

## Research Article

# Ellagic Acid Treatment Improves Postharvest Quality of Tomato Fruits by Enhancing the Antioxidant Defense System

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Ellagic acid (EA) is a bioactive polyphenol compound with numerous biological activities, such as anti-inflammatory, anti-estrogenic, antioxidant, and anticancer activities. In this research, we investigated the effect of postharvest tomato fruits with EA treatment. Our results showed that at 25°C for 20 days, compared to a control group, the weight loss rate, titratable acidity, and soluble solid concentration of tomato fruits treated with 50  $\mu$ M EA were lower. The content of soluble protein in the EA-treated group was approximately 1.65 times higher than in the control group, and EA treatment greatly inhibited the changes in lycopene and vitamin C content. Moreover, EA treatment reduced malondialdehyde content, electrolyte leakage, and the production of reactive oxygen species. Furthermore, EA treatment upregulated the expression of antioxidant-related genes and induced the activities of the antioxidant enzyme. In summary, our results showed that EA could retard senescence and preserve the quality characteristics of harvested tomato fruits via enhancing the antioxidant responses.

## 1. Introduction

Tomato fruits (*Solanum lycopersicum* L.) are one of the major food crops grown worldwide. Tomato fleshy fruits are an essential source of dietary antioxidants, including vitamin C, lycopene, and flavonoids [1–3]. However, their postharvest life is relatively short, especially in summer, which significantly reduces their nutritional value and economic efficiency. Thus, developing an efficient strategy to curb the deterioration of harvested tomato fruits is important for both farmers and purchasers.

Senescence is a genetically determined process of oxidation, characterized by the general decline of physiological functions [4, 5]. Senescence is an oxidation process determined by the genes of *LeCAT1*, *LeCAT2*, and *LeAPX1*. The accumulation of reactive oxygen species (ROS) leads to lipid peroxidation and oxidative damage to membranes of plant cells and finally expedites the insenscence of vegetables and fruits [6].

Postharvest treatments, such as gas, low temperature, and chemical preservation, have been used to extend the freshness time of tomato fruits. However, fruits stored at

low temperature may exhibit cold damage and this incurs high running costs [7]. The change in the storage environment has strict equipment requirements, as gas fluctuation can affect the storage of tomato fruits [8]. The use of chemical compounds for preserving tomato fruits can easily cause environmental pollution. Therefore, exploring a cheaper alternative preservation method for reducing production costs and extending postharvest life is necessary.

Ellagic acid (EA) is a dimeric derivative of gallic acid found in the vacuole of plants [9, 10]. EA is a bioactive polyphenol compound derived from fruits, seeds, and nuts, such as pomegranates and walnuts [11, 12]. Recently, EA has attracted increasing attention owing to its numerous biological activities, including anti-inflammatory, anticancer, and free-radical scavenging activities [13–16]. Moreover, in the food industry, EA has been widely used as a food additive, because in human diet, its polyphenol constituents are the main source of antioxidants. However, to our understanding, no studies have been published in terms of using EA to preserve fresh vegetables and fruits and to extend their postharvest storage time.

In this study, we investigated EA for activities of delaying harvested tomato fruit senescence and preserving its quality characteristics. Our study shows that EA can maintain the quality of harvested tomato fruits by enhancing the antioxidant defense system and upregulating antioxidant-related genes to reduce ROS accumulation.

## 2. Materials and Methods

**2.1. Materials and Treatments.** The tomato fruits (*Solanum lycopersicum* L.) were obtained from a commercial supplier in Hefei city, Anhui Province, China. Red ripening tomato fruits of uniform size, without blemishes and fungal infection, were screened and immediately sent for subsequent experiments. The tomato fruits were washed with purified water and air-dried at ambient temperature before treatment.

The tomato fruits were divided into five groups randomly, with each group containing 24 tomatoes. The control group was soaked in distilled water, and the other four groups were immersed in EA solutions of different concentrations (25, 50, 75, and 100  $\mu$ M) for 10 min [17–19]. After the fruits were treated, they were dried at 25°C, loaded into a food box (12 fruits per box), at 25°C  $\pm$  2°C and 60–70% relative humidity, and stored for 20 days. The data were recorded at every 5 d intervals from day 0 of the treatment to day 20 of the treatment. All the experiments and treatments were repeated 3 times.

**2.2. Weight Loss.** The fruit weight loss of three tomato treatment replicates between day 0 and the end of each storage period was determined using the formula as follows:

$$WL = \frac{(W_a - W_b)}{W_a} \times 100\%, \quad (1)$$

where  $W_a$  is the initial weight and  $W_b$  is the final weight.

**2.3. Titratable Acidity, Soluble Solids, and Soluble Protein.** The treated tomatoes were crushed in a shredder, and the titratable acidity (TA) was calculated by referring the method of Ranggana [20]. Peeled tomato pulp (10 g) was homogenized at 80°C for 0.5 h and cooled to room temperature. Afterward, we diluted the homogenized sample with 30 ml distilled water and filtered. Then, three drops of phenolphthalein were added to 10 ml of the diluted filtrate and titrated with 0.1 M NaOH until it became faint red and did not fade for 30 s. The average value of three titrations was taken. The soluble solid concentration (SSC) of the fruits was measured on the first day and at the end of the storage period, using a digital Brix meter (FNV-32). The machine was standardized and treated with purified water before taking the reading. The soluble protein assay was performed as described by Bradford [21]. Peeled tomato pulp (2 g) was homogenized, diluted with 10 ml of distilled water, and centrifuged at 8000 rpm for 20 min, and the supernatant was the protein extract. Coomassie brilliant blue G-250 was used for protein staining, and we measured the absorbance value at 595 nm, to calculate the protein concentration.

**2.4. Lycopene Content and Vitamin C.** The content of lycopene was calculated based on the method of Xu et al. [22]: Peeled tomato pulp (2 g) was homogenized and shifted to a centrifuge tube, and 5 ml of absolute ethanol solution was added and then thoroughly mixed. The mixed solution was centrifuged at 4°C at 6 000 rpm for 20 min, and the supernatant was discarded. Then, we added 20 ml of ethyl acetate into the precipitate, thoroughly mixed the sample, and placed it in a 50°C water bath for 2 h. The supernatant was centrifuged, and the absorbance was measured at 474 nm. The content of vitamin C was calculated based on the method of Kampfenkel et al. [23]: 5 ml of oxalic acid (2%) solution was added to the 5 g of homogenized peeled tomato pulp, with a fixed volume of 50 ml. After the extract was filtered, we titrated the filtrate with 2,6-dichlorophenol indophenol. Oxalic acid (2%) solution as a control was also titrated.

**2.5. Cell Membrane Permeability and ROS Assay.** The H<sub>2</sub>O<sub>2</sub> content was detected in the sample using an assay kit (Jiancheng Bioengineering, Nanjing, China). The production rate of superoxide anion was calculated based on the method of Wang and Luo [24]: Peeled tomato (0.5 g) was homogenized. Then, 2 ml of 0.05 M PBS (PH 7.5) and 10 ml of 10 mM hydroxylamine hydrochloride were added to the homogenized sample, and it was allowed to stand at 25°C for 60 min. Afterward, 1 ml of 17 mM p-aminobenzenesulfonic acid and 1 ml of 7 mM of naphthylamine were added to the mixed sample, and it was allowed to stand at 25°C for 30 min. Then, we measured the absorbance at 530 nm. Electrolyte leakage was calculated based on the method of Wang et al. [25] with some modifications. The production rate of superoxide anion was determined as follows: we washed the tomato fruit slices of each replica with distilled water and cultured them at 25°C for 3 h, and the conductivity meter (DDS-307A) was used to measure the conductivity (A0) of each sample. We boiled the solution containing tomato slices for 20 min and cooled it under running water to room temperature and then measured the final conductivity (A1). We calculated the electrolyte leakage as follows:

$$\text{electrolyte leakage (\%)} = \frac{A0}{A1} \times 100\%. \quad (2)$$

The malondialdehyde (MDA) content was calculated based on the method of Liu et al. [26, 27]. Peeled tomato (2 g) was homogenized in an ice bath. Then, 5 ml of precooled 50 mM (pH 7.2) PBS was added to the homogenized sample to a constant volume of 25 ml. Then, the sample was centrifuged at 12 000 rpm and 4°C for 20 min, and the enzyme extract was the supernatant. Precisely, 0.5 ml of TBA solution was added to 0.3 ml of the enzyme extract and mixed. We placed the mixed sample at 98°C water bath for 20 min and a rapid iced bath for 10 min. After centrifugation of the sample, at 450, 532, and 600 nm, we measured the absorbance with the enzyme plate analyzer.

**2.6. Assay of Enzyme Activity.** The ascorbate peroxidase (APX) activity of the sample was calculated based on the method of Nakano and Asada [28]. Peeled tomato pulp (1 g)

was homogenized, and precooled 50 mM of  $K_2HPO_4$ - $KH_2PO_4$  buffer was added to the homogenized sample at 1 : 3 (W/V). The end volume of the mixed solution was diluted to 10 ml, and the sample was filtered with two layers of gauze. The filtrate was centrifuged (4 000 rpm) for 30 min. The enzyme extract is the supernatant, to determine the reaction mixture (2 ml) containing 50 mM  $K_2HPO_4$ - $KH_2PO_4$  flushing solution (pH 7.2); 1 M of EDTA- $Na_2$ , 0.06 mM of  $H_2O_2$ , and 0.3 mM of ASA were added to 0.1 ml of the enzyme extract. After  $H_2O_2$  was added to the extract, the change in A290 within 10 to 30 s was measured at 20°C and the amount of AsA and enzyme activity were measured per unit hour. The activity of catalase (CAT) was determined by using the reaction mixture containing 3% (v/v)  $H_2O_2$  through the absorption of  $H_2O_2$  at 240 nm [29–31]: Peeled tomato pulp (2 g) was homogenized, and the end volume of the homogenized sample was reduced to 10 ml. The extract was centrifuged (8 000 rpm) for 30 min. The supernatant was used as a crude enzyme extract for the determination of CAT. Then, we added 1 ml of 0.5 M Tris-HCl enzyme extract and 1.7 ml of distilled water to the reaction solution. Precisely, we added 200  $\mu$ l of 0.2 M  $H_2O_2$  to the mixed solution, and the sample was placed in a water bath at 25°C and soaked for 3 min. Then, A240 readings were taken every 30 s for 3 min. The boiled enzyme extract was the control group. The activity of peroxidase (POD) was measured by catalyzing  $H_2O_2$  and the absorbance change at 420 nm. 20 mM  $KH_2PO_4$  was added to 2 g of homogenized peeled tomato fruit to the total volume of 5 ml and then centrifuged at 12 000 rpm for 20 min. The enzyme extract was the supernatant. We added guaiacol (28 ml) to the reaction mixture (containing 50 ml of 100 mM PBS (PH6.0)). Then, the mixed solution was heated and stirred until a uniform solution was formed. Afterward, 30% hydrogen peroxide of 19 ml was added to the resultant solution after it was cooled. Then, we added 3 ml of the reaction mixture to 1 ml of the enzyme extract. Superoxide dismutase (SOD) concentration in the enzyme extract was the same as that of APX. Precisely, 20 ml of potassium PBS was used as the reaction system solution; 13 mM of Met, 1.3  $\mu$ M of riboflavin, 0.1 mM of EDTA, and 0.063 mM of NBT were added to the reaction system solution. Then, we added 2 ml of reaction system solution and 20  $\mu$ l of enzyme extract to each group. After 20 min of light irradiation at room temperature, the absorbance value at 560 nm was measured. The activity of SOD was measured by the reduction inhibition of nitrogen blue tetrazole (NBT) under light at 450 nm light absorption. The reaction system without enzyme extract was used as control. The activity of polyphenol oxidase (PPO) was detected using an assay kit (Jiancheng Bioengineering, Nanjing, China). The LOX activity was calculated based on the method of Axelrod [32]: 10 ml of precooled 0.5 M PBS (pH 7.2) was added to 2 g of homogenized tomato pulp, and the mixture was centrifuged at 8 000 rpm for 20 min. The enzyme extract was the supernatant. The reaction system (2 ml) contained 25  $\mu$ l of 10 mM sodium linoleate, 2.775 ml of buffer, and 0.2 ml of enzyme extract. The change in 280 nm within 1 min was recorded 15 s after the addition of enzyme solution.

**2.7. Gene Expression Analysis.** Using an assay kit, tomato fruit juices were extracted for RNA isolation (Jiancheng Bioengineering, Nanjing, China). The reverse transcriptase SuperMix is used to reverse transcribe RNA into cDNA. According to the instructions provided for the Bio-Rad iCycler iQ system, platinum SYBR Green qPCR SuperMix UDG was used for quantitative real-time PCR (Invitrogen). Table 1 lists the primers of gene-specific for real-time PCR. At least three replicates of each sample were quantified and standardized using *LeACTIN41* as an internal control. The PCR thermal cycling program procedure was as follows: initial denaturation for 30 s at 95°C and then by 40 cycles at 95°C for 15 s, followed by 30 s at 60°C. Each treatment was technically and biologically repeated thrice.

**2.8. Statistical Analysis.** SPSS 14.0 was used for Student's *t*-test to compare the mean values of independent groups ( $P < 0.05$ ).

### 3. Results

**3.1. Effect of EA on the Deterioration of Tomato Fruits.** To analyze the influences of EA on the quality of postharvest tomato fruits, freshly harvested tomatoes were treated with EA (25, 50, 75, and 100  $\mu$ M) or without EA for 20 days. Compared to the control group, the fruit treated with EA maintained high quality throughout the experimental period, especially at 50 and 75  $\mu$ M. After the treatment, the fruit exhibited good firmness and its life span was extended. In addition, we investigated the weight loss of the fruit, which is the main quality attribute of postharvest tomato fruits [33, 34]. During storage, the weight loss of the control group and EA-treated tomato fruits exhibited an increasing trend [35, 36]. However, the weight loss of EA-treated tomatoes was significantly reduced, and 50  $\mu$ M EA-treated tomato fruits had the lowest weight loss rate, followed by 75  $\mu$ M EA (Figure 1(a)). Thus, these results showed that EA positively affected the postharvest fruit quality.

**3.2. Effect of EA on the Ripening and Senescence of Tomato Fruits.** To explore the effect of EA on physiology and quality of tomato fruits ripening, we measured the SSC, TA, and soluble protein of tomato fruits subjected to EA treatment. The SSC showed a linear increase in the early stage of storage due to the enhanced respiration and hydrolysis rates [34]. According to our results, the SSC level of the EA-treated groups was significantly lower than that of the control group. Among the EA-treated fruits, 50  $\mu$ M EA-treated fruits had the lowest SSC level, and it was 34.4% lower than that of the control at the end of the experiment (Figure 1(b)). Furthermore, organic acids were continuously consumed as substrates for respiration during storage. However, the TA displayed a downward trend during the storage [37]. The initial baseline TA of control and EA-treated tomato fruits was 0.506%. At the end of the storage, the TA of the control and 50  $\mu$ M EA-treated groups was 0.102% and 0.277%, respectively. EA treatment decreased TA, indicating that EA inhibited the utilization of organic acids by reducing the

TABLE 1: Primers used in RT-qPCR analysis for gene expression.

Gene name	GenBank no	Primer sequence
<i>LeAPX1</i>	NC_015443	F: gtgctctattatgctccgctc R: gagtctgagagcaatgtcaagac
<i>LeCAT1</i>	NC_015449	F: gtctatcagggacattcgtg R: cagggacgacttagcatcac
<i>LeCAT2</i>	NC_015439	F: cactaattctgggtcctcctg R: gcatgaacaacacgttctgc
<i>LeACTIN41</i>	NC_015441	F: ctactgtatgccagtggctg R: caagacggagaatggcatg

respiration rate, thereby delaying the ripening of tomato fruits (Figure 1(c)). In addition, the senescence of plant cells can be evaluated by a decrease in soluble protein [38]. The content of soluble protein in the control groups was evidently lower than that of the EA-treated groups (Figure 1(d)). After 20 days of storage, fruits treated with 50  $\mu\text{M}$  EA had the highest soluble protein content, which was 1.65-fold higher than the control groups. The result shows that with EA treatment, the harvested tomato fruits senescence can be delayed.

### 3.3. Effect of EA of Tomato Fruits on the Nutritional Quality.

Vitamin C, representing tomato fruits in the nutritional quality, is one of the main components of vitamins [23, 39]. During the storage period, in both EA-treated and control groups, the content of vitamin C first increased and then decreased (Figure 2(a)). The maximum content of vitamin C was obtained on the 5th day and then, by degrees, decreased till the end of storage. Compared to the control group, the fruit groups treated with EA concentration reduced the loss of vitamin C. When the EA concentration was between 50 and 75  $\mu\text{M}$ , the effect of EA was significant (Figure 2(a)).

Lycopene is characterized by superior antioxidant and antiaging effects, and it contributes to the red color of tomato fruits as one of the most significant nutrients [9, 40]. Figure 2(b) shows that the content of lycopene increased during storage. The content of lycopene in the control group was higher than that in the EA-treated groups. At the end of storage, the 50  $\mu\text{M}$  EA-treated fruit exhibited the lowest level of lycopene, which was 15.9% lower than in the control group.

### 3.4. Effect of EA on the ROS Production and the Permeability of Cell Membrane of Tomato Fruits.

ROS can lead the plant cell membranes to oxidative damage and lipid peroxidation, which eventually expedites the senescence of vegetables and fruits [41, 42]. Thus, we measured the  $\text{H}_2\text{O}_2$  and  $\text{O}_2^{\bullet-}$  production rate in tomato fruits. The change in  $\text{H}_2\text{O}_2$  content in tomato fruits is presented in Figure 3(a). During the storage of the fruits, the  $\text{H}_2\text{O}_2$  content in harvested tomato fruits increased. Before 15 days of fruit storage, EA treatment obviously inhibited the accumulation of  $\text{H}_2\text{O}_2$  content. Afterward, the increase in  $\text{H}_2\text{O}_2$  content in the treated groups was lower than that of the control group. Compared with the control group, the  $\text{O}_2^{\bullet-}$  production rate

of EA-treated fruits was significantly lower during storage. Figure 3(b) shows that the  $\text{O}_2^{\bullet-}$  production rate of the EA-treated tomato fruits declined and then increased, whereas the control group continuously increased.

The amount of electrolyte leakage can be used to evaluate the changes in tomato fruit membrane permeability. In both treated and untreated fruits, the electrolyte leakage was observed. However, the leakage of EA-treated groups was significantly less than of the control group (Figure 3(c)). MDA is another major product of membrane lipid peroxidation, which indicates membrane damage in vegetables and fruits [6]. In both the treated groups and the control group, no significant difference was observed, before day 5 of the storage. After 5 days of storage, the content of MDA in the control groups and the EA-treated groups was increased. The content of MDA in the treated groups was remarkably lower than in the control group (Figure 3(d)). Thus, these results indicated that the treatment of EA could reduce the production of ROS and the cell membrane permeability of tomatoes.

### 3.5. Effect of EA on the Antioxidant Activity of Tomato Fruits.

Enzymatic browning of fruits and vegetables is indicated by PPO [43]. Figure 4(a) shows the trends in PPO enzyme activity in tomato fruits induced by EA treatment. The minimum PPO activity was exhibited in 50  $\mu\text{M}$  EA-treated groups and then in 75  $\mu\text{M}$  EA. In addition, LOX can catalyze free unsaturated fatty acids to produce lipid peroxidation free radicals, which destroy the phospholipid bilayer of the cell membrane [44]. The maximum LOX activity in treated fruits was observed on the 15th day of fruit storage, which was 5 days later than in the control group. The control group of LOX activity was much higher than that in the EA-treated groups during storage (Figure 4(b)). A complex antioxidative defense system of plants has been evolved for removing the ROS [42, 45]. To investigate the involvement of ROS metabolism during tomato ripening and ageing, we measured numerous enzymes associated with ROS. During 15 days of storage, the APX activity of tomato fruits in both the EA-treated and untreated groups was maximum and subsequently decreased until the end of storage (Figure 4(c)). The EA treatment upregulated APX activity of tomato fruits to 10.03–22.57% compared with the control at day 15. The SOD activity effects of the enzymatic activities of the EA treatment are shown in Figure 4(d). The maximum SOD activity in the control group was observed on the 15th day,

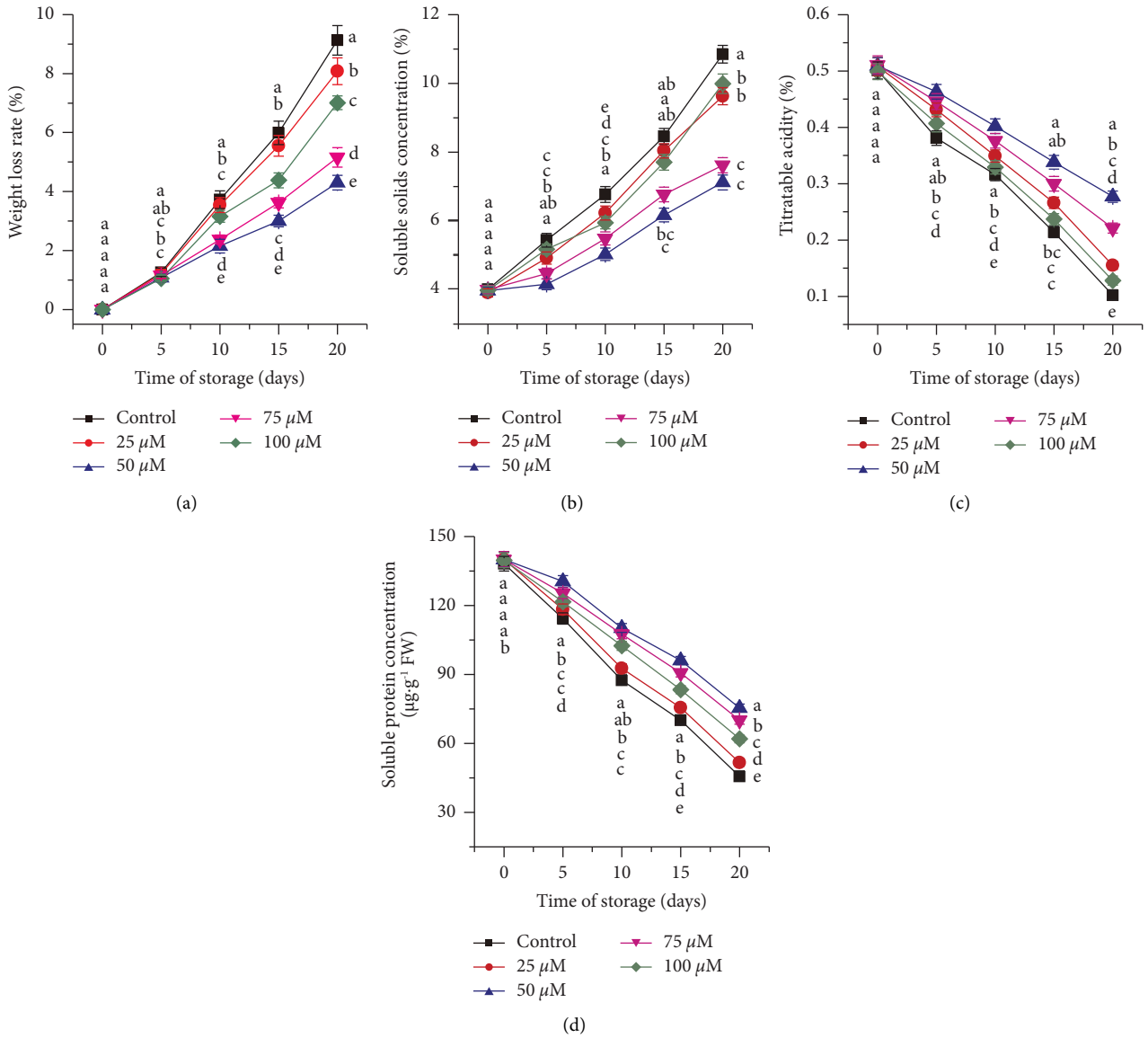


FIGURE 1: Effects of EA treatment on (a) weight loss, (b) soluble solids concentration, (c) titratable acidity, and (d) soluble protein of tomato fruits during storage periods (20 days). Data are the mean ± SE of three independent replicates. Statistical significance is determined using ANOVA. Significant differences ( $P < 0.05$ ) are indicated by different error bars.

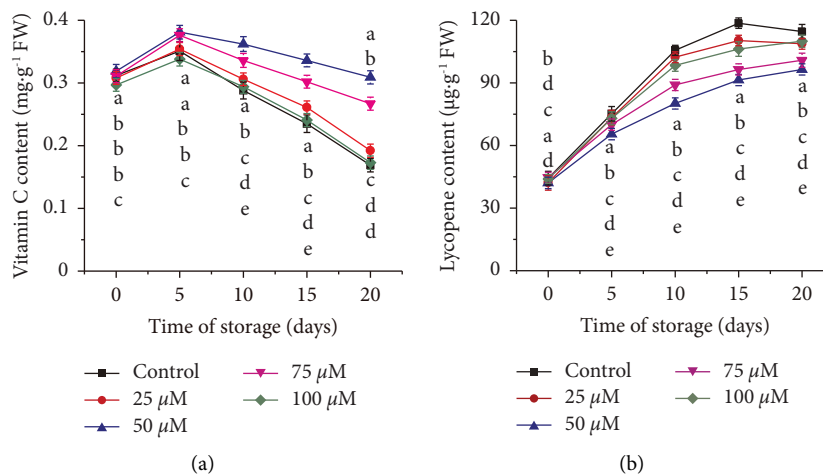


FIGURE 2: Effects of EA treatment on (a) vitamin C content and (b) lycopene content of tomato fruits during 20 days of storage. Data are the mean ± SE of three independent replicates. Statistical significance is determined using ANOVA; significant differences ( $P < 0.05$ ) are indicated by different error bars.

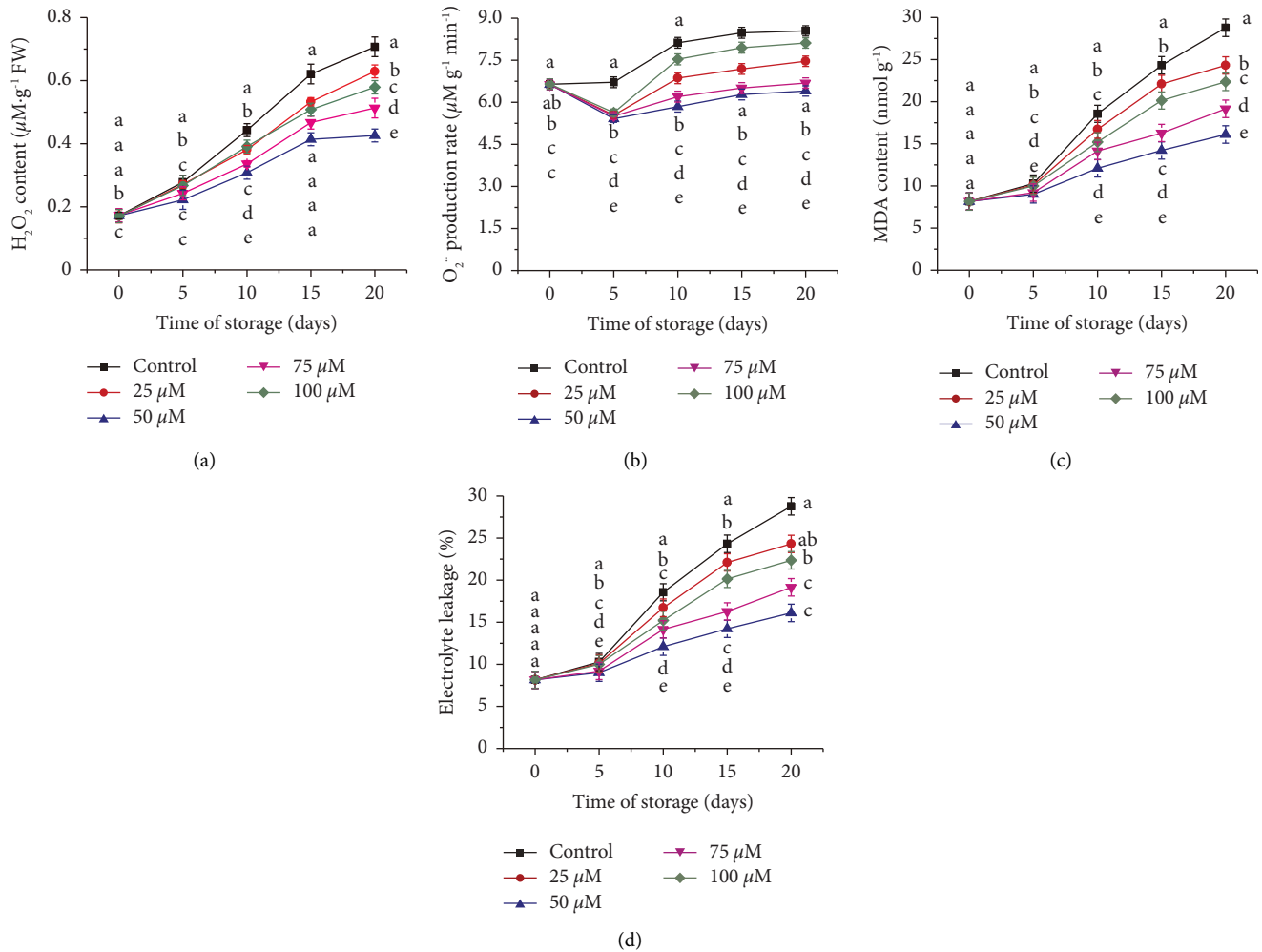


FIGURE 3: Effects of EA treatment on (a)  $H_2O_2$  content, (b)  $O_2^{\bullet-}$  production rate, (c) electrolyte leakage of tomato fruits during 20 days of storage, and (d) MDA content. Data are the mean  $\pm$  SE of three independent replicates. Statistical significance is determined using ANOVA; significant differences ( $P < 0.05$ ) are indicated by different error bars.

which was 5 days later than in the treated groups. Moreover, in both the control and EA-treated groups, the time when POD activity reached its maximum was consistent with the time when SOD activity increased (Figure 4(e)). Compared to the control group, the maximum activity of POD in EA-treated tomato fruits was 7.46–13.21% higher. Furthermore, the CAT activity in tomatoes showed a downward trend increase in time (Figure 4(f)), and it was much higher in EA-treated fruits than in the control groups ( $P < 0.05$ ), particularly after 5 days of storage (Figure 4(f)). The activity of CAT level in EA-treated fruits was 1.35–2.01-fold higher than in the control group at the end of storage. These results indicated that EA treatment enhanced the postharvest tomato fruits of antioxidant enzyme activities.

In addition, we detected the *LeCAT1*, *LeCAT2*, and *LeAPX1* transcription levels in treated and untreated fruits. The results indicated that the transcription levels of *LeCAT1*, *LeCAT2*, and *LeAPX1* in the fruits treated with EA were obviously higher than in the control (Figures 5(a)–5(c)). Moreover, the levels of transcription of *LeCAT1*, *LeCAT2*, and *LeAPX1* reached their peaks at day 10 (*LeCAT1* and *LeCAT2*)

and day 15 (*LeAPX1*) and then decreased at the terminal point of storage. We also measured the expression levels of ethylene pathway-related genes *LeACO1*, *LeACS2*, and *LeACS4*. The results showed that the transcription levels of *LeACO1*, *LeACS2*, and *LeACS4* between EA-treated fruits and the control groups had no obvious difference (Figures S1A–1C) [46–48]. The previous results showed that EA treatment could enhance antioxidant enzyme activities and upregulate the expression of antioxidant-related genes and could not enhance the expression of ethylene biosynthetic related genes.

#### 4. Discussion

The tomato is a representative respiratory transgressive fruit [49]. Fleishy fruits are an essential source of dietary antioxidants, such as flavonoids, vitamin C, and lycopene [1–3]. However, on account of its high respiration rate and short postharvest life, the fruit is likely to wilt and rot, which leads to bad taste and poor consumer acceptance. In this research, we assessed the effects of EA on postharvest fresh tomato fruits and their quality and physiological metabolism during

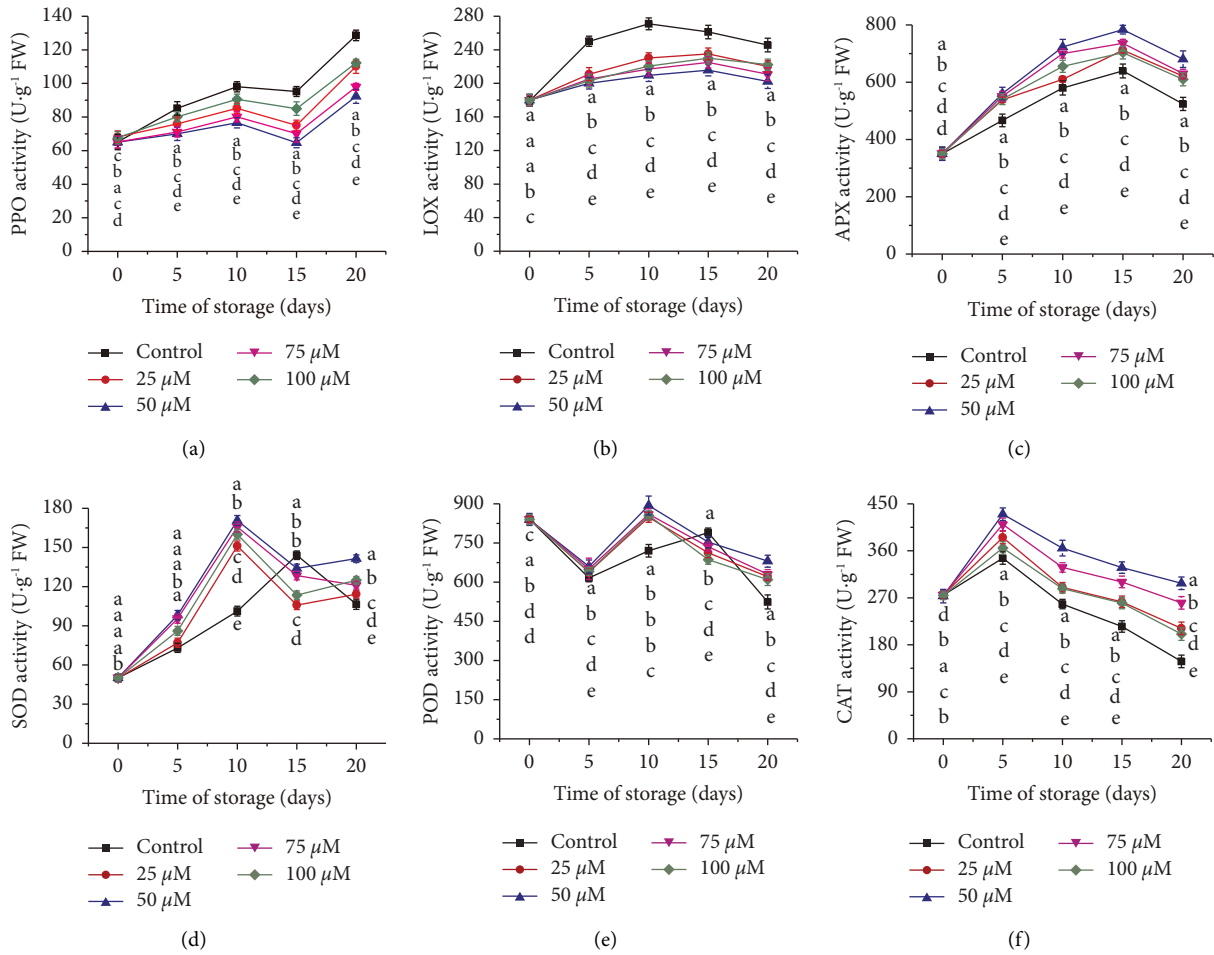


FIGURE 4: Effects of EA treatment on (a) PPO activity, (b) LOX activity, (c) APX activity, (d) SOD activity, (e) POD activity, and (f) CAT activity of tomato fruits during 20 days of storage. Data are the mean  $\pm$  SE of three independent replicates. Statistical significance is determined using ANOVA; significant differences ( $P < 0.05$ ) are indicated by different error bars.

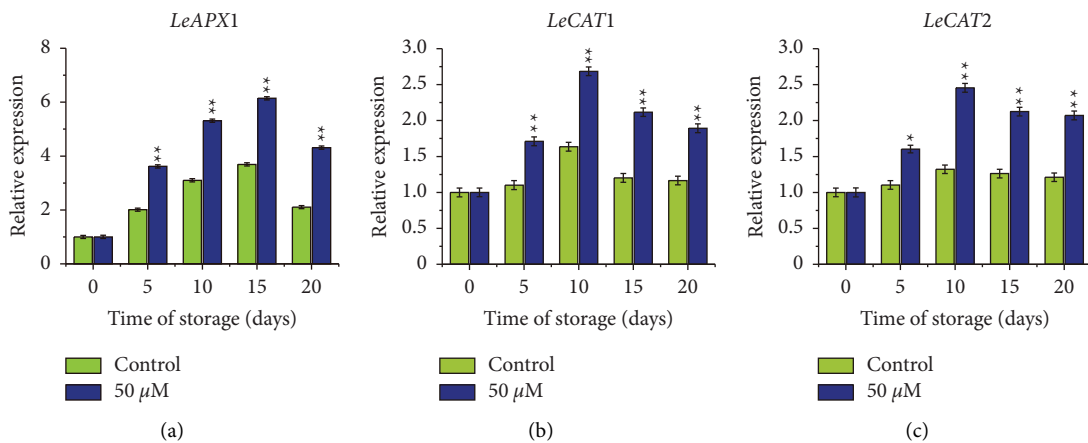


FIGURE 5: qRT-PCR analysis of antioxidant-related genes. (a) *LeAPX1*, (b) *LeCAT1*, and (c) *LeCAT2* were subjected to 50  $\mu$ M EA during storage for 20 days. *LeACTIN41* was used as the internal control. Data are the mean  $\pm$  SE of three independent replicates. Statistical significance is determined using ANOVA; significant differences ( $P < 0.05$ ) are indicated by different error bars.

storage. The results indicated that the shelf-life of post-harvest tomato fruits could be extended by EA treatment.

In fresh vegetables and fruits, the fundamental mechanisms of weight loss are respiration and transpiration [33, 34]. In this research, we found that EA treatment considerably alleviated the weight loss of the tomato fruits, indicating that EA could prevent weight loss by reducing the respiration rate, thereby inhibiting the fruit deterioration during storage (Figure 1(a)). Our findings agree with the results of Ali et al. [50] that gum Arabic-coated tomatoes can modify the internal atmosphere and reduce weight loss during storage.

Tomatoes usually undergo several postharvest physiological changes, such as soluble protein, TA, and SSC. These indices are essential quality attributes of fruit senescence and ripening [38, 51]. In addition, the amount of nutrients in tomato fruits changes considerably with shelf life. Natural conditions result in severe losses of many important nutrients in tomatoes, such as vitamin C and lycopene, which directly affects the business value of tomato fruits [9, 23, 39, 40]. In this research, the changes in common phenomena during the ripening of these fruits were delayed after EA treatment (Figures 1(b)–1(d)), thus maintaining the nutritional quality of tomatoes (Figures 2(a) and 2(b)). The delay in fruit ripening indicates EA can delay the senescence and loss of essential nutrients in harvested tomatoes.

The increase in the ROS level can cause lipid peroxidation and oxidative damage in plant cell membranes, which can lead to a change in intrinsic membrane properties, thereby accelerating fruit senescence and eventually leading to the death of cell. The commonest ROS includes  $^1\text{O}_2$ ,  $\bullet\text{OH}$ ,  $\text{H}_2\text{O}_2$ , and  $\text{O}_2^{\bullet-}$  [45]. The plant cell membrane damage degree can be affected by the MDA content [27]. In all case, electrolyte leakage has been considered as a direct indicator of plant membrane damage and is often associated with postharvest senescence of fruits and vegetables [52]. Our data demonstrated that EA treatment significantly reduced the increase in  $\text{H}_2\text{O}_2$ , MDA, electrolyte leakage, and  $\text{O}_2^{\bullet-}$ , suggesting that EA treatment could reduce cell membrane damage and maintain membrane integrity (Figures 3(a)–3(d)).

EA treatment induces a complex series of antioxidant defence mechanisms to eliminate ROS when excessive ROS accumulates in plants [3]. In this research, the ability of some enzymes involved in ROS metabolism during tomato senescence and ripening to be induced after EA treatment was analysed by measuring the activity of related enzymes and the expression levels of genes for synthesis-related enzymes. EA treatment could upregulate the activities of SOD, APX, CAT, and POD enzymes during storage in tomato fruits (Figures 4(c)–4(f)). In addition, the transcription levels of genes for synthesis-related enzymes, such as *LeCAT1*, *LeCAT2*, and *LeAPX1*, were considerably increased (Figures 5(a)–5(c)). The transcription levels of ethylene biosynthetic-related genes have no significant change (Figures S1A–1C, Table S1). Ellagic acid has a lot of phenolic hydroxyl groups and can chelate with metals [53, 54] that are easily oxidized, so it protects other things from being oxidized. Ellagic acid had a good scavenging effect on DPPH free radicals and showed a concentration-dependent relationship [55, 56].

However, the EA treatment on tomatoes of antioxidant effects has not been reported. Thus, we provide the first evidence that EA can enhance the enzyme and antioxidant activities to restrain oxidative damage and delay the deterioration of harvested tomato fruits.

## 5. Conclusion

In summary, our findings revealed that 50  $\mu\text{M}$  EA-treated fruits maintained high quality compared with the control group during storage periods. EA treatment inhibited the decline in SSC, soluble protein, and TA in tomato fruits. It inhibited the rise in the lycopene content and the decrease in vitamin C content, thereby delaying the aging of postharvest tomato fruits. In further experiments, we explored the mechanism related to EA stimulation. The contents of  $\text{H}_2\text{O}_2$  and MDA, the amount of  $\text{O}_2^{\bullet-}$  produced, and the electrolyte leakage were lower in EA-treated tomato fruits than that in the control group, indicating that EA treatment reduced the production of ROS and cell membrane permeability of tomato fruits. During the storage periods, the activity of PPO and LOX decreased, while the contents of APX, SOD, POD, and CAT increased. The expression of APX- and CAT-related genes was upregulated in the middle and late stages of the storage periods. Thus, the EA treatment improved the antioxidant defense system, upregulated antioxidant-related genes, and inhibited ROS accumulation, further indicating that the postharvest quality of good tomato fruits was associated with enhanced antioxidant defense systems. Hence, this study shows that EA treatment of tomato fruits can delay the senescence and preserve the postharvest quality.

## Data Availability

The data used to support the findings of this study are included within the article.

## Additional Points

Tomato fruits are susceptible to pathogenic bacteria during postharvest storage, resulting in extensive fruit rot. In addition, tomato fruits have soft flesh and are rich in water content, making them more vulnerable to damage and difficult to store. Thus, it is necessary to develop an efficient strategy to maintain tomato fruit quality.

## Disclosure

Junxuan Cao should be considered as the first author.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

## Acknowledgments

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## Supplementary Materials

Supplemental Figure S1: expression profile of *LeACS2*, *LeACS4*, and *LeACO1*. Table S1: primer sequences used for qRT-PCR. (*Supplementary Materials*)

## References

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