Impact of UV-C Irradiation on the Cell Wall-Degrading Enzymes during Ripening of Tomato (Lycopersicon esculentum L.) Fruit

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The effect of a hormic dose of UV-C (254 nm) on changes in fruit firmness and cell wall-degrading enzyme (CWDE) activity was determined using tomato fruit. Throughout the storage period, a decrease in firmness was jointly observed with an increase of the CWDE (polygalacturonase, pectin methyl esterase, cellulase, xylanase, β-D-galactosidase, and protease) activity for all treatments, suggesting the involvement of these enzymes in the ripening process. However, the enhancement in the activity of the CWDE was significantly less in fruit subjected to the hormic dose of UV-C. This reduction may explain why irradiated fruit were firmer than control and consequently may explain how UV-C could delay the ripening and senescence process. We suggest that the CWDE are one of the targets of the UV-C, and by this action, irradiation contributed to a delay of the cell wall degradation and consequently retarded softening of the tomato fruit tissues.

Keywords: *Tomato; ultraviolet radiation; cell wall-degrading enzymes; ripening.*

INTRODUCTION

The major post-harvest problem with storage of tomatoes, as with other fruits and vegetables, is the excessive softening. Ripening of climacteric fruit such as the tomato (*Lycopersicon esculentum* Mill.) is mainly orchestrated by the biosynthesis of ethylene that triggers a serial biochemical and physiological process inducing the softening in texture. While the mechanisms regulating changes in firmness during ripening are not fully understood, the softening of stored tomato fruit flesh has been associated with alterations in pericarp cell wall (Sethu et al., 1996), and specifically the breakdown of middle lamella pectins (Trinchero et al., 1999).

Polygalacturonase (PG) and pectin methylesterase (PME) were considered the primary hydrolase involved in the softening process (King and O'Donoghue, 1995; Sethu et al., 1996). In addition, a variety of other enzymes have been assigned roles in fruit cell wall metabolism such as xylanase and glycosidase (Ahmed and Labavitch, 1980), and cellulase (Awad and Young, 1979). It has been suggested that the complex series of modifications in ripening fruit cell walls may be the result of an orchestrated action of several classes of attack against cell wall integrity during the fruit ripening (Fisher and Bennet, 1991).

A recent application of ultraviolet light is the use at beneficial doses (hormic) to induce an accumulation of phytoalexins (Devlin and Gustine, 1992) and activate genes encoding pathogenesis-related proteins (Green and Fluhr, 1995). This phenomenon is known as hormesis (hormesis is defined according to Calabrese et al. (1987) as the stimulation of beneficial responses by low levels of stressors which are otherwise harmful). The potential of irradiation light (254 nm) was used to extend the shelf life of various fruit and vegetables (Liu et al., 1993; Maharaj et al., 1999). A dose of 3.7 kJ m^{-2} was defined as the hormic dose of UV-C required to delay ripening and senescence of tomato fruit (Maharaj et al., 1999). The observed phenomenon was accompanied by a delay in the climacteric (CO_2 and C_2H_4 emission) by 7 days. The decline in chlorophyll content and the development of the red color and lycopene were also significantly retarded during storage (Maharaj et al., 1999). Recently we observed an increase in the lipid peroxidation markers in tomato fruit during the 5 days following irradiation with the hormic dose (Ait Barka et al., 1999). Beyond this period the levels of those markers become lower in UV-C-treated fruit than in control, suggesting the induction of a defense mechanism, probably by an activation of an antioxidative enzyme (Ait Barka et al., 1999). However, the precise mode of action of irradiation on the softening enzymes and their relationship during the fruit ripening is largely unknown.

This study provides information on changes of several enzymes potentially involved in cell wall degradation during tomato ripening in relation to the UV-C exposure. The fruit firmness was followed as a marker of fruit ripening. The results show an increase in cell wall-degrading enzyme (CWDE) activity during the storage period for all treatments, but with a marked increase in the control compared to UV-C-treated fruit, suggesting that CWDE may be one of the targets of UV-C irradiation.

MATERIALS AND METHODS

All chemicals used in this study were purchased from Sigma Chemical Co. (St. Louis, MO).

Plant Material. Greenhouse-grown tomato (mature-green tomato) fruit (*Lycopersicon esculentum* Mill cv. Trust) were harvested manually at mature-green stage. The tomato maturity stages were determined as color changes in the exocarp

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according to Salunkhe and Desai (1984). Fruit of uniform shape and size were selected, washed with 1% sodium hypochlorite, rinsed with distilled water, and air-dried at 25 °C using a fan ventilator.

UV-C Irradiation and Storage Conditions. UV-C radiation was provided by fluorescent germicidal lamps (GE 30 W) with peak emission at 254 nm. The intensity of radiation was measured by a portable digital radiometer (UVX Digital radiometer UVP Inc., San Gabriel, CA). The fruit were irradiated with a predetermined dose (3.7 kJ m^{-2}) as described by Maharaj et al. (1999). Fruit were placed in opened plastic containers and stored in the dark at 16 °C for 25 days in controlled chambers with 95% of relative humidity. In the first experiment, destructive analysis of fruit firmness was carried out periodically using five fruit per treatment. In the second experiment, five fruits were randomly sampled from each treated and untreated (control) group, and their pericarp tissue was frozen in liquid nitrogen (liq N_2) and stored at −80 °C. Before analysis, the stored pericarp tissue was prepared by grinding the frozen tissue to a fine powder with liq N_2.

Firmness. The force required to puncture the skin of whole tomato fruit placed on a stationary steel plate was determined according to the method of Holt (1970) using the Instron Universal Testing Instrument (Model TA-Xt2 Texture Analyzer, New York). A cylindrical plunger with a 4 mm diameter probe was attached to the crosshead and driven, at a constant speed of 10 cm/min, vertically into the tomato fruit. Four determinations were made on each fruit away from the locular ridge. The fruit firmness recorded was the maximum force (*N*) reached during tissue breakage. Results presented were the means of five fruits for each sampling period and for each treatment.

Protein Extraction. All steps described for extraction were carried out at 4 °C. Proteins were extracted by grinding tomato pericarp tissue to a fine powder in a small mortar and pestle precooled in liq N_2 according to Ait Barka et al. (in press). The protein content was analyzed according to the method of Bradford (1976) using a calibration curve made with bovine serum albumin.

Cell Wall-Degrading Enzymes. Polygalacturonase (EC 3.2.1.15) activity was assayed by measuring the reducing groups released from polygalacturonic acid according to the method of Yoshida et al. (1984). One unit of enzyme activity was defined as the amount of enzyme which liberated 1 μmol of reducing group per hour at pH 4.5 at 35 °C.

Pectin methylesterase (EC 3.1.1.11) activity was determined by the decrease of absorbance at 620 nm according to Hagerman and Austin (1986). One unit of enzyme activity will liberate 1 microequiv of acid from pectin per minute at pH 7.5 at 30 °C.

Cellulase (EC 3.2.1.4) activity was determined by measuring the reducing groups released from carboxymethyl cellulose according to Sethu et al. (1996). One unit is defined as the amount of the enzyme that catalyzed the formation of 1 μmol of reducing groups per hour at pH 5 at 37 °C.

β-galactosidase (EC 3.2.1.23) activity was assayed by following the amount of *p*-nitrophenol released from β-D-galactopyranoside used as substrate using the method of Sozzi et al. (1996). One unit will hydrolyze 1.0 μmol of *p*-nitrophenyl β-D-galactoside to *p*-nitrophenol and D-galactose per minute at pH 4.7 at 37 °C.

Xylanase (EC 3.2.1.8) activity was analyzed according to the method of Bhaskara et al. (1998). One unit will liberate 1 μmol of reducing sugar measured as xylose equivalents from xylan per minute at pH 4.8 at 30 °C.

Protease (EC 3.4.24.32) activity determined according to the method of Pagel and Heitefuss (1990). One unit will hydrolyze 1 μmol of gelatin per minute at pH 7.0 at 30 °C.

Statistical Analysis. All experiments were done in triplicate. The data presented were analyzed using analysis of variance (ANOVA), with treatment and sampling day (1−25) as class variables, and means were separated using least-squares (LS) means analysis. Differences were considered statistically significant at $P < 0.05$.

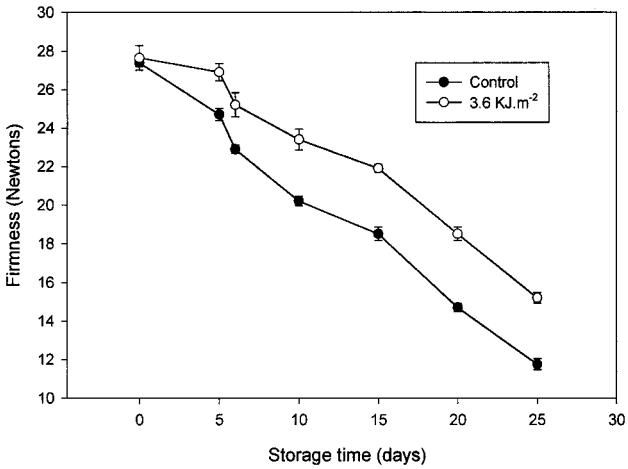

Figure 1. Change in firmness of tomato fruit stored at 16 °C after irradiation exposure to a 0 or 3.7 kJ m^{-2} dose of UV-C. Bars are standard errors, $n = 5$. Each result is the mean of five replicates. Variance is represented by standard errors of the mean ($P < 0.05$).

RESULTS AND DISCUSSION

Firmness. Firmness was significantly ($P < 0.05$) affected by storage time and irradiation exposure (Figure 1). It decreased significantly during storage period in the both control and UV-C treated fruit, showing a marked fruit deterioration. However, in contrast to control, tomato fruit exposed to UV-C were significantly ($P < 0.05$) firmer and more resistant to penetration, indicating that irradiation retards fruit softening. Softening of tissues during ripening results in part from progressive changes in cell wall composition as well as cell separation (Sethu et al., 1996). Firmness values derived from puncture tests reflect the integrity of pericarp tissue (Holt, 1970). In this study, the increased firmness of UV-treated fruit when compared to the control could be associated with enhanced levels of polyamines (Maharaj et al., 1999). Effectively, Kramer et al. (1989) observed that exogenous PAs suppressed cell wall softening and activity of polygalacturonase. Recently, Maharaj et al. (1999) suggested that PAs were assumed to function via a mechanism similar to that of calcium, involving the formation of cation cross-links with pectic acid and other polysaccharides, thus limiting accessibility of the cell wall to degradative enzymes.

Changes in Cell Wall-Degrading Enzyme Activities. A rapid increase was obseved for polygalacturonase activity during the first days after storage for all treated and control samples. Beyond this period, the PG activity continued to increase in the control fruit until day 15 and then started to decrease until the end of storage period. On the other hand, this activity tended to stabilize in UV-C-treated fruit until day 20, where a decline appeared (Figure 2). The results showed that irradiation retards and reduces significantly ($P < 0.05$) the increase of PG activity compared to nontreated fruit. It has been suggested that PG protein is associated with the cell wall/middle lamella of ripening tomato (Crookes and Grierson, 1983). PG activity was reported to increase at onset of ripening in pears (Ahmed and Labavitch, 1980), tomato (Yoshida et al., 1984), blueberries (Proctor and Miesle, 1991), and mango (Abu-Sarra and Abu-Ghoukh, 1992). However, recent studies indicated that the blockage of PG expression by transformation with antisense gene for PG does not prevent (Sheehy et al., 1988) or prevents only partially (Carrington et

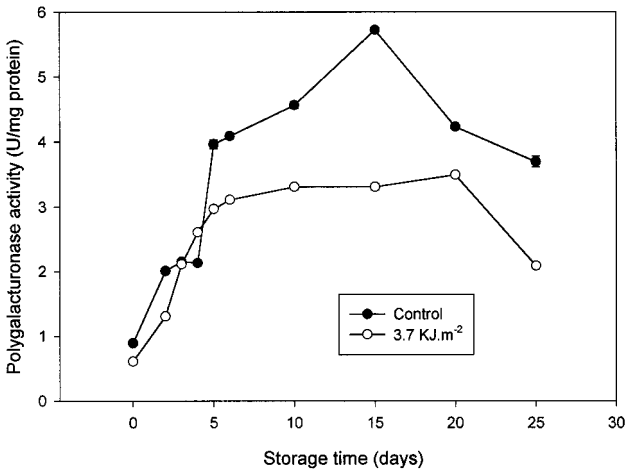

Figure 2. Activity of polygalacturonase in pericarp tissue from tomato fruit stored at 16 °C after irradiation exposure to a 0 or 3.7 kJ m^{-2} dose of UV-C. One unit of enzyme activity was defined as the amount of enzyme which liberated 1 μmol of reducing group per hour at pH 4.5 at 35 °C. Bars are standard errors, $n = 5$. Each result is the mean of five replicates. Variance is represented by standard errors of the mean ($P < 0.05$).

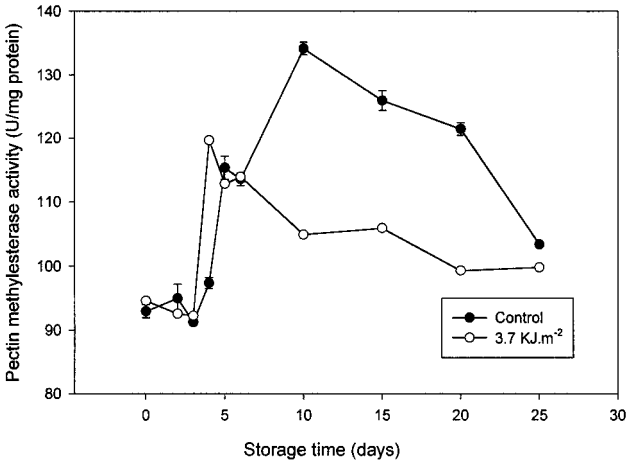

Figure 3. Activity of pectin methylesterase in pericarp tissue from tomato fruit stored at 16 °C after irradiation exposure to a 0 or 3.7 kJ m^{-2} dose of UV-C. One unit of enzyme activity will liberate 1 microequiv of acid from pectin per minute at pH 7.5 at 30 °C. Bars are standard errors, $n = 5$. Each result is the mean of five replicates. Variance is represented by standard errors of the mean ($P < 0.05$).

al., 1993) softening in tomato fruit. In our study the onset of the increase in PG activity was correlated with the onset of ripening and was enhanced as ripening of fruit progressed, supporting the concept of its involvement in cell wall dissolution. Similar results were reported by Ahrens and Huber (1990) in tomato fruit. In accordance with results observed by Barret and Gonzalez (1994), the PG activity was associated with the climacteric event noted by Maharaj et al. (1999). From our results, we suggest that UV-C treatment induced the reduction of PG activity, contributing thereby to a delay in fruit ripening. Similar results were observed in tomato fruit stored at 33−35 °C (Yoshida et al., 1984; Picton and Grierson, 1988) and in avocado fruit subjected to low oxygen stress (Kanellis et al., 1991).

Pectin methylesterase activity was storage time and UV-C treatment dependent (Figure 3). In control fruit, PME increased during ripening, reached the maximum of its activity after 10 days of storage, and then started

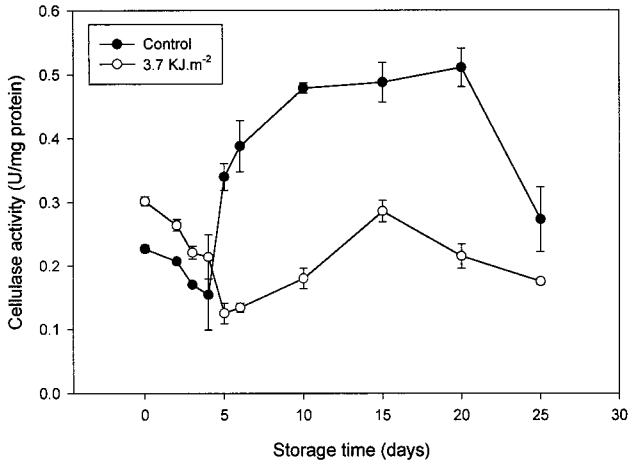

Figure 4. Activity of cellulase in pericarp tissue from tomato fruit stored at 16 °C after irradiation exposure to a 0 or 3.7 kJ m^{-2} dose of UV-C. One unit is defined as the amount of enzyme that catalyzed the formation of 1 μmol of reducing groups per hour at pH 5 at 37 °C. Bars are standard errors, $n = 5$. Each result is the mean of five replicates. Variance is represented by standard errors of the mean ($P < 0.05$).

to decrease until the end of the storage time. In treated fruit, the maximum PME activity was observed after 4 days of storage and then declined progressively throughout the storage period. As noted for PG, the enhancement of the PME activity was significantly reduced by the UV-C exposure ($P < 0.05$). Pectin methylesterase was originally thought to have a support role in fruit ripening (Fisher and Bennet, 1991). However, results observed in an experiment with an antisense PME gene in a transgenic tomato suggest that this enzyme plays a role in determining tissue integrity and fruit texture during late senescence (Tieman and Handa, 1994; King and O'Donoghue, 1995). The decrease observed in control after the 10 days of storage was also noted in avocado (Awad and Young, 1979) and sweet pepper (Jen and Robinson, 1984). Proctor and Miesle (1991) suggested that PME action was required before PG could be fully effective. Our results showed that PME and PG activity rise at the same time as observed also by Koch and Nevins (1990). In contrast, it was reported that PG activity increased, while that of PME decreased during ripening of bell pepper (Sethu et al., 1996) and mango (Abu-Sarra and Abu-Ghoukh, 1992).

In comparison to the control, in treated fruit cellulase activity started off at a high level, followed by a decrease to a minimum after 5 days of storage, and then a second enhancement was observed with a maximum at day 15 (Figure 4). In control fruit, cellulase activity was less important during the first 4 days ($P < 0.05$), and then an increase was observed, reaching the maximum after 20 days of storage. Results showed that UV-C treatment reduces the cellulase activity during the second period of storage. In accordance with our results, an increase in cellulase activity during ripening was detected in avocado (Awad and Young, 1979), mango (Abu-Sarra and Abu-Ghoukh, 1992), and tomato fruit (Hobson and Harman, 1986). This increase of cellulase activity was detected around the climacteric event observed by Maharaj et al. (1999). Similar results were noted in avocado fruit (Awad and Young, 1979; Kanellis et al., 1991) and sherry fruit (Barret and Gonzalez, 1994). The decrease observed in cellulase activity in the late period of storage was also detected in pears (Ben-Arie and Sonego, 1980), tomato (Hobson and Harman, 1986), and

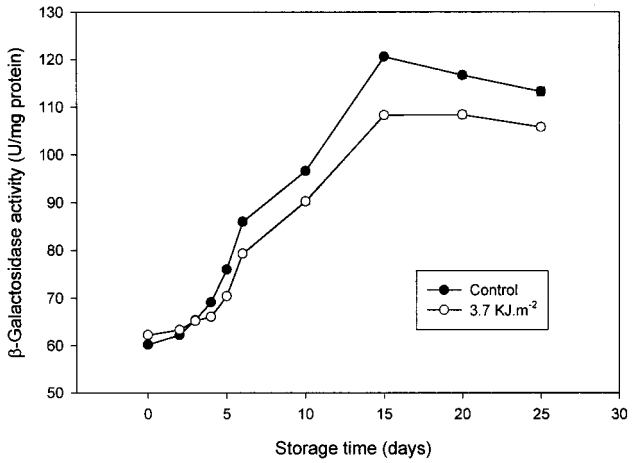

Figure 5. Activity of β-galactosidase in pericarp tissue from tomato fruit stored at 16 °C after irradiation exposure to a 0 or 3.7 kJ m^{-2} dose of UV-C. One unit will hydrolyze 1.0 μmol of *p*-nitrophenyl β-D-galactoside to *p*-nitrophenol and D-galactose per min at pH 4.7 at 37 °C. Bars are standard errors, $n = 5$. Each result is the mean of five replicates. Variance is represented by standard errors of the mean ($P < 0.05$).

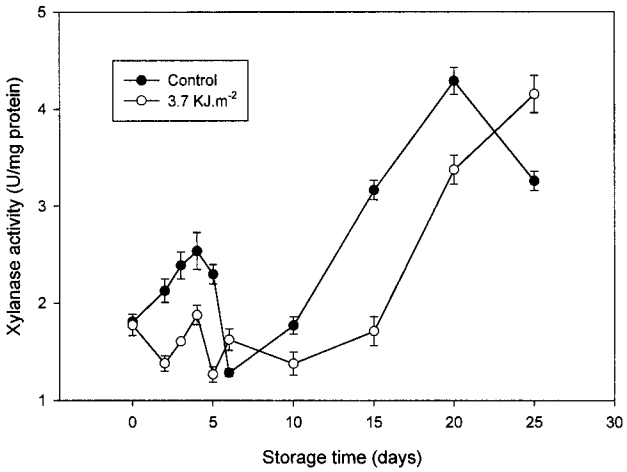

Figure 6. Activity of xylanase in pericarp tissue from tomato fruit stored at 16 °C after irradiation exposure to a 0 or 3.7 kJ m^{-2} dose of UV-C. One unit will liberate 1 μmol of reducing sugar measured as xylose equivalents from xylan per minute at pH 4.8 at 30 °C. Bars are standard errors, $n = 5$. Each result is the mean of five replicates. Variance is represented by standard errors of the mean ($P < 0.05$).

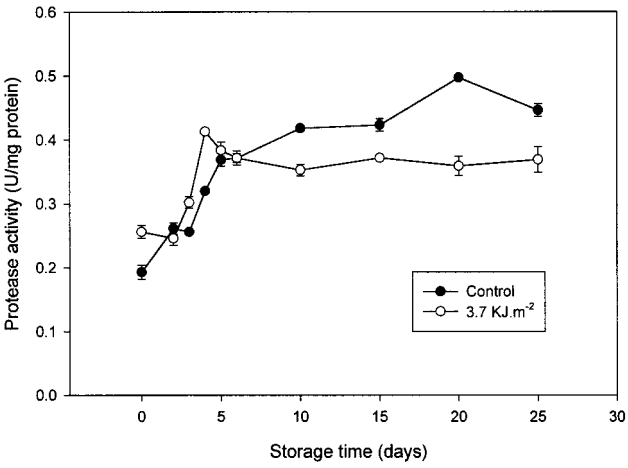

Figure 7. Activity of protease in pericarp tissue from tomato fruit stored at 16 °C after irradiation exposure to a 0 or 3.7 kJ m^{-2} dose of UV-C. One unit will hydrolyze 1 μmol of gelatine per minute at pH 4.7 at 37 °C. Bars are standard errors, $n = 5$.

bell pepper (Sethu et al., 1996). Those authors noted that generally cellulase activity reached a peak and thereafter declined as the fruit continued to soften. A hormic dose of UV-C induced a reduction in the cellulase activity. A similar result was obtained in avocado fruit subjected to low oxygen stress (Kanellis et al., 1991).

This study showed an increase of β-galactosidase activity throughout the storage period. During the first 4 days of storage, no significant difference was observed in the β-galactosidase activity between the control and treated fruit (Figure 5). However, beyond the fifth day, the activity increased significantly in both treated and control samples, but with marked effect in control fruit ($P < 0.05$). A possible relationship between β-D-galactosidase and a substantial loss of wall galactosyl residues was hypothesized in tomato fruit ripening (Pressey, 1983). The ability of this enzyme to degrade and solubilize pectin was recently reported in different fruit, indicating a possible explanation of how fleshy fruit may soften, in the absence of PG (Sozzi et al., 1996). Our results showed that β-D-galactosidase increased with the tomato ripening process, suggesting its role during fruit ripening and softening, and confirming the results of Sozzi et al. (1998). In meantime, Carrington and Pressey (1996) showed that softening began when β-D-galactosidase was detected, suggesting that the earliest phase of softening is orchestrated by some other enzyme or mechanism.

An increase in xylanase activity was observed during the first days in the control fruit with a maximum of its activity after 4 days of storage and then dropped 2 days later, followed by a second enhancement with a peak after 20 days of storage (Figure 6). Similar results were reported in ripening banana fruit by Prabha and Bhagyalakshmi (1998). For UV-C treated fruit, there was no initially significant increase in the xylanase activity until the 15th day after irradiation where an enhancement of the activity started. When compared to control, UV-C induced a significant ($P < 0.05$) reduction in the activity of xylanase. Protease have been reported to be stress-induced wall proteins (Showalter, 1993). This may explain the general increase observed in protease activity as tomato fruit ripening progressed (Figure 7). However, during the first 5 days, there was no significant difference between control and treated tomato fruit, but then a significant increase was observed in the both treated and control samples, but with a greater increase occurring in the control fruit.

During ripening process, the tomato fruit firmness decreased jointly with an enhancement in the activity of all cell wall-degrading enzymes, suggesting that none of the hydrolase enzymes act alone to regulate fruit softening (King and O'Donoghue, 1995). On the contrary, we observed that exposure to UV-C induced a reduction in fruit firmness decay and in the activity of the enzymes involved in cell wall degradation. This study confirmed the role of the cell wall-degrading enzymes in the tomato fruit ripening process as shown by the increase of their activity throughout the storage period, suggesting that fruit ripening is orchestrated by different enzymes. However, UV-C irradiation induced a significant reduction of the CWDE activity when compared to control, suggesting that CWDE may be one of the targets of UV-C irradiation by inducing their proteolysis or the reduction of their de novo synthesis, and explaining how a hormic dose of UV-C triggered the delay of ripening and senescence processes.

ABBREVIATIONS USED

Bovine serum albumin, BSA; cell wall-degrading enzyme, CWDE; pectin methylesterase, PME; polygalacturonase, PG.

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