

Chemical Composition and Physical Properties of Pericarp, Locule, and Placental Tissues of Tomatoes with Internal Bruising

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ABSTRACT. 'Solar Set' tomatoes (*Lycopersicon esculentum* Mill.) were harvested at the mature-green stage of development and treated with 50 $\mu\text{L}\cdot\text{L}^{-1}$ ethylene at 20 °C. Breaker-stage fruit were dropped from 40 cm onto a solid surface to induce internal bruising and held along with undropped fruit at 20 °C. At the ripe stage, pericarp, locule, and placental tissues were analyzed for soluble sugars, vitamin C, pigments, titratable acidity, soluble solids content, pericarp electrolyte leakage, extractable polygalacturonase activity, and locule tissue consistency. Bruising significantly affected chemical composition and physical properties of pericarp and locule tissues, but not placental tissue. For bruised locule tissue, carotenoids, vitamin C, and titratable acidity were 37%, 15%, and 15%, lower, respectively, than unbruised fruit. For bruised pericarp tissue, vitamin C content was 16% lower than for unbruised tissue, whereas bruising increased electrolyte leakage and extractable polygalacturonase activity by 25% and 33%, respectively. Evidence of abnormal ripening following impact bruising was confined to locule and pericarp tissues and may be related to the disruption of cell structure and altered enzyme activity.

During harvesting and handling operations, tomatoes are subjected to different mechanical injuries such as cuts, punctures, and impact, compression and vibration bruising. Physical impacts have also been associated with impaired ripening leading to the development of a physiological disorder known as internal bruising (IB). Halsey (1955) noted that impacted fruit showing no external evidence of damage had internal cellular breakdown in radial pericarp and locular tissues. Hatton and Reeder (1963) observed that bruised locule tissue appeared cloudy and collapsed, showing a yellow-greenish color. In severe cases, locule tissue showed accelerated shrinkage and became dry, and the placental tissue had watersoaked areas.

Incidence and severity of internal bruising are influenced by several factors such as variety, impact energy, number and site of impact, and fruit maturity. Increased CO₂ and ethylene evolution in tomatoes in response to increased number of impacts were observed by MacLeod et al. (1976). Sargent et al. (1992) observed that bruise severity is cumulative during commercial handling and noted that an impact directly over the locule, avoiding pericarp radial walls, will transmit maximum energy to the underlying locule tissue resulting in disruption of ripening and expression of internal bruising. Studies of mechanically injured cucumbers

(Miller et al., 1987) demonstrated increased extractable polygalacturonase, pectin methylesterase, peroxidase, and xylanase activities after impact bruising. In the apple industry, bruising lowers fruit grade, increases decay, and ultimately results in lost revenue (Guyer et al., 1991).

Numerous reports on internal bruising in tomato have focused on the physiological and metabolic effects on whole tomatoes. The purpose of this work was to investigate chemical composition and physical properties of pericarp, locule, and placental tissues of tomatoes in response to internal bruising.

Material and Methods

PLANT MATERIAL, TREATMENTS, AND STORAGE CONDITIONS. 'Solar Set' tomatoes, were harvested in Fall 1996, at the mature-green stage (USDA, 1976) at the Univ. of Florida's North Florida Research and Education Center located in Quincy, Fla., for the first experiment and at a commercial field in Ruskin, Fla., for the second experiment. Beginning with harvest, fruit were carefully handled to avoid mechanical damage and transported the same day to the laboratory in Gainesville. After sorting for existing blemishes and grading for weight (140 ± 10 g), unwashed fruit were gassed for ≈ 48 h with 50 $\mu\text{L}\cdot\text{L}^{-1}$ of ethylene in a flow-through system (flow rate = 50 $\text{mL}\cdot\text{s}^{-1}$) at 20 °C. When fruit reached the breaker stage (USDA, 1976), they were dropped from 40 cm onto a smooth, massive metallic surface. Each tomato was held by vacuum at the desired height to avoid fruit rotation during the drop test and to ascertain that impacts avoided radial pericarp walls. Each fruit was dropped twice, one impact at each of two equidistant points on the fruit equator. Tomatoes were then stored along with undropped fruit at 20 °C and 85% to 90% relative humidity until

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they reached the table-ripe stage. The table-ripe stage was defined as the point when fully red fruit had a noticeable deformation (3 to 4) mm as determined by a static force of 9.8 N applied to the equator with a metallic, convex probe (11 mm in diameter) for 5 s using a Cornell firmness device (Hamson, 1952) as modified by Gull et al. (1980).

CHEMICAL AND PHYSICAL ANALYSES. At the table-ripe stage, pericarp, locule, and placental tissues from impact areas were excised and analyzed for total soluble sugars, vitamin C, total carotenoids, total chlorophyll, titratable acidity, soluble solids content, locule tissue consistency, pericarp electrolyte leakage, and pericarp polygalacturonase activity. Only the outer pericarp wall was analyzed. Locule tissue included the jelly-like parenchymatous tissue, along with seeds. Seeds remained intact during locule tissue processing. Placental samples were excised at the interface with the inner pericarp wall (Hobson and Davies, 1971).

CARBON DIOXIDE AND ETHYLENE EVOLUTION. Upon reaching breaker stage, fruit were placed in 1.45-L glass jars (eight fruit/jar; four jars/treatment) under a continuous flow of humidified air ($1.03 \text{ mL}\cdot\text{s}^{-1}$) at 20°C for an 8-h equilibrium period. After initial carbon dioxide and ethylene readings were recorded, fruit were removed from the jars and impacts applied to half of the sample. Following impact, ethylene and CO_2 production were monitored with a gas chromatograph fitted with a flame ionization detector and alumina packed column every 24 h until fruit reached the table-ripe stage (model 5890, series II; Hewlett Packard, Avondale, Pa.). Carbon dioxide was monitored using a gas chromatograph (series 580; Gow Mac, Lehigh Valley, Pa.) fitted with a 80- to 100-mesh Poropak Q column.

TOTAL SOLUBLE SUGARS. Total soluble sugars were analyzed using 10 g of tissue homogenized at high speed in a commercial blender (Hamilton Beach/Proctor Silex, Inc., Washington, N.C.) for 3 min and centrifuged for 20 min at $18,000 g_n$ (JA-20 rotor). The supernatant was used to measure the concentration of total soluble sugars according to the method of Dubois et al. (1956), with glucose as the standard. Absorbance was determined at 490 nm and the concentration of total soluble sugars was expressed as grams glucose equivalents per kilogram fresh tissue.

VITAMIN C. For vitamin C analysis, 2 g homogenized tissue were combined with 20 mL of 6% metaphosphoric acid in 2 N acetic acid and centrifuged for 20 min at $18,000 g_n$ (rotor JA-20) at 4°C . The analysis was performed by the dinitrophenylhydrazine method of Terada et al. (1978). The concentration of total vitamin C was calculated in milligrams per kilogram of fresh weight from absorbance measured at 540 nm using pure ascorbic acid as a standard.

PIGMENT ANALYSIS. Total carotenoids were analyzed according to methods modified from Lime et al. (1957) and Umiel and Gabelman (1971). Pigment was extracted by homogenizing 15 g of fresh tissue with 30 mL of acetone in a Polytron (PT 1200; Brinkmann Instruments, Westbury, N.Y.) for 1 min at a speed setting of 5. The acetone-pigment extract and 45 mL of hexane were mixed in a separatory funnel and, after phase separation, the lower phase was discarded and the pigment-hexane extract was washed three times with 100 mL of deionized water. After the final wash, the extract was transferred to a 100-mL volumetric flask and the volume was brought up with hexane. Absorbance was read at 451 and 503 nm. Total carotenoids were expressed as milligrams per kilogram of fresh tissue.

Total chlorophyll was determined according to Inskeep and Bloom (1985). Two grams of tissue was added to aluminum foil-covered vials containing 5 mL of *N,N*-dimethylformamide (DMF). After 10 d at 4°C , solutions were filtered through Miracloth and absorbance read at 647 and 664.5 nm. Pigments were expressed as milligrams per kilogram of fresh tissue.

TITRATABLE ACIDITY AND SOLUBLE SOLIDS. For the determination of titratable acidity and soluble solids content, 40 g fresh tissue were homogenized in a commercial blender at high speed and centrifuged for 20 min at $18,000 g_n$ (JA-20). Aliquots of the supernatant were diluted with 50 mL of deionized water and titrated with 0.1 N NaOH to an end point of pH 8.2 using an automatic titrimer (model 395; Fisher Scientific Co, Pittsburgh). The amount of NaOH was converted to milliequivalents of citric acid per kg of fresh weight ($\text{mL NaOH} \times 0.1 \text{ N} \times 0.064$). Soluble solids content was determined using an ABBE refractometer (model MARK II; Cambridge Instruments Inc., New York).

ELECTROLYTE LEAKAGE. Electrolyte leakage was determined according to Whitlow et al. (1992). Discs (1 cm in diameter) from the equatorial region of pericarp tissue were removed with a sterilized brass cork borer (1 cm in diameter), washed briefly with deionized water, blotted dry on paper towels, and then placed in 15 mL of 300 mM mannitol. After 4 h at 20°C , the electrolyte content of the bathing solution was determined using a conductivity bridge (model 31A; Yellow Springs Instruments Co., Yellow Springs, Ohio). After 12 h incubation at 20°C , the solution was thawed at 5°C , heated in a boiling water bath for 25 min and total electrolyte content measured. Electrolyte leakage was expressed as a percent of total electrolyte content.

EXTRACTABLE POLYGALACTURONASE ACTIVITY. Extractable polygalacturonase (PG) activity was measured as described by Huber and O'Donoghue (1993). Briefly, 10 g of fresh pericarp tissue were homogenized with 95% ethanol in a Polytron and centrifuged ($18,000 g_n$) for 20 min at 4°C . The supernatant was discarded and the pellet was washed with 80% ethanol and centrifuged under the same conditions. The pellet was suspended for 30 min in an ice bath in 15 mL of a buffer solution (50 mM Trisma and 1.2 M NaCl) at pH 7.0. After centrifugation, the supernatant was filtered through Miracloth and maintained in an ice bath until assayed for enzyme activities.

Polygalacturonase activity was measured using polygalacturonic acid (Sigma Chemical Co., St. Louis) in 30 mM sodium acetate, pH 4.5. Activity was determined reductometrically by measuring uronic acid reducing sugars according to Milner and Avigad (1967). Polygalacturonase activity was expressed as μmol of galacturonic acid equivalents per g fresh tissue per hour. PG activity was measured only in the pericarp tissue since this protein was not detected in locule tissue (Cheng and Huber, 1996).

CONSISTENCY. Locule tissue consistency was measured according to a modification of the method of Askar and Treptow (1993)

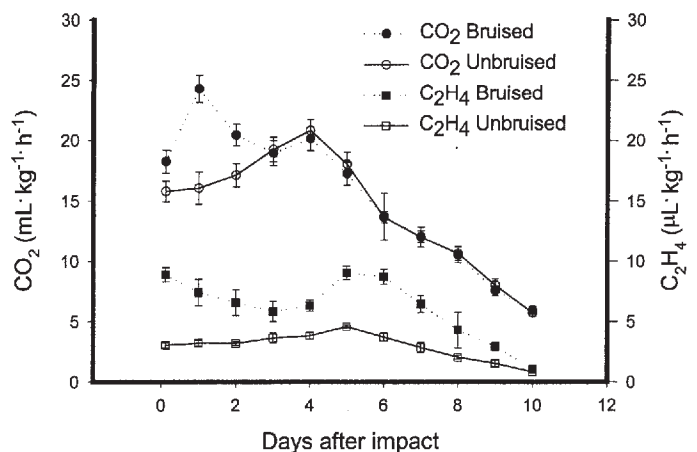


Fig. 1. Carbon dioxide and ethylene evolution of impacted and nonimpacted 'Solar Set' tomatoes during ripening at 20°C . Vertical bars indicate sd.

Table 1. Chemical composition of pericarp tissue in tomatoes with internal bruising.

Parameter	Bruised	Unbruised
Total soluble sugars (g·kg ⁻¹)	16.90 ± 0.37 a ^z	18.90 ± 0.83 a
Vitamin C (mg·kg ⁻¹)	162.80 ± 1.23 b	194.90 ± 2.13 a
Total carotenoids (mg·kg ⁻¹)	104.87 ± 1.14 a	108.03 ± 2.22 a
Total chlorophyll (mg·kg ⁻¹)	0.95 ± 0.05 a	0.40 ± 0.03 b
Titrateable acidity (CA meq·kg ⁻¹) ^y	168.75 ± 6.25 a	160.94 ± 1.56 a
Soluble solids content (%)	4.25 ± 0.21 a	3.98 ± 0.07 a
Electrolyte leakage (% total)	34.76 ± 2.06 a	27.76 ± 3.34 b
Polygalacturonase(GA μmol·kg ⁻¹ ·s ⁻¹) ^y	0.18 ± 0.01 a	0.13 ± 0.01 b

^zMeans ± SD within each quality attribute row with different letters are significantly different at $P < 0.05$ (F test).

^yCA = citric acid; GA = galacturonic acid.

Table 2. Chemical composition and physical properties of locule tissue in tomatoes with internal bruising.

Parameter	Bruised	Unbruised
Total soluble sugars (g·kg ⁻¹ ·kg ⁻¹)	14.90 ± 0.98 a ^z	12.50 ± 0.87 a
Vitamin C (mg·kg ⁻¹ ·kg ⁻¹)	193.30 ± 8.20 b	228.90 ± 5.44 a
Total carotenoids (mg·kg ⁻¹ ·kg ⁻¹)	55.24 ± 5.01 b	87.84 ± 2.23 a
Total chlorophyll (mg·kg ⁻¹)	0.71 ± 0.11 a	0.33 ± 0.06 b
Titrateable acidity (CA meq·kg ⁻¹) ^y	215.63 ± 20.31 b	254.69 ± 6.25 a
Soluble solids content (%)	4.18 ± 0.07 a	3.95 ± 0.07 a
Consistency (mm·s ⁻¹)	2.30 ± 0.10 b	3.70 ± 0.50 a

^zMeans ± SD within each quality attribute row with different letters are significantly different at $P < 0.05$ (F test).

^yCA = citric acid.

Table 3. Chemical composition of placental tissue in tomatoes with internal bruising.

Parameter	Bruised	Unbruised
Total soluble sugars (g·kg ⁻¹)	23.60 ± 0.98 a ^z	19.90 ± 2.50 a
Vitamin C (mg·kg ⁻¹)	151.60 ± 18.24 a	169.50 ± 18.10 a
Total carotenoids (mg·kg ⁻¹)	61.79 ± 9.73 a	61.09 ± 6.10 a
Total chlorophyll (mg·kg ⁻¹)	0.30 ± 0.09 a	0.22 ± 0.03 a
Titrateable acidity (CA meq·kg ⁻¹) ^y	210.94 ± 18.75 a	234.38 ± 20.31 a
Soluble solids content (%)	4.35 ± 0.14 a	4.20 ± 0.07a

^zMeans ± SD within each quality attribute row with different letters are significantly different at $P < 0.05$ (F test).

^yCA = citric acid.

using a Bostwick consistometer. Table-ripe fruit at 20 °C were sliced longitudinally and locule tissue along with seeds (30 mL) were carefully excised with a metal spatula and gently transferred into a gated chamber in the consistometer. The gate was opened and the flow rate (mm·s⁻¹) was determined.

STATISTICAL ANALYSIS. Analyses were performed using a completely randomized design, with two treatments (bruised and unbruised fruit) and four replications ($n = 10$ fruit). Data were subjected to analysis of variance using the general linear model (GLM) procedure of the Statistical Analysis System package. Differences between treatments were determined by F test. All comparisons were made at $P = 0.05$.

Results and Discussion

Dropped fruit showed internal symptoms similar to those described by Halsey (1955), MacLeod (1976), and Sargent et al. (1992). Locule tissue was completely collapsed and disorganized and seeds had apparently no physical contact with placental tissue. Placental tissue had almost no visual damage, although watersoaked areas were observed in a few fruit.

CARBON DIOXIDE AND ETHYLENE EVOLUTION. A transient increase in carbon dioxide and ethylene evolution was observed following impact. After 24 h, carbon dioxide increased from 16.4 to 24.5

mL·kg⁻¹·h⁻¹, while ethylene production tripled within 3 hours after impact (Fig. 1). These results are in agreement with the work of MacLeod et al. (1976) for impact bruising but are in contrast to the work of Silva and Calbo (1992), who observed a decrease in CO₂ evolution after compression bruising.

TOTAL SOLUBLE SUGARS. Unbruised placental tissue showed the highest concentration of total soluble sugars (TSS) among the three tissues studied, having 5.3% and 59.2% more TSS than pericarp and locule tissues, respectively (Tables 1–3). When bruised and unbruised tissues were compared, there was no statistical difference among the TSS of pericarp, locule, and placental tissues, although trends differed among tissues. Unbruised pericarp had 11.8% higher TSS than bruised pericarp. In contrast, bruised locule tissue had 19.2% higher TSS than unbruised tissue. Bruised placental tissue had 18.6% higher TSS than the unbruised tissue. The higher TSS in placental tissue may be related to its role in providing carbohydrates for the growth and development of seeds in the locule tissue.

VITAMIN C CONTENT. Unbruised locule tissue had the highest concentration of vitamin C, being 17% and 34% higher than unbruised pericarp and placental tissues, respectively (Tables 1–3). These results are in agreement with the work of Brecht et al. (1976) in studies with several unbruised tomato varieties.

Impact significantly altered vitamin C content in pericarp

(Table 1) and locule (Table 2) tissues. Bruised pericarp and locule tissues had $\approx 16\%$ less vitamin C than unbruised tissues. Although bruised placental tissue had 10.5% less vitamin C than unbruised tissue, differences were not significant statistically.

In his review of the role of vitamins in fresh fruit and vegetables, Watada (1987) noted that ascorbic acid is associated with quenching of free oxygen radicals. Perhaps, antioxidant protective systems associated with ascorbic acid are damaged by impact bruising, allowing oxidative depletion of ascorbic acid.

TOTAL CAROTENOIDS AND TOTAL CHLOROPHYLL. The highest concentration of total carotenoids was observed in the unbruised pericarp tissue, with 19% more pigment than unbruised locule tissue and $\approx 77\%$ more pigment than unbruised placental tissue (Tables 1 and 2). Impact significantly affected carotenoid content in locule tissue, with bruised fruit exhibiting 37% less pigment than unbruised fruit (Table 2). The decreased levels of carotenoids may contribute to the reduced production of certain volatiles noted in response to impact bruising. Moretti et al. (1997) observed that the concentration of 6-methyl-5-hepten-2-one, a compound suspected to be a lycopene-degradation product (Petró-Turza, 1987), was dramatically reduced in bruised locule tissue. Unbruised locule tissue had 34% more of this compound than bruised tissue.

Total chlorophyll content was $\approx 20\%$ higher in unbruised pericarp tissue than in unbruised locule tissue (Tables 1 and 2). Impact significantly disrupted chlorophyll degradation in both of these tissues but not in the placental tissue (Table 3). Bruised pericarp tissue had 137% higher chlorophyll levels ($0.95 \text{ mg}\cdot\text{kg}^{-1}$) than unbruised tissue ($0.40 \text{ mg}\cdot\text{kg}^{-1}$) (Table 1) and bruised locule tissue had 115% higher chlorophyll levels ($0.71 \text{ mg}\cdot\text{kg}^{-1}$) than the unbruised tissue ($0.33 \text{ mg}\cdot\text{kg}^{-1}$) (Table 2). The yellow-greenish color observed in bruised locule tissue during sample preparation was indicative of higher levels of total chlorophyll when compared to unbruised tissue. Whether the retention of chlorophyll levels in bruised tissues was due to inactivation of chlorophyll-degrading enzymes is not known.

TITRATABLE ACIDITY AND SOLUBLE SOLIDS. Unbruised locule tissue had the highest concentration of citric acid equivalents, being about 8.6 and 58.3% more than that of placental and pericarp tissues, respectively. Locule tissue from impacted fruit showed a 15.3% decrease in citric acid equivalents as compared with tissue from nonimpacted fruit. Pericarp and placental tissues, however, were not significantly affected. Unbruised placental tissue had the highest total soluble solids content among the different tissues (Tables 1–3). It was 5.5 and 6.3% higher than pericarp and locule tissues, respectively. These data are in agreement with the work of Stevens et al. (1977).

ELECTROLYTE LEAKAGE AND EXTRACTABLE POLYGALACTURONASE ACTIVITY. Impact significantly affected pericarp electrolyte leakage (Table 1). Bruised pericarp had 25.2% higher electrolyte leakage than unbruised tissue and water-soaked areas were observed around impact sites.

The increase in electrolyte leakage was probably due to disruption of cells at the impact site. Increased electrolyte leakage is a common response of plant tissues to stresses, including irradiation, chilling injury, high temperature injury, and other changes related to senescence (El Assi et al., 1997; King and Ludford, 1983). Elkashif and Huber (1988) reported that placental tissue from watermelon fruit exposed to $50 \mu\text{L}\cdot\text{L}^{-1}$ ethylene had significantly higher electrolyte leakage than tissue from fruit not exposed to ethylene. El Assi et al. (1997) observed that irradiation enhanced electrolyte leakage in both mature-green and pink tomatoes. Studies conducted by Legge et al. (1986) have also shown that disruption of cellular membranes leads to increases in electrolyte leakage.

Bruised pericarp had higher extractable PG activity (33.3%) than unbruised tissue (Table 1). Miller et al. (1987) observed that extractable PG activity increased 2-fold in the mesocarp of mechanically stressed cucumbers. They also observed that the activity of pectin methylesterase and xylanase increased after impact bruising. The mechanism involved in the stimulation of these enzymes in response to cellular injury is unknown.

CONSISTENCY. Bruised locule tissue was significantly more consistent than unbruised tissue (Table 2), requiring 60% more time to flow the same distance as unbruised locule tissue. It had a cloudy appearance and was almost totally separated from the outer pericarp tissue. Locule tissue volume in bruised fruit was lower than that for unbruised fruit (data not shown). This probably indicates that bruised locule tissue lost more water and/or solutes during ripening than the unbruised tissue. The physiological and metabolic significance of these phenomena is not known.

Consistency is the resistance to flow due to internal friction when fluids are in motion (Askar and Treptow, 1993). The most important contributors to variation in consistency are the pectins (Stevens, 1976). The composition and structure of tomato locule tissue pectins are different from those in tomato pericarp (Cheng and Huber 1996; Huber and Lee 1986) and may contribute to the higher bruise susceptibility of this tissue.

Changes in extractable PG activity and electrolyte leakage in bruised pericarp tissue were observed. Following impact, increased electrolyte leakage and extractable PG activity may increase ionic concentration and pectins in solution, with the resultant increase in locule tissue consistency.

Locule tissue was the most visibly affected of the three tissues studied. Since it is more fluid than the other tissues, internal bruising may have a dramatic effect on flavor acceptance by consumers. Locule tissue components are more readily perceived by taste receptors indicating that it has a greater contribution to tomato flavor than either pericarp or placental tissue (Stevens et al., 1977).

In summary, tomatoes exhibiting internal bruising showed lower quality than unbruised ones, suggesting a potential reduction in fruit grade and market price, which affects the entire tomato industry. The nature of bruise-induced ripening disruption, the interactions between the different fruit tissues, and the enzyme activities involved in the expression of bruise injury are issues that should be addressed in future studies.

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