Modulation of tomato pericarp firmness through pH and calcium: Implications for the texture of fresh-cut fruit

Susana C.F. Pinheiro^a, Domingos P.F. Almeida^{a,b,*}

^a Faculdade de Ciências, Universidade do Porto, Praça Gomes Teixeira, 4099-002 Porto, Portugal ^b CBOF, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, R. Dr. António Bernardino de Almeida, 4200-072 Porto, Portugal

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Abstract

The effect of pH and calcium on pericarp firmness and pectin solubility was investigated in tomato fruit (*Lycopersicon esculentum* Mill. 'Tavira'). Pericarp disks were vacuum-infiltrated with 50 mM CaCl₂ or with distilled water and incubated for 4 h in buffer solutions at pH 4.5 and 7.0, and subsequently stored at 2 °C for 5 days. CaCl₂ treatment had a significant effect on firmness retention in disks from turning and ripe fruit. Pericarp disks from mature-green fruit infiltrated with CaCl₂ were firmer than untreated tissue after a 4 h incubation period, but the effect of calcium did not persist during storage at 2 °C. pH had a significant effect on the firmness of pericarp disks excised from turning and ripe fruit, but not on mature-green tissue. Treatments at pH 7.0 caused a reduction of the softening rate in disks from turning and ripe fruit, but had no significant effect at the mature-green stage. Water-soluble pectins decreased significantly in mature-green and ripe pericarp tissue following treatment with CaCl₂ at pH 7.0, suggesting that pH affects pectin dissolution. Firmness changes induced by pH and calcium after a 4 h incubation treatment were highly correlated with pectin dissolution. The results indicate that, besides calcium, pH contributes to textural changes in tomato fruit pericarp. Since wounding inflicted during processing and acidic solutions used to prevent enzymic browning and microbial growth are likely to acidify the apoplast of fresh-cut fruit, the ability to maintain an apoplastic pH near 7.0 can significantly contribute to enhanced firmness of fresh-cut fruit.

Introduction

Fresh-cut fruit is a growing segment of the fresh food market. Although convenient, fresh-cut fruit are highly perishable and a good understanding of the physiological basis for quality loss is needed to support the development and correct application of technologies for quality maintenance.

The manufacturing process of fresh-cut fruit invariably involves tissue wounding. Wounding hastens senescence and induces tissue softening, which is considered a major limitation of shelf-life in fresh-cut fruit (Beaulieu and Gorny, 2001; Soliva-Fortuny and Martín-Belloso, 2003). Softening is attributed to changes in turgor pressure (Beaulieu and Gorny, 2001) and in the structure and composition of cell walls, including disassembly of the pectic matrix (Rose et al., 1998), mediated, at least in part, by the sequential action of pectinmethylesterase (PME;

Several authors have suggested that the apoplastic environment, namely its pH and mineral composition, may affect the catalytic activity of cell wall enzymes (Huber and O'Donoghue, 1993; Chun and Huber, 1998; Almeida and Huber, 1999). There is evidence that the apoplastic pH of tomato fruit decreases from pH 6.7 in mature-green fruit to 4.4 at the ripe stage (Almeida and Huber, 1999). The acidification of the apoplast over the pH range can provide a mechanism for the regulation of the catalytic activity of cell wall enzymes. The relevance of the changes

EC 3.1.1.11) and endopolygalacturonase (PG; EC 3.2.1.15) (Brummell and Harpster, 2001). Wounding affects the levels of pectic enzymes present in fresh-cut fruit. In papaya the levels of PG and β -galactosidase (β -gal; EC 3.2.1.23) are increased after cutting and remain higher than in intact fruit during subsequent storage (Karakurt and Huber, 2003). In tomato, wounding is reported to have the opposite effect, at least in ripening fruit (Chung et al., 2006). In slices prepared from ripening tomato fruit, the activities of PG and β -gal were lower compared with intact fruit, but no changes in pectic enzymes of fully ripe fruit were observed in response to wounding (Chung et al., 2006).

in the apoplastic environment is even higher in fresh-cut fruit, where the effects of wounding and acidic additives are superimposed on the ripening-relating changes in pH that occur in whole fruit. Wounding caused during processing certainly modifies the apoplastic environment of fresh-cut fruit due to leakage of the acidic vacuolar contents in the vicinity of the cut surface. Moreover, treatments with acidic solutions are often applied to fresh-cut fruit to prevent browning and control microbial growth (Beaulieu and Gorny, 2001).

Few studies have addressed the influence of pH on the physical properties of fruit tissue. Infiltration of pear tissue with a buffer solution at pH 8 improved firmness as compared with a treatment at pH 3, presumably because acidic conditions favor pectin dissolution via calcium displacement (Knee, 1982). Application of calcium is effective in reducing softening in whole and fresh-cut fruit (Dong et al., 2000; Luna-Guzmán and Barrett, 2000; Saftner et al., 2003; Lamikanra and Watson, 2004). Exogenously applied calcium binds the negative charges of the de-esterified uronic acid residues that are generated by PME during ripening, enhancing the tissue's mechanical strength (Mignani et al., 1995; Magee et al., 2003). Since pH affects PME activity (Giovane et al., 1994) and PG-mediated pectin depolymerization and dissolution (Chun and Huber, 1998; Almeida and Huber, 2007), interactions between pH and calcium treatments are likely to occur, and should be taken into consideration in developing additive or coating treatments for fresh-cut fruit.

The objectives of this study were to investigate the effect of pH and calcium on firmness and on disassembly of the pectic matrix of tomato pericarp, and to evaluate whether the control of apoplastic pH can contribute to improve the texture of fresh-cut fruit.

Materials and methods

Plant material

Tomato (*Lycopersicon esculentum* Mill. 'Tavira') fruit were harvested at the mature-green stage, sanitized with NaOCl (50 mg L⁻¹), washed, and allowed to ripen at 15 °C, 90% RH. Outer pericarp disks, 15 mm in diameter, were excised with a sharp cork borer from the equatorial region of the fruit at the mature-green, turning and ripe stages, as determined by the tomato classification chart (USDA, 1991). After excision the disks were thoroughly washed with distilled water to remove remains of the locular gel and cellular contents of the wounded area, and blot-dried prior to incubation.

pH and calcium treatments

Disks were vacuum-infiltrated with $50 \, \text{mM}$ CaCl $_2$ or distilled water for $30 \, \text{s}$ at $5 \, \text{kPa}$. The negative pressure was applied and released gradually, in a constant manner in all treatments. No signs of tissue injury were observed throughout the experiments. After infiltration, groups of five disks with an approximate fresh weight of $7 \, \text{g}$ were incubated in $20 \, \text{mL}$ of buffer solution for $4 \, \text{h}$ at $20 \, ^{\circ}\text{C}$ with continuous agitation at $40 \, \text{rpm}$. Two buffer

solutions with pH 4.5 (100 mM citric acid-sodium citrate and 100 mM acetic acid-sodium acetate), and two buffers with pH 7.0 (100 mM potassium phosphate and 40 mM Hepes) were used.

Disks were analyzed immediately after the 4h incubation period or after storage. The stored disks were incubated for 4h as described and then transferred to Petri dishes, previously sanitized with ethanol (0.70 mL $^{-1}$), and maintained at $2\,^{\circ}C$ for 5 days.

Firmness assessment

At the end of the incubation period or after a 5-day storage period, each disk was cut to the uniform height of 5 mm, and the endocarp excised with a razor blade and the disk placed with the exocarp facing down. Firmness measurements were performed with a universal testing machine (Instron, model 4501, Canton, Mass., USA) equipped with a 100 N load cell and operated at a crosshead speed of 10 mm min⁻¹. The force needed to compress the disk 2.5 mm with a flat-plate probe (30 mm diameter) was registered. All measurements were performed at 20 °C.

Preparation of ethanol-insoluble solids

Ethanol-insoluble solids (EIS) were extracted from 50 g of disks. The tissue was homogenized for 2 min (Ultra-Turrax T25, IKA, Germany) in 200 mL of absolute ethanol at $-20\,^{\circ}$ C. The resulting suspension was boiled under reflux for 20 min to inactivate cell wall enzymes, and kept overnight at $-20\,^{\circ}$ C to assure complete precipitation of cell wall polymers. The suspension was filtered through Miracloth (Calbiochem Corporation, La Jolla, CA, USA) and the solid residue washed twice with 200 mL of acetone. The resulting EIS were oven-dried at 34 $^{\circ}$ C for 12 h and stored in a dessicator at room temperature until analyzed.

Pectin extraction and determination of uronic acids

Total pectin content was determined in the EIS as described by Ahmed and Labavitch (1977). Water-soluble pectins were extracted by suspending 20 mg of EIS in 7 mL of distilled H₂O and incubating the suspension for 6h at 22 °C with agitation. The suspension was then filtered through a GF/C glass fiber filter (Whatman, Springfield, UK) and the filtrate collected for uronic acid determination. After extraction of water-soluble pectins, the solid residue was suspended in 7 mL of a solution of 50 mM sodium acetate, 50 mM trans-cyclohexane-1,2-diaminetetraacetate (CDTA), pH 6.5, and incubated for 6 h to extract the CDTA-soluble fraction. The suspension was filtered through a GF/C glass fibre filter and the uronic acids quantified in the filtrate. The concentration of uronic acids was determined by the *m*-phenylphenol method (Blumenkrantz and Asboe-Hansen, 1973), as modified by Filisetti-Cozzi and Carpita (1991), using D-galacturonic acid as the standard.

Data analysis

The experiments were conducted in a completely randomized block design with six blocks. The two buffer solutions with

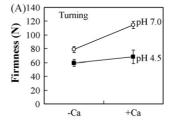
the same pH were considered as replicates and were replicated three times. Each block consisted of a set of 15 disks, excised from different fruit, treated and handled together. Data were analyzed using two-way ANOVA, with pH and calcium treatment as factors. Relationships between firmness and pectin solubility were analyzed using a simple linear regression model. All data analyses were performed with the statistical software SPSS for Microsoft Windows, v. 11.5 (SPSS Inc., Chicago, USA).

Results and discussion

Effect of calcium and pH on pericarp firmness

Calcium treatments are widely used to improve the texture of fresh-cut fruit (Beaulieu and Gorny, 2001). At the end of the incubation period, calcium-treated disks were, on average, 5, 20, and 31% firmer than untreated control disks, at the maturegreen, turning, and ripe stages, respectively (Table 1). The effect of calcium on the firmness of mature-green pericarp did not persist after 5 days at 2 °C (Table 1). However, in disks from turning and ripe fruit, the effect of calcium on firmness retention was enhanced during storage. Firmness in turning and ripe disks was, respectively, 34 and 241% higher in calcium-treated than in untreated disks (Table 1). Positive effects of calcium on the firmness of fresh-cut fruit have been previously documented (Dong et al., 2000; Luna-Guzmán and Barrett, 2000; Beaulieu and Gorny, 2001; Magee et al., 2003; Saftner et al., 2003; Lamikanra and Watson, 2004), but the interaction with pH is seldom considered.

The significant interactions between the effect of calcium and that of pH on the firmness of disks from turning and ripe tomato fruit after a 5-day storage period (Table 1) are depicted in Fig. 1. Although calcium improved firmness at both neutral and acidic pH, higher disk firmness was achieved when calcium-treated disks were incubated at pH 7.0, indicating that apoplastic pH can affect the effectiveness of the calcium solutions used to treat fresh-cut fruit.



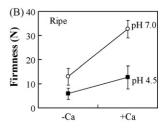


Fig. 1. Interaction between the effects of calcium and pH on the firmness of tomato pericarp disks. Disks were excised from fruit at the turning (A) and ripe (B) stages, infiltrated with distilled water (-Ca) or $50 \, \text{mM} \, \text{CaCl}_2$ (+Ca), incubated for 4 h in buffers with pH 4.5 or 7.0, and stored at 2 °C for 5 days. Each data point is the mean \pm S.E. (n = 6).

Studying the effects of pH on fruit texture poses methodological problems that are not easy to overcome for a number of reasons. No single solution provides the same buffering capacity over the wide pH interval 4.5-7.0 (Lide, 2003) relevant in ripening tomato fruit (Almeida and Huber, 1999). Even if the same buffer solution is used, differences in pH value are invariably associated with differences in the relative balance between anionic species and undissociated acid. Moreover, the chemical composition of the buffer solution per se is likely to interfere with the metabolism (e.g. citrate could chelate cell wall calcium and small uncharged molecules could be taken up by the cell). We have addressed these challenges using two buffers for each pH value studied. By considering the two buffers as replicates, the putative effects of the buffer's chemical nature were included in the experimental error, thus increasing the noise against which the effects of pH were tested. The significant effects of pH on firmness (Table 1) and on pectin dissolution (Tables 2 and 3) provide statistical support for the interpretation that the effects observed are mainly attributed to the concentration of hydrogen ions, despite the fact that the chemical composition of the buffer also affected firmness and pectin dissolution (Figs. 2 and 3).

pH had a significant effect on disk firmness at the turning and ripe stages, but not at the mature-green stage (Table 1).

Table 1 Effect of pH and calcium on firmness of tomato pericarp disks at the end of a 4 h incubation period and following 5 days at 2° C

Factor	Firmness (N)							
	After 4 h			After 5 days				
	Mature-green	Turning	Ripe	Mature-green	Turning	Ripe		
Initial value	141.0 ± 10.9	83.3 ± 3.6	59.1 ± 4.6	199.9 ± 2.4	115.0 ± 3.8	65.6 ± 4.0		
CaCl ₂ (mM)								
0	153.0 ± 2.6	50.7 ± 3.2	35.4 ± 3.2	134.7 ± 4.0	69.2 ± 4.2	9.4 ± 2.3		
50	161.1 ± 1.8	61.0 ± 2.8	46.3 ± 2.7	139.0 ± 3.4	91.5 ± 8.7	22.7 ± 4.1		
pН								
7.0	160.2 ± 2.3	61.6 ± 2.9	47.4 ± 3.0	137.5 ± 3.7	96.7 ± 6.2	22.8 ± 3.8		
4.5	153.9 ± 2.5	50.2 ± 2.9	34.3 ± 2.4	136.2 ± 3.8	63.9 ± 5.3	9.3 ± 2.7		
P-value								
Calcium	0.017	0.003	0.002	0.803	0.002	0.002		
pН	0.056	0.002	0.000	0.403	0.000	0.000		
Calcium × pH	0.982	0.519	0.904	0.141	0.039	0.031		

Disk firmness was measured before the treatments (initial value) and following a 4h incubation period (after 4h) or a 5-day storage period at 2° C (after 5 days). Values are means \pm S.E. (n = 12).

Table 2
Uronic acids extracted from tomato disks at the mature-green and ripe stages before the treatments (initial value), infiltrated with calcium or with distilled water and incubated for 4 h in buffers with pH 4.5 or 7.0

Factor	Uronic acids ($\mu g g^{-1}$)							
	Mature-green			Ripe				
	Total	WSP	CSP	Total	WSP	CSP		
Initial	$266.2 \pm 12.8 \ (100.0)$	$32.8 \pm 1.4 (12.3)$	$87.0 \pm 2.7 (2.7)$	$271.1 \pm 1.3 (100.0)$	$61.1 \pm 2.2 (22.5)$	$113.5 \pm 4.8 (41.9)$		
CaCl ₂ (mM)								
0	$271.3 \pm 10.4 (100.0)$	$26.4 \pm 3.1 (9.7)$	$90.2 \pm 4.8 (33.2)$	$279.1 \pm 8.7 (100.0)$	$61.8 \pm 4.0 (22.1)$	$105.1 \pm 5.8 (37.7)$		
50	$248.3 \pm 4.2 (100.0)$	$20.4 \pm 2.6 \ (8.2)$	$89.1 \pm 3.1 (35.9)$	$265.2 \pm 3.2 (100.0)$	$46.3 \pm 5.9 (17.5)$	$117.6 \pm 5.0 (44.3)$		
pН								
7.0	$265.1 \pm 10.7 (100.0)$	$16.9 \pm 1.2 (6.4)$	$95.0 \pm 3.0 (35.8)$	$272.8 \pm 6.5 (100.0)$	$41.6 \pm 4.1 (15.2)$	$120.9 \pm 4.6 (44.3)$		
4.5	$254.5 \pm 6.4 (100.0)$	$29.9 \pm 2.3 \ (11.7)$	$84.3 \pm 3.9 (33.1)$	$271.6 \pm 6.6 (100.0)$	$66.5 \pm 2.6 (24.5)$	$101.8 \pm 4.7 (37.5)$		
P-value								
Calcium	0.568	0.046	0.311	0.563	0.000	0.002		
pН	0.366	0.000	0.298	0.421	0.000	0.001		
Calcium × pH	0.314	0.978	0.856	0.659	0.073	0.478		

Values are means \pm S.E. (n = 12) and within parenthesis are expressed as a percentage of the total uronic acids. WSP, water-soluble pectins; CSP, CDTA-soluble pectins.

After incubation in buffers with pH 7.0 the disks were 23, and 38% firmer than at pH 4.5, at the turning and ripe stages, respectively. Following storage at 2 °C substantial firmness retention was achieved in disks from turning and ripe fruit incubated at pH 7.0 as compared to pH 4.5 (Table 1).

Effect of calcium and pH on pectin dissolution

Calcium ameliorates fruit firmness by binding to the carboxyl groups of the pectic homogalacturonan backbone, as postulated by the egg-box model (Grant et al., 1973), and may protect the pectic backbone from PG-mediated depolymerization (Wehr et al., 2004). The total amount of pectins was unaffected by pH or by the calcium treatments (Tables 2 and 3). In ripe fruit, calcium reduced the amount of water-soluble pectins and increased the ionically bound fraction at the end of a 4 h incubation period

(Table 2), as reported elsewhere (Mignani et al., 1995; Magee et al., 2003). In mature-green disks, calcium slightly reduced the water-soluble fraction but did not affect CDTA-soluble pectins (Table 2). Interestingly, the effects of calcium on pectin solubility were largely attenuated during storage. After 5 days, the effect of calcium infiltration on pectin solubility was generally non-significant, except in the case of water-soluble pectins from ripe fruit (Table 3). However, a decrease in the amount of water-soluble pectins during storage of calcium-treated disks was observed during storage at 2 °C (Tables 2 and 3), presumably due to the progressive integration of calcium in the cell wall matrix (Magee et al., 2003).

The water-solubility of pectins was significantly enhanced by acidity, especially in ripe tissue after a 4 h incubation period (Table 2). The effect of pH on the water-solubility of pectins from ripe disks can be attributed, at least in part, to the activity

Table 3
Uronic acids extracted from tomato disks at the mature-green and ripe stages treated with calcium and buffers of different pH and stored for 5 days at 2 °C

Factor	Uronic acids ($\mu g g^{-1}$)							
	Mature-green			Ripe				
	Total	WSP	CSP	Total	WSP	CSP		
Initial	$237.5 \pm 3.5 \ (100.0)$	$20.7 \pm 3.2 \ (8.7)$	$72.0 \pm 5.3 (30.3)$	$248.7 \pm 4.8 \ (100.0)$	43.6 ± 8.5 (17.5)	91.7 ± 11.9 (36.9)		
CaCl ₂ (mM)								
0	$238.7 \pm 3.8 \ (100.0)$	15.1 ± 2.7 (6.3)	$70.9 \pm 4.7 (29.7)$	$237.3 \pm 3.1 (100.0)$	$48.1 \pm 6.1 (20.3)$	$82.1 \pm 5.8 (34.6)$		
50	$238.0 \pm 2.9 \ (100.0)$	$11.1 \pm 2.4 (4.7)$	$78.4 \pm 3.8 \ (32.9)$	$234.7 \pm 3.1 \ (100.0)$	$33.8 \pm 4.3 \ (14.4)$	$91.6 \pm 6.1 \ (39.0)$		
pН								
7.0	$233.3 \pm 1.0 (100.0)$	$11.2 \pm 1.5 (4.8)$	$71.8 \pm 3.5 (30.8)$	$237.7 \pm 3.6 (100.0)$	$29.2 \pm 2.4 (12.3)$	$91.2 \pm 6.5 (38.4)$		
4.5	$243.5 \pm 3.8 \ (100.0)$	$15.0 \pm 3.3 \ (6.2)$	$77.6 \pm 5.1 \ (31.9)$	$234.3 \pm 2.2 \ (100.0)$	$52.8 \pm 5.0 \ (22.5)$	$82.6 \pm 5.5 \ (35.3)$		
P-value								
Calcium	0.490	0.292	0.177	0.342	0.007	0.226		
pН	0.320	0.378	0.665	0.346	0.000	0.399		
Calcium × pH	0.504	0.603	0.693	0.370	0.439	0.334		

Values are means \pm S.E. (n = 12) and within parenthesis are expressed as a percentage of the total uronic acids. WSP, water-soluble pectins; CSP, CDTA-soluble pectins.

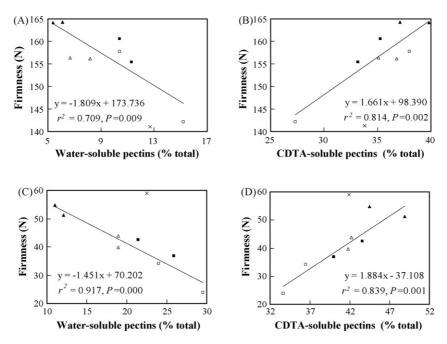


Fig. 2. Relationship between pectin (water and CDTA-soluble) and firmness of mature-green disks (A and B) and ripe (C and D) after a 4-h incubation at pH 4.5 ($\blacksquare \Box$) and pH 7 ($\blacktriangle \Delta$), with previous infiltration with CaCl₂ ($\blacksquare \blacktriangle$) or distilled water ($\Box \Delta$). Untreated disks (\times). The two identical symbols represent two different buffer solutions with the same pH. Each data point is the mean of three measurements.

of PG. This enzyme, present in large amounts in ripe tomato (Della Penna et al., 1986), has optimal activity at pH 4.5–5.0 and is nearly inactive at pH 7.0 (Chun and Huber, 1998). However, the effect of pH on the water-solubility of pectins was also observed in mature-green fruit, a stage of development in which PG is absent (Della Penna et al., 1986), suggesting that pH is influencing a PG-independent mechanism for pectin dissolution.

Collectively, the results reported here support the existence of a relationship between firmness, pectin dissolution, and apoplastic pH, in agreement with the idea that apoplastic pH plays a role in regulating fruit softening (Huber and O'Donoghue, 1993; Chun and Huber, 1998; Almeida and Huber, 1999; Manganaris et al., 2005; Almeida and Huber, 2007). In contrast with the effects on firmness, no significant interactions were observed between the effects of calcium and pH

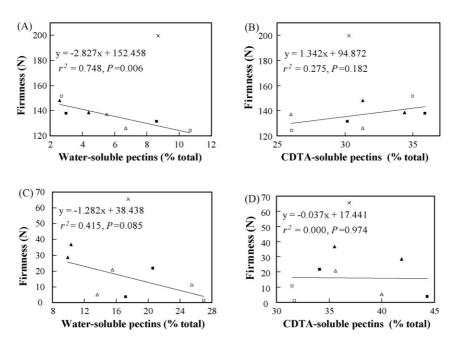


Fig. 3. Relationship between pectin (water and CDTA-soluble) and firmness of mature-green disks (A and B) and ripe (C and D) after a 5-day storage period, following incubation at pH 4.5 (\blacksquare \square) and pH 7 (\blacktriangle \triangle), with previous infiltration with CaCl₂ (\blacksquare \blacktriangle) or distilled water (\square \triangle). Non-treated disks (\times). The two identical symbols represent two different buffer solutions with the same pH. Each data point is the mean of three measurements.

on pectin solubility, indicating that their effects were exerted independently.

Relationship between pericarp firmness and pectin solubility

Clearly, both calcium and pH affected pectin dissolution and pericarp softening. The nature of the relationship between firmness and pectin dissolution under the different experimental conditions was explored by regression analysis. At the end of the 4h incubation period, significant linear relationships were observed between firmness and pectin dissolution in maturegreen and ripe fruit (Fig. 2). Disk firmness was negatively related with the percentage of water-soluble pectins and positively associated with the percentage of the CDTA-soluble fraction. Although the disassembly of the pectic matrix is a late occurring event during normal fruit ripening and softening (Rose et al., 1998), the linear relationships found between pectin dissolution and pericarp firmness suggest that pectin disassembly may be relevant to the texture of fresh-cut tomato, not only in the ripe stage, but also in mature-green fruit (Fig. 2A and B).

Surprisingly, after 5 days at 2 °C the initial relationship between firmness and pectin dissolution became weaker or disappeared completely (Fig. 3). The reasons why strong associations between firmness and pectin solubility at the end of short-term incubations were lost during subsequent storage are not clear, but a homeostatic response to low-temperature stress could be involved. Processing and subsequent handling of freshcut produce must be performed at low-temperature to maintain quality, even in chilling sensitive commodities such as tomato. Chilling injury can occur when tomato fruit is exposed to temperatures below 8-12 °C, with chilling-sensitivity decreasing as ripening progresses (Autio and Bramlage, 1986). The 5-day storage period at 2 °C imposes a chilling stress on the tomato pericarp disks. Under these conditions ion leakage (Autio and Bramlage, 1986) is likely to induce changes in the apoplastic solution that in turn influence the catalytic behavior of cell wall hydrolases. Moreover, chilling alters the activity of pectic enzymes. PG activity is strongly limited, whereas PME activity increases in chilled tomato fruit as compared to non-chilled fruit (Jackman et al., 1992; Marangoni et al., 1995). The chilling effects on cell wall metabolism and rearrangements of the wall structure may have contributed to reduce the initial effect of pH and calcium.

Implications for the texture of fresh-cut fruit

Although the treatments imposed in our experiments were very stringent and do not relate directly to the practice of freshcut processing, the results support the concept that apoplastic acidification can be detrimental to the texture of tomato, and likely to that of other fruit. However, despite the evidence that pH affects fruit texture (Knee, 1982) and cell wall metabolism (Chun and Huber, 1998), the effects of pH on softening remain largely ignored in fresh-cut fruit. Acidification of the apoplast in fresh-cut fruit occurs as a result of contamination with the vacuolar contents released during cutting and due to the

acidic solutions (e.g. citrate, ascorbate) widely used to inhibit polyphenoloxidase-mediated browning and reduce microbial growth (Beaulieu and Gorny, 2001; Lamikanra and Watson, 2001).

The results reported here suggest acidic conditions may have a detrimental effect on texture, which is not completely annulled by calcium. Localized apoplastic acidification resulting from cutting is probably impossible to avoid, but can be minimized by reducing the number of damaged cells through the usage of sharp blades. Moreover, if enzymatic browning is not the main factor limiting the shelf-life of fresh-cut fruit (e.g. tomato) acidic treatments can unnecessarily compromise texture.

Since PG only hydrolyses homogalacturonan regions whose uronic acid residues have been previously demethylated by PME (Koch and Nevins, 1989), and since pectins are synthesized and deposited on the cell wall largely esterified (Staehelin and Moore, 1995), the negative charges generated by PME are necessary for calcium binding to the cell wall and to bring about calcium's firming effects. Thus, the ability to maintain an apoplastic pH simultaneously favorable to the catalytic activity of PME and unfavorable to the activity of PG (e.g. pH 6.5–7.0), associated with calcium applications, can significantly contribute to texture retention in fresh-cut fruit.

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