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Original Research

Combined Application of Methyl Salicylate and L-Arginine Alleviates Chilling Injury, Potentiates Antioxidant System and Maintains Quality of Sweet Pepper (*Capsicum Annum L.*) Fruits cv. 'Winner'

Nida Akram[a]*, Mahmood Ul Hasan[a], Rana Naveed Ur Rehman[a], Rana Muhammad Ateeq Ahmad[a], Zeeshan Ahmed[a], Ummara Khan[b], Faisal Hayat[c]

[a] Postharvest Research and Training Centre, University of Agriculture Faisalabad, 38040, Pakistan
[b] Key Laboratory of Food Processing and Quality Control, College of Food Science and Technology, Nanjing Agricultural University, Nanjing 210095, PR China
[c] Laboratory of Fruit Tree Biotechnology, Nanjing Agricultural University, Nanjing 210095, China

ABSTRACT

Chilling injury is the critical issue in sweet pepper fruit under low temperature storage. Present work was aimed to evaluate the effect of different concentration of methyl salicylate (MS) and L-arginine (Arg) on chilling injury and overall quality of sweet pepper fruits cv. 'Winner'. The treatments were T_1 = Control, T_2 = 0.01mM MS, T_3 = 0.05 mM MS, T_4 = 1mM Arg, T5 = 1.5mM Arg and T6 = 0.01mM MS+1.5mM Arg. After respective treatment (for 10 min) fruits were kept at 5 ± 1 °C with 85-90 % RH for 28+2 days of storage. Physical, biochemical, and phytochemical parameters were studied at 7 days interval followed by two days of reconditioning at ambient conditions (25 ± 2 °C). On last removal (28+2days), sweet pepper fruits treated with combined MS and Arg treatment (T6) showed highly significant results in lower fruit weight loss (8.3%), maintained fruit colour (0.3 score) and firmness (13.4N), and reduced wrinkling (2 score), disease incidence (0.4 score), ion leakage (45.4%), alleviated chilling injury (1.7 score), retained total antioxidants (49.1%) and total phenolic content (74.4 mg 100 g^{-1} GAE FW) as compared to control. In addition, the ascorbic acid content was observed higher in all treatments in comparison with untreated control fruits. In conclusion, MS and Arg combine treatment improved storage potential with reduced chilling injury by maintaining higher total phenolic concentrations, ascorbic acid content and total antioxidants in terms of DPPH radical scavenging activities, and markedly maintained overall quality of sweet pepper under cold storage condition at 5 °C for 28 days.

Keywords: *Cold storage, chilling injury, L-arginine, methyl salicylate, sweet pepper.*

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INTRODUCTION

Sweet pepper (*Capsicum annum L.*) is popular and high value crop grown in tropical and subtropical regions of the world (Lim et al., 2007). It is commonly known as capsicum and locally known as *Shimla Mirch*. It contains non-pungent capsaiciniods hence also called bell pepper. It has high nutritive value and available in various colours and flavours. Due to its crispy texture, sweet pepper is one of the famous vegetables among consumers. Fresh sweet peppers contain high amounts of ascorbic acid content, carotenoid pigments, flavonoids and rich in different phenolic compounds (Ghasemnezhad et al., 2011). Due to immediate water loss in sweet pepper fruit is characterized by shallow cavity and delicate thin layer which reduced shelf life after harvest. Botanically, it is small perennial shrub in the nightshade family of horticultural crops. Sweet pepper is a warm season crop. One of the major problems of tropical and subtropical vegetables as these are highly sensitive to low temperature which induces chilling injury (Imanishi et al., 1998).

Extreme low temperature causes chilling injury in warm season crops and adversely affect plant flowering, pollination and fruiting as earlier reported in pomegranate (Babalar et al., 2018). Low temperature negatively influences sweet pepper crop and fruits causes chilling injury with continuous exposure to temperature of 0-10° C (Rehman et al, 2021). Most common chilling injury symptoms are discoloration of calyx and development of sunken areas and pitting on the fruit surface (Hasan et al., 2019). Ethylene biosynthesis, respiration rate, leakage of electrolytes increased during chilling injury which leads to reduced storage life of sweet pepper (Purvis, 2002).

* Corresponding author
Email: nidaakram951@gmail.com (N. Akram)
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Chilling injury reduces the membrane stability and causes the leakage of ions and intracellular water in sweet pepper (Fung et al., 2004). To overcome this critical issue in horticultural crops, many chemical and non-chemical approaches have been tested (Ali et al., 2021a; Ali et al., 2021b; Hayat et al., 2017; Maryam et al., 2021).

Methyl salicylate (MeSA) is an organic compound synthesized from a phytohormone salicylic acid (SA). SA is naturally occurring and widely distributed in plants (Klessig and Malamy, 1994). Salicylic acid has influence on different physiological parameters such as germination of seed, stomata activity, establishment of seedling and fruit production (Valero et al., 2011). It is an abundant phytohormone essential for stress resistance, plant development and growth. Earlier studies revealed that treatment with SA alleviates CI in peaches by increasing the endogenous SA contents and inducing the regulation of antioxidant enzymes (Yang et al., 2012).

Methyl salicylate acts as a volatile compound helps to enhance resistance and involved directly to respond stress conditions (Tieman et al., 2010). Methyl salicylate is main compound in shikimate pathway (Dicke and Hilker, 2003) and shows a significant role in signal transduction to activate plant resistance. It was reported that MeSA encourages the disease resistance and activated the genes which performed a key role in defense action (Xinhua et al., 2011). Exogenous application of MeSA reduced chilling injury in tomato (Zhang et al., 2011) and mango (Fung et al., 2006) by reducing lipid peroxidation and assisted to stabilize the cell membrane and cell wall structure (Sayyari et al., 2011). Preharvest application of salicylic acid on fruits markedly retained higher bioactive compounds and firmness which leads to increase its storability as earlier reported in Murcott mandarin (Ennab et al., 2020), pomegranate (García-Pastor et al., 2020) and cherries (Gimenez et al., 2015). Postharvest treatment assisted to maintain antioxidant activities, change in colour, loss of intracellular water and essential nutrients as earlier reported in sweet cherries (Valero et al., 2011).

Arginine is a nitrogen containing amino acid. It has high amount of nitrogen as compared to carbon. L-arginine is a molecule with immense quality control and clinical significance. It is mainly found in active sites of various proteins. Its structure is advantageous to help catalyses phosphorylation reactions and binding of phosphate anion (Fuhrmann et al., 2009). Arginine also plays important role in maintaining charge of numerous proteins. By the action of arginase, arginine hydrolysed into ornithine and urea during nitrogen metabolism. Arginine also helps in maintenance of the immune system, hormone secretion and ammonia detoxification (Coman et al., 2008).

Arginine initiates the biosynthesis of Amagtine, polyamines and enhance signal transduction by synthesis of nitric oxide, glutamate, and butyric acid (Gao et al., 2012). It is involved in various plant metabolic reactions and enhance chilling tolerance during storage (Zhang et al., 2010). Applications of Arginine enhanced potential level of antioxidant and reduce chilling injury (CI) in tomatoes, cherries, and cucumbers in cold storage (Zhang et al., 2010; Hasan et al., 2019). Currently, no research has been done on the use of methyl salicylate and Arginine pre-storage application on locally grown sweet pepper fruit for reducing chilling injury and extension of marketable life. Hence, present work was planned with aim to study the potential of MS and Arg treatments in reducing chilling injury, control of disease spread and possible effects in green life extension during cold storage.

MATERIALS AND METHODS

Plant source and treatments

Physiologically mature green coloured sweet pepper cv. 'Winner' was harvested from the peri-urban fields of Faisalabad and instantly transferred to Postharvest Laboratory (PRTC), IHS, UAF by using reefer van (10-12 °C). Fruits were sorted to remove those with diseases, decay and selected based on uniform size and shape, washed, air-dried, and used in the study. The fruits were divided into six groups. The first one was control (T1) and just dipped in distilled water. The second and third group was treated with 0.01mM (T2) and 0.05mM methyl salicylate (MS) (T3) as mentioned by Rehman et al. (2021). Fourth and fifth treatments were consisted of arginine dipping having 1mM Arg (T4) and 1.5mM Arg (T5) concentrations optimized in preliminary trial (data not shown), respectively. Sixth group of pepper fruits were treated with combined application of methyl salicylate and arginine 0.01mM MS+ 1.5mM Arg (T6). Fruits were dipped in respective treatments for 10 mins. In the earlier optimization trials, the results revealed that combination of methyl salicylate and arginine 0.01mM MS+ 1.5mM Arg were most effective to decrease the chilling injury in sweet pepper and hence this adjusted application was used in the present study. Treated and untreated control pepper fruits after 30 min of air drying were kept in cool storage dark rooms at 5 ± 1 °C, 85–90 % RH for 28 days of storage. Fruit sampling was done at 7 days interval from cold storage rooms followed by 2 days of reconditioning at ambient conditions (25 ± 2 °C).

Fruit weight loss, chilling injury, and electrolyte leakage

Digital weight balance was used to calculate the loss of fruit weight and measured as percentage difference between the initial fruit and final weight of the sweet pepper fruit (Malik et al., 2021). The weight loss was determined using the given formula:

$$\text{Fruit weight loss} = (\text{Initial fruit weight} - \text{fruit weight after storage}) / \text{Initial fruit weight} \times 100$$

CI was visually evaluated based on the occurrence of surface pitting, discoloration of calyx and sunken lesions by using prescribed 1-5 scale where, 1= no infected area, 2 = 5% area infected, 3 = 15% area infected, 4 = 30% area infected, 5 = Up to 30% area infected as earlier described by Dong et al. (2012). Electrolytic leakage was determined by the procedure earlier outlined in our paper of Rehman et al. (2021) for sweet pepper fruits and final value expressed as percent.

Fruit colour, disease incidence, firmness, and wrinkling

The fruit colour was assessed by visual appearance of fruit and scored from 0-4 where, 0= dark green (no colour change), 1= light green (25%), 2= green-red (25-50%), 3= red-green (50-75%) and 4= red (100%). Disease incidence was examined by using the scale from 0-5 defined by Babalar et al. (2007) where, 0 = no disease/ symptoms, 1= 1-6% fruit area, 2= 6.1-12% fruit area, 3= 12.1-25% fruit area, 4= 25.1-40% fruit area, 5= Up to 50% fruit area infected. Fruit firmness was measured by penetrometer, by pushing its probe (0.8mm dia.) at the basal side of the fruit and note the reading (Rehman et al., 2021). Wrinkling was evaluated based on the visual signs of dehydration and scale from 0 to 4 where, 0= no sign, 1= some, 2= slightly moderate, 3= moderate, 4= severe (Hasan et al., 2021a).

Total soluble solids, titratable acidity, and sugar acid ratio

Total soluble solids (TSS) of sweet pepper juice were determined using digital refractometer RX 5000 (Atago, Japan) (Maryam et al., 2021). Additionally, titratable acidity (TA) was assessed by using the titration method as described by Ali et al. (2016). Sugar acid ratio was calculated by dividing value of TSS over the value of TA (Hasan et al., 2019).

Ascorbic acid, total phenolic content, and total antioxidants

Ascorbic acid content was determined according to the titration method by using 2, 6 dichlorophenol-indophenol indicator, and was expressed as mg 100 g^{-1} fresh weight (Hasan et al., 2020). TPC was assessed by employing the protocol of Ainsworth and Gillespie (2007) using Folin-Ciocalteu reagent and expressed its value as mg GAE100 g^{-1}. The DPPH (1,1-diphenyl-2 picrylhydrazyl) radical scavenging was assessed according to the procedure devised by Ali et al. (2016) by observing the variation in absorbance at 517 nm. Total antioxidants were expressed in % DPPH inhibition activity.

Data analysis

Present experiment on sweet pepper was conducted in controlled lab conditions under completely randomized design (CRD) with factorial arrangements while two factors are treatments and storage period (days). Collected data for this postharvest trial was subjected to analysis of variance (ANOVA) and LSD (least significance test) was used to comparing means using computer operated software STATISTIX 8.1®.

RESULTS AND DISCUSSION

Fruit weight loss, chilling injury, and electrolyte leakage

Fruit weight loss in sweet pepper fruits was observed reducing during the entire period of storage till 28 days irrespective of treatments. However, mean comparison showed statistically significant results for treated fruits as compared to untreated control fruits. After 28+2 days of storage period, the weight loss of combined application of methyl salicylate and arginine treatment (0.01 mM Ms + 0.01 mM Arg) on sweet pepper was 8.3% as compared to uncoated control sweet pepper (19%) (Fig. 1a). Fruit weight loss is one of the key parameters which is primarily assessed for horticultural crops in postharvest trials

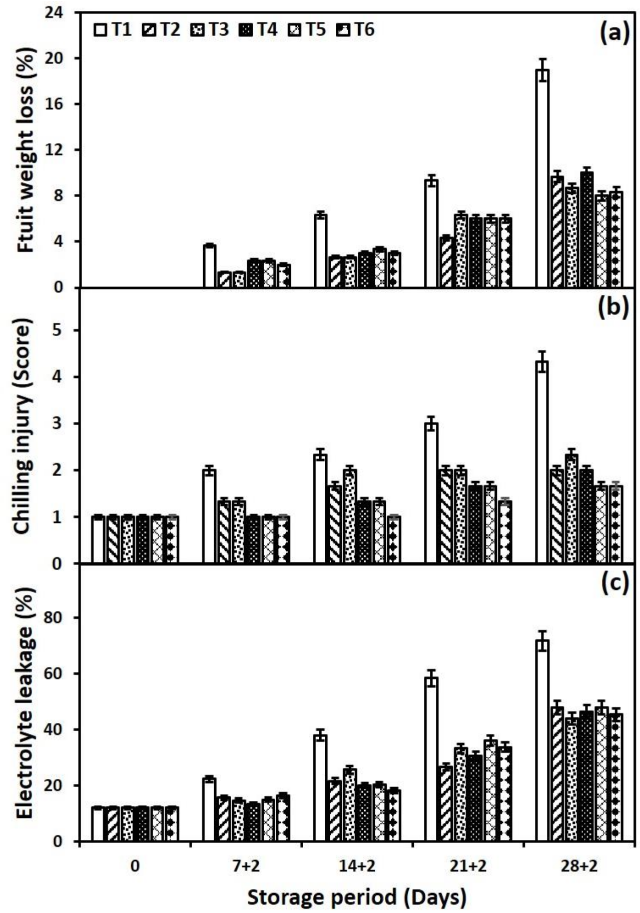

Figure 1: Effect of methyl salicylate and L-arginine treatments on fruit weight loss (a), chilling injury (b) and electrolyte leakage (c) of sweet pepper during low temperature storage. Vertical bars indicate average ± standard error of means. Treatments: T1 = Control, T_2= 0.01mM MS, T_3= 0.05mM MS, T_4 = 1mM Arg, T_5 = 1.5mM Arg and T_6 = 0.01mM MS+1.5mM Arg. 7 days interval in storage and 2 days at shelf for conditioning.

(Hasan et al., 2021b). Our results for weight loss in sweet peppers were observed in-line with the findings of Wang et al. (2017) reported that L-arginine treatment reduced weight loss of fresh asparagus for 24 days of storage. In addition, cucumbers treated with 0.5 mM L-arginine markedly reduced moisture loss during 16 days of low temperature storage (Hasan et al., 2019). Similarly, the sweet peppers treated with methyl salicylate 0.05 mM in combination with short hot water treatment exhibited lower weight loss during entire period of storage (Rehman et al., 2021).

As expected, the chilling injury was significantly pronounced in control fruits (4.3 score) in contrast with combined application of MS and Arg treatment (1.6 score) on sweet pepper fruit after 28+2 days of storage period (Fig. 1b). Ding et al. (2001) reported tomatoes treated with methyl salicylate and kept at 5 °C for 21 days showed reduced chilling injury (1.42 score) as compared to control fruits (3.55 score). Our findings of reduced chilling injury in sweet pepper fruits are supported with earlier reports on cherry tomatoes and pomegranate fruits during entire period of storage by maintaining higher membrane integrity (Sayyari et al., 2011; Zhang et al., 2011).

Methyl salicylate and arginine treatment significantly reduced the electrolyte leakage (EL) of sweet pepper kept under cold storage conditions. On 28+2 days, combined application of MS and Arg treatment (0.01 mM Ms + 0.01 mM Arg) on sweet pepper showed lower EL (45.3 %) as compared uncoated control fruits (71.6%) (Fig. 1c). Membrane damage frequently occurs through environmental oxidative stress in fresh fruits and vegetables and EL which significantly reduced the marketable life during storage (Li et al., 2019). Hasan et al. (2019) noted that L arginine treatment on cucumber fruits cv. 7003 and kept at 5 °C for 16 days, showed gradually decrease EL in cucumber treated with 0.5 mM (57.48%) followed by 1 mM (59.85) and 1.5 mM (51.9%) as compared to control (73.23%).

Fruit colour, disease incidence, firmness, and wrinkling

Fruit colour, disease incidence, firmness and wrinkling in terms of cosmetic appearance were significantly affected by arginine and methyl salicylate treatments, storage periods and their interaction. After 28+2 days of storage, sweet pepper fruits treated with combined application of methyl salicylate and arginine treatment (0.01 mM Ms + 0.01 mM Arg) significantly ($P \leq 0.05$) retained green colour in treated in contrast with control which represented poor quality and marketability (Fig. 2a). White button mushrooms treated with arginine showed better fruit colour retention as compared to control during storage at 4 °C for 8 days (Li et al., 2019). Sweet pepper fruits treated with arginine and methyl salicylate showed significant reduction in disease incidence during the entire storage period. Compared to uncoated control sweet pepper, combined application of arginine and methyl salicylate showed less disease incidence after 28+2 d of storage (Fig. 2b). Postharvest rot in horticulture commodities including sweet pepper is the key issue in the supply chains resulted in lower market returns (Hasan et al., 2021b). Wang et al. (2017) investigated that after 18 days of storage period, no sign of disease was observed in l-arginine treated asparagus as compared to control. Sweet pepper fruits treated with arginine and methyl salicylate were firmer as compared to non-treated ones. However, fruits treated with combined application of methyl salicylate and arginine treatment (0.01 mM Ms + 0.01 mM Arg) had higher firmness throughout the storage period. On 28+2 days of storage, peppers treated with combined treatment application showed higher firmness (13.3 N) as compared to untreated control fruits (7.1 N) (Fig. 2c). Our results are in line with the finding of Hasan et al. (2019) stated that arginine treated cucumbers showed higher firmness during entire storage of 16 days. Likewise, wrinkling and firmness are closely associated parameters observed decreasing with increasing storage period. Fig. 2d displayed that wrinkling in sweet pepper decreasing in treated fruits as compared to control. Hasan et al. (2021a) revealed that hot pepper treated with 50 % aloe vera gel coating significantly reduced wrinkling during cold storage. Furthermore, Ochoa et al. (2011) reported that candelilla wax based coatings assisted in decreasing changes of appearance (wrinkling) in apples.

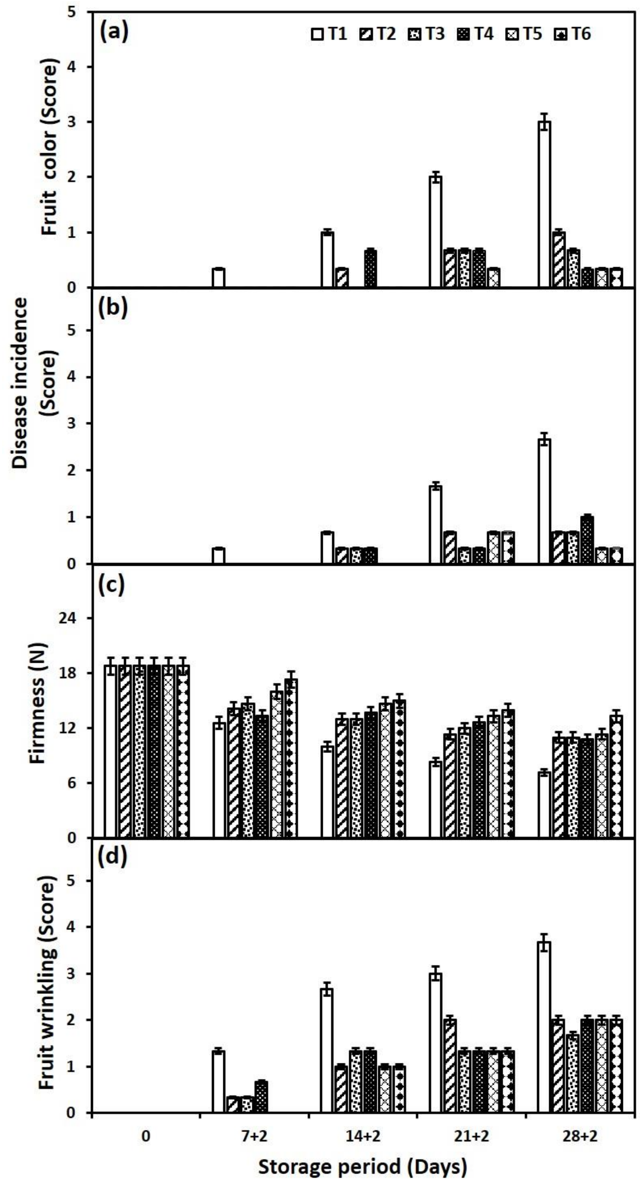

Figure 2: Effect of methyl salicylate and L-arginine treatments on fruit color (a), disease incidence (b), fruit wrinkling (c) and firmness (d) of sweet pepper during low temperature storage. Vertical bars indicate average ± standard error of means. Treatments: T1 = Control, T_2= 0.01mM MS, T_3= 0.05mM MS, T_4 = 1mM Arg, T_5 = 1.5mM Arg and T_6 = 0.01mM MS+1.5mM Arg. 7 days interval in storage and 2 days at shelf for conditioning.

Total soluble solids, titratable acidity, and sugar acid ratio

Total soluble solids of sweet pepper in all treatments showed an overall increasing trend with storage time as shown in Fig. 3a. At 28+2 days of storage, both arginine and methyl salicylate treatment significantly lowered total sugar levels compared with untreated fruit. Results revealed that both arginine and methyl salicylate treatment can slow down the reduction of soluble sugar. Our results are supported with earlier findings

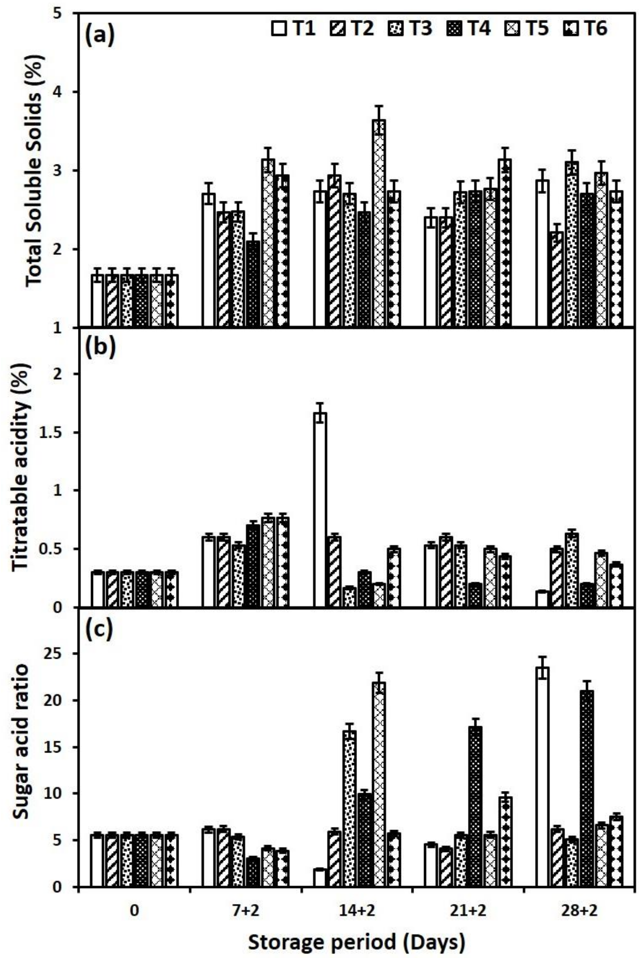

Figure 3: Effect of methyl salicylate and L-arginine treatments total soluble solids (a), titratable acidity (b) and sugar acid ratio (c) of sweet pepper during low temperature storage. Vertical bars indicate average ± standard error of means. Treatments: T = Control, T_2= 0.01mM MS, T_3= 0.05mM MS, T_4 = 1mM Arg, T_5 = 1.5mM Arg and T_6 = 0.01mM MS+1.5mM Arg. 7 days interval in storage and 2 days at shelf for conditioning.

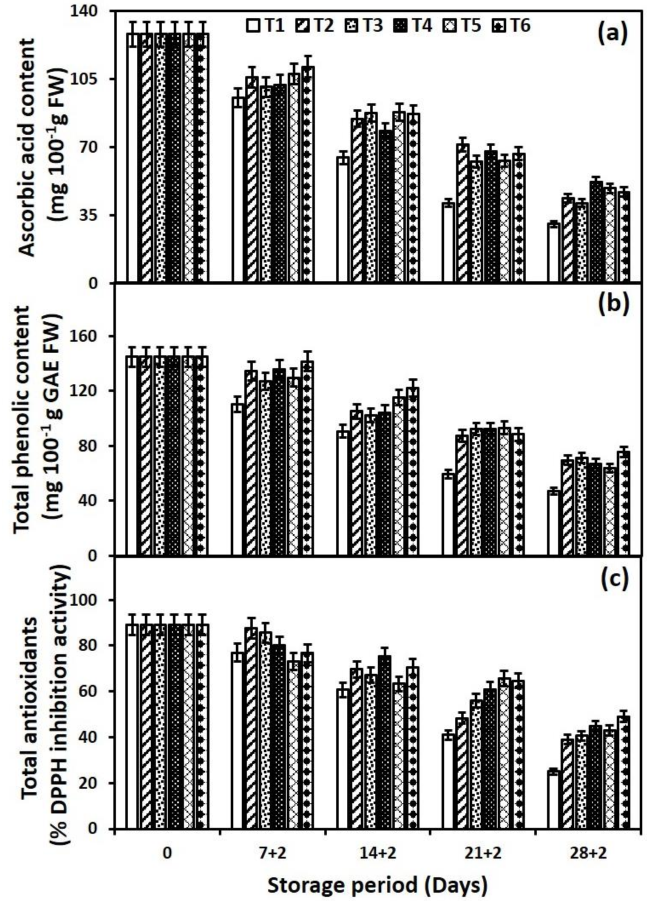

Figure 4: Effect of methyl salicylate and L-arginine treatments ascorbic acid content (a), total phenolic content (b) and total antioxidants (c) of sweet pepper during low temperature storage. Vertical bars indicate average ± standard error of means. Treatments: T = Control, T_2= 0.01mM MS, T_3= 0.05mM MS, T_4 = 1mM Arg, T_5 = 1.5mM Arg and T_6 = 0.01mM MS+1.5mM Arg. 7 days interval in storage and 2 days at shelf for conditioning.

such as cherries cv. Sweet Heart treated with methyl salicylate kept at 2 °C for 28+2 days of storage period showed maximum TSS concentrations as compared to control fruit. Similarly, another cherry cultivar 'Sweet Late' fruits treated also observed maximum content of TSS as compared to non-treated fruit (Gimenez et al., 2015). Nevertheless, after 28+2 days of storage, TA was slightly increased for sweet pepper treated with different concentration of arginine and methyl salicylate as compared to control (Fig. 3b). Sugar: acid ratio with reference to arginine and methyl salicylate treatments and treatments × storage days were found non-significant ($P \leq 0.05$) in sweet pepper fruit (Fig. 3c) while TA (%) progressively decreased with the increase in storage duration. Our results were similar with the findings of Hasan et al. (2019) who reported that cucumbers treated with arginine showed maximum sugar acid ratio in 1.5 mM as compared to control fruit.

Ascorbic acid, total phenolic content, and total antioxidants

During the storage period contents of ascorbic acid were positively affected by treatments and its interaction with storage period. The ascorbic acid content in control sweet pepper fruits were reduced from 0 to 28+2 days of storage. Nevertheless, the sweet pepper which were treated with Arg + MS treatment were significantly reduced loss the ascorbic acid contents (Fig. 4a). Ascorbic acid is one of the organic compounds plays an important role in reducing plant cells ROS. Ascorbic acid act as antioxidant and helped to detoxify the free radicals (Ali et al., 2021b). The preservation of ascorbic acid content might be due to less destruction of cell wall integrity associated with reduced chilling injury and quality retention of treated sweet pepper fruits. Our recorded data supported with the results of Zhang et al. (2019) revealed that during the cold storage of zucchini fruits, ascorbic acid contents were reduced. Similarly, Huang et al. (2008) explained that if navel orange fruit is treated with pre-storage salicylic acid application showed high ascorbic acid contents during storage as compared to untreated control fruits. Furthermore, ascorbic acid contents observed retained in sweet pepper fruit treated with combined application of calcium chloride and salicylic acid might be due reduction in oxidation process (Rao et al., 2011). Earlier findings of Rehman et al. (2021) showed higher retention of ascorbic acid content in sweet pepper fruits treated with combined application of methyl salicylate and short hot water dip treatment. In the present trial, the application of arginine and methyl salicylate significantly decreased the reduction in TPC of sweet pepper fruits than those of untreated control fruits. After 28+2 days of storage, higher TPC (75.3 mg GAE 100 g^{-1}) were recorded in combined application of arginine and methyl salicylate in sweet pepper as compared to untreated control (47 mg GAE 100 g^{-1}) (Fig. 4b). Our results are in-line with the findings of Li et al. (2019) who reported that mushrooms treated with 10mM arginine showed higher phenolic content during storage at 4 °C for 8 days in comparison with control fruits. Rehman et al. (2021) reported that sweet peppers treated with combined application of methyl salicylate and hot water dip treatment displayed higher phenolic concentrations as compared to control. In addition, higher phenolic content also increased disease resistance as earlier reported for mango and guava fruits (Hasan et al., 2020; Malik et al., 2021).

Total antioxidants in terms of DPPH scavenging activity gradually decreased in treated as well as untreated control sweet pepper fruits. However, sweet pepper treated with arginine and methyl salicylate displayed higher DPPH-RSA retention as compared to untreated control fruits. On 28+2 days of storage, the DPPH-RSA was higher (49%) in sweet pepper when treated with combined application of arginine and methyl salicylate as well as uncoated control fruits (25%) (Fig. 4c). The alleviation in oxidative stress is possible through retaining maximum DPPH-RSA during storage period (Ali et al., 2016). Earlier reports also support our findings, button mushrooms treated with L-arginine during storage at 4 °C, the total antioxidants were increased as compared to control (Li et al. 2019).

CONCLUSION

In conclusion, the application of methyl salicylate (MS) and L-arginine (Arg) markedly enhanced the storability of sweet pepper by delaying senescence, reduced weight loss, wrinkling, electrolyte leakage, disease incidence and maintained firmness and fruit colour and alleviates chilling injury and retained higher non-enzymatic antioxidants (ascorbic acid, total antioxidants, and phenolic concentrations) quality of sweet pepper during storage. MS and Arg conserved visual quality with higher marketability index. So, pre-storage combined treatment MS and Arg could be considered potentially suitable for alleviating CI and quality management of sweet peppers during low temperature (5°C) storage for 28 days.

DECLARATION OF COMPETING INTERESTS

All authors declare no conflict of interest for this publication.

AUTHOR CONTRIBUTION STATEMENT

Nida Akram: Conceptualized idea and conducted this research work and collected data. **Mahmood Ul Hasan and Rana Naveed Ur Rehman:** Conceptualized idea, designed this research work and collected data.**:** **Ummara Khan:** Assisted in validation, editing, and reviewing. **Rana Muhammad Ateeq Ahmad and Zeeshan Ahmed:** Assisted in laboratory analysis and data collection; **Faisal Hayat:** Editing, reviewing, software analysis and improving of the manuscript.

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