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Original Research

Pre-storage Application of L-arginine Alleviates Chilling Injury and Maintains Postharvest Quality of Cucumber (*Cucumis sativus*)

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ABSTRACT

Chilling injury in cucumber is the major issue under low temperature storage, which substantially affects cosmetic quality and market value of fruits. Present study was aimed to assess the effect of L-arginine to alleviate surface pitting caused due to chilling injury and response of various quality attributes during cold storage (5 ± 1 °C; RH 90 ± 5%). Cucumber cv. '7003' fruits were treated with different concentrations of L-arginine (0.0, 0.5, 1.0 and 1.5 mM) and stored for 16 days. Fruits were removed at 4 days storage interval followed by 1 day of conditioning (22 ± 2 °C) before quality analysis. Visual quality, fruit colour, decay, fruit weight loss, firmness, chilling injury (CI), electrolyte leakage, taste, texture, flavour, aroma, soluble solids content (SSC), titratable acidity (TA), sugar: acid ratio (SSC/TA ratio) and ascorbic acid content were measured for cucumber fruits. Overall, it was noted that L-arginine treated fruits showed lower fruit weight loss and electrolyte leakage, and maintained taste, texture, aroma, SSC, and sugar: acid ratio during storage. Fruits treated with 0.5 mM L-arginine had significantly reduced chilling injury and decay, maintained fruit colour, firmness and flavour, and displayed higher ascorbic acid content compared with control. Conclusively, pre-storage application of L-arginine (0.5 mM) can be employed as promising technique to alleviate postharvest chilling injury and maintain fruit quality of cucumber under cold storage.

Keywords: *Ascorbic acid content, cosmetic quality, fruit quality, surface pitting.*

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INTRODUCTION

Cold storage techniques have been extensively used for shelf life improvement and quality maintenance of fruits and vegetables (Aghdam and Bodbodak, 2014). Low temperature storage not only lowers respiration rate of stored commodities but also reduces chances of fungal diseases. However, a critical issue is the development of chilling injury in tropical and subtropical commodities under low temperature storage for extended period, which may lead to morphological and physiological changes (Tsuchida et al., 2010) and quality deterioration. Cucumber is an important vegetable crop, which is in demand year-round but has limited shelf life at ambient conditions. It is also prone to chilling injury (CI) when stored below 7 °C (Cen et al., 2016). Some of the CI's symptoms are discoloration, pitting, internal breakdown and decay, which can result in huge postharvest loss. The symptoms may vary, depending on temperature, storage duration and fruit's maturity stage (Chen and Yang, 2013). A range of chemicals such as 6-benzyl aminopurine (Chen and Yang, 2013); methyl jasmonate (Aguilar et al., 2004); methyl salicylate (Ding et al., 2001; Fung et al., 2004); nitric oxide (NO) (Singh et al., 2009); abscisic acid (ABA) (Wang, 1991); glycine betaine (Pan et al., 2019); melatonin (Zhao et al., 2017); chitosan (Zhang et al., 2015); γ-aminobutyric acid (Shang et al., 2011) and oxalic acid (Li et al., 2014) have been used to manage chilling injuries in different vegetables and fruits.

L-arginine is a metabolically active amino acid and precursor of many polyamines (spermine, spermidine and putrescine), proline as well as nitric oxide (NO) (Jubault et al., 2008). The production of polyamines is an adaptive strategy by higher plants against chilling stress (Nasibi et al., 2011; Zhang et al., 2011). Previous reports have revealed that L-arginine is one of the most effective agents against postharvest quality maintenance and reduction of chilling injury in fruits and vegetables such as pomegranate (Babalar et al., 2018), asparagus (Wang et al., 2017) and tomato (Zhang et al., 2013). However, according to our knowledge, no work has already been carried out regarding optimization of L-arginine in cucumber fruit for alleviation of chilling injury and quality maintenance under delayed low temperature storage. Moreover, L-arginine has been acknowledged as safe food additive for human use as employed by Wills and Li (2016) for fresh cut apple and lettuce. Thus, its use in the world's fruit and vegetable industry would be considered safe without any potential health hazards and seems to be acceptable treatment by regulatory authorities due to its status of generally regarded as safe (GRAS).

Keeping in view, the possible involvement of polyamines and

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proline in plant tolerance to chilling injury, it was hypothesized that L-arginine is involved in the stress resistance mechanism. This work was done aiming at exploring the potential effect of L-arginine to mitigate chilling injury and quality maintenance of cucumber.

MATERIALS AND METHODS

Fruit material

Cucumber cv. '7003' fruits were harvested at commercial maturity (colour = 95-100% green) from commercial vegetable tunnels installed in Vegetable Research Area (31.4339, 73.0769), University of Agriculture, Faisalabad (Pakistan). After harvest, fruits were sorted and those free from blemishes, diseases and insect attacks were selected for the experiment. The selected fruits were then packed in plastic fruit crates with paper lining and transferred to the Postharvest Research and Training Centre (PRTC), Institute of Horticultural Sciences, University of Agriculture Faisalabad.

Treatments

At PRTC, fruits were washed with tap water to remove dirt from their surfaces. All the fruits were segregated randomly into four groups with three replications according to research plan and immersed in the aqueous solutions of different concentrations of L-arginine (0.5, 1.0 and 1.5 mM) with 0.01% Tween-20 as surfactant for 5 min., while untreated fruits (control) were dipped in distilled water. After application of L-arginine treatments, the fruits were allowed to dry at room temperature for 10 min. Samples were placed in open top crates and stored in the cold storage at 5 °C with relative humidity of 90% up to 16 days. For quality assessment, fruits were removed at 4 days of storage interval followed by 1 day at shelf life for conditioning.

External quality parameters (visual quality, fruit colour and decay)

During the storage period, all treatments were assessed at each 4+1 days interval for physical parameters including visual quality, fruit colour and decay percentage. Visual quality and fruit colour were evaluated using the similar rating score, where 9 represents excellent appearance, 7 = very good, 5= good, 3= fair and 1 score depicted as poor (Koukounaras et al., 2008; Nasef, 2018). On each sampling interval, fruits were assessed for the signs of rotting using 5-1 score whereby 5 expressed as severe decay, 4 = moderately severe, 3 = moderate, 2 = slight and 1 represented for no signs (Kader et al., 1973).

Fruit weight loss

Cucumber fruits were weighed using a digital weighing balance (Setra, BL-4100S) at the beginning of the experiment (0 day) for all the treatments as initial weight and then measured at each removal (final weight) till the end of study. The data were calculated by following formula and expressed as the percentage weight loss of cucumber fruit (Ali et al., 2016).

$$\text{Fruit weight loss (\%)} = \frac{\text{Initial weight before storage} - \text{Final weight after storage}}{\text{Initial weight before storage}} \times 100$$

Fruit firmness and chilling injury

The fruits firmness was measured by fruit hardness tester (Lutron Electronic, FR-5120) with penetrometer tip while three fruits per replication were tested. Chilling injury was apparently evaluated by the extent of signs as surface pitting according to ascribed 1-5 rating scale such as 1 = no signs, 2 = slight (up to 20%), 3 = moderate (21-40%), 4 = moderately severe (61-80%) and 5 = severe (81-100%) of surface area (Imahori et al., 2008).

SSC, TA, Sugar: acid ratio and ascorbic acid content

Mesocarp tissues of cucumber were blended using electrical kitchen blender machine (MX-GX 1021-Panasonic) and juice from each sample was placed on the prism of digital refractometer (PAL-1, Atago, Japan) to determine soluble solids content which expressed its value in °Brix. Titratable acidity (%) was determined by titration method using 0.1N NaOH with addition of 3 drops of phenolphthalein indicator till pink colour appeared. Sugar: acid ratio was simply analysed by dividing SSC (°Brix) over TA (%) (Khan et al., 2018). However, ascorbic acid content was determined by titration of the cucumber juice against 2, 6-dichlorophenolindophenol as method described by Nasir et al. (2016) for Kinnow mandarin fruit and results were expressed as mg/100g fresh weight.

Electrolyte leakage

Electrolyte leakage was analysed by randomly selecting fruits from each treatment at each removal from storage. The mesocarp tissue samples (3 g) having five discs of same size, were removed from each equatorial portion of cucumber fruit and dipped in 50 mL of double distilled water at room temperature (25 ± 2 °C). The electrolyte leakage was measured with a digital EL meter (HI-98304, Hanna Instruments Inc., Mauritius), initially from pulp tissues while its final reading was observed after one-hour from 10 min boiled samples. Total electrolyte leakage was measured by following the given formula and expressed in % (Huan et al., 2017; Nasef, 2018; Ali et al., 2019).

$$\text{Electrolyte Leakage (\%)} = \frac{\text{(Final reading} - \text{Initial reading)}}{\text{Final reading}} \times 100$$

Sensory Evaluation

A panel of five members comprising of postgraduate students of PRTC was constituted. The panel was presented with coded fruits from each treatment at the end of every storage period for the assessment of taste, texture, flavour and aroma. Panellists rated for all sensory characters of fruits from each replicate using 1-9 hedonic scale (Peryam and Piligram, 1957; Ali et al., 2016).

Statistical analysis

Collected data from current postharvest study was subjected to analysis of variance (ANOVA) with computer operated Statistix-8.1® software (Analytical Software, Tallahassee, USA). Data were analysed using two factors factorial arrangement including treatment application and storage periods. To assess the level of significance at $P \leq 0.05$, least significant differences (LSD) test was employed.

RESULTS

External quality parameters (visual quality, fruit colour and decay)

Visual quality in terms of cosmetic appearance, fruit colour and decay were significantly affected by L-arginine treatments, storage periods and their interaction. After 16+1 days of storage, cucumber fruits treated with 1.5 mM of L-arginine retained significantly ($P \leq 0.05$) better visual quality, fruit colour and scored higher (5 in both cases) in contrast with control score (1 and 1.6, respectively), which represented poor quality and marketability. While other L-arginine treatments (0.5 and 1 mM) showed almost similar trend for visual quality and fruit colour from day 0 till the end of experiment (Fig. 1A & B). Decay was observed by 1-5 rating scale. Slight decay with non-significant ($P \leq 0.05$) difference was observed till 8+1 days of storage. As storage period approached to 12+1 days, decay severity

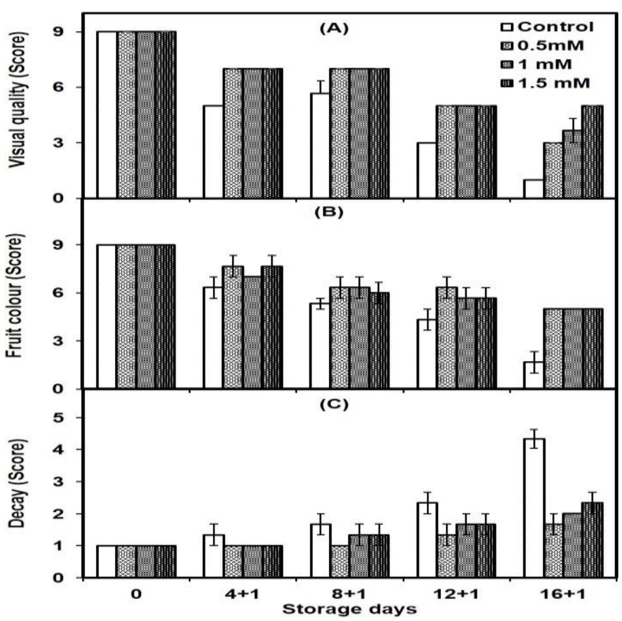

Figure 1: Effect of pre-storage application of L-arginine on visual quality (A), fruit colour (B) and decay (C) of cucumber fruits under cold storage at each time interval. Vertical bars represent standard error (±) of means while invisible bars showed smaller difference in the error means. n = 3. LSD ($P \leq 0.05$) values for visual quality of fruit: treatments (T) = 0.2695, storage intervals (SI) = 0.3013 and T × SI = 0.6026; fruit colour: treatments (T) = 0.6101, storage intervals (SI) = 0.6821 and T × SI = 1.3641; decay: treatments (T) = 0.3565, storage intervals (SI) = 0.3986 and T × SI = 0.7971.

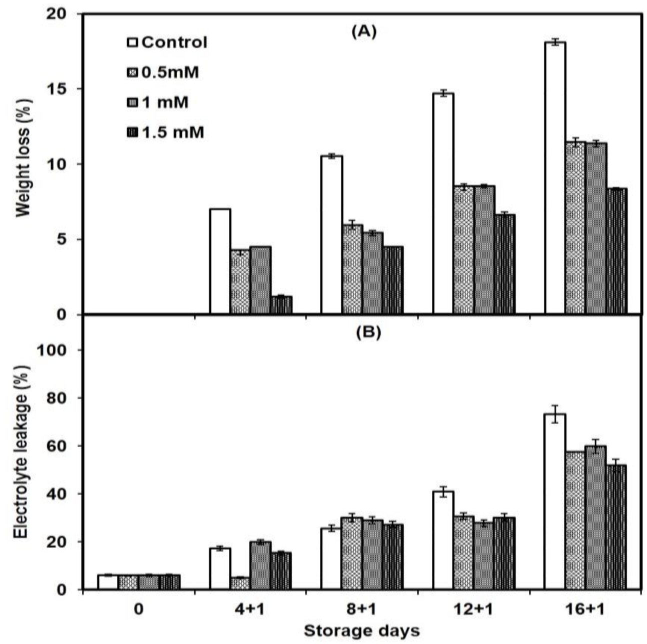

Figure 2: Effect of pre-storage application of L-arginine on weight loss (A) and electrolyte leakage (B) of cucumber fruits under cold storage at each time interval. Vertical bars represent standard error (±) of means while invisible bars showed smaller difference in the error means. n = 3. LSD ($P \leq 0.05$) values for fruit weight loss: treatments (T) = 0.1946, storage intervals (SI) = 0.2175 and T × SI = 0.4350; electrolyte leakage: treatments (T) = 0.8337, storage intervals (SI) = 0.9321 and T × SI = 1.8641.

increased from moderate to moderately severe in control. However, control fruits showed moderately severe to severe decay (4.33) in comparison with fruits treated with L-arginine concentrations 0.5 mM (1.6), followed by 1 mM (2) and 1.5 mM (2.33) at 16+1 days of storage (Fig. 1C).

Weight loss (%)

Weight loss of the fruits constantly increased in all the treatments including control during the whole storage period (Fig. 2A). However, the pre-storage L-arginine application significantly ($P \leq 0.05$) reduced weight loss as compared to control. After 16+1 days of storage, the fruits treated with 1.5mM were significantly different and reduced weight loss (8.35%) was observed in contrast with control (18.10%). Moreover, 0.5 mM and 1 mM treatments showed almost similar (non-significant) loss of fruit weight (12.15% and 11.94%, respectively), but markedly lesser as compare to control.

Electrolyte leakage (%)

The effect of L-arginine treatments, storage periods and treatments × storage periods were found significant ($P \leq 0.05$) for ion leakage in cucumber fruits under study. As storage period increased, ion leakage from cucumber tissues also gradually increased in all the treatments. However, ion leakage was significantly lower (51.9%, 57.48%, 59.85%) in fruits treated with 1.5 mM, 0.5 mM and 1 mM L-arginine, respectively as compared to control (73.23%) (Fig. 2B).

Chilling injury and firmness

Chilling injury is considered a critical issue in cucumber at low temperature storage. There were non-significant ($P \leq 0.05$) differences in chilling injury till 12+1 days of storage and all the L-arginine treatments scored 2.33 to 2.66 representing as moderate injury (21-40%). However, at the end of storage, treated fruits depicted moderate injury, which was lower in 0.5 mM treatment (2.33) as compared to moderately severe (4) in control (highest score of pitting on fruit surface) (Fig. 3A). Cucumber fruits treated with L-arginine were firmer as compared to non-treated ones. However, fruits treated with 0.5 mM L-arginine had higher firmness throughout the storage till 16+1 days of storage (66.08 N), while control treatment showed gradual decline in firmness and at 16+1 days of storage, it reduced 1.5 time (42.62 N) as compared to other corresponding treatments (Fig. 3B).

SSC, TA, Sugar: acid ratio and ascorbic acid content

The effect of L-arginine treatments and their interactions with storage periods showed statistically non-significant ($P \leq 0.05$) results for SSC and TA (%). With the increase in storage period, soluble solids content (SSC) depicted continuous increasing trend till 12+1 days and abruptly decreased at 16+1 days of storage. However, the maximum SSC (4.93 °Brix) was found in L-arginine (0.5 mM) treated fruits at 8+1 days of storage as compared to the minimum (3.2 °Brix) in control treatment at 16+1 days of storage (Fig. 4A). Similarly, results for total acidity (%) and sugar: acid ratio with reference to L-arginine treatments and treatments × storage periods were non-significant ($P \leq 0.05$), while TA (%) gradually decreased with the increase in storage duration. In addition to this, sugar: acid ratio showed unprecedented but significantly ($P \leq 0.05$) different trend throughout the storage period which increased in some ways and decreased at the mid and at the end of the study (Fig. 4B and C).

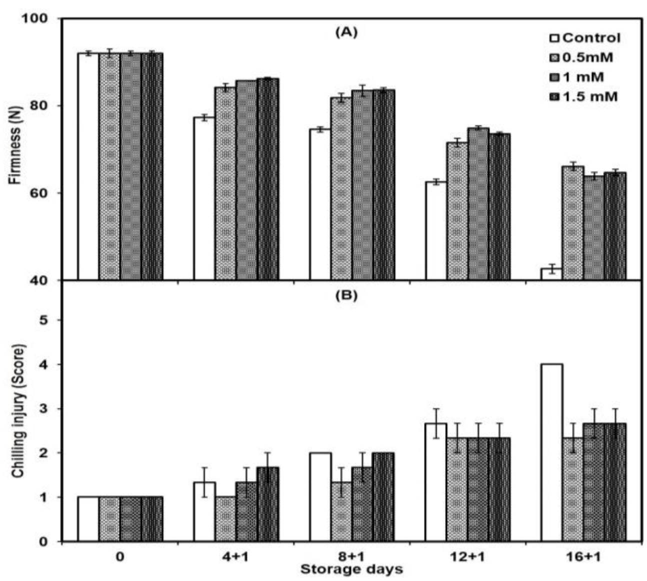

Figure 3: Effect of pre-storage application of L-arginine on firmness (A) and chilling injury (B) of cucumber fruits under cold storage at each time interval. Vertical bars represent standard error (±) of means while invisible bars showed smaller difference in the error means. n = 3. LSD ($P \leq 0.05$) values for fruit firmness: treatments (T) = 0.8428, storage intervals (SI) = 0.9423 and T × SI = 1.8845; chilling injury: treatments (T) = 0.3300, storage intervals (SI) = 0.3690 and T × SI = 0.7380.

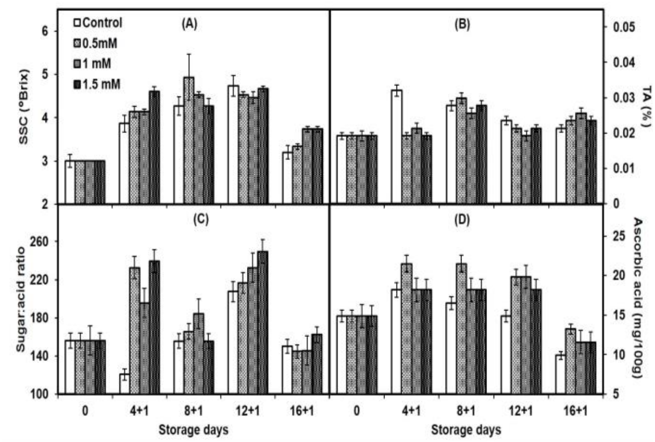

Figure 4: Effect of pre-storage application of L-arginine on SSC (A), TA (B), sugar: acid ratio and ascorbic acid content (D) of cucumber fruits stored under cold storage conditions at each time interval. Vertical bars represent standard error (±) of means while invisible bars showed smaller difference in the error means. n = 3. LSD ($P \leq 0.05$) values for fruit SSC: treatments (T) = 0.1275, storage intervals (SI) = 0.1425 and T × SI = 0.2850; TA: treatments (T) = 0.4469, storage intervals (SI) = 0.7380 and T × SI = 1.3641; Sugar: acid ratio: treatments (T) = 0.3665, storage intervals (SI) = 0.4098 and T × SI = 0.8195; ascorbic acid content: treatments (T) = 2.1650, storage intervals (SI) = 2.4205and T × SI = 4.8410.

Ascorbic acid content

Ascorbic acid content showed statistically significant ($P \leq 0.05$) results for L-arginine treatments and storage periods while their interaction was found statistically non-significant (Fig. 4D). However, the maximum ascorbic acid content (21.48 mg/100 g) was observed at 4+1 and 8+1 days of storage in L-arginine (0.5 mM) treated fruits, while the minimum (9.91 mg/100 g) was recorded in control fruits after 16 days of storage and 1 day of shelf life. Initially, ascorbic acid content increased during storage at 5 °C in treated and non-treated fruits, but sequentially decreased with storage prolongation. These findings depicted that pre-storage application of L-arginine at the concentration of 0.5 mM displayed higher accumulation and maintained ascorbic acid content as compared with control till 16+1 days of storage (Fig. 4D).

Sensory evaluation

As storage progressed, L-arginine treated and non-treated cucumber fruits exhibited gradual but significant ($P \leq 0.05$) decrease in taste, texture, flavour and aroma. However, decrease in sensory characters was markedly lowered in L-arginine treated fruits in contrast with control or untreated fruits (Fig. 5A-D). On 16+1 days of storage, mean scores of taste, texture, flavour and aroma in L-arginine treated cucumber fruits were

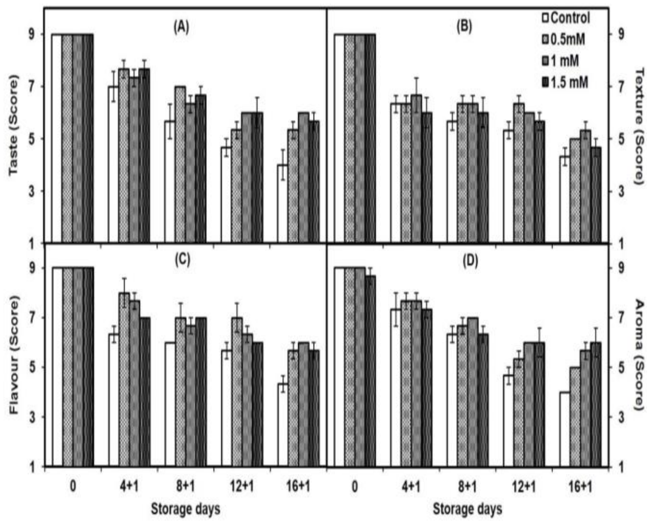

Figure 5: Effect of pre-storage application of L-arginine on sensory characters including taste (A), texture (B), flavour (C) and aroma (D) of cucumber fruits stored under cold storage conditions at each time interval. Vertical bars represent standard error (±) of means while invisible bars showed smaller difference in the error means. n = 3. LSD ($P \leq 0.05$) values for fruit taste: treatments (T) = 0.4469, storage intervals (SI) = 0.4996 and T × SI = 0.9992; texture: treatments (T) = 0.4366, storage intervals (SI) = 0.4881 and T × SI = 0.9763; flavour: treatments (T) = 0.3928, storage intervals (SI) = 0.4392 and T × SI = 0.8784; aroma: treatments (T) = 0.4261, storage intervals (SI) = 0.4764 and T × SI = 0.9527.

around 5.66, 5.33, 6.00 and 6.00, respectively (score falls in neither like nor dislike to like slightly), higher than the control (4.00, 4.33, 4.00 and 4.00, respectively) depicted as dislike slightly, following hedonic scale of organoleptic/sensory evaluation.

DISCUSSION

The shelf life of freshly harvested cucumber (*Cucumis sativus* L.) fruits can be extended by storing the fruits at low temperature. However, storage at below optimum temperature (7–10 °C) leads to appearance of watery patches and surface pitting that are remarkable symptoms of chilling injury. Recently several methods have been identified to retard the chilling injury development in cucumber fruits such as application of plant growth regulators, modified atmospheric packaging (MAP), heat treatment, and nitric oxide (NO) fumigation (Wang and Qi, 1997; Mao et al., 2007; Yang et al., 2011).

Arginine being a versatile amino acid, acts as a source of stress related signalling molecule notably NO. Exogenous application of arginine initiates the biosynthesis of NO by improving activity of nitric oxide synthase (NOS) (EC: 1.14.13.39) (Morris, 2007). Endogenously produced NO ameliorates chilling injury incidence by suppressing accumulation of reactive oxygen species (ROS) in cellular membranes and hinders structural damages to the membranes. Our results showed that during 16+1 day's storage of cucumber fruits, chilling injury incidence was inhibited up to 50% in fruits dipped in 0.5 mM arginine solution. These findings were consistent with Babalar et al. (2018) who reported that pre-harvest application of arginine significantly suppressed chilling injury in pomegranate fruits by reducing electrolyte leakage and maintaining higher levels of ascorbic acid. Similar observations have been reported by Song et al. (2016) who found that chitosan treatment hindered the chilling injury incidence by reducing malondialdehyde (MDA) content and suppressing the accumulation of ROS in loquat fruits.

Regarding electrolyte leakage, our results were consistent with Zhang et al. (2010) that arginine application significantly lowered the electrolyte leakage by attenuating oxidative reactions in cellular membranes (Fig. 1). NOS and other related enzymes oxidize arginine into L-citrulline that is further catalysed into endogenous NO (Wills and Li, 2016). NO is a gaseous signalling molecule that plays significant role in delaying senescence and improving cellular permeability by maintaining fruit quality for longer durations (Wills, 2015; Song et al., 2016). Hence, fruit firmness was significantly maintained in arginine treated fruits as compared to untreated control fruits.

In addition to this, our results of ascorbic acid content were consistent with findings of Babalar et al. (2018) who suggested that maintenance of higher levels of ascorbic acid is correlated with reduced activities of ascorbic acid oxidase (AAO) enzyme (Rao et al., 2011). Besides this, exogenous application of NO improves antioxidant enzymes (such as catalase, peroxidase, and superoxide dismutase) that play significant roles in amelioration of oxidative damage by scavenging free radicals (Yang et al., 2011). Since arginine is NO-donor compound that improves biosynthesis of endogenous NO, hence membrane disruption is significantly hindered (Morris, 2007; Yang et al., 2011; Liu et al., 2016; Babalar et al., 2018).

Maintenance of consistent levels of SSC, TA and sugar: acid ratio is considered as key indicators for fruit senescence (Ali et al., 2019). The consistently higher levels of SSC and TA in arginine treated cucumber may be attributed to delayed senescence and reduced oxidative reactions (Shah et al., 2017). Weight loss is crucial parameter as it is being translated into an actual economical loss (Bico et al., 2009). Since arginine treatment delays senescence by reducing respiration rate and improves cellular impermeability thus weight loss was significantly inhibited. Similar observations have been reported for green asparagus (Morris, 2007; Wang et al., 2017).

The visual quality and fruit colour were maintained for longer duration in arginine treated fruits as compared to control. These results were consistent with the earlier reports of Wang et al. (2017), who suggested that arginine treatment-maintained chlorophyll content by inhibiting degradation of soluble proteins and thus maintained appearance and quality of green asparagus (*Asparagus officinalis* L.) for longer duration under cold storage. In addition to this, decay process has been hindered due to biosynthesis of phenolic compounds and improvement in antioxidant capacity (Nasef, 2018) . The results of sensory evaluations were consistent to the biochemical parameters suggesting that 1 mM arginine concentration has significant impact on the maintenance of fruit quality, taste, flavour, aroma and appearance of cucumber fruits. Wills and Li (2016) also reported that arginine solution dip significantly improved sensory qualities of fresh cut apple and lettuce as compared to calcium ascorbate treatment.

CONCLUSION

Pre-storage application of L-arginine resulted in improved visual quality and overall acceptability during cold storage. Application of 0.5 mM L-arginine significantly reduced chilling injury, fruit decay and improved the bioactive compounds of cucumber fruit under low temperature storage.

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