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Short communication

Putrescine treatment suppresses PPO activity and retards fruit browning during ambient storage of pear

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ABSTRACT

The efficacy of putrescine (PUT) application in regulating the polyphenoloxidase (PPO) and peroxidase (POD) enzymatic activity and internal fruit browning of "Punjab Beauty" pear (*Pyrus* spp. cv. Punjab Beauty) was examined during the ambient storage. Postharvest dip treatments of PUT (1mM, 2mM or 3mM) were given for 5 min to the pear fruit. Control fruit were dipped in distilled water only. Fruit were kept at ambient conditions (31 ± 2°C, 78 ± 5% RH) for 15 days. Fruit were analyzed for physical and enzymatic observations on 0 day (before treatment) and at 3, 6, 9, 12 and 15 days of storage. PUT @ 3 mM effectively retained the higher total phenols content (TPC) and POD activity, while lowered the carotenoids content of fruit during storage. Moreover, 3mM PUT significantly delayed the PPO activity along with a reduction in the internal browning of fruit. As compared to the control, PUT treatment maintained the peel colour (*L, *a** and *b**) and 'chlorophyll a and b' content of pear fruit for a longer duration. The present findings exhibited the potential of PUT dip treatment as an effective tool to maintain the postharvest quality by enhancing the TPC, POD and carotenoids content, while reducing the PPO activity and internal browning of pear fruit during ambient storage.**

Key words: *Pyrus pyrifolia*, Internal browning, peroxidase, polyphenol oxidase, putrescine.

Subtropical pears occupy an important place in the horticulture industry of northwestern India. However, a distinctive type of damage in pear fruit is internal flesh browning incidence causes a major problem during storage. Internal browning is characterized by flesh brown colour without a visual appearance on surface traits which is difficult to investigate visually. Internal browning is owing to the cell membrane damage occurs due to the enzymatic oxidation of phenolics substrates to *o*-quinones by activity of PPO enzyme located in the cytoplasm which formed the brown polymer in the cortex (Sun *et al*., 12). Reduction in the capability of antioxidant metabolism such as POD in pear also led to occurrence of internal browning (Lentheric *et al*., 8). Pears are climacteric fruit and lead to enhanced ethylene production during storage of fruit. Generally, exposure of fruit to ethylene for a longer period led to higher browning intensity along with PPO activity and reduction in phenolics content (Couture *et al*., 4).

Polyamines (PAs), a new class of growth substances has anti-stress and anti-senescence properties by their antioxidant behaviour with ability to stabilized cell membrane and cell wall (Velikova *et al*., 14). Among the PAs, PUT is dominant polyamine and antagonist to ethylene production, as it shares the common biosynthesis precursor i.e. *S*-Adenosyl Methionine. In literature, the role of PUT in extending the shelf-life of fruit like apricot (Koushesh Saba *et al*., 7) and mango (Malik and Singh 10) during storage is well documented. To the best of our knowledge, no information is accessible regarding the role of PUT in modulating the PPO, POD activity and phenolics content along with internal browning of sub-tropical pears during ambient storage. Therefore, aim of the present study was to investigate the effect of PUT on PPO, POD enzymatic activity and phenolics content also with internal browning in pear fruit cv. Punjab Beauty during storage at ambient conditions.

Uniform, healthy and free from any visual defect fruit of pear cv. Punjab Beauty were harvested at a commercial maturity (135 days after fruit set) from Fruit Research Farm, Punjab Agricultural University, Ludhiana (30.90°N, 75.79°E). Harvested fruit were immediately shifted to the Post-Harvest Laboratory, and sanitized with 100 ppm chlorinated water. Fruit were immersed in different concentration of PUT (1mM, 2mM or 3mM) for 5 min, while the control fruit were give water dip only. Afterwards, fruit were packed in three-ply corrugated fiber board (CFB) boxes (5% perforation) with paper lining and kept at ambient conditions (31 ± 2°C, 78 ± 5% RH). Each treatment consists of 4 replications and each replication contains 1.0 kg fruit for every storage interval, and a total of 16 kg fruit per treatment were used during the study. Periodical observations were made on 0, 3rd, 6th, 9th, 12th and 15th day of storage. Total phenolics content was estimated as per the method given by Swain and Hills (13). For estimating PPO activity, 2.5g frozen

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core tissue of pear were homogenized in 10mL of 100mM phosphate buffer (pH 7.8) in 1.0g polyvinyl pyrrolidone (PVP), then centrifuged at 10,000rpm for 30 min at 4°C. One unit of PPO activity was considered against 0.01 changes in A410 per min normalized total protein content, and the PPO activity was expressed as U mg^{-1} protein (Bradford 3). For estimation of POD activity, 5.0g tissue sample of pulp was homogenised with 5mL of 100mM sodium acetate buffer (pH 5.5) having 1mM polyethylene glycol (PEG-4000), 1% (v/v) Triton X-100 and 8% (v/v) PVP. The homogenate was centrifuged at 10,000 rpm for 30 min at 4°C. The supernatant was used to analysis the POD activity and expressed as unit/min/g FW (Liu *et al*., 9).

Internal browning of pear fruit was calculated on the number basis by counting the fruit have internal browning in the pulp and expressed in percentage by dividing the number of fruit with internal browning to the total number of fruit. For estimation of chlorophyll and carotenoids content, 0.2g tissue was taken and dipped in 5mL DMSO solution and placed overnight for pigment extraction. The absorbance was read at 480, 645 and 663nm. Chlorophyll and carotenoids contents were expressed as mg/g FW tissue (Barnes *et al*., 1). The peel colour of pear was noted down from both sides of fruit by the help of Colour Flex 45°/0° spectrophotometer (Hunter Lab Colour Flex, Hunter Associates Inc., Reston, VA, USA). The colour was expressed in CIE scale *L** (lightness/darkness), *a** (red/green) and *b** (yellow/blue) (Hunter, 6).

The experiment was conducted during the years 2016 and 2017 and laid out in Completely Randomized Design with four replicates. The data were pooled and analyzed by one-way analysis of variance (ANOVA) and means were separated using LSD test. Differences were considered statistically significant at the level $p \leq 0.05$ using statistical software SAS (version 9.3 for windows). Experimental data were presented as the mean ± standard error.

Total phenolics content in the fruit tissue decreased steadily during the storage in PUT treated fruit (Fig 1). However, a rapid reduction in TPC was observed in

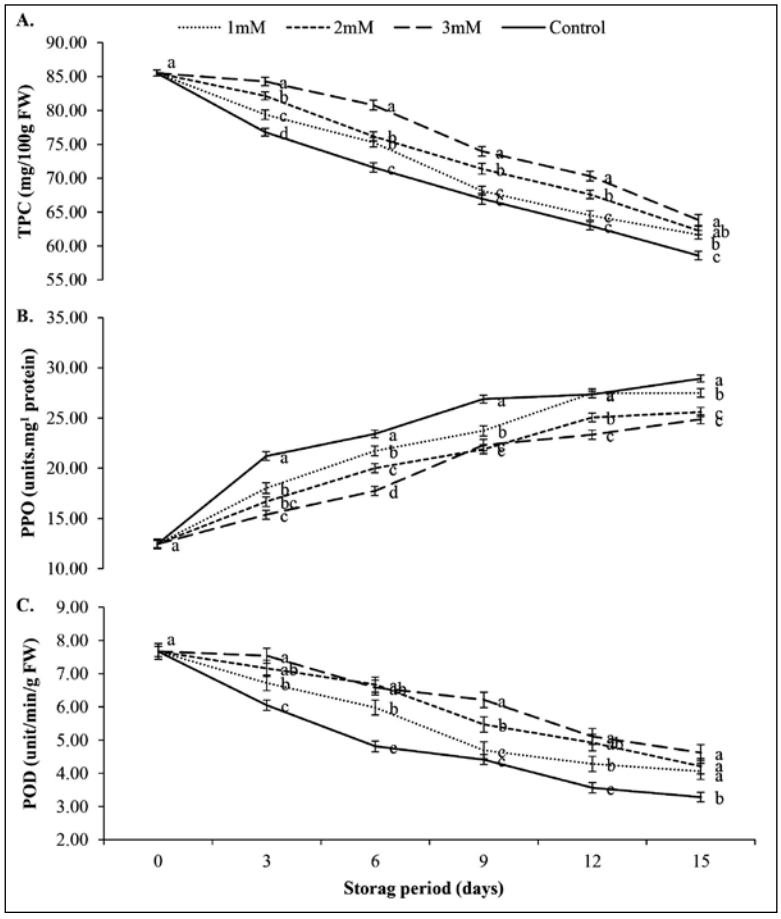

Fig. 1. Changes in TP content (A), PPO (B) and POD activity (c) of pear fruit under ambient conditions in relation to PUT treatments. Vertical bars represent ± S.E. of means for 4 replicates. Mean values followed by same letters on storage day are not significantly different at $*p \leq 0.05$.

the untreated fruit. During the entire storage, control fruit registered 31.50 % reduction in TPC while this reduction was only 25.34 % in 3mM PUT treated fruit. Similarly in apricot, KousheshSaba *et al*. (7) observed a slower reduction in TPC in polyamines treated fruit compared with untreated fruit. The reduction in TPC during storage is due to increased PPO activity; however, polyamines hindered the enzymatic activity of PPO that led to more retention of TPC during storage. A similar effect of PUT on PPO activity of pear fruit was observed in the current study (Fig 1). PUT treatment of 3mM effectively reduced the PPO activity during storage of fruit compared with control. Polyamines have an inhibitory effect on the PPO activity of the fruit (KousheshSaba *et al*., 7). POD is the main enzyme involved in the antioxidant metabolism of fruit. PUT had a significant effect on POD activity of pear fruit during the ambient storage (Fig 1). Enzymatic activity of POD was decreased during the storage of fruit. However, the lowest reduction in activity was recorded in 3mM PUT treated fruit. At the end of storage, fruit treated with 3mM PUT retained 29% higher POD activity compared with control. KousheshSaba *et al*. (7) reported similar results in apricot with the application of polyamines. Clearly, in this investigation, PUT treatment delayed the pear fruit senescence by inducing higher POD activity.

During storage, fruit showed the symptoms of IB (Table 1). Pear fruit during storage at ambient conditions showed no IB up to 12th day of storage. However, at the end of storage, all the PUT treated and control fruit showed an incidence of internal browning. PUT treatments significantly ($p \leq 0.05$) influenced the internal browning incidence, fruit treated with 3mM PUT showed minimum internal browning comparatively 26.75% less than control. Similar to our study, KousheshSaba *et al*. (7) reported a lower incidence of IB in apricot fruit with polyamines treatment. The reduction in internal browning by polyamines application might be due to low enzymatic oxidation by PPO to produce quinones from phenolics substrates in the presence of oxygen (Friedman, 5). The chlorophyll content of treated as well as control group of pear fruit declined

Table 1. Changes in IB, 'Chl a', 'Chl b' and carotenoids content of pear fruit under ambient conditions in relation to different PUT treatments.

Parameters	PUT Treatment	Storage period (days)				
		3	6	9	12	15
IB (%)	1mM (14DBH)	-	-	-	-	12.00±0.44^{b}
	2mM (14DBH)	-	-	-	-	10.90±0.28^{c}
	3mM (14DBH)	-	-	-	-	10.65±0.27^{c}
	Control	-	-	-	-	14.54±0.41^{a}
	Base value: 0.00±0.00					
Chl a (mg/g FW)	1mM (14DBH)	1.75±0.06^{b}	1.42±0.06^{c}	1.14±0.05^{b}	0.92±0.04^{b}	0.80±0.05^{b}
	2mM (14DBH)	1.94±0.06^{a}	1.70±0.07^{b}	1.48±0.06^{a}	1.18±0.06^{a}	0.89±0.05^{b}
	3mM (14DBH)	2.09±0.07^{a}	1.87±0.05^{a}	1.59±0.07^{a}	1.31±0.05^{a}	1.09±0.04^{a}
	Control	1.58±0.06^{b}	1.15±0.05^{d}	0.94±0.07^{c}	0.74±0.05^{c}	0.58±0.03^{c}
	Base value: 2.27±0.05					
Chl b (mg/g FW)	1mM (14DBH)	1.68±0.05^{b}	1.19±0.05^{b}	0.93±0.04^{b}	0.72±0.06^{b}	0.56±0.04^{bc}
	2mM (14DBH)	1.83±0.06^{ab}	1.56±0.07^{c}	1.29±0.04^{a}	0.98±0.05^{a}	0.69±0.05^{b}
	3mM (14DBH)	1.95±0.06^{a}	1.76±0.05^{d}	1.40±0.08^{a}	1.08±0.06^{a}	0.87±0.05^{a}
	Control	1.35±0.05^{c}	0.90±0.06^{a}	0.74±0.05^{c}	0.53±0.04^{c}	0.46±0.04^{c}
	Base value: 2.08±0.05					
Carotenoids (mg/g FW)	1mM (14DBH)	1.47±0.04^{b}	1.69±0.03^{a}	1.81±0.03^{b}	1.86±0.03^{b}	1.88±0.04^{b}
	2mM (14DBH)	1.33±0.03^{c}	1.55±0.03^{b}	1.66±0.04^{c}	1.75±0.03^{c}	1.81±0.04^{bc}
	3mM (14DBH)	1.27±0.02^{c}	1.44±0.03^{c}	1.57±0.03^{c}	1.68±0.03^{c}	1.75±0.03^{c}
	Control	1.59±0.03^{a}	1.77±0.04^{a}	1.91±0.04^{a}	1.97±0.02^{a}	2.03±0.04^{a}
	Base value: 1.20±0.02					

Mean values followed by same superscript within a column are significantly at par ($p \leq 0.05$), each point represents the mean ± S.E. of 4 replicates.

continuously during ambient storage (Table 1). There was a slow and gradual decline in chlorophyll content in PUT treated samples, while it decreased sharply in the control group. For instance, in 3mM PUT treatment, the 'chlorophyll a and b' content reduced only to 47.85% and 55.38%, respectively, relative to initial chlorophyll content, but it was reduced to 63.29% and 65.93% in control. Results indicated that the PUT effectively suppressed the chlorophyll loss in postharvest pears. Beigbeder *et al*. (2) reported that polyamines stabilized the photo-system complexes during storage, which could reduce the chlorophyll loss and carotenoids synthesis in thylakoid membranes. A significant difference of carotenoids content between PUT treatment and control was observed during the storage (Table 1). The rate of carotenoids synthesis in control was higher compared to the PUT treated fruit. At the end of storage, the 3mM PUT treated fruit registered 13.79% less carotenoids content synthesis than in control. Malik and Singh (10) also observed the low carotenoids content synthesis in PUT treated mango fruit during storage along with low chlorophyll degradation compared with control.

Fruit colour is a vital factor reflecting the maturity and quality of fruit. The effect of PUT on colour changes of pear fruit during ambient storage is presented in Fig 2. Colour values (*L**, *a** and *b**) of

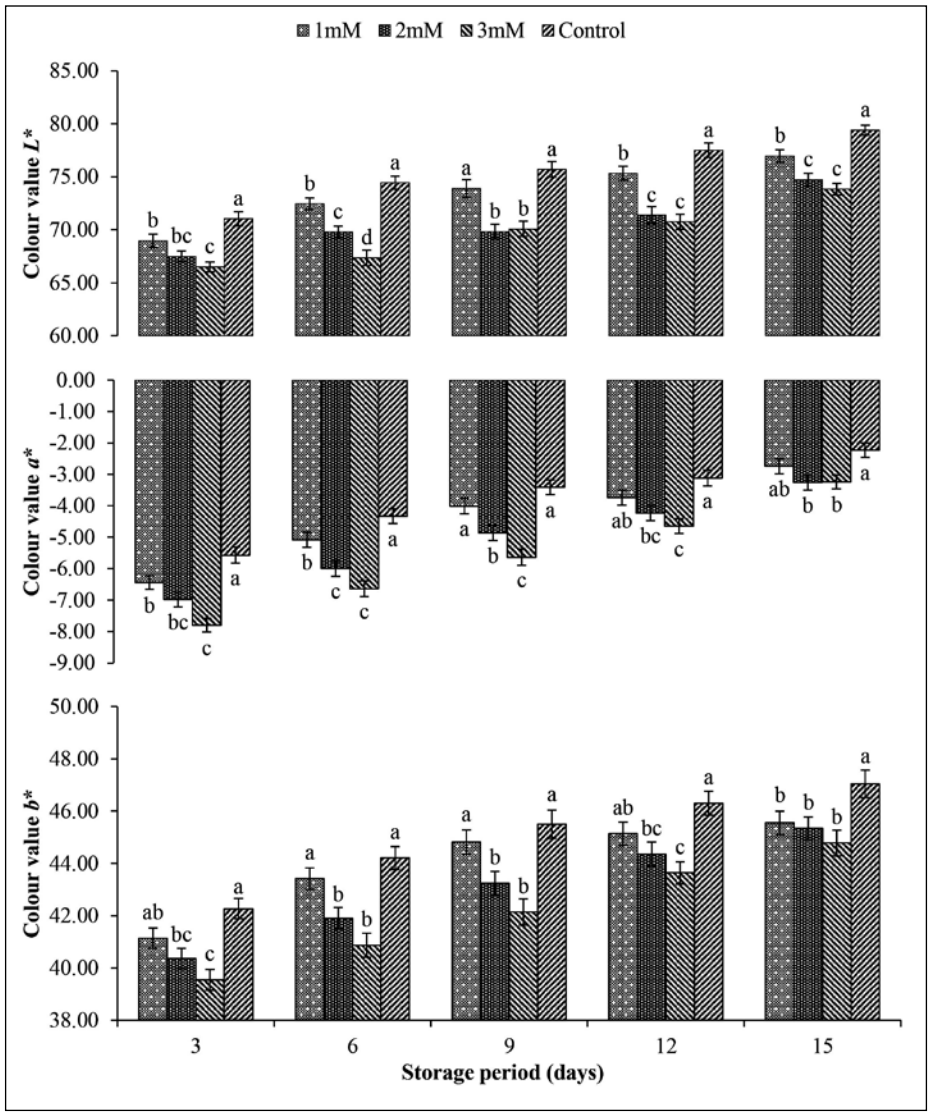

Fig. 2. Changes colour value *L**, *a** and *b** of pear fruit during ambient storage in relation to different PUT treatments. Vertical bars represent ± S.E. of mean for 4 replicates. Base value: *L**: 65.68, *a**: -7.70 and *b**: 38.42

Mean values followed by same superscript within an interval are not significantly different at **p* ≤0.05.

treated and untreated fruit increased continuously during storage. However, the changes in colour coordinates were significantly ($p \leq 0.05$) lower in 3mM PUT treated fruit followed by 2mM PUT treatment while the maximum variations are recorded in control fruit. The lower value of *L** and *b** while higher *a** value indicating that 3mM PUT treated fruit were greener than the lower concentration of PUT treatment and control. Malik and Singh (10) also reported lower fruit colour development in PUT treated mango fruit. Lower chlorophyll degradation and carotenoids synthesis retarded the fruit colour development with PUT application. Exogenous polyamines application retards the hydrolytic activities of chloroplast thylakoid membranes, which reduced the chlorophyll content degradation and delayed the peel changes during storage and ripening of fruit (Popovic, 11).

In conclusion, our investigation showed that 3mM PUT significantly prolonged the shelf life of pear fruit of Punjab Beauty cultivar. The pear fruit of PUT treatment had lower internal browning with reduced PPO and higher POD activity and total phenolics content. PUT also reduced the chlorophyll degradation along with colour changes during storage.

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REFERENCES

1. Barnes, J. D., Balaguer, L., Manrique, E., Elvira, S. and Davison, A. W. 1992. A reappraisal of the use of DMSO for the extraction and determination of chlorophylls a and b in lichens and higher plants. *Environ. Exp. Bot.* **32**: 85-100.

2. Beigbeder, A., Vavadakis, M., Navakoudis, E. and Kotzabasis, K. 1995. Influence of polyamine inhibitors on light-independent and light-dependent chlorophyll biosynthesis and on the photosynthetic rate. *J. Photochem. Photobiol.* **28**: 235–42.

3. Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem.* **72**: 248–54.

4. Couture, R., Cantwell, M. I., Ke D. M. E. and Saltveith M. E. J. 1993. Physiological attributes related to quality attributes and storage life of minimally processed lettuce. *HortSci.* **28**: 723–25.

5. Friedman, M. 1996. Food browning and its prevention: An overview. *J. Agric. Food Chem.* **44**: 631-53.

6. Hunter, S. 1975. *The Measurement of Appearance*. John Wiley and Sons, New York. 304-05p.

7. KousheshSaba, M., Arzani, K. and Barzegar, M. 2012. Postharvest polyamine application alleviates chilling injury and affects apricot storage ability. *J. Agric. Food Chem.* **60**: 8947−53.

8. Lentheric, I., Pinto, E., Graell, J. and Larrigaudiere, C. 2003. Effect of CO_2 pretreatment on oxidative metabolism and core-browning incidence in controlled atmospheric pear. *J. Hortic. Sci. Biotech.* **78**: 177-81.

9. Liu, H., Jiang, W., Zhou, L., Wang, B. and Luo, Y. 2005. The effects of 1-methylcyclopropene on peach fruit (*Prunus persica* L. cv. Jiubao) ripening and disease resistance. *Int. J. Food Sci. Technol.* **40**: 1–7.

10. Malik, A. U. and Singh, Z. 2005. Pre-storage application of polyamines improves shelf-life and fruit quality of mango. *J. Hort. Sci. Biotech.* **80**: 363-69.

11. Popovic, R. B., Kyle, D. J., Cohen, A. S. and Zalik, S. 1979. Stabilization of thylakoid membranes by spermine during stress induced senescence of barley leaf discs. *Plant Physiol.* **64**: 721-26.

12. Sun, J., You, X. R., Li, L., Peng, H. X., Su, W. Q., Li, C. B., He, Q. G. and Liao, B. 2011. Effects of a phospholipase D inhibitor on postharvest enzymatic browning and oxidative stress of litchi fruit. *Postharvest Biol. Tec.* **62**: 288–94.

13. Swain, T. and Hills, W. E. 1959. The phenolics constituents of *Prunus domestica* in the quantitative analysis of phenolic constituents. *J. Sci. Food. Agric.* **10**: 63-68.

14. Velikova, V., Yordanov, I. and Edreva, A. 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants. Protective role of exogenous polyamines. *Plant Sci*. **151**: 59–66.

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