

Using Chitosan Combined Treatment with Citric Acid as Edible Coatings to Delay Postharvest Ripening Process and Maintain Tomato (*Solanum lycopersicon* Mill) Quality

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Abstract Physicochemical properties of citric acid and chitosan treated fresh tomato fruits were investigated in the present study. The experimental results showed that control fruits had higher weight loss, lower firmness and pH, but tomatoes-treated with chitosan and citric acid had no significant effect on pH, Vc, TSS and firmness, but significantly increased the SOD, POD, CAT and PAL activities of fresh tomatoes. All these treated tomato fruits maintained lighter color and higher contents of total phenolics and flavonoids, significantly decreased weight loss and MDA content of fresh tomato fruits compared to control. Of all these treated tomato fruits, citric acid + chitosan treatment maintained higher fruit quality, reduced the undesirable changes and protected the fruit tissues from injury. In short, the experiment datum indicated that citric acid + chitosan might be applied in a safe and effective preservative of fresh tomato fruits.

Keywords: chitosan, citric acid, water loss, firmness, MDA, total phenolics

Cite This Article: Jihong ZHANG, Li ZENG, Helong SUN, Junhong ZHANG, and Shaoyang CHEN, "Using Chitosan Combined Treatment with Citric Acid as Edible Coatings to Delay Postharvest Ripening Process and Maintain Tomato (*Solanum lycopersicon* Mill) Quality." *Journal of Food and Nutrition Research*, vol. 5, no. 3 (2017): 144-150. doi: 10.12691/jfnr-5-3-1.

1. Introduction

Fresh tomato fruits (*Lycopersicon esculentum*) are one of popular horticultural products worldwide, and highly appreciated by people for their health-related compounds such as ascorbic acid, flavonoids and phenolic acids, etc [1,2]. However, tomato is a seasonal fruit, because it has a shorter postharvest storage period due to several factors such as higher weight loss, rate of respiration and enhanced ripening [3,4]. Therefore, it is necessary to find out an effective method for postharvest preservation to maintain tomato fruits' shelf-life quality.

The natural occurrences of citric acid as metabolic intermediate product exist in mostly all organisms, which is generally recognized as safe (GRAS) for use as a food preservative material and is often used to reduce the microbial contamination of fresh-cut vegetables [5,6]. Citric acid has been used for preventing browning, maintaining the quality and prolonging fresh fruits' shelf-life [7,8].

Chitosan is a natural biopolymer having non-toxic in nature, unique polycationic, chelating and film forming properties. Owing to these unique properties, chitosan is widely used in diverse fields ranging from medicine, pharmaceutical, cosmetics, food and nutrition and agriculture [9,10]. Many literatures had reported that chitosan had

anti-browning virtue and can reduce microbial decay and maintain tissue hardness for postharvest fruits to prolong shelf-life periods which had been used for numerous horticultural commodities as a protective edible film [11,12,13]. In addition, chitosan can also be combined with various functional compounds that have antioxidant, antimicrobial or other activities to enhance the product quality [14,15]. The treatment of chitosan plus citric acid can be used for controlling the browning and improving the storage life of fresh and fresh-cut fruits [13,16,17]. Yet, there are few reports on citric acid and chitosan coatings for extending the shelf life of tomato.

The purpose of the study was conducted to assess the efficiency of citric acid treatment combined with chitosan on reducing the undesirable changes of tomato fruits in color, weight loss and firmness during storage at 28°C, and develop a novel preservation method to improve the stored tomatoes' quality.

2. Material and Methods

Fresh tomato (*Lycopersicon esculentum*, Zhongshu No.5) fruits were obtained from Yangmei farm in Xiangtan city. Fruits were picked by hand with maximum care to minimize mechanical damage. Uniformity of size, shape and color of fruits were used for the further experiment.

2.1. Citric Acid and Chitosan Coating Treatment

Tomato fruits were surface-sterilized with 1% sodium hypochlorite solution (v/v) immersing for 2 min. Subsequently, sterile water rinsed fruits three times, and left to air-dried. In preliminary experiment, a series of concentration of citric acid and chitosan solutions, 0, 0.5, 1.0 and 1.5% (w/v) for citric acid and 0, 0.5, 1.0 and 1.5% (w/v) for chitosan were used, respectively. According to preliminary experimental results, 1.0% citric acid and 1.0% chitosan were selected as the treatment concentration.

The dip treatment was according to the method of Ducamp-Collin *et al.* [16]. This was prepared by dissolving 10.0g/L molecular weight (MW 40 000) chitosan was added in boiling water, and then citric acid was dissolved in the above solution to a final concentration of 1.0g/100 mL, which was cooled to room temperature. Each tomato fruit was dipped in the solution, drained, dried naturally and then stored at 28±2°C and 35-45% relative humidity (RH). Each group comprised 20 fruits with three replications.

2.2. Color Changes

Surface colors of fruits were measured using a minoltachromameter CR400 (Konica Minolta, Ramsey, NJ, USA) by averaging the values at three locations of each fruit around the equator [18]. The chroma value was expressed as a^* (green to red), b^* (blue to yellow), and L^* (lightness) values. The a/b ratio was calculated as an index of maturation [19].

2.3. Fruit Firmness and Weight Loss

A hand-held digital force gauge (GY-J, Top Instrument Co., Ltd., Zhejiang, China) was used for determining fruit firmness. Cut a 2cm×2cm square wound at equatorial region of tomato fruits with a blade. Firmness was taken as the force (N) required to puncture the wound 1cm with a 2-cm probe. By measuring the weight of whole tomato fruit at the beginning and each storage period, weight loss was expressed relative to the initial weight in percent.

2.4. TSS, pH and Ascorbic Acid Content

A portable refractometer (Atago PAL-1, Japan) was used for determining the total soluble solids (TSS) of the juice. A Metron model pH meter (WTW 526, Germany) was used for measuring the pH values at room temperature.

Phenolindo-2, 6-dichlorophenol (DPIP) was used to determine ascorbic acid (Vc) by titration [20]. Samples were protected from light during all procedures. The analysis was performed in triplicate for experimental point. Vc contents were expressed as mg per 100 g FW.

2.5. Flavonoids, Total Phenolics and MDA Contents

Flavonoid and total phenolic compounds were measured on the basis of a previously described method [21]. The absorbances were measured at 280 and 325 nm,

respectively. Phenolic compounds and flavonoid compounds were expressed as $OD_{280} g^{-1} FW$ and $OD_{325} g^{-1} FW$, respectively. 1% HCl-methanol solution was used as blank control. MDA contents were assayed on the basis of a previously described method [22] with some modifications. The absorbances were measured at 450 nm, 600nm and 532nm, respectively. The MDA contents were expressed as $nmol \cdot g^{-1} FW$.

2.6. Assay of SOD, POD, CAT and PAL Enzyme Activity

Superoxide dismutase. Superoxide dismutase (SOD) was assayed according to the method of Wang and Chen [23] with some modifications. One unit of superoxide dismutase activity was defined as the amount of enzyme required to cause 50% inhibition of the reduction of NBT under the assay condition, and the enzyme units were defined as U/g·min FW.

Peroxidase. POD enzyme activities were measured on the basis of a previously described method [24]. The changes in absorbance (OD) at 470 nm were measured by a spectrophotometer. The POD enzyme activities were expressed as OD value' differences per minute per gram fresh weight.

Catalase. CAT enzyme activities were determined on the basis of the method of Aebi [25] with some modifications. One unit of catalase enzyme activity was expressed as 0.01 change of absorbance value at 240 nm per minute. CAT activity was defined as enzyme units per gram fresh weight.

Phenylalanine ammonia lyase. PAL enzyme activity was assayed according to the methods of Zucker [26]. One enzyme unit was expressed as the amount of enzyme that brought about the increase in absorbance of 0.01 at 290 nm per 1 hour under the specified conditions.

2.7. Statistical Analysis

Values presented are an average of three replicates along with the standard deviation ($\pm SD$), and data were analyzed by one-way ANOVA. Unless noted otherwise, only significant results at $p < 0.05$ were discussed in accordance with Duncan's multiple range tests.

3. Results and Discussion

Color is an important external indicator of tomato fruit quality. The L^* values generally presented a declining trend, while the L^* values in tomatoes treated with citric acid and chitosan coating were significantly higher than those in uncoated tomatoes, which may be related to the higher anthocyanin content in pericarp (Figure 1A). These experimental results are consistent with previous studies, in which coating treatment delayed fruits' color changes [27,28]. In general, the tomatoes for control had significantly ($P < 0.05$) higher a/b ratios than those treated tomatoes after 9 days of storage, pointing to faster ripening (Figure 2B), the similar results had been reported by García-García *et al.* [19]. The treatment with chitosan and citric acid effectively fixed the red colouration of the litchi fruit [7].

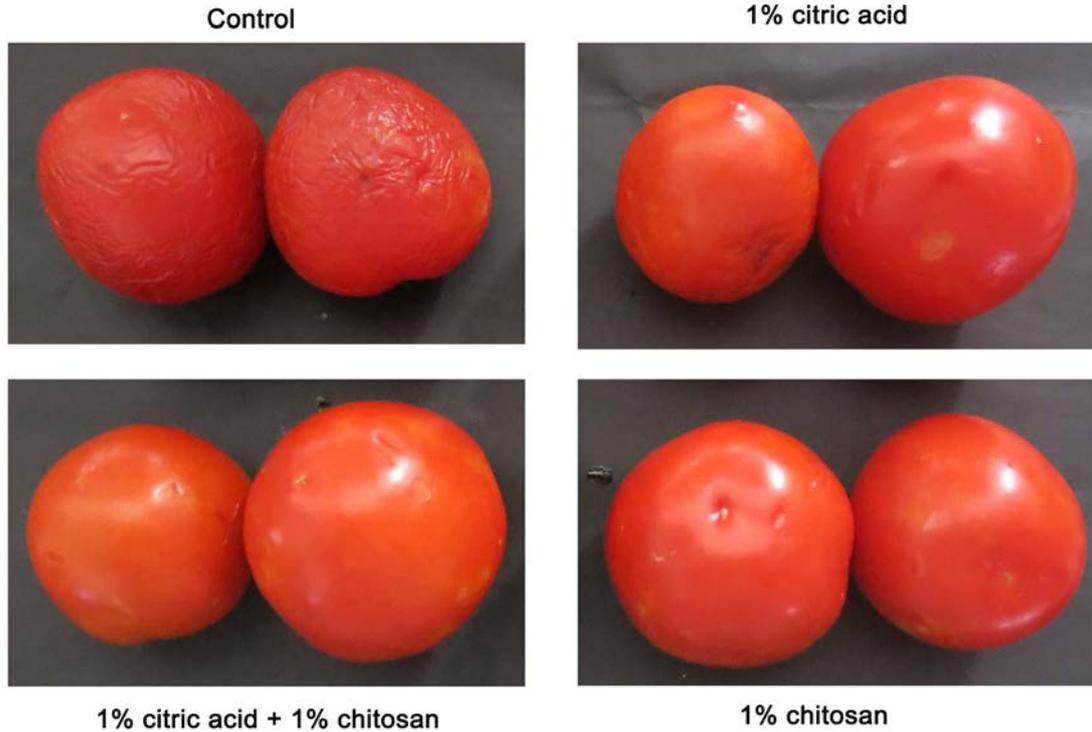


Figure 1. Appearance of tomatoes treated with 0, 1% citric acid, 1% citric acid + 1% chitosan and 1% chitosan, and then stored at 28°C for 15 days

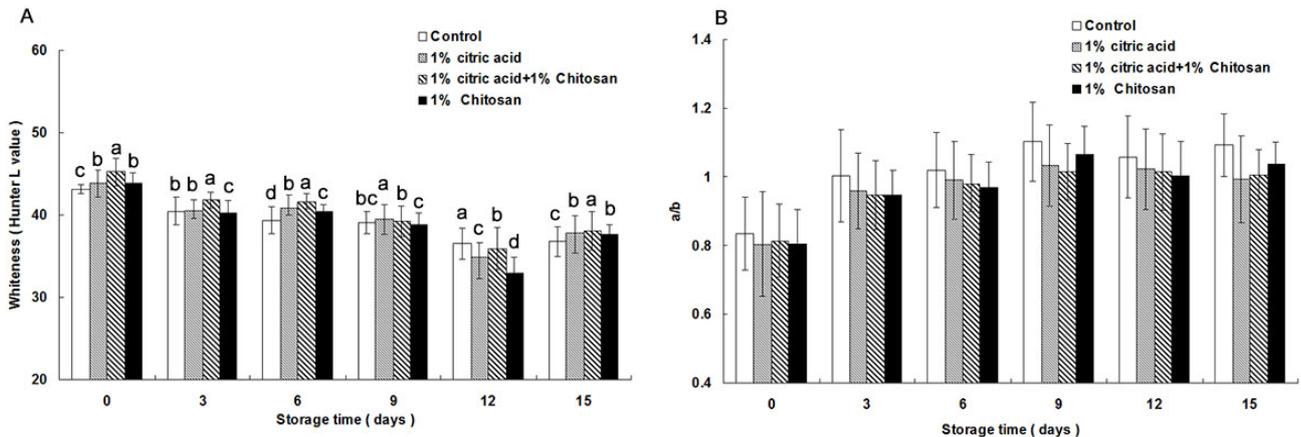


Figure 2. Changes in colour parameters [L^* (A), a/b (B)] in treated and untreated tomato fruits over 15 days storage at 28°C. Each data point is the mean of three replicate samples ($n=20$). Bars marked with different letters in each column indicate significantly different values at $p < 0.05$

At the end of storage time, the highest weight loss ($\approx 20.40\%$) was recorded in control fruits, while the minimum weight loss was 14.86% for the combination treatment, which indicated that chitosan and citric acid treatment can effectively reduce weight loss of tomato fruits (Figure 3A). Higher weight loss of tomato fruits happened in the later storage condition which was associated with faster metabolism and ripening at higher temperature, increased cell wall degradation and higher membrane permeability leading to exposure of cell water for easy evaporation [29] [Lee et al. 1995]. Chitosan coating played an obvious effect in preventing weight loss, and the effects could be increased by mixing with citric acids [8]. However, the increase of weight loss of fresh-cut products treated with citric acid has been also reported in fresh-cut potatoes [30] and mango [31]. These differences may be attributed to different fruit types, different treatment methods, and or other unknown reasons.

Softening is generally with the dissolution of pectin, involving many enzyme actions including pectin-esterase, polygalacturonase and pectate lyases [32]. Firmness rapidly decreased for control fruits, which lost about 36.42% of firmness within 15 days (Figure 3B). Simultaneously, the combined citric acid and chitosan coating had greater firmness than that of control, possibly because of citric acid and chitosan treatment of synergistic effect.

Quality parameters of tomato fruits after 15 days storage at 28°C were summed up in Table 1. There were no obvious differences in TSS percent, pH and Vc content at the end of storage in control and treated fruits. However, chitosan and citric acid delayed the natural physiological process like ripening, senescence and respiration, responsible for increase of pH values as well as decrease of soluble solid and total sugars, which was due to inhibitory effect on the activities of hydrolysis [33].

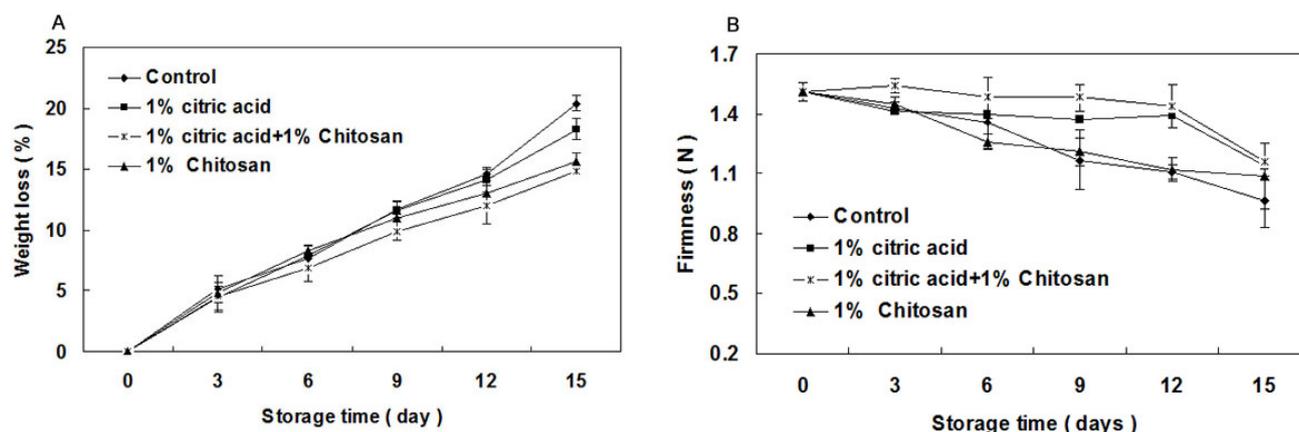


Figure 3. Changes in firmness (A) and weight loss (B) for different treatments of tomato fruits during 15 days storage at 28°C. Each data point is the mean of three replicate samples

Table 1. Changes in quality parameters (pH, TSS, Vc, Total phenolics and Flavonoids content) in treated and untreated tomato fruits after 15 days storage at 28°C. Values within the same column with different letters for each parameter are significantly different at $p < 0.05$

physiological index	Treatments	Storage time (days)					
		0	3	6	9	12	15
pH	Control	4.25±0.10a	4.23±0.06a	4.21±0.07a	4.41±0.15a	4.40±0.09a	4.45±0.05a
	1%citric acid	4.25±0.10a	4.22±0.11a	4.21±0.10a	4.33±0.07a	4.36±0.11ab	4.48±0.04a
	1% citric acid+1% Chitosan	4.25±0.10a	4.31±0.21a	4.19±0.03a	4.31±0.02a	4.09±0.06c	4.45±0.03a
	1% Chitosan	4.25±0.10a	4.35±0.04a	4.08±0.17a	4.32±0.10a	4.14±0.13bc	4.43±0.11a
TSS	Control	3.67±0.07a	3.63±0.27a	3.03±0.07b	3.30±0.25ab	3.20±0.16a	3.53±0.42ab
	1%citric acid	3.67±0.07a	3.15±0.50b	3.65±0.18a	3.15±0.15bc	3.00±0.08a	3.58±0.23a
	1% citric acid+1% Chitosan	3.67±0.07a	3.13±0.19b	3.78±0.17a	3.03±0.03c	3.20±0.10a	3.50±0.20b
	1% Chitosan	3.67±0.07a	2.93±0.03b	2.78±0.18b	3.45±0.05a	3.23±0.19a	3.48±0.25ab
Total phenolics content (OD ₂₈₀ /g FW)	Control	6.40±0.98a	5.99±0.02d	6.16±0.05c	6.33±0.01c	8.22±0.01d	7.55±0.10c
	1%citric acid	6.40±0.98a	6.89±0.17c	9.13±0.05a	7.92±0.01b	10.92±0.01b	8.27±0.05b
	1% citric acid+1% Chitosan	6.40±0.98a	7.35±0.26a	8.79±0.02b	9.03±0.01a	11.96±0.01a	12.32±0.23a
	1% Chitosan	6.40±0.98a	8.04±0.69b	8.77±0.01b	8.06±0.02b	8.58±0.01c	8.17±0.07b
Vc (mg/100g)	Control	32.94±0.88a	30.96±0.29c	29.52±1.05d	32.40±0.14c	28.08±0.68c	32.16±0.26a
	1%citric acid	32.94±0.88a	30.6±0.36d	30.60±0.55c	32.64±0.65c	31.18±0.61b	33.12±0.51a
	1% citric acid+1% Chitosan	32.94±0.88a	33.36±0.36a	35.04±0.24a	37.80±1.01a	32.62±0.36a	33.36±0.45a
	1% Chitosan	32.94±0.88a	32.4±1.01b	31.12±0.65b	33.36±0.72b	31.46±0.34b	33.12±0.72a
Flavonoids content (OD ₃₂₅ /g FW)	Control	2.92±0.01a	2.37±0.17d	2.80±0.01d	2.70±0.01c	3.98±0.02c	3.16±0.05c
	1%citric acid	2.92±0.01a	4.40±0.20a	3.92±0.02c	3.45±0.01b	4.89±0.01b	5.78±0.11a
	1% citric acid+1% Chitosan	2.92±0.01a	3.78±0.30b	4.74±0.01a	3.82±0.01a	6.62±0.01a	3.57±0.03b
	1% Chitosan	2.92±0.01a	3.44±0.11c	4.42±0.03b	3.36±0.01b	3.83±0.01c	3.30±0.01c

Data are the mean±SE. (n≥3).

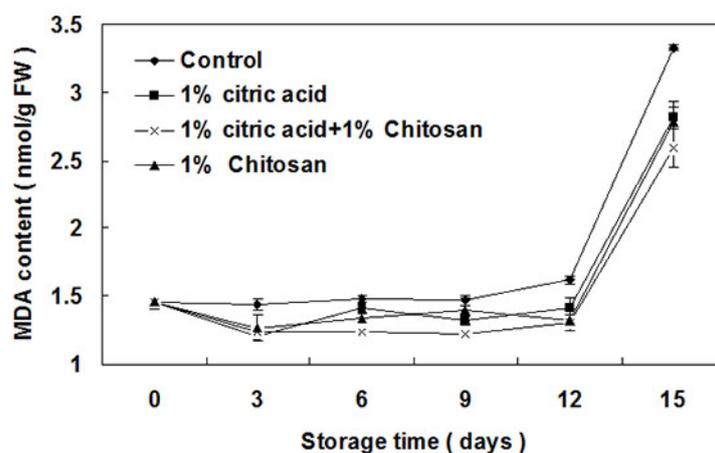


Figure 4. Effect of chitosan and citric acid on MDA in tomato fruits during storage at 28°C for 15 days. Data are means of three replicates ± SE

It was known that MDA was a peroxidation product for lipids in the cell membrane, as well as the accumulation could indirectly cause a reduction in membrane integrity [34]. The results showed the phenolic compounds in citric acid + chitosan treated samples continuously increased for the initial levels of 6.40 ± 0.98 to 12.32 ± 0.23 OD₂₈₀/g-FW at the end of experiment. The changes of the flavonoids fluctuated greatly, and fruits coating treatments significantly maintained higher the flavonoids than control at the end of storage. The similar results had been previously reported by Ramandeep *et al.* [35] and Liu *et al.* [36], soluble phenolic content increased slightly during storage at different temperatures (7°C or 15°C), from the first eight days, and showed a little decline toward the end of storage period.

The results indicated that MDA contents in the combined treatment of fruits stored at 28°C were significantly lower compared to control (Figure 4). The result explained that citric acid plus chitosan-treated fruits could retard senescence and reduce MDA accumulation in tomato fruits, suggesting that the combination treatment played positive roles in maintaining membrane integrity.

SOD enzyme can convert O₂^{·-} to H₂O₂, which are removed by CAT and POD enzymes [37]. The SOD levels in the combined treatment group were higher than those in other groups during majority storage periods (Figure 5A). The POD activities in the combined treatment of fruits were significantly higher than those in control during the

whole storage (Figure 5B).

The results were consistent with the previous study, and tomato fruits coated with higher concentrations of gum arabic retarded ripening process by delaying physiological and biochemical changes occurring during storage [38].

As shown in Figure 6A, CAT activities of tomato fruits tended to change differently at various storage periods, and fruits treated with chitosan and citric acid exhibited a higher CAT activity than those in control fruits. For PAL activity, the combined treatment tomato fruits showed a significantly higher activity compared with other treatment group fruits, and at the 9th day reached a peak value (315.03 ± 2.79 U/g FW), which was more than 8.0-fold higher with respect to the same period of control (39.17 ± 1.18 U/g FW) (Figure 6B).

The changes of total phenolics content may be related to fluctuations of PAL enzyme activity during cold storage, which is directly involved in the phenylpropanoid pathway and phenolic compounds biosynthesis [39]. In plums, PAL is one of the key enzymes involved in the biosynthesis of anthocyanins, and PAL activities are recognized as a response to anthocyanin accumulation [40].

POD, CAT and PAL antioxidant enzymes in tomato fruits were enhanced by the combined treatment (Figure 5 and Figure 6), which may be related to protect the fruit tissues from oxidative injury for citric acid and chitosan combination-coated fruit caused by superoxide anion, prolong the shelf-life and maintain fruit quality.

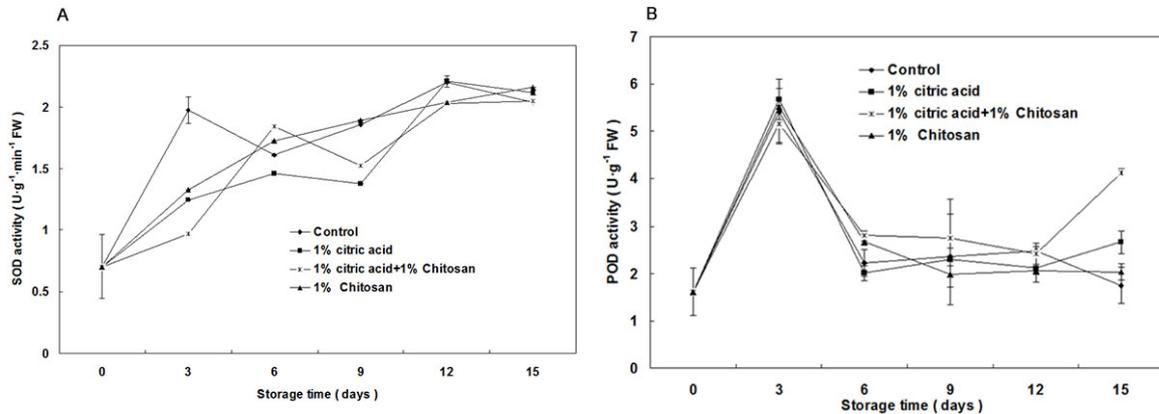


Figure 5. Changes of SOD (A), POD activities (B) in tomato fruit. Fruit were treated with chitosan coating, citric acid and the combined citric acid and chitosan coating, stored at 28°C for 15 days. Bars represent standard deviations

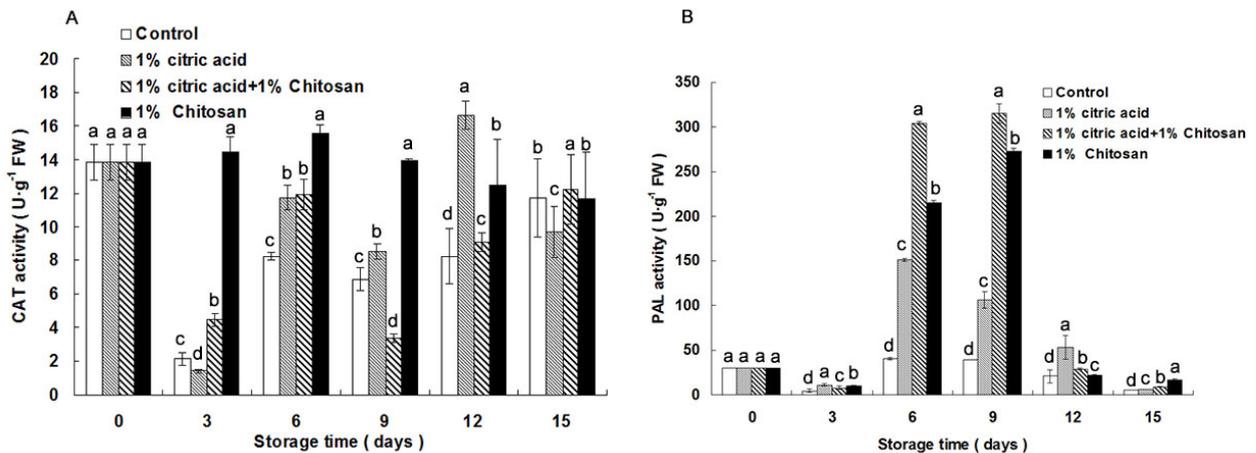


Figure 6. Changes of CAT (A), PAL activities (B) in tomato fruit. Fruit were treated with chitosan coating, citric acid and the combined citric acid and chitosan coating, stored at 28°C for 15 days. Bars marked with different letters in each column indicate significantly different values at $p < 0.05$

4. Conclusions

The combined treatment with chitosan and citric acid is an effective way to retard postharvest tomato fruits ripening process demonstrated by reducing many physiological changes, for example, colour, firmness, total soluble solids and weight losses, as well as maintaining higher bioactive substances including total phenolics and flavonoids content. The results of parallel experiments with citric acid and chitosan demonstrated that the combination treatment was superior to citric acid and chitosan alone (Figure 1).

The mechanism may be through improving antioxidant enzymes activities, reducing the accumulation of H₂O₂, O₂⁻ and lipid peroxidation product MDA. Overall, the results suggest that fresh tomatoes coated with chitosan and citric acid could be stored with optimal quality and be useful in selecting post-harvest storage method for tomatoes.

Acknowledgments

This research was financially supported by Hunan Provincial Natural Science Foundation of China (No.2015JJ6108) and Doctoral Start-up Funding of Xiangtan University of China (12DQ27).

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