




Genetic and environmental factors contribute to variation in cell wall composition in mature desi chickpea (*Cicer arietinum* L.) cotyledons

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Abstract

Chickpea (*Cicer arietinum* L.) is an important nutritionally rich legume crop that is consumed worldwide. Prior to cooking, desi chickpea seeds are most often dehulled and cleaved to release the split cotyledons, referred to as dhal. Compositional variation between desi genotypes has a significant impact on nutritional quality and downstream processing, and this has been investigated mainly in terms of starch and protein content. Studies in pulses such as bean and lupin have also implicated cell wall polysaccharides in cooking time variation, but the underlying relationship between desi chickpea cotyledon composition and cooking performance remains unclear. Here, we utilized a variety of chemical and immunohistological assays to examine details of polysaccharide composition, structure, abundance, and location within the desi chickpea cotyledon. Pectic polysaccharides were the most abundant cell wall components, and differences in monosaccharide and glycosidic linkage content suggest both environmental and genetic factors contribute to cotyledon composition. Genotype-specific differences were identified in arabinan structure, pectin methylesterification, and calcium-mediated pectin dimerization. These differences were replicated in distinct field sites and suggest a potentially important role for cell wall polysaccharides and their underlying regulatory machinery in the control of cooking time in chickpea.

KEYWORDS

arabinan, cellulose, cooking time, pectin, polysaccharide, starch

1 | INTRODUCTION

Chickpea (*Cicer arietinum* L.) is a significant and valuable source of protein, energy, minerals, and vitamins in the diets of many consumers

around the world. Kabuli chickpea are generally larger, have thin white/cream seed coats, and are often canned, cooked whole, or prepared as hummus. In contrast, desi chickpea are normally smaller with a thicker, darker seed coat that is often removed before cooking as

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dhal. Both whole kabuli seeds and desi chickpea dhal can be milled for use as a flour, known as besan. Processing performance of desi chickpea is an important target of international chickpea breeding programs (Gaur et al., 2016; Rubio, Gil, Cobos, & Millán, 2011), and there is increasing interest in cotyledon composition for nutritional benefits (Ashokkumar et al., 2015; Frimpong et al., 2009; Gaur et al., 2016; Patane, 2006; Rubio et al., 2011; Vaz Patto, 2015). The contribution of composition to processing performance in cereals is well established (D'Appolonia, Gilles, Osman, & Pomeranz, 1964; Fitzgerald, McCouch, & Hall, 2009; Sissons, 2012) but has only recently started to attract research attention in pulses (Wang, Hou, Santos, & Maximiuk, 2016; Wood, Knights, Campbell, & Choct, 2014a; Wood, Knights, Campbell, & Choct, 2014b; Wood, Knights, Campbell, & Choct, 2014c; Wood, Knights, Campbell, & Choct, 2014d; Wood, Knights, Campbell, & Choct, 2017).

The chemical composition of whole seeds from chickpea and other pulses has been reported in a number of studies (Petterson, Sipsas, & Mackintosh, 1997; Rincón & Martínez, 1998; de Almeida Costa, da Silva Queiroz-Monici, Reis, & de Oliveira, 2006; Nikolopoulou, Grigorakis, Stasini, Alexis, & Iliadis, 2007; Zia-UI-Haq et al., 2007; Wang, Hatcher, Tyler, Toews, & Gawalko, 2010; Wood et al., 2014a-d; Ghribi, Maklouf, Blecker, Attia, & Besbes, 2015). However, because desi chickpea are predominantly dehulled before further processing and consumption, the whole seed composition is less relevant as it is significantly influenced by the seed coat. The chemical composition of desi chickpea dhal (dehulled and split seeds, i.e., the cotyledons) is less commonly reported (Attia, El-Tabey Shehata, Aman, & Hamza, 1994; Iqbal, Khalil, Ateeq, & Khan, 2006; Frimpong et al., 2009; Wood et al., 2014a-d) and has generally focussed on major components such as protein, starch, minerals, and some antinutrients. The composition of dhal is important for nutrition but is also likely to contribute to the cooking time, which is defined as softening, most often through a hydrothermal process, which improves dhal texture, palatability, and digestibility (Sasikala, Ravi, & Narasimha, 2011; Shiga, Cordenunsi, & Lajolo, 2009; Wood, 2016). In general, plant tissue softening is due to weakening or dissociation of intercellular connections between cells through solubilization, depolymerization, and/or the loss of pectic polysaccharides (Brummell & Harpster, 2001; Paciulli et al., 2016; Vicente, Ortugno, Powell, Greve, & Labavitch, 2007).

Cooking times of pulses are known to be influenced by the environment, both in the field and in postharvest storage (Berry et al., 2016; Castellanos Ramos et al., 1994; Ghaderi, Hosfield, Adams, & Uebersax, 1984; Hentges, Weaver, & Nielsen, 1990; Morais, Valentini, Guidolin, Baldissera, & Coimbra, 2010; Paredes-López, Maza-Calviño, & González-Castañeda, 1989; Paredes-López, Reyes-Moreno, Montes-Rivera, & Carabez-Trejo, 1989; Perez Herrera, Acosta Diaz, Padilla Ramirez, & Acosta Gallegos, 1999; Reyes-Moreno, Okamura-Esparza, Armienta-Rodelo, Gómez-Garza, & Milán-Carrillo, 2000; Stanley, Michaels, Plhak, & Caldwell, 1990). In particular, high heat and humidity can cause what is known as the hard-to-cook defect, a condition where pulse seeds hydrate but do not soften even after prolonged cooking (Hohlberg & Stanley, 1987; Reyes-Moreno, Paredes-López, & Gonzalez, 1993; Sefa Dedeh, Stanley, & Voisey, 1979; Wood, 2016). Several hypotheses have been put forward to explain the hard-to-cook mechanism, including restricted starch

gelatinization (by protein insolubilization, pectin β -elimination, degradation, or lipid oxidation) and reduced pectin solubility (through the binding activity of phytates, phenolics, or lignification). However, the evidence is limited and further research into these theories has led to mixed results (Farinelli & Lemos, 2010; Garcia, Filisetti, Udaeta, & Lajolo, 1998; Liu & Bourne, 1995; Liu, Phillips, & McWatters, 1993; Mafuleka, Ott, Hosfield, & Uebersax, 1993; Martin-Cabrejas et al., 1995; Nasar-Abbas et al., 2008; Njoroge et al., 2016; Reyes-Moreno et al., 1993; Reyes-Moreno et al., 2000; Srisuma et al., 1989; Stanley, 1992).

Recently, Njoroge et al. (2014), Njoroge et al. (2015), and Njoroge et al. (2016) examined differences in pectic polysaccharides in relation to cooking time for common beans. Their results suggest that a hard-to-cook variety generally had lower pectin solubility and more arabinans (suggesting higher amounts of branched pectin) with lower amounts of acetylation, but no significant difference in methylesterification compared with an easy-to-cook bean (Njoroge et al., 2014). In addition, Njoroge et al. (2016) concluded that the development of the hard-to-cook property was due to the release of Ca^{2+} into the middle lamella where it cross-links low methoxyl pectin. Pectins are a central component of dicot plant cell walls that accumulate in the middle lamella (Daher & Braybrook, 2015). The cell wall composition of pulses has been examined previously (Bhatty, 1990; Brillouet & Carre, 1983; Brillouet & Riochet, 1983; Matsuura & Hatanaka, 1990; Njoroge et al., 2014; Njoroge et al., 2015; Parker, 1984a, 1984b; Rozo, Bourne, Hood, & Van Soest, 1990; Shiga et al., 2009; Shiga & Lajolo, 2006; Shiga, Lajolo, & Filisetti, 2004; Stolle-Smits, Beekhuizen, Recourt, Voragen, & van Dijk, 2000) but has received limited attention in relation to variation in cooking time, particularly in chickpea (Bhatty, 1990; Clemente, Sánchez-Vioque, Vioque, Bautista, & Millán, 1998; Matsuura & Hatanaka, 1990; Reyes-Moreno et al., 2000; Reyes-Moreno, Rouzaud-Sandez, Milán-Carrillo, Garzón-Tiznado, & Camacho-Hernández, 2001; Rozo et al., 1990; Shiga et al., 2004; Shiga et al., 2009; Shiga & Lajolo, 2006; Stolle-Smits et al., 2000). This is despite the availability of desi chickpea genotypes that shows distinct differences in cooking time (Wood, 2016). Because cooking implies softening of the dhal structure, it almost certainly requires loosening and some degree of dissociation of cell walls within the cotyledon parenchyma. Hence, the composition of cell walls and the middle lamella are potential factors affecting the speed at which cell dissociation can occur (i.e., cooking and softening). The objective of this study was to explore the impact of genotype and growth environment on the chemical composition of chickpea cotyledon cell walls and to identify differences between samples that might contribute to variation in cooking time.

2 | MATERIALS AND METHODS

2.1 | Biological material and sample preparation

The 12 samples used in this study were selected from trials conducted by Pulse Breeding Australia (PBA) and Tamworth Agricultural Institute (New South Wales Department of Primary Industries) agronomy researchers in Northern NSW, Australia. They consisted of three

environments (1997 Spring Ridge, 1997 Moree, and 2010 Tamworth) and seven genotypes including four cultivars (Amethyst, Norwin, Kyabra, and PBA HatTrick) and three breeding lines (Rounded isoline, Angular isoline, and a *Cicer echinospermum* derived line; Table S1). Two genotypes grown in both the Spring Ridge and Moree trials (Amethyst and Norwin) were selected based on differences in cooking times. The four cultivars and three breeding lines were included to examine a wider range of genetic diversity at the same site and year.

Seeds of each sample were repeatedly passed through the "pitter" component of an SK Engineering Mill (SK Engineering, India) to gently remove the seed coat and split the cotyledons, followed by aspiration, to produce dhal for investigation. Seeds and dhal were stored in sealed containers at 4 °C prior to analysis.

2.2 | Cooking time determination

Cooking times of pulses are difficult to precisely quantify (Wood, 2016) but were estimated using two different methods. The first method was the tactile (finger and thumb) method APQ-102.1 (Burridge, Hensing, & Petterson, 2001; Williams, El-Haramain, Nakkoul, & Rihawi, 1988; Wood, 2016). Briefly, dhal (20 g) was placed in boiling water and a timer started. At regular time periods, dhal was withdrawn from the water and squashed between the finger and thumb. When the sample was close to being soft (i.e., cooked) 10 dhal were tested at a time, and the sample was deemed to be cooked when 90% of the dhal were soft to squash and showed no white core (Wood, 2016). If a dhal sample did not cook within 60 min, the test was stopped and the sample was labelled as "hard-to-cook."

The second method was the Mattson Cooker method (Wang & Daun, 2005; Wood, 2016). This method is normally used for whole pulse seeds; however, on this occasion, the method was adapted to obtain cooking performance of dhal samples. Briefly, 25 individual dhal samples were placed in the apparatus saddles, centred with their convex side up. This orientation was preferred as the dhal was found to slip out from under the plunger more often when placed with their convex side down. A plunger (100 g weight; 2.0 mm rounded tip) was carefully positioned on the top centre of each individual dhal, before introducing the entire Mattson apparatus into a large vessel of boiling water. The time after immersion at which each plunger fell through the softening dhal was recorded as the cooking time for that individual dhal. The resulting 25 cooking time values for each dhal were then used to compare the cooking performance of each sample, such as the mean cooking time or the time taken to cook 80% or 90% of the dhal (Wood, 2016).

Both cooking methods were performed in duplicate, producing similar results. For comparative purposes, we arbitrarily classified the cooking times used in this work as slow (>40 min), medium (30–40 min), or fast (<30 min) cooking (see Table S1).

2.3 | Monosaccharide analysis by high performance liquid chromatography

To prepare the ground chickpea cotyledons for monosaccharide analysis, 2 × 20 mg aliquots of flour were weighed accurately into 2 ml tubes. One aliquot was used directly for monosaccharide analysis.

The remaining aliquot was washed with ethanol (500 µl, 70%) at 100 °C for 15 min, followed by two further washes (100%) at room temperature. The ethanol washes were pooled and dried under vacuum.

Monosaccharide analysis was performed as described in Comino, Shelat, Collins, Lahnstein, and Gidley (2013). The alcohol insoluble and soluble compounds were hydrolysed in 1 ml of 1 M sulfuric acid at 100 °C for 3 hr. The hydrolysates were diluted (20×) and derivatized with 1-phenyl-3-methyl-5-pyrazolone (PMP). The PMP-monosaccharides were quantified by high performance liquid chromatography on an Agilent 1200 liquid chromatograph fitted with a Phenomenex Kinetex C18 reversed phase column (particle size: 2.6 µm; pore size: 100 Å; dimensions: 100 × 3 mm). Peak areas were compared with areas obtained from standard curves constructed using mannose, ribose, rhamnose, galacturonic acid, glucuronic acid, fucose, xylose, arabinose, mannose, glucose, and galactose for calibration.

Between two and four replicates of each sample were analysed. Correlation coefficients and significant differences were calculated in R as described earlier (Wilkinson & Tucker, 2017), using one-way analysis of variance and the Tukey–Kramer test in Genstat or Student's *t* test in Microsoft Excel.

2.4 | Starch and cellulose analysis

Starch analysis was performed on 40 mg of flour using a scaled-down version of the Megazyme Total Starch assay (amyloglucosidase/α-amylase method) for samples containing D-glucose (McCleary, Solah, & Gibson, 1994). The samples were initially washed with 80% ethanol at 85 °C for 5 min, followed by a second wash with 80% ethanol at room temperature. Standards (Megazyme, 96% starch) were included with every batch and analysis was performed in duplicate. Cellulose was quantified on 75 mg of flour (duplicates) according to the Updegraff method (Updegraff, 1969).

2.5 | Glycosidic linkage analysis

To prepare alcohol insoluble residues (AIR) for glycosidic linkage analysis, 300 mg of flour was shaken three times in 10 ml of each of the following solvents, hexane and ethyl acetate (2 hr each); 80% ethanol for 8 hr; and acetone and methanol (20 min each). The samples were centrifuged at 3,000 g for 30 min after each wash, and the supernatant was discarded. The residues corresponding to the AIR preparations were vacuum dried before de-starching. Approximately 20 mg of each AIR sample was gently mixed with 0.5 ml of DMSO at 80 °C for 1 hr. Another 0.5 ml of DMSO was added in each tube, and the AIR samples were successively placed at 100 °C for 5 min and into a bath at 70 °C. A solution of thermostable α-amylase (Megazyme, enzyme from *Bacillus licheniformis*, 100 U/ml in 100 mM sodium acetate buffer pH 5.0 containing 5 mM CaCl₂) was added (1.5 ml per tube), and the samples were gently stirred for 8 hr at 70 °C. The de-starched AIR residues were cooled to 50 °C and 1 ml of 200 mM sodium acetate buffer pH 4.5 was added, followed by incubation with 1 ml of amyloglucosidase solution (Megazyme, 3 mg/ml in 200 mM sodium acetate buffer pH 4.5) at 50 °C for 2 hr. The samples were dialysed (molecular weight cut-off 3,600 Da) against de-ionized water

for 48 hr before freeze-drying. The cell wall material was subsequently precipitated in ethanol. Treatment with α -amylase revealed no significant difference in the yield of cell wall material isolated from the desi chickpea dhal samples. Uronic acids in the sample wall material (~2 mg) were converted to their 6,6-dideuterio neutral sugar counterparts using carbodi-imide activation at pH 4.75 followed by sodium borodeuteride (NaBD₄) reduction at pH 7.0 (Kim & Carpita, 1992). Glycosidic linkage analysis by methylation was performed as described in Xing et al. (2017) to produce permethylated alditol acetates. These derivatives were analysed using an Agilent 7890B/5977B GC-MS fitted with an Agilent J&W VF-23 ms GC (30 m \times 0.25 mm, film thickness 0.25 μ m) capillary column. Analysis was performed in duplicate.

2.6 | Tissue fixation, embedding, and sectioning

Chickpea cotyledons were fixed in 0.25% (v/v) glutaraldehyde, 4% (w/v) paraformaldehyde, and 4% (w/v) sucrose in phosphate buffered saline, pH 7.2, dehydrated and embedded in LR White resin according to Aditya et al. (2015). Sections (1 μ m thickness) were cut on a Reichert-Jung Ultracut ultramicrotome using a diamond knife and dried onto SuperFrost polysine coated 1 mm microscope slides (ThermoFisher Scientific, Australia).

2.7 | Immunolabelling and staining of cell walls

Immunohistochemical analysis of cell walls was performed according to Burton et al. (2010) with the exception of the immunodetection with the 2F4 antibody. Dilutions of the primary antibody (1:50) were applied to sections followed by a dilution (1:100) of the appropriate secondary antibody as listed in Table S2. For the 2F4 primary antibody, a similar method was followed using TcaS buffer (20 mM Tris-HCl, pH 8.2, 0.5 mM CaCl₂, 150 mM NaCl) and skimmed milk for blocking, a 1:5 dilution of the primary antibody and 1:100 dilution of the secondary antibody (Guillemin et al., 2005). All images were captured using a Zeiss AxioImager M2 (Carl Zeiss, Oberkochen, Germany)

equipped with an AxioCam MRm camera. All primary antibodies were from Plant Probes (Leeds, UK) and secondary antibodies from Invitrogen™ (ThermoFisher Scientific, Australia). Appropriate negative controls were included to verify the absence of cross reactivity and eliminate false positives. Sections were also stained with the general stain Toluidine Blue (30 s with 0.01% [w/v] stain) and Pontamine Fast Scarlet (Sigma-Aldrich, Cat #: S479896; 20 min with 0.1% [w/v] stain) to detect cellulose.

3 | RESULTS

In this study, we aimed to identify differences in chickpea cotyledon composition, polysaccharide content, and structure that accompany differences in environment, genotype, and cooking time. Twelve desi chickpea samples were selected for analysis from different field trials (Table S1).

3.1 | Chickpea cotyledon flour is composed of diverse monosaccharides whose abundance vary in samples grown at different field sites

The sugar composition of chickpea cotyledons was analysed by acid hydrolysis and monosaccharide profiling as a first step to identify potential quantitative differences in polysaccharide content between the different samples. Four independent replicates were prepared for each of the 12 samples using total cotyledon flour (Table 1), in addition to flour separated into alcohol insoluble and soluble residues (Figure 1a, Tables S3 and S4). The most abundant monosaccharide detected in the cotyledon flour and insoluble residue was glucose, which contributed approximately 43% (w/w) of the mass (Table 1 and Figure 1a). Much smaller amounts of arabinose, galactose, xylose, and galacturonic acid were also detected, contributing altogether ~5% of the total mass (Table 1). In the soluble fraction, relatively low amounts

TABLE 1 Monosaccharide, starch, and cellulose compositional analysis of desi chickpea cotyledons

Genotype (Site, CT)	Cotyledon flour monosaccharide analysis (% w/w)									
	Total flour						Total flour			
	Gluc	Sig.	Gal	Sig.	Ara	Sig.	Starch (% w/w)	Sig.	Cellulose (% w/w)	Sig.
Kyabra (TA, S)	44.1 \pm 0.5	b	0.9 \pm 0.4	a	3.0 \pm 0.1	abcd	51.5 \pm 0.7	a	1.9 \pm 0.3	abcd
PBA HatTrick (TA, S)	45.1 \pm 2.7	b	0.6 \pm 0.3	a	3.1 \pm 0.2	bcd	48.8 \pm 2.5	ab	1.6 \pm 0.1	bc
PBA HatTrick (TA, F)	44.6 \pm 3.5	b	0.7 \pm 0.4	a	3.1 \pm 0.2	abcd	49.8 \pm 1.6	ab	1.8 \pm 0.1	abcd
Kyabra (TA, M)	44.8 \pm 3.2	b	0.8 \pm 0.4	a	3.0 \pm 0.2	abcd	48.8 \pm 2.8	ab	2.2 \pm 0.2	ad
Rounded isoline 1290 (TA, S)	40.8 \pm 2.4	ab	0.8 \pm 0.3	a	3.2 \pm 0.2	bcd	47.8 \pm 0.0	ab	1.0 \pm 0.1	abcd
PBA HatTrick (TA, M)	44.8 \pm 3.0	b	0.7 \pm 0.3	a	3.4 \pm 0.2	d	46.6 \pm 0.0	ab	1.8 \pm 0.3	abcd
57Q (TA, M)	42.5 \pm 2.5	ab	0.7 \pm 0.3	a	3.1 \pm 0.2	bcd	46.3 \pm 1.7	ab	2.1 \pm 0.2	abcd
Angular isoline 1220 (TA, F)	40.0 \pm 1.8	ab	0.8 \pm 0.4	a	3.1 \pm 0.2	bcd	44.3 \pm 1.1	b	1.9 \pm 0.1	abcd
Norwin (SR, F)	41.2 \pm 2.0	ab	0.8 \pm 0.3	a	2.7 \pm 0.1	abc	46.1 \pm 0.6	ab	1.6 \pm 0.1	bc
Amethyst (SR, S)	37.7 \pm 2.7	a	0.7 \pm 0.4	a	2.6 \pm 0.2	ac	45.4 \pm 0.9	b	1.7 \pm 0.2	abcd
Norwin (MO, F)	43.9 \pm 2.2	ab	0.8 \pm 0.3	a	2.9 \pm 0.1	abcd	49.5 \pm 0.3	ab	2.2 \pm 0.3	a
Amethyst (MO, S)	42.6 \pm 2.4	ab	0.9 \pm 0.3	a	3.2 \pm 0.1	bd	47.7 \pm 0.3	ab	2.0 \pm 0.3	abcd
Average	42.7 \pm 3.1		0.8 \pm 0.4		3.0 \pm 0.3		47.8 \pm 2.3		1.9 \pm 0.3	

Note. See Table S1 for additional details regarding genotypes and field sites. Site SR = Spring Ridge; MO = Moree; TA = Tamworth. CT = cooking time, where S = slow, M = medium, F = fast. Gluc = glucose, Gal = galactose, Ara = arabinose, Sig = significant differences as determined by one-way analysis of variance and the Tukey-Kramer test ($p < .05$).

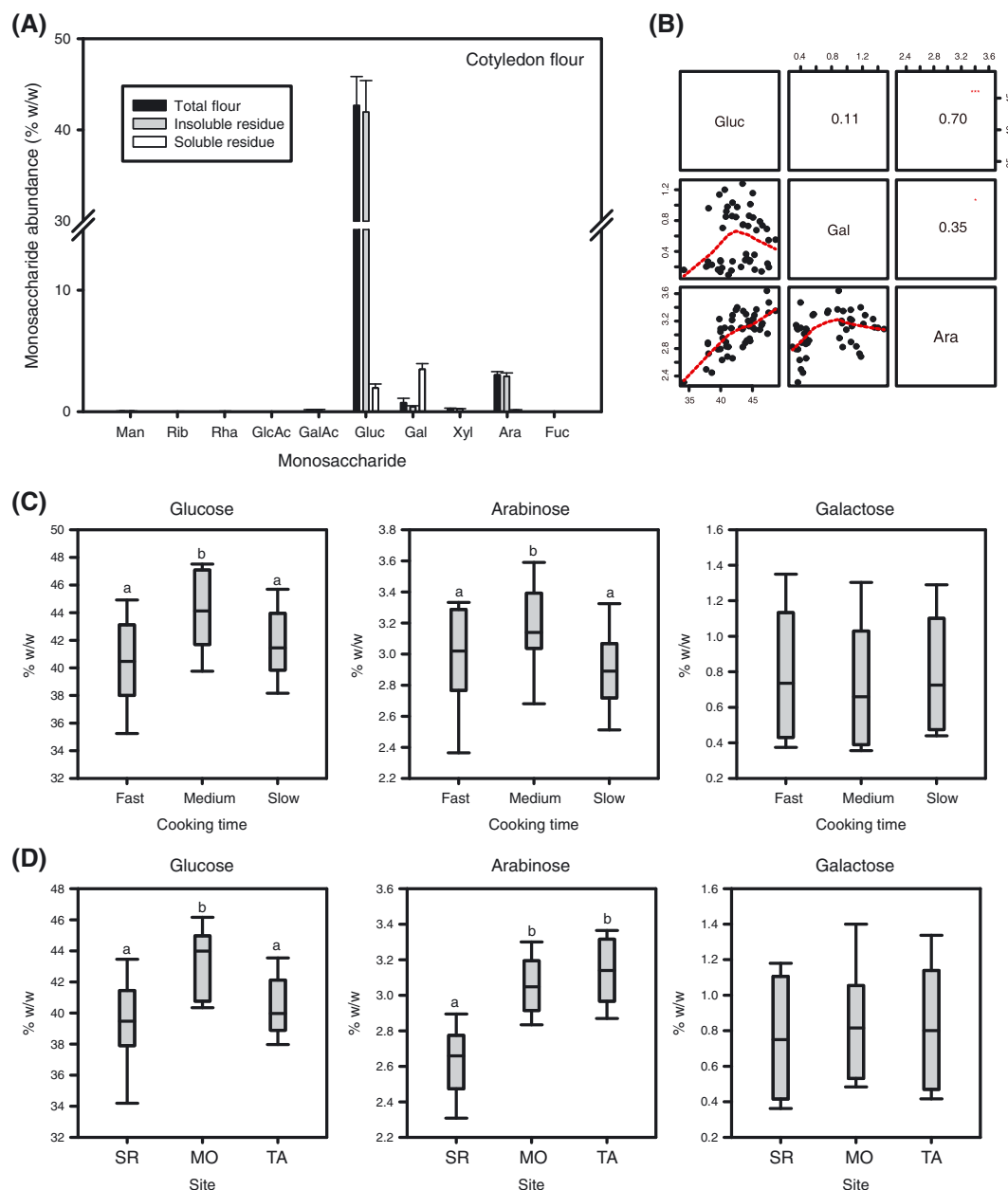


FIGURE 1 Monosaccharide analysis of chickpea cotyledon flour samples. (a) Average composition of unfractionated (total), alcohol insoluble, and soluble residues in all 12 chickpea cotyledon samples. (b) Correlations between monosaccharide abundance in total flour (four replicates of 12 chickpea samples). Numbers indicate the value (correlation coefficient), and the significance level is indicated; * $p < .05$, *** $p < .001$. (c) Monosaccharide abundance in fast, medium, and slow-cooking samples (in total cotyledon flour) shows significant differences in glucose and arabinose content ($p < .05$). Significant differences are indicated by the symbols a and b. (d) Monosaccharide abundance (in total cotyledon flour) also shows significant differences depending on the field site ($p < .05$). SR = Spring Ridge, MO = Moree, TA = Tamworth

of galactose and glucose were detected, contributing ~7% of the total flour mass (Figure 1a).

Monosaccharide levels were compared to identify similar trends in abundance (Figure 1b). A significant positive correlation was identified between glucose and arabinose content, whereas galactose content appeared to vary independently of the other monosaccharides. This may indicate that some changes in polysaccharide abundance are interrelated, even though the monosaccharides may not necessarily be derived from the same polymer.

The abundance of the three most prevalent monosaccharides was analysed to assess a putative association with cooking time variation

(Figure 1c). No significant differences were identified between the fast- and slow-cooking samples, whereas the medium samples showed some differences in glucose and arabinose content compared with fast- and/or slow-cooking varieties. To test whether monosaccharide composition might be impacted by site, samples from Spring Ridge, Moree, and Tamworth were examined (Figure 1d). Samples from Spring Ridge and Moree showed significant differences in glucose and arabinose content, with Spring Ridge samples tending to show lower levels of both monosaccharides.

Although details of all 12 samples are provided (Tables 1 and S3), for the remainder of this study, we focussed predominantly on

analysis of the Norwin, Amethyst, and PBA-HatTrick genotypes. The reasons for this were twofold (a) significant differences were identified in arabinose content between PBA-HatTrick (TA, M; $3.4 \pm 0.2\%$ w/w) and Amethyst (SR, S; $2.6 \pm 0.2\%$ w/w) genotypes, possibly as a result of differences in cell wall composition and (b) the Norwin and Amethyst genotypes grown at Moree and Spring Ridge provided an opportunity to assess differences in cell wall composition that might contribute to variation in cooking time.

3.2 | Chickpea cotyledon cell walls contain arabinan, cellulose, and pectin

Based on the monosaccharide analysis (Table 1 and Figure 1a), polysaccharides present in chickpea cotyledon cell walls might include cellulose (Glc), xyloglucan (Glc, Xyl, and Gal), arabinan (Ara), and pectin (Ara, Rha, GalA, and Gal). Updegraff assays confirmed that crystalline, acid-resistant cellulose, was present at low levels ranging from 1.6% to 2.2% (w/w). By contrast, starch was abundant in all samples, varying from 44% to 52% (w/w) with an average content of $48 \pm 2\%$ (w/w). These complementary assays are consistent with previous studies and indicate that most of the Glc detected in the monosaccharide profiling (Table 1 and Figure 1a) arises from starch.

To complete our compositional analysis and gain further insight into the identity of the nonstarchy polymers present in the cotyledons, methylation analysis was undertaken on de-starched samples. This method provides quantitative details of different glycosidic linkages and the nature of different polysaccharides present (Tables 2 and 3). The assays were performed on Norwin and Amethyst samples

TABLE 2 Linkage analysis of desi chickpea cotyledon samples

Linkage	Abundance (Mol%)			
	Norwin (SR)	Norwin (MO)	Amethyst (MO)	Amethyst (SR)
t-Araf	26.0 ± 0.9	26.7 ± 2.1	27.2 ± 1.8	24.8 ± 2.7
5-Araf	21.6 ± 1.7	20.7 ± 1.2	20.8 ± 1.3	20.5 ± 1.3
2,5-Araf	2.2 ± 0.1	2.5 ± 0.6	2.6 ± 0.4	2.5 ± 0.4
3,5-Araf	6.4 ± 0.5	6.2 ± 0.7	6.6 ± 0.8	6.4 ± 0.6
2,3,5-Araf	8.6 ± 0.7	8.5 ± 0.4	7.5 ± 1.2	7.4 ± 1.4
t-Xylp	1.9 ± 0.4	1.7 ± 0.3	2.3 ± 0.3	2.3 ± 0.3
2-Xylp	0.7 ± 0.1	0.6 ± 0.2	0.7 ± 0.0	0.8 ± 0.2
4-Xylp	1.6 ± 0.3	1.4 ± 0.3	1.3 ± 0.4	1.4 ± 0.4
2-Rhap	0.2 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 0.3
2,4-Rhap	1.1 ± 0.2	1.2 ± 0.2	1.2 ± 0.2	1.6 ± 0.3
t-Manp	2.2 ± 0.9	3.8 ± 0.4	3.9 ± 0.7	4.2 ± 2.5
2-Manp	0.7 ± 0.4	1.0 ± 0.2	0.9 ± 0.2	1.1 ± 0.4
4-Manp	1.5 ± 0.1	1.6 ± 0.4	1.4 ± 0.4	1.5 ± 0.2
t-Galp	1.1 ± 0.1	1.4 ± 0.3	1.6 ± 0.1	1.6 ± 0.2
3-Galp	1.2 ± 0.4	1.2 ± 0.2	1.0 ± 0.3	0.9 ± 0.3
4-Galp	1.2 ± 0.3	1.4 ± 0.2	1.4 ± 0.2	0.9 ± 0.2
4-GalAp	0.7 ± 0.1	1.0 ± 0.2	1.3 ± 0.2	1.8 ± 0.7
t-Glcp	1.3 ± 0.1	1.4 ± 0.2	1.5 ± 0.3	2.0 ± 0.5
4-Glcp	18.1 ± 4.4	15.6 ± 2.4	14.5 ± 0.3	16.0 ± 1.1
4,6-Glcp	1.8 ± 0.4	1.8 ± 0.3	1.7 ± 0.1	1.9 ± 0.0

Note. SR = Spring Ridge; MO = Moree.

TABLE 3 Predicted desi chickpea cotyledon cell wall polysaccharide composition based on linkage analysis

Polysaccharide	Mol%
Arabinan	53.8
Type I arabinogalactan	1.2
Type II arabinogalactan	1.1
Rhamnogalacturonan I	2.9*
Homogalacturonan	1.2*
Heteroxylan	1.6
Heteromannan	3.2
Xyloglucan	6.2
Cellulose	12.9
Unassigned	10.6

Note. The average abundance of each linkage was determined across all Norwin and Amethyst samples and replicates. Polysaccharide composition was estimated following the protocol of Pettolino, Walsh, Fincher, and Bacic (2012). The asterisk indicates that the exact composition of several pectic polysaccharides is unclear due to a lack of data regarding the identity of side-chains on rhamnogalacturonan I.

collected from Moree and Spring Ridge, which represent genotypes showing distinct differences in cooking time. Three biological and two technical replicates were prepared for each sample (24 samples in total). Ethanol precipitation and α -amylase treatment removed ~80% of the flour weight, thereby increasing the relative abundance of nonstarch polysaccharides. Consistent with the monosaccharide profiling, linkage analysis of the residual material revealed an abundance of linkages containing arabinose (ranging from 61 to 65 Mol%) and glucose (ranging from 17 to 22 Mol%; Table 2).

Detailed analysis of individual linkage types showed a large proportion of terminal arabinose (t-Ara) and 5-linked arabinose (5-Ara), consistent with the presence of branched arabinan polysaccharides (Tables 2 and 3). These may be present as branches on rhamnogalacturonan, which is part of the pectin fraction, although rhamnose (2-Rha and 2,4-Rha) was present only at low levels (Table 2). Low levels of 4-galacturonic acid (4-GalA; ~1.2%) were assigned to rhamnogalacturonan based on the presence of 2,4-Rha branches, but the side chain composition is unclear, and it is also possible that the 4-linked galacturonosyl residues (4-GalA) arise from low levels of homogalacturonan (HG). Some substitution by galactan chains was also confirmed by the presence of terminal galactosyl residues (t-Gal), which may be derived from arabinogalactan proteins (AGPs), although only low levels of 3-Gal were present that are typical of arabinogalactans. The detection of 4-linked glucosyl residues (4-Glc) confirms the presence of linear 1,4-linked glucan that is likely to correspond to cellulose in these de-starched samples. This is further supported by the cellulose assays mentioned above. Some xyloglucan was also detected, as judged by the trace amounts of 4,6-glucosyl residues (4,6-Glc) and terminal xylosyl residues (t-Xyl; Tables 2 and 3). The linkage analysis is also consistent with a low abundance of heteromannan and heteroxylan in the samples (Table 3). In summary, the glycosidic linkage analysis indicates arabinan and cellulose are the main cell wall polysaccharides present within chickpea cotyledon flour.

3.3 | Genotypes showing differences in cooking time exhibit differences in the abundance of glycosidic linkages

The glycosidic linkage data were also used to identify relationships between polysaccharide compositional changes, field site, and genotypes showing differences in cooking time. First, differences between

the samples were assessed in terms of site, irrespective of genotype. Significant differences were identified in the abundance of t-Ara, 2,5-Ara, and 4-Gal ($p < .05$), indicating that environmental variation has an impact on chickpea polysaccharide structure. For these three linkages, levels were reduced in samples from Spring Ridge compared with Moree (Table 2). Second, differences between the samples were assessed in terms of genotype, irrespective of site. Significant

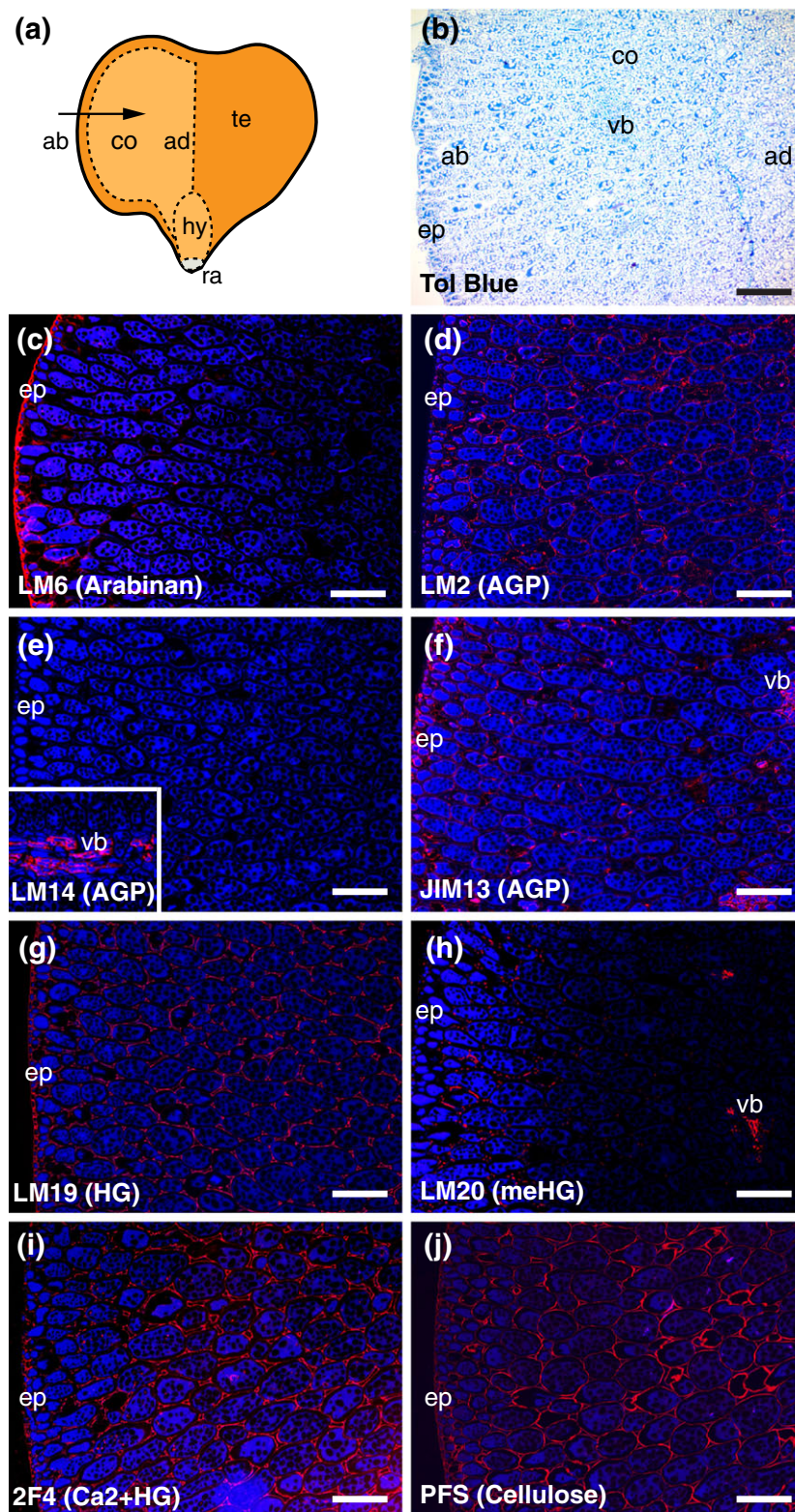


FIGURE 2 Immunolabelling and histological staining of cell wall components in chickpea cotyledons. (a) Schematic representation of a chickpea showing the direction of sectioning (arrow) through a cotyledon. (b) A representative thin section of a cotyledon stained with Toluidine blue. (c) LM6 labelling of arabinan epitopes. (d) LM2 labelling of AGP epitopes. (e) LM14 labelling of AGP epitopes. (f) JIM13 labelling of AGP epitopes. (g) LM19 labelling of homogalacturonan epitopes. (h) LM20 labelling of methylesterified homogalacturonan epitopes. (i) 2F4 labelling of calcium bridges between homogalacturonan residues. (j) Pontamine Fast Scarlet staining of cellulose. All panels show sections from the Amethyst (SR, S) genotype except for h and i, which show sections from the Norwin (MO, F) genotype. ab = abaxial; ad = adaxial; co = cotyledon; ep = epidermis; hy = hypocotyl; ra = radicle; te = testa; vb = vascular bundle. Bar = 120 μ m

differences were observed in the relative abundance of 2,3,5-Ara, t-Xyl, t-Gal, 4-GalA, and t-Glc residues ($p < .05$; Figure S1). The most abundant of these was 2,3,5-Ara, which varied from 4 to 9 Mol%, and was more abundant in the flour of cotyledons from fast-cooking Norwin compared with slow-cooking Amethyst, independent of field site (Table 2). By contrast, levels of t-Xyl were significantly higher in Amethyst compared with Norwin (similar to t-Gal, 4-GalA, and t-Glc), although overall abundance was low (varying from 1.2 to 2.8 Mol%). These results suggest that differences in polysaccharide structure accompany differences in genotype in the two different environments tested.

3.4 | Immunolabelling confirms the presence of diverse polymers in chickpea cotyledon cell walls

Although polysaccharide composition and abundance are known factors affecting cell wall strength and adhesion, the distribution of polymers within the cell wall might also impact traits such as cooking time or softening. Previous studies of chickpea (Wood, Knights, & Choct, 2011) investigated the morphology of cells within the cotyledon, providing evidence of pectic polysaccharides and low levels of glucan, which is consistent with the chemical analysis reported here. To address the spatial distribution of different cell wall-related polymers, chickpea cotyledon samples were embedded in LR-white resin, sectioned and labelled with a diverse set of antibodies and dyes (Figure 2) that recognize arabinan (LM6), cellulose (Pontamine Fast Scarlet), homogalacturonan (HG; LM19), methylesterified homogalacturonan (meHG; LM20), calcium-linked homogalacturonan (Ca^{2+} HG; 2F4), and AGPs (LM2, LM14, JIM13). Toluidine blue staining revealed the different morphology of cell types within the cotyledon, gradually changing from small starch-free cells at the adaxial cotyledon epidermis through to large, round starch-filled sub-epidermal (parenchyma) cells and internal vascular elements (Figure 2a,b).

In general, epitopes showed similar distribution patterns in most of the analysed genotypes (Figure 2). An antibody recognizing arabinan epitopes (LM6) predominantly labelled epidermal cells at the cotyledon surface (Figure 2c). Vascular tissues also showed labelling, whereas relatively weak labelling was detected in the walls of large starch-filled sub-epidermal cells (Figure S2A–F). Different AGP epitopes showed distinct labelling patterns. Binding of LM2 antibodies was evident at the edge of the autofluorescent starch-enriched vacuoles in a punctate pattern (Figure 2d) whereas labelling with LM14 antibodies was absent from most cell types except those associated with the vasculature (Figure 2e). Similar to LM2, JIM13 was distributed at the periphery of the cells but with fewer punctate foci and appeared to be most abundant in epidermal and vascular cell types (Figure 2f; Figure S2G–L). LM19, which recognizes homogalacturonan, was detected in the middle lamella of epidermal and sub-epidermal cell types and was particularly abundant at cell junctions (Figure 2g; Figure S2M–R). LM20 recognizes meHG and was preferentially detected in a punctate pattern in the middle lamella of epidermal or sub-epidermal cotyledon cells, depending on the genotype (Figure 2h; Figure S2S–X). Labelling was also detected in vascular elements (Figure S2T,W). The 2F4 antibody, which detects dimeric association of pectic chains

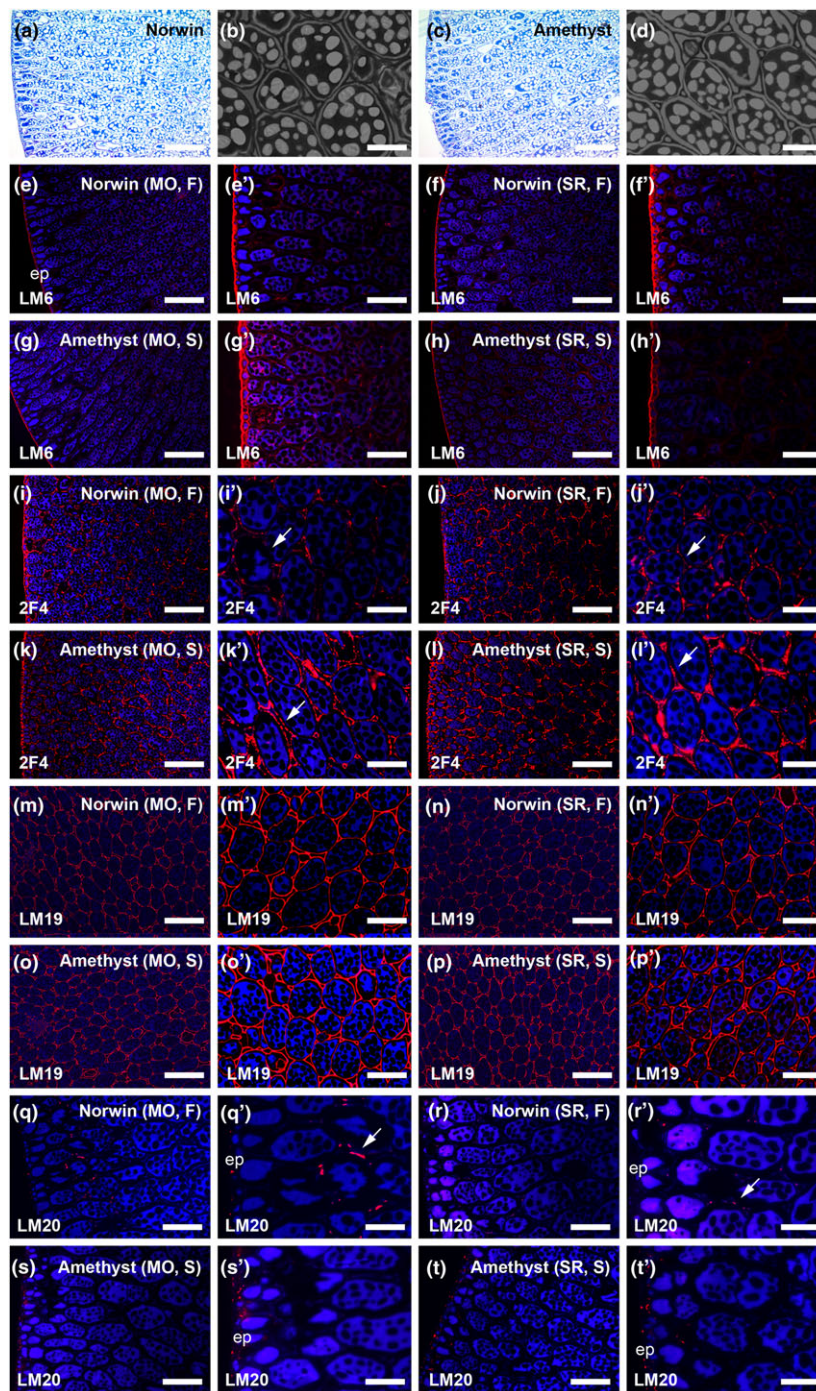
through calcium ions, labelled the cells in a similar pattern to LM19 (Figure 2i). This is consistent with homogalacturonan molecules being linked together through calcium bridges, forming a robust matrix in the mature cotyledon cells. Finally, Pontamine Fast Scarlet staining was used to detect cellulose and showed an even distribution around the periphery of most cell types (Figure 2j; Figure S3). In summary, these data provide information regarding the location of polymer deposition and differences in labelling efficiency between different cell types. These assays provide additional support for the chemical assays, suggesting the cell walls of chickpea cotyledons comprise a complex mixture of arabinan, AGPs, cellulose, and pectin that may influence the physicochemical properties of different cell types during growth and subsequent processing.

3.5 | Differences in cell wall labelling between genotypes and samples with different cooking times

The distinct patterns revealed by immunolabelling provided an opportunity to investigate specific differences in sample composition. First, two genotypes (PBA HatTrick [TA, M] and Amethyst [SR, S]) that showed significant differences in total flour arabinose content (Table 1; $3.4 \pm 0.2\%$ [w/w] vs. $2.6 \pm 0.2\%$ [w/w]) were analysed to determine if differences in monosaccharide abundance might equate to different antibody labelling efficiencies (Figure S2). Samples were compared using the LM6 (arabinan), JIM13 (AGP), LM19 (HG), and LM20 (meHG) antibodies. The LM6 and JIM13 antibodies showed similar labelling efficiencies in PBA HatTrick (TA, M) and Amethyst (SR, S) samples (Figure S2A–L). LM19 labelled epidermal and sub-epidermal cell types within both samples (Figure S2M–R), although labelling at the periphery was weaker in Amethyst (SR, S; Figure S2P–U). Punctate LM20 labelling was detected in the epidermis of PBA HatTrick (TA, M) cotyledons (Figure S2S–U). A similar pattern was detected in Amethyst (SR, S) samples, with the addition of a few punctate spots in sub-epidermal cells (Figure S2V–X). LM20 immunolabelling of vascular tissues was similar in PBA HatTrick (TA, M) and Amethyst (SR, S) samples (Figure S2T,W). These results indicate that epidermal and sub-epidermal cells from PBA HatTrick (TA, M) and Amethyst (SR, S) show subtle differences in pectin composition. However, it is unclear whether these differences relate specifically to differences in monosaccharide abundance, genotype, cooking time, or environmental conditions.

To consider the contribution of genotype to composition, we also investigated the Norwin and Amethyst genotypes, both of which were grown at two different field sites. As described above, these genotypes show distinct differences in cooking time, no consistent difference in monosaccharide composition, and small but significant differences in the abundance of several glycosidic linkage types, including 2,3,5-Ara (Figures 1 and S1 and Tables 2 and 3). The general morphology of sup-epidermal cotyledon cells appeared similar in the two samples (Figure 3a–d). Immunolabelling experiments detected LM6 (Figure 3e–h'), 2F4 (Figure 3i–l'), LM19 (Figure 3m–p'), and LM20 (Figure 3q–t') epitopes, whereas Pontamine Fast Scarlet staining detected cellulose in the cell walls of both samples (Figure S3A–D'). In the majority of cases, labelling patterns appeared to be indistinguishable between samples. However, consistent

FIGURE 3 Immunolabelling of cell wall epitopes in thin sections of chickpea cotyledons from genotypes showing differences in cooking time. (a) A section of a cotyledon from the fast-cooking Norwin genotype stained with Toluidine blue. (b) A grayscale image showing sup-epidermal starch-filled cotyledon cells in Norwin. (c) A thin section of a cotyledon from the slow-cooking Amethyst genotype stained with Toluidine blue. (d) A grayscale image showing sup-epidermal starch-filled cotyledon cells in Amethyst. (e–f') LM6 labelling of arabinan epitopes in Norwin samples collected from Moree and Spring Ridge. (g–h') LM6 labelling of arabinan epitopes in Amethyst samples collected from Moree and Spring Ridge, showing no clear differences in labelling efficiency compared with Norwin. (i–j') 2F4 labelling of calcium-linked homogalacturonan (Ca²⁺ + HG) epitopes in Norwin samples collected from Moree and Spring Ridge. (k–l') 2F4 labelling of calcium-linked homogalacturonan (Ca²⁺ + HG) epitopes in Amethyst samples collected from Moree and Spring Ridge. Labelling intensity appears to be weaker and distributed in a more punctate in the Norwin samples compared with Amethyst (see arrows). (m–n') LM19 labelling of homogalacturonan (HG) epitopes in Norwin samples collected from Moree and Spring Ridge. (o–p') LM19 labelling of homogalacturonan epitopes in Amethyst samples collected from Moree and Spring Ridge, showing no consistent differences in labelling efficiency compared with Norwin. (q–r') LM20 labelling of methylesterified homogalacturonan (meHG) epitopes in Norwin samples collected from Moree and Spring Ridge. (s–t') LM20 labelling of methylesterified homogalacturonan (meHG) epitopes in Amethyst samples collected from Moree and Spring Ridge. Labelling is less abundant in the epidermis and more obvious in sub-epidermal cells of Norwin samples compared with Amethyst (see arrows). Images are shown in the same orientation as Figure 2. Bar in g, i, k, m, o, f, h, j, l, n, p = 200 μ m, in e', g', m', o', f', h', j', l, n, p = 200 μ m, in i', k', j', l', q', s', r', t' = 80 μ m. ep = cotyledon epidermis; MO = Moree; SR = Spring Ridge; S = slow-cooking; F = fast-cooking



differences were detected using 2F4 antibodies, whose labelling appeared to be more sporadic in fast-cooking Norwin compared with slow-cooking Amethyst (compare Figure 3i',j' to k',l'). In addition, differences were observed with LM20 antibodies. In both Norwin samples, the use of LM20 antibodies revealed a punctate pattern in sub-epidermal cells but barely any labelling was detected in the epidermis (Figure 3q–r'). In contrast, the epidermis of both Amethyst samples

was labelled with the LM20 antibody, but this was infrequent or lacking in the sub-epidermal cells (Figure 3s–t'). Despite no obvious difference in LM19 immunolabelling (HG; Figure 3m–p'), differences in LM20 signals may indicate changes in wall flexibility between the samples, whereas increased 2F4 labelling is potentially indicative of more calcium dimerization of non-methyl-esterified galacturonic acid blocks and potentially stiffer cell walls.

4 | DISCUSSION

Chickpea is an important nutritionally rich legume crop that is consumed worldwide, particularly in the Indian subcontinent (FAO, 2017). Two distinct chickpea seed types are utilized for different purposes; kabuli chickpea are normally cooked and consumed whole, canned, or as hummus, whereas desi seeds are most often decorticated and cleaved to release the split cotyledons (referred to as dhal) prior to cooking (Wood & Grusak, 2007). Here, we investigated compositional variation in desi chickpea genotypes that exhibit differences in cooking time. In particular, we focussed on the desi cultivars Norwin, Amethyst, and PBA HatTrick. The aim was to characterize the major cell wall-related polysaccharides in cotyledons, gain an understanding of their structure, identify their location within the cotyledon, and determine if any differences in deposition or abundance might associate with variation in cooking time.

The starch content of the desi chickpea dhal ranged from 44.3% to 51.5%, similar to previous studies (Wood et al., 2014a; Wood et al., 2014d; Wood & Grusak, 2007), whereas cellulose content ranged from 1.6% to 2.2%. The cellulose content of chickpea wholegrain flour has been reported to vary from 4% to 13% (Wood & Grusak, 2007), and the current results are consistent with the majority being derived from the seed coat. In addition to starch and cellulose, monosaccharide profiling indicated that other polysaccharides are present that contain arabinose, galactose, and xylose monomers. This is in agreement with the report of Wood et al. (2014c) who previously examined nonstarch polysaccharides from chickpea cotyledons after the removal of starch and found that arabinose was the most abundant monosaccharide of cotyledon cell walls, followed by glucose, galactose, and xylose.

Methylation-based linkage analysis of cotyledon cell wall material showed a similar trend in monosaccharide abundance, ranging from abundant arabinose to glucose, mannose, galactose, xylose, and rhamnose in decreasing quantities. The third most abundant linkage correspond to 4-linked glucopyranosyl residues (4-Glc), which is the sole monomer present in cellulose, but can also occur in glucomannans and xyloglucans. Together, the Pontamine Fast Scarlet staining (Figure 2j), immunostaining and linkage results (Tables 2 and 3) suggest that chickpea cotyledon cell walls are composed of cellulose coated and/or cross-linked with xyloglucan and embedded within a significant pectic matrix comprising galacturonans (both rhamnogalacturonan and homogalacturonan), arabinan, and arabinogalactan (possibly as AGPs). Small amounts of mannan are also likely to be present. This composition is similar to that of other legumes, including beans and lupins, where cotyledon primary cell walls contain a mixture of cellulose, arabinogalactan, arabinan, pectin, xyloglucan, and galactan (Shiga et al., 2009).

Although the cotyledon cell wall polymers make up only a small fraction (~5–6%) of the total mass compared with starch (~40–50%) and protein (~20–30%; Singh, 1985; Miao, Zhang, & Jiang, 2009; Wood et al., 2014a), studies in bean previously suggested a link between cell wall composition and differences in cooking time. For example, lower pectin solubility, higher arabinan content, and release of Ca^{2+} into the middle lamella all correlate with the hard-to-cook

defect (Njoroge et al., 2014). We considered several aspects of chickpea cell wall composition in terms of cooking time variation. Of these, monosaccharide abundance did not appear to be associated with differences in cooking time and tended to differ more between samples grown at different field sites. In terms of unfractionated cotyledon flour, variation in glucose content across field sites is consistent with previous studies showing the effect of environment on amylose content in pulses (Bhatty, 1988; Frimpong et al., 2009). The significant changes in arabinose content suggest a similar environmental effect on nonstarch polysaccharide composition. This is consistent with the effect of different environments on monosaccharide levels in *Arabidopsis* (Duruflé et al., 2017) and many other species.

Although variation in overall monosaccharide levels did not correlate with genotypic differences and cooking time, this does not exclude the possibility that cell wall polysaccharide structure might contribute to cooking-related properties. Indeed, specific glycosidic linkage types were identified that showed genotype-dependent variations in abundance. The fast-cooking cultivar Norwin showed higher levels of 2,3,5-Ara residues (likely derived from arabinan) and reduced levels of t-Xyl, t-Gal, t-Glc, and 4-GalA residues (possibly derived from mannan, arabinogalactan, rhamnogalacturonan, or homogalacturonan) relative to the slow-cooking cultivar Amethyst. Levels of these linkage types and the inferred polysaccharides were low relative to other cell wall components, but the results were consistent across multiple replicates and field sites. In the case of arabinan, increased branching may be present within the Norwin genotype. Arabinans are cell wall polysaccharides that show great structural diversity during development and between species, but in general contain a 1,5-arabinan main chain that is substituted at O-2 or O-3 by single arabinosyl residues or short side chains (Caffall & Mohnen, 2009). In bean, changes in arabinan branching are not associated with the development of the hard-to-cook property (Shiga et al., 2009). However, in some species such as apple, loss of branching in arabinans occurs in advance of the loss of firm texture (Peña & Carpita, 2004). Although increased branching appears to contrast the fast-cooking nature of Norwin compared with Amethyst, so little is known about the role of arabinans in modulating cell wall flexibility or stiffness (Verhertbruggen, Marcus, Chen, & Knox, 2013) that the effect of altered arabinan branching on chickpea cotyledon softening requires further investigation. It is interesting to note that arabinans were predominantly located to the desi cotyledon epidermis, as detected by LM6 antibody, which may suggest a cell type specific function in mechanical reinforcement.

In general, cell wall immunolabelling is a useful method to reveal the location of different cell wall polymers within complex tissues. Although there are some caveats, particularly in regard to masking of polysaccharides by other wall polymers (Xue, Bosch, & Knox, 2013), different labelling efficiencies can also highlight important changes in polysaccharide distribution and abundance (Aditya et al., 2015; Chowdhury et al., 2014). Comparisons between the fast-cooking Norwin and slow-cooking Amethyst genotypes revealed no clear differences in the distribution of AGP, arabinan, cellulose, or HG epitopes in cotyledon sections. However, differences in LM20 (meHG) and 2F4 (Ca²⁺ + HG) labelling were identified between Norwin and Amethyst samples, and these were conserved across different field sites. The

LM20 antibody detected meHG epitopes in the epidermis of slow-cooking Amethyst. This pattern was different in fast-cooking Norwin, where epitopes were sporadically detected in epidermal walls but more prevalent in sub-epidermal cotyledon cells. Different degrees of HG methylesterification impact the mechanical and physiological properties of pectin gels (Willats et al., 2001). In particular, stretches of un-methyl-esterified galacturonic acid residues may promote the formation of the so-called "egg-box" model structure through Ca^{2+} cross-linking, which is assumed to induce gel formation and thus strengthen the wall (Liners, Letesson, Didembourg, & Van Cutsem, 1989). During cadmium stress in flax hypocotyls, an increase in blockwise de-esterified homogalacturonan and Ca^{2+} cross-linking was detected by 2F4 immunolabelling and was proposed as a change that might oppose cell separation (Douchiche, Driouich, & Morvan, 2010). Consistent with this, the 2F4 antibody, which recognizes dimeric association of pectic chains through calcium ions, revealed a more intense and even distribution around sub-epidermal cells in slow-cooking Amethyst compared with fast-cooking Norwin. This is also consistent with studies in lentil that suggest the formation of hard-to-cook legume seeds may involve interactions among divalent cations, phytates, and pectic compounds (Galiotou-Panayotou, Kyriakidis, & Margaritis, 2008). We propose a model whereby sub-epidermal cotyledon cell walls in Amethyst contain lower levels of HG methyl-esterification, thereby allowing more prevalent calcium-mediated associations between pectin molecules and formation of stronger cell walls. This may explain some differences between the slow-cooking phenotype in Amethyst and fast-cooking phenotype in Norwin. In future studies, we plan to investigate these relationships in greater detail using a larger panel of cultivars showing differences in cooking time.

Taken together, the results from this study provide evidence of variation in arabinan structure, pectin methylesterification, and dimerization between fast- (Norwin) and slow-cooking (Amethyst) desi chickpea genotypes. The more prevalent pectin dimerization in Amethyst cell walls is likely to require a higher energy input (such as a longer cooking time) to weaken or break these intercellular bonds necessary for cotyledon softening. Superimposed over this, environmental factors influence multiple aspects of chickpea cotyledon polysaccharide composition, as indicated by monosaccharide abundance and immunolabelling efficiency. The specific effect of this variation on the properties of cotyledon cell walls and downstream food processing remains unclear at present, as does the genetic basis for variation in cooking time. However, these findings provide some support for theories that suggest despite being a minor component of the chickpea cotyledon, cell wall polysaccharides fulfil an important role in downstream processing-related applications.

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REFERENCES

- Aditya, J., Lewis, J., Shirley, N. J., Tan, H. T., Henderson, M., Fincher, G. B., ... Tucker, M. R. (2015). The dynamics of cereal cyst nematode infection differ between susceptible and resistant barley cultivars and lead to changes in (1,3;1,4)-beta-glucan levels and *HvCsIF* gene transcript abundance. *New Phytologist*, 207(1), 135–147.
- Ashokkumar, K., Diapari, M., Jha, A. B., Tar'an, B., Arganosa, G., & Warkentin, T. D. (2015). Genetic diversity of nutritionally important carotenoids in 94 pea and 121 chickpea accessions. *Journal of Food Composition and Analysis*, 43, 49–60.
- Attia, R. S., El-Tabey Shehata, A. M., Aman, M. E., & Hamza, M. A. (1994). Effects of cooking and decortication on the physical properties, the chemical composition and the nutritive value of chickpea (*Cicer arietinum* L.). *Food Chemistry*, 50, 125–131.
- Berry, M., Wiesinger, J., Nchimbi-Msolla, S., Miklas, P., Porch, T., Fourie, D., & Cichy, K. (2016). Breeding for a fast cooking bean: A study of genotypes across environments to determine stability of the cooking time trait in *Phaseolus vulgaris*. *Bean Improvement Cooperative. Annual Report*, 33–34.
- Bhatty, R. S. (1988). Composition and Quality of Lentil (*Lens culinaris* Medik): A Review. *Canadian Institute of Food Science and Technology Journal*, 21(2), 144–160.
- Bhatty, R. S. (1990). Cooking quality of lentils: The role of structure and composition of cell walls. *Journal of Agricultural and Food Chemistry*, 38, 376–383.
- Brillouet, J. M., & Carre, B. (1983). Composition of cell walls from cotyledons of *Pisum sativum*, *Vicia faba* and *Glycine max*. *Phytochemistry*, 22, 841–847.
- Brillouet, J. M., & Riochet, D. (1983). Cell wall polysaccharides and lignin in cotyledons and hulls of seeds from various lupin (*Lupinus* L.) species. *Journal of Agricultural and Food Chemistry*, 34, 861–868.
- Brummell, D. A., & Harpster, M. H. (2001). Cell wall metabolism in fruit softening and quality and its manipulation in transgenic plants. *Plant Molecular Biology*, 47(1–2), 311–340.
- Burridge, P., Hensing, A., Petterson, D. (2001). Method APQ-102.1: Cooking time of pulse seeds and dhal In *Australian Pulse Quality Laboratory Manual*. SARDI Grain Laboratory for GRDC, Urrbrae, SA.
- Burton, R. A., Ma, G., Baumann, U., Harvey, A. J., Shirley, N. J., Taylor, J., ... Dhugga, K. S. (2010). A customized gene expression microarray reveals that the brittle stem phenotype *fs2* of barley is attributable to a retroelement in the *HvCesA4* cellulose synthase gene. *Plant Physiology*, 153(4), 1716–1728.
- Caffall, K. H., & Mohnen, D. (2009). The structure, function, and biosynthesis of plant cell wall pectic polysaccharides. *Carbohydrate Research*, 344(14), 1879–1900.
- Castellanos Ramos, J. Z., Guzman Maldonado, S. H., Gonzalez de Mejia, E., Acosta Gallegos, J. A., Ochoa, R., Mejia Avila, C., ... Grajeda, O. (1994). Effect of site on protein content and other quality characteristics of *Phaseolus vulgaris* L. *Agricultura Tecnica en Mexico*, 20, 73–83.
- Chowdhury, J., Henderson, M., Schweizer, P., Burton, R. A., Fincher, G. B., & Little, A. (2014). Differential accumulation of callose, arabinoxylan and cellulose in nonpenetrated versus penetrated papillae on leaves of barley infected with *Blumeria graminis* f. sp. hordei. *New Phytologist*, 204(3), 650–660.
- Clemente, A., Sánchez-Vioque, R., Vioque, J., Bautista, J., & Millán, F. (1998). Effect of processing on water absorption and softening kinetics in chickpea (*Cicer arietinum* L.) seeds. *Journal of the Science of Food and Agriculture*, 78, 169–174.

- Comino, P., Shelat, K., Collins, H., Lahnstein, J., & Gidley, M. J. (2013). Separation and purification of soluble polymers and cell wall fractions from wheat, rye and hull less barley endosperm flours for structure-nutrition studies. *Journal of Agricultural and Food Chemistry*, 61(49), 12111–12122.
- Daher, F. B., & Braybrook, S. A. (2015). How to let go: Pectin and plant cell adhesion. *Frontiers in Plant Science*, 6, 523.
- D'Appolonia, B. L., Gilles, K. A., Osman, E. M., & Pomeranz, Y. (1964). In I. Hlynka (Ed.), *Wheat, chemistry and technology* (p. 603). St. Paul, Minnesota, USA.
- de Almeida Costa, G. E., da Silva Queiroz-Monici, K., Reis, S. M. P. M., & de Oliveira, A. C. (2006). Chemical composition, dietary fibre and resistant starch contents of raw and cooked pea, common bean, chickpea and lentil legumes. *Food Chemistry*, 94, 327–330.
- Douchiche, O., Driouch, A., & Morvan, C. (2010). Spatial regulation of cell-wall structure in response to heavy metal stress: Cadmium-induced alteration of the methyl-esterification pattern of homogalacturonans. *Annals of Botany*, 105(3), 481–491.
- Durulé, H., Hervé, V., Ranocha, P., Balliau, T., Zivy, M., Chourré, J., ... Jamet, E. (2017). Cell wall modifications of two *Arabidopsis thaliana* ecotypes, Col and Sha, in response to sub-optimal growth conditions: An integrative study. *Plant Science* 263, 183–193.
- FAO (2017). FAOSTAT database. In *Food and agriculture organization of the United Nations* (ed.). Food and Agriculture Organization of the United Nations. Italy: Rome.
- Farinelli, R., & Lemos, L. B. (2010). Nutritional and technological quality of common bean genotypes cultivated in different seasons. *Bragantia*, 69, 759–764.
- Fitzgerald, M. A., McCouch, S. R., & Hall, R. D. (2009). Not just a grain of rice: The quest for quality. *Trends in Plant Science*, 14, 133–139.
- Frimpong, A., Sinha, A., Tar'an, B., Warkentin, T. D., Gossen, B. D., & Chibbar, R. N. (2009). Genotype and growing environment influence chickpea (*Cicer arietinum* L.) seed composition. *Journal of the Science of Food and Agriculture*, 89, 2052–2063.
- Galiotou-Panayotou, M., Kyriakidis, N. B., & Margaritis, I. (2008). Phytase-phytate-pectin hypothesis and quality of legumes cooked in calcium solutions. *Journal of the Science of Food and Agriculture*, 88, 355–361.
- Garcia, E., Filisetti, T. M. C. C., Udaeta, J. E. M., & Lajolo, F. M. (1998). Hard-to-cook beans (*Phaseolus vulgaris*): Involvement of phenolic compounds and pectates. *Journal of Agricultural and Food Chemistry*, 46, 2110–2116.
- Gaur, P. M., Singh, M. K. K., Samineni, S., Sajja, S. B. B., Jukanti, A. K. K., Kamatam, S., & Varshney, R. K. K. (2016). Inheritance of protein content and its relationships with seed size, grain yield and other traits in chickpea. *Euphytica*, 209, 253–260.
- Ghaderi, A., Hosfield, G. L., Adams, M. W., & Uebersax, M. A. (1984). Variability in culinary quality, component interrelationships, and breeding implications in navy and pinto beans. *Journal of the American Society for Horticultural Science*, 109, 85–90.
- Ghribi, A. M., Maklouf, I., Blecker, C., Attia, H., & Besbes, S. (2015). Nutritional and compositional study of Desi and Kabuli chickpea (*Cicer Arietinum* L.) flours from Tunisian cultivars. *Advances in Food Technology and Nutritional Sciences*, 1, 38–47.
- Guillemain, F., Guillon, F., Bonnin, E., Devaux, M. F., Chevalier, T., Knox, J. P., ... Thibault, J. F. (2005). Distribution of pectic epitopes in cell walls of the sugar beet root. *Planta* 222(2), 355–371.
- Hentges, D. L., Weaver, C. M., & Nielsen, S. S. (1990). Reversibility of the hard-to-cook defect in dry beans (*Phaseolus vulgaris*) and cowpeas (*Vigna unguiculata*). *Journal of Food Science*, 55, 1474–1474.
- Hohlberg, A. I., & Stanley, D. W. (1987). Hard-to-cook defect in black beans. Protein and starch considerations. *Journal of Agricultural and Food Chemistry*, 35, 571–576.
- Iqbal, A., Khalil, I. A., Ateeq, N., & Khan, M. S. (2006). Nutritional quality of important food legumes. *Food Chemistry*, 97, 331–335.
- Liners, F., Letesson, J. J., Didembourg, C., & Van Cutsem, P. (1989). Monoclonal antibodies against pectin: Recognition of a conformation induced by calcium. *Plant Physiology*, 91(4), 1419–1424.
- Liu, K., & Bourne, M. C. (1995). Cellular, biological, and physicochemical basis for the hard-to-cook defect in legume seeds. *Critical Reviews in Food Science and Nutrition*, 35, 263–298.
- Liu, K., Phillips, R. D., & McWatters, K. H. (1993). Mechanism of pectin changes during soaking and heating as related to hard-to-cook defect in cowpeas. *Journal of Agricultural and Food Chemistry*, 41, 1476–1480.
- Mafuleka, M. M., Ott, D. B., Hosfield, G. L., & Uebersax, M. A. (1993). The role of phytase and lignin in decorticated dry bean (*Phaseolus vulgaris*) hardening during storage. *Journal of Food Processing and Preservation*, 17, 1–20.
- Martin-Cabrejas, M. A., Esteban, R. M., Waldron, K. W., Maina, G., Grant, G., Bardocz, S., & Pusztai, A. (1995). Hard-to-cook phenomenon in beans: Changes in antinutrient factors and nitrogenous compounds during storage. *Journal of the Science of Food and Agriculture*, 69, 429–435.
- Matsuura, Y., & Hatanaka, C. (1990). Matrix polysaccharides involved in adhesion of the cell walls of kidney bean cotyledon tissues. *Agricultural and Biological Chemistry*, 54, 3013–3014.
- McCleary, B. V., Solah, V., & Gibson, T. S. (1994). Quantitative measurement of total starch in cereal flours and products. *Journal of Cereal Science*, 20, 51–58.
- Miao, M., Zhang, T., & Jiang, B. (2009). Characterisations of kabuli and desi chickpea starches cultivated in China. *Food Chemistry*, 113(4), 1025–1032.
- Morais, P. P. P., Valentini, G., Guidolin, A. F., Baldissera, J. N. d. C., & Coimbra, J. L. M. (2010). Influence of the period and storage conditions of beans at the time of cooking. *Revista Ciencia Agronomica*, 41, 593–598.
- Nasar-Abbas, S. M., Plummer, J. A., Siddique, K. H. M., White, P., Harris, D., & Dods, K. (2008). Cooking quality of faba bean after storage at high temperature and the role of lignins and other phenolics in bean hardening. *LWT - Food Science and Technology*, 41, 1260–1267.
- Nikolopoulou, D., Grigorakis, K., Stasini, M., Alexis, M. N., & Iliadis, K. (2007). Differences in chemical composition of field pea (*Pisum sativum*) cultivars: Effects of cultivation area and year. *Food Chemistry*, 103, 847–852.
- Njoroge, D. M., Kinyanjui, P. K., Chigwedere, C. M., Christiaens, S., Makokha, A. O., Sila, D. N., & Hendrickx, M. E. (2016). Mechanistic insight into common bean pectic polysaccharide changes during storage, soaking and thermal treatment in relation to the hard-to-cook defect. *Food Research International*, 81, 39–49.
- Njoroge, D. M., Kinyanjui, P. K., Christiaens, S., Shpigelman, A., Makokha, A. O., Sila, D. N., & Hendrickx, M. E. (2015). Effect of storage conditions on pectic polysaccharides in common beans (*Phaseolus vulgaris*) in relation to the hard-to-cook defect. *Food Research International*, 76(1), 105–113.
- Njoroge, D. M., Kinyanjui, P. K., Makokha, A. O., Christiaens, S., Shpigelman, A., Sila, D. N., & Hendrickx, M. E. (2014). Extraction and characterization of pectic polysaccharides from easy- and hard-to-cook common beans (*Phaseolus vulgaris*). *Food Research International*, 64, 314–322.
- Paciulli, M., Ganino, T., Carini, E., Pellegrini, N., Pugliese, A., & Chiavaro, E. (2016). Effect of different cooking methods on structure and quality of industrially frozen carrots. *Journal of Food Science and Technology*, 53(5), 2443–2451.
- Paredes-López, O., Maza-Calviño, E. C., & González-Castañeda, J. (1989). Effect of the hardening phenomenon on some physicochemical properties of common bean. *Food Chemistry*, 31, 225–236.
- Paredes-López, O., Reyes-Moreno, C., Montes-Rivera, R., & Carabez-Trejo, A. (1989). Hard-to-cook phenomenon in common beans- influence of growing location and hardening procedures. *International Journal of Food Science and Technology*, 24, 535–542.

- Parker, M. L. (1984a). Cell wall storage polysaccharides in cotyledons of *Lupinus angustifolius* L. II. Mobilization during germination and seedling development. *Protoplasma*, 120, 233–241.
- Parker, M. L. (1984b). Cell wall storage polysaccharides in cotyledons of *Lupinus angustifolius* L. I. Deposition during seed development. *Protoplasma*, 120, 224–232.
- Patane, C. (2006). Variation and relationships among some nutritional traits in Sicilian genotypes of chickpea (*Cicer arietinum* L.). *Journal of Food Quality*, 29, 282–293.
- Peña, M. J., & Carpita, N. C. (2004). Loss of highly branched arabinans and debranching of rhamnogalacturonan I accompany loss of firm texture and cell separation during prolonged storage of apple. *Plant Physiology*, 135(3), 1305–1313.
- Perez Herrera, P., Acosta Diaz, E., Padilla Ramirez, S., & Acosta Gallegos, J. A. (1999). Effect of drought stress on seed quality of common bean (*Phaseolus vulgaris* L.). *Agricultura Tecnica en Mexico*, 25, 107–114.
- Petterson, D. S., Sipsas, S., & Mackintosh, J. B. (1997). *The chemical composition and nutritive value of Australian pulses*. 2nd Edn., Grains Research and Development Corporation. Australia: Kingston ACT.
- Pettolino, F. A., Walsh, C., Fincher, G. B., & Bacic, A. (2012). Determining the polysaccharide composition of plant cell walls. *Nature Protocols*, 7(9), 1590–1607.
- Reyes-Moreno, C., Okamura-Esparza, J., Armienta-Rodelo, E., Gómez-Garza, R. M., & Milán-Carrillo, J. (2000). Hard-to-cook phenomenon in chickpeas (*Cicer arietinum* L.): Effect of accelerated storage on quality. *Plant Foods for Human Nutrition*, 55, 229–241.
- Reyes-Moreno, C., Paredes-López, O., & Gonzalez, E. (1993). Hard-to-cook phenomenon in common beans—A review. *Critical Reviews in Food Science and Nutrition*, 33, 227–286.
- Reyes-Moreno, C., Rouzaud-Sandez, O., Milán-Carrillo, J., Garzón-Tiznado, J. A., & Camacho-Hernández, L. (2001). Hard-to-cook tendency of chickpea (*Cicer arietinum* L.) varieties. *Journal of the Science of Food and Agriculture*, 81, 1008–1012.
- Rincón, F., Martínez, B., & Ibáñez, M. V. (1998). Proximate composition and antinutritive substances in chickpea (*Cicer arietinum* L.) as affected by the biotype factor. *Journal of the Science of Food and Agriculture*, 78, 382–388.
- Rozo, C., Bourne, M. C., Hood, L. F., & Van Soest, P. J. (1990). Effect of storage time, relative humidity and temperature on the cookability of whole red kidney beans and on the cell wall components of the cotyledons. *Canadian Institute of Food Science and Technology Journal*, 23, 72–75.
- Rubio, J., Gil, J., Cobos, M. J., & Millán, T. (2011). Chickpea. In C. Kole (Ed.), *Genetics, genomics and breeding of cool season grain legumes* (pp. 205–236). Science Publishers.
- Sasikala, V. B., Ravi, R., & Narasimha, H. V. (2011). Textural changes of green gram (*Phaseolus aureus*) and horse gram (*Dolichos biflorus*) as affected by soaking and cooking. *Journal of Texture Studies*, 42, 10–19.
- Sefa Dedeh, S., Stanley, D. W., & Voisey, P. W. (1979). Effect of storage time and conditions on the hard-to-cook defect in cowpeas (*Vigna unguiculata*). *Journal of Food Science*, 44, 790–796.
- Shiga, T. M., Cordenunsi, B. R., & Lajolo, F. M. (2009). Effect of cooking on non-starch polysaccharides of hard-to-cook beans. *Carbohydrate Polymers*, 76, 100–109.
- Shiga, T. M., & Lajolo, F. M. (2006). Cell wall polysaccharides of common beans (*Phaseolus vulgaris* L.)—Composition and structure. *Carbohydrate Polymers*, 63, 1–12.
- Shiga, T. M., Lajolo, F. M., & Filisetti, T. M. C. C. (2004). Changes in the cell wall polysaccharides during storage and hardening of beans. *Food Chemistry*, 84, 53–64.
- Singh, U. (1985). Nutritional quality of chickpea (*Cicer arietinum* L.): Current status and future research needs. *Plant Foods for Human Nutrition*, 35, 339–351.
- Sissons, M. J. (2012). *Durum wheat: chemistry and technology*, 2nd edition ed. AACC International, St. Paul, Minnesota, USA, 300 pages.
- Srisuma, N., Hammerschmidt, R., Uebersax, M. A., Ruengsakulrach, S., Bennink, M. R., & Hosfield, G. L. (1989). Storage induced changes of phenolic acids and the development of hard-to-cook in dry beans (*Phaseolus vulgaris* var. Seafarer). *Journal of Food Science*, 54, 311–314.
- Stanley, D. W. (1992). A possible role for condensed tannins in bean hardening. *Food Research International*, 25, 187–192.
- Stanley, D. W., Michaels, T. E., Plhak, L. C., & Caldwell, K. B. (1990). Storage-induced hardening in 20 common bean cultivars. *Journal of Food Quality*, 13, 233–247.
- Stolle-Smits, T., Beekhuizen, J. G., Recourt, K., Voragen, A. G. J., & van Dijk, C. (2000). Preheating effects on the textural strength of canned green beans. 1. Cell wall chemistry. *Journal of Agricultural and Food Chemistry*, 48, 5269–5277.
- Updegraff, D. M. (1969). Semimicro determination of cellulose in biological materials. *Analytical Biochemistry*, 32, 420–424.
- Vaz Patto, M. C. (2015). Grain legumes: Missing links for delicious health... *Legume. Perspectives*, 9, 4. <http://ils.nsseme.com/#journals>
- Verherbruggen, Y., Marcus, S. E., Chen, J., & Knox, J. P. (2013). Cell wall pectic arabinans influence the mechanical properties of Arabidopsis thaliana inflorescence stems and their response to mechanical stress. *Plant Cell Physiology*, 54(8), 1278–1288.
- Vicente, A. R., Ortugno, C., Powell, A. L., Greve, L. C., Labavitch, J. M. (2007) Temporal sequence of cell wall disassembly events in developing fruits. 1. Analysis of raspberry (*Rubus idaeus*). *Journal of Agricultural and Food Chemistry*, 55(10), 4119–4124.
- Wang, N., & Daun, J. K. (2005). Determination of cooking times of pulses using an automated Mattson cooker apparatus. *Journal of the Science of Food and Agriculture*, 85, 1631–1635.
- Wang, N., Hatcher, D. W., Tyler, R. T., Toews, R., & Gawalko, E. J. (2010). Effect of cooking on the composition of beans (*Phaseolus vulgaris* L.) and chickpeas (*Cicer arietinum* L.). *Food Research International*, 43, 589–594.
- Wang, N., Hou, A., Santos, J., & Maximiuk, L. (2016). Effects of cultivar, growing location, and year on physicochemical and cooking characteristics of dry beans (*Phaseolus vulgaris*). *Cereal Chemistry*, 94, 128–134.
- Wilkinson, L. G., & Tucker, M. R. (2017). An optimised clearing protocol for the quantitative assessment of sub-epidermal ovule tissues within whole cereal pistils. *Plant Methods*, 13, 67.
- Willats, W. G., Orfila, C., Limberg, G., Buchholt, H. C., van Alebeek, G. J. W., Voragen, A. G., ... Knox, J. P. (2001). Modulation of the degree and pattern of methyl-esterification of pectic homogalacturonan in plant cell walls, implications for pectin methyl esterase action, matrix properties, and cell adhesion. *Journal of Biological Chemistry*, 276(22), 19404–19413.
- Williams, P., El-Haramein, F. J., Nakkoul, H., & Rihawi, S. (1988). Crop quality evaluation methods and guidelines. In *ICARDA Technical Manual, International Center for Agricultural Research in the Dry Areas*. No.14, Ed. 2. Syria
- Wood, J. A. (2016). Evaluation of cooking time in pulses: A review. *Cereal Chemistry*, 94, 32–48.
- Wood, J. A., & Grusak, M. A. (2007). Nutritional value of chickpea. In S. S. Yadav, R. Redden, W. Chen, & B. Sharma (Eds.), *Chickpea breeding and management* (pp. 101–142). Wallingford, UK: CAB International.
- Wood, J. A., Knights, E. J., Campbell, G. M., & Choct, M. (2014a). Differences between easy- and difficult-to-mill chickpea (*Cicer arietinum* L.) genotypes. Part I: Broad chemical composition. *Journal of the Science of Food and Agriculture*, 94, 1437–1445.
- Wood, J. A., Knights, E. J., Campbell, G. M., & Choct, M. (2014b). Differences between easy- and difficult-to-mill chickpea (*Cicer arietinum* L.) genotypes. Part II: Protein, lipid and mineral composition. *Journal of the Science of Food and Agriculture*, 94, 1446–1453.
- Wood, J. A., Knights, E. J., Campbell, G. M., & Choct, M. (2014c). Differences between easy- and difficult-to-mill chickpea

- (*Cicer arietinum* L.) genotypes. Part III: Free sugar and non-starch polysaccharide composition. *Journal of the Science of Food and Agriculture*, 94, 1454–1462.
- Wood, J. A., Knights, E. J., Campbell, G. M., & Choct, M. (2014d). Erratum to: Differences between easy- and difficult-to-mill chickpea (*Cicer arietinum* L.) genotypes. Part I: Broad chemical composition. *Journal of the Science of Food and Agriculture*, 94, 3305–3306.
- Wood, J. A., Knights, E. J., Campbell, G. M., & Choct, M. (2017). Near-isogenic lines of desi chickpea (*Cicer arietinum* L.) that differ in milling ease: Differences in chemical composition. *Journal of Food Science and Technology*, 54, 1002–1013.
- Wood, J. A., Knights, E. J., & Choct, M. (2011). Morphology of chickpea seeds: Comparison between desi and kabuli types. *International Journal of Plant Sciences*, 172, 632–643.
- Xing, X., Hsieh, Y. S. Y., Yap, K., Ang, M. E., Lahnstein, J., Tucker, M. R., ... Bulone, V. (2017). Isolation and structural elucidation by 2D NMR of planteose, a major oligosaccharide in the mucilage of chia (*Salvia hispanica* L.) seeds. *Carbohydrate Polymers*, 175, 231–240.
- Xue, J., Bosch, M., & Knox, P. (2013). Heterogeneity and glycan masking of cell wall microstructures in the stems of *miscanthus x giganteus*, and its parents *M. sinensis* and *M. sacchariflorus*. *PLoS One*, 8, e82114. <https://doi.org/10.1371/journal.pone.0082114>
- Zia-Ul-Haq, M., Iqbal, S., Ahmad, S., Imran, M., Niaz, A., & Bhanger, M. I. (2007). Nutritional and compositional study of desi chickpea (*Cicer arietinum* L.) cultivars grown in Punjab, Pakistan. *Food Chemistry*, 105, 1357–1363.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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