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**Research Article**

# Enhancing shelf life and antioxidant capacity in nectarine fruit with threonine under low temperature

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## Abstract

**Chilling injury limits the storage life of nectarine fruit at low temperatures. Therefore, increasing the resistance to chilling injury will provide long-term maintenance of nectarine fruit at low temperatures. In this research, the effect of threonine at 250 and 500 µM on chilling injury and fruit quality of nectarine during storage at 1°C with 95% relative humidity for 30 days was studied. The measured traits included chilling injury, hydrogen peroxide, lipid peroxidation, TSS, organic acids, antioxidant capacity and antioxidant enzyme activity. The results showed that chilling injury decreased hydrogen peroxide production and lipid peroxidation in treated fruits with threonine compared to control during storage. The amount of organic acids, TSS, antioxidant capacity and activity of antioxidant enzymes in treated fruits increased until the end of storage. In general, the 500 µM threonine had the most significant effect on maintaining the quality of nectarine fruits during storage.**

**Keywords: Amino Acid, Decay, Quality, Storage, Stress**

## Introduction

Peaches (*Prunus persica* L. Batsch) and nectarines are widely consumed and favored because of their good nutrition and flavor, which is attributed to their compositions and interrelationships of soluble sugars, organic acids, amino acids, and polyphenols. In addition, peaches and nectarines are rich in polyphenols, including chlorogenic acid, catechin, neochlorogenic acid and quercetin, indicating that they have good antioxidant capacity (Liao *et al.*, 2019). Organic acids, amino acids and sugars also play crucial roles in the antioxidant properties of peaches and nectarines (Kim *et al*., 2003). Nectarine fruit is climacteric and produces a lot of ethylene when ripe. Nectarines ripen quickly at room temperature and eventually decay.

Cold storage was used to reduce the rate of maturation and the development of decay (Watkins and Jackie, 2004). However, chilling injuries limit the shelf life of nectarines at low temperatures .Chilling injury was influenced by genetics, temperature and storage time (Lurie and Crisosto, 2005). Despite these limitations, the use of refrigeration technology as a tool to increase the shelf life of fruits and vegetables is important. For this purpose, various techniques have been developed to reduce the amount of chilling injury (Watkins and Jackie, 2004). The elevated accumulation of metabolites in response to abiotic stress is an important aspect of plant amino acid metabolism. Resistance to environmental stresses such as cold and heat, drought and dehydration has led to an increase in the importance of the use of amino acids in plants (Thomas *et al.*, 2009). Amino acids are involved in growth, respiration, and photosynthesis and are precursors to plant hormones and growth factors (Hounsome *et al*., 2008). Amino acids are essential components in the early stages of protein synthesis. Studies in recent years have shown that foliar application of amino acids can have a direct impact on vital activities and plant structures (Cao *et al*., 2010). Foliar application of amino acids, especially when the ambient temperature of the plant is suitable, provides the absorption of these compounds through plant stomata, contributes significantly to the synthesis of proteins, and also meets the nutritional needs of the plant (Botta, 2013).

Threonine plays an essential role in accepting the signals from receptors that sense the phytohormones, adverse environmental conditions, and other physicochemical factors and translating them into specific functional outputs such as plant growth, development, and changes in metabolism, gene expression, seed development, storage protein gene expression, abiotic stress tolerance, cell growth and division (Joshi *et al*., 2010). Threonine deaminase and methionine c-lyase transcription, as well as isoleucine accumulation, are induced in response to osmotic stress (Jander and Joshi, 2009). In recent years, the use of low-risk compounds such as amino acids due to their ability

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to be used in organic agriculture and food production has become well known and is now commercially used in agriculture (Joshi *et al*., 2010). The purpose of this study was to investigate the effect of threonine as a safe compound and a healthy alternative for chemical substances in reducing chilling damage and maintaining the quality of fruit stored at low temperatures.

## Materials and methods

**Nectarine fruit and treatments:** Nectarine (L.) fruits were harvested at commercial maturity from a commercial orchard in Kerman, Iran, and transported to the laboratory on the same day. Nectarine fruits without wounds or rot were selected based on uniformity of size and the absence of physical injury or disease. The harvested fruits were disinfected with 1% sodium hypochlorite (v/v) for 2 minutes, washed, and dried in the air. Subsequently, they were randomly divided into 3 groups. Based on our preliminary experiments, two of the groups were immersed in an aqueous solution containing, respectively, 250 and 500 µM of threonine for 5 min. The third group (control) was immersed in distilled water for 5 minutes. All fruits were enclosed in plastic boxes with polyethylene film bags to maintain the relative humidity at about 95% and were stored at 1˚C.

**Evaluation of chilling injury (CI):** Chilling injury (%) was calculated by the following formula (Obenland *et al*., 2008): [(Total number of fruits in each treatment - number of fruits with no chilling injury) / (total number of fruits in the treatment)]×100.

**Measurement of lipid peroxidation content:** Lipid peroxidation content was determined and expressed as malondialdehyde (MDA) equivalents, according to the method of Rajinder *et al*. (1981), with slight modifications. Pulp and peel tissue (4.0 g) from nectarine fruit were homogenized with 20 mL of 10% trichloroacetic acid and then centrifuged for 10 min at 5000 × g. One mL of the supernatant was mixed with 3 mL of 0.5% thiobarbituric acid (TBA) dissolved previously in 10% trichloroacetic acid. The reaction mixture was heat-treated for 20 min at 95˚C, quickly cooled, and then centrifuged for 10 min at 10,000 × g to clarify precipitation. The absorbance at 532 nm was measured and subtracted from the nonspecific absorbance at 600 nm. The amount of MDA was calculated using an extinction coefficient of 155 $mM^{-1}$ $cm^{-1}$ and expressed as mM $g^{-1}$ FW.

**Hydrogen peroxide assay:** The assay for $H_2O_2$ content was carried out according to the procedure described by Prassad (1996). Fresh tissues (2 g) were homogenized with 10 ml of acetone at 0˚C. After centrifugation for 15 min at 6000g at 4˚C, the supernatant phase was collected. The supernatant (1 m1) was mixed with 0.1 ml of 5% titanium sulphate and 0.2 ml of ammonia, and then centrifuged for 10 min at 6000g and 4˚C. The pellets were dissolved in 3 ml of 10% (v/v) $H_2SO_4$ and centrifuged for 10 min at 5000g. The absorbance of the supernatant phase was measured at 410 nm. $H_2O_2$ content was calculated using $H_2O_2$ as a standard and then expressed as µg $g^{-1}$ FW (Prassad, 1996).

**Assay of antioxidant enzyme activityY; Catalase and peroxidase activity:** Catalase and peroxidase activity were analyzed according to Xing *et al*. (2007), with a slight modification. The reaction mixture consisted of 2 mL sodium phosphate buffer (50 mM, pH 7.0), 0.5 mL $H_2O_2$ (40 mM), and 0.5 mL enzyme extract. The decomposition of $H_2O_2$ was measured by the decline in absorbance (A) at 240 nm. CAT specific activity was expressed as U $kg^{-1}$ of FW, where U = Δ-A at 240 nm per s. For POD determination, 0.5 mL of enzyme extract was incubated in 2 mL of buffered substrate (100 mM sodium phosphate, pH 6.4, and 8 mM guaiacol) for 5 min at 30˚C, and the increasing absorbance was measured at 460 nm every 30 s for 120 s after adding 1 mL of $H_2O_2$ (24 mM). POD and CAT activity were expressed as U $mg^{-1}$ proteins.

**Superoxide dismutase:** The activity of superoxide dismutase was assayed according to Misra and Fridovich (1972). About 200 mg of fresh tissue was homogenized in 5 ml of 100 mM K-phosphate buffer (pH 7.8) containing 0.1 mM EDTA, 0.1% (v/v) Triton X-100 and 2% (w/v) polyvinyl pyrrolidone (PVP). The extract was filtered through muslin cloth and centrifuged at 22000/g for 10 min at 4-8˚C. The supernatant was dialyzed in cellophane membrane tubing against the cold extraction buffer for 4 h with carbonate/bicarbonate buffer and then used for the assay. The assay mixture, in a total volume of 3 ml, contained 50 mM sodium carbonate/bicarbonate buffer (pH 9.8), 0.1 mM EDTA, 0.6 mM epinephrine, and enzyme. Epinephrine was the last component to be added. The adrenochrome formation in the next 4 minutes was recorded at 475 nm in a UV-Vis spectrophotometer. One unit of SOD activity is expressed as the amount of enzyme required to cause 50% inhibition of epinephrine oxidation under experimental conditions. The specific activity of enzymes is expressed as the U $mg^{-1}$ protein.

**Ascorbate peroxidase:** Ascorbate peroxidase was assayed according to Nakano and Asada (1981). The reaction mixture in a total volume of 1 ml contained 50 mM K-phosphate buffer (pH 7.0), 0.2 mM ascorbic acid, 0.2 mM EDTA, 20 mM $H_2O_2$ and enzyme. $H_2O_2$ was the last component to be added, and the decrease in absorbance was recorded at 290 nm (extinction coefficient of 2.8 $mM^{-1}$ $cm^{-1}$) using a UV-Vis spectrophotometer. A correction was made for the low, non-enzymic oxidation of ascorbic acid by $H_2O_2$. The specific activity of enzymes is expressed as the U $mg^{-1}$ protein.

**DPPH radical scavenging activity:** DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was done according to Oliveira *et al*. (2009) with some modifications. Fresh tissue (0.5g) from each treatment sample was homogenized in 4 ml absolute methanol at 4˚C. After centrifugation, an aliquot (0.3 ml) of the

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methanol extract was mixed with 2.7 ml of a methanolic solution containing DPPH radicals (0.1 mmol/l). The reaction mixture was shaken and incubated for 120 min at room temperature, and the absorbance was read at 517 nm against a blank. The scavenging ability was calculated using the following equation, scavenging activity = [($A_{517}$ of control-A517 of sample0/A517 of control)]×100. α-tocopherol was used as a standard antioxidant analyzed at the same time. The final results were calculated and expressed as α-tocopherol equivalents per gram on a fresh weight basis.

**Data analysis:** The experiments were performed using a completely randomized design with three replicates per treatment. All statistical analyses were performed with SPSS version 13.0 (SPSS Inc., Chicago, IL, USA). Data at each time point were analyzed by one-way ANOVA, and mean separations were performed by Duncan's new multiple range test. Differences at $P<0.05$ were considered significant. Each treatment consisted of three replicates, and the experiment was repeated six times.

## Result

The results of the analysis of variance show the significant effect of threonine on the quality of nectarine fruits (Table 1). The results showed (Fig. 1) that the percentage of chilling injury increased during storage. But in the fruits treated with threonine, the chilling injury decreased with increasing threonine concentration. The lowest value of chilling injury was observed in fruits treated with threonine 500 mM during storage time.

The amount of hydrogen peroxide and lipid peroxidation at the beginning of storage is the lowest in both controlled and treated fruits. However, their amount has gradually increased until the 30th day of storage. The highest amount of hydrogen peroxide and lipid peroxidation was observed in control fruits, and the lowest amount was observed in fruits treated with threonine 500 µM. Using the treatment of threonine compared to the control causes a decrease in the amount of hydrogen peroxide and lipid peroxidation.

However, the use of 250 µM threonine also reduced the rate of lipid peroxidation and hydrogen peroxide in nectarine fruits (Figures 2 and 3).

The results of Figure 4 show that the amount of antioxidant capacity in fruits treated with threonine increased with increasing storage time. So, in every period of storage, the highest amount was related to fruits treated with threonine 500 µM and the lowest amount was observed in control fruits (Figure 4).

At the beginning of storage, there was no difference between the amount of TSS in the control and treated fruits. But in the continuation of storage, the amount of TSS in fruits treated with 500 µM threonine was higher than in other treatments. At the end of storage times, the amount of TSS in the control was significantly lower than that in fruits treated with threonine (Figure 5).

At the beginning of storage time, the highest amount of organic acids was observed in the treated fruits compared to the control. As the highest amount is observed in fruits treated with 500 µM threonine. Threonine 500 µM-treated fruits maintain an increasing trend linearly until the end of storage. However, control and threonine 250 µM fruits, showed a decreasing trend until the 30$^{th}$ day (Figure 6).

The amount of ascorbate peroxidase in treated and control fruits is not different at the beginning of storage. However, after ten days from the beginning of storage until the end of storage, the amount of ascorbate peroxidase in treated fruits was higher than the control (Figure 7).

The activity of the peroxidase enzyme in fruits treated with threonine 500 µM is the highest until the end of storage. However, there is not much difference between fruits treated with threonine 250 µM and controls (Figure 8).

With increasing storage days, the amount of superoxide dismutase enzyme in fruits treated with threonine 500 µM has increased. Although treated fruits with threonine 250 µM have higher levels of enzymes than controls, they are not statistically significant (Figure 9).

The results showed that activity of catalase enzyme in treated fruits increased compared to the control. The highest amount in fruits treated with threonine is 500 µM (Figure 10).

## Discussion

Peaches and nectarines ripen rapidly at room temperature. Cold storage is used to slow these processes and develop decay. However, low temperature disturbances, damage caused by colds, which is classified as internal breakdown, limit the shelf life of peaches and nectarines in cold storage (Lurie and Crisosto, 2005). Chilling injury is one of the most common disorders occurring in stone fruits. This physiological damage occurs at any time under adverse environmental conditions, such as during the growing season, transportation, distribution or even storage (Watkins and Jackie, 2004). The present results showed that with increasing storage time, the amount of chilling injury and the production of hydrogen peroxide and lipid peroxidation increased in nectarine fruits. The use of the amino acid threonine reduced chilling injury, produced hydrogen peroxide and lipid peroxidation, and increased resistance during storage in fruits. It is likely that the amino acid joined the cell membrane to prevent membrane degradation and provide resistance during storage. Amino acids also have several other roles in plants. For example, they regulate ion transport and stomatal opening and affect the synthesis and activity of enzymes, gene expression, and redox homeostasis, helping the plants cope with the harmful effects of osmotic stress (Rai, 2002). Stress conditions, such as cold, salinity, UV radiation, drought, heavy metals, and extreme temperatures, disturb the defense mechanisms in plants, which leads to ROS accumulation inside the

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**Table 1. Analysis of variance of effect of threonine on shelf life and antioxidant capacity in nectarine fruit**

| Variation Sources | df | Average of squares | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Chilling injury | $H_2O_2$ | Lipid peroxidatiob | DPPH | TSS | Organic Acid | Accorbate peroxidase | Peroxidsa | Superoxide dismutase | Catalase |
| Treatment | 4 | 4 | 0.06* | 40.22* | 29.18* | 8.01* | 54.23* | 211.05* | 1.01* | 0.03* | 208.23* |
| Error | 10 | 10 | 0.012 | 1.31 | 1.09 | 2.28 | 10.22 | 31.02 | 0.018 | 0.005 | 35.01 |
| cv | | | 3.21 | 9.14 | 11.02 | 6.12 | 10.25 | 8.29 | 4.18 | 9.32 | 11.02 |

**P≤ 5% significant probability level*, P≤% significant probability level**, ns= not significant**

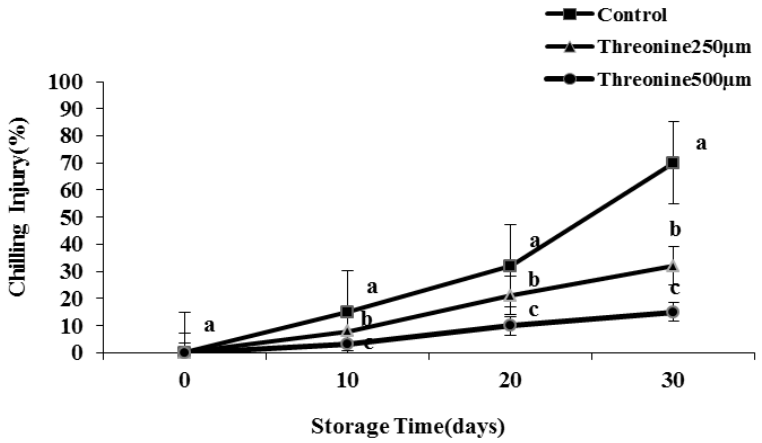



**Figure 1. The effect of different concentrations of threonine on chilling injury in nectarine fruits during storage at 1˚C**

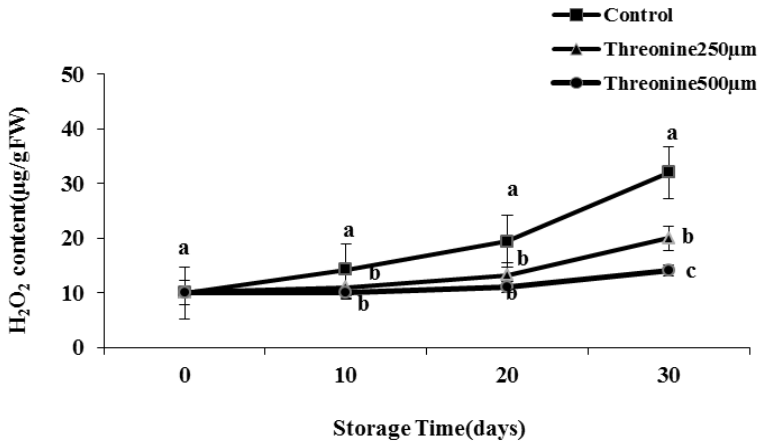



**Figure 2. The effect of different concentrations of threonine on $H_2O_2$ in nectarine fruits during storage at 1˚C.**

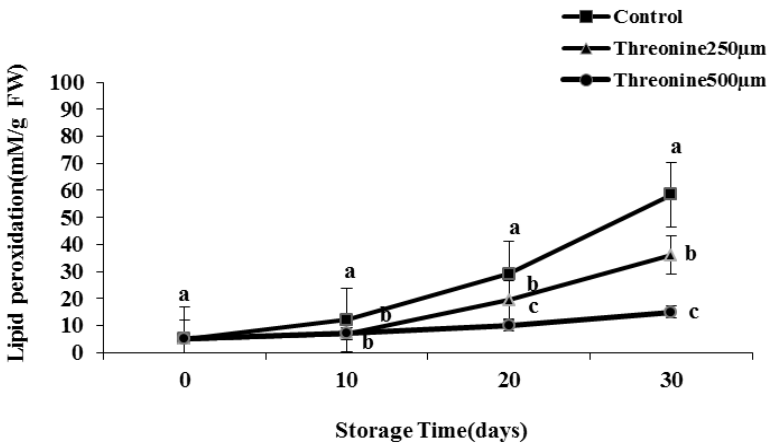



**Figure 3. The effect of different concentrations of threonine on lipid peroxidation in nectarine fruits during storage.**

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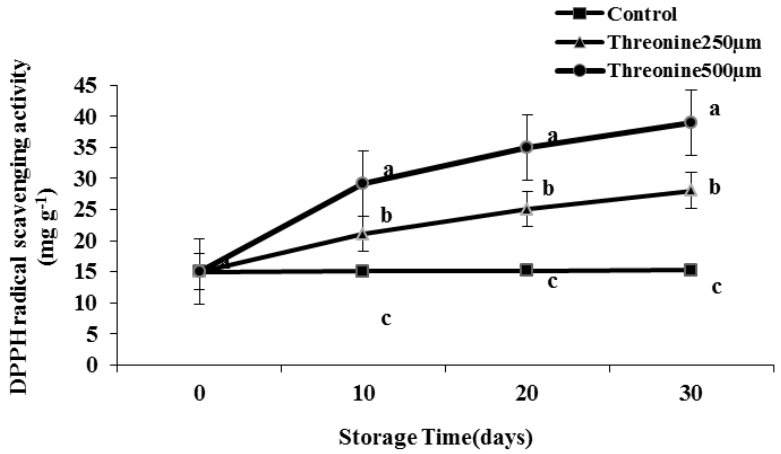



**Figure 4. The effect of different concentrations of threonine on antioxidant capacity in nectarine fruits during storage.**

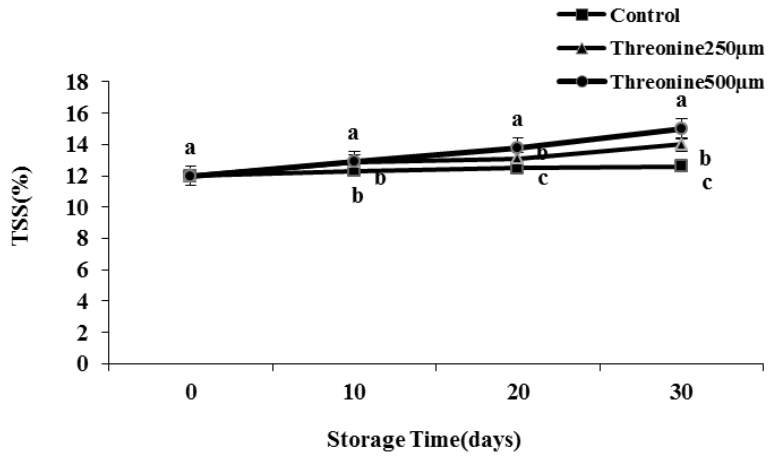



**Figure 5. The effect of different concentrations of threonine on level of TSS in nectarine fruits during storage.**

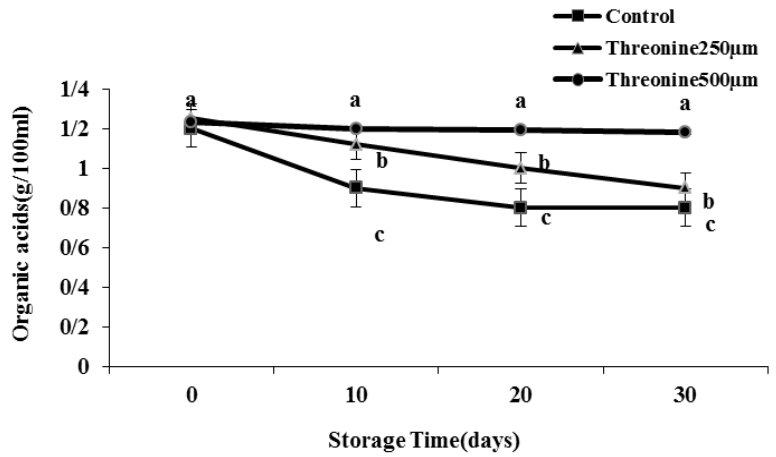



**Figure 6. The effect of different concentrations of threonine on level of organic acids in nectarine fruits during storage.**

cells. Singlet oxygen, superoxide ($O^{2-}$), hydrogen peroxide ($H_2O_2$), and hydroxyl radicals are toxic to plants and damage proteins, lipids, carbohydrates, and DNA, resulting, in extreme cases, in cell death. Excessive ROS accumulation as a result of various environmental stresses is the main reason for agricultural yield loss worldwide (Gill and Tuteja, 2010).

The exogenous application of different antioxidants in plants might help minimize the harmful effects of stress. Many substances are useful in the way that they provide crucial protection against oxidative damage, and, many of them, when applied in small quantities, are also able to enhance the plant's ability to assimilate applied nutrients, or provide benefits to plant development (Calvo *et al*., 2014).

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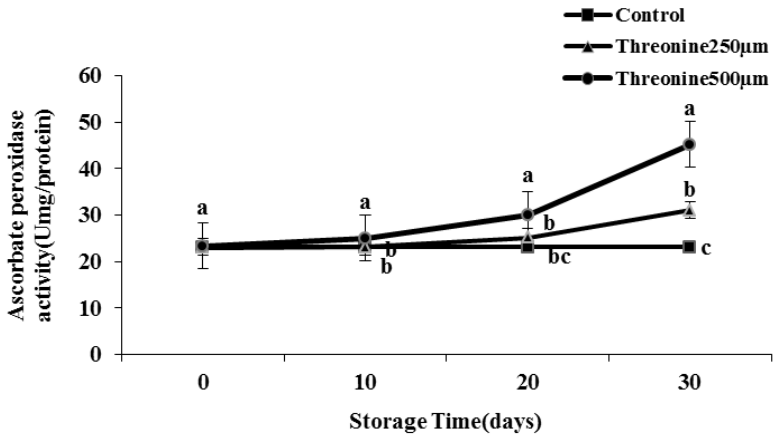



**Figure 7. The effect of different concentrations of threonine on level of ascorbate peroxidase in nectarine fruits during storage.**

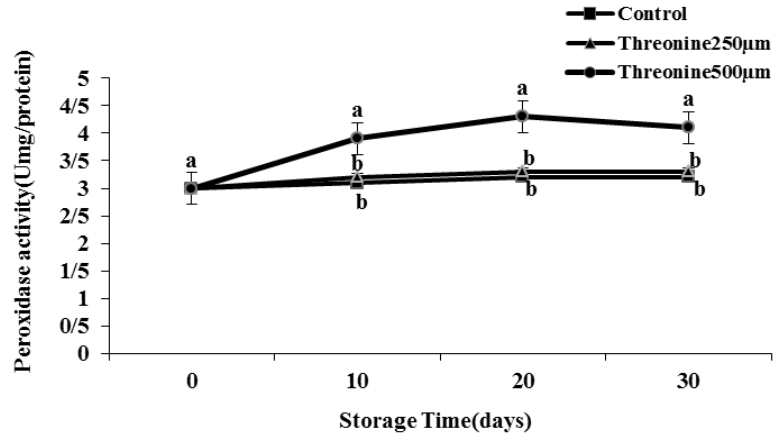



**Figure 8. The effect of different concentrations of threonine on level of peroxidase in nectarine fruits during storage.**

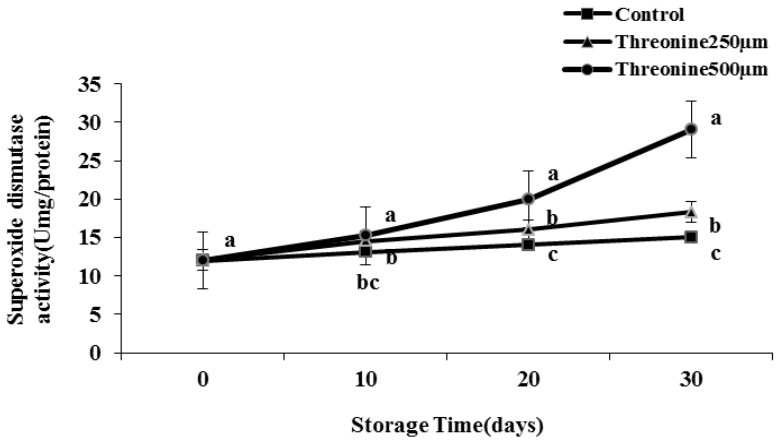



**Figure 9. The effect of different concentrations of threonine on level of superoxide dismutase in nectarine fruits during storage.**

Plants with antioxidant systems that contain enzymatic compounds including superoxide dismutase, catalase, peroxidase, glutathione peroxidase, ascorbate peroxidase and glutathione reductase, balance the levels of reactive oxygen species in the cell (AL-Aghabary *et al*., 2004).

In addition to their obvious role in protein synthesis, amino acids perform essential functions in both primary and secondary plant metabolism. Some amino acids serve to transport nitrogen from sources to sinks; others serve as precursors to secondary products such as hormones and compounds involved in plant defense (Coruzzi and Last, 2000). Physiologically, amino acids account for inducing growth and protection against

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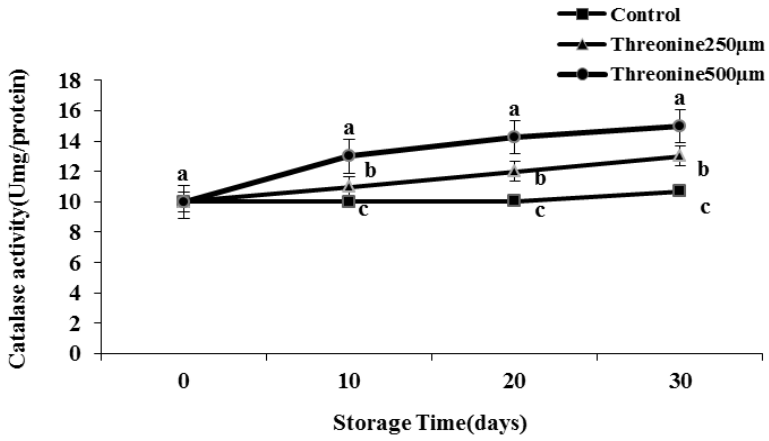



**Figure 10. The effect of different concentrations of threonine on level of catalase in nectarine fruits during storage**

ammonia toxicity and act as a source of carbon and energy (Abdel Aziz *et al*., 2010).

Tajik and Danaee (2016) studied the effect of glotamine, arginine, and phenylalanine (50 or 100 ppm) pre-harvest spray on some physicochemical and enzymatic traits and longevity of *Gerbera jamesonii* cv. Sorbet flowers. They showed that the glutamine treatment at 100 ppm had the greatest effect on improving fresh weight, dry weight, relative water content, the number of flowers, the diameter of the flower, flowering stem length, bent neck, leaf area, cell membrane stability index, anthocyanin content, total chlorophyll of leaves, proline, protein, superoxide dismutase, and phenylalanine ammonia-lyase activity. Threonine is an endogenously produced in all plant species (Abdel Aziz *et al*., 2010; Botta, 2013). As a healthy ingredient contained in the diet, many fruits and vegetables, including tomatoes, apples, cherries, bananas, and strawberries, provide natural amino acids (Botta, 2013; Joshi *et al*., 2010). As a safe and beneficial material, perhaps, threonine acts not only as a signaling molecule for enhancing the resistance of plants to biotic and abiotic stresses, but also as a powerful free-radical scavenger and has direct antioxidant activity (Botta, 2013).

## Conclusion

In this study, exogenous threonine treatment has been tested as an effective postharvest treatment to promote ripening and improve the quality of nectarine fruit, delay postharvest senescence and increase the chilling tolerance of peach fruit. However, little information is available regarding the effects of threonine as a postharvest treatment on the postharvest life and quality of peaches and ece. This study may promote the application of threonine to the postharvest quality of peach fruit as well as other fruits and vegetables in the future. It can be concluded from this study that 250 or 500 µm postharvest threonine treatment was most effective in delaying senescence of peach fruit by reducing chilling injury, $H_2O_2$ and MDA content. Furthermore, postharvest treatment of 250 or 500 µm threonine promoted the activity of antioxidant enzymes, resulting in higher antioxidant capacity. Moreover, 250 or 500 µm threonine postharvest treatment enhanced the expression of antioxidant capacity and consequently increased the shelf life of nectarines during storage.

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