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

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ABSTRACT

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

1. Introduction

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

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

β-glucan is mainly composed of two major building blocks: cellotriose (DP3) and cellotetraose (DP4) units linked β 1-3 (Cui & Wang, 2009; Wood, 2010; Woodward, Fincher, & Stone, 1983; Woodward, Phillips, & Fincher, 1988). The ratio of DP3/DP4 is used as a fingerprint for identifying various types of cereal β-glucans, and is considered to be related to polymer solubility. The further this ratio deviates from 1.0, the higher the relative amounts of either trisaccharide or tetrasaccharide units, which favours more intermolecular associations of regular repeat regions within the β-glucan chains, thus decreasing stability of aqueous solutions (Izydorczyk, Biliaderis, Macri, & MacGregor, 1998; Izydorczyk, Macri & MacGregor, 1998).

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

The AX structure also has occasional arabinofuranosyl residues esterified at O-5 with ferulic acid (Collins et al., 2010; Muralikrishna, Rao, & Subba, 2007; Nino-Medina et al., 2010; Saulnier et al., 2007). The amount of ferulic acid linked to AX is low and represents 0.2–0.4% of water-extractable AX (WEAX) (w/w) and 0.6–0.9% of water-unextractable AX (WUAX) in wheat (Saulnier et al., 2007). The insoluble forms of arabinoxylans within the cell walls thus contain higher levels of bound phenolic acids which may form oxidative cross-links (Muralikrishna et al., 2007) and restrict extractability (Saulnier et al., 2012).

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

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

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

2. Materials and methods

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

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

Phenolic assay Materials: 2 M sodium hydroxide, 12 M hydrochloric acid, ethyl acetate, and internal standard – 3,4-dimethoxycinnamic acid, Tri-Sil (1-Trimethylsilyl imidazole – Pyridine mixture) were purchased from Sigma–Aldrich, St Louis, MO, USA. Standards – o-coumaric acid, syringic acid, ferulic acid, p-coumaric acid, trans-cinnamic acid, were purchased from Sigma–Aldrich, St Louis, MO, USA.

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

2.1. Separation of water extractable and insoluble (cell wall) fractions from endosperm flours

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

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

2.2. Total phenolic acid profile by GC–MS

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