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Post-storage quality and physiological responses of tomato fruits treated with polyamines

J. Javanmardi[(1)], M. Rahemi, M. Nasirzadeh
Department of Horticultural Sciences, College of Agriculture, Shiraz University, Shiraz, Iran.

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Abstract: Two greenhouse F1 tomato cultivars, M19 and M79, were grown hydroponically and the mature green fruits were harvested and subjected to eight polyamine (PA) treatments including 1 and 2 mM putrescine (Put), spermidine (Spd) and their combination before being placed at 3°C for 15 and 25 days. Electrolyte leakage, weight loss, fruit firmness, decay percentage, chilling injury index, titratable acidity, total soluble solid content and ascorbic acid content were then measured after keeping at 20°C for 3 days and compared to control. The Put:Spd (2:2 mM) treatment decreased electrolyte leakage (over 50%), chilling injury index and fruit decay percentage. Combinations of PAs caused greater total soluble solids and greater effect on decreasing weight loss during storage when compared to their sole PA application. PAs caused a net increase in fruit firmness during post-harvest life. Titratable acidity increased with increasing duration of low temperature storage for all treatments. Ascorbic acid in fruits stored at low temperature for 25 days was greater than those stored for 15 days. The effects of exogenous PAs on reducing chilling-related disorders decreased with time. Correlations among weight loss, electrolyte leakage, chilling injury, decay percentage and fruit firmness during low temperature storage were positive and significant, but they were non-significant or significantly negative when compared against ascorbic acid, titratable acidity and TSS.

1. Introduction

Tomato as a plant indigenous to tropical regions is susceptible to chilling injury when subjected to low temperature storage (Saltveit, 2001). Chilling injury limits tomato storage life and leads to significant degradation of fruit quality and decreases the market value. It can increase membrane permeability, and a resultant increase in leakage of ions from cell membrane, surface pitting, susceptibility to decay and diseases, weight losses, abnormal ripening, change in respiration, ethylene production and senescence. Chilling injury symptoms mainly develop during shelf life following cold storage (Candan *et al.*, 2007).

Numerous attempts, such as breeding for increased chilling tolerance, genetic engineering, modifying crop management practices and application of chemicals, have been made to increase chilling tolerance and avoid chilling injury (Baninasab, 2009). Chilling alleviation in fruits and vegetables has been attributed to several factors including accumulation of polyamines, nitric oxide and proline (Aghdam and Bodbodak, 2013).

Over the years, studies have shown the involvement of polyamines (PAs) in a wide array of processes in plants, ranging from triggering organogenesis to protecting against stress (Walden *et al.*, 1997). PA accumulation occurs under abiotic stresses including drought, salinity, extreme temperatures, UV-B, heavy metals, mechanical wounding and herbicide treatment (Hussain *et al.*, 2011). Amongst different kind of PAs, the diamine putrescine (Put), triamine spermidine (Spd), and tetramine spermine (Spm) are the most common PAs in plant cells, while others are of more limited occurrence (Galston and Sawhney, 1990; Valero *et al.*, 2002). Distribution of these biogenic amines differs between species with Put and Spd being particularly abundant and Spm the least abundant in plant cells. These amines are important for cell viability and their intracellular levels are tightly regulated, making it difficult to characterize individual effects of Put, Spd and Spm on plant growth and developmental processes (Mattoo *et al.*, 2010).

It has been reported that exogenous PA application leads to an inhibition of ethylene emission rate in the climacteric fruits (Valero *et al.*, 2002) and delays ripening and fruit abscission (Paksasorn *et al.*, 1995). On the other hand, PAs are precursors of many important secondary metabolites and changes in PA biosynthesis appear to have a reciprocal effect on ethylene biosynthesis (Walden *et al.*, 1997). Accumulation of Put in tissues seems to be a general response of plants to chilling temperatures (Faust and Wang, 1992). Accordingly, we hypothesized that exogenous application of PAs may have effects on the postharvest physiology of fruits, especially on chilling tolerance. An experiment was arranged to study the effects of exogenous PA application

[(1)] Corresponding author: javanm@shirazu.ac.ir

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on the level of chilling injury, ripening, shelf life and some quality factors of tomato fruit (as a model plant) after a period of low temperature storage. Another goal of the study was to determine which polyamine, concentration and/or their combination is more effective. Two greenhouse tomato cultivars were selected to evaluate whether PAs play the same role in different cultivars or not.

2. Materials and Methods

Plant material and polyamine treatments

F1 hybrid seeds of tomato (*Solanum lycopersicom* L.) cultivars M19 and M79 (Tropica Seeds, IndoSem Ltd. India) were sown in 30 × 30 × 60 mm/cell plastic plug trays filled with peat and perlite (1:1 v:v). Seedlings were grown for five weeks in a polycarbonate greenhouse (25/21°C and 60-70% relative humidity). Plugs were then transplanted into 12-l pots filled with a peat:perlite (60:40 v/v) mixture. Pots were kept in the same greenhouse with an average 60% relative humidity and 25±5°C temperature until the end of the experiment. Cultural practices consisting of standard recommendations for growing tomato seedlings and plants in a hydroponic system were according to Papadopoulos (1991). Fruits for study were harvested at mature green stage based on the "Color Classification Requirement in United States Standards for Grades of Fresh Tomatoes" chart, published by the USDA.

Eight polyamine treatments consisted of immersing same-sized, selected mature green fruits in 1 and 2 mM putrescine (Put), spermidine (Spd) and their combination for 4 min as indicated by Mirdehghan *et al.* (2007 a). Immersion in distilled water for the same period was taken as control (Table 3). Fruits were then held at 3°C in a temperature-controlled chamber in darkness with relative humidity of 90%. After 15 and 25 days, five fruits from each treatment replicate were sampled and stored at 20°C for 3 days. Electrolyte leakage, weight loss, fruit firmness, decay percentage, chilling injury index, titratable acidity, total soluble solid content (TSS) and ascorbic acid content of fruits were measured as described below.

Electrolyte leakage

Electrolyte leakage was used to assess membrane permeability. The procedure was based on Lutts *et al.* (1996) with slight modification. Briefly, five tomato fruit pericarp discs (10 mm diameter) per replicate from randomly chosen fruits were taken and placed in test tubes containing 10 mL of distilled water followed by three washes with distilled water to remove surface contamination. Samples were incubated at room temperature on a shaker for 24 h. Electrical conductivity (EC) of the bathing solution (EC1) was read after incubation. The samples were then placed in a boiling water bath for 20 min and the second reading (EC 2) was determined after cooling the solution to room temperature. The electrolyte leakage was calculated using EC1/EC2 and expressed as a percentage. All leakage data were expressed as a percentage of the total electrolyte readings.

Weight loss

Weight loss during postharvest storage of individual replicates was determined by subtracting sample weights on sampling dates (day 15 or 25 of low temperature storage) from their initial weight on day 0 and presented as percent of weight loss compared to initial weight.

Fruit firmness

Fruit firmness was determined according to Ben-Yehoshua *et al.* (1983). A compression tester using a 1 kg weight centered over a locule on the equatorial region of each tomato fruit was used. Full deformation was measured 30 s after exerting the force on the fruit, then the weight was removed and residual deformation was measured 15 s later. Lower readings denoted firmer fruit. Five fruits were measured for each treatment replicate. Firmness was expressed as mm deformation.

Chilling injury index

Chilling injury (CI) was evaluated according to Ding *et al.* (2002) at 20°C for 3 days, following 15- and 25-day low temperature storage period. Briefly, tomato fruit surface pitting was considered as CI symptom. The severity of CI symptoms was assessed visually according to a four-stage scale: 0= no pitting; 1= a few scattered pits; 2= pitting covering up to 5% of the fruit surface; 3= extensive pitting covering 5-25% of the fruit surface, and 4= extensive pitting covering more than 25% of the fruit surface. The average extent of CI damage was expressed as a CI index and calculated using the following formula:

$$CI\ index = \frac{\sum(\text{injury classification level x number of fruit at that level})}{\text{total number of friut at the treatment}}$$

Decay percentage

Extent of decay was assessed according to González-Aguilar *et al.* (2000), based on the area of decay and the surface area with microorganisms growing on it. Decay was rated for each replicate of treatments at the end of the 15- and 25-day cold storage periods after an additional 3 days at 20°C. The values were expressed as decay percentage.

Total soluble solids, titratable acidity and ascorbic acid content

Total soluble solids concentrations (TSS) of fruit juice were determined using a digital refractometer (Pal-3, ATAGO Co., Ltd. Tokyo, Japan) at 20°C and presented as °Brix. Fruit juice titratable acidity (TA) was determined by titration of 1 mL juice in 25 mL distilled water with 0.1N NaOH until the pH reached 8.1, according to El Ghaouth *et al.* (1992). Results were expressed as gram of citric acid equivalent per 100 g fresh weight (g CAE/100 g fw).

Fruit ascorbic acid content was determined according to the method described by the AOAC (1984).

Statistical analysis

The experiment was arranged in a completely randomized design. Each polyamine treatment consisted of three replicates; each replicate consisted of 10 plants. Data were analyzed separately for tomato cultivars and days of low temperature incubation using one-way analysis of variance. Means for each low temperature storage duration and cultivar were compared separately using the least significant differences (LSD) test at p≤0.01. All data analyses including correlation analysis were performed using SPSS21 (SPSS Inc., Chicago, IL) computer software for Windows.

3. Result and Discussions

One-way analysis of variance showed significant differences for all measured characteristics in both cultivars except for ascorbic acid content in cv. M79 after 15 days of low temperature storage (Table 1 a, b, and Table 2 a, b).

Electrolyte leakage

Application of PAs decreased electrolyte leakage of low-temperature incubated tomato fruits. The highest

Table 1 a - One way analysis of variance for characteristics of polyamine-treated tomato cultivar M19 after 15 and 25 days storage at 3°C

SOV	df	Mean Squares							
		Electrolyte leakage		Weight loss		Fruit firmness		Chilling injury	
		15	25	15	25	15	25	15	25
Polyamines	8	88.692**	163.37**	5.193**	3.055**	0.755**	0.87**	114.593**	65.167**
Error	18	3.751	3.731	0.161	0.283	0.051	0.047	3.625	16.759
Total	26	29.887	52.848	1.709	1.136	0.268	0.3	37.767	31.654

NS, *, ** non-significant and significant at 0.05 and 0.01, respectively.

Table 1 b - One-way analysis of variance for characteristics of polyamine-treated tomato cultivar M19 after 15 and 25 days storage at 3°C

SOV	df	Mean Squares							
		Decay percentage		TSS		Titratable acidity		Ascorbic acid	
		15	25	15	25	15	25	15	25
Polyamines	8	370.37 **	902.759 **	0.832 **	0.592 **	0.028 **	0.033 **	1.697 **	15.319 **
Error	18	37.037	65.852	0.167	0.079	0.002	0.003	0.185	0.999
Total	26	139.601	323.362	0.369	0.236	0.01	0.012	0.650	5.406

NS, *, ** non-significant and significant at 0.05 and 0.01, respectively.

Table 2 a - One-way analysis of variance for characteristics of polyamine-treated tomato cultivar M79 after 15 and 25 days storage at 3°C

SOV	df	Mean Squares							
		Electrolyte leakage		Weight loss		Fruit firmness		Chilling injury	
		15	25	15	25	15	25	15	25
Polyamines	8	48.792**	101.07**	4.593**	4.708**	0.564**	0.749**	80.624**	135.995**
Error	18	0.948	0.673	0.034	0.087	0.017	0.046	5.701	10.417
Total	26	15.669	31.564	1.437	1.509	0.185	0.262	28.754	49.056

NS, *, ** non-significant and significant at 0.05 and 0.01, respectively.

Table 2 b - One-way analysis of variance for characteristics of polyamine-treated tomato cultivar M79 after 15 and 25 days storage at 3°C

SOV	df	Mean Squares							
		Decay percentage		TSS		Titratable acidity		Ascorbic acid	
		15	25	15	25	15	25	15	25
Polyamines	8	193.667**	424.833**	0.431**	0.951**	0.021**	0.029**	1.043ns	3.987**
Error	18	13.852	29.667	0.026	0.029	0.003	0.002	0.989	0.822
Total	26	69.179	151.256	0.150	0.313	0.009	0.01	1.005	1.795

NS, *, ** non-significant and significant at 0.05 and 0.01, respectively.

electrolyte leakage was observed in control treatments as almost 100% greater than those treated with the highest PA concentrations in combined treatments on both cultivars (Table 3). Pretreatment of cucumber plants with PAs diminished the increased electrolyte leakage caused by chilling in the leaves (Gill and Tuteja, 2010). The exogenous application of polyamines on pomegranate (*Punica granatum* L.) protected the membrane lipid from being converted from liquid crystalline to a solid-gel state (induced by chilling) through preventing lipid peroxidation (Mirdehghan *et al*., 2007 b). Previously, Put has been reported to act as protective toward cold stress in tomato plants, since reduced cold-induced electrolyte leakage in leaves due to its application was observed (Kim *et al*., 2002). In addition, PA application induced cold acclimation through maintenance of membrane fluidity at low temperatures and reduced electrolyte leakage, skin browning, and thus the severity of CI symptoms (Mirdehghan *et al*., 2007 a).

In general, the effect of Spd on decreasing electrolyte leakage was significantly greater than Put but the differences were not significant when they were applied in combinations (Table 3). In agreement with our results, Gill and Tuteja (2010) found different patterns of Put and Spd action in different cucumber cultivars. Accordingly, it seems the mode of action of PAs may differ within species.

A large amount of evidence showed that exogenous application of PAs plays a role in stabilizing plant cell membranes and protecting them from damage under stress conditions (Liu *et al*., 2007; He *et al*., 2008; Gill and Tuteja, 2010). PAs in their free forms have been described as anti-senescence agents (Valero *et al*., 2002) due to their capacity to preserve membrane stability, which is crucial in plant adaptation to temperature stresses (Oufir *et al*., 2008). Their attachment to membranes by way of phospholipids results in altered patterns of solute permeation through those membranes and decreased fluidity of membrane components (Galston and Sawhney, 1990). They are involved in the regulation of many basic cellular processes, including cellular cation-anion balance and membrane stability (Gill and Tuteja, 2010). In the present experiment, tomato cv. M19 showed greater electrolyte leakage for all PA and low temperature storage treatments compared to cv. M79 (Table 3). Possibly the PAs pattern of action in the studied tomato cultivars was at different rates, as previously found for cucumber cultivars (Gill and Tuteja, 2010).

Increasing the duration of low temperature storage from 15 to 25 days increased electrolyte leakage in both cultivars (Table 3). This could be an indicator that the effects of exogenous PAs on lowering electrolyte leakage decrease with time.

Weight loss

It has been reported that tomatoes at room temperature showed greater weight loss than those stored in cold storage (Javanmardi and Kubota, 2006). At least 50% greater weight loss was found in control treatments compared to those with PA applied in both cultivars and low temperature durations (Table 3). Transpiration has been considered the main cause of weight loss during tomato storage (Javanmardi and Kubota, 2006). Reduction in weight loss and respiration rate due to Put and Spd application in mango has been reported (Malik and Singh, 2005).

The differences between combined PA treatments were not significant in the studied cultivars for the two low temperature durations, however they showed less weight loss when PAs were applied singularly (Table 3).

It has been reported that chilled fruits had a greater weight loss rate than non-chilled fruits after transfer to non-chilling conditions. This is due to the development of microscopic cracks in peel tissue (Cohen *et al*., 1994), cellular breakdown and loss of membrane integrity which have an important role in water exchange through the rind (González-Aguilar *et al*., 2000). Storage conditions or treatments that reduce fruit water loss have been shown

Table 3 - Effect of polyamine application and duration (15 and 25 days) of low-temperature (3°C) storage on electrolyte leakage and fruit weight loss in tomato cultivars M19 and M79

Treatment	Electrolyte leakage				Weight loss			
	M 19		M 79		M 19		M 79	
	15	25	15	25	15	25	15	25
Control	30.06 a	42.43 a	24.40 a	35.64 a	5.48 a	5.22 a	5.27 a	5.87 a
Put 1 mM	19.55 b	24.96 b	15.43 b	20.13 b	3.13 b	3.27 b	2.52 b	3.22 b
Put 2 mM	18.50 b	22.77 bc	14.20 b	19.78 b	2.54 bc	2.90 bcd	2.40 b	2.86 bc
Spd 1 mM	16.43 bc	22.98 bc	14.20 b	19.47 bc	2.40 cd	3.17 bc	2.14 bc	2.96 b
Spd 2 mM	14.93 bc	21.83 bc	13.91 b	17.73 bc	1.92 cde	2.48 bcd	1.90 cd	2.40 cd
Put 1 mM + Spd 1 mM	15.56 bc	21.31 bc	13.13 bc	18.81 bc	1.69 de	2.23 cd	1.59 de	2.20 de
Put 1 mM + Spd 2 mM	14.98 bc	21.99 bc	12.97 bc	17.74 bc	1.61 de	2.13 d	1.61 de	2.00 de
Put 2 mM + Spd 1 mM	11.45 c	18.19 c	10.32 c	17.31 c	1.19 e	2.17 cd	1.19 e	1.93 de
Put 2 mM + Spd 2 mM	13.01 c	18.05 c	11.41 c	17.30 c	1.40 e	1.97 d	1.27 e	1.79 e
LSD value 0.01	4.55	4.54	2.29	1.92	0.94	1.25	0.43	0.69

Means in columns followed by the same letter are not significantly different, $P \leq 0.05$, LSD test. Means for each column were compared separately.

to alleviate CI (Wang, 1993). In our experiment, PA application resulted in less membrane permeability (less electrolyte leakage) and therefore less water loss than control fruits. Also storage duration affected weight loss: the longer fruits remained in low-temperature storage, the greater their weight loss.

Fruit firmness

Fruit firmness was affected by PA application (Table 4). All PA-treated fruits (except for Put 1 mM after 15 days of storage in M19) showed firmer fruit (less compression) than control fruits (Table 4). The differences between combined PA treatments in each low temperature storage duration were not significant. However, when compared to the control they showed at least 43 and 69% greater firmness in M19 tomato, and 80 and 45% in M79 tomato for 15 and 25 days storage, respectively. It has been shown that fruits and vegetables infiltrated with PAs had a net increase in firmness during post-harvest life. This effect of PAs on fruit firmness has been attributed to the cross linking to the COO^- group of the pectic substance and changes in polygalacturonic acids in the cell wall (Valero *et al.*, 2002). Retarded fruit softening due to Put and Spd application in mango has been reported (Malik and Singh, 2005). Although it is believed that the overall softening process results from a number of changes in turgor pressure, cell wall and membrane composition and degradation, but cell wall modifications have been implicated to be the major determinant of fruit softening (Smith *et al.*, 2002). Put and Spd have anti-senescence properties (Saftner and Baldi, 1990), and are able to retard the maturation process (including softening) in a wide range of climacteric and non-climacteric fruits (Valero *et al.*, 2002). The ethylene production in tomato fruits, enhances softening, but its effect may decrease due to increased polyamine level (Tiecher *et al.*, 2013). Increased duration of low temperature storage decreased fruit firmness for all treatments. Fruit firmness of control plants M19 and M79 showed 52 and 56% decrease, respectively, when 15 days of low temperature storage values were compared width 25 days. The values obtained from 2.2 mM, Put:Spd tratment were 108 and 148% for M19 and M79, respectively (Table 4). PAs with higher number of available cations have greater effect on fruit firmness, as Spm+4>Spd+3>Put+2 (Valero *et al.*, 2002). According to our results, the PA concentration would also affect the fruit firmness.

Chilling injury index

Chilling injury index drastically increased with time of low temperature storage in both cultivars (Table 4). The increased percentages of 25 days compared to 15 days of low temperature storage for the control treatment were 64 and 102% in M19 and M79, respectively. The combined PA treatments resulted in greater CI index (but less than control) after 25 days of low temperature storage than 15 days, especially when 2:2 mM Put:Spd was used (Table 4). The effects of PA on 15 days low temperature stored tomatoes showed significant decrease in CI index, but its impact for a longer period (25 days) was not significant, except for 1 mM Put (Table 4). The lowest CI index after 15 days of low temperature storage was found in Put:Spm (2:2 mM) treated tomatoes in both cultivars (Table 4). It is possible that the impact of PAs on lowering CI index in tomato are time as well as species dependent. Pre-storage application of PAs improved shelf-life of pomegranate (*Punica granatum* L.) stored at chilling temperature by increasing endogenous polyamine levels (Mirdehghan *et al.*, 2007 a). Although increased endogenous Put in tomato due to low temperature storage is considered to act as protective toward cold stress, the reported mechanism is unclear (Gonzalez-Aguilar *et al.*, 1998). The involvement of polyamines in reducing chilling injury has been related to reducing oxidative damage via increases of antioxidant or reducing the activity of oxidative enzyme (Oufir *et al.*, 2008).

Table 4 - Effect of polyamine application and duration (15 and 25 days) of low-temperature (3°C) storage on fruit firmness and chilling injury index in tomato cultivars M19 and M79

Treatment	Fruit firmness (mm deformation)				Chilling injury index (%)			
	M 19		M 79		M 19		M 79	
	15	25	15	25	15	25	15	25
Control	2.41 a	3.67 a	2.19 a	3.43 a	19.17 a	31.50 a	16.42 a	33.33 a
Put 1 mM	2.04 ab	2.95 b	1.69 b	2.85 b	16.67 ab	25.67 ab	13.33 ab	20.00 b
Put 2 mM	1.82 b	2.48 c	1.45 c	2.52 bc	15.50 b	24.17 abc	9.17 bc	16.67 bcd
Spd 1 mM	1.88 b	2.49 c	1.54 bc	2.79 b	9.17 c	21.67 bc	10.00 bc	17.50 bcd
Spd 2 mM	1.68bc	2.24 cd	1.40 c	2.37 cd	5.83 c	19.18 bc	7.67 cd	14.17 bcd
Put 1 mM + Spd 1 mM	1.68bc	2.16 cd	1.21 d	2.35 cd	6.67 c	22.50 bc	4.17 def	15.00 bcd
Put 1 mM + Spd 2 mM	1.16 c	1.94 d	1.03 d	2.04 de	1.75 d	15.83 c	2.50 ef	11.67 cd
Put 2 mM + Spd 1 mM	1.18c	1.99 d	1.04 d	2.19 cde	0.75 d	21.33 bc	6.67 cde	14.17 bcd
Put 2 mM + Spd 2 mM	0.92 c	1.92 d	0.78 d	1.94 e	0.42 d	17.67 bc	0.0f	10.83 d
LSD value 0.01	0.53	0.49	0.31	0.37	4.47	9.62	5.61	7.85

Means in columns followed by the same letter are not significantly different, P ≤ 0.05, LSD test. Means for each column were compared separately

Decay percentage

Decay incidence in all control treatments was significantly greater than in PA-treated fruits (Table 5). Prolonging low temperature storage from 15 to 25 days resulted in greater decay incidence. Although the differences between PA-treated fruits were not significant, the combined PA treatments could be considered more effective since 0% decay decreases possible further contamination and decay. As a susceptible crop to chilling injury, tomato shows increased susceptibility to decay when stored at low temperatures after harvest (Ding *et al*., 2002). In the present study, treatment with Put:Spd (2:2 mM) was very effective in alleviating chilling injury and decreasing the incidence of decay in tomato fruits (Tables 4, 5). This result suggest that PA application enhances the natural resistance of the fruits to chilling injury and decaying agents. Taken together, results obtained in this study indicate that the higher levels of PAs reduce CI and decay of tomato fruit. The reduction in CI symptoms by PAs has been related to their capacity to preserve membrane integrity, both by lowering the membrane phase-transition temperature fluidity and by retarding lipid peroxidation, resulting in increased cell viability (González-Aguilar *et al*., 2000).

Total soluble solids and titratable acidity

In all cases the highest values for TSS were observed in Put:Spd (2:2 mM), however the differences among other PA combinations were also not significant (Table 5). The differences in TSS between 15 and 25 days of low temperature storage were not significant in the two cultivars (data not shown). The earlier experiment showed no significant changes in TSS between room temperature and low temperature stored tomatoes (Javanmardi and Kubota, 2006). It has been reported that TSS remains unchanged after chilling and reconditioning (Luengwilai and Beckles, 2010), however it is possible that individual sugars and the sugar-acid balance may be adversely affected (Beckles, 2012). Harvesting riper fruit (i.e. those with already well-developed sugar profiles) would reduce the harm caused by chilling injury due to lower temperature (Beckles, 2012).

Titratable acidity in combined PA treatments for fruits stored 15 days at low temperature were significantly greater than other treatments in both cultivars. Treated fruits with Put:Spd (2:2 mM) showed the highest titratable acidity after 25 days of storage (Table 6). Titratable acidity increased with increasing duration of low temperature storage for all treatments (Table 6).

It is reported that increased endogenous Pas, spermine and spermidine, through transgenic manipulation of tomato showed similar levels of juice TSS, pH and titratable acidity in transgenic, azygous, and wild-type fruits (Mehta *et al*., 2002). Application of Put and Spd on apricot did not affect TSS and TA (Koushesh Saba *et al.*, 2012).

Ascorbic acid content

All treated fruits showed greater ascorbic acid in fruits stored for 25 days than 15-day low temperature storage group (Table 6). The pattern of changing ascorbic acid level in tomato fruit varies with the physiological ripening stages as it increases slowly reaching a maximum and then declines slowly coinciding with the initiation of ripening, as indicated by color change, and an increase in the activity of ascorbate oxidase (Yahia *et al*., 2001). In this experiment the mature green fruits treated and stored at low temperature showed the same increasing pattern until 25 days of storage. At that time the color had not started to change. Most likely PA application does not change the ascorbic acid pathway at least until ripening symptoms (color change) appear. It is reported that during the ripening process, the levels of Spd and Spm decline

Table 5 - Effect of polyamine application and duration (15 and 25 days) of low-temperature (3°C) storage on fruit decay percentage and total soluble solid content in tomato cultivars M19 and M79

Treatment	Decay percentage				Total soluble solid content (°Brix)			
	M 19		M 79		M 19		M 79	
	15	25	15	25	15	25	15	25
Control	33.33 a	60.00 a	25.00 a	40.00 a	4.01 b	4.28 b	4.80 b	4.13 c
Put 1 mM	13.33 b	16.33 b	9.67 b	9.67 b	4.50 b	4.85 ab	5.18 ab	4.73 bc
Put 2 mM	6.67 b	11.37 b	3.33 b	4.00 b	5.43 ab	5.13 ab	5.43 ab	5.20 b
Spd 1 mM	3.33 b	12.33 b	3.33 b	6.33 b	5.35 ab	5.08 ab	5.25 ab	5.00 bc
Spd 2 mM	0.00 b	10.00 b	2.33 b	5.00 b	5.55 a	5.25 ab	5.33 ab	5.55 ab
Put 1 mM + Spd 1 mM	0.00 b	6.00 b	0.00 b	3.33 b	5.35 ab	5.30 a	5.35 ab	5.50 ab
Put 1 mM + Spd 2 mM	0.00 b	5.00 b	0.00 b	1.67 b	5.60 a	5.50 a	5.73 a	5.63 ab
Put 2 mM + Spd 1 mM	0.00 b	6.67 b	0.00 b	6.67 b	5.28 ab	5.48 a	5.68 a	5.60 ab
Put 2 mM + Spd 2 mM	0.00 b	5.67 b	0.00 b	3.33 b	5.75 a	5.68 a	5.88 a	5.85 a
LSD value 0.01	14.3	19.07	8.74	12.78	0.96	0.66	0.38	0.40

Means in columns followed by the same letter are not significantly different, $P \leq 0.05$, LSD test. Means for each column were compared separately.

in fruits (Mattoo and Handa, 2008). The ripening process has been shown to be delayed in tomato fruits by infusing them with Put (Saftner and Baldi, 1990). Polyamines and ethylene are known to have opposite effects in relation to fruit ripening and senescence (Saftner and Baldi, 1990). Free polyamines inhibit ethylene production in a variety of tissues (Suttle, 1981) and the elevated level of free polyamines may be responsible for the reduction in both ethylene production and ripening processes of tomato fruits (Saftner and Baldi, 1990). Elevated levels of polyamines help maintain cellular vitality and longer life of ripening tomato (Mattoo *et al*., 2010).

Correlations among fruit characteristics

The correlations among weight loss, electrolyte leakage, chilling injury, decay percentage and fruit firmness for 15 and 25 days of low temperature storage in both cultivars were positive and significant, but they were either non-significant or significantly negative when analyzed against ascorbic acid, titratable acidity and TSS (Tables 7-10). The greatest impact of electrolyte leakage was found on weight loss and decay percentage for the studied

Table 6 - Effect of polyamine application and duration (15 and 25 days) of low-temperature (3°C) storage on fruit juice acidity and ascorbic acid content in tomato cultivars M19 and M79

Treatment	Titratable acidity (g CAE/100 g fw)				Ascorbic acid (mg/100ml)			
	M 19		M 79		M 19		M 79	
	15	25	15	25	15	25	15	25
Control	0.34 b	0.43 c	0.37 bc	0.47 b	9.09 b	11.01 c	11.88 a	12.07 c
Put 1 mM	0.35 b	0.41 c	0.31 c	0.37 c	9.74 b	11.97 bc	10.53 a	12.12 c
Put 2 mM	0.41 b	0.44 c	0.31 c	0.42 c	9.81 b	11.02 c	11.35 a	13.19 bc
Spd 1 mM	0.37 b	0.43 c	0.36 bc	0.39 c	9.60 b	11.97 bc	11.25 a	12.25 c
Spd 2 mM	0.39 b	0.50 b	0.40 bc	0.44 bc	11.01 a	12.44 bc	10.63 a	13.07 bc
Put 1 mM + Spd 1 mM	0.52 a	0.56 b	0.48 ab	0.53 b	10.40 a	13.47 b	11.39 a	15.00 a
Put 1 mM + Spd 2 mM	0.57 a	0.63 a	0.43 abc	0.69 a	11.32 a	17.91 a	12.40 a	15.60 a
Put 2 mM + Spd 1 mM	0.54 a	0.60 a	0.46 ab	0.54 b	11.05 a	12.06 bc	11.88 a	13.62 abc
Put 2 mM + Spd 2 mM	0.58 a	0.70 a	0.56 a	0.65 a	11.80 a	13.60 b	12.15 a	14.03 ab
LSD value 0.01	0.11	0.13	0.13	0.10	1.01	2.35	2.29	2.13

Means in columns followed by the same letter are not significantly different, P ≤ 0.05, LSD test. Means for each column were compared separately.

Table 7 - Correlation analysis between fruit characteristics of tomato cv. M19 treated with different polyamines after 15 days storage at 3°C

	Weight loss	Electrolyte leakage	Chilling injury	Decay percentage	Fruit firmness	Ascorbic acid	Titratable acidity
Electrolyte leakage	0.927 **						
Chilling injury	0.745 **	0.623 **					
Decay percentage	0.769 **	0.803 **	0.694 **				
Firmness	0.753 **	0.761 **	0.741 **	0.699 **			
Ascorbic acid	-0.332 NS	-0.367 NS	-0.258 NS	-0.340 NS	-0.380 NS		
Titratable acidity	-0.679 **	-0.629 **	-0.680 **	-0.555 **	-0.726 **	0.377 NS	
TSS	-0.701 **	-0.719 **	-0.642 **	-0.701 **	-0.618 **	0.083 NS	0.421 **

NS, *, ** non-significant, significant at 0.05 and 0.01, respectively.

Table 8 - Correlation analysis between fruit characteristics of tomato cv. M79 treated with different polyamines after 15 days storage at 3°C

	Weight loss	Electrolyte leakage	Chilling injury	Decay percentage	Fruit firmness	Ascorbic acid	Titratable acidity
Electrolyte leakage	0.950 **						
Chilling injury	0.716 **	0.737 **					
Decay percentage	0.894 **	0.909 **	0.735 **				
Firmness	0.861 **	0.825 **	0.806 **	0.815 **			
Ascorbic acid	-0.018 NS	0.023 NS	-0.162 NS	0.003 NS	-0.166 NS		
Titratable acidity	-0.353 NS	-0.378 NS	-0.664 **	-0.314 NS	-0.617 **	0.291 NS	
TSS	-0.642 **	-0.633 **	-0.632 **	-0.624 **	-0.806 **	0.415 *	0.548 **

NS, *, ** non-significant, significant at 0.05 and 0.01, respectively.

Table 9 - Correlation analysis between fruit characteristics of tomato cv. M19 treated with different polyamines after 25 days storage at 3°C

	Weight loss	Electrolyte leakage	Chilling injury	Decay percentage	Fruit firmness	Ascorbic acid	Titratable acidity
Electrolyte leakage	0.954 **						
Chilling injury	0.625 **	0.714 **					
Decay percentage	0.912 **	0.859 **	0.536 **				
Firmness	0.849 **	0.845 **	0.721 **	0.731 **			
Ascorbic acid	-0.346 NS	-0.287 NS	-0.539 **	-0.400 *	-0.576 **		
Titratable acidity	-0.485 *	-0.532 **	-0.600 **	-0.464 *	-0.664 **	0.545 **	
TSS	-0.837 **	-0.806 **	-0.624 **	-0.710 **	-0.821 **	0.428 *	0.583 **

NS, *, ** non-significant, significant at 0.05 and 0.01, respectively.

Table 10 - Correlation analysis between fruit characteristics of tomato cv. M79 treated with different polyamines after 25 days storage at 3°C

	Weight loss	Electrolyte leakage	Chilling injury	Decay percentage	Fruit firmness	Ascorbic acid	Titratable acidity
Electrolyte leakage	0.946 **						
Chilling injury	0.884 **	0.884 **					
Decay percentage	0.860 **	0.912 **	0.764 **				
Firmness	0.839 **	0.787 **	0.793 **	0.752 **			
Ascorbic acid	-0.429 *	-0.433 *	-0.518 **	-0.415 *	-0.636 **		
Titratable acidity	-0.331 NS	-0.232 NS	-0.344 NS	-0.237 NS	-0.604 **	-0.549 **	
TSS	-0.846 **	-0.795 **	-0.807 **	-0.717 **	-0.862 **	0.651 **	0.605 **

NS, *, ** non-significant, significant at 0.05 and 0.01, respectively.

cultivars and low temperature durations.

4. Conclusions

The results of this experiment indicate that exogenous Put and Spd application on tomato fruit could maintain or even improve fruit quality during low temperature storage. The combined application of Put with Spd (2:2 mM) could be recommended for low temperature and long duration storage of tomato fruits.

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