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Quantitative structural organisation model for wheat endosperm cell walls: Cellulose as an important constituent

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ABSTRACT

The cell walls of cereal endosperms are a major source of fibre in many diets and of importance in seed structure and germination. Cell walls were isolated from both pure wheat endosperm and milled flour. ^{13}C CP/MAS NMR in conjunction with methylation analysis before and after acid hydrolysis showed that, in addition to arabinoxylan (AX) and (1, 3; 1, 4)-β-D-glucan (MLG), wheat endosperm cell walls contain a significant proportion of cellulose (ca 20%) which is tightly bound to xylans and mannans. Light microscopy showed that the cellulose was relatively evenly distributed across the grain endosperm. The cell walls contain a fibrous acid-resistant core structure laminated by matrix polysaccharides as revealed by AFM imaging. A model for endosperm cell wall structural organisation is proposed, based on a core of cellulose and interacting non-cellulosic polysaccharides which anchors AX (with very occasional diferulic acid cross-linking) that in turn retains MLGs through physical entanglement.

1. Introduction

Wheat is one of the world's major staple crops, together with rice and corn. It forms the basis of a number of diets of different cultures across the globe, for example in pasta, noodles or bread. However, the nutritional functionality of wheat-based foods is often considered lower than other cereals such as oats and barley. This has been attributed in part to the low solubility of the dietary fibre as well as lower levels of (1, 3; 1, 4)-β-D-glucan (mixed-linkage glucan; MLG) in wheat. Refined (white) wheat flour is the basis for many foods and contains largely the central starchy endosperm with some residual outer layer material (aleurone, pericarp etc). Although the level of dietary fibre in wheat endosperm is relatively low compared to that of outer (bran) layers, yeast bread and rolls largely made from refined flour provided the largest single source of dietary fibre in US diets from 2003-2006 (Neil, Keast, Fulgoni, & Nicklas, 2012). Understanding the chemical composition and architecture of wheat endosperm cell walls is important to identify opportunities for enhancing the quality and quantity of dietary fibre that wheat can provide.

The dominant polysaccharide in wheat endosperm cell walls has been reported to be arabinoxylan (AX) then MLG, and an unusually low level (< 5%) of cellulose compared to other cell walls (Bacic & Stone, 1980; Collins et al., 2010). It is the large proportion of water insoluble AX that is thought to reduce the overall solubility of wheat fibre compared with either oat or barley. It has been proposed that AX cross-linking via ferulic acid residues is responsible for the limited swelling and solubility of wheat endosperm cell walls (Grabber, Hatfield, & Ralph, 1998; Kamisaka, Takeda, Takahashi, & Shibata, 1990). Ferulic

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acid is ester-linked to the C-5 position of some arabinose units in wheat (and other cereals) AX. Its concentration is much higher in the cell walls of aleurone and pericarp than the starchy endosperm of mature grain (Antoine, Peyron, Lullien-Pellerin, Abecassis, & Rouau, 2004; Barron, Surget, & Rouau, 2007). Some of the ferulic acid residues form a range of dehydrodiferulic acid cross-links, which are considered to be important for cell wall integrity. The external layers of the grain (aleurone and pericarp) exhibit higher levels of the cross-links than the starchy endosperm (Antoine et al., 2004; Lempereur, Surget, & Rouau, 1998). Among the peripheral layers, aleurone is the richest in ferulic acid, while the content of dehydrodimers is high in the pericarp (Antoine et al., 2003). Dimers form a bridge between the cell wall polymers (Bunzel, 2010; Hatfield, Ralph, & Grabber, 1999) which is proposed to affect wall properties such as extensibility, plasticity and degradability (Hatfield et al., 1999).

Most studies of wheat grain cell walls use milled flour as a starting material. Whilst this is rich in endosperm tissue, in a recent study (Gartaula, Dhital, Pleming, & Gidley, 2017) we showed that the cell walls isolated from wheat flour are different to the cell walls from pure starchy endosperm, in both microstructure and chemical composition. Wheat flour contains a small proportion of material from peripheral layers such as aleurone and pericarp that have much thicker cell walls than endosperm cells, and so can have a disproportionate influence on the analysis of flour cell walls compared to pure endosperm cell walls. While the composition and properties of cell walls isolated from wheat flour have been studied extensively (Bacic & Stone, 1980; Mares & Stone, 1973; Ordaz-Ortiz & Saulnier, 2005), less is known about endosperm cell walls. Pure endosperm cell walls can be obtained by dry heating grains to inactivate endogenous enzymes, soaking in water and then 'popping' the endosperm out manually after cutting the distal end of the grain (Gartaula et al., 2017). In this study, we used cell walls isolated from pure starchy endosperm 'popped' from whole grains and compared them with cell walls isolated from milled flour of the same variety for detailed chemical and microstructural characterisation. As 'popped' endosperm cell walls have a glucose content of 33.8% (Gartaula et al., 2017) which is greater than reported levels of MLG (Bacic & Stone, 1980), we hypothesize that there is a substantial amount of cellulose which could provide a scaffold for the cell wall.

2. Materials and methods

2.1. Materials

Whole wheat grains (*Triticum aestivum* var Lincoln) were donated by Pacific Seeds, QLD, Australia. Wheat flour (*T. aestivum* var Lincoln; 75% extraction rate) was obtained by milling the grains as described in Gartaula et al. (2017).

Thermostable alpha-amylase from *Bacillus licheniformis* (E-BLAAM; EC 3.2.1.1) and amyloglucosidase from *A. niger* (E-AMGDF; EC 3.2.1.3) were purchased from Megazyme International (Wicklow, Ireland). Protease from *B. licheniformis* (P4860; EC 3.4.21.62) was purchased from Sigma-Aldrich (Lane Cove, NSW, Australia). Feruloyl esterase (*Clostridium thermocellum*) (E-FAEZCT) was purchased from Megazyme International. Phosphate buffered saline (PBS) tablets were purchased from ThermoFisher Scientific Australia Pty Ltd. (Scoresby, Vic, Australia). All other chemicals and reagents were purchased from Sigma-Aldrich and Merck (Bayswater, Vic, Australia).

2.2. Isolation of cell walls

The endosperm of the whole grain was 'popped' out as described in Gartaula et al. (2017) by heating the grain to 130 °C for 90 min, then soaking for 5 days to moisten the endosperm, which allowed the endosperm to be 'popped' easily from the surrounding tissues. The isolation of cell walls from 'popped' endosperm and milled flour was carried out as detailed in Gartaula et al. (2017). Briefly, the flour or isolated endosperms were suspended in a PBS buffer (pH 7.2) and incubated with thermostable alpha-amylase, amyloglucosidase and protease to hydrolyse the starch and protein. The hydrolysis was carried out at an elevated temperature (70 °C) as well as at 37 °C. After complete digestion, the hydrolysed suspension was wet-sieved through a 20 μm screen under running water. The non-digestible cell walls remain on the screen and were centrifuged at 3200*g* for 10 min. The residue was washed twice with 70% ethanol followed by acetone, and left to air dry at room temperature.

2.3. Mixed-linkage glucans

The MLG content in the isolated cell walls was determined by the Streamlined Method (McCleary & Codd, 1991) using a mixed-linkage beta-glucan assay kit (AOAC Method 995.16) from Megazyme International. Analyses were performed in duplicate and the enzymes and standards from the assay kit were used. Briefly, samples were treated with lichenase and β-glucosidase and the D-glucose thus produced was assayed using a glucose oxidase/peroxidase reagent. The absorbance was measured at 510 nm.

2.4. Updegraff treatment

The cellulosic fraction of cell walls was isolated by hydrolysis with Updegraff reagent (Updegraff, 1969), a mixture of acetic acid, water and nitric acid in 8:2:1 ratio. Briefly, about 20 mg of the cell walls were placed in a glass tube with a screw cap and 1 ml Updegraff reagent added. The tubes were placed in a boiling water bath for 30 min with intermittent vortex mixing. Upon cooling, the acid-resistant residue was collected by centrifugation (10,000*g*, 10 min) and washed with MilliQ water. Washing and centrifugation was done until neutral. A final washing was done with acetone, followed by centrifugation before leaving the pellet to dry by evaporation overnight. The dried residue was weighed and then subjected to solid state ^{13}C NMR and linkage analysis. Analyses were performed in duplicate.

2.5. NMR analysis

The whole cell wall isolates were examined using solid-state ^{13}C CP/MAS and SP/MAS NMR experiments at a ^{13}C frequency of 75.46 MHz on a Bruker MSL-300 spectrometer (Bruker, Billerica, MA, USA). The cell wall isolates were packed in a 4 mm diameter, cylindrical, PSZ (partially-stabilized zirconium oxide) rotor with a KelF end cap. For hydrated CP/MAS and all SP/MAS spectra, water was added 1:1 to make a paste and this was then packed in the rotor. The rotor was spun at 5 kHz at the magic angle (54.7°). The 90° pulse width was 5 μs and a contact time of 1 ms was used for all samples with a recycle delay of 3 s. The spectral width was 38 kHz, acquisition time 50 ms, time domain points 2 k, transform size 4 k and line broadening 50 Hz. At least 5000 scans were accumulated for each spectrum. Chemical shifts were referenced to external adamantane. The percentage of cellulose in each sample (Updegraff-treated residue) was estimated by calculating the relative areas of the peaks at 105 ppm (cellulose C-1) and 102 ppm (xylan and mannan). To examine the mobile components of the samples, single pulse direct polarization (SP/MAS) spectra were recorded. The recycle time was 60 s and 20 k spectra were accumulated for each sample.

2.6. Phenolic acids by UPLC

The extraction and quantification of esterified phenolic acids and dimers was done by sequential enzymatic and alkali treatment of the sample (Bartolomé et al., 1997; Waldron, Parr, Ng, & Ralph, 1996). For each sample, three sequential enzymatic treatments, followed by four sequential alkali treatments were performed, with each of the seven fractions analysed separately. Briefly, about 20 mg cell walls in 1000 μl