Structural and chemical differences in the cell wall regions in relation to scale firmness of three onion (Allium cepa L.) selections at harvest and during storage

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Abstract

BACKGROUND: Firmness in vegetables is an important textural attribute affecting consumer attitudes toward freshness and quality. Firmness, structural carbohydrates, polygalacturonase (PG), and pectin methylesterase (PME) activity were measured in three onion (Allium cepa L.) lines at harvest and after 4, 8, and 12 weeks of storage.

RESULTS: The high-dry-matter onion, MBL87-WOPL, had the firmest bulbs at harvest and delayed softening during storage. MBL87-WOPL had the thickest cell wall/middle lamella region, and highest levels of dry matter and total uronic acid. Furthermore, MBL87-WOPL had the lowest levels of PG and PME activity during storage. Pegasus, a poor-storing cultivar, had the softest bulbs at harvest, lowest levels of uronic acid, and thinnest cell wall/middle lamella. A good storing, moderately firm onion cultivar (MSU4535B) presented intermediate levels of firmness and total uronic acid content. Differences in uronic acid in water-soluble pectin accounted for much of the difference in total uronic acid among lines. Cellulose concentrations were similar among all lines at harvest. In addition, cellulose concentrations decreased in all lines during storage. Transmission electron microscopy performed on bulbs at harvest and after 12 weeks of storage indicated that degradation of the middle lamella had occurred during storage, leading to cell separation.

CONCLUSION: Our results suggest that differences in onion scale firmness at harvest may be due to differences in water-soluble pectin uronic acid concentrations. Furthermore, the rate of bulb softening during storage at 6.6 °C was greater in onion lines with higher levels of PME and PG activity in storage.

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Keywords: cellulose; uronic acid; pectin methylesterase; polygalacturonase; middle lamella

INTRODUCTION

Onions (Allium cepa L.) have been cultivated by humans for thousands of years. Though primarily consumed for their flavor, the textural characteristics of onions also contribute to the consumer’s perception of bulb quality.1,2 Texture is an aggregate quality composed of several measurable parameters, including firmness, crispness, elasticity, and gumminess.3 Owing to ease of use and cost-effectiveness, firmness values in fruits and vegetables are often measured using a hand-held or press-mounted penetrometer.4 Measurements of firmness in fruit and vegetables can be affected by a number of factors including cell size and arrangement, cell wall and middle lamella strength and cell turgor.4,5

The physiochemical properties of the cell wall and middle lamella in fruits and vegetables have been extensively investigated.6 However, unlike fruits such as apple, for which the relationship between cell wall/middle lamella and firmness has been thoroughly investigated, much less is known about the physiochemical properties of onion.2,4,5,7 There have been several studies reporting on the cell wall chemistry of onion.8–11 One study correlated mechanical properties of bulbs to cell wall chemistry, demonstrating that the tensile strength of tightly packed epidermal cells was many times greater than that of loosely spaced parenchyma cells in bulb scales in the onion cultivar ‘Sturon’.10 In addition, the hard, papery, outer scales of the bulbs had significantly higher concentrations of galactose-rich pectin than softer interior scales. This suggests that the physical arrangement of cells and chemical composition of the cell wall/middle lamella could affect the mechanical properties of bulbs.10 Recent studies have indicated

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that significant softening occurs in onion tissue during refrigerated storage.\textsuperscript{12} The composition of pectic polysaccharides may affect the texture of onion.\textsuperscript{10} Therefore it is possible that changes in firmness during storage could be affected by the activity of pectinases. Pectin methylesterase (PME) (EC 3.1.1.11) is responsible for demethylesterification of pectic polygalacturonic acid chains. Once demethylesterified these polymers can bind to free calcium ions, resulting in cross-linking of adjacent pectin chains, increasing firmness. However, if native polygalacturonases (PG) (EC 3.2.1.15) are present, the galacturonic acid chains may be hydrolyzed, resulting in depolymerization of the pectin and potential softening.\textsuperscript{13,14} PME activity, which has been detected in fresh and dehydrated onion tissue, has been proposed to affect the firmness of thermally processed onion.\textsuperscript{15,16} This suggests that PME may play an active role in modifying the texture of onion. Although PG activity has not been investigated in onion, it has been correlated to a loss of cellular adhesion in the roots of Leek (\textit{Allium porrum} L.), a species related to onion.\textsuperscript{17} Thus, although there are many factors that may influence texture in fruits and vegetables, it is probable that PME and PG activity may affect texture in onion tissue during storage.\textsuperscript{18–20}

The roles of pectin, cellulose, PME, PG, and cell wall/middle lamella architecture in relation to onion scale firmness at harvest and during storage are unknown. Therefore, the objectives of this study were to determine the relationship between firmness at harvest and during storage and uronic acid concentrations in pectin, cellulose content, and PME and PG activity. Cell wall/middle lamella architecture was also investigated to determine whether changes in firmness could be correlated to changes in cell wall morphology.

**MATERIALS AND METHODS**

**Plant material**

Three onion selections with contrasting firmness values were selected for the current study: (a) the short-day cultivar ‘Pegasus’ (Seminis seeds, Oxnard, CA, USA) represented a soft, poor-storing bulb grown for the sweet onion market; (b) the long-day breeding line, MSU4535B, has good storage potential and is moderately firm; and (c) the open pollinated selection MBL 87-WOPL and MSU4535B were harvested on 25 May 2006 and 16 June 2006, respectively, and were cured and placed into storage as described previously. Due to the presence of disease, only three replications of MBL 87-WOPL and MSU4535B were harvested and stored. Bulbs were analyzed at harvest and after 4, 8, and 12 weeks in storage. All subsequent analyses were conducted on 15 bulb composite samples with four replications of Pegasus and MSU4535B and three replications of MBL 87-WOPL.

**Sprouting and rooting**

As an indicator of the state of dormancy, the percentage of bulbs that could resume root or shoot growth was measured. To do this, 2–3 cm thick cross-sections of 15 bulbs containing the root plate and apical meristem were placed in boxes filled with Fafard Super Fine Germinating Mix (Fafard Inc.). Bulb slices were watered daily and maintained in a greenhouse with day/night temperature set points of 28/20 \degree C under natural light intensities and photoperiods. After 10 days the percentage of bulbs exhibiting rooting and sprouting was recorded.

**Soluble solids and firmness**

Total soluble solids (TSS) were measured by taking 1–2 mm longitudinal slices from bulbs at each sampling time and crushing them in a pneumatic press. Several drops of the juice were placed on a hand-held refractometer (Kernco, Tokyo, Japan) to determine TSS. Bulb scale firmness was measured by cutting a 2 × 4 cm slice from the first fully fleshy scale (usually the second or third scale from the outside of the bulb) at the equatorial region of each bulb. Firmness was measured as the force required to penetrate the scale using a 1 mm diameter probe coupled to a fruit penetrometer mounted on a motorized press operated at a speed of 1.5 mm \textit{s}\textsuperscript{−1} (Model 327, McCormick Fruit Tech, Yakima, WA, USA). Firmness for each 2 × 4 cm slice was measured three times and averaged.

**Alcohol-insoluble solids, pectin fractioning, and total pectin determination**

The alcohol-insoluble solids (AIS) residue was prepared from onion tissue according to a modification...
of the method of Huber and Lee. Longitudinal slices, 5–10 mm thick, were cut from bulbs. Slices were homogenized in a blender for 60 s with four volumes (w/v) of 95% ethanol. Two more volumes of 95% ethanol were added and the homogenate was boiled at 100 °C for 20 min with slow stirring. The homogenate was cooled in an ice-water bath for 30 min. The cooled residue was filtered under vacuum through glass fiber filters (APFF, 0.7 µm, Millipore, Billerica, MA, USA). Based on the initial sample weight the residue was sequentially washed with six volumes of 95% ethanol, four volumes of 100% ethanol, and four volumes of acetone. The residue was dried overnight in a fume hood. The dried AIS residue was weighed and ground to a fine powder using a coffee grinder and stored at −20 °C until analysis.

The AIS was sequentially fractioned into water-, chelator-, acid-, and alkali-soluble pectin. Approximately 30 mg of onion AIS was extracted at 60 °C for 90 min in 40 mL of 0.05 mol L⁻¹ sodium acetate buffer, pH 5.2, to obtain water-soluble pectin (WSP). The WSP was obtained by centrifuging the extract at 30 000 × g for 15 min and filtering the supernatant through one layer of Miracloth (CalBiochem, EMD Biosciences, San Diego, CA, USA). The remaining pellet was resuspended in 40 mL of 0.05 mol L⁻¹ sodium oxalate, 0.05 mol L⁻¹ ammonium oxalate, and 0.05 mol L⁻¹ sodium acetate pH 5.2 and incubated for 90 min at 60 °C to obtain chelator-soluble pectin (CSP). The remaining pellet was again suspended in 40 mL of HCL pH 2.5 and incubated for 90 min at 60 °C. The extract was centrifuged and filtered as previously to obtain the acid-soluble pectin (ACSP). The remaining pellet was resuspended in 40 mL of 0.05 mol L⁻¹ NaOH and incubated for 90 min at 60 °C, centrifuged, and filtered as previously in order to obtain the alkaline-soluble pectin (AKSP). The uronic acid content of each pectin fraction was determined using the m-hydroxydiphenol method. Fructans with a degree of polymerization longer than three are co-extracted in the AIS. Although fructans do react with the m-hydroxyphenol used to determine uronic acid concentrations, their contribution to the total absorption of each fraction was minimal and did not significantly affect the results of the assay.

Total uronic acid content of the AIS was also determined. Approximately 5 mg of AIS was weighed into a 50 mL beaker to which 5 mL of concentrated cold sulfuric acid was added. The beaker was stirred in an ice bath for approximately 10 min until the AIS dissolved. Then 5 mL of cold deionized water was added in 1 mL increments. After 10 min cold deionized water was added to bring the solution to a volume of 25 mL in a volumetric flask. An aliquot of the solution was analyzed for galacturonic acid.

Cellulose concentrations in the AIS residue were determined. Approximately 30 mg of AIS residue was weighed into glass test tubes and dissolved with 3.0 mL of 10:1 (80% acetic acid:concentrated nitric acid) and heated in a boiling water bath at 100 °C for 30 min, centrifuged for 10 min at 5000 × g and the supernatant was discarded. The pellet was washed with 10 mL of deionized water and centrifuged at 5000 × g for 10 min and repeated twice. The washed pellet was dissolved in 10 mL of 67% sulfuric acid, mixed and diluted to 100 mL with deionized water. An aliquot of the solution was pipetted into a glass test tube to which 3.5 mL of deionized water and 10 mL of cold anthrone reagent (0.2 g anthrone in 100 mL concentrated sulfuric acid) (Sigma, St Louis, MO, USA) was added. Tubes were mixed and placed in a boiling water bath for 18 min. After cooling in an ice bath for 5 min, samples were read on a spectrophotometer at 620 nm and concentrations calculated based on a standard curve developed using purified cellulose (Sigma). Spiked samples containing purified cellulose averaged 90% recovery.

Enzyme activity and protein determination

All enzyme extractions and purification steps were performed in a cold room at 4 °C. Enzyme extracts were boiled for 5 min prior to assaying to serve as blanks for each sample. PG activity was determined according to a modification of the 2-cyanoacetamide method. PG was extracted from approximately 15 g fresh tissue obtained from the equatorial region of bulbs using a 6 mm cork borer and homogenized in 45 mL of cold 1 mol L⁻¹ NaCl solution pH 6.0 for 1 min using a Waring blender at high speed (Waring Laboratory and Science, Torrington, CT, USA). The homogenate was extracted overnight at 4 °C. The homogenate was filtered through two layers of Miracloth and centrifuged at 4 °C and 10 000 × g for 10 min. A 0.5 mL aliquot of the supernatant was concentrated and reducing sugars removed using centrifugation and filtration at 12 000 × g and 4 °C for 1 h (Microcon YM-10, Millipore). Then 0.1 mL of 50 mmol L⁻¹ sodium acetate pH 4.4 was added to the retentate and the solution was re-centrifuged by centrifugation and filtration at 12 000 × g and 4 °C for 40 min. The retentate was redissolved in 0.1 mL 50 mmol L⁻¹ sodium acetate pH 4.4 and galacturonic acid content determined. One unit of PG activity was determined to be the amount of enzyme that released 1 µmol of reducing sugar (galacturonic acid) 60 s⁻¹.

PME activity was determined by titration at pH 7.5. PME was extracted from 15 g of fresh tissue homogenized with 60 mL of ice-cold extraction buffer (0.1 mol L⁻¹ NaCl, 0.25 mol L⁻¹ Tris-base, pH 8.0) for 1 min using a Waring blender at high speed. The homogenate was extracted for 3 h at 4 °C, after which the mixture was filtered through two layers of Miracloth and centrifuged for 10 min at 4 °C and 10 000 × g. The supernatant was collected and 30%
ammonium sulfate added. The solution was allowed to precipitate overnight. The dispersion was centrifuged for 15 min at 4 °C and 10 000 × g and the supernatant collected. The enzyme assay was carried out using a pH stat titrator (Brinkmann, Westbury, NY, USA). 0.4 mL of extract was added to 20 mL of a solution of 1.0% high-methoxy citrus pectin with 0.1 mol L⁻¹ NaCl at pH 7.5 and 37 °C. The assay was conducted for 20 min. One unit of PME activity was determined to be the amount of enzyme that released 1 µmol of carboxylic acid 60 s⁻¹.

Protein analysis
Total protein concentrations used in each enzyme assay were determined using the bicinchoninic acid method. A commercially available kit was used and manufacturer’s instructions followed using bovine serum albumin protein as a standard (Pierce BCA Protein Assay Kit, Pierce, Rockford, IL, USA).

Microscopy
Samples were analyzed using light microscopy and transmission electron microscopy (TEM) to determine whether any structural changes could be observed in the cell wall/middle lamella region between cultivars at harvest and during storage. All samples were prepared in a cold room at 4 °C. At harvest and after 12 weeks of storage, bulbs were sampled. Five, 1–2 mm³ samples were cut from each of 15 bulbs and immediately placed into Sorensen’s phosphate buffer, pH 7.2, with 4% glutaraldehyde fixative for 8 h (EMS, Hatfield, PA, USA). The fixative solution was decanted and samples washed twice for 30 min and once overnight in Sorensen’s phosphate buffer. Samples were decanted and post-fixed in Sorensen’s phosphate buffer containing 1% osmium tetraoxide for 2 h. Samples were washed for 20 min in Sorensen’s buffer and repeated twice. Samples were dehydrated in a graded ethanol series (20%, 30%, 50%, 70%, 95%, 100% ethanol) for 20 min at each step. Propylene oxide was used as a transitional solvent prior to embedding. Samples were embedded in Spurr’s low-viscosity embedding resin (EMS). Sections for light (1–2 µm) and TEM (90 nm) microscopy were cut on a Reichert Jung ultracut E ultramicrotome (Reichert Microscope Services, Depew, NY, USA) using a diamond knife (Diatome Histo 5100 and Ultra 45°; Diatome, Hatfield, PA, USA). Sections for light microscopy were dried on glass slides and stained with 0.5 g L⁻¹ toluidine blue (Sigma) and visualized on an Olympus Bx51 microscope (Olympus America Inc., Center Valley, PA, USA). Cell wall/middle lamella thickness was determined by measuring cell walls of four cells per bulb at points where adjacent cells made contact (20–25 measurements per bulb) and averaging them. Ultrathin sections were cut for TEM and placed on 200 mesh copper grids and stained with uranyl acetate and lead citrate. Sections were visualized on a Zeiss 902A TEM (Carl Zeiss Microimaging, Thornwood, NY, USA). Five samples for each onion line per sampling time were tested.

Statistical analysis
All data was subjected to the GLM procedure testing for significance of main effects and interactions between cultivar and storage time using SAS statistical software (SAS v. 9.1.3, SAS institute, Cary, NC, USA). Percentage data were transformed using arc–sin transformations. Data were considered significant when \( P < 0.05 \).

RESULTS
Firmness
Onion scale firmness was affected by cultivar and storage time (Fig. 1). There was also a significant interaction among cultivars and storage time for onion scale firmness. The interaction was the result of firmness decreasing at different rates in the cultivars during storage. The high-dry-matter line MBL87-WOPL had the highest average firmness at harvest (4.17 N), which decreased by 0.34 N over 12 weeks in storage. The cultivar Pegasus had the lowest average firmness readings at harvest (2.96 N). Scale firmness decreased by 0.44 N after 12 weeks in storage. The long-day line, MSU4535B, had an average firmness of 3.75 N at harvest and an average decrease of 0.52 N in scale firmness during storage.

Dry matter, TSS, and weight loss
The percentage dry matter in bulbs differed significantly by cultivar and storage time (Fig. 2(A)). Bulb dry matter concentrations in MBL87-WOPL ranged from 15% to 18%. The fresh-market sweet onion Pegasus had levels of bulb dry matter between 9% and 10.5%. A significant interaction between cultivar and storage time regarding dry matter concentration was
Differences in the cell wall regions in relation to scale firmness in *Allium cepa* L. monitored. This resulted from the increase in dry matter content in MBL87-WOPL during storage, while the other cultivars presented slight differentiation. Total TSS was affected by cultivar, and was highest in MBL87-WOPL and lowest in Pegasus (Fig. 2(B)). Total TSS was also affected by storage time. Onion line and storage time significantly interacted to affect bulb TSS. While TSS declined slightly during storage in Pegasus and MSU4535, TSS increased slightly in MBL87-WOPL, resulting in a small, but significant interaction. Weight loss in bulbs, measured as a percentage of fresh weight at harvest, differed among lines and increased during storage (Fig. 2(C)). There was also a significant interaction between onion line and storage time for weight loss. Much of this interaction is the result of the large weight loss that occurred in MBL87-WOPL between 4 and 8 weeks of storage, compared to the smaller weight losses incurred by the other cultivars during that time. MSU4535B lost the least amount of its harvest weight during storage (6%), while MBL87-WOPL had the largest weight loss, eventually losing 16% of its initial weight after 12 weeks in refrigerated storage.

### Rooting and sprouting

Breaking of dormancy as measured by the ability of the harvested bulbs to resume growth by sprouting new shoots or roots was affected by cultivar and storage duration (Fig. 3). There was also a significant interaction between cultivar and storage duration regarding sprouting and rooting. At harvest the onion cultivar Pegasus exhibited rooting or sprouting in roughly 60% of bulbs, while the other varieties required 8 weeks of storage to reach similar levels.

### Pectins and cellulose

Total uronic acid content differed among onion lines, but did not change during storage (Fig. 4(A)). No interactions were present. Average total uronic acid content ranged from 51 to 54 mg g\(^{-1}\) dry weight in MBL87-WOPL, followed by MSU4535B with 42–47 mg g\(^{-1}\) dry weight, and Pegasus, with 36–38 mg g\(^{-1}\) dry weight. There was a significant interaction between cultivar and storage time affecting WSP uronic acid concentrations. The interaction was the result of a significant increase in WSP uronic acid concentrations between harvest and 4 weeks storage in MSU4535B, while the WSP uronic acid concentrations in other onion lines remained unchanged. Second, there was an increase in WSP uronic acid concentrations in MBL87-WOPL between 8 and 12 weeks of storage, while the WSP uronic acid concentration in other cultivars remained stable. Although there was a significant interaction between onion line and storage duration for average WSP uronic acid concentrations, it is important to note the significant differences in WSP uronic acid concentrations among the different onion lines at harvest and during storage. The difference in WSP uronic acid concentrations accounted for much of the differences observed for total pectin concentrations.

Storage time and cultivar interacted to affect CSP uronic acid concentrations (Fig. 4(C)). This interaction was the result of some cultivars experiencing larger changes in storage than others. There was also a significant decrease in CSP uronic acid concentration during storage, most noticeably in MBL87-WOPL.

The ACSP uronic acid concentration differed among onion lines and during storage, though no interactions were present (Fig. 4(D)). Pegasus and MBL87-WOPL had the highest concentrations of

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**Figure 2.** (A) Percentage dry matter content; (B) percentage total soluble solids (TSS); (C) percentage fresh weight loss from harvest. Each data point represents the mean (± s.e.) of four replications for Pegasus and MSU4535B and three replications of MBL87-WOPL onion (*Allium cepa* L.) lines measured at harvest, 4, 8 and 12 weeks of storage.
ACSP uronic acid during storage, while MSU4535B had the least. The ACSP uronic acid concentration decreased in all onions during storage. The AKSP uronic acid fraction, composed primarily of hemicellulose, was not affected by onion line or storage time and averaged 17.3 mg g\(^{-1}\) dry weight for all cultivars.

Cellulose concentrations decreased in all lines during storage, falling by nearly 40% in MBL87-WOPL. There was also significant interaction between storage time and cultivar for bulb cellulose concentrations (Fig. 4(E)).

**Enzyme analysis**

Storage time and onion line interacted to affect PME activity (Fig. 5(A)). Bulb PME activity varied widely in Pegasus during storage, resulting in a cultivar \(\times\) storage interaction. Bulb PME activity decreased significantly during storage for MSU4535B and MBL87-WOPL. Although similar to Pegasus at harvest, MBL87-WOPL consistently had the lowest level of PME activity during storage, averaging between 0.1 to 0.2 units mg\(^{-1}\) protein. Onion type and storage time also interacted to affect PG activity (Fig. 5(B)). The interaction was the result of an increase in PG activity in Pegasus accompanied by a decrease in PG activity in MSU4535B after 8 weeks in storage. In addition to the interaction, there were significant differences in PG activity among onion lines, with Pegasus and MSU4535B having the highest activities and MBL87-WOPL having the lowest level of activity.

**Microscopy**

Differences in cell wall/middle lamella thickness were observed in onions at harvest (Table 1). Significant changes in the thickness of the cell wall and middle lamella region were not observed during storage. Light micrographs of onion cells illustrate visible differences in cell wall/middle lamella thickness at harvest for the onions tested (Fig. 6(A–C)). Changes to the middle lamella region were observed frequently during storage using TEM (Fig. 7(A–F)). In many cases the middle lamella appeared to be disrupted in adjacent cells. Structures resembling carbohydrate chains were frequently observed in the middle lamella after cell separation (Fig. 7(B,C)).

**DISCUSSION**

Bulb firmness was highest in MBL87-WOPL, followed by MSU4535B and Pegasus at harvest. In addition, MBL87-WOPL remained firm for the first 8 weeks of storage, whereas both MSU4535B and Pegasus experienced declines in firmness during the first 8 weeks of storage. Such results prompted the following questions. What properties at harvest could be responsible for the differences in bulb firmness, and what could account for the varying rates of softening between MBL87-WOPL and Pegasus or MSU4535B during storage?

Firmness at harvest correlated well with bulb dry matter content and TSS. The differences in dry matter content, which may be due differences in fructan concentration, could result in an increase in cell water potential and cell turgor, which may lead to firmer plant tissue and increased postharvest lift.\(^{13,32,33,34}\) In addition, nearly 90% of weight loss in onion bulbs during storage has been attributed to water loss.\(^{35}\) Thus it may be possible that changes in firmness could be due to water loss and changes in turgor. However, MBL87-WOPL was the firmest at harvest and throughout storage, and experienced the largest percentage of weight loss of cultivars tested (Fig. 2(C)). The weight loss that occurred between 4 and 8 weeks of storage did not lead to a concomitant decrease in firmness.

Uronic acid concentrations in pectin correlated well with bulb firmness levels at harvest. Pectin accounts for about 30% of the polysaccharides in the primary cell wall and middle lamella.\(^{36}\) At harvest, MBL87-WOPL had the highest total uronic acid concentration and had the firmest bulbs. Pegasus had the lowest total uronic acid concentration at harvest and produced the

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**Table 1.** Mean cell wall and middle lamella thickness for three onion (*Allium cepa* L.) lines measured at harvest and after 12 weeks in storage using light microscopy. Each mean represents five individual bulb replications.

<table>
<thead>
<tr>
<th>Onions</th>
<th>Harvest (µm)</th>
<th>12 weeks (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBL87-WOPL</td>
<td>4.19a</td>
<td>3.81a</td>
</tr>
<tr>
<td>MSU4535B</td>
<td>3.07ab</td>
<td>3.91a</td>
</tr>
<tr>
<td>Pegasus</td>
<td>2.29b</td>
<td>1.97b</td>
</tr>
</tbody>
</table>

\(^{a}\) Different letters in the same column indicate significant differences at \(P < 0.05\).
Differences in the cell wall regions in relation to scale firmness in *Allium cepa* L.

**Figure 4.** (A) Total uronic acids (UA); (B) uronic acids in water-soluble pectin (WSP); (C) uronic acid in chelator-soluble pectin (CSP); (D) uronic acid in acid-soluble pectin (ACSP); (E) uronic acids in alkaline-soluble pectin (ASP); (F) cellulose, measured at harvest, 4, 8, and 12 weeks of storage. Data points represent the mean (± SE) in mg g\(^{-1}\) dry weight (DW) of four replications for Pegasus and MSU4535B and three replications of MBL87-WOPL onions (*Allium cepa* L.).

**Figure 5.** Enzyme activities measured for three cultivars of onion (*Allium cepa* L.) at harvest, 4, 8, and 12 weeks of storage. Activities of (A) pectin methylesterase (PME) and (B) polygalacturonase (PG). Each data point represents mean (± SE) in activity units mg\(^{-1}\) protein for four replications of Pegasus and MSU4535B and three replications of MBL87-WOPL. One activity unit is equivalent to 1 \(\mu\)mol of product produced 60 s\(^{-1}\) from a given substrate.
softest bulbs. Much of the difference in total uronic acid concentrations can be attributed to differences in WSP uronic acids among the three cultivars. Although there were significant differences among onions in concentrations of CSP and ACSP uronic acids, they were generally minor. The uronic acids isolated in the WSP fraction are generally shorter and less branched than the other pectin fractions.\textsuperscript{11,37} Nonetheless, WSP uronic acids contribute to cellular adhesion and the strength of the middle lamella.\textsuperscript{37} It is possible that differences in WSP uronic acids at harvest may be responsible for the measured differences in onion scale firmness. Light microscopy (Fig. 6(A–C)) and subsequent measurements of the middle lamella suggest that the structure of the middle lamella contributed to the observed differences in scale firmness among the onion lines measured (Table 1). MBL87-WOPL had the thickest cell wall/middle lamella, highest concentration of WSP and was firmest at harvest. Pegasus had the softest scales at harvest, thinnest cell wall/middle lamella and lowest concentration of uronic acids in the WSP fraction.

No differences were monitored in cellulose concentrations among the cultivars at harvest. Because cellulose is the principal structural unit of the primary cell wall, it would be expected to be most concentrated in the firmest bulbs.\textsuperscript{38} It has been reported that higher concentrations of cellulose were detected in a firm onion cultivar ‘Pukekohe Longkeeper’ than in a softer ‘Houston Grano’ bulb.\textsuperscript{39} However, there were no differences in cellulose concentrations among the cultivars tested in this experiment. Furthermore, all cultivars experienced a significant decrease in cellulose concentrations between harvest and 4 weeks of storage, but only bulbs from Pegasus and MSU4535B underwent significant softening during the same period. This suggests that changes in cellulose concentration were not responsible for loss of firmness during storage.

Firmness in the three onion lines tested in this experiment declined significantly during storage. However, Pegasus and MSU4535B had a greater rate...
of softening in storage than MBL87-WOPL. Results obtained with TEM suggested that degradation of the pectin-rich middle lamella during storage was common. As such, the pectin-modifying enzymes PME and PG were measured.

There was significant variation in the level of PME and PG activity in Pegasus and MSU4535B bulbs during storage, resulting in a significant storage duration × variety interaction. However, the level of PME and PG activity in these two lines was generally higher than in MBL87-WOPL during storage. The level of PME and PG activity in MBL87-WOPL was relatively stable and low for the duration of the experiment. The combined actions of PME and PG in plants results in the degradation of galacturonic acid polymers, resulting in cell separation along the middle lamella. Thus, the higher levels of PME and PG activity in Pegasus and MSU4535B could have led to a decrease in cellular adhesion, resulting in the rapid softening that occurred in these bulbs. Previously, PG activity was linked to disruption in cellular adhesion in roots of A. porrum. Although it is clear that many enzymes are involved in softening of fruits and vegetables, our data suggest that PME and PG activity during storage may be related to rate of softening in onion bulbs. Comparisons with rooting and sprouting indices suggest that changes in dormancy were not directly related to PME and PG activity.

Decreases in onion scale firmness during storage also appeared to be related to the integrity of the middle lamella. Previous results obtained using an environmental scanning electron microscope demonstrated that onion cells, mechanically separated using a specially designed tensometer, fractured within the middle lamella material and not at the middle lamella cell wall interface. Using TEM, changes were observed in the structure of the middle lamella during storage. After 12 weeks of storage, disruption of the middle lamellae region was frequently observed (Fig. 7). In some samples structures resembling carbohydrate chains were observed between adjacent cells. Since TEM was only performed on samples at harvest and after 12 weeks in storage we cannot speculate on the rate of degradation observed during storage. However, the results demonstrate that degradation of the middle lamella during storage was common in the onions tested.

Our results indicate that uronic acid concentration in the WSP may be a factor affecting bulb firmness in the onions analyzed. Pegasus had the lowest concentration of uronic acids in the WSP and the softest bulbs at harvest, while MBL87-WOPL had the highest concentration of WSP uronic acids and were the firmest bulbs at harvest. Furthermore, light microscopy of onion cells indicated large differences in cell wall/middle lamella region, with MBL87-WOPL having the thickest cell wall/middle lamella region and Pegasus the thinnest. In addition, degradation of the middle lamella during storage appeared to be relatively common. To our knowledge this is the first attempt to correlate bulb softening to changes in pectin and cellulose concentrations as well as PME and PG activity in onion during storage. The results obtained here should provide researchers with more insight regarding what factors affect bulb texture at harvest and during storage.

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