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Effect of Dough Mixing on Wheat Endosperm Cell Walls

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Supporting Information

ABSTRACT: Dough-derived cell wall fragments isolated by ultracentrifugation were largely derived from the starchy endosperm, with some fragments deriving from the aleurone and outer layers, as indicated by fluorescence microscopy. Dough mixing had little effect on the structure and composition of cell wall fragments compared to thin grain sections, as determined by Fourier transform infrared (FTIR) and ^{1}H nuclear magnetic resonance (NMR) spectroscopy. These analyses confirmed that the fragments largely comprised water-unextractable arabinoxylan and β-glucan. FTIR microspectroscopy of dough-derived cell wall fragments prepared from five bread wheat cultivars showed that two largely comprised highly substituted arabinoxylan (cv. Manital and San Pastore), one comprised a mixture of low, medium, and highly substituted arabinoxylan (cv. Hereward), and the remaining two comprised a greater proportion of low substituted arabinoxylan (cv. Claire and Yumai 34). Yumai 34 yielded a greater mass of cell wall material, and its cell walls comprised a high proportion of medium substituted arabinoxylan. Such methods will allow for the impact of bakery ingredients and processing on endosperm cells, including the addition of xylanases, to be investigated in the future to ensure any potential health benefits arising from wheat breeding are realized in the food that reaches the consumer.

KEYWORDS: *Arabinoxylan, endosperm cell walls, FTIR, ^{1}H NMR, wheat flour*

INTRODUCTION

The starchy endosperm cell walls in wheat grains are known to impart beneficial health effects to cereal products. These benefits include generating viscosity, which may slow the rate of gastric emptying, reducing mobility in the small intestine and lowering postprandial glucose and insulin responses in humans.[1] Arabinoxylan, the main component of wheat endosperm cell walls, may also dilute the energy density of the diet, therefore prolonging intestinal digestive processes, which in turn aids the control of satiety.[2] In addition, the arabinoxylan content of wheat grain and flour affects their functionality during processing, including brewing and biofuel production,[3] breadmaking,[4,5] and gluten−starch separation.[6,7] One of the difficulties in improving the dietary intake of cereal fiber and whole grains is that many consumers prefer the textural and flavor attributes of white compared to wholemeal bread. In response to this, food manufacturers have sought to supplement cereal foods with fiber-rich ingredients, using particular milling fractions or using white wheat cultivars to increase the fiber content while maintaining the consumer appeal of white bread.

An alternative strategy is to source wheat cultivars with enhanced fiber content, which is a heritable characteristic and shows extensive diversity in composition and structure within modern cultivated wheats.[8,9] In wheat, about 14−15% of the endosperm cell walls comprises protein, with the remaining 75% being non-starch polysaccharides, of which about 70% is arabinoxylan, 20% is (1−3)(1−4)-β-D-glucan, 7% is β-glucomannan, and 2% is cellulose.[10,11] Cereal arabinoxylan consists of backbone chains of β(1−4)-linked D-xylopyranosyl residues, to which α-L-arabinofuranose units are linked as side chains. In common with many other plant polysaccharides, arabinoxylan also exhibits a high degree of endogenous micro-heterogeneity, making it impossible to assign a single structure. The degree and distribution of side chains are important factors in the physiochemical properties of arabinoxylan, such as water solubility, viscosity, and gelation properties, as well as their physiological functions in the gastrointestinal (GI) tract. About 35% of the total arabinoxylan in the starchy endosperm of wheat is classified as water-soluble/extractable, and the remainder is classified as water-insoluble/unextractable.[12] Continuous segments of unsubstituted xylose residues permit intermolecular realignments and interchain associations, while arabinosyl moeties appear to stiffen the molecules; therefore, water-extractable arabinoxylans exhibit high viscosities in aqueous solutions.[13]

Many studies of the arabinoxylan structure have relied on fractions extracted from flour using various aqueous, alkali, or enzymatic extraction methods[13] or have used grain sections and

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infrared mapping.[14,15] However, this is not the form in which arabinoxylan is consumed in foods, where it is present largely in fragments of endosperm cell wall and residual bran particles, resulting from the milling process. We have therefore developed a procedure to isolate largely intact endosperm cell wall fragments from wheat flour dough and have used light microscopy, ^{1}H nuclear magnetic resonance (NMR) and Fourier transform infrared (FTIR) spectroscopy, and spectroscopic imaging to characterize the effects of the dough mixing process on endosperm cell walls using wheat cultivars, which were previously shown to differ in their composition of cell wall arabinoxylan.[9]

■ MATERIALS AND METHODS

Wheat Cultivars and Arabinoxylan Samples. White flour was prepared from the following hard bread wheat cultivars: Hereward, Yumai 34, Manital, San Pastore, and Claire. All cultivars were grown at the Agricultural Research Institute of the Hungarian Academy of Sciences, Martonvásár, Hungary (latitude, 47° 21′ N; longitude, 18° 49′ E; altitude, 150 m) in 2004/2005 under controlled conditions and provided by the EC FP6 project HEALTHGRAIN (Food-CT-2005-514008).[16] These cultivars were known to differ in their total contents of water-extractable and total arabinoxylan[8] and in the extent of substitution of the arabinoxylan.[9] The levels of endogenous xylanase in flours of the cultivars grown in this year were low.[17] Analysis of the HEALTHGRAIN data set showed a clear correlation between the activity of xylanase and the content of WE-AX in flour.[17] Hence, the effect of endogenous xylanase on AX composition in the samples studied should be low.

Extraction of Cell Wall Fragments from Wheat Flour. Dough was prepared by mixing 3.75 g of wheat flour with ~1.5−2.5 mL of water (depending upon the water absorption value of the flour, calculated as water absorption/100 × flour weight), using a mini mixer[18] that was able to produce ~6 g of dough per run. The water absorption was determined to the 600 BU line using the industry standard Farinograph method (Brabender, Duisberg, Germany). The miniature dough mixer provided a means of mixing small dough samples (2−12 g) in compliance with the Chorleywood bread process, which requires an energy input of 40 J g^{-1}. It consisted of essentially a miniature Tweedy-type mixer assembly [rotor, mixing bowl, mixing guard, stand, and direct current (DC) electric motor, with DC tachometer for monitoring the rotational speed], with an analogue control system (motor drive module and connections to the computer ACL-8112PG interface card). A prototype "flour chute" was constructed using a Wavetek oscillator and a loud speaker transducer to add the flour to the mixer gradually and without clogging.

Polycarbonate ultracentrifuge tubes (16 × 76 mm) with screw-on caps (Beckman, U.K.) were filled to the rim with approximately 12 g of dough per sample and ultracentrifuged in a fixed angle rotor at 200000*g* [53 000 rpm using a Ti70.1 rotor (Beckman, U.K.)] for 30 min at 25 °C.[19] At the same time, 12 g of flour combined with water (with minimum mechanical input) was also centrifuged to compare this to the dough. After centrifugation, the lipid pellicle and dough liquor (~2 mL) were carefully poured from the top of the separated layers and the underlying cell wall fragment layer was scraped from each tube. These were placed into 15 mL centrifuge tubes (Corning, Tewksbury, MA) and washed 3 times with 10 mL of water, by shaking to disperse the cell walls and remove any residual soluble material (sugars, soluble proteins, and water-extractable arabinoxylan), and then centrifugation (MSE Mistral 2000, DJB Labcare, U.K.) at 3000*g* for 10 min. After dispersion in 5 mL of water, cell walls were freeze-dried using a VirTis Advantage 2.0 bench top freeze drier (SP Scientific, Gardiner, NY) and stored in sealed freeze-drier vials. Freeze-dried cell wall fragments (0.001 g) were redispersed in 1.5 mL of water, shaken, and left overnight to rehydrate prior to use. The resulting suspension (100 μL) was pipetted onto a barium fluoride disc and allowed to air-dry at room temperature for FTIR spectroscopic imaging.

FTIR Spectroscopy. *Attenuated Total Reflectance (ATR).* FTIR (ATR) spectra were measured, between 700 and 4000 cm^{-1}, using a Digilab FTS 6000 spectrometer (Digilab, Cambridge, MA), by placing a small amount of each sample of cell wall fragments onto the diamond crystal (1.5 × 1.5 mm^2) of a Golden Gate single-reflection ATR accessory (Specac, U.K.). The spectral resolution was 2 cm^{-1}, and 128 scans were co-added for each spectrum. The empty crystal was used as a background, and the spectrum for water was measured and subtracted from each sample spectrum. This method was used to analyze the extracted cell wall fragments and a reference arabinoxylan/β-glucan mixture, made up by mixing 75% water-unextractable arabinoxylan with 25% β-glucan (both Megazyme, Bray, Ireland).

FTIR Spectroscopic Imaging. FTIR spectroscopic maps were collected using a Nicolet iN10 MX imaging spectrometer. An area of approximately 3000 × 3000 μm was selected for each barium fluoride disc (coated with a dried down layer of freeze-dried and then redispersed cell wall fragments). The imaging detector had an automatic aperture of 25 × 25 μm per pixel, and 3 s scans (16 scans per spectrum at 8 cm^{-1} resolution) were collected. Because the detector consists of a linear array, the collection time was approximately 1 h.

All data analysis was performed using the image analysis software ENVI 4.3 (Research Systems, Inc., Boulder, CO). To avoid the influence of variation in sample thickness and, therefore, spectral intensity, all spectra were baseline-corrected and normalized. This was performed by converting all spectra to transmittance units, correcting the baseline to 1 (using the continuum-removal function), returning the spectra to absorbance units, and finally, conducting an area normalization to the maximum carbohydrate peak at 1047 cm^{-1} (dividing by the height of the band and then multiplying by a factor).

To provide spectroscopic images, an ENVI classification method was devised, which was similar to that used previously[9,14,15] during the determination of the spatial distribution of arabinoxylan structures within the endosperm cell wall network. Here, however, five classes were defined (rather than three), according to the height of the spectral shoulder at 1075 cm^{-1} relative to the main carbohydrate (arabinoxylan) peak at 1047 cm^{-1}. The total range of the peak height ratios within an image was between ~55 and 80%; hence, the specific levels chosen were as follows: low substituted arabinoxylan, <60% peak height, color-coded dark green; medium−low substituted arabinoxylan, 60−65%, colored light green; medium substituted arabinoxylan, 65−70%, colored yellow; medium−highly substituted arabinoxylan, 70−75%, colored light blue; and highly substituted arabinoxylan, >75%, colored dark blue. Histogram plots were also produced, using the heights of the shoulder at 1075 cm^{-1} for each pixel, to provide an indication of the distribution of substitution levels across the cell wall spectral images. The number of pixels, depicted by each color in the images, was determined and used to calculate the percentages of each class of arabinoxylan in each spectral image; these were also plotted as histograms.

Microscopy. The cell wall fragments (freshly prepared, before and after "washing", and reconstituted freeze-dried samples) were smeared onto a glass slide with a drop of water or sodium hydroxide solution (1%, v/v) and viewed using a light microscope (Olympus BX60, Japan) at magnifications of 10×, 20×, and 40×. An ultraviolet (UV) lens was also used to visualize the autofluorescence generated by the phenolic compounds, namely, ferulic acid residues, which are covalently linked through ester linkages to C(O)-5 of some of the arabinose residues. In white flour, about a fifth of the ferulate in WE-AX is present as 5,5′, 8-O-4′, and 8,5′ dimers.[20]

^{1}H NMR Analysis. Approximately 0.001 g of freeze-dried endosperm cell wall fragments was mixed with 600 μL of a solution of D_2O containing sodium 3-(trimethylsilyl)-propionate-d_4 (TSP) as a reference and stirred using a spatula at room temperature for ~10 s. A total of 550 μL of the whole mixture was then transferred to a 5 mm NMR tube for analysis. ^{1}H NMR spectra were recorded at 70 °C on a 600 MHz Bruker Avance spectrometer (Bruker Biospin, Rheinstetten, Germany) according to the method described previously.[9,15,21]

The arabinose/xylose (A/X) ratio, the ratio of mono- to disubstituted xylopyranosyl (Xyl*p*) residues, and the proportions of mono-, di-, and unsubstituted Xyl*p* residues were determined.[9,21] The signal assignments were based on detailed ^{1}H NMR data for arabinoxylan oligosaccharides.[22,23]

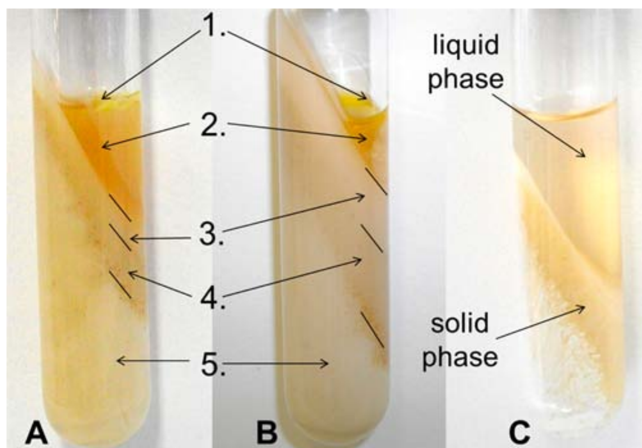

Figure 1. (A) cv. Hereward and (B) cv. Yumai 34 ultracentrifuged dough consisting of five separate layers: (1) a lipid pellicle, (2) the "dough liquor", containing soluble proteins and carbohydrates, (3) the "gel-like" layer, composed of endosperm cell wall fragments, (4) a gluten network, and (5) starch granules. (C) Ultracentrifuged combined flour and water showing no separate cell wall fragment layer.

■ RESULTS

Characterization of Cell Wall Fragments from Dough. To characterize the effects of processing procedures, such as dough mixing, on the arabinoxylan structure, a method was developed to extract endosperm cell wall fragments from wheat flour both quickly and efficiently, with minimally destructive procedures. Wheat flour dough prepared using the appropriate amount of water, determined by the flour water absorption values,[16] and mixed using a mini-mixer, which mimics commercial dough mixers,[18] was subjected to ultracentrifugation at 200000*g*. The dough phase-separated to form five clear layers (panels A and B of Figure 1), as observed by others:[24] (1) a creamy lipid pellicle floating at the top, (2) a straw-colored viscous material called "dough liquor",[19] which contained the soluble proteins and carbohydrates (including water-extractable arabinoxylan), (3) a thin opaque "gel-like layer", which consisted of the endosperm cell wall fragments, (4) a viscoelastic layer containing the gluten protein network, and (5) a deposit of starch granules at the bottom of the tube. Figure 1B shows that a smaller volume of dough liquor and a far greater mass of cell wall fragments (~4-fold more) were recovered from dough prepared

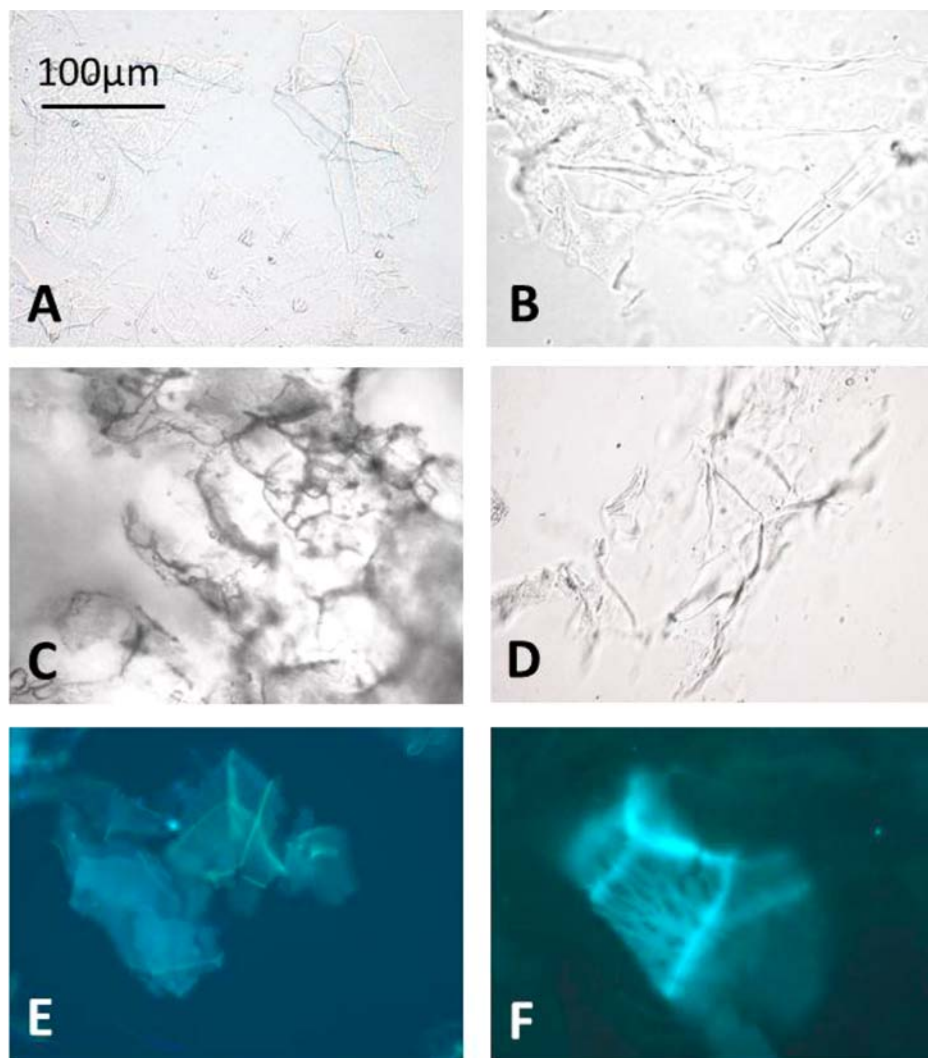

Figure 2. Light microscope images of the extracted cell wall fragments: (A) directly after extraction (i.e., layer 3 in Figure 1), (B) following a "washing" process, (C) freeze-dried, and (D) freeze-dried and then redispersed in water. (E) UV microscopic image of a cell wall fragment directly after extraction, showing increased levels of phenolic compounds in blue. (F) UV image of a fragment of the aleurone cell wall, showing increased levels of cell wall phenolic compounds forming a mesh-like structure.

using flour from cv. Yumai 34, a cultivar know to have a high content of cell wall arabinoxylan, compared to the classical bread-making cultivar, Hereward, which contains lower levels of AX (Figure 1A) and was representative of the other cultivars investigated. This phase separation was not achieved when the flour was simply combined with water and centrifuged in the same way (Figure 1C), which gave only solid and liquid phases rather than separated dough components. Thus, the formation of the gluten network within the dough was essential to enable the separation of the cell wall fragments from the other cell contents (i.e., starch granules and the protein matrix) by centrifugation.

Initially, the cell wall fragments in the "gel-layer" (panels A and B of Figure 1) were observed using light microscopy (Figure 2) and found to consist entirely of endosperm cell wall fragments, formed as a consequence of fracturing and shearing of the wheat grain during the milling process. There was no protein visible in the sample, and the addition of iodine confirmed that there was also no starch present. The formation of the dough did not appear to have visually altered the cell wall fragments formed following milling,[25] although they lacked the fragments of the protein matrix and clusters of starch granules embedded in the protein matrix that have been reported to be attached to fragments of the cell wall in flour.[25,26] They also resembled cell wall fragments extracted from flour using more invasive methods.[12,27]

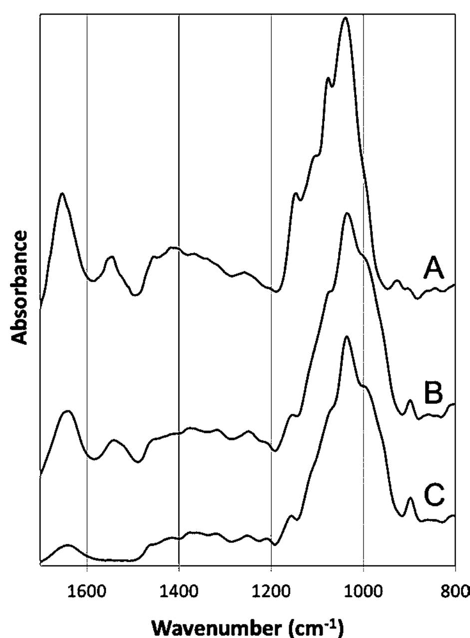

Figure 3. Typical FTIR (ATR) spectra for (A) unwashed endosperm cell wall fragments as extracted from cv. Hereward flour dough, (B) washed and freeze-dried endosperm cell wall fragments (cv. Hereward), and (C) standard mixture of water-unextractable arabinoxylan and β-glucan to a ratio of 75:25.

To remove any soluble sugars, the cell wall fragments were washed, a process that did not alter their structure visually, apart from slight swelling because of further retention of water (Figure 2B), perhaps a result of the extremely high water-holding capacity of wheat endosperm cell wall arabinoxylan.[28] Upon air drying, the cell wall fragments aggregated into a solid translucent film, while freeze-dried preparations had a crumb-like structure (Figure 2C), with the cell wall fragments being loosely attached to one another. Unlike air drying, the freeze-drying process enabled the cell walls to be redispersed into water whenever required, and allowed them to retain the same visual appearance as the freshly prepared cell wall fragments (Figure 2D), thus allowing them to be stored pending chemical analyses.

4-Hydroxy-3-methoxycinnamic (ferulic) acid is known to occur in high concentrations in the aleurone cell walls of wheat, to a lesser extent in the seed coat and embryo, and in only trace amounts in the endosperm.[29−31] Ferulic acid autofluoresces in the blue region of the spectrum, and fluorescence microscopy using a UV filter is therefore used routinely to determine its distribution in wheat grain.[32,33] Most of the cell wall fragments prepared from wheat doughs showed an even distribution of fluorescence and, hence, feruloylation (Figure 2E), with slightly increased levels being visible where cell wall fragments overlapped. However, some fragments showed a much greater level of fluorescence (Figure 2F), suggesting that they were fragments of the aleurone cell wall. Far higher levels of fluorescence were seen at the cell junctions, which was in line with studies carried out using polyclonal antibodies.[34] A "mesh-like" structure could also be seen across aleurone cell walls (Figure 2F), indicating that ferulic acid may contribute to the structural organization of the wall.

The composition of the cell wall fragments isolated from dough was then determined using FTIR (ATR) spectroscopy. The spectra of freshly prepared endosperm cell wall fragments (flour cv. Hereward) and fragments following the "washing" and freeze-drying processes are shown in Figure 3, with a reference spectrum determined for a mixture of arabinoxylan and β-glucan at a ratio of 75:25. The spectrum of the cell walls taken immediately after extraction from the dough (Figure 3A) showed high levels of residual starch oligosaccharides and polysaccharides remaining in the sample from the dough liquor, with bands at 1148, 1106, 1077, and 1040 cm^{-1}, all characteristic of cell wall polysaccharides.[19,35−38] This was removed by washing (Figure 3B), as indicated by the absence of spectral features related to soluble sugars. Apart from the additional amide I and II peaks between 1500 and 1700 cm^{-1}, resulting from the presence

Table 1. Percentages of Various Arabinoxylan Components Determined Using FTIR Spectroscopic Imaging and Gas Chromatography of Alditol Acetates[41] [a]

	FTIR spectroscopic imaging					arabinoxylan content[a]		
cultivar	LS	MLS	MS	MHS	HS	tot-AX	WE-AX	WU-AX
Manital	0.086	4.52	24.75	37.09	33.55	2.03	0.57	1.57
San Pastore	0.014	3.58	26.05	41.44	28.91	1.77	0.40	1.46
Hereward	0.116	9.48	42.24	35.64	12.53	1.70	0.41	1.42
Claire	1.483	28.33	40.87	21.33	7.98	1.75	0.44	1.43
Yumai 34	0.014	7.91	65.59	24.76	1.74	2.74	1.38	1.99

[a]From ref 9 (see the Supporting Information). LS, low substituted arabinoxylan; MLS, medium−low substituted arabinoxylan; MS, medium substituted arabinoxylan; MHS, medium−highly substituted arabinoxylan; HS, highly substituted arabinoxylan; tot-AX, total arabinoxylan; WE-AX, water-extractable arabinoxylan; and WU-AX, water-unextractable arabinoxylan.

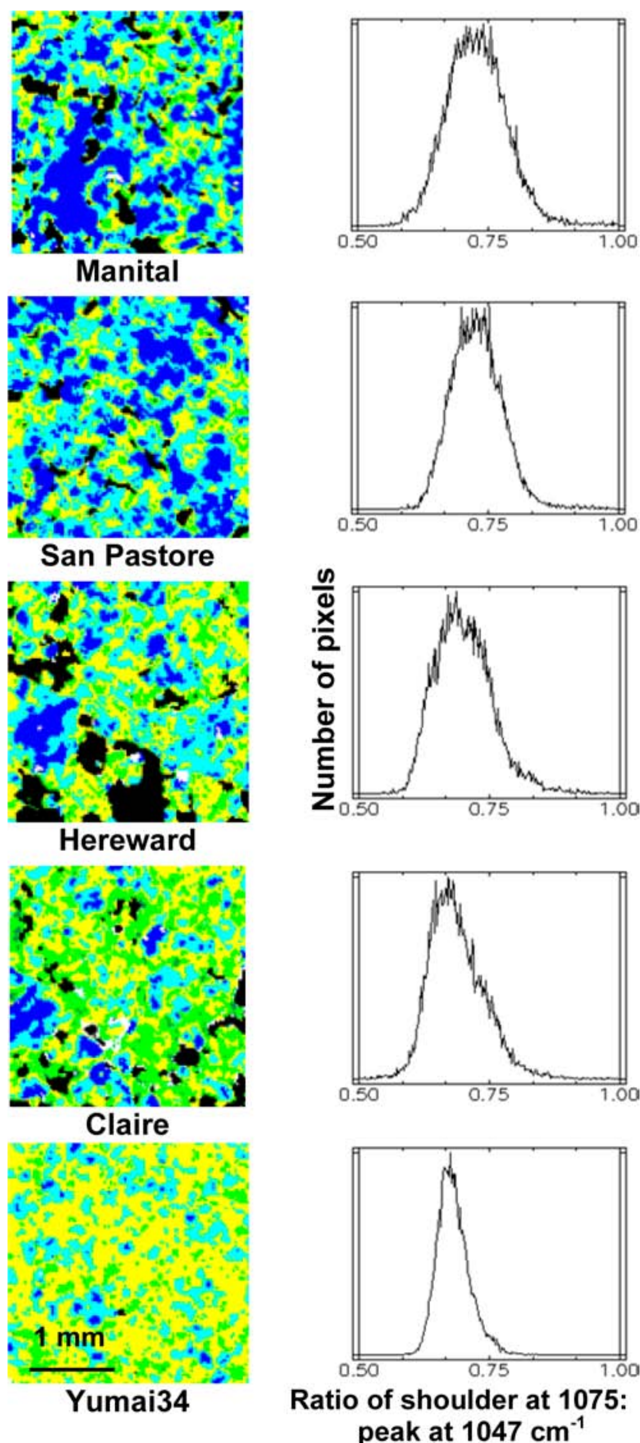

Figure 4. Spectroscopic images color-coded according to levels of arabinose substitution in the cell wall arabinoxylan for each cultivar: low substituted arabinoxylan, dark green; medium−low substituted arabinoxylan, light green; medium substituted arabinoxylan, yellow; medium−highly substituted arabinoxylan, light blue; and highly substituted arabinoxylan, dark blue. Alongside, histograms show the distributions for the shoulder height at 1075 cm^{-1} (x, ratio of the shoulder height at 1075 cm^{-1} to the peak height at 1047 cm^{-1}; y, number of spectral pixels).

of intrinsic cell wall proteins, the spectrum for the cell wall fragments showed the same spectral features as a reference spectrum (Figure 3C) of a mixture of pure water-unextractable arabinoxylan and β-glucans, in a ratio of 75:25, which is the

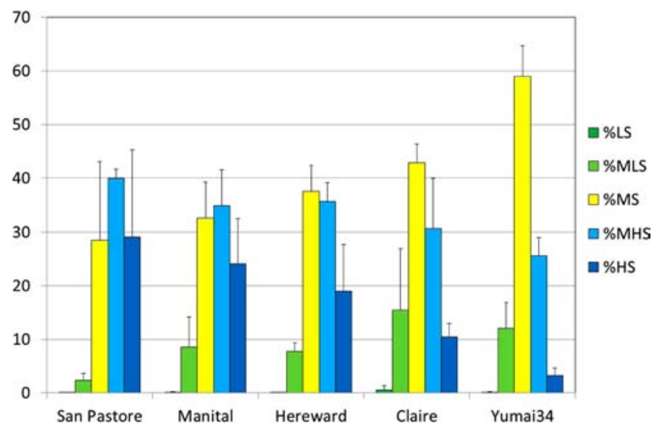

Figure 5. Average percentage of pixels (not including those for starch or holes) depicting each level of arabinoxylan substitution for three replicate extractions of each of the five cultivars (±standard deviation).

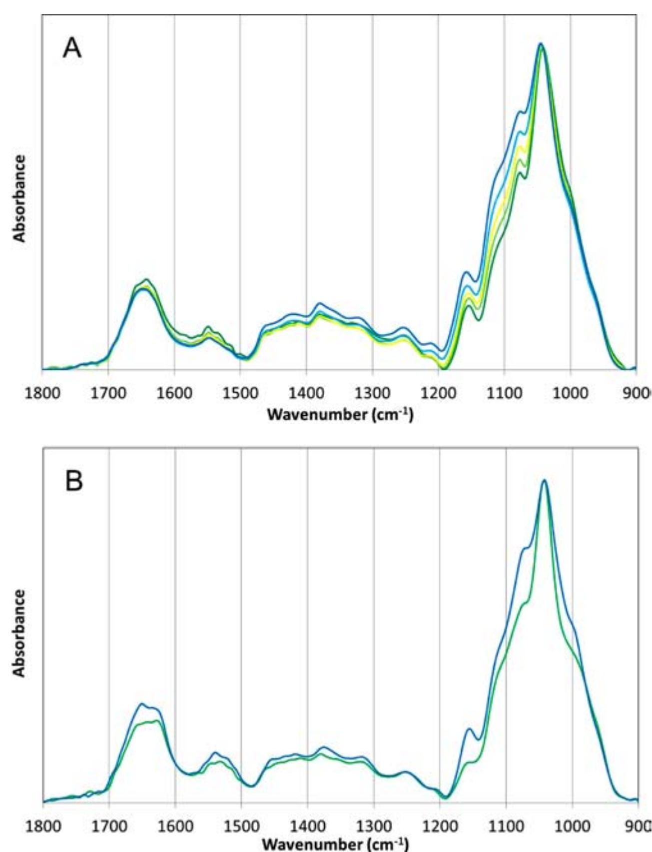

Figure 6. Average FTIR spectra (in a spectral image) at each arabinose substitution level for endosperm cell walls of cv. Hereward: (A) following extraction from a flour dough and (B) using cell wall only thin sections.[9] Low substituted arabinoxylan, dark green; medium−low substituted arabinoxylan, light green; medium substituted arabinoxylan, yellow; medium−highly substituted arabinoxylan, light blue; and highly substituted arabinoxylan, dark blue.

approximate ratio of these components in the starchy endosperm cell walls of wheat[10] (the spectra for six other mixtures of arabinoxylan and β-glucan with differing ratios is provided in the Supporting Information). These data therefore confirmed that the cell wall fragments from dough comprised mainly arabinoxylan and (1,3;1,4)-β-glucan. Because the water-extractable arabinoxylan should have been solubilized during the

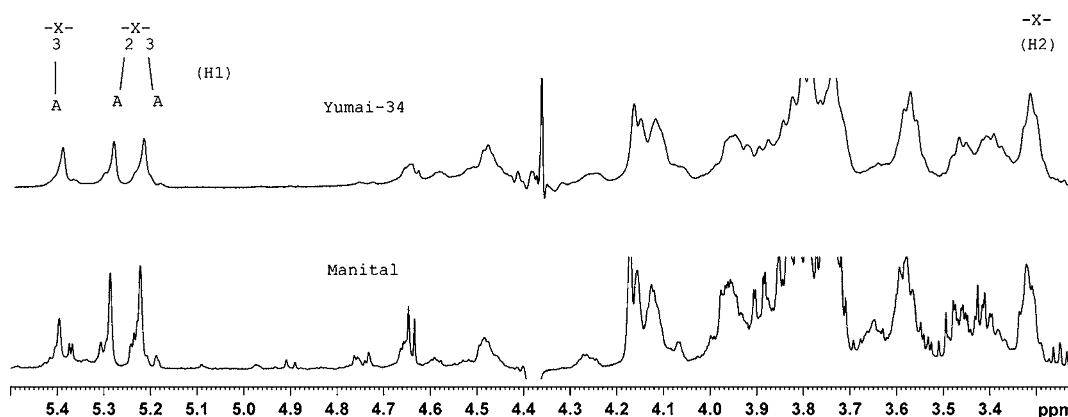

Figure 7. ^{1}H NMR spectra for the cell walls of two wheat cultivars following extraction from a flour dough.

formation of the dough and, therefore, present in the dough liquor phase,[19] the arabinoxylan in the cell wall fragments should correspond to the water-unextractable fraction.

Variation in Cell Wall Fragments from Different Wheat Cultivars. Cell wall fragments were then compared from five bread wheat cultivars with differing levels of arabinose substitution in their cell wall water-unextractable arabinoxylan.[9] The amounts of total and water-extractable arabinoxylan (Table 1) in Yumai 34 were far higher than in the other cultivars,[8] a difference that is highly heritable.[39] The cell wall fractions were characterized using infrared spectroscopic imaging, with the shoulder height at 1075 cm^{-1} in the FTIR spectrum (which relates to the extent of substitution with arabinose) being calculated as a percentage of the total height of the main carbohydrate (arabinoxylan) peak (at 1047 cm^{-1}) (Figure 4). In addition, the proportions of spectral pixels (not including the spectra depicting holes or starch) for each cultivar and each substitution level (low, <60% of peak height; medium−low, 60−65%; medium, 65−70%; medium−high, 70−75%; and highly substituted arabinoxylan, >75%) were also calculated (Table 1 and Figure 5). The mean shoulder height was greatest for cv. Manital and San Pastore, which indicated that the arabinoxylan in the cell wall fragments was more highly substituted with arabinose. In contrast, the mean shoulder height was lower for cv. Hereward, which comprised a mixture of high and low substitution arabinoxylan. The spread of the distribution was narrowest for cv. Claire and Yumai 34, showing that the arabinoxylan in these cultivars varied less in the degree of substitution, although cv. Claire had a greater proportion of low substituted arabinoxylan than Yumai 34. The cell wall fragments of Yumai 34 differed from those from the other lines in having a very high proportion of medium substituted arabinoxylan.

FTIR spectra, obtained using the imaging detector, had identical spectral features for endosperm cell walls of cv. Hereward extracted from either (A) dough or (B) using cell wall only thin sections of grain[9] (Figure 6). The relative height of the shoulder at 1075 cm^{-1} was an indication of the levels of arabinose substitution; the higher the shoulder, the higher the level of substitution,[9,14,15] confirming that the preparation procedure resulted only in the loss of highly soluble arabinoxylan in the dough liquor.[19]

The cv's Manital and Yumai 34 previously showed the greatest differences in their arabinose/xylose ratio, the ratio of mono- to disubstituted Xyl*p* residues (M/D), and relative proportions of xylose residues (un-, mono-, and disubstituted).[9] Cell wall fragments from doughs of these cultivars were therefore studied in more detail using ^{1}H NMR spectroscopy (Figure 7).[9,15,21] The values determined from the spectra for the ratio of A/X (Table 2) are consistent with those determined previously.[9]

Table 2. Arabinose/Xylose Ratio (A/X), Percentages of Unsubstituted (U), Monosubstituted (M), and Disubstituted (D) Xyl*p*, and Ratio of Mono- to Disubstituted Xyl*p* Residues (M/D) Determined during ^{1}H NMR Experiments

	arabinoxylan ratios		xylose residues		
cultivar	A/X	M/D	U	M	D
Yumai 34	0.49	0.88	0.68	0.18	0.17
Manital	0.63	0.55	0.62	0.13	0.25

The proportions of mono-, di-, and unsubstituted xylose residues were also similar in that the proportion of unsubstituted residues was around 3 times higher than that for mono- or disubstituted residues for both Manital and Yumai 34. The ratio of mono- to disubstituted xylose residues differed between the two procedures because of slight differences in the proportions of monosubstituted arabinoxylan residues, which were slightly lower in Manital in the dough samples, and disubstituted residues, which were slightly higher for both cultivars in the dough samples (Table 2). These differences may have been due to the use of grain and flour from very different sources.

■ DISCUSSION

Analyses of cell wall fragments prepared from wheat doughs showed that the variation in the composition and structure of the endosperm cell wall arabinoxylan between bread wheat cultivars observed from grain sections was retained after dough mixing. The dough preparations used in this study were based on simplified recipes and did not include the enzyme improvers, such as xylanases, which are frequently used to enhance the baking performance of bread wheats. Such enzymes may modify the arabinoxylans in the cell wall fragments and could potentially facilitate release of fragments. The method used here was comparable to the Chorleywood bread process and was therefore too short for the actions of any endogenous enzymes within the flour to have any effect. However, other bread-making processes, with longer fermentation times and the addition of lactic acid fermentation in sour doughs, could maybe modify the cell wall components. The methods described here are sensitive enough to be applied to these as well.

A much higher amount of cell wall material was recovered from the cultivar Yumai 34, which was previously shown to have

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very high levels of both water-extractable and total arabinoxylan by analysis of flours,[8] and this comprised a higher proportion of medium substituted arabinoxylan compared to the other cultivars. Previous studies have shown that the contents of total and water-extractable arabinoxylan in white flour (i.e., in starchy endosperm cell walls) are highly heritable, with genotype accounting for about 70 and 60% of the variation in these fractions, respectively.[40] This high heritability indicates that it will be possible to develop new types of wheat with contents and compositions of arabinoxylan that are optimized for different end use requirements, such as improving the health-related and technological functionality of wheat by, for example, increasing the water-extractable arabinoxylan content and, hence, the viscosity of grain products for human nutrition and reducing it in grain for animal feed. Such approaches will need to be taken forward with due consideration of the effects of the baking processing on the cell wall composition to ensure any potential health benefits resulting from increasing the contents of total and water-extractable arabinoxylan by breeding are realized in foods reaching the consumer.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

FTIR spectra for β-glucan, arabinoxylan, and six mixtures with varying ratios of both (arabinoxylan/β-glucan). This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

ATR, attenuated total reflectance; A/X, arabinose/xylose; TSP, sodium 3-(trimethylsilyl)-propionate-d_4; Xyl*p*, xylopyranosyl

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