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Effects of putrescine postharvest dips and refrigerated storage temperature on quality attributes and shelf-life of 'Solo' papaya fruit

Eulenda Mabunda[1], Tieho Mafeo[1], Nhlanhla Mathaba[1], Duduzile Buthelezi[2], Thabiso Satekge[1*]

[1]University of Limpopo, Department of Plant Production, Soil Science and Agriculture Engineering, Private Bag X1106, Sovenga, 0727, South Africa
[2]Sefako Makgatho Health Sciences University, School of Science and Technology, Private Bag X 180 Rankuwa, 0208, South Africa

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***Corresponding author:**
[1]University of Limpopo, Department of Plant Production, Soil Science and Agriculture Engineering, Private Bag X1106, Sovenga, 0727, South Africa.

Email: thabiso.satekge@ul.ac.za

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ABSTRACT

Purpose: Low temperature storage is commonly used to extend papaya (*Carica papaya* L.) fruit storability. The optimal recommended storage temperature is below 10 ˚C for export and distant markets. However, chilling injury (CI) disorder occurs at 10 ˚C or lower temperatures (5-8 °C) during prolonged cold storage. Chilling injury affects fruit quality and consumer preference. Therefore, the study investigated the potential of postharvest polyamine dips to improve the quality and shelf-life of 'Solo' papaya fruit. **Research Method**: Mature papaya fruit were treated with putrescine (PUT) dips (0, 1, 2 or 3 mM) and stored for 21 days at 7.5 ˚C plus 6 days at ambient temperature. **Findings**: The results showed that 2 and 3 mM PUT treatment significantly ($P<0.05$) reduced mass and firmness loss compared to 1 mM PUT dips and untreated fruit. The same trend was observed in peel colour change. Furthermore, the results showed that 2 mM PUT treatment retained lower titratable acid and total soluble solids values compared to control fruit. **Research limitations**: The study did not focus on Put mode of action including antioxidant system response. **Originality/Value**: The study demonstrated that 2 and 3 mM PUT postharvest dips reduce 'Solo' papaya pathological and physiological disorders during low temperature long storage. Therefore, 2 mM has the potential to improve postharvest quality by reducing the onset/development of pathological and physiological disorders under low temperature storage thereby benefitting exporters.

INTRODUCTION

Papaya fruit (*Carica papaya* L.) is native to Southern Mexico and neighbouring Central America (Sharma, 2015). In tropical and subtropical zones, papaya is popular; and economically important fruit (Silva et al., 2007). It is high in health promoting compounds such as vitamin C, vitamin A, riboflavin, folate, calcium, thiamine, iron, niacin, potassium and fibre (Pawase et al., 2018). However, papaya is a typical climacteric fruit with a short shelf-life at ambient storage, leading to a high postharvest loss (Lanka et al., 2011; Marpudi et al., 2011). During postharvest storage, papaya fruit undergoes physico-chemical changes including moisture loss, softening, sugars and acid changes and subsequently quality deterioration (Ahmad & Siddiqui, 2015; Lata, 2017; Ngnamba, 2013; Workneh et al., 2012). These changes lead to a reduced shelf-life of the fruit, which may vary between 3-6 days, depending on the cultivar (Jayathunge et al., 2011; Parven et al., 2020). To delay these changes and prolong papaya fruit shelf-life, cold storage has been used as a postharvest technique (Ahmad & Siddiqui, 2015; Ayomide et al., 2019; Jawandha et al., 2012).

The optimum temperature for cold storage depends on maturity stage. For instance, fruit harvested at mature-green to one-fourth yellow stage are stored at 13 $^{\circ}$C for up to 21 days, whereas for export and distant market, partially ripe fruit showing one-fourth to one-half yellow colour is stored at 10 $^{\circ}$C or lower temperatures for 16 days (Kader, 2006; Workneh et al., 2012). However, low temperatures for papaya storage may be limited due to the fruit susceptibility to chilling injury leading to poor quality and short shelf-life (Ahmad and Siddiqui, 2015; Ngnamba, 2013; Perez et al., 2004; Workneh et al., 2012). To overcome this, constrain, several postharvest treatments like chemicals, hot water treatment and the application of wax and wraps have been used in the past (Lata, 2017). Generally, the use of fungicides have been the most common and effective technique to reduce postharvest disorders in papaya fruit during storage (Hanif et al., 2020). However, it has been criticised due to its detrimental effect on human and the environment, therefore, environmentally friendly techniques are needed. Recently, postharvest application of polyamines (PAs) is merging as a natural technique used to improve the shelf-life of fruits under cold storage (Bhat et al., 2013; Koushesh-Saba et al., 2012; Razzaq et al., 2014).

Putrescine (PUT), spermine (SPM), cadaverine (CAD) and spermidine (SPD) are biologically active forms of PAs that can regulate various physical, physiological and biochemical processes of fruits (Fawole et al., 2020; Khan et al., 2007). Studies showed that postharvest application of PAs reduced chilling injury, colour and firmness loss in 'Native' and 'Cavendish' banana (Hosseini et al., 2018), 'M19' and 'M79' tomato (Javanmardi et al., 2013) and 'Langra' mango fruit (Jawandha et al., 2012). Similarly, in 'Samar Bahisht Chaunsa' mango fruit stored at 11±1 $^{\circ}$C, 2 mM PUT delayed fruit ripening and senescence (Razzaq et al., 2014). Recently, it was shown that 2 mM PUT is the most effective concentration for extending the shelf life of 'Red lady' papaya fruit at 12 $^{\circ}$C (Hanif et al., 2020). However, there is limited information on the use of PUT to prolong 'Solo' papaya shelf-life and maintain quality at 10 $^{\circ}$C, a recommended temperature for fruit harvested at 25%. The use of PUT to inhibit CI in papaya could be a future tool to prolong its marketing potential through standardization with recommended harvest maturity and storage conditions. Therefore, the study investigated the potential of postharvest PUT dips to prolong 'Solo' papaya fruit shelf-life and maintain quality during cold storage.

MATERIALS AND METHODS

Plant materials and study sites

Matured and uniform-sized 'Solo' papaya fruits were harvested at 25% yellow colour break from Kudu farm, Low`s Creek, Nelspruit, Mpumalanga, South Africa (25°58'07'' S, 31°30'04'' E). Fruits were transported in a ventilated vehicle to the Agricultural Research Council (ARC-TSC) postharvest laboratory in Nelspruit (25°28'0'' S, 30°58'0'' E) for postharvest PUT treatment, storage and analysis.

Postharvest procedures, treatment and design

In the laboratory, fruit without bruises, damage, punctures and diseases were randomly selected and dipped in different solutions of Putrescine (0, 1, 2 or 3 mM) for 60 minutes. Thereafter, treated fruits were allowed to air-dry for 30 minutes at ambient temperature. Subsequently, 16 fruits per treatment were stored at 7.5 °C and 90±5% relative humidity (RH) for 21 days. Fruits were then transferred to shelf-life condition (ambient storage) for 6 days. During shelf-life, mass loss, firmness, colour attributes, total soluble solids, titratable acidy, chilling injury and anthracnose incidence were determined at 1-day interval until day 6. The experiment was carried out as a completely randomized design with treatment arranged in a factorial manner, 4 polyamine dips (0,1, 2 and 3 mM) and 6 shelf-life days.

Determination of fruit mass loss

Fruits were weighed using a digital weighing balance (SBA 61, Scaltec instruments, Heiligenstadt, Germany). The percentage of mass loss was calculated as the difference between initial fruit mass and final mass to the initial fruit mass. Mass loss percentage was calculated using Eq. (1) as follows (Gharezi et al., 2012):

$$\text{Mass loss (\%)} = \frac{Initial\ mass - final\ mass}{Initial\ mass} x\ 100\% \qquad (1)$$

Determination of firmness

Fruit firmness was determined using a Sinclair IQ™ automated desktop machine (Model: 53524, Bareiss, Oberdischingen, Germany). The firmness of each fruit was determined by taking the mean of three readings at the equatorial region and expressed as newton (N) (Hanif et al., 2020).

Determination of peel colour

Peel colour was determined using a handheld Minolta chromameter (Minolta CR-400 Corp, Ramsey, NJ, USA) with a white calibration plate (Y = 87.00; x = 0.3146; y = 0.3215). Chromameter was initially calibrated and the colour parameters readings, L* value (lightness), chroma (C*), a* (greenness), b* (yellowness) and hue angle were displayed automatically and recorded (Chepngeno et al., 2016).

Determination of total soluble solids and titratable acidity

A total number of four fruits per treatment were used for determining total soluble solids (TSS) and titratable acidity (TA). A digital refractometer (121, Yagami International Ltd, Tokyo, Japan) was used to determine the TSS from papaya juice. The TSS was measured before and after storage until the end of the shelf-life using a drop of juice, and values were expressed in °Brix (Pila et al., 2010). The method used to determine TA was described by Fadanelli et al. (2019). In brief, 10 g of papaya juice were diluted in 40 ml of distilled water

and titrated with 0.1N sodium hydroxide (NaOH) to pH 8.1. The TA was expressed as g citric acid/kg papaya, using the following equation (2):

$$\text{TA (g citric acid/kg of papaya)} = \frac{VX0.1X1000X0.064}{M} \quad (2)$$

Where: 0.1 is the normality of NaOH (N), 0.064 is the conversion factor for citric acid, V is the volume of NaOH required (mL) and m is the mass of papaya juice sample used (g).

Determination of chilling injury

Using a visual scale, chilling injury was determined using a three-category chilling injury index (CII): 0 = no chilling injury; 1 = slight chilling injury, 2 = moderate chilling injury; and 3 = severe chilling injury. The CII was calculated using the following formula (3) (Herrera, 2007):

$$\text{CII} = \sum \frac{No\ of\ fruit\ in\ a\ scale X Scale\ value}{Total\ no\ of\ evelauted\ fruit} \quad (3)$$

Determination of anthracnose

Anthracnose was characterised by round brownish depressed lesions (Moraes et al., 2013). Using a visual scale, fruit were classified into four categories: 0 = no mould growth; 1 = slightly visible mould growth; 2 = 10-40% surface area covered with mould growth and 3; when greater than 40% fruit surface area of the fruit was covered with mould growth (Lata, 2017).

Data analysis

The analysis of variance (ANOVA) was carried out using GenStat® 21th version computer-based statistical software (VSN international Hemel Hempsted, UK). The significant difference between treatments means was separated using Least Significant Difference (LSD) at $P \leq 0.05$.

RESULTS AND DISCUSSION

Fruit mass loss

All the treatments affected mass loss in papaya fruit during the entire storage duration and shelf-life days. In general, mass loss increased irrespective of the treatment (Fig. 1). However, untreated fruit showed the highest mass loss compared with PUT treated fruit throughout shelf-life. Generally, 3 mM PUT exhibited the lowest cumulative mass loss during shelf life of 6 days compared to the control. Additionally, smaller mass loss was reported on 'Native' and 'Cavendish' banana fruit treated with 2 mM PUT when compared with control fruit at 2 °C (Hosseini et al., 2018). Mass loss in stored papaya fruit is mainly due to rapid respiratory activities, water loss (through the skin) and the consumption of stored metabolites during metabolic activities and it becomes apparent as shriveling (Fawole et al., 2020). Mass loss in papaya fruit has been reduced with the application of PUT dips. The lower mass loss in PUT treated fruit could be attributed to stabilization or consolidation of both cell integrity and the permeability of tissues. Furthermore, PUT prevents the loss of water during metabolic processes such as respiration and transpiration, consequently reducing mass loss in papaya during storage.

Firmness

Figure 2 showed that firmness of 'Solo' papaya fruit decreased during ripening irrespective of the treatment. In general, control fruit exhibited the lowest firmness throughout shelf-life days when compared with PUT treated fruit. Contrary, 2 mM PUT treated fruit were firmer than untreated fruit throughout shelf-life. Fruit softening results from the activity of hydrolyzing enzymes (such as pectinesterase (PE), pectinmethylesterase (PME) and polygalacturonase (PG) and rapid production of reactive oxygen species (ROS) during storage (Cheng et al., 2008). The results suggest that PUT dips delayed 'Solo' papaya fruit softening at ambient temperature. Similar results were reported by Javanmardi et al. (2013), who found that 'M19' and 'M79' tomato treated with 2 mM had higher firmness during storage at 13 °C. The effect of polyamines (PAs) on fruit softening or firmness reduction augmentation is thought to be a result of their bonds with pectin in the cell wall leading to a physically stabilized cell wall, which is detectable immediately after treatment (Hosseini et al., 2018). The bonds between PAs and pectin also inhibit the activity of wall-degrading enzymes; thus, reducing fruit softening (Fawole et al., 2020). Additionally, the use of PUT dips enhanced firmness retention, probably to reduced respiration rate and water loss (Fig. 1).

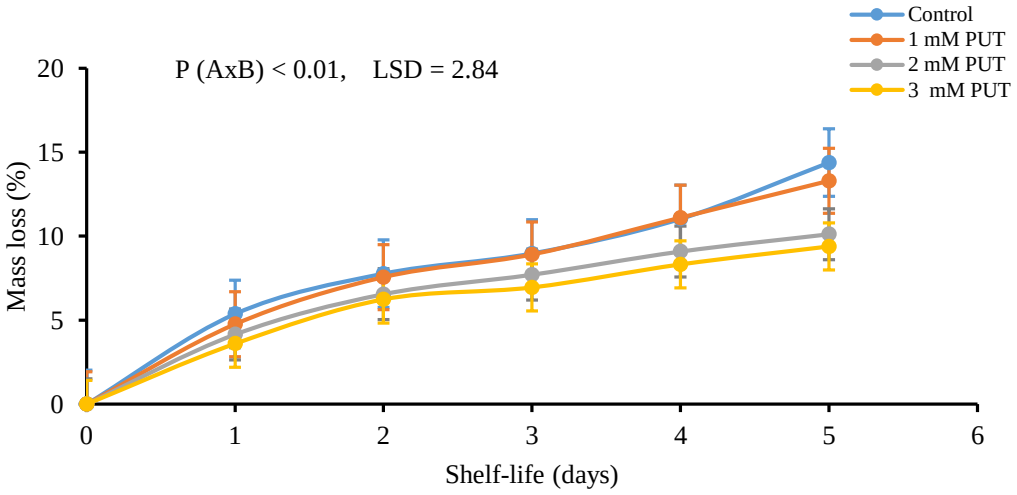


Fig. 1. Mass loss (%) of putrescine treated 'Solo' papaya fruit after 21 days' storage at 7.5 °C plus 6 days shelf-life (n = 8). Error bars indicate ±SE of means at P ≤ 0.05. A = treatment, B = shelf-life days, A x B = interaction of treatment and shelf-life days. LSD, least significant difference.

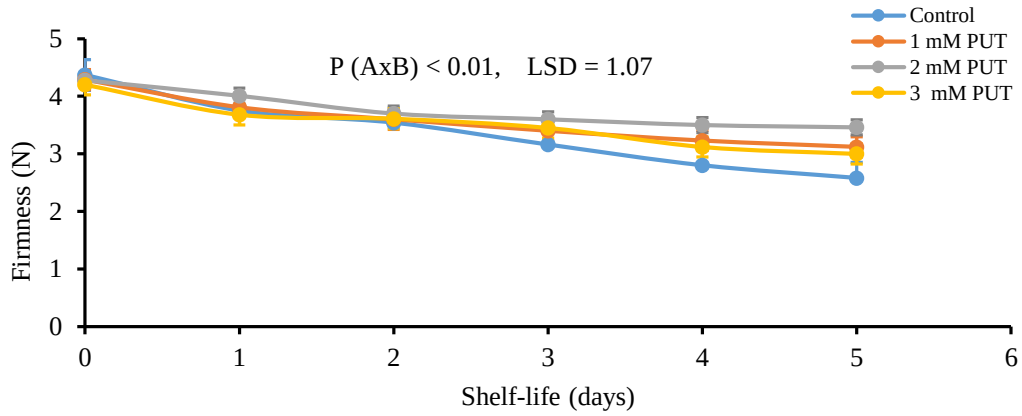


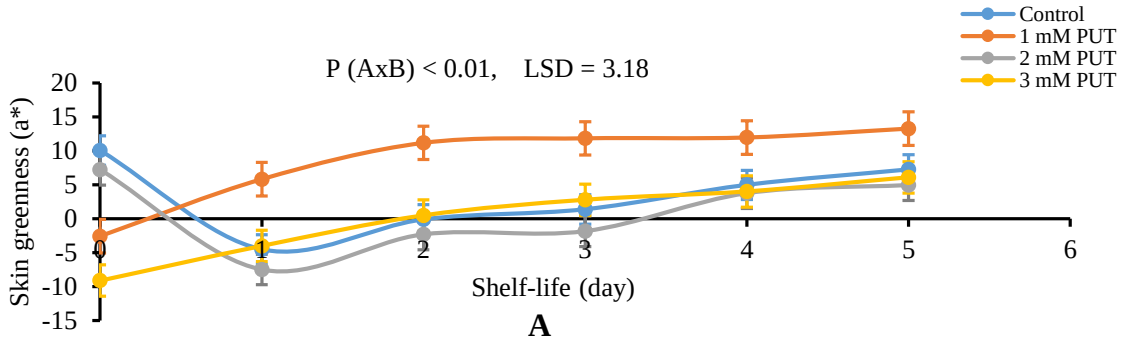
Fig. 2. Firmness (N) of putrescine treated 'Solo' papaya fruit after 21 days' storage at 7.5 °C plus 6 days' shelf-life (n = 8). Error bars indicate ±SE of means at P ≤ 0.05. A = treatment, B = shelf-life days, A x B = interaction of treatment and shelf-life days. LSD, least significant difference.

Peel colour change

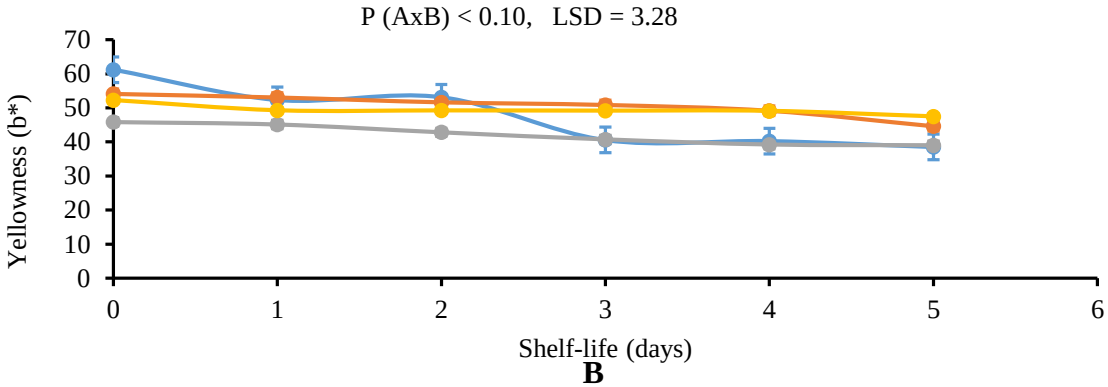
Generally, control fruit exhibited rapid yellow colour development when compared with PUT treated fruit during shelf-life. Additionally, untreated fruit showed significantly higher a* values when compared with PUT treated fruit throughout shelf-life (Fig. 3A). Similarly, lower b*, C* and L* values were observed on PUT-treated fruit compared to respective controls (Fig. 3 A, C and E). In contrast, PUT treatment retained maximum h° values when compared to untreated fruit during shelf-life. A decrease in h° is characteristic of ripening development and colour change from green to yellow due to chlorophyll degradation and the progressive increase in tissue softness (Fig. 3) during storage in papaya fruit (Abbasi et al., 2019). The results showed that PUT significantly decreased rapid yellow colour development on papaya peel. Similarly, the effect of 2 mM PUT on delaying colour change during ripening was reported in 'Langra' mango fruit at 13 $^{\circ}$C (Jawandha et al., 2012). The retardation of green peel colour development by PUT treatment indicates lower chlorophyll degradation and carotenoids biosynthesis due to respiration rate (Fig. 1) and ethylene production suppression (Fig. 2); and consequently, a delayed senescence (Razzaq et al., 2014; Jawandha et al., 2012). It has also been suggested that suppression in ethylene production in PUT treated fruit may also be ascribed to the reduction in the activities of 1-aminocyclopropane-1-carboxylic acid synthase (ACS) and 1-aminocyclopropane-1-carboxylic acid oxidase (ACO) enzymes (Razzaq et al., 2014). Additionally, Figure 3 (D) shows that PUT treatment resulted in a reduced or slow green colour change in papaya fruit due to inhibited ethylene synthesis.

Titratable acidity (TA)

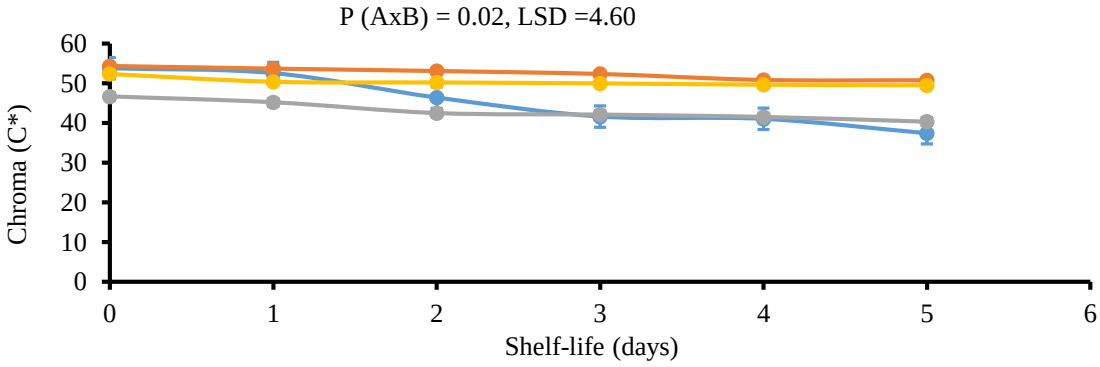
In general, there was a gradual decrease in TA across all the treatments. However, 3 mM PUT was efficient in maintaining the highest TA throughout storage and during shelf-life days when compared with untreated fruit (Fig. 4). The above findings are in conformity with Hosseini et al. (2018) work on 'Native' and 'Cavendish' banana and Jawandha et al. (2012) on 'Langra' mango fruit. In papaya fruit, there is a decrease in TA during ripening associated with the conversion of organic acids into sugars and their derivatives or their utilization in respiration as respiratory substrate (Lata, 2017). Therefore, an increase in TA related to PUT exogenous application is attributed to its role on delaying respiration rate (Fig. 1) and ethylene production (Fig. 3D) in papaya fruit during ripening. Additionally, lower acidity rate in PUT treated papaya fruit could also be due to the suppression of ascorbate oxidase activity by PUT (Malik et al., 2006).


Control
1 mM PUT
2 mM PUT
3 mM PUT
P (AxB) < 0.01, LSD = 3.18
Skin greenness (a*)
Shelf-life (day)

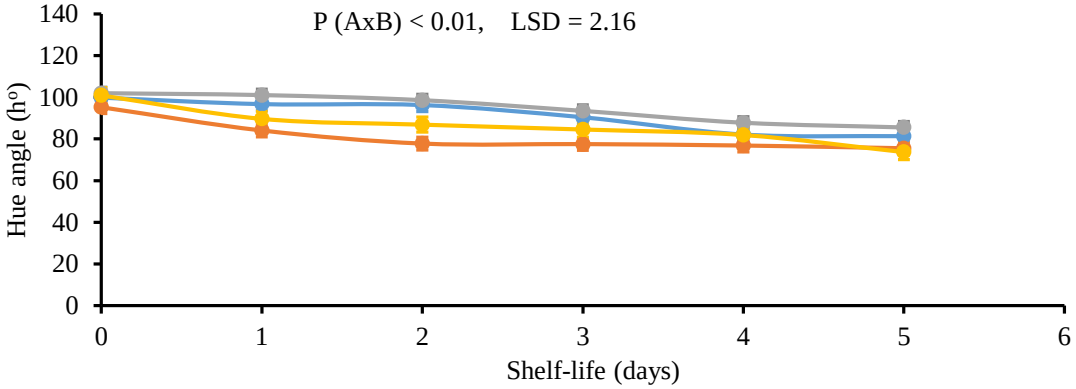
A


P (AxB) < 0.10, LSD = 3.28
Yellowness (b*)
Shelf-life (days)

B


P (AxB) = 0.02, LSD =4.60
Chroma (C*)
Shelf-life (days)

C


P (AxB) < 0.01, LSD = 2.16
Hue angle (h°)
Shelf-life (days)

D

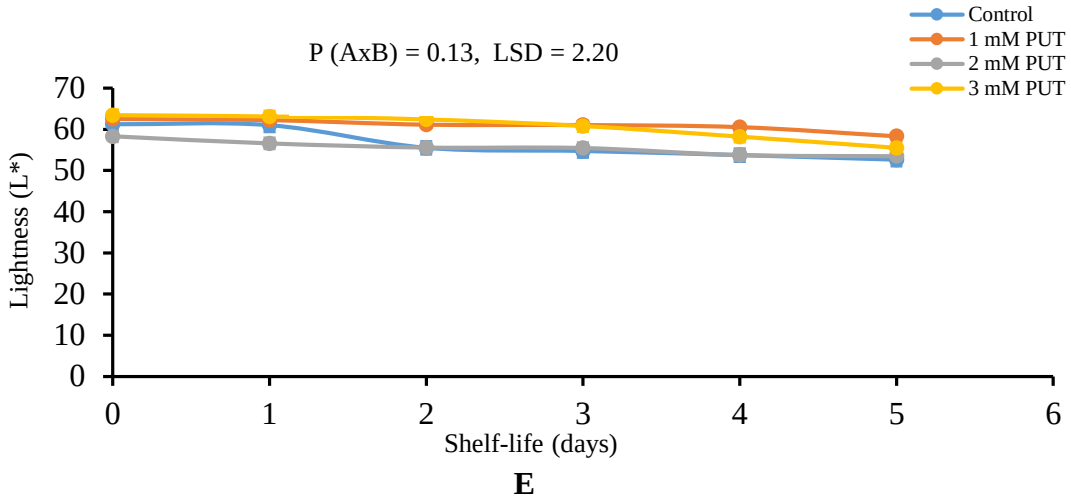


Fig. 3. Change in (A) a* values; (B) b* values; (C) C* values; (D) h˚ values and (E) L* values of putrescine treated 'Solo' papaya fruit after 21 days of storage at 7.5 ˚C plus 6 days shelf-life (n = 8). Error bars indicate ±SE of means at P ≤ 0.05. A = treatment, B = shelf-life days, A x B = interaction of treatment and shelf-life days. LSD, least significant difference.

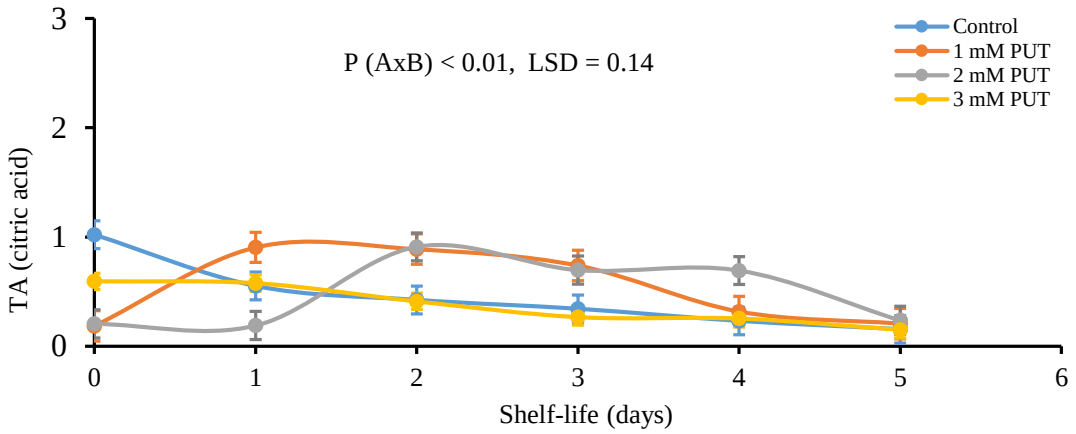


Fig. 4. Titratable acidity (TA) of PUT treated 'Solo' papaya fruit after 21 days of storage at 7.5 ˚C plus 6 days shelf-life (n = 8). Error bars indicate ±SE of means at P ≤ 0.05. A = treatment, B = shelf-life days, A x B = interaction of treatment and shelf-life days. LSD, least significant difference.

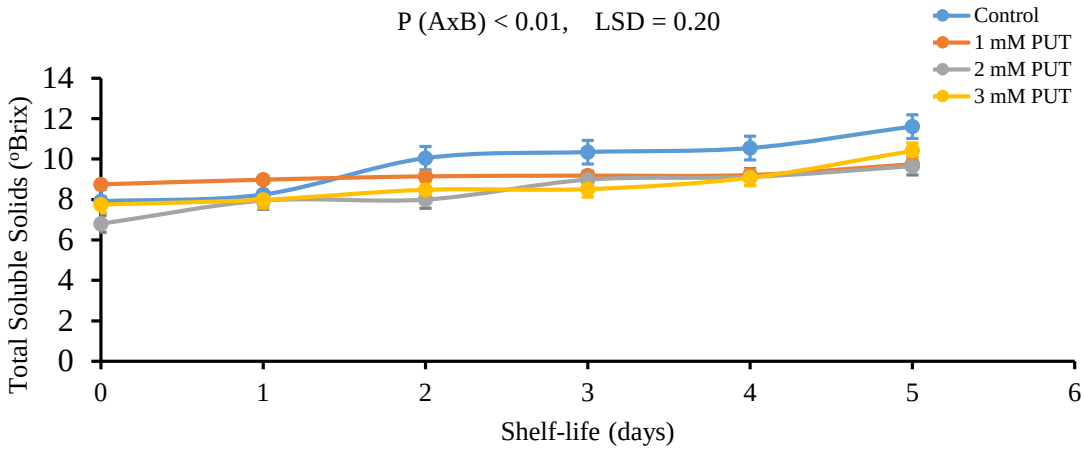


Fig. 5. Total Soluble Solids (TSS) of putrescine treated 'Solo' papaya fruit after 21 days of storage at 7.5 °C plus 6 days' shelf-life (n = 8). Error bars indicate ±SE of means at P ≤ 0.05. A = treatment, B = shelf-life days, A x B = interaction of treatment and shelf-life days. LSD, least significant difference.

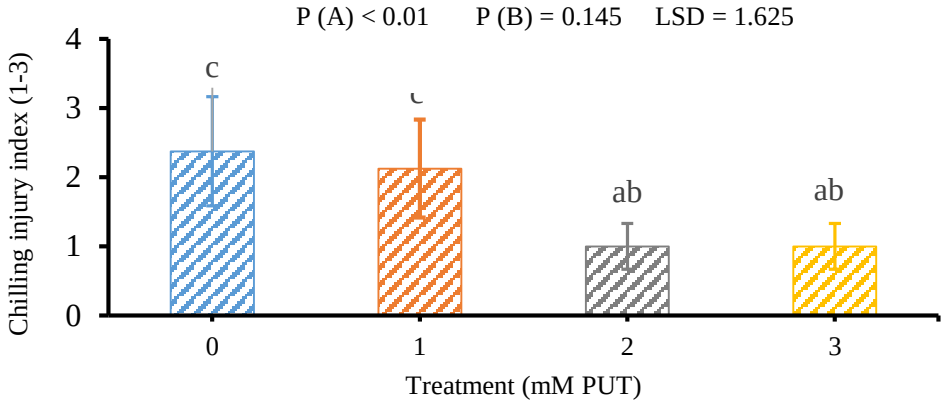


Fig. 6. Chilling injury index of putrescine treated 'Solo' papaya fruit after 21 days of storage at 7.5 °C plus 6 days' shelf-life (n = 8). Error bars indicate ±SE of means at P ≤ 0.05. A = treatment, B = shelf-life days. LSD, least significant difference.

Total soluble solids (TSS)

In the current study, total soluble solids revealed highly significant (P < 0.01) results between putrescine treated and untreated 'Solo' papaya fruit (Fig. 5). In general, TSS was increased in all the treatments during ripening. Regarding treatment effects, TSS was increased in control fruit throughout shelf-life days. Contrarily, fruit treated with 2 mM PUT had a minimum TSS throughout storage and shelf-life when compared control fruit. The increase in TSS with the onset of ripening could be related to several factors, including starch decomposition into sugars, increased respiration rate, sugar transformation into carbon dioxide and water and cell wall polysaccharide hydrolysis (Eshghi et al., 2014; Fawole & Opara, 2013). Figure 5 showed that an increase in TSS accumulation was considerably reduced by PUT treatment. Similarly, lower TSS was found in 'Native' and 'Cavendish' banana fruit treated with 2 mM PUT at 2 °C (Hosseini et al., 2018). Therefore, PUT was efficient in reducing respiration rate (Fig. 1) in papaya fruit consequently slowing down the breakdown of starch into sugar content due to retarded ripening process (Fig. 1, 2 and 3).

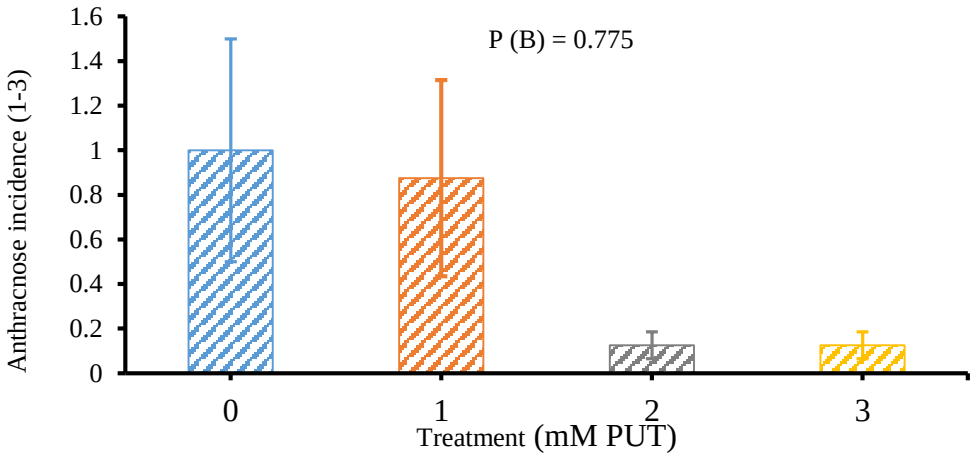


Fig. 7. Anthracnose incidence of PUT treated 'Solo' papaya fruit after 21 days of storage at 7.5 °C plus 6 days' shelf-life (n = 8). Error bars indicate ±SE of means at P ≤ 0.05. A = treatment, B = shelf-life days. LSD, least significant difference.

Chilling injury index (CII)

Figure 6 showed that chilling injury developed when 'Solo' papaya fruit was stored at 7.5 °C, and symptoms were visible as external discolouration and pitting on the fruit peel. In general, CI was more prominent on 1 mM PUT and control fruit rather than 2 and 3 Mm PUT treated fruit after cold storage (Fig. 6). Therefore, the results show that postharvest PUT treatment significantly reduced CI in papaya fruit. Similar findings were observed by Javanmardi et al. (2013) in 'M19' and 'M79' tomato fruit treated with 2 mM PUT. Furthermore, exogenous PUT induces cold adaptation by improving membrane fluidity at low temperature, therefore, minimizing electrolyte loss and skin browning (Barman et al., 2011). Additionally, PUT primarily inhibits lipid peroxidation and thereby preserves the membrane from physical state conversion (Mirdehghan et al., 2007).

Anthracnose incidence

The current study showed that anthracnose incidence differed significantly (P < 0.01) in papaya fruit irrespective of the treatment. In general, disease incidence increased with progression in ripening (Fig. 7). Anthracnose symptoms were more prominent in control fruit rather than in fruit treated with PUT. However, anthracnose infection on 2 mM PUT treated papaya fruit was less than control treatments; therefore, PUT was effective in reducing anthracnose (Fig. 8). Furthermore, in 'Native' and 'Cavendish' banana fruit, 2 mM PUT significantly decreased microbial population (Hosseini et al., 2018). Putrescine makes strong bond with phenols and hydroxycinnamic acid amide both of which induce resistance against pathogens, ultimately, reducing decay incidence (Fawole et al., 2020). Another factor for reducing the decay of PUT treated papaya fruit may also be associated with the strong defence mechanism against fungal attack (Hanif et al., 2020).

Fig. 8. Anthracnose incidence of PUT treated 'Solo' papaya fruit on day 6 of shelf-life (A=Control, B=1 mM PUT, C= 2 mM PUT, D= 3 mM PUT).

CONCLUSION

The study revealed that exogenous putrescine application, especially at higher concentrations (2 and 3 mM) improved quality and shelf-life of papaya fruit by reducing changes in physical (mass, firmness, colour) and biochemical (TA and TSS) parameters and inhibiting CI and anthracnose during storage. Therefore, putrescine could be a promising technique to significantly improve fruit quality and prolong the post-harvest storage life of papaya under cold storage temperatures.

Conflict of interest

The authors declare no conflict of interest.

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