30	0.55	0.003
P (<0.05 values differ significantly)	0.9225	0.0100	0.0380	0.1704	0.2162	0.1005	0.5891

[a,b] values within a single column, within the same cereal type, with different superscripts differ significantly ($p<0.05$)

n= 10 per cereal type; μ= mean; σ= standard deviation; SEM = Standard Error of the Mean

Table 4: Arabinoxylan and β-glucan analysis of insoluble cell wall fraction of processed foods (means of duplicate samples)

Sample	Monosaccharide HPLC %w/w					Total AX	A/X Ratio	% β-glucan Megazyme (AOAC 995.16)	DP3/DP4 ratio
	Man	Gluc	Gal	Xyl	Ara				
Barley Hull less Flour CW	2.71	72.93	0.00	12.83	10.09	20.17	0.79	68.19	2.50 [b]
Barley Hull less Dough CW	2.15	59.85	0.10	15.70	10.10	22.70	0.64	69.25	2.60 [a]
Barley Hull less Bread CW	2.22	62.94	0.10	15.95	10.24	23.04	0.64	64.10	2.65 [a,b]
Barley Hull less Noodle CW	2.25	60.10	0.15	15.85	10.35	23.06	0.65	66.20	2.65 [a,b]
Barley Hull less Extruded CW	2.16	61.06	0.10	15.84	10.17	22.89	0.64	60.90	2.60 [a]
μ	2.19	60.99	0.11	15.83	10.22	22.92	0.65	65.11	2.63
σ	0.06	1.45	0.06	0.11	0.11	0.17	0.01	3.42	0.05
SEM	0.02	0.51	0.02	0.04	0.04	0.06	0.00	1.21	0.02
P (<0.05 values differ significantly)	0.0563	0.2428	0.9148	0.8704	0.8904	0.6411	0.5891	0.1209	0.0274
Rye Flour CW	2.40	10.90	0.45	42.90	23.05	58.04	0.54	10.62	2.20
Rye Dough CW	1.45	10.45	0.30	42.60	22.70	57.46	0.53	10.85	2.25
Rye Bread CW	2.15	9.50	0.55	43.52	22.91	58.46	0.53	10.55	2.30
Rye Noodle CW	2.70	11.05	0.50	41.75	22.85	56.85	0.55	10.45	2.20
Rye Extruded CW	2.13	11.33	0.45	41.55	22.67	56.51	0.55	10.50	2.25
μ	2.16	10.65	0.45	42.46	22.83	57.46	0.54	10.59	2.24
σ	0.47	0.81	0.11	0.80	0.29	0.84	0.01	0.39	0.05
SEM	0.15	0.26	0.03	0.25	0.09	0.27	0.00	0.12	0.02
P (<0.05 values differ significantly)	0.0591	0.1333	0.6634	0.5870	0.8904	0.6792	0.6023	0.7209	0.0518
Wheat Flour CW	3.55 [a]	19.45	0.75	47.70	28.25	66.84	0.59	19.20	2.30
Wheat Dough CW	2.85 [b]	19.75	0.85	51.30	28.50	70.22	0.56	20.70	2.10
Wheat Bread CW	3.19 [a,b]	19.84	0.80	48.68	26.97	66.57	0.55	19.20	2.10
Wheat Noodle CW	3.70 [a]	20.45	1.25	48.00	25.65	64.81	0.53	19.60	2.15
Wheat Extruded CW	3.18 [a,b]	18.61	0.00	50.53	27.59	68.75	0.55	19.75	2.15
μ	3.29	19.62	0.73	49.24	27.39	67.44	0.56	19.69	2.16
σ	0.36	0.66	0.43	1.55	1.13	2.03	0.02	0.59	0.08
SEM	0.11	0.21	0.14	0.49	0.36	0.64	0.01	0.19	0.03
P (<0.05 values differ significantly)	0.0094	0.0816	0.4840	0.1367	0.3324	0.2412	0.5891	0.7629	0.0591

[a,b] values within a single column, within the same cereal type, with different superscripts differ significantly p<0.05)
n= 10 per cereal type; μ= mean; σ= standard deviation
SEM= Standard Error of the Mean

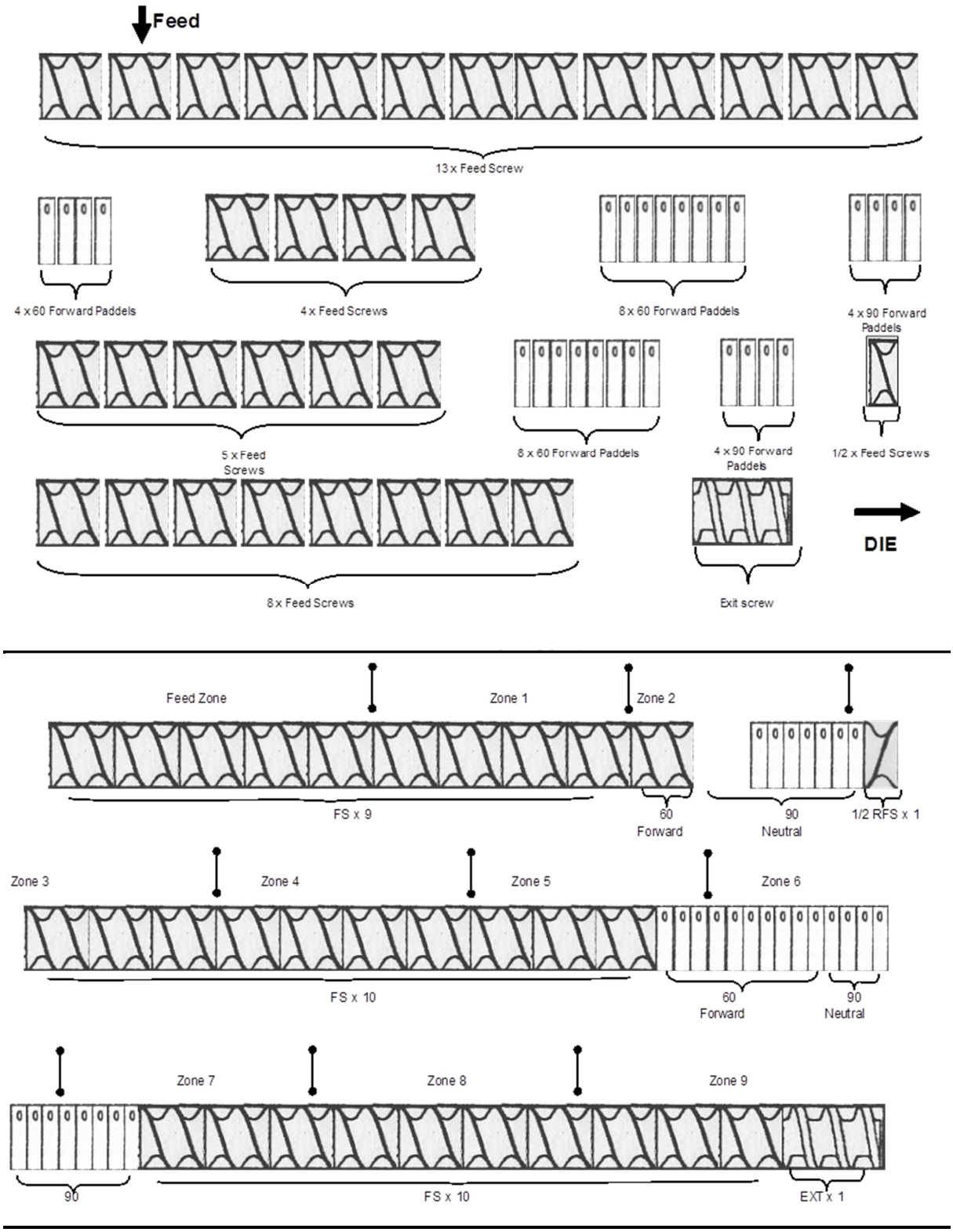
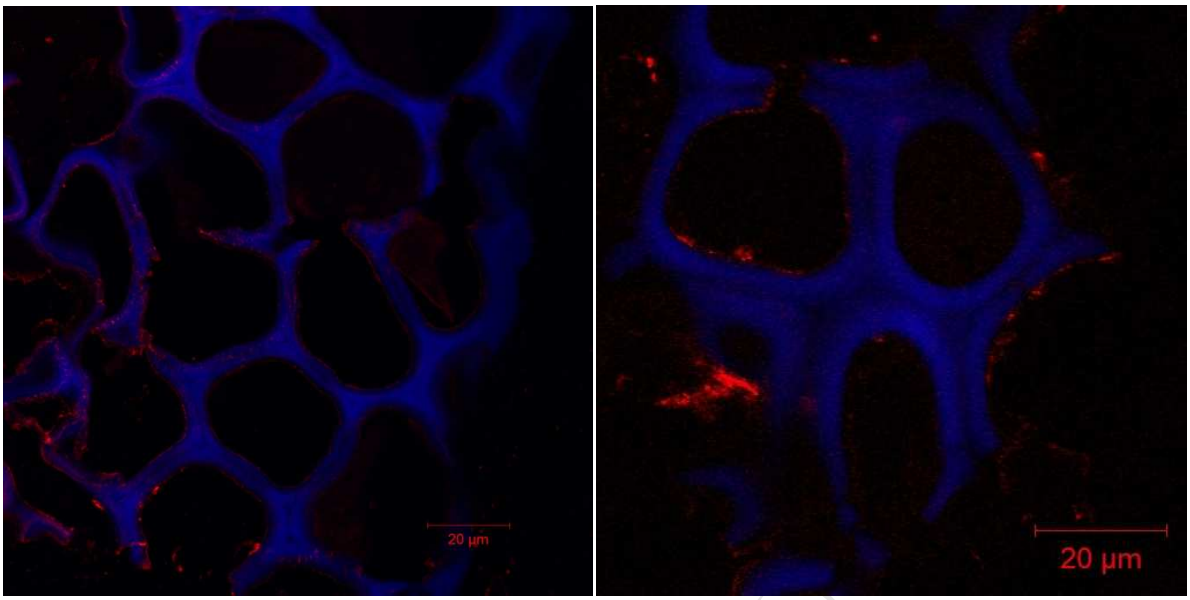


Figure 1: Prism EuroLabKX16 Co-Rotating Screw Configuration. Temperatures achieved in zones 1 -10 were measured to be 36oC, 49oC, 66ºC, 101ºC, 120ºC, 119ºC, 119ºC, 120ºC, 130ºC, and 93ºC



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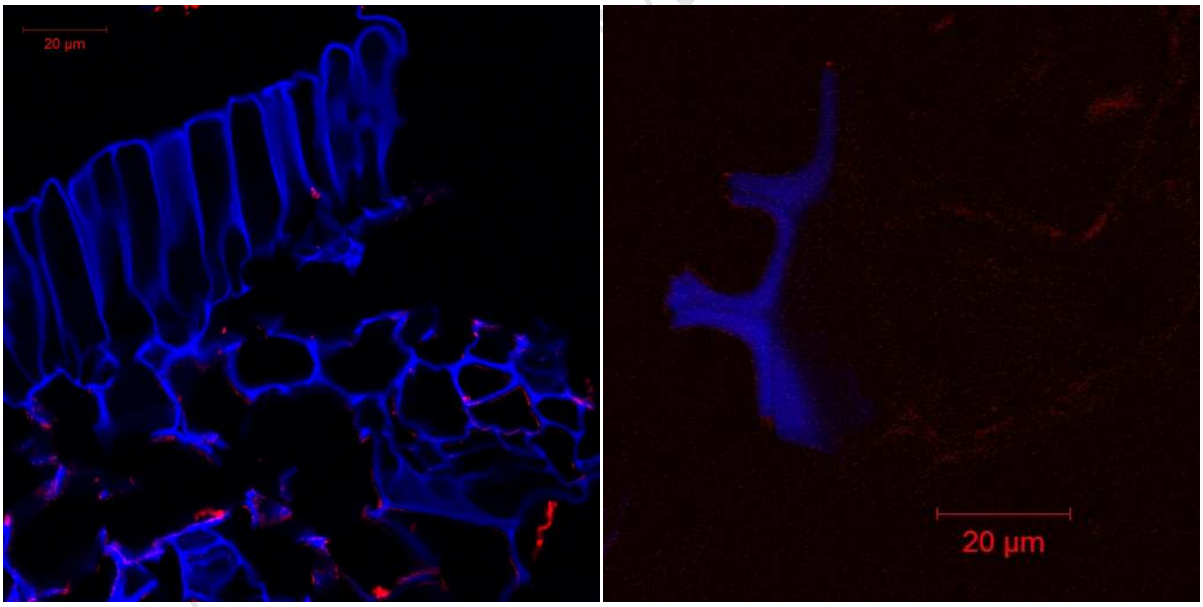

C D

Figure 2: Confocal microscope images of processed foods with fluorescent antibodies against β-glucan (red) and arabinoxylan (blue), (A) rye extruded food cell wall (B) cooked wheat noodle cell wall (C) wheat dough cell wall (D) hull less barley extruded food cell wall.

Highlights

- Effects of four food processing conditions on each of three cereal flours studied
- Structure and yield of soluble/insoluble dietary fibre determined for all treatments
- Consistent increase in soluble fibre yields across three flours after each treatment
- Polysaccharide chemical structure very similar for soluble and insoluble fractions
- Diverse food treatments increase fibre solubility through a common mechanism