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# Polygalacturonase Activity Does Not Fully Explain Textural Differences of Melting Flesh versus Non-melting Flesh Peaches

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**Texture is the main distinction between melting-flesh (MF) and non-melting flesh (NMF) peach cultivars. MF peaches soften extensively toward the end of the ripening process while NMF peaches soften more slowly and lack the "melting" stage of fruit softening. Two pectolytic enzymes thought to be involved in peach softening are terminal cleaving exo-polygalacturonase (exo-PG) and random cleavingendo-PG. The decreased capacity of NMF peaches to degrade cell walls (i.e., soften) is thought to be related to a deletion of endo-PG gene or a truncation of the mRNA. Thus, NMF cultivars would be expected to possess lower endo-PG activity than MF cultivars. In this study, the extractable PG activity of two MF cultivars, 'Flordaprince' and 'TropicBeauty', and two NMF cultivars, 'UFSun' and 'Gulfking', during the climacteric ripening stage were determined. 'Flordaprince' possessed similar endo- and exo-PG activities as the two NMF cultivars while 'TropicBeauty' had the highest endo- and exo-PG activities of the four cultivars. Surprisingly, the endo-PG activity of NMF 'Gulfking' was significantly higher than its exo-PG activity and was also higher than that of MF 'Flordaprince'. However, the higher endo-PG activity of 'Gulfking' was not reflected in its texture since it was almost five times firmer when ripe than 'Flordaprince'(10.77 N vs. 2.34 N), which implies that endo-PG activity does not fully explain the textural differences between MF and NMF peaches. Since cell wall disassembly presumably involves concerted and synergistic action of several different enzymes, other cell wall modifying enzymes may have a more crucial role than PG during peach fruit softening.**

Peaches are economically valuable, but excessive softening of traditional melting flesh (MF) types at the final stages of ripeness significantly reduces postharvest life due to increased mechanical injuries. Thus, non-melting flesh (NMF) peaches that soften relatively slowly and lack the "melting" phase are better suited for shipping and storage.

The texture difference between MF and NMF peaches is related to a reduced capacity for cell wall degradation in the NMF fruit (Shewfelt et al., 1971), which could be attributed to either a partial or complete deletion (Callahan et al., 2004), or mutation of the endopolygalacturonase (endo-PG) gene (Morgutti et al., 2006). Endo-PG (E.C. 3.2.1.15) is a cell wall modification enzyme that cleaves the pectin chain in arandom fashion and effectively reduces its molecular size (Pressey and Avants, 1978). Since endo-PG mRNA is highly expressed after the ethylene climacteric rise and the increasedenzyme activity is accompanied by rises in water-soluble pectin during ripening,especially in the MF peaches (Orr and Brady, 1993; Pressey and Avants, 1978), endo-PG is regarded as the primary enzyme responsible for peach softening (Lester et al., 1994). The changes to pectin in MF but not in NMF peaches in the final stage of ripening was considered by Fishman et al. (1993) to demonstrate that endo-PG is more active when extensive softening occurs.

In contrast to endo-PG, exo-PG (PG, E.C. 3.2.1.67) removes monomer units from the non-reducing end of the pectin chain and has a minimal effect on the size of the macromolecule (Pressey and Avants, 1978). According to Pressy and Avants (1973a) and Manganaris et al. (2006), exo-PG activity can be similar or higher in NMF than in MF peaches during ripening. Two forms of exo-PG in the mesocarp tissue of ripe MF peaches were distinguished, and the increased enzyme activity occurred only when the mesocarp tissue was very soft (Downs and Brady, 1990). Therefore, exo-PG may not be have an important role in initiation or promotion of fruit softening during ripening but may act together with endo-PG to produce the MF texture (Orr and Brady, 1993).

Based on previous research, a direct relationship between endo-PG and peach softening may be assumed; however, antisense RNA work in transgenic tomato did not support a direct relationship between endo-PG and softening(Carrington et al., 1993). Furthermore, ripening inhibition of avocados using 1-methylcyclopropene (1-MCP) also showed that endo-PG is not required for the extensive softening that occurs in ripening avocado fruit (Jeong et al., 2002).

Using two MF and two NMF low-chill subtropical peach cultivars, the relationship between PG activityand fruit softening after storage at 20 °C for 7 d was examined. It was expected that the two MF cultivars, 'Flordaprince' and 'TropicBeauty', would have higher endo-PG activities and similar or lower exo-PG activities than the two NMF cultivars, 'UFSun' and 'Gulfking'.

## Materials and Methods

**PLANT MATERIAL.** Fruit of the MF cultivars, 'Flordaprince' and 'TropicBeauty', and the NMF cultivars, 'Gulfking' and 'UFSun', were collected from the University of Florida Plant Science Research Unit at Citra, FL, in 2007 and 2010. Initial peel ground

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color (C.I.E. L*, a*, and b* values), measured objectively using a reflectance colorimeter (Minolta CR-400, Konica Minolta, Japan) on the greenest portion of the peel,was used to determine the maturity stage of the peaches. For all peach genotypes, the chromaticity a*-value (green-red) of the peel ground color (GCa*- value) increases the most with increasing maturation and ripening, whereas L* (lightness) and b* (yellow-blue) values change only slightly with maturation and ripening (Delwiche and Baumgardner, 1985). Since PG activity is higher at more advanced ripeness stages, at least nine fruit from each of the four cultivars with initial GC a*-values ≥15 were pooled together from three harvests in 2007. In 2010, fruit were collected from one harvest. 'Flordaprince' and 'Gulfking' were compared separately from 'TropicBeauty' and 'UFSun' since the former had initial GC a*-values ≥15 and the latter had a*- values <15. All the fruit were ripened at 20 °C for 7 d before firmness measurements and tissue collection. Fruit tissue was diced and stored at –20 °C until enzyme analyses were conducted.

**Flesh firmness determination.** Flesh firmness was measured with a texture analyzer model 1132 from Instron (Canton, MA) that applied a compressive force from a 50-kg load cell. A convex tip probe (Maness-Taylor type), 7.9 mm in diameter, was attached to the load cell moving at a speed of 12 cm/min. Flesh firmness was measured on the cheeks of the fruit on both sides with peel-removed and was expressed as the maximum bioyield force (N).

**Preparation of cell-free protein extract.** Enzyme extracts were prepared similarly to Jeong et al. (2002). Partially thawed mesocarp tissue (15 g) was homogenized with 25 mL of ice-cold 95% ethanol for 1 min in an Omnimixer (Model GLH-01, Newtown, CT) and centrifuged at 15,000 $g_n$ for 10 min at 4 °C. The supernatant was discarded and the pellets were resuspended in 25 mL of ice-cold 80% ethanol for 1 min and centrifuged at 15,000 $g_n$ for 10 min at 4 °C. The pellets were transferred to 10 mL of 50 mM sodium acetate buffer, pH 5, containing 0.5 M NaCl, for 30 min in an ice-cold water bath followed by centrifugation at 15,000 $g_n$ for 10 min at 4 °C. The supernatant was analyzed for enzyme activities. Total protein was measured using the bicinchoninic acid method with bovine serum albumin as the standard (Smith et al., 1985).

**Polygalacturonase activity.** Endo-PG activity was assayed by mixing 250 µL of enzyme extract with 250 µL of 0.5% polygalacturonic acid (from orange peel; Sigma Chemical Co., St. Louis, MO) in 50 mM sodium acetate buffer (pH 4.4) and incubated at 30 °C for 16 h according to Pressey and Avants (1973a). For measurement of exo-PG activity, 250 µL of enzyme extract was mixed with 250 µL of 0.5% polygalacturonic acid in 50 mM sodium acetate buffer (pH 5.5) containing 2 mM $CaCl_2$, and incubated at 30 °C for 16 h. Uronic acid (UA) reducing groups released were measured using the method of Milner and Avigad (1967) with mono-D-galacturonic acid as the standard. One unit of activity was defined as 1 µg ofgalacturonic acid produced per milligram of protein per hour.

**Statistical analysis.** One way ANOVA was used to detect significant differences in flesh firmness and enzyme activities among the cultivars. The least significant difference (LSD) test was used for mean separation.

## Results and Discussion

In 2007, 'TropicBeauty' was the only MF cultivar with a higher level of endo-PG activity than the two NMF cultivars after being stored at 20 °C for 7 d (Table 1a). The NMF cultivar 'Gulfking' had endo-PG activitythat was similar to MF 'Flordaprince', but 'Gulfking' was approximately 5 times firmer than 'Flordaprince'. In 2010, there were no differences in endo-PG activity for any MF and NMF cultivars paired by initial ground color although flesh firmness of each pair was significantly different (Table 2a). Since MF and NMF cultivars can have similar endo-PG activities, lack of endo-PG mRNA accumulation does not fully explain the delayed softening characteristic of NMF peaches. Furthermore, no direct relationship between endo-PG activity and flesh softening was found in this study. This confirms the report of Morgutti et al. (2006), who observed that peach fruit with essentially the same value of flesh firmness (46 N) showed barely detectable accumulation of endo-PG polypeptide in NMF 'OroA' and much higher accumulation in MF 'Bolero'.

Exo-PG activities of the two NMF cultivars and MF 'Flordaprince' were not significantly different after being stored at 20 °C for 7 d in 2007 (Table 1a). In 2010, NMF 'Gulfking' had significantly higher exo-PG activity than MF 'Flordaprince', whereas exo-PG activity was not significantly different for NMF 'UFSun' and MF 'TropicBeauty' (Table 2a), thus confirming the results of Pressey and Avant (1978) and Manganaris et al. (2006) that exo-PG activities of NMF fruit can be similar or higher than that of MF fruit after ripening.When comparing the endo- and exo-PG activities for each cultivar, NMF 'Gulfking' results differed between the two seasons in that it had lower exo-PG than endo-PG activity in 2007 (Table 1b). Therefore, it is possible to have lower, similar or higher exo-PG activity than endo-PG activity in ripe NMF fruit.

The level of endo-PG activity in peaches is relatively low compared to that of ripe tomatoes (Pressey and Avants, 1973a, 1973b). In this study, endo-PG and exo-PG activities of all peach cultivars were approximately 6–10 times and 3–8 times lower, respectively, than that of ripe tomatoes (Table 2b). This may reflect differences in the softening mechanism, cell wall structure, or the instability of enzyme in vitro (Orr and Brady, 1993).

## Conclusion

Based on the results presented, the previously reported low level of endo-PG mRNA and polypeptide cannot fully explain the delayed softening characteristics of peaches exhibiting the

Table 1a. Cultivar comparison after ripening at 20 °C for 7 days in 2007.

| Cultivar | Endo-PG activity (units)[z] | Exo-PG (units)[z] | Firmness (N) |
|---|---|---|---|
| Flordaprince (MF) | 0.67 bc | 0.73 b | 2.34 b |
| TropicBeauty (MF) | 2.86 a | 2.17 a | 2.86 b |
| UFSun (NMF) | 0.12 c | 0.25 b | 11.40 a |
| Gulfking (NMF) | 1.08 b | 0.63 b | 10.77 a |
| Significance | * | * | * |

[z]1 unit of PG activity = 1 µg galacturonic acid $mg^{-1}$ protein $h^{-1}$.
NS, *Nonsignificant or significant, respectively, at $P \leq 0.05$.

Table 1b. Endo- and exo-PG activity comparison for each cultivar in 2007.

| PG activity (units)[z] | Flordaprince (MF) | TropicBeauty (MF) | UFSun (NMF) | Gulfking (NMF) |
|---|---|---|---|---|
| Endo-PG | 0.67 | 2.86 | 0.12 | 1.08 |
| Exo-PG | 0.73 | 2.17 | 0.25 | 0.63 |
| Significance | NS | NS | NS | * |

[z]1 unit of PG activity = 1 µg galacturonic acid $mg^{-1}$ protein $h^{-1}$.
NS, *Nonsignificant or significant, respectively, at $P \leq 0.05$.

Table 2a. Cultivar comparison after ripening at 20 °C for 7 d in 2010.

| Initial GC a* value ≥ 15 | Endo-PG activity (units)[z] | Exo-PG activity (units)[z] | Firmness (N) |
|---|---|---|---|
| Flordaprince (MF) | 1.64 | 0.48 | 2.97 |
| Gulfking (NMF) | 1.29 | 1.22 | 8.03 |
| Significance | NS | * | * |
| **Initial GC a* value < 15** | **Endo-PG activity (units)[z]** | **Exo-PG activity (units)[z]** | **Firmness (N)** |
| TropicBeauty (MF) | 2.14 | 0.86 | 2.89 |
| UFSun (NMF) | 1.91 | 0.96 | 8.62 |
| Significance | NS | NS | * |

[z]1 unit of PG activity = 1 µg galacturonic acid $mg^{-1}$ protein $h^{-1}$.
NS, *Nonsignificant or significant, respectively, at $P \leq 0.05$.

Table 2b. Endo- and exo-PG activity comparison for each cultivar in 2010.

| PG activity (units)[z] | Flordaprince (MF) | TropicBeauty (MF) | UFSun (NMF) | Gulfking (NMF) | Ripe tomato |
|---|---|---|---|---|---|
| Endo-PG | 1.64 | 2.14 | 1.91 | 1.29 | 13.16 |
| Exo-PG | 0.48 | 0.86 | 0.96 | 1.22 | 3.69 |
| Significance | * | * | NS | NS | * |

[z]1 unit of PG activity = 1 µg galacturonic acid $mg^{-1}$ protein $h^{-1}$.
NS, *Nonsignificant or significant, respectively, at $P \leq 0.05$.

NMF trait since MF and NMF cultivars can have similar endo-PG and exo-PG activities. Endo-PG activity may not have a direct relationship with peach softening during ripening since no consistent relationship was found between endo-PG activity and flesh firmness. Other cell wall modifying enzymes may have a more crucial role than endo-PG during peach fruit softening.

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