Effect of Polyamines Application on Reducing Chilling Injury Incidence in Okra Pod (*Abelmoschus esculentus*) Stored at Low Storage Temperature

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Abstract

Generally, immature okra pod is perishable and sensitive to chilling when exposed to temperature below 10 °C. Polyamines application has been claimed to be able to cope with low temperature stress due to their polycationic and antioxidant properties. In the present study, the effects of putrescine, spermidine and spermine on maintaining quality of stored okra pod were investigated. Immature okra pods were treated with putrescine, spermidine and spermine at two different concentrations (0.5 and 1.0 mM) with four replications per treatment. On the other hand, the control okra pods were only dipped in distilled water. All the pods were stored at 4 °C with 85 ± 5% relative humidity for 12 days. Results showed that the okra pods treated with putrescine at both concentrations were significantly lower in chilling injury (CI) incidence (46 to 56%) and weight loss (51 to 68%) than the control. While spermidine and spermine showed no differences with control after 8 storage days. Exogenous putrescine application resulted in a higher DPPH scavenging activity as well as antioxidant enzymes activity of catalase and peroxidase with respect to control after 12 days of storage. These responses could possibly be involved in chilling tolerance in okra pod during cold storage.

Keywords: Catalase, Chilling injury, Okra, Peroxidase, Putrescine, Weight loss

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1. Introduction

The consumption of okra pods has increased over time due to its nutritional values and high antioxidants content (Lin *et al.*, 2014; Sreeshma and Bindu, 2013). However, considering its tropical origin, storage of okra pods at low but non-freezing temperature is limited by development of physiological disorder. This physiological disorder is known as chilling injury (CI) which cause the degeneration of okra pods quality by increasing weight loss, decay and therefore, reduces overall consumer acceptance (Perkins-Veazie and Collins, 1992).

Many postharvest treatments were applied to reduce CI in okra pod such as heat treatment (Rebeiro *et al.*, 2017), intermittent warming (Perkins-Veazie and Collins, 1995), fumigation of 1-methylcyclopropene (1-MCP) (Huang *et al.*, 2012) and modified atmosphere (MA) (Perkins-Veazie and Collins, 1992). However, heat treatment, MA and intermittent warming was not able to prevent CI to occur and only able to extent the shelf to 4–8 days. Additionally, 1-MCP fumigation is time consuming, which required about 16 to 20 h for the treatment to be beneficial. Hence, there is a need to look for a better and more effective method to alleviate CI in okra pod.

Polyamines (PAs) are naturally occurring compounds found in growing cells in all organisms. Three common PAs found in the cell are putrescine (Put), spermidine (Spd) and spermine (Spm) (Serrano *et al.*, 1996). It is reported that PAs has the ability to protect the plant cell from varies stresses through binding with negative charged molecules like phospholipids as well as exhibited antioxidant properties (Gupta *et al.*, 2013; Serrano *et al.*, 1996). The exogenous application of these PAs to cope with plant stresses and maintain the quality of agricultural produces is increasing. Studies have proven that these PAs are able to maintain the postharvest quality of fruits such as application of 2 mM Put maintained strawberry fresh weight (Khosroshahi *et al.*, 2007), 1 mM Put or Spd extend storage life of pomegranate (Mirdehghan *et al.*, 2007), 1 mM Put or Spm reducing CI in zucchini *(Palmaa et al.*, 2015) as well as in 'Valencia' orange when used 5 mM Put (Mohammadrezakhani and Pakkish, 2014).

Nonetheless, little works have reported on the application of these PAs on reducing CI in okra pods. Thus, the objective of this study was to investigate the effect of Put, Spd and Spm on maintaining quality of okra pods stored at 4 °C. The effectiveness of PAs varies in their concentrations with the produces, hence, two difference concentrations (0.5 and 1.0 mM) of each treatment were used to find out which concentration is able reduce CI in okra pod.

2. Materials and Methods

2.1 Plant materials and treatment

Okra pods were harvested from a farm and transported to laboratory within 2 h. The okra pods were then sorted for uniform sizes, mechanical and diseases free. The sorted okra pods were randomly chosen and immersed for 10 min in Put, Spd and Spm at two different concentrations of 0.5 and 1.0 mM each or distilled water as control at which all solutions contained 0.02% Tween-80. There were four replications per treatment and each replication had 10 pods. The treated pods were air dried, weighed for 95 ± 5 g and packed into oriented polypropylene bag (OPP) that being used by exporting company and stored at 4 °C. The treated pods were evaluated for CI index and fresh weight loss every 4 days for 12 storage days. On day 12, the peel was separated, chopped and frozen to check for total phenolics content, antioxidant capacity and antioxidant enzymes.

2.2 Chilling injury index

Chilling injury index was determined according to method described by Huang et al. (2012). The CI index was assessed based on the area of pitting, translucency or discoloration on the surface of the stored okra pods using the scale as follows :0, 0% injury; 1, injury □ 25%; 2, 25% < injury □ 50%; 3, 50% < injury □ 75%, and 4, 75% < injury □ 100%. The CI index was calculated using the formula as per cited.

2.3 Water loss

The pods of each replication stored at 4 °C and 13 °C as well as the pods removed from both storage temperature and held at 25 ± 1 °C for 16 h were weighed before and after storage for every 0, 4, 8 and 12 days .The percentage of weight loss was calculated based on the equation: % Weight loss = [(initial weight – final weight)/initial weight] × 100.

2.4 Total phenolic content (TPC)

Two g of frozen peel was homogenized with 15 mL 80% (v/v) ethanol and followed by centrifuged at 12,000 × g for 10 min at 4 °C .The supernatant was then used to determine total phenolic content and antioxidant capacity.

Total phenolic content was determined using method of Toor and Savage (2005) by adding 1 mL of supernatant into 5 mL Folin-Ciocalteu reagent, followed by 4 mL of 7.5% (w/v) sodium carbonate. The mixture was incubated at 30 °C for 1 h and brought to room temperature for another 15 min. Absorbance was read at 765 nm and the results were obtained by comparing with a standard curve of gallic acid which was expressed as mg gallic acid equivalent (GAE) 100 g^{-1} fresh weight (FW).

2.5 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity

The total antioxidant activity was estimated using DPPH radical scavenging activity following method of Rajurkar and Hande (2011). The DPPH scavenging activity was determined by mixing the extract with ethanolic DPPH (0.1 mM) and incubated in the dark for 30 min. Absorbance was read at 515 nm and the antioxidant activity was calculated.

2.6 Antioxidant enzymes activity

Cold extraction was carried out by homogenizing 2 g of sample with 100 mM sodium phosphate buffer at pH 7.0, containing 1 mM ethylenediaminetetra acetic acid (EDTA) and 1% polyvinylpolypyrrolidone (PVPP). The homogenate was centrifuged at 12,000 × g at 4 °C for 20 min. The supernatant was used to analyze the activities of peroxidase (POD) and catalase (CAT).

The activity of POD was estimated by following method of (Wang *et al.*, 2005). The reaction mixture containing 100 mM phosphate buffer, 24 mM hydrogen peroxide, 8 mM guaiacol and crude enzyme.

CAT activity was measured according to Aebi (1984) with some modification by monitoring the decrease of absorbance at 240 nm. The reaction mixture contained 50 mM phosphate buffer (pH 7.0), 40 mM hydrogen peroxide and enzyme extract. The activity was expressed as mmol H_2O_2 min^{-1} mg^{-1} protein.

2.7 Experimental design and statistical analysis

The experiment was conducted in randomized complete block design with four replications in each treatment and 10 fruits per replication. Data were subjected to a statistical analysis of variance (ANOVA). Means were compared using Tukey test when F-test was significant at p □ 0.05 or p □ 0.01.

3. Results and Discussion

3.1 Chilling injury index and weight loss

The occurrence of physiological disorder during storage of okra at chilling temperature (4 °C) is seen as pitting, water-soaked lesions and surface browning. This physiological disorder is known as CI which is more severe as the storage periods prolonged (Wang, 1994). As shown in Figure 1, CI increased as storage day progress from day 0 to 12. Nonetheless, CI progress slowly for the treated pods with 0.5 mM and 1.0 mM Put, 1.0 Spd and 0.5 Spm on day 8. While on day 12, CI was highly suppressed in pods treated with Put at both concentrations. Similar studies reported that exogenous application of Put suppressed CI in pomegranate (Mirdehghan *et al.*, 2007), 'Valencia' orange (Mohammadrezakhani and Pakkish, 2014) and zucchini (Palmaa *et al.*, 2015). Stored okra pods at 4 °C showed an increased in

weight loss during storage for both control and treated ones. However, the treated pods of Put at both concentrations showed lower percentage of weight loss throughout storage period than control, Spd- and Spm- treated pods (Figure 2). Application of Put is also found to prevent water loss in apricot (Davarynejad *et al*., 2013), mango (Jawandha *et al*., 2012) and strawberry (Khosroshahi *et al*., 2007). There were no significant differences between the pods treated with 0.5 mM and 1.0 mM Put. The polycationic properties of PAs protect cell membranes and maintain cell integrity, thus prevent the cell membrane damage when exposed to chilling temperature (Serrano *et al*., 1996) which could explain the ability of Put to suppressed CI and weight loss in okra pods. Tissue water loss is positively correlated with CI in bell pepper, whereby bell peppers stored at 1 °C lose more water than at 10 °C which due to CI development which causes deterioration of epicuticular wax and cell membrane) Lim *et al*., 2007.

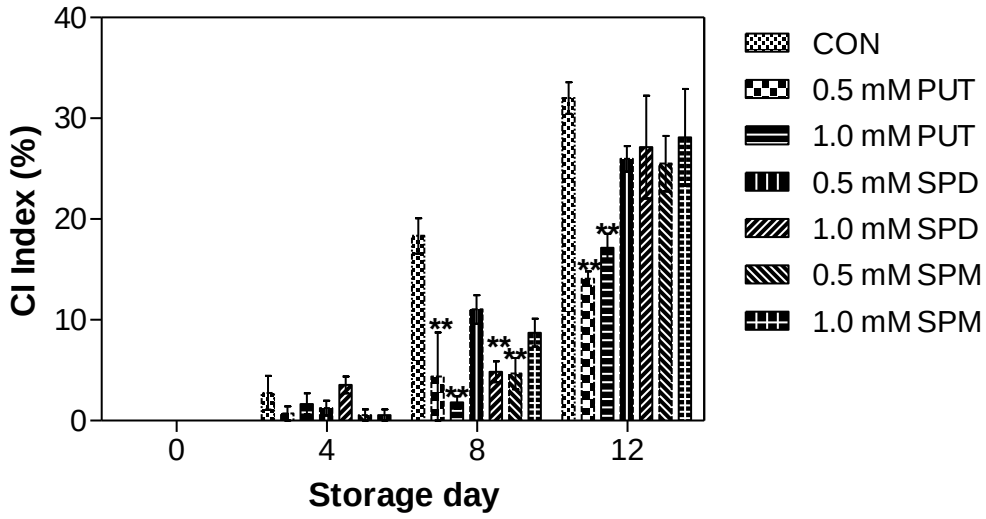

Figure 1 Effect of polyamines treatment on chilling injury index of okra pods stored at 4 °C for 12 days. Data are means ± S.E. of four replicates. Asterisks indicate statistical difference by Tukey test at $p \leq 0.01$ (**)

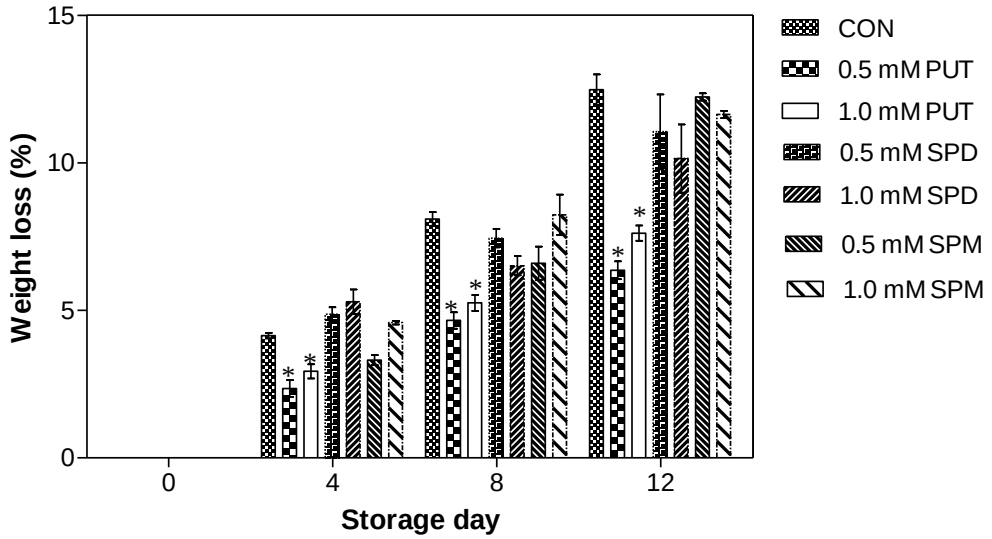

Figure 2 Effect of polyamines treatment on fresh weight loss of okra pods stored at 4 °C for 12 days. Data are means ± S.E. of four replicates. Asterisks indicate statistical difference by Tukey test at $p \leq 0.05$ (*)

3.3 TPC, DPPH scavenging and antioxidant enzymes activity

Antioxidant defense systems play a crucial part in plant to cope with stresses (Sharma *et al.*, 2012). The TPC was affected by treatment whereby 0.5 mM Put showed a significantly higher TPC than 0.5 mM Spd and both concentrations of Spm. Nonetheless, there were no significant differences found among the treated pods of Put (both 0.5 and 1.0 mM), 1.0 mM Spd and control (Figure 3A). Davarynejad *et al.* (2013) reported that TPC is maintain when treated with 4.0 mM Put in apricot, while Mirdehghan *et al.* (2007) reported that the Put treated apricot through immersion technique did not show difference in TPC at the end of storage when compared to pressure infiltration technique. Pressure infiltration technique which force the Put solution directly into fruits is more effective in respect to dipping technique. Thus, the TPC vary according to concentration used as well as technique applied.

Contrary, Figure 4B shows that the treated pods of 0.5 mM and 1.0 mM Put, the DPPH scavenging activity was higher than control. Figure 4A shows that after 12 days of storage, POD activity in Put-treated (0.5 mM and 1.0 mM) and 0.5 mM Spd-treated pods were significantly higher than control pods (36%, 34% and 35%, respectively). While for CAT activity, we found that Put- and Spd-treated pods at both concentrations were significantly higher than control pods after stored at 4 °C for 12 days (86%, 99%, 132% and 95%, respectively) as shown in Figure 3B. Higher CAT, POD and SOD activities were also found in Put- and/or Spd-treated apricot (Davarynejad *et al.*, 2013; Mirdehghan *et al.*, 2007) and kiwifruit (Yang et al., 2016). Elevation of these DPPH scavenging activity and antioxidants enzymes in PAs treatment could be helping okra pods to tolerate chilling stress.

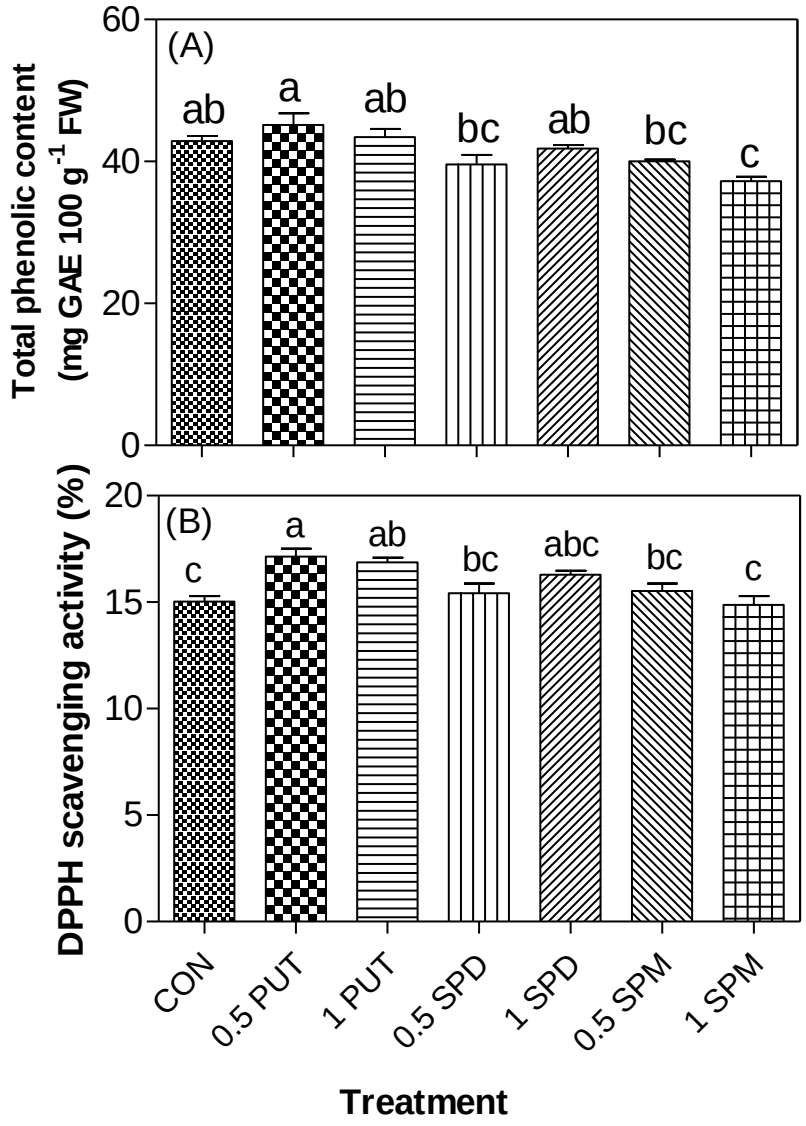

Figure 3 Effect of polyamines treatment on (A) total phenolics content and (B) antioxidant capacity by DPPH scavenging activity assay of okra peel stored at 4 °C on 12 days of storage. Data are means ± S.E. of four replicates. Means with the same letters are not significant different by Tukey test at p □ 0.05.

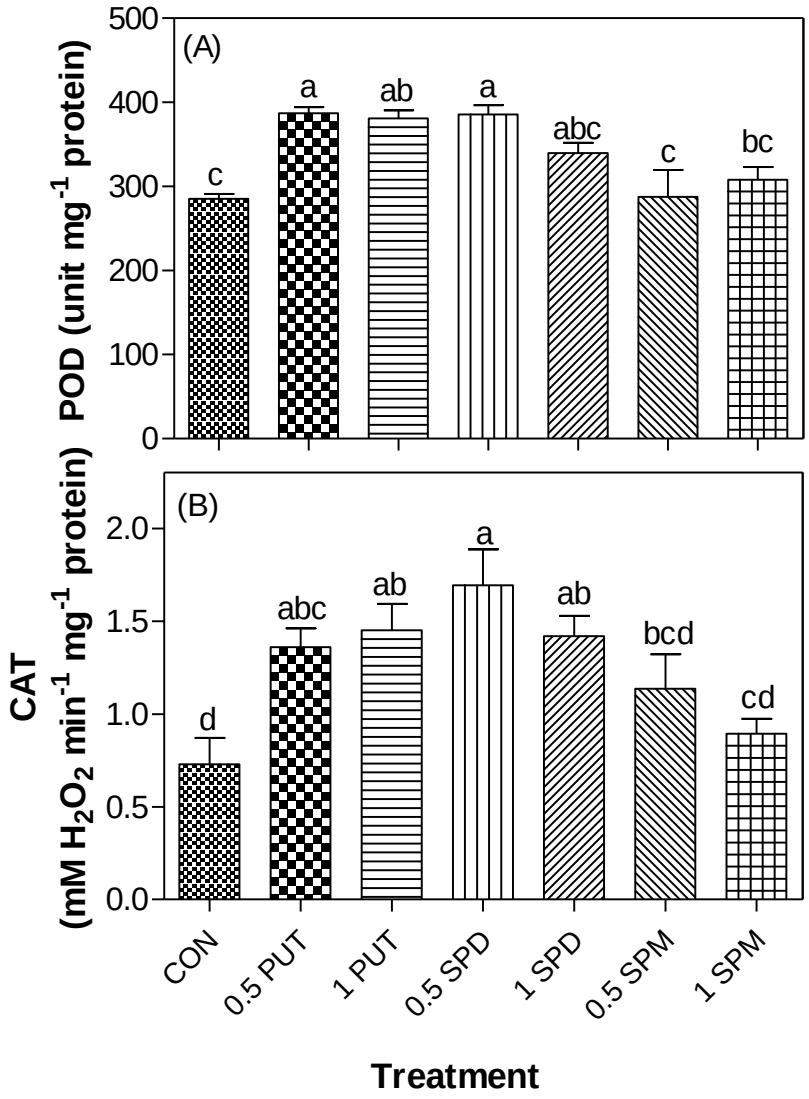

Figure 4 Effect of polyamines treatment on (A) peroxidase and (B) catalase activities of okra peel stored at 4 °C on 12 days of storage. Data are means ± S.E. of four replicates. Means with the same letters are not significant different by Tukey test at p □ 0.05.

4. Conclusion

In conclusion, Put treatment at 0.5 and 1.0 mM using dipping method could be used to maintain quality of okra pods during storage at 4 °C as the Put treatments suppressed CI. This response could be due to the increase in antioxidant activity and antioxidants enzymes.

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