

RESEARCH PAPER

Potato tuber pectin structure is influenced by pectin methyl esterase activity and impacts on cooked potato texture

Heather A. Ross¹, Kathryn M. Wright², Gordon J. McDougall¹, Alison G. Roberts², Sean N. Chapman², Wayne L. Morris¹, Robert D. Hancock¹, Derek Stewart¹, Gregory A. Tucker³, Euan K. James⁴ and Mark A. Taylor^{1,*}

¹ Plant Products and Food Quality Programme, Scottish Crop Research Institute, Dundee DD2 5DA, UK

² Cell Biology and Imaging, Plant Pathology Programme, Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, UK

³ School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, Leicestershire LE12 5RD, UK

⁴ College of Life Sciences, University of Dundee, Dundee DD1 5EH, UK

* To whom correspondence should be addressed: E-mail: Mark.Taylor@scri.ac.uk

Received 15 June 2010; Revised 18 August 2010; Accepted 19 August 2010

Abstract

Although cooked potato tuber texture is an important trait that influences consumer preference, a detailed understanding of tuber textural properties at the molecular level is lacking. Previous work has identified tuber pectin methyl esterase activity (PME) as a potential factor impacting on textural properties. In this study, tuber PME isoform and gene expression profiles have been determined in potato germplasm with differing textural properties as assessed using an amended wedge fracture method and a sloughing assay, revealing major differences between the potato types. Differences in pectin structure between potato types with different textural properties were revealed using monoclonal antibodies specific for different pectic epitopes. Chemical analysis of tuber pectin clearly demonstrated that, in tubers containing a higher level of total PME activity, there was a reduced degree of methylation of cell wall pectin and consistently higher peak force and work done values during the fracture of cooked tuber samples, demonstrating the link between PME activity, the degree of methylation of cell wall pectin, and cooked tuber textural properties.

Key words: Cell wall, pectin, pectin methyl esterase, potato, texture, tuber.

Introduction

Potato tuber texture is a key quality determinant of cooked potato and a major trait that influences consumer preference (McGregor, 2007). Tuber texture is also a key issue in potato processing and is known to be affected by pre-processing procedures such as blanching and peeling, and by storage (Shomer and Kaaber, 2006; Thybo *et al.*, 2006). Factors that may impact on cooked potato texture include starch content and distribution within the tuber, starch swelling pressure, cell size, cell-wall structure and composition, and the breakdown of the cell wall middle lamella during cooking (reviewed in Taylor *et al.*, 2007). The relative importance of the different factors involved is not yet clear. Several studies have described potato germplasm

that produces tubers with markedly different textural properties (Van Marle *et al.*, 1994; Ducreux *et al.*, 2008; Ross *et al.*, 2010). In particular, members of the *Solanum tuberosum* group Phureja (Phureja) have been identified which exhibit a boiled tuber texture described as extremely floury or crumbly (De Maine *et al.*, 1993, 1998). The cooking time of Phureja tubers is generally in the order of half that taken for typical *Solanum tuberosum* group Tuberosum (Tuberosum) tubers at the same developmental stage (Ducreux *et al.*, 2008; Ross *et al.*, 2010).

Mechanical methods have been developed for the quantitative assessment of textural properties. A wedge fracture test was first developed by Vincent *et al.* (1991) to provide

a method that reflects the sensory experience of the consumer during consumption. Good correlations between the wedge fracture and panel sensory tests for a range of different food types were demonstrated. More recently, the method has been developed to assess cooked potato tuber texture (Ross *et al.*, 2010) and it was demonstrated that tubers from the Phureja group, when cooked (by steaming or boiling) gave significantly lower peak force and work done values during fracture than typically observed for those from Tuberosum. Apart from the stem end of the tuber, which is enriched in vascular tissues, there was little variation in tuber textural properties throughout the rest of the tuber. Interestingly, the dry matter content of the pith region of the tuber was 25% lower than in the perimedullary region and yet the measured textural properties were very similar, suggesting that dry matter content did not have a great influence on tuber texture, at least in the potato lines assessed by this method. Other textural properties such as the degree of sloughing, defined as the flaking and disintegration of the outer layers of potato tubers cooked in water, also show considerable variation between potato cultivars (Hejlova and Blahovec, 2008).

A recently developed 44 000-element potato microarray was used to identify tuber gene expression profiles that correspond to differences between Phureja and Tuberosum germplasm differentiated in a range of traits including tuber textural properties (Ducreux *et al.*, 2008). Based on differential expression patterns between Phureja and Tuberosum tubers, several genes related to cell wall metabolism are candidates that may account for tuber textural differences. The most clear-cut example is a gene encoding pectin methyl esterase (*PME*, MICRO.4403.C2_728). Orthologous *PME* genes have been shown to impact on the texture of fruit from many species (reviewed in Fischer and Bennett, 1991). As pectin is a major component of the cell wall and the middle lamella, its structure is likely to be an important factor in texture in potato tubers as well as other plant tissues (Fischer and Bennett, 1991). The pectin molecule consists of a backbone of α -(1-4)-D-galacturonic acid and contains some regions with alternating L-rhamnose and D-galacturonic acid (Voragen *et al.*, 2001). Twenty to eighty per cent of the rhamnose units in the backbone can be branched, with side chains composed of galactose, arabinose, and small amounts of fucose and mannose. Some of the galacturonic acids in pectin are methyl esterified or acetylated. Non-methylated D-galacturonic acid sequences are sites for cross-linking polymeric chains through the site-specific interaction with Ca^{2+} (Pilnik and Voragen, 1991). The content of unesterified uronic acid residues can be increased by the action of *PME*. The binding of pectin chains has a co-operative nature and an increase in Ca^{2+} binding efficiency contributes to higher tissue firmness (Andersson *et al.*, 1994). The much higher (*c.* 10-fold) expression level of the *PEST1* gene and total *PME* activity in Tuberosum tubers may underpin changes in pectin structure and contribute to the differences in textural properties compared with Phureja (Ross *et al.*, 2010). In all plants studied to date, several *PME* genes are expressed

(reviewed in Pelloux *et al.*, 2007). For example, tomato fruit contains at least three isoforms of *PME*, which have been termed PE1, PE2, and PE3 (Tucker *et al.*, 1982), while at least five isoforms have been detected in leaf tissue (Gaffe *et al.*, 1994). Detailed analysis has identified differences in the temporal and spatial patterns of expression of the tomato *PME* genes. Different functions for the isoforms are implied by the results of silencing experiments. For example, *PE1* down-regulation leads to an increase in the rate of fruit softening (Phan *et al.*, 2007) whereas *PE2* down-regulation has no appreciable effect in this regard (Hall *et al.*, 1994).

In view of the reported differences in texture between Phureja and Tuberosum, an objective of this study was to investigate the activity profile of *PME* isoforms in tuber extracts from the Phureja and Tuberosum types. A further objective was to associate the changes in *PME* expression and activity with differences in pectin structure, revealed both biochemically and by the use of monoclonal antibodies specific for different pectic epitopes.

Materials and methods

Plant material

Commercially-available fresh market potato cultivars examined in this study (Phureja cultivars Mayan Gold and Inca Sun and Tuberosum cultivars Desiree, Montrose, and Pentland Dell) were grown in field trials during 2007 at Gourdie Farm, Dundee, UK (56°29' N 3°3' W) using standard agronomic practices. The trial design was a randomized complete block (three blocks) with plots consisting of 20 plants. Tubers for each cultivar were harvested at maturity for each plot within the three blocks. Representative-sized tubers were selected and formed the basis for all the analysis described in this paper.

Tuber texture measurements

All tests were carried out on a QTS 25 Texture Analyser (Brookfield Engineering, Harlow, UK). The potato samples were boiled for 10 min and the wedge fracture test (as outlined by Vincent *et al.*, 1991) was used as described by Ross *et al.* (2010). Briefly, using an acrylic wedge descending through the cooked tuber cube at 5 mm min⁻¹, two measurements were recorded for each sample; work done is the energy required to penetrate to 10 mm while peak force is the maximum force required for the wedge initially to cut and then force the tissue apart and propagate a crack in the cube ahead of the wedge.

Tuber PME isoform analysis

Tubers were extracted in 2 vols ice-cold 20 mM potassium phosphate buffer (pH 7.0) containing 1 mM PMSF, 1 M NaCl, and 10 mM Na₂SO₃ in a cooled Waring Blender. The extracts were centrifuged at 4 °C at 20 000 g for 30 min and the volume of the removed supernatant was measured. Protein from the supernatant fractions was precipitated by the addition of (NH₄)₂SO₄ to 80% saturation at 4 °C and resuspended in 10 mM TRIS-HCl (pH 7.5). The protein extracts were then dialysed overnight against 10 mM TRIS-HCl (pH 7.5) (2 × 4.0 l) prior to column separation of the isoforms. The separation of the *PME* isoforms was carried out using Affi-Gel Heparin Gel (Bio-Rad, Hemel Hempstead, UK) affinity chromatography (20 ml column volume) similar to that described by Phan *et al.* (2007). A HPLC 432 UV/VIS detector (Kontron, Chichester, UK) was used along with a Kontron HPLC

pump 420 and HPLC gradient former 425. The column was maintained at 4 °C using a cooled water bath pumped to a sleeve surrounding the column. The cooled column was equilibrated with buffer A (10 mM TRIS-HCl, 10 mM NaCl, pH 7.5). Prior to loading, the dialysed protein extracts were centrifuged for 5 min at 16 000 g, and 2 ml samples were applied in buffer A. The column was eluted at 1 ml min⁻¹ with buffer A for 20 min and then a linear gradient was applied to 25% buffer B (10 mM TRIS-HCl, 1 M NaCl, pH 7.5) over 100 min. Finally, step gradients were applied to 50% and 100% buffer B. Following each step, elution was continued until all protein had been washed off the column as determined by eluent absorbance at 280 nm. Fractions (96×2 ml) were collected on a Frac-100 fraction collector (Pharmacia, Stockholm, Sweden) and kept on ice prior to assay. The assay procedure was as described by Phan *et al.* (2007) using 20 µl of each fraction in a 96-well microtitre plate assay. Assay buffer (200 µl) consisting of 0.5% citrus pectin, 2 mM TRIS-HCl, 150 mM NaCl, and 0.002% phenol red (pH 8.2) was added into each well. Absorbance at 405 nm was read on a Spectra-Max M5 plate reader (Molecular Devices, Sunnyvale, CA, USA).

Test for salt sensitivity

The PME activity of total protein samples prepared as described above, were assayed over a range of NaCl concentrations from 10 mM to 180 mM using the method described by Ross *et al.* (2010).

Sloughing test

Tubers from each of the potato lines harvested at maturity from the field were used for testing the degree of sloughing during boiling. The tubers were cut into seven 20 mm cubes to ensure all areas of the tuber were tested and the group of seven cubes weighed prior to boiling. The cubes were placed in a wire basket in 1.0 l of vigorously boiling water in a pan. A pretest of cooking times for each of the lines was carried out by subjective assessment and this showed that the Phureja lines were adequately cooked after 5–6 min, whereas the Tuberosum lines required 14 min. Consequently, Inca Sun and Mayan Gold were tested over 4, 6, 8, and 10 min and Desirée and Pentland Dell over 10, 14, 16, and 18 min. At each of the time points, the cubes in the wire baskets were removed and the boiling water containing any sloughed material was poured through double, preweighed coffee filters. Four tubers of each line for each time period were used. The filters were placed in polythene bags and frozen at –20 °C along with blank control filters through which 1.0, l of boiled water had been passed. The opened polythene bags containing the filters were then lyophilized (Christ Gamma 1-16 LSC, Shrewsbury, UK) and reweighed to obtain weight gain due to freeze-dried sloughed material.

To show the location of the sloughing across the tuber, cubes of tuber were cooked and photographed. Pentland Dell, Montrose, Inca Sun, and Mayan Gold tubers were cut to produce a 20 cm wide ring of tissue across the centre of the tuber. This was further dissected to produce 20 cm ‘cubes’ of tuber that incorporated the epidermis. Tissue samples were cooked for 10 min in boiling water, cooled rapidly in chilled water, and photographed using a digital SLR camera (EOS 350D; Canon, Woodhatch, UK).

Quantitative PCR using the universal probe library

Reverse transcription of 10 µg of RNA was performed using Invitrogen Superscript™ II reverse transcriptase (www.invitrogen.com) using random hexamers as primer. cDNA (25 ng) was used as template for real-time PCR using the Universal Probe Library System (<https://www.roche-applied-science.com/sis/rtqpcr/upl/index.jsp>). Reactions were performed in 25 µl containing 1× FastStart TaqMan® Probe Master (supplemented with ROX reference dye). Gene-specific primers and probe were used at a concentration of 0.2 µM and 0.1 µM, respectively. Thermal cycling conditions were: 10 min denaturation at 95 °C followed by

40 cycles (15 s at 94 °C, 60 s at 60 °C). The reactions were repeated in triplicate with independent cDNAs. Relative expression levels were calculated and the primers validated using the $\Delta\Delta C_t$ method (Livak and Schmittgen, 2001) using data obtained with the elongation factor-1 alpha specific primers as an internal reference control. In the case where relative efficiencies of the target and reference amplicons were not within recommended limits, an alternative method for calculating relative quantification was used (Pfaffl, 2001). Universal probe library (UPL) primer and probe sequences were as follows: EF1 alpha fwd, 5'-CTTGACGC-TCTTGACCAGATT-3', EF1 alpha rev, 5'-GAAGACGGAGG-GGTTTGTCT-3', UPL probe number 117 (5'-AGCCCAAG-3'); Micro.2764.c1 fwd, 5'-GGACTGAAACCAGATGTGACAG-3', Micro.2764.c1 rev, 5'-CGTCTCATAATCGCAACCAC-3', UPL probe number 66 (5'-GGCTGCTG-3'); Micro.2676.c2 fwd, 5'-GGAACTTACAAAGAAAATGTTGAG-3', Micro.2676.c2 rev, 5'-GCCATCACCCACAAGCATA-3', UPL probe number 143 (5'-AGAAGAAGA-3'); Pest1 fwd, 5'-TGGGTGAGTTC-GAGGATAG-3', Pest1 rev, 5'-TGCATTGCTTTGAT-GTCCT-3', UPL probe number 156 (5'-GCTGATGG-3'); Pest2 fwd, 5'-TGAGGCCCAAGCTTATACTCC-3', Pest2 rev, 5'-CCAGTGGAGCTCAACCAACT-3', UPL probe number 67 (5'-TGCTGGAG-3').

Tuber cell wall preparation

Cell walls were prepared from tuber samples collected within 1 week of harvest at full maturity. Cell walls were prepared from frozen tuber material largely following the method of Jardine *et al.* (2002). Briefly, 100 g of tubers were chopped with knives, whilst still frozen, and placed into 500 ml ice-cold mixed cation buffer (MCB) consisting of 10 mM sodium acetate, 3 mM KCl, 2 mM MgCl₂, and 1 mM CaCl₂, pH 6.5 containing 0.5 ml Triton X-100 then homogenized using an Ultra-Turrax (IKA, Staufen, Germany; 10×1 min bursts; 3/4 power). All extractions were carried out in triplicate using tubers from the three separate field plots for each tuber type. Insoluble material was collected by sieving on 53 µm nylon mesh and washed with 500 ml MCB to remove Triton X-100. All of the wash was collected as Wash A. The crude cell wall material (CWM) was washed with 500 ml of 50% acetone/ultra pure water (UPW) then recovered by filtration on a grade 2 glass sinter and weighed.

The CWM was deproteinized by extraction in 4 vols of 80% (w/v) saturated phenol for 30 min at room temperature with stirring at 80 rpm. After extraction, the CWM was recovered by filtration on a glass sinter then washed with 500 ml ice-cold MCB.

After frozen storage, the CWM was de-starched. The material was suspended in MCB preheated to 80 °C and incubated at 80 °C in a water bath for 45 min to gelatinize the starch. The material was then cooled rapidly to 20 °C by placing in cold water. Starch digestion was initiated by adding 1 g of pancreatic amylase powder (Sigma type VI-B) and 25 µl of pullulanase suspension (Sigma P-5420) and incubating overnight at 25 °C with rotary shaking at 80 rpm.

Starch digestion was monitored by removing small aliquots of the insoluble material and staining with iodine. Samples were satisfactorily de-starched following the addition of another 0.5 g of amylase and further incubation for 8 h. The de-starched CWM was recovered on a glass sinter and the filtrate collected as Wash B. The CWM was washed with excess MCB then dried by washing in 50% acetone then from water. Samples were frozen then lyophilized.

Cell wall recovery ranged from 2–5 g dry weight per 100 g tuber material and the residual starch content was below 5% (Karkalas, 1985). Samples of washes A and B were centrifuged at 15 000 g for 10 min at 4 °C to remove insoluble starch. Soluble pectins in the wash A and wash B supernatants were recovered by precipitation with 3 vols of ice-cold ethanol and centrifugation at 15 000 g for 15 min at 4 °C. The pelleted polysaccharides were re-suspended in

UPW and the galacturonic content assessed (Filisetti-Cozzi and Carpita, 1991). The wash A samples contained very little pectin whereas substantial amounts were present in Wash B.

Tuber cell wall analysis

The degree of pectin methylation was determined for the cell wall samples and the solubilized pectins. After saponification, released methanol was measured using the alcohol oxidase/Purpald method as described by Anthon and Barrett (2004). Fourier transform infra-red (FT-IR) spectroscopy was performed using the DRIFT method on a Bruker IFS-66 spectrometer (Bruker UK Ltd, Coventry, UK) as described previously (Stewart *et al.*, 1997). Triplicate spectra were obtained for each sample and averaged, then normalized using the OPUS 5.5 software. Spectra were also obtained from commercially-available pectin standards with different degrees of methylation to direct assignment of spectral peaks.

Tissue preparation for light microscopy immunocytochemistry

Wedges of potato tuber tissue 2–3 mm thick were cut from mature tubers of Phureja cultivar Inca Sun and Tuberosum cultivar Pentland Dell. Using a TP1020 tissue processor (Leica, Milton Keynes, UK), the samples were fixed in 4% paraformaldehyde in PEM buffer (0.1 M PIPES pH 6.95, 1 mM EGTA, 1 mM MgSO₄) for 6 h, washed in 0.85% NaCl (30 min), dehydrated in an ethanol series (70% for 1 h, 80% for 1.5 h, 90% for 2 h, 3 × 100% for 1 h, 1.5 h, and 2 h) and xylene (100% for 1 h and 1.5 h) before infiltration with Paramat extra wax (VWR-Gurr, Lutterworth, UK) (2 h and 4 h) at 65 °C. Following embedding, 10 µm thick sections were cut using a RM2265 rotary microtome (Leica, Milton Keynes, UK) and mounted on Polysine slides (VWR, Lutterworth, UK). The sections were dewaxed in Histo-Clear II (National Diagnostics, Hessele, UK) prior to rehydration in an ethanol series (100%, 95%, 85%, 70%, 40%, 0%) made in the appropriate buffer (see below).

Antibody labelling for light microscopy

Sections to be incubated with monoclonal antibodies (mAbs) JIM5, JIM6, LM5, and LM6 were rehydrated in PBS buffer (pH 7.4) before blocking with 1% BSA in PBS. Sections were incubated overnight at 4 °C in this blocking buffer including mAbs diluted 1:20, rinsed in blocking buffer and incubated in Alexa 488 nm goat anti-rat (Invitrogen, Paisley, UK) diluted 1:100 in blocking buffer for 3 h at 20 °C. Sections to be incubated with mAb 2F4 were rehydrated in TCaS buffer (20 mM TRIS-HCl, pH 8.2, 1 mM CaCl₂, 150 mM NaCl) and blocked in 1% BSA in TCaS. The 2F4 antibody was diluted 1:75 in this blocking buffer before incubating as described above and the sections were then treated with Alexa 488 nm goat anti-mouse (Invitrogen) diluted 1:100 in blocking buffer. The sections were rinsed in blocking buffer and the appropriate individual buffer prior to staining with 0.25% Alcian blue in 3% acetic acid for 10 min and mounting in Fluoromount G (Southern Biotech, Birmingham, Alabama, USA).

Imaging

Confocal sections were collected using a Leica SP2 confocal laser scanning microscope (Leica Microsystems, Heidelberg GmbH, Germany) fitted with a HC PL Fluotar 10.0 × 0.30 dry objective, with 488 nm excitation and emission collected between 500 nm and 530 nm. The laser intensity and gain settings were maintained at the same levels for all samples. Multiple z-stacked images were collected across the whole of the tissue using the tile-scan function and motorized stage of the microscope, maximum intensity projections were then produced from the z-stacks and stitched together using Leica LCS software to create maps of the whole tissue with microscopic resolution.

Electron microscopy

Pieces of tuber tissue were fixed, dehydrated, and embedded in LR White resin as described by Bush and McCann (1999). Ultrathin sections were collected on pyroxylin-coated nickel grids. Detection with antibody LM6 was performed as described by Bush and McCann (1999), except that grids were blocked on Goat Gold Conjugate Blocking Solution (Aurion, Wageningen, Netherlands) and LM6 was diluted in 0.1% BSA-c (Aurion) in phosphate-buffered saline (PBS). Probing with antibody 2F4 was performed as described by Bush and McCann (1999), except a dilution of 1:50, instead of 1:300, in 1% (w/v) skimmed milk in TCaS buffer was used. Following incubations with primary antibodies, grids were washed five times with the same buffer used for dilution of the primary antibody. Grids were then incubated with 10 nm gold conjugates (British BioCell International, Cardiff, UK) diluted 1:50 in the same buffer as used for the primary antibody. Subsequently, grids were washed five times on the buffers used for antibody dilution, three times on PBS or TCaS buffers, and twice on distilled water. Ultrathin sections were counterstained with uranyl acetate and lead citrate and examined in a JEM1400 (Jeol UK Ltd, Welwyn Garden City, UK) transmission electron microscope.

Results

Tuber textural properties

Recently, the textural characteristics of potato tubers during the boiling process have been described, comparing tuber textural properties in three Phureja cultivars (Inca Sun, Mayan Gold, and breeding line DB333/16) with those from four table Tuberosum cultivars (Pentland Dell, Montrose, Maris Piper, and Desiree) (Ross *et al.*, 2010). Using a wedge fracture test, for an equivalent cooking time (10 min), it was demonstrated that the Phureja types required a substantially (3–5-fold) lower peak force to initiate fracture and required a lower total amount of work done to propagate a fracture of 10 mm. Similar differences in textural properties were measured in the Phureja and Tuberosum samples used in this study, demonstrating that this trait was stable year-to-year (data not shown). As a further measurement of the differences in textural properties between the Phureja and Tuberosum types, the degree of sloughing (Hejlova and Blahovec, 2008) from cubes of potato during boiling was measured (Table 1). Comparing values after 10 min of boiling, the Phureja samples had sloughed appreciably whereas the amount of material sloughed from the Tuberosum samples was at least 8-fold lower. Tuberosum tubers (Fig. 1A, B) were largely intact after 10 min cooking whereas Phureja tubers (Fig. 1C, D) showed marked separation of the tissue in the region of the vascular ring and cortex resulting in sloughing of material from the cortical/epidermal layers.

Tuber pectin methyl esterase activity

The total pectin methyl esterase activity was significantly (3–5-fold) lower in Phureja than Tuberosum tubers (Fig. 2A). PME isoform profiles were investigated in tubers from the Phureja (Inca Sun and Mayan Gold) and Tuberosum (Pentland Dell and Desiree) cultivars using heparin

Table 1. Weight (g) of insoluble material sloughed from *Solanum tuberosum* and *Solanum phureja* lines during boiling

Values are represented as mean \pm SE ($n=4$). Initial weight of potato material was 58.43 ± 0.02 g.

Boiling time (min)	Solanum phureja		Solanum tuberosum	
	Inca Sun	Mayan Gold	Desirée	Pentland Dell
4	0.29 \pm 0.18	0.11 \pm 0.04		
6	0.60 \pm 0.18	0.61 \pm 0.23		
8	2.05 \pm 0.33	0.45 \pm 0.10		
10	4.11 \pm 1.03	0.76 \pm 0.30	0.09 \pm 0.03	0.08 \pm 0.01
14			0.08 \pm 0.04	0.04 \pm 0.00
16			0.06 \pm 0.01	0.04 \pm 0.01
18			0.08 \pm 0.02	0.06 \pm 0.02

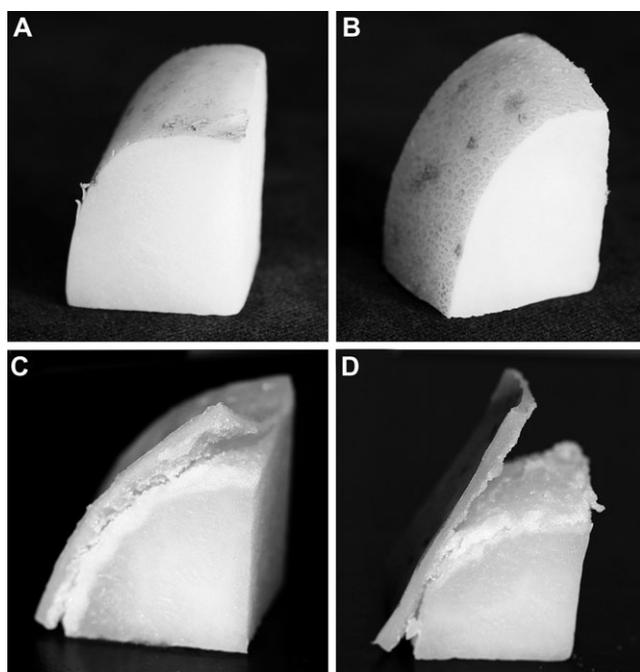


Fig. 1. Effect of cooking on sloughing in Tuberosum and Phureja tubers. Tuberosum tubers Pentland Dell (A) and Montrose (B) show no evidence of tissue sloughing after 10 min cooking while Phureja tubers Mayan Gold (C) and Inca Sun (D), cooked for the same time, show marked loosening of the tissue in the region of the vascular ring and cortex and the cortical/epidermal region sloughs off as the tissue cooks. Each cube is 2 cm across the base.

column chromatography. Profiles from Pentland Dell and Inca Sun are shown (Fig. 2B (i) and (ii), respectively), but similar profiles were obtained for extracts from Mayan Gold and Desirée. Although three peaks of activity could be resolved in samples from all four cultivars, much greater activity was found in the Tuberosum samples. Therefore, Tuberosum profiles were obtained after 100 min but 600 min for the Phureja profiles. The Phureja and Tuberosum profiles were consistently qualitatively similar and further analysis, perhaps using transgenic approaches

to manipulating individual isoforms, would be required to confirm whether the peaks represent the same isoforms. The salt sensitivity of the three resolved isoforms was determined as both salt-sensitive and insensitive isoforms have been identified in tomato (Phan *et al.*, 2007). In contrast to tomato fruit, no salt-insensitive PME activity could be detected. Activity associated with peak 1 required the lowest salt concentration for maximal activity (40 mM), whilst that in peaks 2 and 3 required 80 mM for maximal activity (data not shown).

PME gene expression

Previous microarray analysis of Phureja and Tuberosum tuber gene expression has identified that five genes, annotated as encoding PME, are expressed in potato tubers (Ducreux *et al.*, 2008). Quantitative RT-PCR analysis was undertaken to investigate the expression levels of these genes in the samples used for this study (Fig. 2C). Genes encoding two PME isoforms were expressed at significantly higher levels in Tuberosum than Phureja tubers (*MICRO.2676* and *PEST1*) whereas two genes *MICRO.2764* and *PEST2* were expressed at higher levels in Phureja tubers than in Tuberosum. It was not possible to associate particular peaks in the isoform profiles with the expression of specific PME genes although in view of the greatly elevated level of PME activity in Tuberosum compared with Phureja tubers, it would appear that the isoforms encoding *MICRO.2764* and *PEST.2* contribute little to total PME activity.

Cell wall composition

The isolated tuber cell wall preparations gave largely similar FT-IR spectra with subtle differences. The main difference between the Phureja and Tuberosum spectra was seen in the region around 1630 cm^{-1} (Fig. 3A, arrow). Absorbance in this region has been associated with differences in the abundance of acid groups within pectins (McCann *et al.*, 1997) and has been assigned to C–O stretching frequencies in acid groups. Indeed, FT-IR spectra of standard pectins with varying degrees of methylation differ in the ratio of this peak to the peak at $\sim 1740\text{ cm}^{-1}$, which is associated with pectate acid groups acylated with methyl groups (results not shown). The monosaccharide composition of the cell walls of Phureja and Tuberosum types was similar with no significant differences in monosaccharide composition (data not shown). The degree of cell wall methylation differed between the Phureja and Tuberosum types (Fig. 3B) with the Phureja cell walls consistently releasing 1.5-fold higher amounts of methanol upon saponification. This confirms the suggestion from the FT-IR spectral data that the Tuberosum walls were less methylated.

The release of soluble pectins during de-starching of the tuber cell walls (wash B), where the material is maintained at $>70\text{ }^{\circ}\text{C}$ to ensure gelatinization, is analogous to cooking. There was a marked difference in the amount of solubilized pectin between the Phureja and the Tuberosum lines

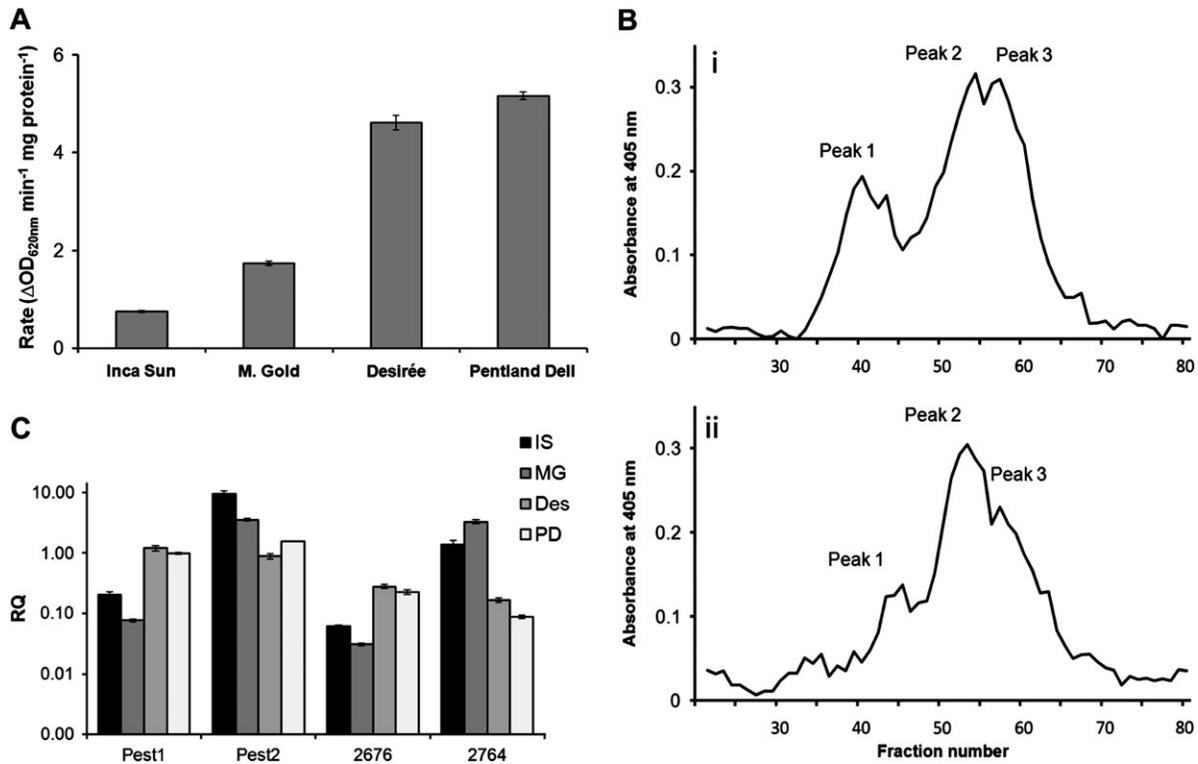


Fig. 2. Analysis of PME activity, isoforms and expression profiles. (A) Total pectin methyltransferase activity for the two Phureja and two Tuberosum lines. Values, expressed as $\Delta OD_{620nm} \text{ min}^{-1} \text{ mg}^{-1} \text{ protein}$, are presented as mean \pm SE ($n=3$). (B) Isoform profiles of PME activity. (i) Pentland Dell measured after 100 min and (ii) Inca Sun measured after 600 min. (C) Expression profiles of *PME* genes in potato tubers as determined by quantitative RT-PCR. Cultivars analysed were Pentland Dell (PD), Desiree (Des), Mayan Gold (MG), and Inca Sun (IS). RQ, relative quantitation. Values are presented as mean \pm SE ($n=3$).

(Table 2). Note that the solubilized pectin fraction from Desiree was not retained but another Tuberosum type (variety Montrose) gave similarly low levels of solubilized pectin. The methanol content of the solubilized pectin fractions was also higher in the Phureja than Tuberosum varieties (Table 2). This suggests that the Phureja types release a higher proportion of easily solubilizable pectin than the Tuberosum types, which could be related to their textural differences.

Epitope mapping of Phureja and Tuberosum tuber types

To study the spatial distribution of pectic polysaccharides *in situ*, indirect immunofluorescence labelling was performed on longitudinal sections of tuber tissue using a range of well-characterized monoclonal antibodies (Bush *et al.*, 2001). JIM5 and JIM7 recognize homogalacturonan (HG), LM5 recognizes (1-4)- β -D-galactan, LM6 recognizes (1-5)- α -L-arabinan, and 2F4 binds to homogalacturonans with degrees of methyl-esterification (DM) up to 40%. All cell walls of the cortex, perimedulla, and vascular bundles were labelled equally strongly along their length with mAb LM6. However, there was stronger labelling of the cortex for the Phureja cultivars (Fig. 4A, and insert) than for the Tuberosum cultivars (Fig. 4B, and insert). At the ultrastructural level, probing of cortical tissue sections from Phureja

and Tuberosum cultivars with mAb LM6 gave gold labelling across the entire width of parenchymal cell walls when imaged using an electron microscope, but with lower abundance in the region of the middle lamella (Fig. 5A). In accordance with the light microscopic analysis, denser gold labelling seen by electron microscopy was obtained for the Phureja tissue than for the Tuberosum tissue. The amount of labelling of the vascular tissue was similar to the surrounding cells in both cultivars.

There was no apparent difference between the Phureja and Tuberosum cultivars in the pattern of labelling with any of the other antibodies tested. Although all the cells were labelled with mAb 2F4, the walls of the cortex and, in particular, the corners were more densely labelled than the cells of the perimedulla (Fig. 4C, D, and inserts). The distribution of mAb 2F4 epitopes was investigated at the ultrastructural level through probing of Phureja and Tuberosum sections from pith and perimedulla tissue. The densest gold labelling was associated with electron opaque material occluding apoplastic spaces at the cell wall junctions with sparse gold labelling along the length of parenchymal cell walls. As reported previously (Bush and McCann, 1999), gold label was concentrated in cell walls at junctions and plasmodesmata (Fig. 5B). The abundance of 2F4 epitopes at cell wall junctions was assessed by counting gold particles in randomly imaged junctions, not including particles in occluded apoplastic spaces. Although labelling was found to

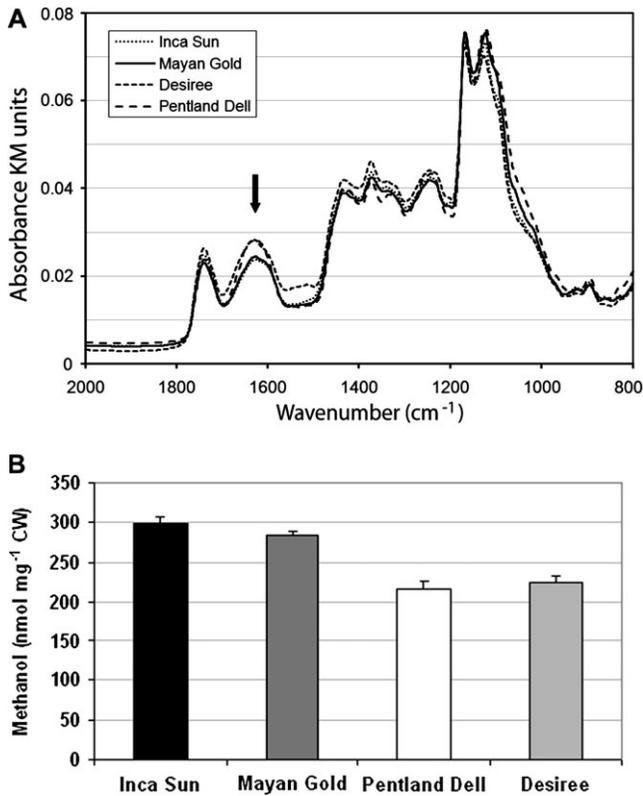


Fig. 3. Analysis of methylation status of isolated tuber cell walls. (A) FT-IR spectra of isolated cell walls from Tuberousum and Phureja tubers. Each spectrum is the average of three replicate scans of three replicate cell wall samples. The arrow indicates the main spectral difference between the Phureja and Tuberousum samples at $\sim 1630\text{ cm}^{-1}$. (B) Methanol content of isolated cell wall preparations from Tuberousum and Phureja tubers. Values are presented as mean \pm SE ($n=3$)

Table 2. Galacturonic acid and methanol content of solubilised pectin fractions isolated from potato tubers

Replicate samples ($n=4$) were extracted from each individual plot as described in the Materials and methods. Data are presented as mean \pm SE.

Sample	Total GalA content ($\mu\text{mol GalA g}^{-1}\text{ FW}$)	Total MeOH content ($\text{nmol MeOH g}^{-1}\text{ FW}$)
Inca Sun 1	291 \pm 13	625 \pm 15
Inca Sun 2	253 \pm 14	520 \pm 3
Inca Sun 3	253 \pm 13	575 \pm 25
Mayan Gold 1	188 \pm 4	383 \pm 16
Mayan Gold 2	219 \pm 7	524 \pm 23
Mayan Gold 3	285 \pm 8	587 \pm 15
Montrose 1	112 \pm 5	241 \pm 22
Montrose 2	107 \pm 6	215 \pm 4
Montrose 3	101 \pm 3	171 \pm 17
Pentland Dell 1	142 \pm 3	116 \pm 12
Pentland Dell 2	174 \pm 1	88 \pm 18
Pentland Dell 3	117 \pm 1	79 \pm 13

be higher in pith tissue than perimedulla tissue, no statistically significant differences were found between Phureja and Tuberousum cultivars (data not shown).

The labelling with mAb JIM5 was higher at the corners of the cell walls compared with the intervening walls and was more prominent in the cells of the cortex than in tissues of the perimedulla (Fig. 4E, F, and inserts). The amount of labelling of the vascular tissue was much higher than the surrounding cells resulting in the appearance of prominent vascular bundles.

The mAbs LM5 (Fig. 4G, H, and inserts) and JIM7 (data not shown) produced a uniform distribution of label, with all areas of the cell walls and all tissues showing a similar intensity of labelling. Light microscopical analysis of antibody distribution was also carried out on cooked tuber sections and showed similar results to the uncooked tissue. Again, there were no differences detected in the localization of each antibody between Phureja and Tuberousum apart from the increased labelling of the cortical tissue of Phureja tubers with LM6 antibody (data not shown).

Discussion

PME activity has been associated with the texture of cooked potato in several previous studies (Ng and Waldron, 1997; Jarvis, 1999; Ross *et al.*, 2010). However, direct evidence that links PME activity, cooked tuber textural properties, and pectin structure has, until now, been lacking. In this study, we have taken advantage of the diverse textural properties exhibited by Phureja potatoes in comparison with the Tuberousum types. Our previous comparison of the textural properties of the Phureja and Tuberousum types revealed a more rapid softening of the Phureja tuber type on boiling and that the total PME activity in Phureja tubers was 2–4-fold lower than could be extracted from the Tuberousum types (Ross *et al.*, 2010).

The mode of action of PME has been described in some detail. Some PME isoforms can remove methyl esters from pectin in a block-wise fashion (Limberg *et al.*, 2000). The demethylated pectin chains can then link via calcium interchelation into egg box structures described by Grant *et al.* (1973), which may lead to strengthening of the cell wall. Alternatively, demethylated pectin may be more susceptible to polygalacturonase-catalysed degradation, particularly pectin that is not demethylated in a blockwise fashion, but in a random pattern as catalysed by some plant PME isoforms (Willats *et al.*, 2001). Thus PME activity can either strengthen or weaken cell wall structure dependent on the pattern of demethylation and the environment in which it acts. In this study, the expression pattern of four *PME* genes was described, with only two exhibiting clear-cut elevated expression levels in the Tuberousum types. Although two *PME* genes were expressed at significantly higher levels in Phureja tubers than Tuberousum, only isoforms with greater activity in Tuberousum could be detected. Thus it would appear that the isoforms encoded by the Phureja up-regulated *PME* genes contribute little to overall PME

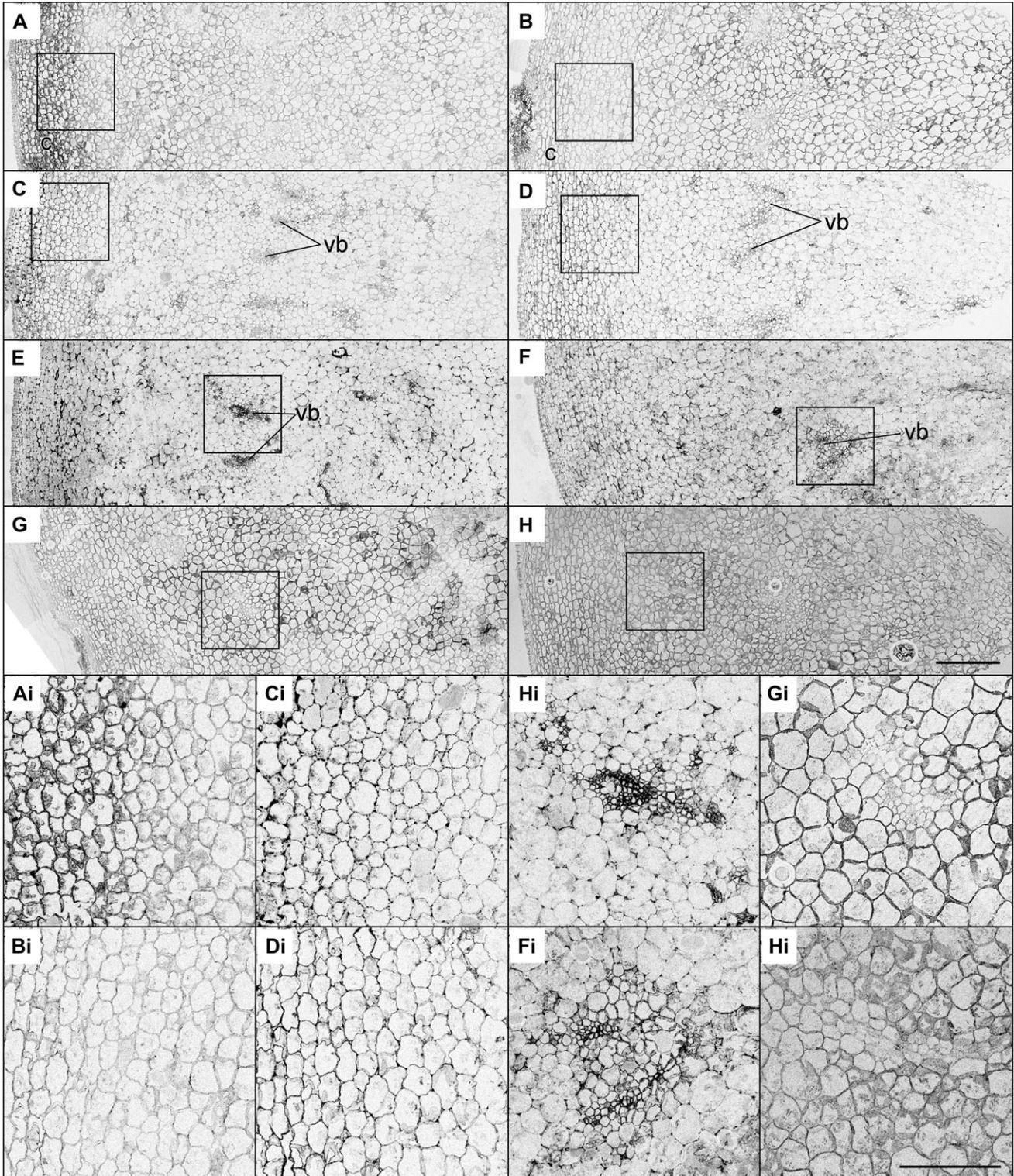


Fig. 4. Cell wall pectic epitope labelling using fluorescently-labelled antibodies. Transverse sections taken through the periderm, cortex (c), perimedulla, and vascular bundles (vb) of *Solanum tuberosum* group Phureja cv. Inca Sun (A, C, E, G) or group Tuberosum cv. Pentland Dell (B, D, F, H) showing preferential accumulation of LM6 in the cortical tissue of Inca Sun (A) but not Pentland Dell (B) and equal labelling along the length of all cell walls (inserts Ai and Bi), accumulation of 2F4 (C, D) particularly at the corners of the cortical cells (inserts Ci and Di) or JIM5 (E, F) at cell corners and within the vascular bundles (inserts Ei and Fi) with reduced labelling of perimedullary tissue, and the uniform distribution of LM5 (G, H) throughout all cell walls (inserts Gi and Hi) and tissue types (scale bar 1 mm for sections A–H, and 0.5 mm for sections Ai–Hi).

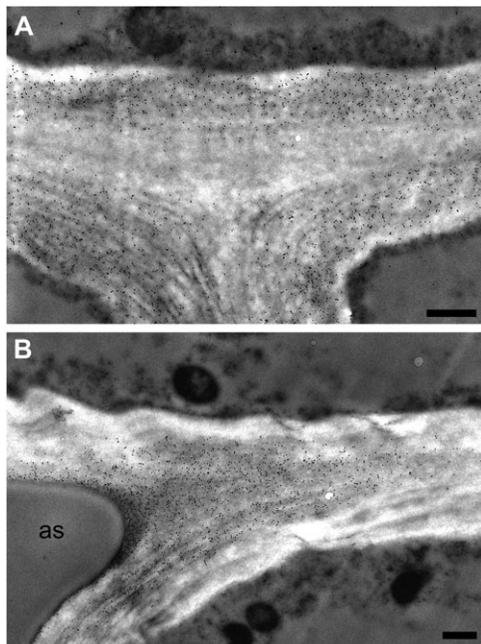


Fig. 5. Ultrastructural analysis of tuber cell walls using immunoelectron microscopy. (A) Cell wall junction from Inca Sun (Phureja) cortical tissue: antibody LM6 labels the width of the cell wall, but predominantly outside the region of the middle lamella. (B) Cell wall junction from Inca Sun pith tissue: antibody 2F4 labelling is concentrated at junctions in the region of the middle lamella and margins of apoplastic spaces (as). Scale bars=0.5 μm for (A) and (B).

activity. It is conceivable that these isoforms may have a cell wall weakening effect and an activity below that detectable in the assay system employed in this study. Further transgenic studies will be required to associate particular *PME* genes with peaks in *PME* activity resolved in the chromatogram of total *PME* activity (Fig. 2B).

Two independent approaches were used to investigate the level of pectin methylation in cell walls from the Tuberosum and Phureja types. Firstly, FT-IR analysis revealed differences consistent with a higher degree of methylation in cell walls from the Phureja type. Secondly, direct chemical analysis of pectin methylation clearly confirmed its elevation in the Phureja type cell walls. Thus the higher levels of pectin methylation consistently measured in the Phureja samples compared with those from Tuberosum are consistent with the hypothesis that pectin methylation depends on the total *PME* activity extracted from the sample. The question then arose as to whether the changes in pectin methylation were reflected in any alteration in either the distribution or levels of different pectin epitopes. Previously, the low methoxyl epitopes labelled by mAbs JIM5 and 2F4 have been shown to be generated in response to *PME* de-esterification (Knox *et al.*, 1990; Morvan *et al.*, 1998). In contrast to previous observations in which JIM5 labelled all cell walls of the cortex and perimedulla equally strongly across their entire width and length (Bush and McCann, 1999), in the present study this antibody showed

enhanced labelling at the cell corners along with prominent labelling of the vascular bundles. This was similar to the 2F4 ('egg-box' dimer) epitope, localized to the middle lamellar particularly at cell corners and the linings of intercellular spaces as has previously been observed (Bush *et al.*, 2001). However, at the light microscopy level, there was no obvious difference between the labelling of the Phureja compared to the Tuberosum tubers with either JIM5 or 2F4, or in the labelling by mAb JIM7 for HGA pectin with a high degree of methylation. Quantification of relatively small changes in localized pectin methylation may be confounded by sample to sample variability and therefore antibody labelling may be difficult to quantify. In addition, at the electron microscopic level, although the pattern of the distribution of the low esterified HG epitope (recognized by the 2F4 antibody) was similar to previous work (Bush *et al.*, 2001), consistent quantitative differences in the level of labelling between Phureja and Tuberosum sections were not evident.

The main difference in pectin epitope distribution observed between the Phureja and Tuberosum types was for the LM6 epitope, (1-5)- α -L-arabinan. The LM6 epitope was present at much higher levels in the cortex of Phureja tubers than in Tuberosum. The outer cell layers (including the periderm and cortex) of Phureja but not Tuberosum tubers were frequently detached during cooking (see Fig. 1) and the difference in arabinan distribution could be involved in this phenomenon. Arabinans are very flexible molecules in aqueous solution (Cros *et al.*, 2004) and they are also very mobile *in muro* (Renard and Jarvis, 1999). Indeed, it has been suggested that arabinans are not structural cell wall components but they act as plasticizers and water-binding agents in the wall (Renard and Jarvis, 1999). Whether the arabinan distribution has an effect on tuber texture or is involved in the sloughing of the outer tissue layers remains to be determined.

Although the focus of this study was on the changes in pectin and pectin methyl esterase in relation to cooked tuber texture, it is likely that the texture trait is also affected by other factors. Our previous study suggested that there was little correlation between starch content and textural properties as the textural properties of the low starch pith were similar to other regions of the tuber containing higher levels of starch (Ross *et al.*, 2010). In addition, there were no obvious correlations between cell size and shape and textural properties in the germplasm used in this study, although this has also been put forward as a factor in cooked tuber texture (Taylor *et al.*, 2007). However, other differences in cell wall structure may well have an influence on cooked tuber texture. A genetic analysis of a potato population that segregated for tuber textural properties identified a gene encoding a cell wall protein as underlying a QTL for tuber texture (Kloosterman *et al.*, 2010). The tyrosine and lysine-rich cell wall protein (StTLRP) was present at higher levels in individuals within the population which exhibited a firmer cooked tuber texture. Interestingly, microarray analysis of gene expression in Phureja and Tuberosum tubers revealed a significantly higher level of

expression of this gene in Tuberosum tubers compared with Phureja (Ducreux *et al.*, 2008). Further genetic and transgenic studies will be required to determine more precisely the combination of gene expression required to achieve different degrees of cooked potato texture, although the evidence presented in this study clearly identifies that modulation of pectin methylation through PME is one of the key mechanisms that impacts on the texture trait.

Acknowledgements

The financial support of the Scottish Government Rural and Environment Research and Analysis Directorate is gratefully acknowledged. We thank Susan Verrall for assistance in preparation of figures.

References

- Andersson A, Gekas V, Lind I, Oliveira F.** 1994. Effect of preheating on potato texture. *Critical Reviews in Food Science and Nutrition* **34**, 229–251.
- Anthon GA, Barrett DM.** 2004. Comparison of three colorimetric reagents in the determination of methanol with alcohol oxidase. Application to the assay of pectin methylesterase. *Journal of Agricultural and Food Chemistry* **52**, 3749–3753.
- Bush MS, McCann MC.** 1999. Pectic epitopes are differentially distributed in the cell walls of potato (*Solanum tuberosum*) tubers. *Physiologia Plantarum* **107**, 201–213.
- Bush MS, Marry M, Huxham IM, Jarvis MC, McCann MC.** 2001. Developmental regulation of pectic epitopes during potato tuberisation. *Planta* **213**, 869–880.
- Cros S, Imberty A, Bouchemal N, Dupenhoat CH, Perez S.** 1994. Modeling of arabinofuranose and arabinan. 2. NMR and conformational-analysis of arabinobiose and arabinan. *Biopolymers* **34**, 1433–1447.
- De Maine M, Carrol CP, Torrance CJW.** 1993. Culinary quality of tubers derived from *Solanum phureja* and *Solanum tuberosum* × *Solanum phureja* hybrids. *Journal of Agricultural Science* **120**, 213–217.
- De Maine M, Lees A, Bradshaw J.** 1998. Soft-rot resistance combined with other tuber characters in long day-adapted *Solanum phureja*. *Potato Research* **41**, 69–82.
- Ducreux LJM, Morris WL, Prosser IM, Morris JA, Beale MH, Wright F, Shepherd T, Bryan GJ, Hedley PE, Taylor MA.** 2008. Expression profiling of potato germplasm differentiated in quality traits leads to the identification of candidate flavor and texture genes. *Journal of Experimental Botany* **59**, 4205–4219.
- Filiseti-Cozzi TMCC, Carpita NC.** 1991. Measurement of uronic acids without interference from neutral sugars. *Analytical Biochemistry* **197**, 157–162.
- Fischer RL, Bennett AB.** 1991. Role of cell wall hydrolases in fruit ripening. *Annual Reviews of Plant Physiology and Plant Molecular Biology* **42**, 675–703.
- Gaffe J, Tiemann DM, Handa AK.** 1994. Pectin methylesterase isoforms in tomato (*Lycopersicon esculentum*) tissue: effects of expression of a pectin methylesterase antisense gene. *Plant Physiology* **105**, 199–203.
- Grant GT, Morris ER, Rees DA, Smith PJC, Thorn D.** 1973. Biological interactions between polysaccharides and divalent cations: the egg-box model. *FEBS Letters* **32**, 195–198.
- Hall LN, Bird CR, Picton SP, Tucker GA, Seymour GB, Grierson D.** 1994. Molecular characterisation of cDNA clones representing pectin esterase isozymes from tomato. *Plant Molecular Biology* **25**, 313–318.
- Hejlova A, Blahovec J.** 2008. Sloughing in potatoes induced by tuber density and affected by variety. *Czech Journal of Food Sciences* **26**, 48–57.
- Jardine WG, Doeswijk-Voragen CHL, MacKinnon IMR, van den Broek LAM, Ha MA, Jarvis MC, Voragen AGJ.** 2002. Methods for the preparation of cell walls from potatoes. *Journal of the Science of Food and Agriculture* **82**, 834–839.
- Jarvis MC.** 1999. Intercellular separation forces generated by intracellular pressure. *Plant, Cell and Environment* **21**, 1307–1310.
- Karkalas J.** 1985. An improved enzymic method for the determination of native and modified starch. *Journal of the Science of Food and Agriculture* **36**, 1019–1027.
- Kloosterman B, Oortwijn M, Celis-Gamboa C, uitdeWilligen J, America T, de Vos R, Visser RGF, Bachem C.** 2010. From QTL to candidate gene: a genetical genomics approach using a pooling strategy for simple and complex traits in potato. *BMC Genomics* **11**, 158.
- Knox JP, Linstead PJ, King J, Cooper Ch, Roberts K.** 1990. Pectin esterification is spatially regulated both within cell walls and between developing tissues of root apices. *Planta* **181**, 512–521.
- Limberg G, Komer R, Buchholt HC, Christensen T, Roepstorff P, Mikkelsen JD.** 2000. Analysis of different de-esterification mechanisms for pectin by enzymic fingerprinting using endopectin lyase and endopolygalacturonase II from *A. niger*. *Carbohydrate Research* **327**, 293–307.
- Livak KJ, Schmittgen TD.** 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* **25**, 402–408.
- McCann MC, Chen L, Roberts K, Kemsley EK, Sene C, Carpita NC, Stacey NJ, Wilson RH.** 1997. Infrared microspectroscopy: sampling heterogeneity in plant cell wall composition and architecture. *Physiologia Plantarum* **100**, 729–738.
- McGregor I.** 2007. The fresh potato market. In: Vreugdenhil D, ed. *Potato biology and biotechnology: advances and perspectives*. Oxford, UK: Elsevier, 3–26.
- Morvan O, Quentin M, Jauneau A, Mareck A, Morvan C.** 1998. Immunogold localisation of pectin methylesterases in the cortical tissues of flax hypocotyl. *Protoplasma* **202**, 175–184.
- Ng A, Waldron KW.** 1997. Effect of steaming on cell wall chemistry of potato (*Solanum tuberosum* cv. Binje) in relation to firmness. *Journal of Agricultural and Food Chemistry* **45**, 3411–3418.

- Pelloux J, Rusterucci C, Mellerowicz EJ.** 2007. New insights into pectin methylesterase structure and function. *Trends in Plant Sciences* **12**, 267–277.
- Pfaffl MW.** 2001. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Research* **29**, e45.
- Phan TD, Bo W, West G, Lycett GW, Tucker GA.** 2007. Silencing of the major salt-dependent isoform of pectin esterase in tomato alters fruit softening. *Plant Physiology* **144**, 1960–1967.
- Pilnick W, Voragen AGJ.** 1991. The significance of endogenous and exogenous pectin enzymes in fruit and vegetable processing. In: Fox PE, ed. *Food enzymology*. London: Elsevier Applied Science, 303–336.
- Renard CM, Jarvis MC.** 1999. A cross-polarization, magic-angle-spinning, ¹³C-nuclear-magnetic-resonance study of polysaccharides in sugar beet cell walls. *Plant Physiology* **119**, 1315–1322.
- Ross H, McDougall GJ, Vincent JFV, Stewart D, Verrall S, Taylor MA.** 2010. Discerning intra-tuber differences in textural properties in cooked *Solanum tuberosum* group Tuberosum and group Phureja tubers. *Journal of the Science of Food and Agriculture* **90**, 1527–1532.
- Shomer I, Kaaber L.** 2006. Intercellular adhesion strengthening as studied through simulated stress by organin acid molecules in potato (*Solanum tuberosum* L.) tuber parenchyma. *Biomacromolecules* **7**, 2971–2982.
- Stewart D, Yahiaoui N, McDougall GJ, Myton K, Marque C, Boudet AM, Haigh J.** 1997. Fourier-transform infrared and Raman spectroscopic evidence for the incorporation of cinnamaldehydes into the lignin of transgenic tobacco (*Nicotiana tabacum* L.) plants with reduced expression of cinnamyl alcohol dehydrogenase. *Planta* **201**, 311–318.
- Taylor MA, McDougall GJ, Stewart D.** 2007. Potato flavor and texture. In: Vreugdenhil D, ed. *Potato biology and biotechnology*. Oxford, UK: Elsevier, 525–540.
- Thybo AK, Christiansen J, Kaack K, Petersen MA.** 2006. Effect of cultivars, wound healing and storage on sensory quality and chemical components in pre-peeled potatoes. *LWT – Food Science and Technology* **39**, 166–176.
- Tucker GA, Robertson NG, Grierson D.** 1982. Purification and changes in activities of tomato pectinesterase isoenzymes. *Journal of the Science of Food and Agriculture* **33**, 396–400.
- Van Marle JT, Van Dijk C, Voragen AGJ, Biekman ESA.** 1994. Comparison of the cooking behaviour of the potato cultivars Nicola and Irene with respect to pectin breakdown and the transfer of ions. *Potato Research* **37**, 183–195.
- Vincent JFV, Jeronimidis G, Khan AA, Luyten H.** 1991. The wedge fracture test: A new method for measurement of food texture. *Journal of Texture Studies* **22**, 45–57.
- Voragen F, Beldman G, Schols H.** 2001. Chemistry and enzymology of pectins. In: McCleary BV, Prosky L, eds. *Advanced dietary fibre technology*. Oxford, UK: Blackwell Science, 379–398.
- Willats WGT, Orfila C, Limberg G.** 2001. Modulation of the degree and pattern of methyl-esterification of pectic homogalacturonan in plant cell walls. *Journal of Biological Chemistry* **276**, 19404–19413.