Irradiation-induced Changes in Tomato Fruit and Pericarp Firmness, Electrolyte Efflux, and Cell Wall Enzyme Activity as Influenced by Ripening Stage

Najib El Assi, Donald J. Huber[1], and Jeffery K. Brecht
Department of Horticultural Sciences, POB 110690, Fifield Hall, University of Florida, Gainesville, FL 32611-0690

ABSTRACT. Mature green and pink tomato (*Lycopersicon esculentum* Mill.) fruit were subjected to ionizing irradiation in the range of 0.7 to 2.2 kGy from gamma-or X-ray sources. Firmness of whole fruit and pericarp tissue, pericarp electrolyte leakage, and pericarp cell wall hydrolase activities were measured following irradiation and during postirradiation ripening at 20 °C. Irradiation-induced softening was evident in mature-green and pink fruit within hours following irradiation, and differences between irradiated and control fruit persisted throughout postirradiation storage. Trends of firmness loss were much more consistent and showed much greater dose dependency in pericarp tissue than whole fruit. Irradiation enhanced electrolyte efflux in fruit of both maturity classes. Fruit irradiated at the mature-green stage softened during postirradiation storage but exhibited an apparently irreversible suppression in polygalacturonase activity, with levels remaining <10% of those of nonirradiated fruit. Polygalacturonase activity was less strongly affected in irradiated pink fruit than in mature-green fruit, but activity remained reduced relative to the controls. Pectinmethylesterase and β-galactosidase activities were significantly enhanced in irradiated fruit of both ripening stages in the early period following irradiation, but reductions were noted after prolonged storage.

Firmness is an important quality factor that influences the manner in which horticultural commodities are handled after harvest. Collectively, firmness directly influences consumer quality perception and is an important determinant of the ability of the commodity to withstand the stresses of postharvest handling.

In addition to the firmness changes characteristic of normal ripening, softening can also occur in response to irradiation treatments used to maintain postharvest quality or in postharvest quarantine operations (Kader, 1986). While irradiation may prolong the shelf life of many fruit, one deleterious consequence is the development of abnormal textural changes that can seriously compromise quality and handling endurance. Irradiation-induced changes in textural properties have been reported for pear (*Pyrus communis* L.), peach (*Prunus persica* Stokes), nectarine (*Prunus persica* L.) (Maxie and Sommer, 1963), apple (*Malus pumila* Mill.) (Boyle et al., 1957; Eric et al., 1970; Glegg et al., 1956), lemon (*Citrus limon* (L.) Burm.f.), orange (*Citrus sinensis* L. Osbeck) (Maxie and Sommer, 1963), grape (*Vitis vinefera* L.) (Maxie et al., 1964), strawberry (*Fragaria ananassa* Duch.) (Brecht et al., 1992; Johnson et al., 1965), sweet cherry (*Prunus avium* L.) (Massey et al., 1965), and in other organs including carrot (*Daucus carota* L.) roots (Skou, 1963) and potato (*Solanum tuberosum* L.) tubers (Hayashi et al., 1992). The irradiation threshold at which deleterious changes are observed varies significantly for various plant tissues (Glegg et al., 1956).

The softening of tomato fruit in response to irradiation is typically evident within hours following treatment, even for mature-green fruit (Abdel-Kader et al., 1968; Ahmed et al., 1972; Bramlage and Lipton, 1965; Yasir et al., 1987). The basis of irradiation-induced firmness loss has not been established but may involve turgor loss due to membrane damage (Hatton et al., 1984;

Srb and Hluchovsky, 1963) or effects on specific cell wall enzymes (Yasir et al., 1987). Enhanced ion efflux was reported in discs from irradiated carrots (Skou, 1963) and potato tubers (Hayashi et al., 1992). Polygalacturonase (PG) activity was not noticeably affected by irradiation of tomato fruit at 1.0 kGy and higher, although PG activity during postirradiation storage was slightly suppressed compared with nonirradiated controls (Yasir et al., 1987).

The objectives of this study were to investigate the influence of irradiation on the electrolyte efflux and firmness of mature green (preripe) and pink (ripening) tomato fruit during postirradiation storage. Firmness was measured with intact fruit and with discs prepared from the outer pericarp tissue. Polygalacturonase, β-galactosidase, and pectinmethylesterase activities were measured in irradiated fruit, since these enzymes are most often implicated in firmness changes occurring during normal ripening (Fischer and Bennett, 1991).

Materials and Methods

PLANT MATERIAL. 'Sunny' tomatoes were harvested at the mature-green and pink stages of development from commercial fields near Ruskin, Fla. In a second experiment, mature-green and pink fruit were harvested from field plantings at the Univ. of Florida Gulf Coast Research and Education Center, Bradenton. In a third experiment, mature-green and pink 'Sunny' fruit were obtained from a commercial packing house in Ft. Pierce, Fla., and transported to Gainesville. Nearly all of the nonirradiated green fruit were at the breaker stage (appearance of lycopene at blossom end) within 48 h at 20 °C, confirming the mature status of these specimens at harvest. Fruit were selected for uniformity of size and freedom from defects, surface sterilized with 1 mM sodium hypochlorite (NaOCl) for 1 min, rinsed in H_2O, and dried using paper towels. Irradiation treatment was performed as described below.

IRRADIATION TREATMENT. Fruit for the first two experiments were irradiated within 3 to 4 h after harvest at Vindicator Food Technology Service Inc. using gamma-rays emitted from Cobalt-60 at doses of 0, 0.73 ± 0.11, or 2.21 ± 0.22 kGy. Tomatoes were placed

in standard shipping cartons that were maintained on a rotating table during irradiation. Following irradiation, fruit from cartons receiving a similar dose were combined and selected randomly for postirradiation measurements. Following irradiation, fruit were maintained at 20 °C and removed at intervals for firmness determinations and other analyses as described below.

In instances where the Vindicator facilities were unavailable for our studies, irradiation was performed using an electron-beam X-ray source at the Florida Dept. of Agriculture and Consumer Services, Division of Plant Industry (DPI) facilities in Gainesville. The third experiment reported in this study used the electron-beam X-ray source at 0.72 ± 0.11 or 1.41 ± 0.15 kGy (dosimetry monitored by DPI staff). Fruit were placed on 24-cell polyester trays, one fruit per cell, and four trays were placed in a standard 11.4-kg tomato carton. Since the DPI facilities did not permit automated rotation of the irradiated target, after receiving one-half of the desired doses, the cartons were rotated 180° before completing the irradiation exposure. Following irradiation, the fruit were transferred to storage at 20 °C. In each experiment, a minimum of 96 fruit of each maturity class (mature green and pink) were used for each irradiation level. Treatment facilities were within the range of 22 to 25 °C and irradiation duration did not exceed 2 h.

DETERMINATION OF WHOLE FRUIT AND PERICARP FIRMNESS. Firmness of whole fruit was determined using a Cornell firmness device (Hamson 1952) as modified by Gull et al. (1980). Individual fruit (24 for each maturity class at each irradiation level) were positioned on their side in a concave rubber ring, a 1-kg mass was applied to the upper fruit surface, and deformation in millimeters was measured after 5 s. Measurements were taken at three positions along the equatorial plane of the fruit, avoiding regions subtended by radial pericarp walls. In the first experiment, the initial firmness determinations were performed within 5 h after irradiation and again at daily intervals for 6 d for the pink and 12 d for the mature-green fruit. In the second experiment, initial measurements were performed within 24 h of irradiation treatment and at daily intervals over 8 d for the pink and 10 d for the mature-green fruit. Measurements of whole fruit firmness were determined using a minimum of 24 fruit from each treatment.

Pericarp firmness was monitored in tissue discs prepared from external pericarp of irradiated and control fruit. Discs (1 cm in diameter) were excised using a cork borer and trimmed of exocarp and endocarp tissue to 3 mm in thickness with razor blades positioned between plexiglass plates (Ahrens and Huber, 1990). Twelve discs from four fruit were used at each sampling time. Discs were tested using a food textural measurement instrument (model 1132; Instron Corp., Canton, Mass.) equipped with a 50-kg mechanical load cell. Crosshead speed was 10 cm·min⁻¹. Discs were compressed to the point of tissue failure, or bioyield, with a 7.5-mm-diameter convex probe. Data are presented as the maximum force recorded during disc compression and failure.

ELECTROLYTE EFFLUX. Electrolyte efflux was determined using a conductivity bridge equipped with a conductivity electrode (models 31A and 3403A, respectively; Yellow Springs Instruments Inc., Yellow Springs, Ohio). Pericarp discs (1 cm in diameter) from the same fruit used for pericarp firmness determinations were excised from the equatorial region (five discs per fruit, four fruit per treatment), rinsed briefly in distilled water, and then transferred to 14 mL of 300 mM mannitol in 50-mL beakers and placed on a shaker. Conductivity of the bathing solution was measured after 1 and 3 h (for fruit irradiated with gamma rays at 0.73 or 2.21 kGy) or 2 and 4 h (X-rays at 0.72 or 1.41 kGy), and total tissue electrolytes were determined after subjecting the discs to a freeze–thaw cycle. Net efflux was expressed as a percentage of total tissue electrolyte content.

EXTRACTION OF POLYGALACTURONASE, PECTINMETHYLESTERASE, AND β-GALACTOSIDASE. Salt-extractable protein was prepared from alcohol-insoluble solids as described (Huber and O'Donoghue, 1993). Briefly, 3 g of external pericarp tissue was homogenized in 15 mL 80% ethanol and centrifuged at 15,000× g for 20 min at 4 °C. The pellet was resuspended in 25 mL cold 80% ethanol and again centrifuged. The final pellet was suspended in 15 mL 50 mM Tris, pH 7.0, containing 1.2 M NaCl for extraction of proteins. After incubating the suspension for 30 min in an ice bath, the sample was centrifuged at 15,000× g for 20 min at 4 °C. The supernatant was filtered through Miracloth, and the filtered extract was maintained on ice until assayed for polygalacturonase (PG, EC. 3.2.1.15), pectinmethylesterase (PME, EC 3.1.1.11), and β-galactosidase (EC 3.2.1.23) activities.

ASSAY OF SALT-EXTRACTABLE ENZYMES. PG activity was determined using polygalacturonic acid substrate (Sigma). One-half milliliter of substrate (1 mg) in 30 mM Na-acetate, 150 mM NaCl, pH 4.5, along with 0.1 mL of active or heat-inactivated protein were incubated for 30 min at 34 °C. The reaction was terminated by adding copper reagent, and activity was determined reductometrically (Milner and Avigad, 1967). Enzyme activity was expressed on a tissue fresh mass basis as micromoles galacturonic acid reducing equivalent per gram per minute. Galacturonic acid was used as the standard.
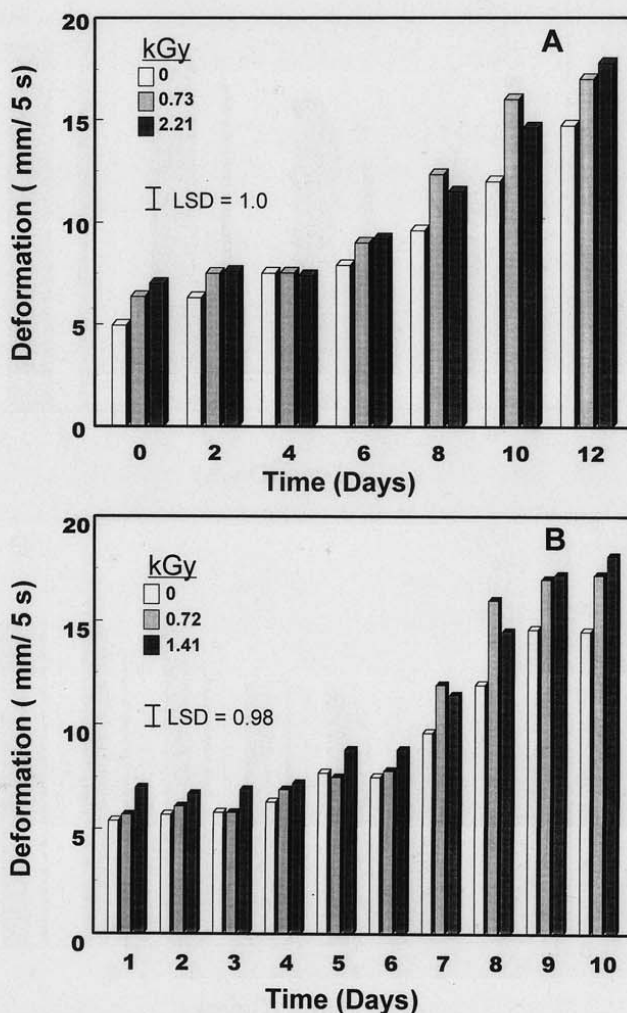

Fig. 1. Firmness of mature-green tomato fruit during storage at 20 °C following irradiation at 0, 0.73, or 2.21 kGy (**A**) and 0, 0.72, or 1.41 kGy (**B**). Vertical bars = LSD ($P < 0.05$).

101

β-Galactosidase activity was determined as described by Huber and Nevins (1981). A 0.1-mL aliquot of the protein extract was added to 0.1 mL substrate (25 mM ρ-nitrophenyl β-D-galactopyranoside) in 10 mM sodium acetate, pH 5.5, along with 0.8 mL of the 10 mM acetate. After 30 min at 30 °C, the reaction was terminated by adding 2 mL of 200 mM sodium carbonate. Absorbance was measured at 400 nm. Activity expressed on tissue fresh mass basis as micromoles nitrophenol per gram per hour. Free nitrophenol (Sigma) was used as standard.

PME activity was assayed by determining the release of methanol from citrus pectin as described by Wood and Siddiqui (1971) with slight modifications. A 0.2-mL aliquot of active or heat-inactivated protein along with 0.3 mL of citrus pectin substrate (600 µg) in 100 mM sodium acetate, pH 6.0, were incubated for 60 min at 30 °C. The reaction was terminated by adding 0.5 mL of 2 N sulfuric acid and 0.2 mL of 2% (w/v) aqueous potassium permanganate and swirling gently. After incubation in an ice bath for 15 min, 0.2 mL of 0.5 M sodium arsenite in 0.12 N sulfuric acid was added followed by 0.6 mL of deionized water. After 1 h at 25 °C, 2.0 mL of 0.02 M acetylacetone in 2 M ammonium acetate and 0.05 M acetic acid were added. After shaking, the tubes were heated at 60 °C for 15 min and then cooled to room temperature. Absorbance was measured at 412 nm using a recording spectrophotom-

eter (UV-160; Shimadzu Corporation, Tokyo). Activity expressed on a tissue fresh mass basis as micromoles methanol released per gram per minute. Methanol was used as standard.

Data were analyzed using the analysis of variance (ANOVA) procedure and means separation with least significance difference (LSD).

Results and Discussion

FIRMNESS OF WHOLE FRUIT FOLLOWING IRRADIATION. A significant decrease in whole fruit firmness was apparent within 5 h following irradiation in the first experiment for mature-green tomato fruit irradiated at 0.73 or 2.21 kGy (Fig. 1A) and within 24 h in the second experiment for fruit irradiated at 0.72 or 1.41 kGy (Fig. 1B). The initial firmness loss in green fruit was not as extensive as was reported for green fruit irradiated at 2.0 to 6.0 kGy (Abdel-Kader et al., 1968). Mature-green fruit subjected to 2.5 or 5.0 kGy exhibited firmness values comparable to fully ripe, nonirradiated fruit (Yasir et al. 1987). Bramlage and Lipton (1965) found that the influence of irradiation (1.25 to 5.0 kGy) on tomato firmness was cultivar dependent, with the effects being significant immediately following irradiation in 'Early-Pak' and 'Pearson' but persistent throughout ripening only in 'Early-Pak'. Irradiated fruit were significantly softer during the late postirradiation period when compared to nonirradiated fruit (Fig. 1).

Figure 2 illustrates the firmness of pink fruit irradiated at 0.73 or 2.21 kGy (Fig. 2A) and 0.72 or 1.41 kGy (Fig. 2B). In the first experiment (Fig. 2A), irradiated pink fruit were significantly less firm than control fruit on the second day following treatment, but this difference did not persist to 4 and 6 d post-treatment. In a second experiment, irradiated fruit were less firm than controls on the third and fifth days for pink fruit irradiated at 0.72 or 1.41 compared with the control fruit (Fig. 2B).

The effect of irradiation on pink fruit was of lower magnitude than observed for mature-green fruit. Abdel-Kader et al. (1968) reported a relative decrease in the effect of irradiation on firmness of tomato fruit ('Early Pak No. 7') at an advanced stage of ripening, whereas Bramlage and Lipton (1965) noted that riper fruit softened more immediately following irradiation than fruit at the green or breaker stage.

Although whole-fruit firmness was clearly influenced by irradiation treatment, the magnitude and significance of the effects were variable within and among different experiments (Figs. 1 and 2). As shown in a study of several tomato cultivars differing in firmness (Ahrens and Huber, 1990), the patterns of decreasing firmness during ripening were much more distinct and consistent when measured with excised pericarp tissue. These results indicated that the locule and other internal tissues may be important factors in the high variability of whole-fruit firmness. Furthermore, in view of the fact that the internal tissues of tomato fruit ripen in advance of external pericarp, the respective tissues may exhibit different responses to irradiation. To investigate the possibility that the development of the internal tissues was obscuring irradiation effects as determined with whole fruit, firmness changes were examined in pericarp excised from irradiated fruit.

FIRMNESS OF PERICARP TISSUE AS AFFECTED BY IRRADIATION. Firmness loss in pericarp tissue was apparent 24 h following irradiation at 0, 0.72, and 1.41 kGy (Fig. 3). In contrast to whole fruit, the effect of irradiation on pericarp firmness for mature-green fruit became more pronounced during fruit storage and was more clearly dose dependent, with the higher dose resulting in significantly enhanced firmness loss (Fig. 3A). In contrast to the progressive loss of pericarp firmness in the control, firmness of pericarp
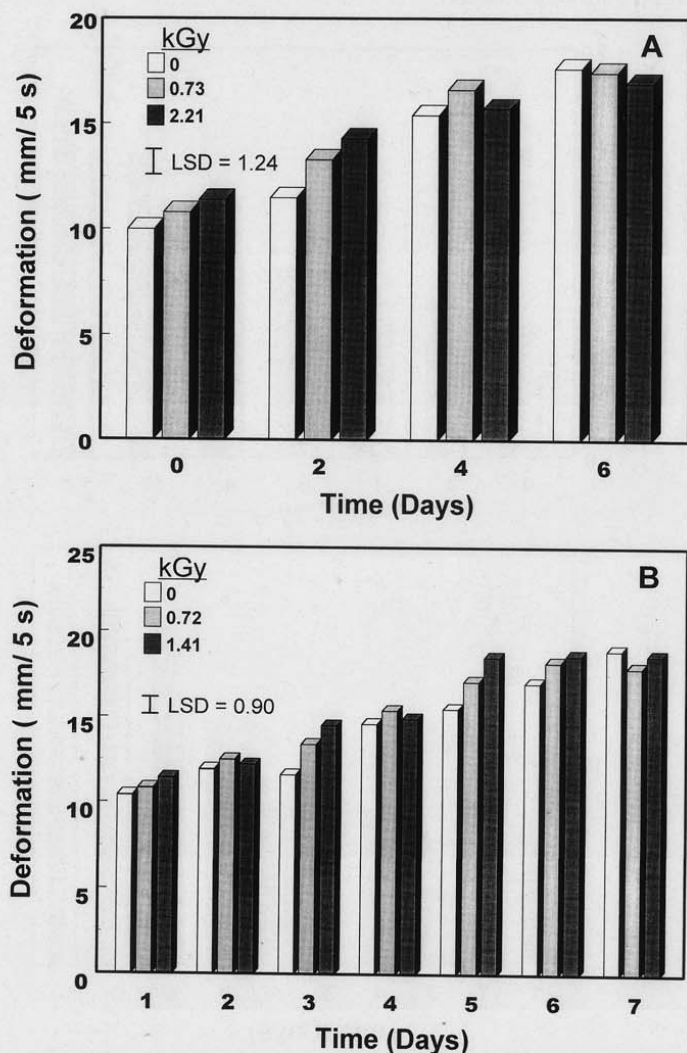

Fig. 2. Firmness of pink tomato fruit during storage at 20 °C following irradiation at 0, 0.73, or 2.21 kGy (A) and 0, 0.72, or 1.41 kGy (B). Vertical bars = LSD (*P* < 0.05).
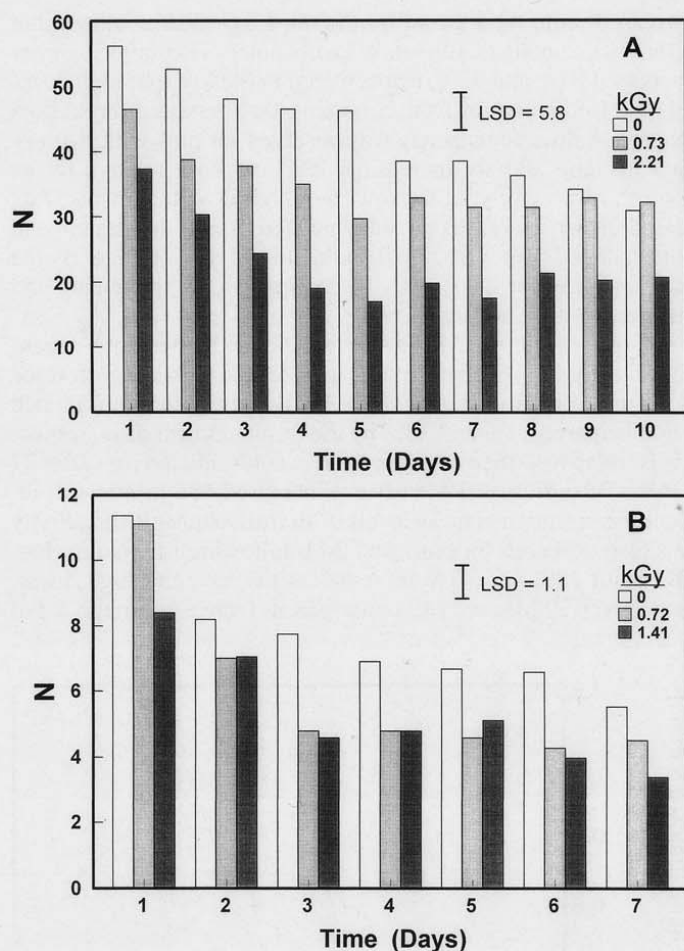
Fig. 3. Firmness of mature-green (A) and pink (B) pericarp tissue following irradiation of fruit at 0, 0.73, or 2.21 kGy. Firmness expressed in Newtons (N). Vertical bars = LSD ($P < 0.05$).

from irradiated fruit decreased more sharply over the initial 5 d of storage than the remaining storage period. By 7 d after treatment, firmness of pericarp from the low-dose treatment was comparable to that of pericarp from the control fruit. Significant differences persisted between control and high-dose treatments through 10 d (Fig. 3A). As observed for mature-green fruit, the effect of irradiation on the firmness of pink fruit was considerably more pronounced in pericarp (Fig. 3B) compared with whole fruit. While irradiation effects on firmness were observed throughout the storage period, no dose response was noted until the sixth and seventh days following irradiation. After 6 to 7 d of storage for pink fruit, at which time values for whole fruit firmness were similar between the irradiated and control fruit (Fig. 2 A and B), irradiation dose effects remained significant in pericarp (Fig. 3B).

ELECTROLYTE EFFLUX. Electrolyte efflux values of pericarp tissue from fruit treated with X-rays at 0.72 or 1.41 kGy or gamma rays at 0.73 or 2.21 kGy are shown in Tables 1 and 2 . No differences in electrolyte efflux were noted between control and irradiated mature-green tomato fruit following 1-h incubations in 300 mM mannitol (Table 1). After 3 h (Table 1) in the first experiment using X-rays or 2 h in a second trial using gamma-rays (Table 2), discs from green fruit exposed to the higher dose exhibited 25% to 37% higher net efflux, respectively, relative to the control treatment. Following longer incubation in mannitol, 21% (Table 1) and 11% (Table 2) higher net efflux were noted for discs from mature-green fruit irradiated at the high doses com-

pared with the controls. The efflux of K^+ (80% of tissue K^+) and amino acids (>50% of total) from mature-green tomato tissue (Yasir et al., 1987) was considerably higher than the overall efflux values for mature-green fruit (about 30%) shown in Tables 1 and 2. Our data, however, reveal a much greater response of ion efflux to irradiation than was noted by Yasir et al. (1987). The use of distilled water as an incubation medium (Yasir et al., 1987) likely contributed to the high efflux values noted in all treatments and, consequently, diminution of the irradiation effects.

Electrolyte efflux from pink fruit was higher than that for mature-green fruit and was also more sensitive to irradiation (Tables 1 and 2), with discs from irradiated pink fruit exhibiting 27% and 60% increases in electrolyte efflux at the low and high X-ray doses, respectively, measured after 1 h of incubation (Table 1). In a second experiment with pink fruit, 62% and 93% increases in efflux were noted for the low and high dose treatments, respectively (Table 2). Generally, irradiation effects persisted during prolonged (3 or 4 h) incubation of discs from pink fruit.

Our data demonstrate significant and rapid effects of irradiation on membrane properties and are consistent with reports for irradiated potato tubers (Hayashi et al., 1992) and carrot roots (Skou, 1963). The inherently higher leakage of pink fruit compared with green fruit is characteristic of electrolyte efflux trends reported for ripening fruit and other senescing tissues (Elkashif and Huber 1988; Ferguson and Watkins, 1981; Palma et al., 1995). The more pronounced effects of irradiation on electrolyte efflux in pink compared with green fruit may be a consequence of increased membrane dysfunction and decreased repair capacity. Voisine et al. (1993) reported that phospholipase D activity increased in gamma-irradiated cauliflower (*Brassica oleracea* L.) florets. Mitochondria from irradiated ripening pear showed a reduced recovery in oxidative competence compared with mitochondria from irradiated preripe fruit (Romani et al., 1968). Electrolyte efflux in response to irradiation may also reflect, in part, radiolytic solubilization of electrolytes originating in the cell wall (Triantaphylidès et al., 1994).

POLYGALACTURONASE, β-GALACTOSIDASE, AND PECTINMETHYLESTERASE ACTIVITIES. The influence of irradiation on firmness of tomato fruit and pericarp tissue prompted an investigation of the effects of irradiation on polygalacturonase (PG), pectinmethylesterase (PME), and β-galactosidase activities. All of these enzymes exist in multiple forms in tomato fruit; however, our study addressed total activities without regard to specific isozymes. PG was not detected in mature-green fruit, either before or within 24

Table 2. Electrolyte efflux of pericarp discs from mature-green and pink tomato fruit irradiated at 0, 0.73, or 2.21 kGy[z].

	Electrolyte efflux (% total)	
Treatment	2 h	4 h
Mature-green		
Control	19.3 a[y]	30.4 ab
0.72 kGy	20.9 a	28.4 a
2.21 kGy	28.4b	33.7 b
Pink		
Control	22.4 a	37.4 a
0.72 kGy	36.3 b	43.0 ab
2.21 kGy	43.4 c	48.4 b

[z]Electrolyte efflux values are expressed as percentage of total electrolyte content.
[y]Values in columns within ripening stage followed by the same letter are not significantly different at $P < 0.05$ (LSD).

Table 1. Electrolyte efflux in pericarp discs from mature-green and pink tomatoes irradiated at 0, 0.72, or 1.41 kGy[z]

Treatment	Electrolyte efflux (% total)	
	1 h	3 h
Mature-green		
Control	15.4 a[y]	24.3 a
0.72 kGy	13.9 a	23.5 a
1.41 kGy	15.2 a	29.4 b
Pink		
Control	24.9 a	22.4 a
0.72 kGy	31.8 b	36.3 c
1.41 kGy	40.0 c	52.0 d

[z]Electrolyte efflux expressed as percentage of total electrolyte content.
[y]Values in columns within each maturity stage followed by the same letter are not significantly different at $P < 0.05$ (LSD).

h of irradiation (Fig. 4A). PG was detected in nonirradiated mature-green fruit by the third day after treatment, at which time these fruit were exhibiting external signs of ripening. Lower PG activity was present in the fruit treated at the low irradiation dose. PG was not detected in green fruit irradiated at the high dose until 5 d after treatment, after which time little further increase in PG activity was observed in fruit from either irradiation treatment. At 9 d after irradiation, PG activity in the low- and high-dose treated fruit was about 9% and 3%, respectively, of PG activity in the nonirradiated fruit. These data contrast with those of Yasir et al. (1987), who observed that PG accumulation in mature-green fruit subjected to 1 kGy irradiation was largely unaffected until after 6 d of storage, when irradiated fruit exhibited about 60% of the activity of control fruit. The data of Yasir et al. (1987) are atypical in that PG levels in mature-green fruit were nearly 40% of the levels found after 6 d of storage, when fruit were nearly ripe. This is unusually high PG for preripe tomato and indicates that these fruit may have been beyond the mature-green stage at the time of irradiation. As shown below, the response of PG activity is quite different for fruit irradiated when mature green versus during ripening.

PG activity was detected in all pink fruit (Fig. 4B), consistent with the accumulation of this protein following the onset of tomato ripening (Tucker and Grierson, 1982). When assayed 24 h after irradiation, the levels of PG were reduced at both irradiation doses compared to nonirradiated fruit. During further ripening, PG activity increased in all fruit, although the levels remained significantly lower in the irradiated than nonirradiated fruit. Except for the determinations at 24 h, the irradiation-induced suppression of PG activity showed no dose dependency. PAGE and Western analysis (data not shown) revealed that irradiation-induced suppression of PG activity was accompanied by a significant reduction in the accumulation of PG protein, particularly in fruit irradiated when mature-green. These data suggest that the capacity of tomato fruit to synthesize and accumulate PG depends on the timing of irradiation treatment and may have implications regarding the relative susceptibility to irradiation of transcriptional versus translational events. In a study of ACC synthase synthesis in γ-irradiated cherry tomatoes, Larrigaudière et al. (1990) speculated that irradiation (1 kGy) temporarily suppressed transcription of some genes and stimulated translation of preexisting transcripts.

The response of β-galactosidase activity in mature-green fruit to irradiation is illustrated in Fig. 5A. β-Galactosidase activity 24 h after irradiation was slightly higher (18%) at the high dose compared with the low dose and control. β-Galactosidase activity

increased more than 2-fold by the third day after treatment but differences among treatments were not noted. Thereafter, activity decreased 37% and 32%, respectively, in the low and high doses relative to the control fruit, a ranking that persisted throughout storage. A dose dependency was observed for pink fruit, with β-galactosidase activity increasing 35% and 86% relative to the control, respectively, at the low- and high-dose treatments (Fig. 5B). Further increases in enzyme activity in irradiated and nonirradiated fruit were noted on the third day of storage. By the fifth and seventh days, activity declined in all treatments and differences were not significant.

Mature-green fruit 24 h after irradiation exhibited dose-dependent increases in PME activity, with levels remaining elevated through 5 d of storage (Fig. 6A). PME activity in nonirradiated fruit decreased 12% and 32% by the third and fifth days, respectively, relative to the activity present in nonirradiated fruit after 24 h. After 7 d of storage, PME activity increased 76% in control fruit, resulting in similar activity levels in all fruit. Higher PME activity was also observed for pink fruit 24 h following irradiation (Fig. 6B), with 18% and 47% increases at the low and high doses, respectively. PME activity continued to increase through a 5-d
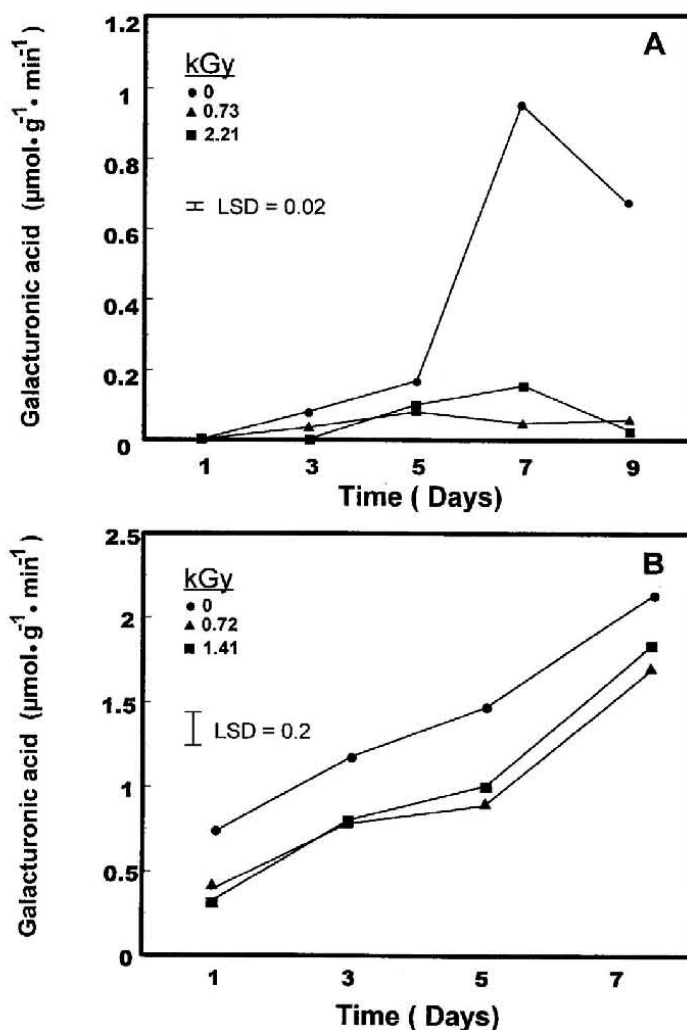
Fig. 4. PG activity of mature-green (A) and pink (B) tomato fruit following irradiation at 0, 0.72, or 1.41 kGy. Samples were prepared 24 h and again at 2-d intervals after irradiation and analyzed for PG activity. Units are expressed as micromoles galacturonic reducing equivalents per gram pericarp fresh mass per minute. Vertical bars = LSD ($P < 0.05$).
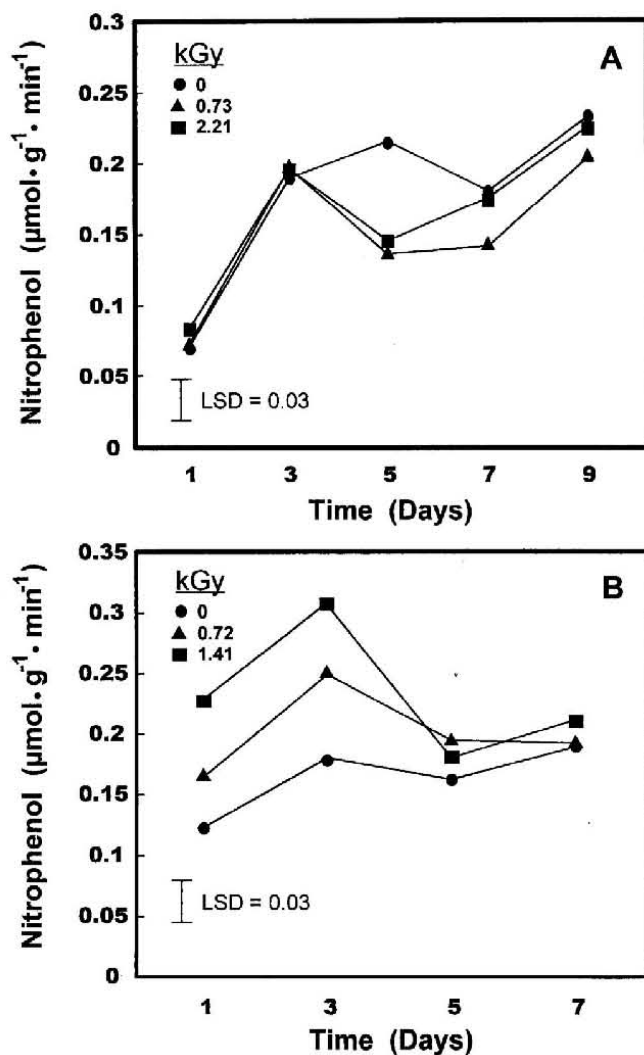
104

Fig. 5. β-Galactosidase activity of mature-green (A) and pink (B) tomato fruit following irradiation at 0, 0.72, or 1.41 kGy. Fruit were selected 24 h and again at 2-d intervals after irradiation and analyzed for *B*-galactosidase activity. Units are expressed as micromoles nitrophenol per gram pericarp fresh mass per minute. Vertical bars = LSD (*P* < 0.05).
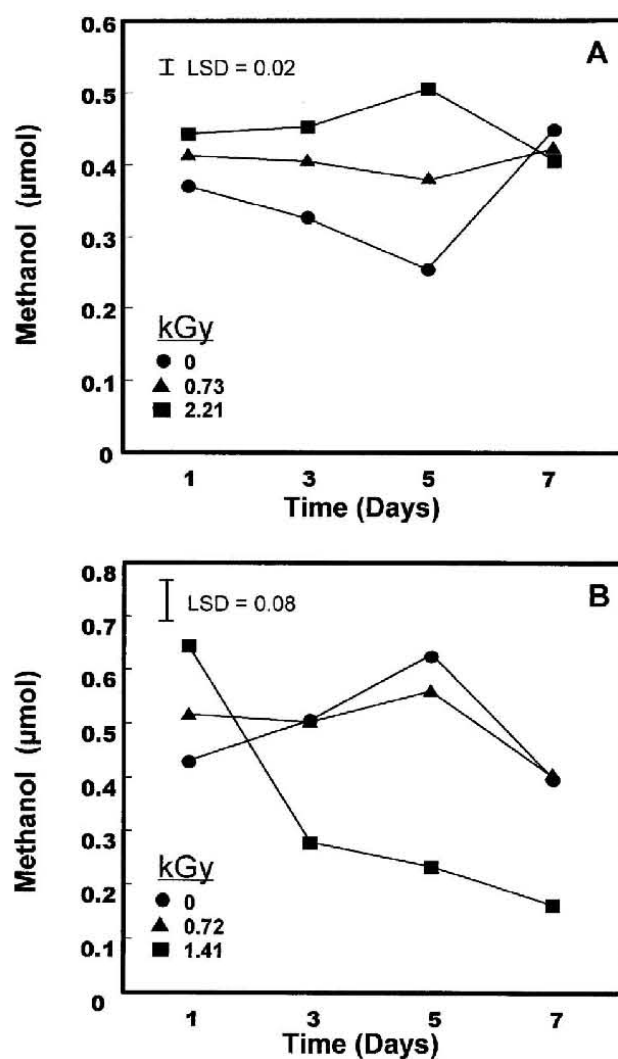
Fig. 6. PME activity of mature-green (A) and pink (B) tomato fruit following irradiation at 0, 0.72, or 1.41 kGy. Fruit were selected 24 h and again at 2-d intervals after irradiation and analyzed for PME activity. Units are expressed as micromoles methanol per gram pericarp fresh mass per minute. Vertical bars = LSD (*P* < 0.05).

period in the control fruit, whereas activity remained relatively constant at the low irradiation dose. In contrast, PME activity declined >50% for fruit at the high irradiation dose relative to levels detected at 24 h. The trend of decreasing PME activity in the high-dose fruit continued through 7 d of storage. A decrease in PME activity was observed in the control (48%) and low irradiation (28%) treatment on the last day, at which time there were no significant differences between the two treatments.

The increase in β-galactosidase and PME activities in response to irradiation is in sharp contrast to the behavior for PG. Increased PME activity was also reported for sweet cherries irradiated at 2.0 and 5.0 kGy (Somogyi and Romani, 1964), and oranges irradiated at 1.0 to 3.0 kGy (Dennison et al. 1967). Other enzymes reported to increase in response to irradiation include ACC synthase in cherry tomato fruit (Larrigaudière et al., 1990), and phenylalanine ammonia lyase and peroxidase in mango (*Mangifera indica* L.) fruit (Frylinck et al., 1987), enzymes known to function in the transduction of or response to stress stimuli. While β-galactosidase is not commonly associated with stress responses, increased PME activity was reported in mechanically stressed cucumber fruit (Miller et al., 1987) and chill-stressed tomato fruit (Marangoni et

al., 1995). Studies of protein synthesis in irradiated tomato fruit (Ferullo et al., 1994) have shown that a short-term, global decrease in protein levels was followed by the renewed synthesis of several classes of proteins, including heat-shock proteins.

The response of PG, PME, and β-galactosidase activities to irradiation did not parallel changes in whole fruit firmness. Cell wall enzyme activities were either suppressed (PG) or showed transient increases in response to irradiation, whereas firmness decreases were enhanced. Similarly, enzyme activities showed no consistent relationship with firmness changes in excised pericarp, which exhibited more consistent decreases and rankings among treatments. PME activity increased sharply in the short term, at which time dose-dependent decreases in pericarp firmness were first noted, but subsequent changes in activity during storage did not parallel the trends in pericarp firmness. The rapidity of the initial firmness decline in irradiated fruit indicates that nonenzymic, radiolytic processes may be involved. Although the enhanced electrolyte efflux observed following irradiation is consistent with membrane damage, we have also observed rapid effects of irradiation on the mol mass properties of cell wall structural polysaccharides (unpublished data).

Literature Cited

Abdel-Kader, A.S., L.L. Morris, and E.C. Maxie. 1968. Physiological studies of gamma-irradiated tomato fruits. III. Effects on ascorbic acid content, acidity and texture. J. Amer. Soc. Hort. Sci. 93:843–852.

Ahmed, E.M., E.C. Fluck, and R.A. Dennison. 1972. Textural properties of irradiated tomatoes. J. Tex. Stud. 3:115–121.

Ahrens, M.J. and D.J. Huber. 1990. Physiology and firmness determination of ripening tomato fruit. Physiol. Plant. 78:8–14.

Boyle, F.P., Z.I. Kertesz, R.E. Glegg, and M.A. Connor, Jr. 1957. Effects of ionizing radiation on plant tissue. II. Softening of different varieties of apples and carrots by gamma rays. J. Food Res. 22:89–95.

Brecht, J.K., S.A. Sargent, J.A. Bartz, K.V. Chau, and J.P. Emond. 1992. Irradiation plus modified atmosphere for the storage of strawberries. Proc. Fla. State Hort. Soc. 105:97–100.

Bramlage, W.J. and W.J. Lipton. 1965. Gamma radiation of vegetables to extend market life. Mktg. Res. Rpt. 703, USDA, Washington, D.C.

Dennison, R.A., E.M. Ahmed, and F.G. Martin. 1967. Pectinesterase activity in irradiated 'Valencia' oranges. J. Amer. Soc. Hort. Sci. 91:163–169.

Elkashif, M.E. and D.J. Huber. 1988. Electrolyte leakage, firmness, and scanning electron microscopic studies of watermelon fruit treated with ethylene. J. Amer. Soc. Hort. Sci. 113:378–381.

Eric, B., J. Le Compte, and R.F. Reeve. 1970. Organoleptic assessment of irradiated 'Granny Smith' apples from western Australia. Food Technol. Austral. 22:298–300.

Ferguson, I.B. and C.B. Watkins. 1981. Ion relations of apple fruit tissue during fruit development and ripening. 1. Cation leakage. Austral. J. Plant Physiol. 8:155–164.

Ferullo, J.-M., L. Nespoulous, and C. Triantaphylidès. 1994. Gamma-ray-induced changes in the synthesis of tomato pericarp protein. Plant Cell Environ. 17:901–911.

Fischer, R. L. and A. B. Bennett. 1991. Role of cell wall hydrolases in fruit ripening. Annu. Rev. Plant Physiol. Plant Mol. Biol. 42:675–703.

Frylinck, L., I.A. Dubery, and J.C. Schabort. 1987. Biochemical changes involved in stress response and ripening behaviour of gamma irradiated mango fruit. Phytochemistry 26:681–686.

Glegg, R.E., F.P. Boyle, L.W. Tuttle, D.E. Wilson, and Z.I. Kertesz. 1956. Effect of ionizing radiations on plant tissues. I. Quantitive measurements of the softening of apples, beets and carrots. Radiation Res. 5:127–133.

Gull, D., D. Cartegena, and E.C. French. 1980. Análisis de calidad de tomate para lograr un mejor producto. IBTA/Prodes/UFLA. Cochabamba, Bolivia.

Hamson, A.R. 1952. Measuring firmness of tomatoes in a breeding program. Proc. Amer. Soc. Hort. Sci. 60:425–433.

Hatton, T.T., R.H. Cubbedge, L.A. Risse, D.W. Hale, D.H. Spaulding, D. von Windeguth, and V. Chew. 1984. Phytotoxic response of Florida grapefruit to low dose irradiation. J. Amer. Soc. Hort. Sci. 109:607–710.

Hayashi, T., S. Todoriki, and A. Nafao. 1992. Effect of gamma-irradiation on the membrane permeability and lipid composition of potato tubers. Environ. Expt. Bot. 32:265–271.

Huber, D.J. and E.M. O'Donoghue. 1993. Polyuronides in avocado (*Persea americana*) and tomato (*Lycopersicon esculentum*) fruits exhibit markedly different patterns of molecular weight downshifts during ripening. Plant Physiol. 102:473–80.

Huber, D.J. and D.J. Nevins. 1981. Partail purification of endo- and exo-β-D-glucanase enzymes from *Zea mays* L. seedlings and their involvement in cell-wall autohydrolysis. Planta 151:206–214.

Johnson, C.F., E.C. Maxie, and E.M. Elbert. 1965. Physical and sensory tests on fresh strawberries subjected to gamma radiation. Food Technol. 19:119–123.

Kader, A. A. 1986. Potential application of ionizing radiation in postharvest handling of fresh fruits and vegetables. Food Technol. 40:117–121.

Larrigaudière, C., A. Latché, J.C. Pech, and C. Triantaphylidès. 1990. Short-term effects of γ-irradiation on 1-aminocyclopropane-1-carboxylic acid metabolism in early climacteric cherry tomatoes. Plant Physiol. 92:577–581

Marangoni AG, R.L. Jackman, and D.W. Stanley. 1995. Chilling-associated softening of tomato fruit is related to increased pectinmethylesterase activity. J. Food Sci. 60:1277–81.

Massey, Jr., L.M., W.B. Robinson, J.F. Spaid, D.F. Splittstoesser, J.P. Van Buren, and Z.I. Kertesz. 1965. Effect of gamma radiation upon cherries. J. Food Sci. 30:759–765.

Maxie, E.C. and N.F. Sommer. 1963. Radiation technology in conjunction with postharvest procedures as a means of extending the shelf life of fruits and vegetables. U.S. Atomic Energy Comm. Res. and Dev. Rpt. UCD-4P80-1.

Maxie, E.C., N.F. Sommer, and D.S. Brown. 1964. Radiation technology in conjunction with postharvest procedures as a means of extending the shelf life of fruits and vegetables. U.S. Atomic Energy Comm. Res. and Dev. Rpt. UCD-34P80-2.

Miller, A.R., J.P. Dalmasso, and D.W. Kretchman. 1987. Mechanical stress, storage time, and temperature influence cell-wall degrading enzymes, firmness, and ethylene production by cucumbers. J. Amer. Soc. Hort. Sci. 112:666–671.

Milner, Y. and G. Avigad. 1967. A copper reagent for the determination of hexuronic acids and certain ketohexoses. Carbohydr. Res. 4:359–361.

Palma, T., A.G. Marangoni, and D.W. Stanley. 1995. Environmental stresses affect tomato microsomal membrane function differently than natural ripening and senescence. Postharvest Biol. And Technol. 6:257–273.

Romani, R.J., I.K. Yu, L.L. Ku, L.K. Fisher, and M. Dehgan. 1968. Cellular senescence, radiation damage to mitochondria, and the compensatory response in ripening pear fruits. Plant Physiol. 43:1089–1096.

Skou, J.P. 1963. Changes in the permeability of carrot tissues due to gamma-irradiation and other physical and chemical treatments. Physiol. Plant 16:423–441.

Somogyi, L.P. and R.J. Romani. 1964. Irradiation-induced textural change in fruits and its relation to pectin metabolism. J. Food Sci. 29:366–371.

Srb, V. and B. Hluchovsky. 1963. Changes in cellular permeability of different coats of *Allium cepa* L. after X-ray irradiation. Expt. Cell Res. 29:261–267.

Triantaphylidès, C., N. Banzet, J.M. Ferullo, C. Larrigaudière, and L. Nespoulous. 1994. Stress response of tomato fruit tissue submitted to massive doses of ionising radiation. Proc. Royal Soc. Edin. 102:97–105.

Tucker, G.A. and D. Grierson. 1982. Synthesis of polygalacturonase during tomato fruit ripening. Planta 155:64–67.

Voisine, R., L-P. Vézina, and C. Willemot. 1993. Modification of phospholipid catabolism in microsomal membranes of γ-irradiated cauliflower (*Brassica oleracea* L.). Plant Physiol. 102:213–218.

Wood, P.L. and I.R. Siddiqui. 1971. Determination of methanol and its application to measurement of pectin ester content and pectinmethylesterase activity. Anal. Biochem. 39:418–428.

Yasir, M.S., K. Chachin, and T. Iwata. 1987. Effects of gamma irradiation on tissue firmness, some cell wall degrading enzymes and pectic substances of tomato fruit. Bull. Univ. Osaka Pref., Ser. 39:9–20.