Effect of post-harvest UV-C irradiation and calcium chloride on enzymatic activity and decay of tomato (*Lycopersicon esculentum* L.) fruit during storage

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Abstract

Tomato is one of the extensively consumed vegetable crops worldwide. The regular consumption of tomato decreases the incidence of chronic degenerative diseases such as certain types of cancer, and cardiovascular diseases. The objective of this study was to find an appropriate method that not only reduces tomatoes decay, but also maintains its post-harvest quality. A factorial experiment based on randomized complete block design with three replications was conducted to evaluate effects of ultraviolet (UV) and calcium chloride (CaCl₂) applications on tomato during storage. The traits studied included ethylene, polygalacturonase (PG) activity, pectin methyl esterase (PME) activity, firmness, total phenol content, and fungal-induced decay were measured weekly during 35 days of storage. Both UV and CaCl₂ treatments had a positive effect on tomato quality as compared to control treatment. The 3 and 4.5 kJ m⁻² levels of UV and 2% CaCl₂ had positive effects on quality characteristics. Fruits treated by UV and CaCl₂ had higher phenol and firmness, and less PME activity, PG activity, ethylene, and decay than the control fruits. In conclusion, increasing in storage duration significantly affected the fruits quality by increasing in ethylene, PME activity, PG activity, decay and decreasing the phenol content and firmness. But UV and CaCl₂ led to significant decrease in this adverse impact relative to control treatment.

Keywords: CaCl₂, ethylene production, firmness, PG, PME, phenol

1. Introduction

Tomato (*Lycopersicon esculentum* L.) is one of the major vegetable components of the human diet worldwide. The fleshy fruits, however, are susceptible to decay and loss of quality once they have been harvested, due to ethylene production and activities of cell wall-degrading enzymes (Zhu et al. 2016). A number of post-harvest treatments have been evaluated for their ability to prevent post-harvest
decay and extending the shelf-life of tomato fruits, including the high-pressure and thermal treatments (Krebbers et al. 2003), 1-MCP (Guillen et al. 2007) and sodium selenite (Zhu et al. 2016).

Among the mineral nutrients, calcium plays an important role in plant cell functions which has a key role in cross linking of cell wall polymers and, hence, cell wall strength, and is determinant in fruit quality and shelf life (Shiri et al. 2014). It is reported that calcium makes fruit more acceptable by reducing color change rate, maintaining membrane permeability and slow ripening processes (Shiri et al. 2014). The calcium bonds as pectate in the middle blades are also necessary to strengthen the cell walls and plant tissues. Polygalacturonase (PG) enzyme degrades pectate and changes insoluble pectic materials to soluble pectin which results in softening fruit tissue (Barka et al. 2000). The calcium also inhibits pectin methyl esterase (PME) which catalyze the de-esterification of pectin into pectate and methanol, and decomposes the pectic compounds and leads to formation of two-phases of fruit juice (Hajjoo et al. 2012).

Calcium reduces respiration and ethylene production (Shiri et al. 2016). Ethylene is a natural plant hormone produced by many fruits and vegetables that enhances the rate of respiration and accelerates the senescence process. High concentration of ethylene in the storage air causes ripening, fruit softening and enhancing the storage diseases. It is reported that foliar application of calcium chloride reduces fungal growth on strawberries, because calcium ions make cross-linking among the pectin polysaccharides in the cell wall and septum, which strengthens the cells and their connection and increases tissue resistance to fungal enzymes activity (Cheour et al. 1991).

UV-C irradiation is a technique that increases resistance to the decay and shelf life of fruits and vegetables (Stevens et al. 2005). However, the effectiveness of UV treatment in controlling decay and accumulating phytoalexins depends on cultivar type, UV irradiation intensity and exposure duration (D’hallewin et al. 2000). It has been reported that mangoes treated with 2.46–4.93 kJ m⁻² UV led to reduction of decay and increase of shelf life which may be because of positive effect of UV in increasing total phenol and flavonoids (Gonzalez-Aguilar et al. 2007). Furthermore, Stevens et al. (1998) observed that peaches irradiated by UV had less ethylene and decay as compared to control fruits. Similarly, Stevens et al. (2004) showed that tomatoes irradiated by UV-C had significantly more firmness and less PG activity than the control fruits. Also, Barka et al. (2000) reported a low cell wall degrading enzymes activity such as PG and PME in tomatoes treated by UV.

On the basis of the above findings, it is crucial to analyze the effects of CaCl₂ and UV-C irradiation on ethylene production and cell wall-degrading enzymes in fruits. Tomato fruit characteristically follows a pattern controlled by ethylene. To our knowledge, little information is available about the comparative effects of post-harvest CaCl₂ treatment and UV-C irradiation on physiological responses of tomato during storage. Therefore, the aim of this study was to find an appropriate method to reduce post-harvest losses in tomatoes and maintain fruits quality by post-harvest CaCl₂ treatment and UV-C irradiation.

2. Material and methods

2.1. Plant material and experiment condition

The tomato fruits (cultivar Valouro) were harvested at the light-red ripening stage (pinkish-red or red color shows on over 60% but red color covers not more than 90% of the tomato surface), with uniform color, sizes, round shape and without bruises or signs of infection, were obtained from a commercial greenhouse Alborz Province, center of Iran. The tomatoes were randomly divided into groups of five fruits and used in factorial experiments based on completely randomized design with three replications in each experiment. In experiment I, treatment factors were CaCl₂ in four levels of 0 (control), 1, 1.5, and 2% and sampling time at the 0, 7, 14, 21 and 35th day. To apply CaCl₂ treatment, fruits were fully immersed in the CaCl₂ solution for 10 min then dried and packed in hermetically sealed containers.

In experiment II, tomato fruits were arranged in a single layer and were subjected to 0 (control), 1.5, 3, and 4.5 kJ m⁻² UV-C radiation for 5, 10 and 15 min, respectively. The tomatoes were placed 25 cm far from the UV light source with 30 W output power and 254 nm wavelength (30 W/G30T8 with spectral peak at 254 nm, Philips). The UV treated tomatoes were packed in hermetically sealed containers. As well as experiment I, sampling times were the 0, 7, 14, 21 and 35th day of storage. Storage conditions of the both experiments were 7°C temperature and 90% relative humidity. To isolate each treatment condition and prevent gas exchange from the containers in both experiments, treatments were performed in 1 L capacity hermetically sealed containers, as previously reported by Guillen et al. (2007).

2.2. Ethylene determination

Ethylene production was estimated according to Bu et al. (2013) with some modifications. Fruits were sealed in a 1.0 L-jar and were kept at 18°C for 1 h. Then, headspace gas was sampled with a 1 ml-syringe and injected to a gas chromatograph. The column oven temperature was held at 90°C for 3 min and then programmed to 130°C at 30°C min⁻¹. The injector and detector temperatures were 120 and 100°C respectively. The injection volume of headspace gas was
Total phenol content was determined using the Folin-Ciocalteau method, as described by Shiri et al. (2016) with some modifications. The absorbance of the samples was measured at 765 nm with a UV/Vis spectrophotometer (model PG Instrument ‘80, Leicester, UK). Catechin was used as a standard curve for obtaining the calibration curve. Data were expressed as milligram of catechin per 100 g of fruit fresh weight (mg 100 g⁻¹ FW).

2.7. Data analysis

Data were analyzed as a two-factor linear model based on a completely randomized design with three replications by SPSS software (Ver. 20), where treatments and storage times were the factors. Before analysis of the variance, data were tested for normality and homoscedasticity using the Kolmogorov–Smirnov and Cochran tests, respectively. Least significant difference (LSD) test was calculated at Ps<0.01 and Ps<0.05 was calculated to compare differences between means following a significant ANOVA effect.

3. Results

3.1. Ethylene production

Ethylene production increased during the first 7 days but thereafter declined with increasing storage duration. However, the ethylene production was less in CaCl₂ or UV treated fruits as compared to control (Fig. 1). As shown in Fig. 1, the maximum ethylene production in control and CaCl₂ treated tomatoes was observed on the 7th day, and CaCl₂ 2% led to minimum ethylene production in all sampling times (Fig. 1-A).

UV treatments were effective in controlling ethylene production and this trend was maintained across the storage duration (Fig. 1-B). On the 35th day, the highest ethylene production was recorded in control treatment, whereas the lowest was observed in 4.5 kJ m⁻² UV treatment (Fig. 1-B). Also, results of t-test showed that UV treatment was significantly more effective than CaCl₂ in scavenging ethylene (Table 1).

3.2. PG activity

Analysis of variance showed that difference between levels of UV-C, CaCl₂, and time, and effect of UV-C×time and CaCl₂×time interactions were significant (Ps<0.01). CaCl₂ significantly decreased the PG activity and led to less PG activity in all levels of CaCl₂ treatment at all sampling dates than those control fruits (Fig. 2-A). The lowest and highest PG activity was observed with 2% CaCl₂ and control, at the end of storage duration, respectively.

UV treatments significantly decreased PG activity com-
pared to the control. On the 7th day of storage, fruits treated by UV had less PG activity compared to the control. By the end of the 35th day of storage, the highest and lowest PG activity was recorded in 1.5 and 4.5 kJ m⁻², respectively, except control treatment. The 4.5 and 3 kJ m⁻² UV had the same effects in all sampling times, but 1.5 and 4.5 kJ m⁻² PG activity values were significantly different (Fig. 2-B). The PG activity of stored tomatoes was not significantly different in UV and CaCl₂ treatments and both treatments had similar effect on PG activity (Table 1).

### 3.3. PME activity

The analysis of variance for PME activity showed that the effect of UV-C×time and CaCl₂×time interactions were significant (P≤0.01) for PME activity. The PME activity significantly decreased with CaCl₂ treatments. The 2% CaCl₂ was the most effective treatment in reducing PME activity, whereas the difference between 1 and 1.5% was not significant (Fig. 3-A).

As shown in Fig. 3-B, there was significant decrease in PME activity of fruits treated with UV in comparison with the control for all times, while there was not significant difference among different treatments of UV for PME activity. The PME activity in UV treatment was significantly higher than those in CaCl₂ (Table 1).

### 3.4. Fruit firmness

The effect of different levels of UV-C, CaCl₂, time, and the interaction of UV-C×time and CaCl₂×time was significant (P≤0.01) for fruit firmness. The firmness of fruits treated with CaCl₂ was more than those treated by UV. Increasing both CaCl₂ and UV resulted in increased the fruit firmness (Fig. 4). The highest and lowest fruit firmness was observed in 2% CaCl₂ (4.4 N m⁻¹) and control fruits (3.9 N m⁻¹), re-

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**Table 1** Comparison means of calcium chloride (CaCl₂) and ultraviolet (UV) treatments for studied characteristics through Student’s t-test¹

<table>
<thead>
<tr>
<th>Statistic</th>
<th>Ethylene</th>
<th>PG activity</th>
<th>PME activity</th>
<th>Fruit firmness</th>
<th>Decay severity</th>
<th>Phenol content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean difference</td>
<td>0.844</td>
<td>–0.117</td>
<td>–0.0011</td>
<td>0.529</td>
<td>0.112</td>
<td>–6.625</td>
</tr>
<tr>
<td>t-test</td>
<td>3.48**</td>
<td>–1.29 ns</td>
<td>–4.46**</td>
<td>5.63**</td>
<td>1.82 ns</td>
<td>–5.02**</td>
</tr>
</tbody>
</table>

¹PG, polygalacturonase; PME, pectin methyl esterase.

**and ns are significant at 1% level of probability and not significant, respectively.**
spectively on the 7th day (Fig. 4-A). The decreasing trend of fruit firmness was observed during storage time because the time has significant decreasing effect on the fruit firmness. At the end of storage time, fruits treated by CaCl₂ and UV were firmer than the control fruits (Fig. 4), and the maximum firmness was observed in 2% CaCl₂. Also, the effect of CaCl₂ on fruit firmness was higher than those of UV (Fig. 4 and Table 1).

3.5. Decay severity

The analysis of variance showed that the effect of time, UV-C×time and CaCl₂×time interactions were significant (P≤0.01) for fruit decay. Decay progress in control and treated fruits increased as the time proceeded. The 2% CaCl₂ on the 7th day was considered as the most appropriate treatment because of the lowest decay in tomato fruits. However, the highest decay was obtained in control fruit on the 7th day. At the end of storage (the 35th day), the 2% CaCl₂ was the most effective treatment to reduce decay (Fig. 5-A). Fruits treated by UV had less decay as compared to the control at each storage interval. On the 7th day, the 4.5 kJ m⁻² UV was the most effective treatment in reducing the decay. The maximum and minimum inhibition of decay compared to the control was observed in 1.5 and 4.5 kJ m⁻² UV, respectively (Fig. 5-B). The decay severity was not significantly different in UV and CaCl₂ treatments, but decay severity in fruits treated with CaCl₂ was higher than UV treated fruits (Table 1).

3.6. Phenol content

According to the analysis of variance, the effects of UV-C, CaCl₂×time, UV-C×time and CaCl₂×time interactions were significant (P≤0.01) for total phenol. Different levels of UV and CaCl₂ had an increasing effect on maintenance phenolic compounds in comparison with control fruits across storage duration (Fig. 6). However, the phenol content generally decreased in storage duration in all treatments (Fig. 6). At the end of Storage, phenolic compounds in the 2% CaCl₂ treatments was more as compared to all treatments (Fig. 6-A). There was an increase in phenol content in treatments fruits with 3 and 4.5 kJ m⁻² on the 7th day. The phenolic compounds were reduced in 3 and 4.5 kJ m⁻² UV on day 14 (Fig. 6-B). At the end of storage duration, the lowest and highest phenol content was recorded in control and fruits treated with 4.5 kJ m⁻² UV, respectively. The 3 and 4.5 kJ m⁻² UV were more effective than the 1.5 kJ m⁻² in all sampling times (Fig. 6-B). Phenol content of the fruits treated with UV was significantly higher than CaCl₂ treated fruits (Table 1).
4. Discussion

Tomato fruit is characterized by a rise in ethylene production reaching the climacteric peak. Similarly to the climacteric fruits, ethylene in the tomato is the dominant trigger for ripening processes, with an acceleration of colour changes, softening and modification in the balance between total soluble solids and acidity (Guillén et al. 2007). Low temperature storage is not enough to inhibit ethylene production and the associated ripening process. Therefore, inhibitors of ethylene production such as 1-MCP (Guillén et al. 2007) and UV-C (Bu et al. 2013) have been used to extend the shelf life and to improve the quality of tomato has been evaluated. In both studies it was concluded that 1-MCP and UV-C treatments improved the tomato shelf life and quality relative to control tomatoes.

In present study, CaCl₂ treated fruits had less ethylene production. In other words, increasing of CaCl₂ resulted in reduction of ethylene in tomato tissues. It is revealed that CaCl₂ treatment improves fruits firmness and reduces the physiological disorders which results in decreases of respiration, reduction of ethylene production, and postpone of fruit ripping Shiri et al. (2016). Luna-Guzma and Barrett (2000) reported that immersion of melon components in CaCl₂ significantly reduced the production of ethylene and respiration during storage. Also, it was observed that ethylene production in UV irradiated tomatoes is significantly lower. This result is in accordance with those obtained by Bu et al. (2013), while Severoa et al. (2015) reported that UV increases ethylene production.

PG is an important enzyme responsible for reducing the firmness of tomato fruits (Ketsa and Daengkanit 1999). Results of the present study show that CaCl₂ has significant inhibitory impact on this enzyme. Li et al. (2014) indicated that the use of 1-MCP and 1% CaCl₂ reduces PG activity in jujube fruit. Furthermore, Stevens et al. (2004) reported that UV treatment led to 40% reduction in PG activity. UV-C decreases the activity of cell wall degrading enzymes such as PG and delay in fruits softening progress (Bu et al. 2013).

Similar to the PG, PME enzyme activity is negatively correlated with tomato fruit firmness (Ketsa and Daengkanit 1999). Hajiloo et al. (2012) showed that application of CaCl₂ significantly reduces PME activity of blueberries during storage. Also, significant reduction of PME activity in tomato fruits treated with UV was reported by Bu et al. (2013).

Fruit firmness is an important quality attribute, especially in stored fruits. Conway and Sam (1984) indicated that the change in firmness is a sign of cell wall degradation caused by metabolism of cell wall carbohydrates. Calcium ion makes cell membrane stable by bonding carboxylate groups, phosphates, phospholipids and proteins of membrane surface (Manganaris et al. 2005). Therefore maintaining the firmness in fruits treated with CaCl₂ is due to production of links between free carboxyl group of cell wall and pectin.

Fig. 5 The mean values of decay severity during the tomato storage period. A, CaCl₂×time interaction; B, UV-C×time interaction. The bars represent list significant difference at 5% level of probability.

Fig. 6 The mean values of Phenol content during the tomato storage period. A, CaCl₂×time interaction; B, UV-C×time interaction. The bars represent list significant difference at 5% level of probability.
chain that stabilizes cell membranes (Manganaris et al. 2007). In addition, irradiation by UV can improve the fruit firmness by decreasing the activity of enzymes catalyzing the cell wall of tomato fruit (Bu et al. 2013).

Results of this study indicated that increasing in calcium concentration reduces the fungal infection of treated fruits. Calcium is an important mineral element that significantly affects health, decay and physiological disorders of fruits Lurie (2009). Fonseca and Rushing (2006) obtained same result in watermelon and reported that UV can reduce the spread of fungal decay. Two reasons for reducing the decay by UV are mentioned: First, UV has germicidal effects by damaging DNA of microorganisms; and second, with induction of resistance by increasing the secondary metabolites such as phytoalexins and phenol, has an eminent role in the control of decay Gonzalez et al. (2007). Secondary metabolites such as phenols have an important role to reduce growth and activity of microbes in plants Gonzalez et al. (2007).

Calcium prevents senescence-induced stress by maintaining the strength of its membrane, thereby the presence of calcium in the cell membrane maintains strength of cell and thus delays the release of phenolic compounds Shirij et al. (2016). Also, UV increases phenylalanine ammonia lyase (PAL) that is a key enzyme in phenol synthesis. Similar to this study, increasing the total phenol content in fruits exposed to UV were reported on mango Gonzalez et al. (2007) and tomato Jagadeesh et al. (2009).

Results of this study show that UV treatment is far superior to CaCl2 treatment. Therefore, UV treatment could be considered not only as favorable tools in tomato shelf life extending but also able to preserve quality characteristics such as phenolic compounds and decay during storage time.

5. Conclusion

Accumulation ethylene during the storage can cause serious damage to the stored vegetables. It plays a significant role in shelf life and can cause a marked increase in respiration rates and enhanced senescence. So remove ethylene might be one of the possible solutions for delaying tomato fruit softening. The present study was conducted to present appropriate methods that not only reduce decay, but also maintain their post-harvest quality. The results of this study demonstrate that the storage life of tomato could be extended through application of UV-C irradiation and CaCl2. Our experiments shows that both UV-C and CaCl2 treatments have positive effect on tomato quality in comparison with control treatment because the treated fruits having higher phenol and firmness, and less PME activity, PG activity, ethylene, and decay. In conclusion, UV-C irradiation and CaCl2 can be an effective method to maintain post-harvest quality and to extend shelf-life. Whereas, increasing storage duration has significantly adverse impact on the evaluated traits due to increase in ethylene, PME activity, PG activity, decay and decrease in phenol content and firmness.

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