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Phenylalanine Alleviates Postharvest Chilling Injury of Plum Fruit by Modulating Antioxidant System and Enhancing the Accumulation of Phenolic Compound

Running title: Phenylalanine Treatment for Alleviating Chilling Injury of Plum Fruit

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SUMMARY

Research background. Low temperature storage causes chilling injury (CI) in plum (*Prunus domestica* L.) fruits. Consequently, any treatments with beneficial effects on these symptoms would achieve attention. For this purpose, phenylalanine treatments were applied on 'Stanley' plum fruits. The main purpose of the present study was to investigate the influence of the exogenous application of phenylalanine on fruit quality, chilling tolerance, and antioxidant capacity of 'Stanley' plums during cold storage.

Experimental approach. Phenylalanine at different concentrations were applied on 'Stanley' plums. Following phenylalanine application, plums were cold stored. Chilling injury, antioxidant capacity, electrolyte leakage, malondialdehyde, proline, and internal contents of anthocyanin, flavonoids, phenols, ascorbic acid, and some antioxidant enzymes were assessed.

Results and conclusions. Phenylalanine treatment significantly alleviated chilling injury in plum fruits by enhancing antioxidant capacity and increasing the activity of phenylalanine ammonia lyase enzyme (PAL). Phenylalanine-treated fruits had higher levels of ascorbic acid, anthocyanin, flavonoids, and phenols, as well as a higher total antioxidant activity, than the control fruits during low temperature storage. Phenylalanine at 7.5 mM was the most effective treatment in enhancing the

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activity of PAL and the accumulation of phenolic compounds and in reducing the severity of chilling injury. Treatments delayed mass loss and maintained fruit firmness. In addition, the application of 7.5 mM phenylalanine improved the activities of antioxidant enzymes (superoxide dismutase, catalase, and ascorbate peroxidase), decreased the accumulation of hydrogen peroxide, and increased the endogenous content of proline. Moreover, phenylalanine maintained membrane integrity, manifested by a reduced electrolyte leakage and malondialdehyde accumulation.

Novelty and scientific contribution. In the current study, chilling injury had a positive correlation with the activities of PAL and antioxidant enzymes. However, negative correlations were observed between chilling injury and ascorbic acid content and antioxidant capacity. Considering the results, phenylalanine treatment could be spotted as an encouraging approach to alleviate the severity of chilling injury and thus preserve nutritional quality of plums during low temperature storage.

Key words: antioxidant capacity, chilling injury, phenylalanine, plum fruit, phenylalanine ammonia-lyase (PAL)

INTRODUCTION

Plum (*Prunus domestica* L.), a temperate fruit tree, is mostly cultivated for its fresh fruits around the world. Plums are considered excellent sources of fibers, carbohydrates, organic acids, potassium, calcium, vitamins C and E, polyphenols, carotenoids, flavonoids, and anthocyanins, all of which are essential/beneficial for human health (*1*). The rapid fruit softening under ambient conditions is one of the most important challenges in the production of plum fruits. This is the main reason for the decrease of fruit quality during storage, transport, and marketing. Therefore, low-temperature treatment is an efficient method for the reduction of the postharvest quality losses. However, plum fruits are highly susceptible to low temperatures, and the chilling injury symptoms show up as flesh browning (FB), translucency, bleeding, and abnormal ripening when the fruits are kept at 0 or 5 °C (*2*). Thus, with the development of chilling symptoms, storage life is limited. However, cultivar, storage temperature, maturity stage, and cultivation conditions affect the rate of CI development in plums (*3*). Candan *et al*. (*4*) reported that the severity of chilling injury in 'Larry Ann' plums was high during cold storage.

Chilling stress triggers the accumulation of reactive oxygen species (ROS) in fruits, which can initiate lipid peroxidation and cause oxidative damage to cell membrane, chloroplasts, mitochondria, and apoplast. Plants have the most effective non-enzymatic (phenolics, flavonoids, carotenoids, ascorbic acid, tocopherols, glutathione, and proline) and enzymatic (catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR), monodehydroascorbate

reductase (MDHAR) and dehydroascorbate reductase (DHAR)) antioxidant defense system to protect cells against oxidative stress (*5*). Thus, for the improvement of antioxidant capacity and the maintenance of the quality of plum fruits during the postharvest life, suitable postharvest technologies should be considered in combination with cold storage.

Recent reports have shown that the administration of some treatments, such as salicylic acid (*6*) and oxalic acid application (*7*), hot water dipping (8), and nitric oxide fumigation (*9*) increase the plum quality for longer periods than cold storage alone. Phenylalanine is an aromatic amino acid used for the biosynthesis of all phenolic compounds via the phenylpropanoid pathway. In this pathway, phenylalanine ammonia lyase (PAL) is the first enzyme, which catalyzes the conversion of phenylalanine to flavonoids, phenolics, and anthocyanins. Various biotic and abiotic stresses may stimulate PAL activity, which results in the accumulation of bioactive compounds (*10*). Recently, the application of phenylalanine solution as pre-harvest and postharvest fruit treatments has been considered for enhancing the nutritional quality of horticultural crops. Pre-harvest spray of phenylalanine improved the content of phenolic compounds, including anthocyanins and flavonoids, in grape fruits (*11*). Aghdam *et al*. (*10*) reported that phenylalanine application significantly decreased chilling injury, membrane lipid peroxidation, and ROS accumulation of tomato fruits during cold storage. Moreover, in fruits treated with 0.5 mM phenylalanine, the contents of phenols and flavonoids and the activity of PAL were more than in those treated with other treatments, including control fruits. Phenylalanine treated fruits also showed higher activities of antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR), concurrent with a higher endogenous accumulation of lycopene and proline. Kumar Patel *et al*. (*12*) showed that the preharvest or postharvest treatment of phenylalanine in mango, avocado, strawberry, and citrus fruits activates the fruits' natural defense responses and thus tolerance to fungal pathogens, leadings to the inhibition of postharvest decay caused by different fungal pathogens. However, few reports have shown the positive potential of phenylalanine in the reduction of chilling injury and maintenance of post-harvest quality in horticultural crops. Therefore, the main purpose of the present study was to investigate the influence of the exogenous application of phenylalanine on fruit quality, chilling tolerance, and antioxidant capacity of 'Stanley' plums during 40 days of cold storage.

MATERIALS AND METHODS

Chemicals

The chemicals used in this study were of analytical grade. Phenylalanine, trichloroacetic acid, 2, 6-dichloroindophenol, metaphosphoric acid, Folin–Ciocalteu, sulfosalicylic acid, ninhydrin, proline, L-methionine, potassium iodide, riboflavin and phosphate buffer were from Merck, Darmstadt,

Germany. Thiobarbituric acid, 2,2-diphenyl-1-picrylhydrazyl, gallic acid, nitroblue tetrazolium, glacial acetic acid, EDTA, bovine serum albumin, PVP (polyvinylpyrrolidone), Na-carbonate and methanol were from Sigma-Aldrich, St. Louis, MO, USA.

Fruits, treatments and storage

'Stanley' plum fruits were hand-picked at the commercial maturity stage (Brix° 12.01) from a commercial orchard in Zanjan province, Iran. Fruits were immediately transferred to the postharvest lab and graded based on the size and the absence/presence of defects. They were rinsed gently with water, dried naturally at room temperature, and randomly divided into four groups: control (distilled water) and phenylalanine ($C_9H_{11}N_1O_2$) treatments (2.5, 5, or 7.5 mM) for 10 min at 25 °C. Fruits were then dried at room temperature for about 30 min and placed in open plastic boxes, each containing 10 plum fruits (120 fruit for each treatments). Then, all fruits were stored at 1 °C and (90±2) % RH for 40 days. Following 10, 20, 30, and 40 days of cold storage, three boxes (three replications of each treatment) for each treatment were transferred to the chamber with a controlled temperature and maintained at 25 °C for one day. Approximately, a total of 40 g fruit tissue was collected from 5 fruits per replication of each treatment, frozen at once in liquid nitrogen, kept at -80 °C for later biochemical measurements.

Fruit quality (mass loss, firmness, soluble solid content)

The fruit mass of each sample (box) was measured at the beginning of the storage and at each sampling time. The results were expressed as the percentage loss of the initial mass. Fruit firmness was evaluated in kg/cm^2, using a texture analyzer (FT011, Facchinil srl, Alfonsine (Ra), Italy), fitted with an 8 mm diameter flat probe. A portable refractometer (PAL-1, Atago Co., Tokyo, Japan) was used to evaluate the soluble solid content (SSC) of plum juice in °Brix.

Chilling injury (CI) and membrane integrity

The CI index was determined after the fruits were kept at 25 °C for one day. The CI symptoms were gel breakdown, flesh woolliness, and flesh browning (*13*), which were assessed visually according to the following five-stage scale: 0=none, 1=slight injury, 2=moderate injury, 3=moderately severe injury and 4=severe injury. The chilling injury index was calculated based on the following formula:

$$CI \text{ index} = \sum [(\text{CI scale}) \times (\text{number of fruit at the CI scale})] / (4 \times \text{total number of fruit in the treatment}) \quad /1/$$

Electrolyte leakage (EL) was assayed using the method of Promyou *et al.* (*14*). The percentage of EL was measured using the following equation:

EL (%) = (Initial electrolyte leakage/Final electrolyte leakage) × 100 /2/

The level of malondialdehyde (MDA) was determined using the procedure developed by Dhindsa *et al.* (*15*) with some modifications. One gram of fruit samples was crushed in 10 mL of 5 % (*m*/*V*) trichloroacetic acid (TCA) and then centrifuged (Z36HK, Hermle Labortechnik GmbH, Wehingen, Germany) at 10 000×*g* for 15 min. The supernatant (2 mL) was mixed with 2 mL of 5 % TCA containing 0.5 % thiobarbituric acid. The reaction solution was heated in a boiling water bath at 100 °C for 30 min. The mixture was cooled quickly and finally centrifuged at 10 000×*g* for 10 min. The absorbance was read at 532 and 600 nm using a spectrophotometer (Specorp 250 Jena-History, Analytik Jena AG, Jena, Germany).

Fruit biochemical factors (total phenolics (TP), favonoids (TF), anthocyanins (TAC) contents, ascorbic acid (AA), total antioxidant activity (TAA), PAL and PPO enzyme activity)

For the determination of TP, TF, and TAC, fruit samples (1 g) were extracted with 0.1 % HCl-acidified 80 % methanol (*V*/*V*) (10 mL) and then centrifuged (12 000×*g*, 20 min, 4 °C). TP value of the extracts was assayed through Folin–Ciocalteu reagent method (*16*), evaluated against a gallic acid standard curve and finally expressed as mg gallic acid (GAE) per 100 g fresh mass. TF of the extracts was evaluated following Bouayed *et al*. (*17*) method with the absorbance read at 510 nm and expressed as mg catechin per 100 g fresh mass. TAC levels were determined following the pH differential method (*18*) and a molar extinction coefficient of 29 600 (cyanidin 3-glucoside) and stated as mg cyanidin-3-glucoside per 100 g fresh mass according to equation below:

Absorbance (A) = (A_{520pH1}−A_{700pH1}) − ($A_{520pH4.5}$−$A_{700pH4.5}$) /4/

The level of ascorbic acid was measured following 2, 6-dichloroindophenol titrimetric method (*19*). Ten grams of fruit samples was mixed uniformly in 3 % (*V*/*V*) metaphosphoric acid and filtered through two layers of cheesecloth. The supernatant (10 mL) was titrated against the standard 2, 6-dichloroindophenol dye until the faint pink color persisted for 5s and the result was expressed as mg of ascorbic acid per 100 g of fresh mass (fm). The antioxidant activities of the methanolic extract of the fruit samples were estimated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay (*20*). An aliquot (0.1 mL) of the extract was added to 1.9 mL of a 0.1 mM methanolic solution of DPPH and incubated for 30 min at room temperature in the darkness. After incubation, the absorbance was read against a blank at 517 nm using a spectrophotometer (Specorp 250 Jena-History, Analytik Jena AG). The percentage of the inhibition of the DPPH radical was calculated by the following formula:

$$\text{Inhibition of DPPH} = [(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100 \quad /3/$$

where A_{control} is the absorbance of DPPH solution without extract.

PAL activity was assayed following the procedure described in Nguyen *et al*. (*21*) indicating katals produced per mass of protein (kat/kg). The activity of polyphenol oxidase (PPO) was assessed following the method described by Kahn (*22*). For this purpose, the increase in absorbance of 1.5 mL of the reaction mixture, including citrate (100 mM), phosphate buffer (200 mM, pH=5), catechol (0.05 M), and the supernatant (100 µL), was measured at 420 nm during 2 min. The records of the enzyme activity (PPO) indicated katals produced per mass of protein, kat/kg.

Proline determination

For the determination of proline, one-gram fruit tissue was uniformly mixed in 10 mL sulfosalicylic acid (3 %) and subsequently centrifuged (10 000×*g*, 15 min). The obtained supernatant (2 mL) reacted with ninhydrin (2 mL, 2.5%) and glacial acetic acid (2 mL), heated at 100 °C for 60 min, and then cooled down in an ice bath. Each tube received toluene (4 mL) and was later shaken dynamically until it was separated into two phases. The absorbance of the proline-containing phase was measured at 520 nm, and proline value (µg/g fresh mass) was calculated with standard curve of known concentrations of proline (*23*).

Extractions and assays of antioxidant enzymes activities and H_2O_2 content

Frozen plum fruits (1 g) were ground and extracted for 30 s with phosphate buffer (50 mM, pH=7.8) containing EDTA (0.2 mM) and PVP (2 %). The homogenates were centrifuged (12 000×*g*, 4 °C, 20 min) to obtain supernatants for enzymatic assays. For the catalase (CAT) assay, to 0.1 mL extract, H_2O_2 (15 mM) and phosphate buffer (pH=7) were added, and the absorbance at 240 nm for 60 s was recorded. For the ascorbate peroxidase (APX), the reaction mixture (2 mL) consisted of 20 µL supernatant, phosphate buffer (pH=7), ascorbic acid (0.5 mM), and H_2O_2 (1 mM). The absorbance of the reaction mixture at 290 nm for 60s was recorded. To measure the activity of superoxide dismutase (SOD), the supernatant (50 µL), phosphate buffer (25 mM, pH=7), L-methionine (12 mM), NBT (1 M), riboflavin (1 M), and Na-carbonate (50 mM, pH=10.2) were mixed to reach a 3 mL reaction mixture. Then, the absorbance was measured at 560 nm after exposure to light for 30 min, demonstrating the enzyme ability to inhibit photochemical reduction of nitroblue tetrazolium (NBT). The only difference for the blank mixture was exposure to the dark for 30 min (*24*). The protein content was determined following Bradford's (*25*) method using bovine serum albumin (BSA) as the standard. For the measurement of H_2O_2 content, 1 g of fruit tissue was homogenized in TCA (5 mL, 1 % *V*/*V*) and centrifuged (10 000×*g*, 5 min). After that, 750 µL phosphate buffer (100 mM) and 1.5 mL

potassium iodide (KI) (1 M) were added into the extract, and the absorbance of the reaction mixture was measured at 390 nm (*26*).

Experimental design and data analysis

The experiments were performed in a completely randomized design (CRD). The statistical analysis was carried out using SPSS software v. 20.0 (*27*). Experimental data were subjected to ANOVA analysis. The treatments and storage were sources of variation. All treatments were conducted with three replications, and each replication contained 10 fruits. Means were compared using Duncan's multiple range test ($p \leq 0.05$).

RESULT AND DISCUSSION

Plum fruit quality

The mass loss of plums increased during cold storage, which was higher in control fruits than those treated with phenylalanine, while there was no significant difference between phenylalanine treated fruits. During 40 days of storage, control fruits lost approximately 6.01 % of their mass, while those treated with phenylalanine lost 4.20 % of their mass ($p<0.05$). During the storage, the firmness of all fruits decreased significantly, but the control fruits had a faster softening rate (Table 1). It seems that the application of phenylalanine could retard tissue softening but significant differences were not observed between phenylalanine treated fruits. Total soluble solids (SSC), as a maturity and quality parameter and harvest index are very important measurements for plum fruits. SSC was 12.01 % at harvest and increased to 15 % during 40 days of storage at 1 °C, but there were no significant differences between the experimental groups. Fruit mass loss is important factors affected by low temperatures during cold storage. The main reason for the mass loss of fruits is the water loss due to physiological activities such as transpiration and respiration (*28*). The harvest time and maturity stage of plums directly affect the firmness of fruits (*29*). Fruit softening during the storage significantly reduces the postharvest life and increases the susceptibility to fungal infection. In this investigation, the application of phenylalanine effectively delayed the softening of plum fruits, thus helping to maintain their quality. During fruit ripening and postharvest life, the mechanical strength of the cell walls and cell to cell stickiness reduces and so does the firmness of the fruits (*30*). Furthermore, Garde-Cerdan *et al.* (*31*) reported that the pre-harvest application of nitrogen composition such as proline and phenylalanine increased amino acid concentrations and fruit quality of 'Tempranillo' grapes. They reported that the foliar application of phenylalanine increased the synthesis of fermentative volatile compounds, thereby improving wine quality. Seemingly, phenylalanine treatment could be an efficient treatment to increase plum fruit quality during cold storage.

Plum fruit chilling injury and membrane integrity

For the first time, the control fruits and those treated with 2.5 mM of phenylalanine showed translucency and internal browning at 1°C after ten days of storage. The rate of cold damage increased in all fruits as time passed (Fig. 1a). However, phenylalanine treatments of 5 and 7.5 mM decreased the chilling index throughout storage and increased the subsequent shelf-life periods. Meanwhile, during storage for 40 days at 1 °C, the plums were chilled, the cell membrane was damaged, and so the electrolyte leakage and MDA accumulation increased. The ion-leakage and the MDA content significantly increased with the passage of time in all fruits (Fig. 1b and 1c). However, the application of a 7.5 mM solution of phenylalanine reduced their rates, but there was no significant difference between phenylalanine treatments regarding electrolyte leakage and MDA content. In this study, CI had a positive correlation with electrolyte leakage and MDA content. However, negative correlations were observed between CI and ascorbic acid and antioxidant capacity (Table 2). The optimal method for the storage of stone fruits is cold storage, but plums are cold susceptible and might exhibit CI disorder (*32*). Thus, with the development of chilling symptoms, the storage life is limited. However, cultivar, storage temperature, maturity stage, and cultivation conditions could affect the rate of CI development in plums (*3*). The chilling injury increased after ten days of cold storage at 1 °C plus one day at 25 °C (Fig. 1a). Candan *et al.* (*4*), working on "Larry Ann" plums, have already reported similar results. Moreover, the measurements of electrolyte leakage and MDA accumulation in cold stressed plants are practical methods for assessing membrane integrity (*33*). Electrolyte leakage is a reliable indicator of cell membrane damage and is widely used for the assessment of fruit chilling injury (*34*). Peroxidation of lipids at low temperatures can modify cell membrane structure. During the cold stress, cell membrane peroxidation occurred, and MDA was produced, which damaged the fruit cell membranes. The existence of both saturated and unsaturated fatty acids in cell membranes might reduce ion leakage and MDA accumulation, thereby preventing membrane lipid peroxidation and cell damage. Furthermore, the accumulation of ROS and the activities of cell wall destructive enzymes damaged cell membranes (*6*). Electrolyte leakage and MDA content showed considerable enhancements in control and treated fruits during cold storage (Fig. 1b and 1c), but phenylalanine treatment reduced the harmful effects of chilling injury, so it could be concluded that 7.5 mM phenylalanine treatment might have decreased CI by preventing the peroxidation of membrane lipids. Additionally, the application of 7.5 mM phenylalanine increased the resistance of tissue to chilling injury and maintained the integrity of the cell membrane, in comparison with control fruits. These were due to the capacity of phenylalanine at increasing the activity of antioxidant enzymes and the accumulation of ROS scavenging agents, such as (ascorbic acid) AA, phenols, flavonoids, and proline. Our results are similar to those of phenylalanine treated tomatoes, which

showed an increase in the membrane integrity and resistance of tissues to chilling injury during storage (*10*). Regarding our results, MDA contents were lower in phenylalanine treated fruits, and therefore they better maintained the integrity of the membrane. This is in agreement with the results from a previous study on the exogenous application of phenylalanine on tomatoes during cold storage (*10*). The increased ROS scavenging activity following phenylalanine application might be a possible positive response of plum fruits to low temperature stress during cold storage. Kusvuran *et al.* (*35*) reported that fruit chilling injury was due to oxidative stress from ROS accumulation, which further damaged cell membrane. Furthermore, Blokhina *et al.* (*36*) have revealed that plant tissues that have better antioxidant systems better resist low temperatures. Nukuntornprakit *et al.* (*37*) reported that the development of CI symptoms was correlated with ROS metabolism, so that it might reduce total antioxidant capacity. The result indicates that the high antioxidative potential of 7.5 mM phenylalanine treated fruits is responsible for the low CI.

Biochemical factors

Total phenol content at harvest was 263.12 mg per 100 g fresh mass based on gallic acid, which was, during storage, quickly reduced in control fruits but significantly increased in treated fruits. After 40 days of cold storage, fruits treated with 7.5 mM phenylalanine (Fig. 2a) had the highest total phenol content. During storage period, the content of total phenol in control fruits decreased from 263.12 to 195.37 mg/100 g fresh mass but increased to 365.14, 406.24, or 442.13 mg/100 g fresh mass in those treated with 2.5, 5, or 7.5 mM phenylalanine, respectively. The amount of total flavonoids in fruits treated with 7.5 mM phenylalanine was higher than in those of the other treatments after 40 days of storage (Fig. 2b). At the beginning of the experiment and after 40 days of storage, total anthocyanin was 83.20 and 58.46 mg/100 g fresh mass of cyanidin 3-glucoside in control fruits, respectively. Total anthocyanin decreased more in control than in the treated fruits (Fig. 2c). After 40 days of storage, fruits treated with 7.5 mM phenylalanine had a higher total anthocyanins than the other experimental groups. During 40 days of storage at 1 °C, the content of ascorbic acid also decreased in all fruits. However, phenylalanine treatments maintained a higher ascorbic acid content during storage (Table 3). Finally, the content of ascorbic acid decreased to 16.67, 18.75, 29.17, and 31.67 mg/100 g fresh mass in control, 2.5, 5, and 7.5 mM phenylalanine treated fruits, respectively. Before the administration of the treatments, antioxidant activity was 79 % based on the fresh mass, which was gradually declined in control fruits during the experiment. The antioxidant activity in the treated fruits increased during the first 20 days of storage but then decreased. After 40 days of storage, the antioxidant activity in fruits treated with 7.5 mM phenylalanine was greater than in those of the other treatments (Table 3). As shown in Fig. 2d, the activity of PAL in all fruit samples enhanced

during storage. However, during storage, PAL activity in the control fruits was lower than in the treated ones. After 40 days of storage, the activity of PAL in 7.5 mM phenylalanine treated fruits was higher than in the other fruits (Fig. 2d). Our results showed that cold storage increased the activity of PPO during storage (Fig. 2e). The activity of PPO in fruits treated with 7.5 mM phenylalanine was significantly lower than in those of the other treatments. On the first day, the activity of PPO was 14.01 kat/kg protein and increased to 27.85 and 22.20 kat/kg protein in control and 7.5 mM phenylalanine treated fruits, respectively. The results showed that flavonoids, phenols, ascorbic acid, and anthocyanin were present at remarkably higher levels in fruits treated with phenylalanine than in untreated fruits. In fruits treated with phenylalanine, a higher activity of PAL is accountable for the higher accumulation of phenolic compounds. In the current study, PAL positively correlated with total phenol, flavonoids, and antioxidant activity (Table 2). The increased PAL activity has been correlated with an increased production of phenylpropanoid (*38*). The phenylpropanoid pathway has been extensively studied concerning the production of a wide variety of natural phenolic compounds such as isoflavonoids, flavonoids, hydroxy-cinnamic acids, coumarins, lignin, and stilbenes. In addition, PAL is an important enzyme during the biosynthesis of anthocyanins in plums, and PAL activities result in the accumulation of anthocyanins (*7*). Therefore, the increased PAL activity promoted the phenylpropanoid pathway (Fig. 2d) in plums in response to the exogenous phenylalanine application at 7.5 mM, which resulted in a higher accumulation of phenolic compounds (Fig. 2a-c), thereby enhancing the DPPH scavenging capacity. These bio compounds might be involved in the antioxidant capacity. It seems that the increased levels of phenolic compounds and anthocyanins following the administration of phenylalanine treatments were probably responsible for the increased antioxidant capacity as measured by DPPH assays. PPO, as a basic enzyme, is responsible for the browning of cold damaged horticultural crops. In the present experiment, during cold storage, the activity of PPO increased in all samples and was greater in control than in phenylalanine treated fruits (Fig. 2e). Martinez and Whitaker (*39*) reported that the activity of PPO increased during storage at 0 °C, and it might be a chilling injury symptom. It was suggested that the increased the activity PPO was in response to the chilling of plum fruits, which implicated a role for PPO in the development of flesh browning. In this study, the activity of PPO had a positive correlation with CI in plum fruits (Table 2). Therefore, a low activity of PPO in phenylalanine treated fruits appears to be related to a less CI occurrence. The membrane penetrability and the interplay of phenols and PPO, which are commonly found in separate cellular compartments, take place throughout CI disturbance (*40*). A relationship between polyphenol content and the activity of PPO was achieved in the early and late-harvested fruits during the postharvest period. In this research, TP reduced during storage in control plums, and a decrease in TP was accompanied by an increase in the activity of PPO. Ascorbic acid is an anti-

browning agent that acts as an inhibitor of PPO activity (*41*). The concentration of ascorbic acid in the fruits decreased during storage, and a supposition has been developed (*42*), which states that a higher ascorbic acid concentration leads to a less susceptibility of fruit to FB. Although PPO has been strongly associated with FB in fruits, other enzymes such as peroxidase (POD) and PAL have also been studied for the development of the disorder. Recently, the association of POD has been reported in several articles, including Pauziah *et al.* (*43*), Zhou *et al.* (*44*), and Selvarajah *et al.* (*45*). POD has been shown to be involved in the discoloration of numerous fruits and vegetables. However, its association with FB is still questioned as both lower and higher POD activities have been reported. Zhou *et al.* (*44*) observed that chilling injury increased the activity of PAL and stimulated the biosynthesis of polyphenol compounds in pineapple. Furthermore, there was no direct correlation between PAL activity and FB development (*45*).

Proline content

Proline contents were increased in fruits during storage at 1 °C for 40 days (Fig. 3). The exogenous application of phenylalanine displayed a statistically superior proline accumulation during storage in the fruits treated with phenylalanine. After 40 days of storage, the proline accumulation in the fruits treated with 7.5 mM phenylalanine was more than in those treated with 2.5 or 5 mM phenylalanine (Fig. 3). The accumulation of proline, as an osmoregulator, alleviated the stress in the fruits. By inhibiting enzymatic deterioration and scavenging hydroxyl radicals via osmoregulation, proline enhanced the endurance of plants against stress (*46*), thus protecting membrane integrity and antioxidant enzymes. The accumulation of proline could improve cold tolerance in cold sensitive plants (*47*). Our results are in line with the previous findings that showed that phenylalanine treatment increased proline content (*10*), which led to an increased cold tolerance in plums.

Activities of antioxidant enzymes and H_2O_2 content

The activities of antioxidant enzymes (SOD, APX, and CAT) in all fruits increased permanently during the storage period. During storage the control fruits showed relatively lowered enzyme activities in comparison with the treated fruits. At the end of the storage, the activities of SOD, APX, and CAT in control fruits were 2.35, 6.58, and 2.12 kat/kg protein, respectively, while they were 3.87, 9.12, and 3.65 kat/kg protein in 7.5 mM phenylalanine treated fruits, respectively (Table 4). During storage, the content of H_2O_2 increased in control and treated fruits (Fig. 4), suggesting the intensification of peroxidation in the fruits and CI development. The highest/lowest content of H_2O_2 were detected in control/7.5 mM phenylalanine treated fruits. Regarding our results, a positive correlation was found between CI and the activities of antioxidant enzymes (SOD, APX, and CAT)

and H_2O_2 content (Table 2). The low temperatures caused CI disturbance in the fruits and increased membrane lipid oxidation, which may be linked to tissue destruction (*13*). The accumulation of ROS accelerates senescence by inducing lipid peroxidation and oxidative damage imposition (*48*). For the removal of these reactive oxygen species, plants use enzymatic (SOD, POX, APX, CAT) and non-enzymatic (antioxidant compounds such as AA) systems (*49*). Increased activities of antioxidant enzymes are one of the protection mechanisms in plants against harmful effects of reactive oxygen species (ROS) produced in several tissues at low temperatures (*5*). SOD is known as the first antioxidant enzyme that acts at the front line of the defense system. Several investigations have shown that CAT may be a necessary antioxidant enzyme in the chilling tolerance of susceptible fruits during low-temperature storage (*50*). APX converts H_2O_2 into water using two molecules of AA as a reducing power with a joint production of two units of monodehydroascorbate. The increased activity of APX, utilizing AA as a substrate in the oxidation reaction to inhibit the accumulation of H_2O_2, has been found to be a mechanism for chilling tolerance in some horticultural crops (*51*,3). Therefore, the alterations in the antioxidant ingredients in plums during cold storage seem to be more significant in the protection against the oxidative damage, chilling injury. It appears that the chilling tolerance in phenylalanine-treated fruits is due to the enhanced activities of SOD, CAT, and APX. In this research, the enhanced activities of these enzymes improved the tissue capacity to detoxify ROS, leading to a delay in ripening and senescence processes. Aghdam *et al.* (*10*) reported that tomato fruits, a cold susceptible crop, treated with phenylalanine exhibited higher activities of antioxidant enzymes such as SOD, CAT, APX, and GR, which led to a higher tolerance to chilling. In this study, the application of phenylalanine significantly reduced hydrogen peroxide (H_2O_2) in plum fruits. Therefore, it is safe to say that treatment with 7.5 mM phenylalanine has prevented membrane lipid peroxidation in plums during the storage period and thus reduced CI. CI may lead to the formation of reactive oxygen species such as H_2O_2, which harms the membrane via lipid peroxidation (*52*). One of the most probable reasons for the decreased H_2O_2 content in phenylalanine-treated plums during storage is the increased activities of the antioxidant enzymes SOD, CAT, and APX, as they are liable for the elimination of a lot of H_2O_2. These researches suggest that phenylalanine-treated plum fruits tolerant to low-temperature stress are equipped with a more effective antioxidative system.

CONCLUSIONS

A positive correlation was found between CI and electrolyte leakage and MDA content. Postharvest treatment of plums with phenylalanine decreased the chilling injury and protected cell membranes during storage. The low-temperature tolerance may be because of a lower accumulation of H_2O_2 as a result of the more active reactive oxygen species (ROS) removing enzymes and

antioxidant systems, along with a more endogenous accumulation of proline. In the current study, PAL had a positive correlation with the antioxidant capacity. However, a negative correlation was found between ascorbic acid and antioxidant capacity and CI index. Consequently, high levels of ascorbic acid and antioxidant capacity were accompanied by a low CI index. Phenylalanine treatments significantly increased the activity of phenylalanine ammonia-lyase (PAL) in plum fruits during cold storage. Phenylalanine treatments maintained more concentrations of total flavonoids, phenolics, and anthocyanins. Our results demonstrated that phenylalanine treatments effectively retarded or avoided tissue softening, maintained a higher ascorbic acid and antioxidant capacity, and decreased the activity of PPO, which is responsible for the browning of plum fruits during storage. Accordingly, phenylalanine treatments may be effective in maintaining the quality of, reduce the severity of the chilling injury in, and extending the postharvest life of plums during cold storage.

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CONFLICT OF INTEREST

The authors have no conflict of interest in this study.

AUTHORS' CONTRIBUTION

O.B. Sogvar participated in processing/interpreting data, preparation of manuscript and writing/ revising the manuscript. V. Rabiei and F. Razavi took part in designing/performing expriments, processing/interpreting data, preparation of manuscript and writing/ revising the manuscript. G. Gohari participated in processing/interpreting data, preparation of manuscript and writing/ revising the manuscript.

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REFERENCES

1. Ertekin C, Gozlekci S, Kabas O, Sonmez S, Akinci I. Some physical, pomological and nutritional properties of two plum (*Prunus domestica* L.) cultivars. J Food Eng. 2006;75(4):508-14. https://doi.org/10.1016/j.jfoodeng.2005.04.034

2. Crisosto CH, Garner D, Crisosto GM, Bowerman E. Increasing 'Blackamber' plum (*Prunus salicina* Lindell) consumer acceptance. Postharvest Biol Technol. 2004;34(3):237-44. https://doi.org/10.1016/j.postharvbio.2004.06.003

3. Singh SP, Singh Z. Postharvest cold storage-induced oxidative stress in Japanese plums (*Prunus salicina* Lindl. cv. Amber Jewel) in relation to harvest maturity. Aust J Crop Sci. 2013;7 (3):391-00. https://doi.org/10.1016/j.foodres.2010.12.027

4. Candan AP, Graell J, Larrigaudière C. Roles of climacteric ethylene in the development of chilling injury in plums. Postharvest Biol Technol. 2008;47(1):107-12. https://doi.org/10.1016/j.postharvbio.2007.06.009

5. Mittler R. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. 2002;7(9):405-10.

6. Luo Z, Chen C, Xie J. Effect of salicylic acid treatment on alleviating postharvest chilling injury of 'Qingnai' plum fruit. Postharvest Biol Technol. 2011;62(2):115-20. https://doi.org/10.1016/j.postharvbio.2011.05.012

7. Wu F, Zhang D, Zhang H, Jiang G, Su X, Qu H, et al. Physiological and biochemical response of harvested plum fruit to oxalic acid during ripening or shelf-life. Food Res Int. 2011;44(5):1299-05. https://doi.org/10.1016/S1360-1385(02)02312-9

8. Abu-Kpawoh JC, Xi YF, Zhang YZ., Jin YF. Polyamine accumulation following hot-water dips influences chilling injury and decay in 'Friar'plum fruit. J Food Sci. 2002;67(7):2649-53. https://doi.org/10.1111/j.1365-2621.2002.tb08793.x

9. Singh S P, Singh Z., Swinny EE. Postharvest nitric oxide fumigation delays fruit ripening and alleviates chilling injury during cold storage of Japanese plums (*Prunus salicina* Lindell). Postharvest Biol Technol. 2009;53(3):101-08. http://dx.doi.org/10.1016/j.postharvbio.2009.04.007

10. Aghdam MS, Moradi M, Razavi F, Rabiei V. Exogenous phenylalanine application promotes chilling tolerance in tomato fruits during cold storage by ensuring supply of NADPH for activation of ROS scavenging systems. Sci Hortic. 2019;246:818-25. https://doi.org/10.1016/j.scienta.2018.11.074

11. Portu J, López-Alfaro I, Gómez-Alonso S, López R, Garde-Cerdán T. Changes on grape phenolic composition induced by grapevine foliar applications of phenylalanine and urea. Food Chem. 2015;180:171-80.

https://doi.org/10.1016/j.foodchem.2015.02.042

12. Kumar Patel M, Maurer D, Feygenberg O, Ovadia A, Elad Y, Oren-Shamir M, Alkan N. Phenylalanine: A promising inducer of fruit resistance to postharvest pathogens. Foods, 2020;9(5):646.

https://doi.org/10.3390/foods9050646

13. Lurie S, Crisosto CH. Chilling injury in peach and nectarine. Postharvest Biol Technol. 2005;37(3):195-08.

https://doi.org/10.1016/j.postharvbio.2005.04.012

14. Promyou S, Ketsa S, van Doorn WG. Salicylic acid alleviates chilling injury in anthurium (*Anthurium andraeanum* L.) flowers. Postharvest Biol Technol. 2012;64(1):104-10.

https://doi.org/10.1016/j.postharvbio.2011.10.002

15. Dhindsa RS, Plumb-Dhindsa P, Thorpe TA. Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. J Exp Bot. 1981;32(1): 93-01.

https://doi.org/10.1093/jxb/32.1.93

16. Singleton VL, Rossi J A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic. 1965;16(3): 144–58.

17. Bouayed J, Hoffmann L, Bohn T. Total phenolics, flavonoids, anthocyanins and antioxidant activity following simulated gastro-intestinal digestion and dialysis of apple varieties: Bioaccessibility and potential uptake. Food Chem. 2011;128(1):14-21.

https://doi.org/10.1016/j.foodchem.2011.02.052

18. Caleb OJ, Opara UL, Mahajan PV, Manley M, Mokwena L, Tredoux AG. Effect of modified atmosphere packaging and storage temperature on volatile composition and postharvest life of minimally-processed pomegranate arils (cvs.‘ Acco’ and ‘Herskawitz’). Postharvest Biol Technol. 2013;79:54-61.

https://doi.org/10.1016/j.postharvbio.2013.01.006

19. AOAC Official method 967.21,45.1.14. Ascorbic acid. Official final action. (16th ed.), Rockville ,MD,USA:AOAC International;1995.

20. Razavi F, Hajilou J. Enhancement of postharvest nutritional quality and antioxidant capacity of peach fruits by preharvest oxalic acid treatment. Sci Hortic. 2016;200:95-01.

https://doi.org/10.1016/j.scienta.2016.01.011

21. Nguyen TBT, Ketsa S, van Doorn WG. Relationship between browning and the activities of polyphenoloxidase and phenylalanine ammonia lyase in banana peel during low temperature storage. Postharvest Biol Technol. 2003;30(2):187-93.

https://doi.org/10.1016/S0925-5214(03)00103-0

22. Kahn V. Polyphenol oxidase activity and browning of three avocado varieties. J Sci Food Agr. 1975;26(9):1319-24.

https://doi.org/10.1002/jsfa.2740260910

23. Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water-stress studies. Plant Soil. 1973;39(1):205-07.

https://doi.org/10.1007/BF00018060

24. Zhang Z, Huber DJ, Rao J. Antioxidant systems of ripening avocado (*Persea americana* Mill.) fruit following treatment at the preclimacteric stage with aqueous 1-methylcyclopropene. Postharvest Biol Technol. 2013;76:58-64.

https://doi.org/10.1016/j.postharvbio.2012.09.003

25. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72:248-54.

https://doi.org/10.1016/0003-2697(76)90527-3

26. Alexieva V, Sergiev I, Mapelli S, Karanov E. The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. Plant Cell Environ. 2001;24(12):1337-44.

https://doi.org/10.1046/j.1365-3040.2001.00778.x

27. IBM SPSS, v. 20.0, IBM Corp, Armonk, NY, USA; 2011. Available from https://www.ibm.com.

28. Magazin N, Gvozdenovic D, Keserovic Z, Milic B. Fruit quality of Granny Smith apples picked at different harvest times and treated with 1-MCP. Fruits. 2010;65(3):191-97.

https://doi.org/10.1051/fruits/2010013

29. Casquero PA, Guerra M. Harvest parameters to optimise storage life of European plum 'Oullins Gage'. Int J Food Sci Technol. 2009;44(10):2049-54.

https://doi.org/10.1111/j.1365-2621.2009.02029.x

30. Valero D, Serrano M. Postharvest biology and technology for preserving fruit quality. CRC press; 2010.

31. Garde-Cerdán T, López R, Portu J, González-Arenzana L, López-Alfaro I, Santamaría, P. Study of the effects of proline, phenylalanine, and urea foliar application to Tempranillo vineyards on grape amino acid content. Comparison with commercial nitrogen fertilizers. Food Chem. 2014;163:136-41.

https://doi.org/10.1016/j.foodchem.2014.04.101

32. Crisosto CH, Mitchell FG, Ju Z. Susceptibility to chilling injury of peach, nectarine, and plum cultivars grown in California. J Am Soc Hortic Sci. 1999;34(6):1116-18.

https://doi.org/10.21273/HORTSCI.34.6.1116

33. Aghdam MS, Bodbodak S. Physiological and biochemical mechanisms regulating chilling tolerance in fruits and vegetables under postharvest salicylates and jasmonates treatments. Sci Hortic. 2013;156:73-85.
https://doi.org/10.1016/j.scienta.2013.03.028

34. McCollum TG, McDonald RE. Electrolyte leakage, respiration, and ethylene production as indices of chilling injury in grapefruit. J Am Soc Hortic Sci. 1997;26(9):1191-92.
https://doi.org/10.21273/HORTSCI.26.9.1191

35. Kusvuran S, Kiran S, Ellialtioglu SS. Antioxidant enzyme activities and abiotic stress tolerance relationship in vegetable crops. Abiotic and biotic stress in plants-recent advances and future perspectives. 2016;17:481-03.

36. Blokhina O, Virolainen E, Fagerstedt KV. Antioxidants, oxidative damage and oxygen deprivation stress: a review. Ann Bot. 2003;91(2):179-94.
https://doi.org/10.1093/aob/mcf118

37. Nukuntornprakit OA, Chanjirakul K, van Doorn WG, Siriphanich J. Chilling injury in pineapple fruit: Fatty acid composition and antioxidant metabolism. Postharvest Biol Technol. 2015;99:20-6.
https://doi.org/10.1016/j.postharvbio.2014.07.010.

38. Ozeki Y, Komamine A. Changes in activities of enzymes involved in general phenylpropanoid metabolism during the induction and reduction of anthocyanin synthesis in a carrot suspension culture as regulated by 2,4-D. Plant Cell Physiol. 1985;26(5):903-11.
https://doi.org/10.1093/oxfordjournals.pcp.a076985

39. Martinez MV, Whitaker JR. The biochemistry and control of enzymatic browning. Trends Food Sci Technol. 1995;6(6):195-200.
https://doi.org/10.1016/S0924-2244(00)89054-8

40. Koushesh saba M, Arzani K, Barzegar M. Postharvest polyamine application alleviates chilling injury and affects apricot storage ability. J Agric Food Chem. 2012;60(36):8947-53.
https://doi.org/10.1021/jf302088e

41. Jimenez A, Hernandez JA, del Río LA, Sevilla F. Evidence for the presence of the ascorbate-glutathione cycle in mitochondria and peroxisomes of pea leaves. Plant Physiol. 1997;114(1):275-84.
https://doi.org/10.1104/pp.114.1.275

42. Veltman RH, Kho RM, Van Schaik ACR, Sanders MG, Oosterhaven J. Ascorbic acid and tissue browning in pears (*Pyrus communis* L. cvs Rocha and Conference) under controlled atmosphere conditions. Postharvest Biol. Technol. 2000;19(2):129-37.
https://doi.org/10.1016/S0925-5214(00)00095-8

43. Pauziah M, Abdullah H, Ahmad Tarmizi S, Mohd Salleh P, Mohamed Selamat M, Norhayati M. Association of polyphenoloxidase (PPO) and peroxidise (POD) with blackheart development of Malaysian pineapples. Mardi Horticulture Research Centre Technical Report. 2005;172-75.

44. Zhou Y, Dahler JM, Underhill SJ, Wills RB. Enzymes associated with blackheart development in pineapple fruit. Food Chem. 2003;80(4):565-72.
https://doi.org/10.1016/S0308-8146(02)00375-8

45. Selvarajah S, Bauchot AD, John P. Internal browning in cold-stored pineapples is suppressed by a postharvest application of 1-methylcyclopropene. Postharvest Biol Technol. 2001;23(2):167-70.
https://doi.org/10.1016/S0925-5214(01)00099-0

46. Kuznetsov VV, Shevyakova NI. Proline under stress: biological role, metabolism, and regulation. Russ J Plant Physiol. 1999;46(2):274-87.

47. Zhao DY, Shen L, Fan B, Liu KL, Yu MM, Zheng Y, *et al*. Physiological and genetic properties of tomato fruits from 2 cultivars differing in chilling tolerance at cold storage. J Food Sci. 2009;74(5): 348-52.
https://doi.org/10.1111/j.1750-3841.2009.01156.x

48. Hariyadi P, Parkin KL. Chilling-induced oxidative stress in cucumber fruits. Postharvest Biol Technol. 1991;1(1):33-45.
https://doi.org/10.1016/0925-5214(91)90017-6.

49. Bian S, Jiang Y. Reactive oxygen species, antioxidant enzyme activities and gene expression patterns in leaves and roots of Kentucky bluegrass in response to drought stress and recovery. Sci Hortic. 2009;120(2):264-70.
https://doi.org/10.1016/j.scienta.2008.10.014

50. Ghasemnezhad M, Marsh K, Shilton R, Babalar M, Woolf A. Effect of hot water treatments on chilling injury and heat damage in ‘satsuma’mandarins: Antioxidant enzymes and vacuolar ATPase, and pyrophosphatase. Postharvest Biol Technol. 2008;48(3):364-71.
https://doi.org/10.1016/j.postharvbio.2007.09.014

51. Ding ZS, Tian SP, Zheng XL, Zhou ZW, Xu Y. Responses of reactive oxygen metabolism and quality in mango fruit to exogenous oxalic acid or salicylic acid under chilling temperature stress. Physiol Plant. 2007;130(1):112-21.
https://doi.org/10.1111/j.1399-3054.2007.00893.x

52. Mascher R, Nagy E, Lippmann B, Hörnlein S, Fischer S, Scheiding W, *et al*. Improvement of tolerance to paraquat and drought in barley (*Hordeum vulgare* L.) by exogenous 2-aminoethanol: effects on superoxide dismutase activity and chloroplast ultrastructure. Plant Sci. 2005;168(3):691-98.

https://doi.org/10.1016/j.plantsci.2004.09.036

Table 1. Mass loss/% and firmness/(kg/cm^2) in plums either control or treated with phenylalanine at different concentrations. Fruit were stored at 1 °C for up to 40 days

t(storage)/day	*c*(Phenylalanine)/mM	mass loss/%	firmness/(kg/cm^2)
0	Control	-	(2.88±0.04)^{a}
	2.5	-	(2.88±0.04)^{a}
	5	-	(2.88±0.04)^{a}
	7.5	-	(2.88±0.04)^{a}
10	Control	(1.24±0.06)^{ef}	(1.73±0.08)^{de}
	2.5	(1.07±0.1)^{efg}	(1.86±0.03)^{cde}
	5	(1.5±0.2)^{ef}	(2.56±0.12)^{ab}
	7.5	(0.48±0.11)^{fg}	(2.95±0.12)^{a}
20	Control	(3.56±0.2)^{bc}	(1.21±0.03)^{fgh}
	2.5	(2.64±0.38)^{cd}	(1.4±0.03)^{efg}
	5	(1.9±0.14)^{de}	(2±0.15)^{cd}
	7.5	(2±0.2)^{de}	(2.5±0.13)^{ab}
30	Control	(4.56±0.19)^{b}	(0.94±0.04)^{gh}
	2.5	(4±0.17)^{b}	(1.22±0.03)^{fgh}
	5	(3.8±0.12)^{b}	(1.56±0.14)^{def}
	7.5	(3.5±0.13)^{bc}	(2.32±0.10)^{bc}
40	Control	(6.01±0.31)^{a}	(0.13±0.01)^{i}
	2.5	(4.33±0.25)^{b}	(0.85±0.10)^{h}
	5	(4.5±0.37)^{b}	(1.01±0.15)^{gh}
	7.5	(4.02±0.32)^{b}	(1.52±0.08)^{def}
Significant	df		
Time	4	**	**
Treatment	3	**	**
T × T	12	Ns	**

Data are presented as means value ± *S.D.* of three replications. Different letters indicate significant differences between treatments according to Duncan's test at p=0.05. * and ** Significant difference at $p < 0.01, 0.05$.

Table 2. Pearson's simple correlation coefficient between Chilling injury (CI), total flavonoid (TF), total phenol (TP), ascorbic acid (AA), antioxidant capacity (AC), PAL, APX, CAT, SOD, PPO, electrolyte leakage (EL), malondialdehyde (MDA), and hydrogen peroxide (H_2O_2) of the phenylalanine treated plum fruit

	CI	TP	TF	AA	AC	PAL	APX	CAT	SOD	PPO	EL	MDA	H_2O_2
CI	1	0.26*	0.34**	-0.77**	-0.41**	0.64**	0.55**	0.43**	0.47**	0.84**	0.68**	0.80**	0.64**
TP	0.26*	1	0.85**	0.10 ^{ns}	0.50**	0.73**	0.65**	0.72**	0.70**	0.06 ^{ns}	-0.07 ^{ns}	0.07 ^{ns}	-0.19 ^{ns}
TF	0.34**	0.85**	1	0.09 ^{ns}	0.43**	0.74**	0.68**	0.76**	0.77**	0.16 ^{ns}	-0.03 ^{ns}	0.17 ^{ns}	-0.09 ^{ns}
AA	-0.77**	0.10 ^{ns}	0.09 ^{ns}	1	0.45**	-0.39**	-0.39**	-0.07 ^{ns}	-0.22 ^{ns}	-0.87**	-0.77**	-0.79**	-0.80**
AC	-0.41*	0.50*	0.43*	0.45*	1	0.24 ^{ns}	0.27*	0.36**	0.38**	-0.40**	-0.39**	-0.40**	-0.40**
PAL	0.64**	0.73**	0.74**	-0.39**	0.24	1	0.88**	0.76**	0.81**	0.51**	0.36**	0.44**	0.29*
APX	0.55**	0.65**	0.68**	-0.39**	0.27*	0.88**	1	0.78**	0.81**	0.46**	0.37**	0.36 ^{ns}	0.27*
CAT	0.43**	0.72**	0.76**	-0.07 ^{ns}	0.36**	0.76**	0.78**	1	0.76**	0.23 ^{ns}	0.11 ^{ns}	0.18 ^{ns}	0.05^{ns}
SOD	0.47**	0.70**	0.77**	-0.22 ^{ns}	0.38**	0.81**	0.81**	0.76**	1	0.31*	0.24^{ns}	0.29*	0.10^{ns}
PPO	0.84**	0.06 ^{ns}	0.16 ^{ns}	-0.87**	-0.40**	0.51**	0.46**	0.23 ^{ns}	0.31*	1	0.70**	0.79**	0.74**
EL	0.68**	-0.07 ^{ns}	-0.03 ^{ns}	-0.77**	-0.39**	0.36**	0.37**	0.11 ^{ns}	0.24^{ns}	0.70**	1	0.75**	0.68**
MDA	0.80**	0.07 ^{ns}	0.17 ^{ns}	-0.79**	-0.40**	0.44**	0.36**	0.18 ^{ns}	0.29*	0.79**	0.75**	1	0.66**
H_2O_2	0.64**	-0.19 ^{ns}	-0.09 ^{ns}	-0.80**	-0.40**	0.29*	0.27*	0.05 ^{ns}	0.10^{ns}	0.74**	0.68**	0.66**	1

* and **Significant difference at $p < 0.01, 0.05$.

Table 3. Ascorbic acid/(mg/100 g fm) and antioxidant activity/(% DPPH inhibition) in plums either control or treated with phenylalanine at different concentrations. Fruit were stored at 1 °C for up to 40 days

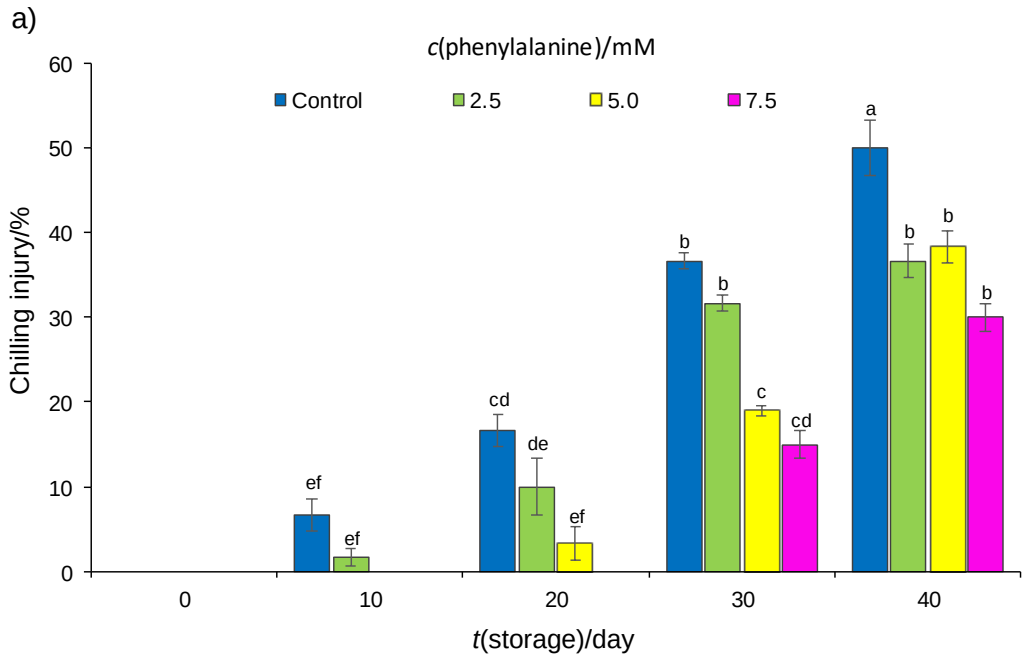
t(storage)/day	*c*(Phenylalanine)/mM	*w*(ascorbic acid/(mg/100 g fm)	antioxidant activity/%
0	Control	(39.17±0.48)^{a}	(79.02±0.25)^{def}
	2.5	(39.17±0.48)^{a}	(79.02±0.25)^{def}
	5	(39.17±0.48)^{a}	(79.02±0.25)^{def}
	7.5	(39.17±0.48)^{a}	(79.02±0.25)^{def}
10	Control	(30±0.83)^{def}	(78.86±0.21)^{def}
	2.5	(35±0.0)^{bc}	(80.05±0.25)$^{b-f}$
	5	(34.17±0.96)^{bc}	(79.84±0.29)$^{b-f}$
	7.5	(35.83±0.96)^{ab}	(81.13±0.55)$^{a-d}$
20	Control	(23.33±0.48)^{gh}	(78.42±0.65)^{f}
	2.5	(26.67±0.48)^{fg}	(80.46±0.32)$^{b-f}$
	5	(34.17±0.48)^{bc}	(81.43±0.55)^{abc}
	7.5	(36.67±1.27)^{ab}	(82.85±0.62)^{a}
30	Control	(23±1.09)^{gh}	(75.62±0.45)^{g}
	2.5	(22±0.74)^{h}	(79.55±0.30)$^{c-f}$
	5	(26.67±0.48)^{fg}	(80.88±0.37)$^{a-e}$
	7.5	(33.25±0.63)^{bcd}	(82.02±0.53)^{ab}
40	Control	(16.67±0.48)^{j}	(75.02±0.36)^{g}
	2.5	(18.75±0.72)^{ij}	(78.67±0.39)^{ef}
	5	(29.17±1.27)^{ef}	(79.27±0.37)$^{c-f}$
	7.5	(31.67±0.48)^{cde}	(80.12±0.32)$^{b-f}$
Significant	Df		
Time	4	**	**
Treatment	3	**	**
T × T	12	**	**

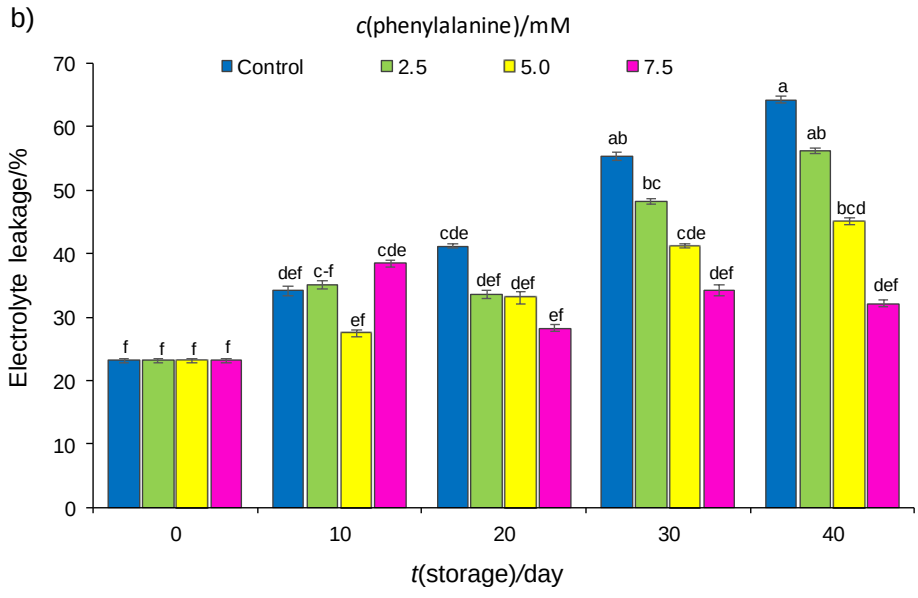
Data are presented as means value ± *S.D.* of three replications. Different letters indicate significant differences between treatments according to Duncan's test at p=0.05. * and ** Significant difference at $p < 0.01, 0.05$.

Table 4. CAT activity/(kat/kg protein), APX activity/(kat/kg protein) and SOD activity/(kat/kg protein) in plums either control or treated with phenylalanine at different concentrations. Fruit were stored at 1 °C for up to 40 days

t(storage)/day	*c*(Phenylalanine)/mM	Enzyme activity/(kat/kg)		
		CAT	APX	SOD
10	Control	(1.71±0.12)^{h}	(4.72±0.44)^{fg}	(2.05±0.08)^{fgh}
	2.5	(2.32±0.10)^{def}	(4.47±0.20)^{gh}	(1.95±0.15)^{gh}
	5	(2.11±0.07)^{fgh}	(5.20±0.30)$^{d-g}$	(2.31±0.17)$^{e-h}$
	7.5	(2.46±0.14)$^{c-}$f	(6.58±0.09)^{cd}	(2.45±0.05)$^{d-g}$
20	Control	(2.01±0.05)^{fgh}	(5.03±0.42)^{efg}	(1.98±0.08)^{gh}
	2.5	(2.15±0.13)$^{e-h}$	(6.35±0.15)^{cde}	(2.52±0.08)$^{d-g}$
	5	(2.35±0.07)^{def}	(6.63±0.04)^{cd}	(2.78±0.12)^{de}
	7.5	(2.62±0.08)^{cdf}	(7.21±0.21)^{bc}	(2.95±0.24)^{cd}
30	Control	(2.25±0.07)$^{d-g}$	(5.96±0.18)$^{c-f}$	(2.44±0.09)$^{d-g}$
	2.5	(2.19±0.09)$^{d-h}$	(6.11±0.11)$^{c-f}$	(2.65±0.07)^{def}
	5	(2.66±0.12)^{cd}	(7.34±0.20)^{bc}	(3.56±0.05)^{ab}
	7.5	(3.11±0.11)^{b}	(8.77±0.25)^{a}	(4.05±0.09)^{a}
40	Control	(2.12±0.06)$^{e-h}$	(6.58±0.42)^{cd}	(2.35±0.16)$^{d-h}$
	2.5	(2.35±0.06)^{def}	(7.11±0.16)^{bc}	(2.85±0.12)^{def}
	5	(2.87±0.09)^{bc}	(8.46±0.21)^{ab}	(3.41±0.12)^{bc}
	7.5	(3.64±0.07)^{a}	(9.12±0.11)^{a}	(3.87±0.09)^{ab}
Significant	df			
Time	3	**	**	**
Treatment	3	**	**	**
T × T	9	**	ns	*

Data are presented as means value ± *S.D.* of three replications. Different letters indicate significant differences between treatments according to Duncan's test at p=0.05. * and ** Significant difference at $p < 0.01, 0.0$





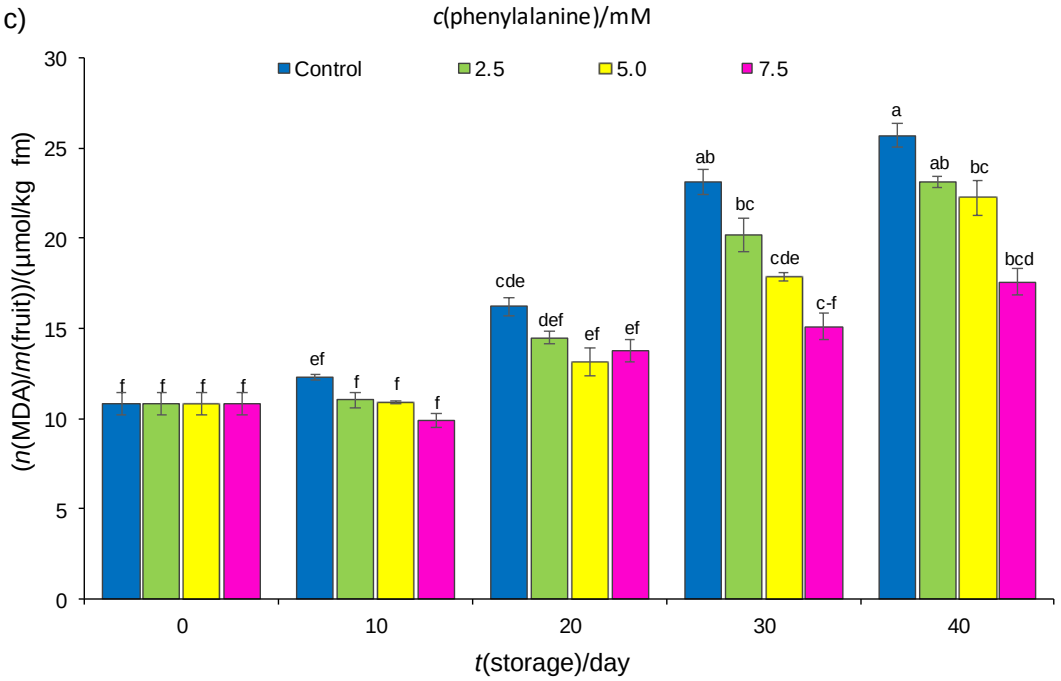
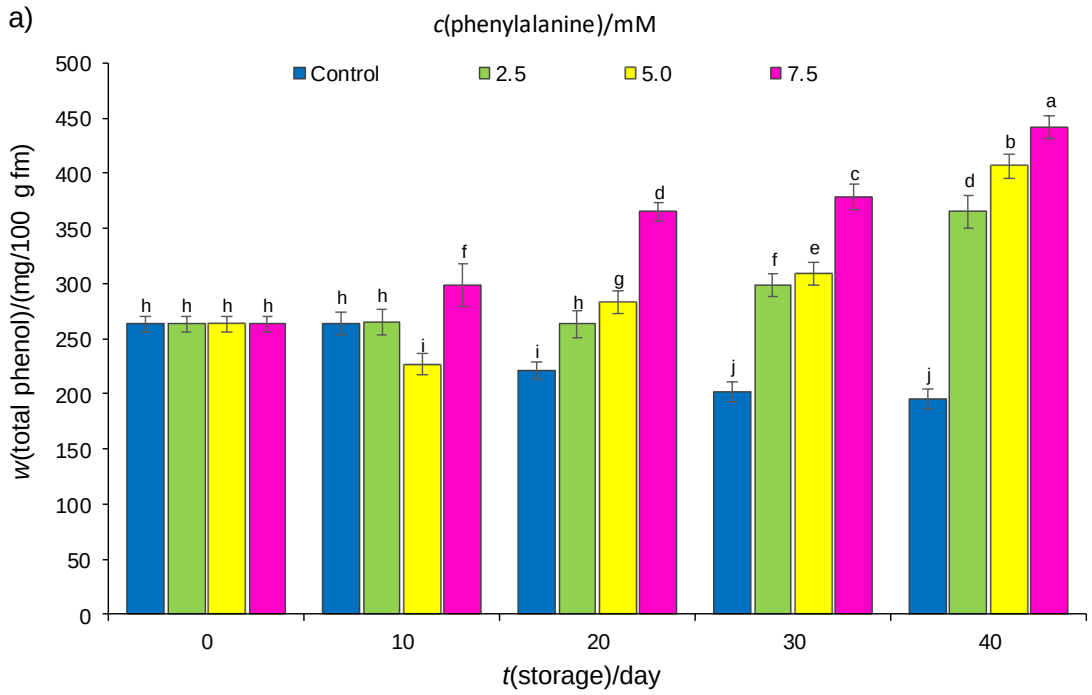
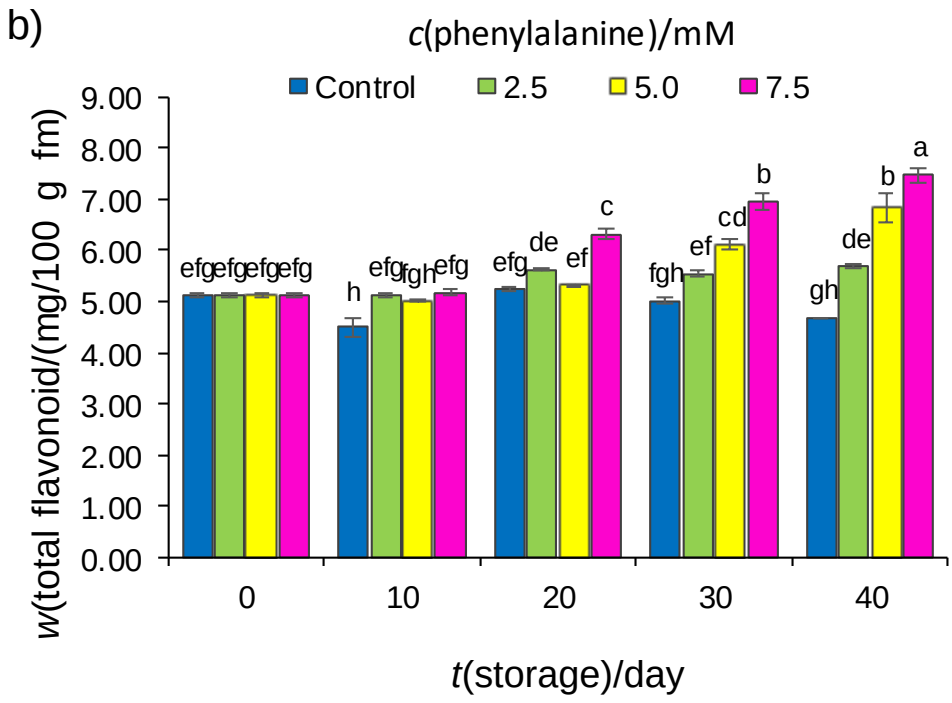
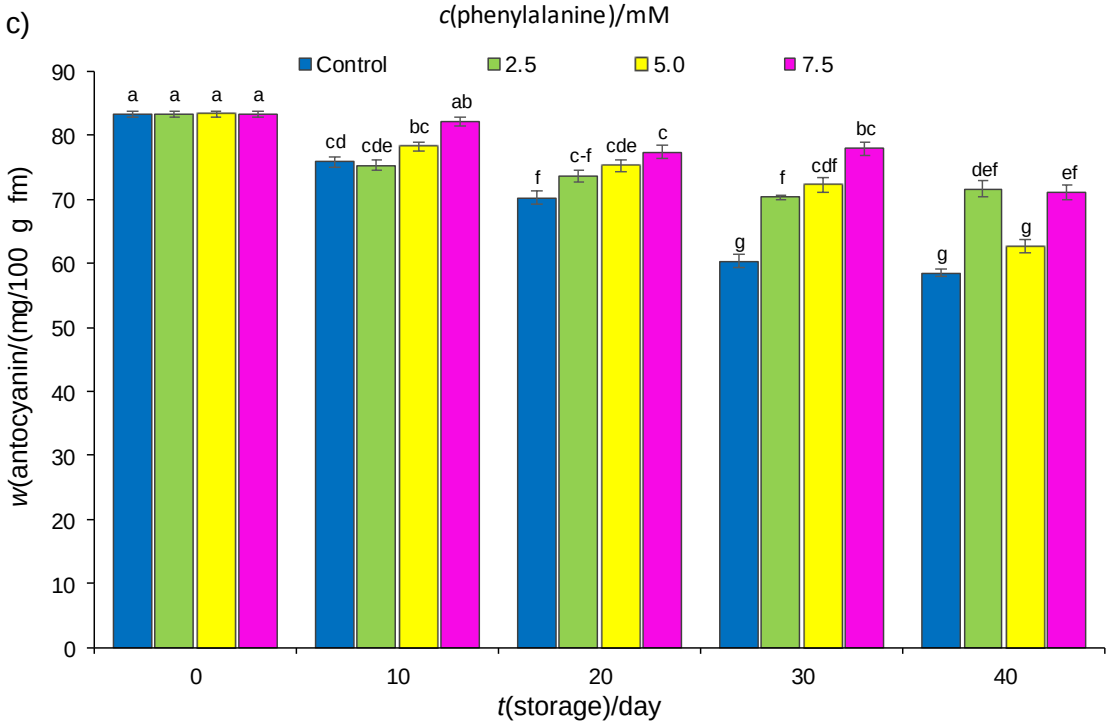


Fig. 1. Chilling injury index/% a), electrolyte leakage/% b) and MDA content/(µmol/kg fm) c) in plums either control or treated with phenylalanine at different concentrations. Fruit were stored at 1 °C for up to 40 days. Data are presented as mean value ± *S.D.* of three replications. Different letters indicate significant differences between treatments according to Duncan's test at p=0.05







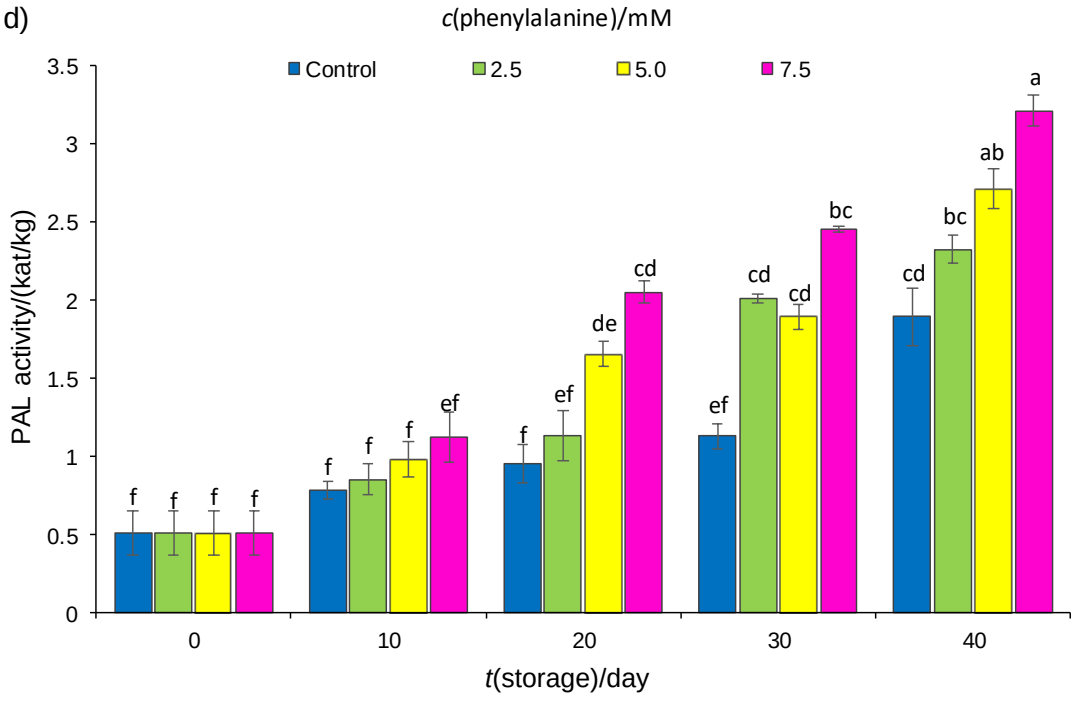


Fig. 2. Total phenol/(mg GAE/100 g fm) a), total flavonoid/(mg/100 g fm) b), anthocyanin/(mg/100 g fm) c), PAL activity/(kat/k*g* protein) d) and PPO activity/(kat/k*g* protein) e) in plums either control or treated with phenylalanine at different concentrations. Fruit were stored at 1 °C for up to 40 days. Data are presented as mean value ± *S.D.* of three replications. Different letters indicate significant differences between treatments according to Duncan's test at p=0.05

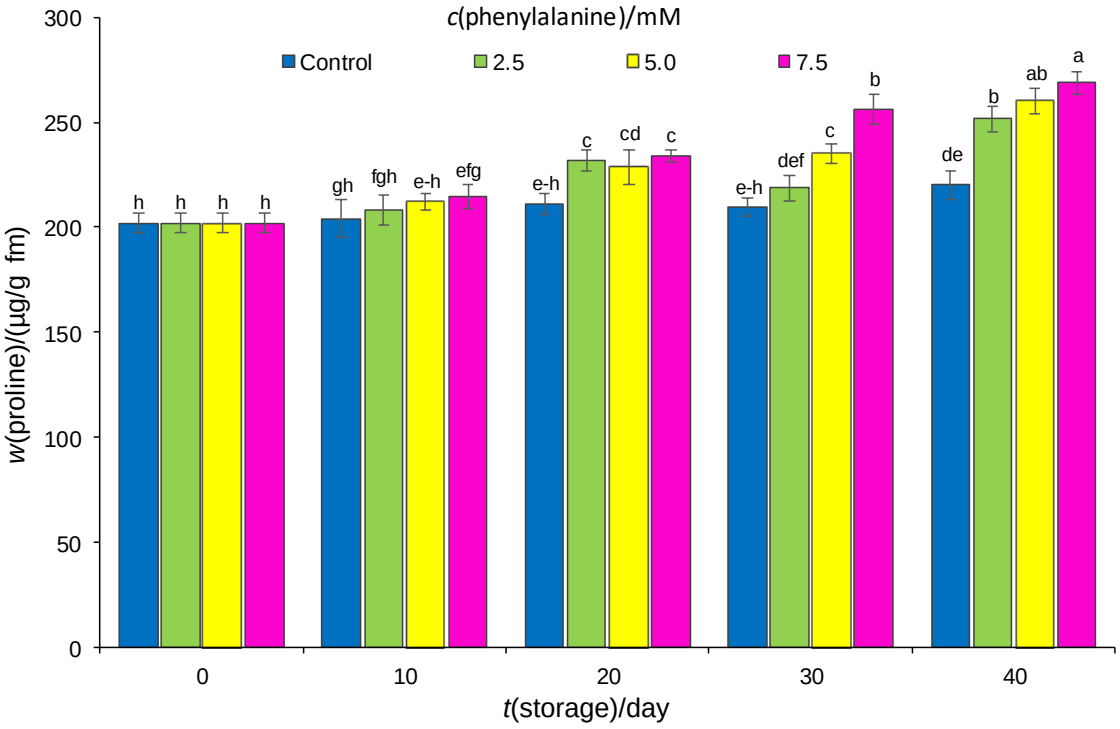


Fig. 3. Proline content/(μg/g fm) in plums either control or treated with phenylalanine at different concentrations. Fruit were stored at 1 °C for up to 40 days. Data are presented as mean value ± *S.D.* of three replications. Different letters indicate significant differences between treatments according to Duncan's test at p=0.05

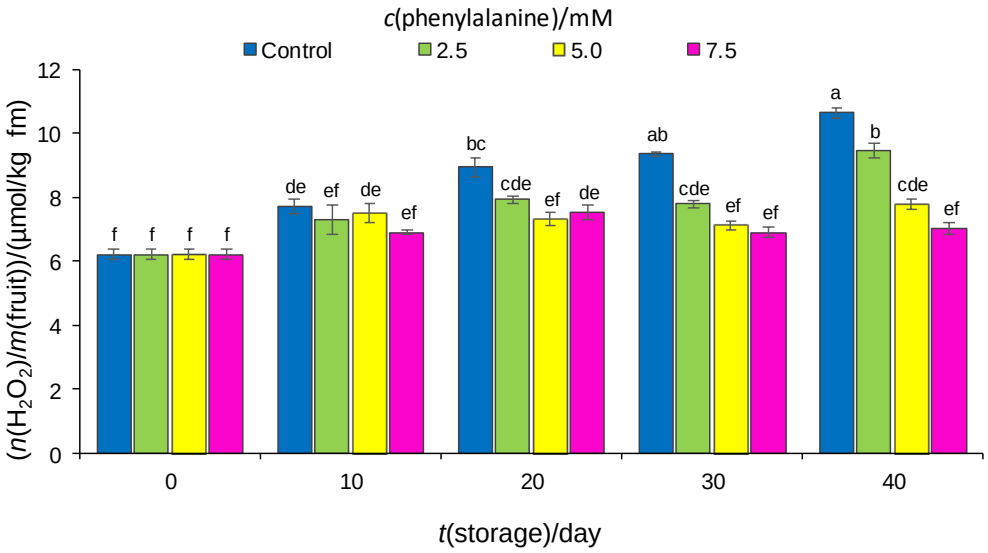


Fig. 4. H_2O_2 content/(μmol/kg fm) in plums either control or treated with phenylalanine at different concentrations. Fruit were stored at 1 °C for up to 40 days. Data are presented as mean value ± *S.D.* of three replications. Different letters indicate significant differences between treatments according to Duncan's test at p=0.05.