

VACUUM INFILTRATION OF 24-EPIBRASSINOLIDE DELAYS CHLOROPHYLL DEGRADATION AND MAINTAINS QUALITY OF LIME DURING COLD STORAGE

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ABSTRACT

Effects of 24-epibrassinolide (EBR) treatments on chlorophyll (Chl) degradation and postharvest quality of two lime ('Persian lime' and 'Tahiti') cultivars during chilling-induced storage were studied in this work. EBR at 0, 2.5, 5 and 10 μM were applied to fruit by vacuum infiltration at 30.63 kPa for 7 min and afterward stored at 4°C for 60 days. Postharvest EBR application effectively maintained greater fruit firmness and lessened weight loss during cold storage. The results displayed that EBR treatments efficiently delayed the reduction of the Chl *a* contents and hue values. EBR treatments significantly increased ascorbic acid content, total phenolic content (TPC), antioxidant activity, and titratable acidity (TA). On the other hand, the treatments reduced soluble solids concentration (SSC), pH, and SSC/TA ratios during cold storage. Overall, EBR application by vacuum infiltration could be an effective and simple method for maintaining postharvest quality of limes during cold storage.

Key words: antioxidant activity, brassinolides, lime, postharvest quality, vacuum infiltration

INTRODUCTION

One the main economically important horticultural produce is Lime (*Citrus latifolia* Tanaka) which is cultivated in the south of Iran with a production season from August to October. When the lime peel is green with high fragrant compounds, harvesting is done. The lime fruit quality characteristics are strongly determinative for its price. After harvest, lime fruit postharvest quality deteriorates rapidly. The loss of rind greenness that commonly arises with chlorophyll degradation is the most dominant deterioration factor [Kaewsuksaeng et al. 2011, Srilaong et al. 2011]. It is necessary to retain the lime rind green color during and even after the cold storage, for the maintenance of postharvest quality.

When the tropical and subtropical origin crops are stored at low temperature, a chilling injury (CI) disorder occurs. The temperatures above the freezing point are the minimum safe temperatures for chilling sensitive crops. The acute temperature for chilling injury changes with the produce. When storage is done at temperatures under 10–13°C, CI usually happens. Accordingly, commodities which are susceptible to chilling injury mostly have a short storage life hence for delaying pathogen growth and deterioration, low temperatures cannot be used. The damage of cell membranes is the main reason of chilling injury in fruits. This deterioration brings a wave of secondary responses, which may contain respiration

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increase, ethylene production, alteration of the cellular structure and accumulation of toxic compounds. Relative humidity from 85 to 95% and temperatures ranging from 10 to 12°C are the best storage conditions for lime. Fruit can be stored for four to eight weeks, under such conditions. Surface pitting and decay incidence enhancement are the main symptoms of chilling injury in fruit under the temperatures below 8°C [Kader and Arpaia 1992, Kluge et al. 2007].

A ubiquitous new class of natural hormones in plants is Brassinosteroids (BRs) [Sasse 2003]. BRs are thought to play fundamental roles in resistance to a variety of environmental stresses and regulating plant growth and development [Bajguz and Hayat 2009]. BRs have been widely utilized to enhance stress resistance and crop production [Xia et al. 2009]. Recently, some studies have reported the impacts of BRs on maintaining postharvest quality of horticultural commodities. Zhu et al. [2010] showed that postharvest brassinolide treatment considerably prevented blue mould rot development also ethylene production and hence hindered jujube fruit senescence. Gao et al. [2016] indicated that brassinolide application might be an efficient manner to induce chilling tolerance in peach fruit. It has been shown that BRs treatment significantly increased chilling tolerance of harvested tomato [Aghdam and Mohammadkhani 2014], green bell pepper [Wang et al. 2012] and mango [Li et al. 2012] fruits by mechanisms that raise enzymatic and non-enzymatic antioxidant activity and retain the high membrane integrity. Wu and Yang [2015] revealed that brassinolide coating could preserve the asparagus spears quality and prolong its postharvest life. Regarding to application method, fruit dipping is fast and easy, however vacuum infiltration is more effective than dipping [Ramezani et al. 2010].

‘Tahiti’ and ‘Persian lime’ are the main lime cultivars in Fars province (southeast of Iran, as subtropical region) and have popularity among consumers due to its juice (high vitamin C concentration) and high quality. Limes are highly susceptible to CI and they can undergo internal metabolic changes during extended storage, resulting in rind disorders and green color fading and loss of fruit acceptance by the market [Kluge et al. 2003]. Therefore, new investiga-

tion is vital to maintain lime quality during cold storage. However, to date, no information has been reported on possible role of BRs application on post-harvest quality of lime fruit. Thus, the aim of this work was to investigate the effect of 24-epibrassinolide (EBR) application on physicochemical changes in two popular lime cv. ‘Tahiti’ and ‘Persian lime’ during cold storage.

MATERIALS AND METHODS

Fruit of two lime cultivars (‘Tahiti’ and ‘Persian lime’) were harvested from a commercial orchard Jahrom county, Fars province (South of Iran) at early of September. Fruit were selected for uniformity in maturity, lack of defects, rind color and shape and size, after transportation to the laboratory. The fruit were submerged in 2% $\text{Ca}(\text{ClO})_2$ solution for two minutes, cleansed with distilled water, and dried at room temperature. Fruit were selected randomly for each cultivar divided into 3 lots of 90 fruit for each treatment. Treatments were distilled water as control, 2.5 μM , 5 μM and 10 μM 24-epibrassinolide (EBR). All treatments were applied using vacuum infiltration at 30.63 kPa for seven minutes, where treating 30 fruit in 7 L of solutions. Taking after treatment, fruit were left to dry at room temperature, then partitioned into sets of three replicates of ten fruit for each sampling time. Each replicate was stored in polyethylene packaging bags containing twelve holes with 3 mm diameter, weighed and stored for up to 65 days at 4°C and 85% RH.

Appraisal of qualitative and quantitative factors were done at the first day (before treatment) and at 20, 40 and 60 days of cold storage. After transporting fruit to laboratory, seven non-treated fruit as a specimen were sampled for first measurements. At the end of each storage cycle, for two days, the fruit were maintained at 20°C to imitate shelf life.

Symptoms of chilling injury (CI) include sunken lesions and pitting on the fruit surface. The CI index was estimated based on the percentage of total fruit surface area containing sunken lesions or surface pitting [Fung et al. 2004]: Grade 0 (no signs of CI), Grade 1 (<25% of the fruit area showing CI), Grade 2 (25–50% of the fruit area showing CI) and Grade 3

(>50% of the fruit area showing CI). The CI index was expressed as: CI index (%) = $\Sigma[(CI \text{ level}) \times (\text{number of fruit at this level})] / (\text{highest level} \times \text{total number of fruit in the treatment}) \times 100$. Three replicates for each treatment were performed, and each replicate contained 30 fruit.

Color was measured on two opposite sides of each fruit (Chroma meter CR400, Minolta, Japan). Chroma and hue parameters were measured for each fruit and presented as color index. Chlorophyll a content was determined using N,N-dimethylformamide [Moran 1982].

Weight loss was calculated during storage by weighing fruit at the initial stage (W_1), and also at the different sampling dates (W_2). The weight loss was calculated using the following equation [Candir et al. 2017]:

$$\text{Weight loss (\%)} = \frac{W_1 - W_2}{W_1} \times 100$$

At the end of each storage time, fruit firmness per treatment was measured using a food texture analyzer, the TA-XT *plus* (Stable Micro System, Godalming, UK). Fruit were compressed by 2 mm with parallel plates at a crosshead speed of 1 mm s⁻¹.

In order to prepare fruit juice, fruit of each replicate were cut separately and the juice was collected using a reamer. Juice pH was measured by pH meter (HANNA edge, HI2202, Italy). Titratable acidity (TA) was determined by titrating 5 mL of juice with NaOH 0.1 mol L⁻¹ until the end point of pH 8.2 and expressed as percentage of citric acid. SSC were ascertained utilizing a hand-held digital refractometer (PAL α , ATAGO Co., Japan) at 20°C and readings are shown as percentage (%).

Total phenolic content (TPC) analyzed according to the Folin Ciocalteu colorimetric method according to the method described by Halicia et al. [2005]. 700 μ L of extract mixed with 900 μ L of 2% sodium carbonate, and 3 min maintained at room temperature. Afterwards 200 μ L of 50% Folin was added. Mixtures were left to remain at room temperature for thirty minutes before measuring absorbance at 750 nm using a spectrophotometer (Varian 220, Aus-

tralia). The concentration of total phenolic content was presented as mg g⁻¹ F.W.

Ascorbic acid concentrations of the lime juice were quantified utilizing the 2,6-dichlorophenol indophenol method as described in AOAC [2000].

By DPPH free radical scavenging method, antioxidant activity of each extract was measured [Burits et al. 2001]. One hundred μ L of extracts with 1 mL DPPH (0.1 Mm) and 1 mL Tris-HCl (pH = 7.5) buffer mixed and for thirty minutes kept at room temperature. The mixture absorbance was read utilizing an ELx808 absorbance microplate reader (BioTek Instruments Inc., USA) at 515 nm. Finally, antioxidant activity was calculated using the following formula:

$$\begin{aligned} \text{Antioxidant activity} &= \\ &= 1 - (A_{\text{sample}(515\text{nm})} / A_{\text{control}(515\text{nm})}) \times 100 \end{aligned}$$

All treatments were performed according to a completely randomized design (CRD) with 3 replicates. Physicochemical data were analyzed using three-factor analysis of variance (treatments, cultivars and storage time) procedures. Statistical analysis was performed using analysis of variance (ANOVA) which was carried out with the SPSS version 19 software. Significant differences between means of data were compared using the Duncan's multiple range test at the 5% level.

RESULTS

In all treatments, weight loss increased during storage in both cultivars, however it was lowest in EBR treated lime fruit (fig. 1A and B). Generally, weight loss was higher in 'Tahiti' than in 'Persian lime' fruit. After sixty days of cold storage, the lowest fruit weight loss was monitored in 'Persian lime' fruit treated with 10 μ M EBR (9.33%), compared with the control (24.71%). In both cultivars, 10 μ M level of EBR demonstrated less weight loss in comparison with the control, at the end of storage (fig. 1A and B). With respect to the initial weight, treated fruit weight loss of both cultivars was less than 13%, however, after 60 days of storage, it was more than 25% in control fruit (fig. 1A and B).

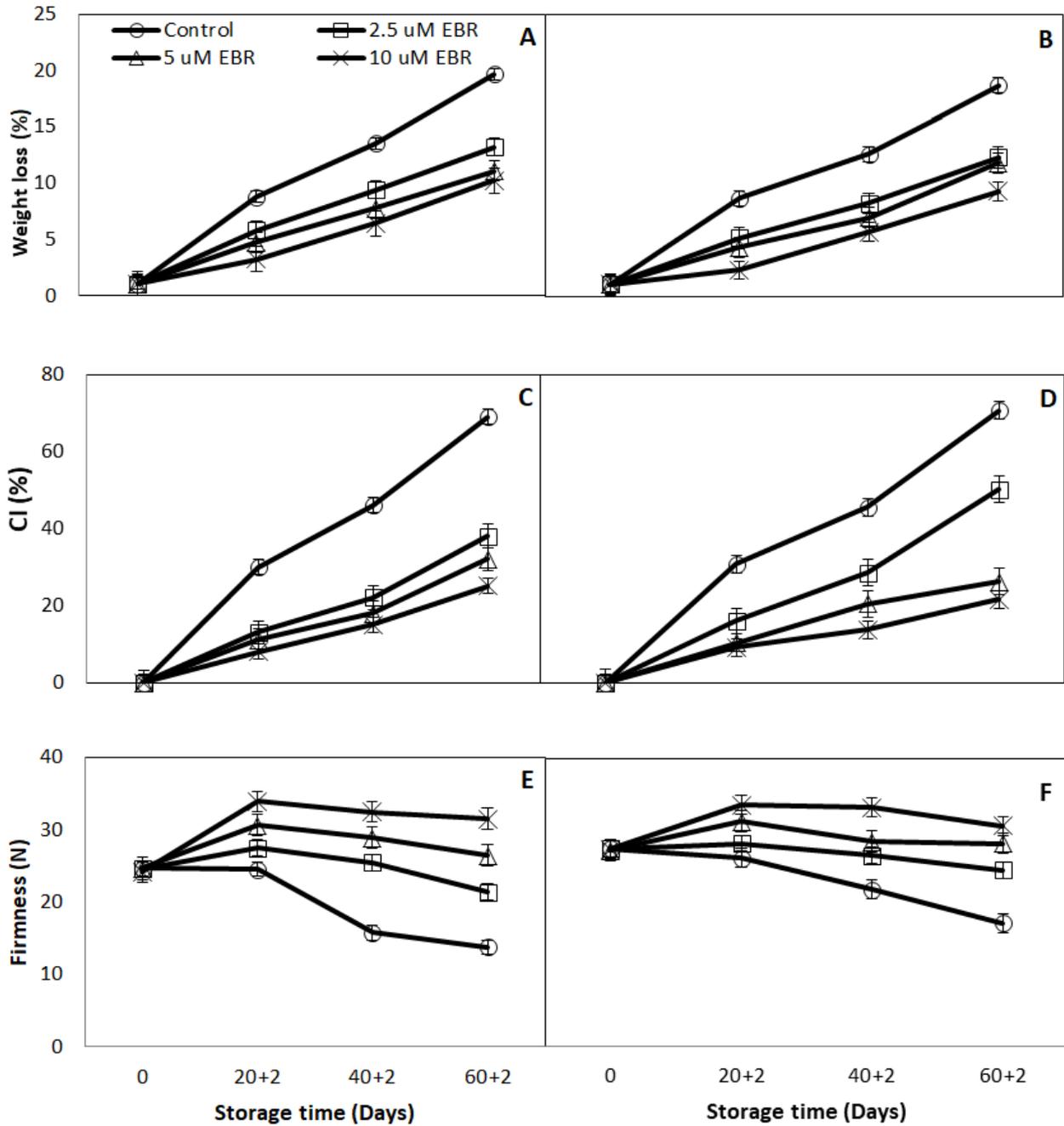


Fig. 1. Changes in weight loss (A. 'Tahiti'; B. 'Persian lime'), chilling injury (CI) index (C. 'Tahiti'; D. 'Persian lime') and fruit firmness (E. 'Tahiti'; F. 'Persian lime') of treated limes with epibrassinolide during storage at 4°C and 85% RH. Vertical bars represent ± standard error of means

First visible CI symptoms in lime fruit were pitting after 2 days of storage at 20°C. As shown in Figure 1C and D, the CI index of control fruit progressed rapidly, especially when the fruit were removed from cold storage and placed at 20°C for 2 days. EBR treatment at 10 µM significantly reduced the increase in the CI index in both cultivars, having a value of about 20% compared with control fruit on day sixty. Physiological decay of fruit was very evident in control fruit after the two days period at room temperature. Overall, the EBR treatment significantly lowered the CI, delaying the appearance of symptoms of CI during the storage of both cultivars of lime fruit at low temperature.

Firmness of ‘Tahiti’ and ‘Persian lime’ fruit was 24.7 N and 27.4 N at harvest time, respectively. In the both cultivars, fruit firmness decreased during storage, however, EBR treatments kept higher

firmness and brought an enhance in fruit firmness until twenty days of cold storage in ‘Tahiti’ and ‘Persian lime’ (fig. 1E and F) and, afterwards declined up to the end of storage. Albeit, the level of softening was greater in ‘Tahiti’ than in ‘Persian lime’. In comparison with the control, fruit firmness, almost to the end of storage was greater in those treated with 5 and 10 µM EBR (fig. 1E and F).

Without EBR treatment, lime fruit remained green for twenty days of storage at 4°C and turned to yellow after that time. The results revealed that the hue value of the lime fruit of both cultivars declined during the cold storage (fig. 2A and B). However, EBR application preserved hue value higher than the control in all storage times. The increment of EBR concentration notably raised hue value in both cultivars. At the end of the storage period, the lowest h° value was found in control fruit of both cultivars.

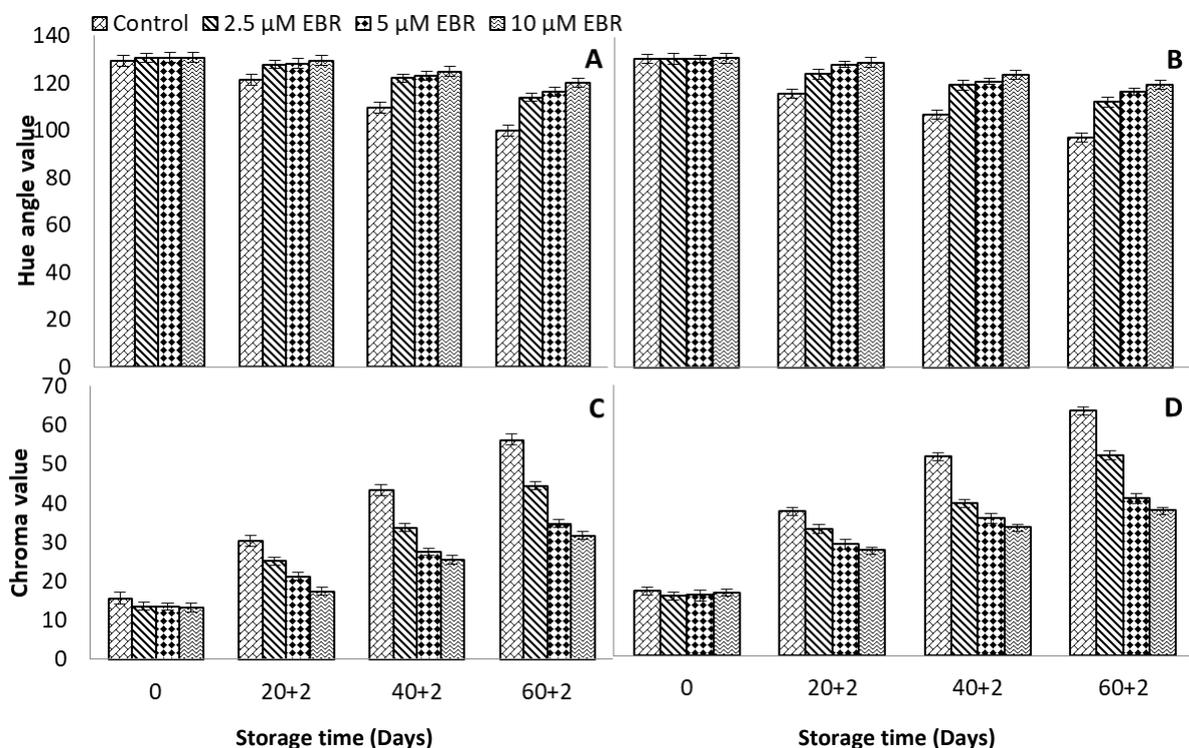


Fig. 2. Changes in hue angle (A. ‘Tahiti’; B. ‘Persian lime’) and chroma value (C. ‘Tahiti’; D. ‘Persian lime’) of treated limes with epibrassinolide during storage at 4°C and 85% RH. Vertical bars represent ± standard error of means

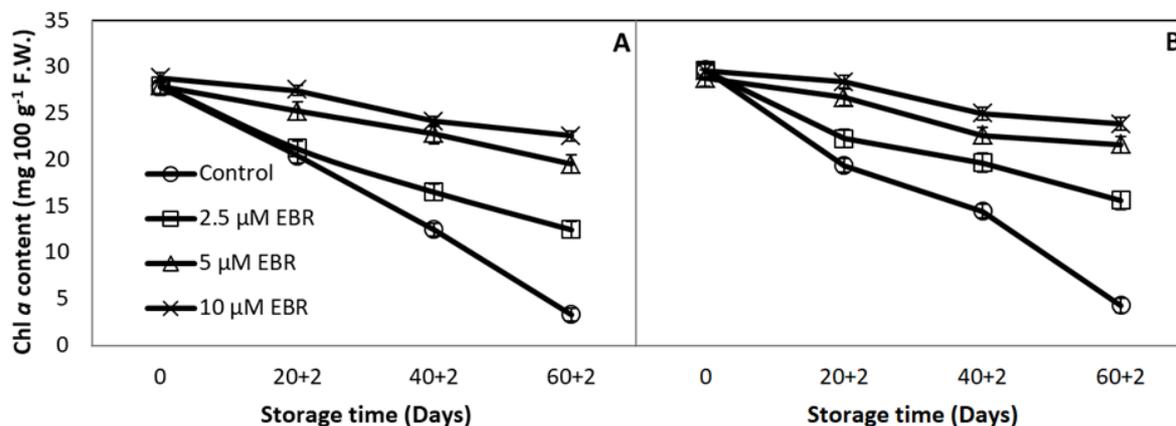


Fig. 3. Changes in chlorophyll *a* content (A. 'Tahiti'; B. 'Persian lime') of treated limes with epibrassinolide during storage at 4°C and 85% RH. Vertical bars represent \pm standard error of means

There were notable difference in chroma value between EBR treated and non-treated lime fruit of both cultivars during cold storage, as a lower chroma value was obtained in EBR treated limes compared to the control up to the end of storage (fig. 2C and D). The increase in EBR concentration significantly decreased the chroma value. Altogether, at the end of cold storage, the lowest value of chroma was achieved in fruit of 'Tahiti' and 'Persian lime' treated with EBR at the rate of 10 μ M (31.78 and 32.16, respectively).

At harvest, Chl *a* content of 'Tahiti' and 'Persian lime' was 27.82 mg 100 g⁻¹ F.W. and 29.73 mg 100 g⁻¹ F.W., respectively (fig. 3A and B). Both cultivars had dark green color at harvest (fig. 2A–D). As displayed in Figure 3A and B, epibrassinolide treatment at 10 μ M in both cultivars of lime fruit lingered the decreasing of chlorophyll *a* content more than any other treatment. During storage, the Chl *a* content in rind of EBR treated fruit retarded the decrease, whilst that in the control severely declined from the beginning until the end of storage. The most pronounced effect occurred at the end of storage with 10 μ M EBR a decrease in Chl *a* content of 'Tahiti' fruit (21.67%) and in 'Persian lime' fruit (19.35%), compared with a decrease (88.03% and 85.43%, respectively) in 'Tahiti' and 'Persian lime' control fruit (fig. 3A and B).

Figure 4 shows the pH, SSC and TA contents of the lime fruit. Throughout storage, pH of juice in-

creased ceaselessly in both cultivars (fig. 4A and B). The highest pH was acquired in control fruit at the end of storage. During storage, epibrassinolide treatment delayed the increase of pH in both cultivars (fig. 4A and B), albeit the level of decrease was larger for 'Tahiti' than 'Persian lime'. The lowest pH was obtained in 'Tahiti' treated with 10 μ M EBR during storage (fig. 4A and B).

SSC and SSC/TA ratios augmented from harvest time to sixty days of cold storage (fig. 4E–H). SSC and SSC/TA ratios in the fruit treated with EBR were lower in comparison with the control fruit. Titratable acidity presented a declining trend until sixty days of storage, although the rate of decrement was more in control than in treated fruit (fig. 4C and D). The decrease of TA in untreated fruit was greater in 'Tahiti' than 'Persian lime'. However, at the end of storage, treated fruit retained higher rates of TA compared with control fruit (56.9% and 36% excess in 'Tahiti' and 'Persian lime', respectively).

Ascorbic acid (vitamin C) content of 'Tahiti' and 'Persian lime' was 40.1 mg 100 mL⁻¹ and 44.5 mg 100 mL⁻¹, respectively, at harvest time. Ascorbic acid content diminished in both cultivars during sixty days of storage exposed to all treatments; however the level of decline was higher in the control than in treated fruit (fig. 5A and B). In regard to various EBR concentrations, 5 and 10 μ M treatments had a significant effect on ascorbic acid content during

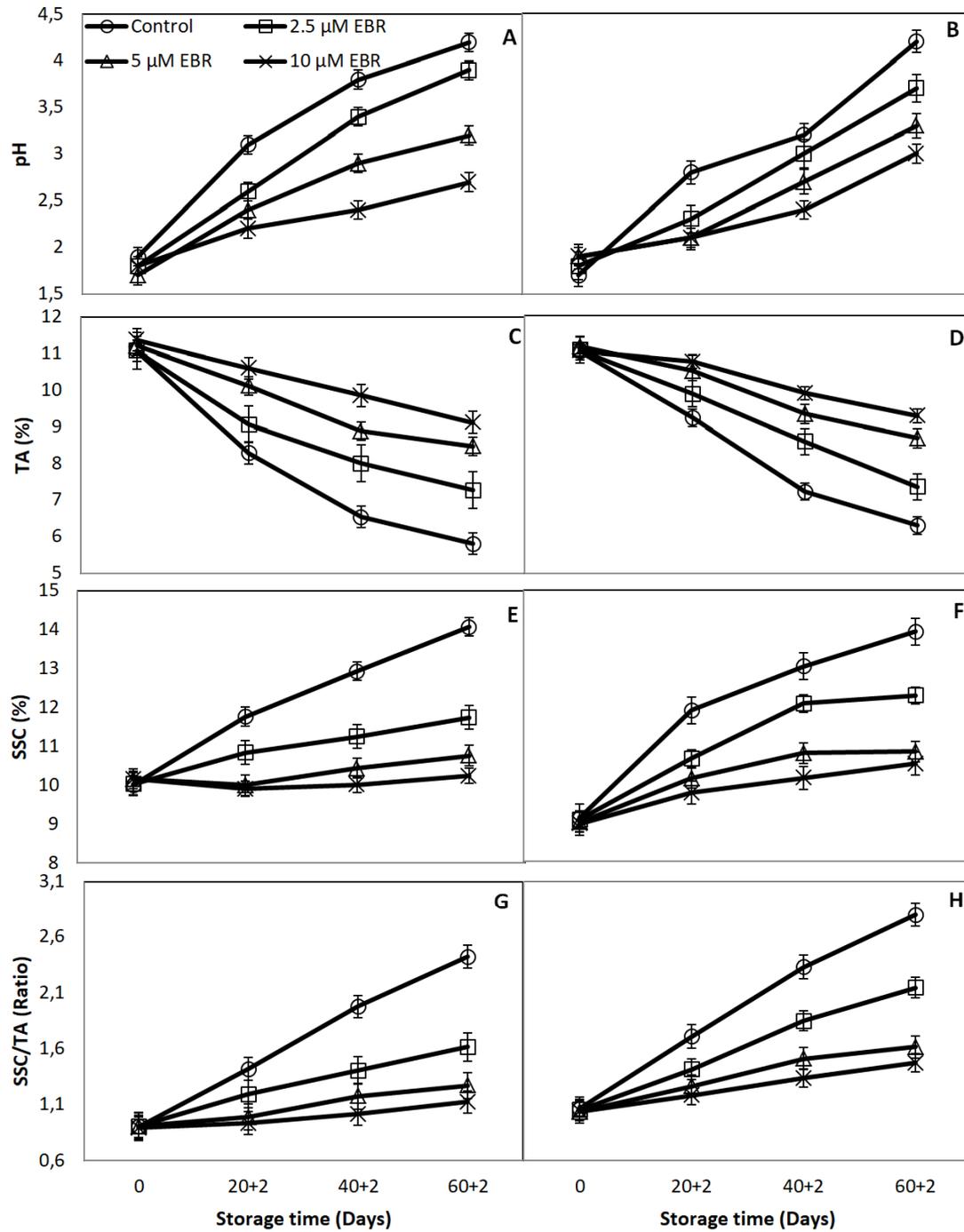


Fig. 4. Changes in pH (A. 'Tahiti'; B. 'Persian lime'), TA (C. 'Tahiti'; D. 'Persian lime'), SSC (E. 'Tahiti'; F. 'Persian lime') and SSC/TA (G. 'Tahiti'; H. 'Persian lime') of treated limes with epibrassinolide during storage at 4°C and 85% RH. Vertical bars represent ± standard error of means

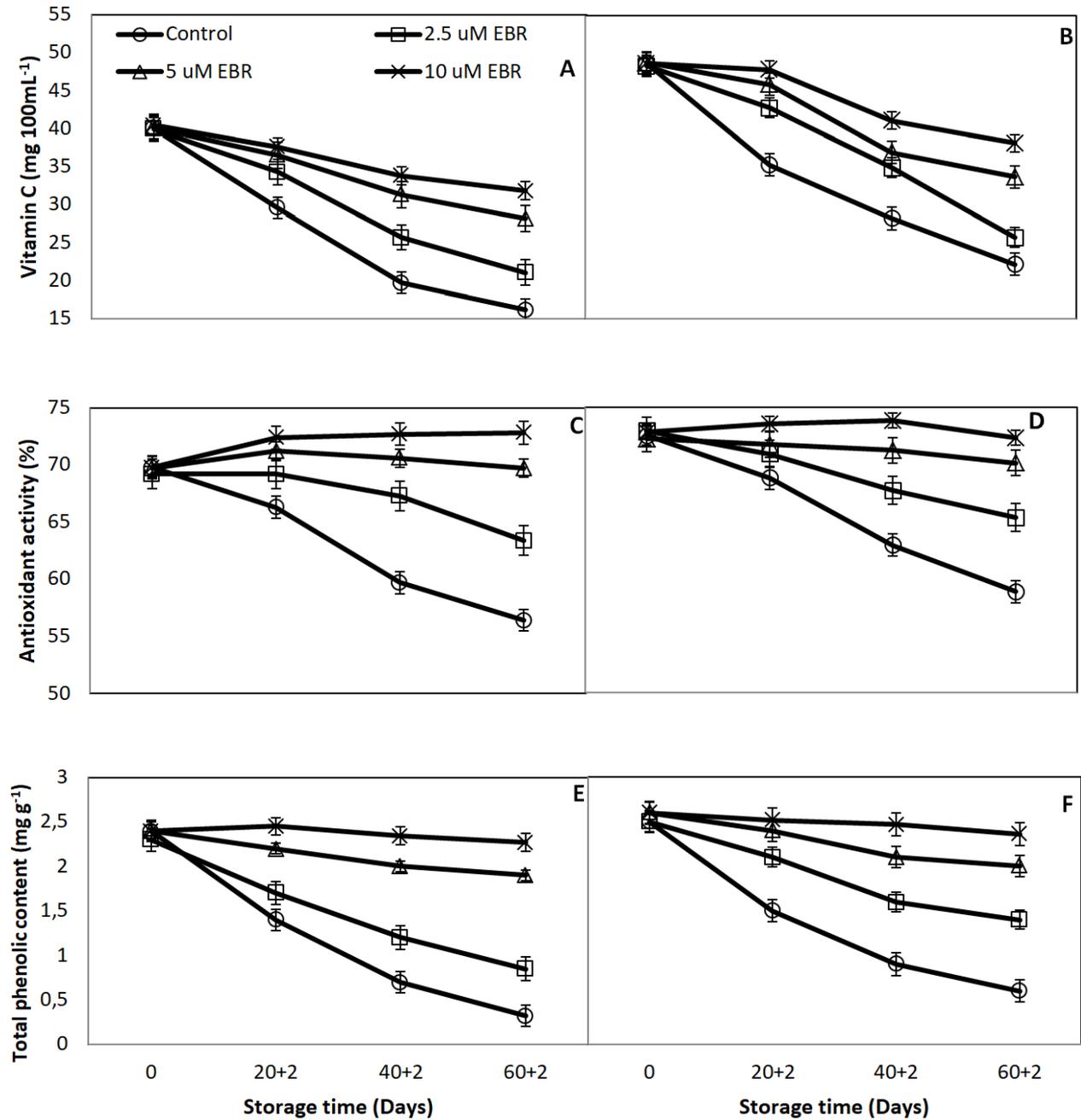


Fig. 5. Changes in ascorbic acid content (A. 'Tahiti'; B. 'Persian lime'), antioxidant activity (C. 'Tahiti'; D. 'Persian lime') and total phenolic content (E. 'Tahiti'; F. 'Persian lime') of treated limes with epibrassinolide during storage at 4°C and 85% RH. Vertical bars represent ± standard error of means

the storage term, albeit 10 μM was more efficient than 5 μM EBR. In untreated and treated fruit, ascorbic acid content reduction in 'Tahiti' was more than in the 'Persian lime'. A higher concentration of EBR (10 μM) displayed less ascorbic acid content reduction in comparison with the controls in both cultivars at the end of storage (fig. 5A and B).

Regardless of treatments in both cultivars, antioxidant activity decreased throughout cold storage, (fig. 5C and D). However, EBR application significantly maintained antioxidant activity at high level compared with control fruit during cold storage in 'Tahiti' and 'Persian lime'. During the storage period, antioxidant activity of 'Persian lime' treated fruit was higher than in 'Tahiti'. Epibrassinolide concentration at 10 μM also brought about the highest increment in antioxidant activity during storage term (fig. 5C and D).

Epibrassinolide application considerably retained total phenolic content (TPC) in treated fruit of both cultivars during the 60 days of storage. Total phenolic content remained constant by using 5 and 10 μM EBR, but in untreated fruit of both cultivars TPC decreased from the beginning until the end of storage (fig. 5E and F). Epibrassinolide at the concentrations of 5 and 10 μM had more considerable effect on increasing TPC in both cultivars. Total phenolic content of treated fruit in 'Tahiti' was lower than in 'Persian lime'. During storage, 10 μM EBR caused the highest increment in total phenolic content in 'Persian lime' (2.36 mg g⁻¹) (fig. 5E and F).

DISCUSSION

During sixty days of storage, epibrassinolide treatment mitigated weight loss in comparison with control fruit in both cultivars (fig. 1A and B). Post-harvest fruit weight loss is induced by water exchange between the external and internal atmosphere, in addition precipitates by cellular collapse [Supavanich et al. 2017]. Our findings are supported by Zhou et al. [2008] where by using brassinolide, Huanghua pears water loss was decreased. Also, Wu and Yang [2016] showed that the asparagus spears treated with brassinolide had a significantly lower

weight loss during storage. Epibrassinolide declined fruit weight loss by retarding the respiration rate and physiological metabolisms during postharvest life of peach and jujube fruit [Zhu et al. 2010, Ge et al. 2016]. Harindra Champa et al. [2015] recognized that in the grape cold storage, EBR-treated clusters resulted in a significantly lower weight loss, which was attributed to the respiration of cluster stem.

EBR has also been shown to result in improved chilling tolerance and to reduce chilling injury in several fruit species when applied exogeneously [Li et al. 2012, Aghdam and Mohammadian 2014, Gao et al. 2016]. In the present study, treatment with 10 μM EBR was shown to be effective in alleviating chilling injury in both cultivars of lime fruit. Untreated (control) fruit stored at 4°C exhibited symptoms of CI after a few days, and the severity of CI increased over time (fig. 1C and D). EBR treated fruit also exhibited symptoms of CI at twenty days at 4°C, but subsequent symptom development during the extent of the experiment was less severe than in the control fruit.

Fruit treated by EBR were firmer than the control ones (fig. 1E and F). Brassinolides enhance fruit firmness by increasing protopectin, Ca²⁺ and pectin of cell walls [Peng et al. 2004]. Harindra Champa et al. [2015] showed that grape clusters treated by BRs maintained higher firmness of berries compared with control. Maintenance of higher firmness with BRs treatment has been reported by Peng et al. [2004] in litchi, Zhu et al. [2010] in jujube, Ahmadipour Roghabadi and Pakkish [2014] in sweet cherry and Zhu et al. [2015] in mandarin. Gao et al. [2016] also reported that peach fruit firmness was about 53% higher in treatment with EBR than that in control fruit at the end of storage. However, Mazorra et al. [2013] were not able to detect consistent differences in papaya firmness among BRs treatments. BRs decreased activity of enzymes that have role in firmness such as polygalacturonase, pectin methylesterase and etc. So, the fruit firmness protected by BR application during storage [Brosa 1999, Zhu et al. 2010].

In the present study, it was shown that EBR-treated lime fruit had better color indices than the control fruit. Surface pitting and peel discoloration are the main symptoms of CI. Rind of lime fruit has

the crucial role in preserving the objective qualities. The loss of green color with chlorophyll depression is the main factor related to quality deterioration for postharvest of horticultural commodities such as limes [Sri-laong et al. 2011, Kaewsuksaeng et al. 2015]. During storage, the green color preservation in the limes peel is needed for fruit to retain their value prices [Kaewsuksaeng et al. 2011]. In agreement with our result, BR treated green bell pepper fruit maintained their green color during cold storage by delaying the decrease of chlorophyll content due to a low respiration rate [Wang et al. 2012]. Wu and Yang [2016] demonstrated that exogenous BR was effective for the retention of green color in green asparagus. They also showed that BR treatment inhibited the degradation of chlorophyll, and a higher level of chlorophyll in BR-coated samples than control was observed after 24 days of storage at 4°C. The loss of chlorophyll was 38.8% in BR-coated asparagus spears, while loss of as much as 54.5% occurred in control over 24 days of storage at 4°C. Findings of Harindra Champa et al. [2016] showed that BRs had a better potential in achieving beneficial effects against browning of detached grape clusters. Green color retention might account by the direct effect of BRs on suppression of ethylene production [Zhu et al. 2010], which is the main hormone triggering chlorophyll loss and color fading [Taiz and Zeiger 2010]. In the current research hue values of fruit peel increased with increasing the concentration of EBR. At the end of storage, the best color quality was obtained at 10 µM in ‘Persian lime’ compared to the ‘Tahiti’ and control fruit. Appropriate concentrations of EBR preserve color quality certainly due to the antioxidant activity enhancement that impedes pigments oxidation during cold storage [Zhang et al. 2014]. Through EBR vacuum infiltration, storing limes at crucially low temperatures is possible with quality preservation and without chilling injury symptoms.

During storage, pH of lime juice augmented in both cultivars continuously, however more in control than in treated fruit (fig. 4A and B). Within cold storage, SSC and SSC/TA ratios raised (fig. 4E–H). In comparison with the control, treated fruit had greater rates of TA (fig. 4C and D). Organic acids diminish in stored citrus fruit may be rather along of

the use as substrate for energy production and alcoholic fermentation. Actually, the synthesis of phenolic compounds appertain to the carbon skeletons which provided by organic acids [Rapisarda et al. 2008]. Synthesis of sugars from organic acids is the possible mechanism by which sugar rates mount in harvested citrus fruit, because of the presence and proliferate of glycolytic enzymes throughout storage of fruit [Rapisarda et al. 2008]. In addition, cell wall components such as polysaccharides of pectins and cellulose, which are digested by the cell wall degrading enzymes, cause an increased level of SSC. By EBR application on lime fruit, these variations were delayed during cold storage (fig. 4E and F). Similarly, Harindra Champa et al. [2015] demonstrated that BRs, at the dose of 0.5 mg l⁻¹ effectively reduced SSC of table grapes up to 45 days of storage. Soluble sugars are probably to increase during fruit storage, as a result of the action of the key enzyme, sucrose-phosphate synthase (SPS), in sucrose biosynthesis [Hubbard et al. 1991]. Ahmadipoor Roghabadi and Pakkish [2014] found that salicylic acid decreased SPS enzyme activity, bringing about a reduction of SSC. As has been reported, higher TA in treated fruit brought a lower SSC/TA ratio in comparison with the controls (fig. 4C and D, G and H). Severe increase in respiration of untreated fruit due to chilling injury might cause organic acids deterioration and TA reduction. Zhu et al. [2015] revealed that brassinolide treatment could delay the decrease of titratable acidity. It has been also shown that BR treated jujube and peach fruit had a significantly higher level of TA during storage compared to control fruit [Zhu et al. 2010, Ge et al. 2016]. Reduced TA and organic acids in untreated sweet cherry fruit, because of the increase in respiration would be due to the ethylene production and fruit senescence [Ahmadipoor Roghabadi and Pakkish 2014]. EBR application reduces fruit respiration and delay organic acids utilization [Zhu et al. 2010].

During sixty days of storage, ascorbic acid content reduced in both ‘Tahiti’ and ‘Persian lime’ exposed to various treatments. Epibrassinolide at the rate of 10 µM had a considerable effect on retaining ascorbic acid during the cold storage term (fig. 5A and B). EBR can alleviate enzymes activity contrib-

uted to ascorbic acid degradation and mitigate chilling injury [Wang et al. 2012]. Besides, it has been found that the ascorbic acid content decreases during cold storage and this decrement is correlated with lessened antioxidant activity and fruit quality [Rapisarda et al. 2008]. In blood orange, ascorbic acid diminution also arisen either at ambient temperatures or during cold storage [Habibi and Ramezani 2017]. EBR-treated fruit had duller loss of vitamin C compared to the control fruit (fig. 5A and B). Post-harvest EBR application which plays a significant role in the system of antioxidative and establish defense to membranes versus the oxidative injury afforded by ROS, may be caused the enhancement in total antioxidant activity [Wang et al. 2012, Zhu et al. 2015]. In line with our results, Guo et al. [2014] reported that ascorbic acid content increased in broccoli sprouts after treatment with 20 nM 24-epibrassinolide. Wang et al. [2012] reported that BRs treatment mitigated CI in green bell pepper, which is accompanied by maintaining ascorbic acid content leading to enhanced pepper nutritional quality. In another study EBR treatment maintained ascorbic acid content in lotus root slices [Gao et al. 2017]. Brassinolide treated mandarin fruit had higher ascorbic acid contents compared with control fruit [Zhu et al. 2015].

There is increasing evidence that fruit and vegetable consumption imparts beneficial health effects based on the content of several compounds with antioxidant activity, including ascorbic acid and phenolic compounds [Valero and Serrano 2011]. In the current study, during cold storage, antioxidant activity and total phenolic content of EBR treated lime fruit of both cultivars enhanced more than the control fruit (fig. 5C–F). Antioxidant activity rose from internal bioactive compounds. In limes, the total antioxidant activity is attributed to the phenolic compounds and ascorbic acid [Rivera-Cabrera et al. 2010]. Formerly, a good correlation between the antioxidant capacity (DPPH scavenging activity) and total phenol content in banana, honey pineapple, and Thai seedless guava was shown [Kahkonen et al. 2001]. The correlation between the phenolic compounds or ascorbic acid and the antioxidant activity pertains to chemical structure and synergistic interplay of distinct con-

stituents [Huang et al. 2005]. Studies in other fruit species have demonstrated that the accumulation of phenolic compounds is highly correlated with chilling resistance [Liu et al. 2014]. Phenolics display a wide range of antioxidant properties that reduce or eliminate excess free radicals generated by cold stress [Pan and Liu 2011]. These antioxidants also restrict the accumulation of reactive oxygen species (ROS) and protect cells from excess ROS produced during senescence [Xia et al. 2009]. Zhang et al. [2010] reported that BR enhanced the enzymatic and non-enzymatic antioxidants in maize leaves. BRs treatment enhanced the antioxidants, and thus mitigated chilling stress in green bell pepper by maintaining membrane integrity. Meng et al. [2009] reported that phenolics have a dual function; first, phenolics can be oxidized by PPO, which leads to flesh browning, the main CI symptom in fruits and vegetables, second, phenolics, which accumulate in fruits and vegetables in response to chilling stress, have antioxidant capacity. Another proposed role for phenolics is its conversion to quinone in the presence of peroxidase (POD) and PPO, which is toxic to the fungus [Meena et al. 2001]. We suggest that the BR induction of antioxidant activity in lime may be a key factor in lowering oxidative damage caused by cold stress, thus improving the cold tolerance and alleviating CI of lime stored at cold storage. Phenolic content of BR treated green asparagus was significantly higher than control. Choudhary et al. [2012] found that 3- and 3.5-fold increase in total phenolics were observed in BR-treated *Raphanus sativus* seedlings subjected to Cu stress compared with Cu stress alone. The increase in total phenolics compounds, and antioxidant activity reported in the present study are also consistent with reports in peach [Liu et al. 2005, Ge et al. 2016], pear [Cao and Jiang 2006], muskmelon [Liu et al. 2014] and mango fruit [Zhu et al. 2008].

CONCLUSION

In conclusion, postharvest epibrassinolide treatments to limes by vacuum infiltration can enhance the storability and quality of fruit stored at chilling injury causing temperatures. EBR application increases rind Chl *a* and preserved objective qualities

and price values relative to control fruit. Overall, 10 μ M EBR brought about the greatest effects. Post-harvest EBR application could be an effective and simple method for maintaining bioactive compounds including ascorbic acid, total phenolics also antioxidant activity in treated lime fruit under cold storage term without chilling injury symptom.

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