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# **Role of Post-harvest Treatment of Polyamines on Physico-physiological Qualities of Papaya (***Carica papaya* **L.) Fruits Var. Red Lady during Ambient Storage**

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#### *Authors' contributions*

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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#### **ABSTRACT**

Polyamines are the natural compound, act as anti-senescent agents, reduce rate of respiration, delay ethylene production, retard colour change, maintain fruit firmness. Show that plays a pivotal role in postharvest life of fruits and vegetables. The objective of this study was to investigate the effect of post-harvest application of polyamines in different concentration on physico-physiological quality parameters of papaya fruits during ambient storage (33-36 $^{\circ}$ C). Papaya fruits are treated with different concentration of polyamines i.e.,  $T_2$ -0.50mM Spermine,  $T_3$ - 1.0mM Spermine,  $T_4$ - 1.5mM Spermine,  $T_5$ - 0.50 mMSpermidine,  $T_6$ - 1 mM Spermidine,  $T_7$ - 1.5mM Spermidine,  $T_8$ - 2 mM Putrescine, T<sub>9</sub>- 3 mM Putrescine, T<sub>10</sub>- 4 mM Putrescine and T<sub>1</sub>-Control. Among the treatments, papaya fruits treated with 4 mM of Putrescine  $(T_{10})$  recorded significantly minimum physiological loss in weight, respiration rate and colourvalues (*L\*, a\*, b\**) of peel and pulp, fruit disease index and maximum firmness, shelf life compared to control  $(T<sub>1</sub>)$ . Hence it is confirmed from the study that Putrescine at 4mM  $(T_{10})$  was found to be effective in delaying the physico-chemical and physiological process of papaya fruit cv. Red Lady.

\_ *Keywords: Polyamines; physiological loss in weight; firmness; respiration rate; colourvalues; percent disease index; shelf life.*

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## **1. INTRODUCTION**

Papaya (*Carica papaya* L.) is also known as pawpaw belongs to the family Caricaceae. It is considered as one of the most popular fruits among the millions of people due to it taste, nutrition value and medicinal use. It is regarded as the wonder fruit of the tropical and subtropical regions. It is originated in Mexico and it was introduced to India during 16<sup>th</sup> century from Malaca (Kumar and Abraham, 1943)<sup>[27]</sup>. India is the main producer of papaya, while Mexico ranks sixth and accounts for about 45.01 per cent and 6.13 per cent of total production, respectively  $($ Anon., 2018)<sup>[3]</sup>. India has approximately 1.38 lakh hectares of land under papaya cultivation and produces around 59.89 million metric tons per year (Anon., 2018)<sup>[4]</sup>. Andhra Pradesh is the leader in papaya production among the Indian states followed by Karnataka, Gujarat and Maharashtra.

It is one of the richest source of vitamin A and a good source of vitamin C besides rich in sugars and pectin content. The red-fleshed varieties contain lycopene as a major pigment [4,5]. Lycopene is vitamin A inactive but is a more efficient antioxidant than β-carotene (Dimascio et al., 1989) and it has been linked with reduction of risk of cancer especially lung, stomach and prostate cancer [6]. All these aspects have made papaya an ideal dessert fruit. The milky latex of unripe papaya fruits contains papain, a proteolytic enzyme that digests proteins. Papain is used as a meat tenderizer and for medical and industrial purposes [7]. It is a common observation that because of the extremely delicate nature of papaya fruits, heavy spoilage is of frequent occurrence before they reach the consumers. In India, the estimated loss is 10 to 25 per cent in ripe and 5 to 10 per cent in green fruits [3].

The major polyamines found in every plant cell are spermidine, spermine and putrescine [8]. Polyamines are the natural compounds that have a specific role in fruits and vegetables in postharvest life. Earlier research suggests that, polyamines in their free forms act as antisenescent agents, reduce rate of respiration, delay ethylene production, retard colour change, maintain fruit firmness, induce mechanical resistance and reduce chilling injury symptoms [9]. Polyamines are known to inhibit the ethylene production as it exerts a competition during its synthesis for a common precursor, S-adenosyl methionine and also provide alternative to use of chemicals to extend the shelf life of many fruits. According to Rahman et al. [10], bioactive products of plants have less negative effect on the environment and are safe for mammals and other non-target organisms for the control of post-harvest losses.

#### **2. MATERIALS AND METHODS**

#### **2.1 Experimental Details**

An experiment was carried out at Department of Post-harvest Technology, College of Horticulture, University of Horticultural Sciences, Bagalkot, Karnataka state during the year 2018-2019 with 10 treatments and 3 replications. The statistical design applied was completely randomized design (CRD). The treatment Details are  $T_1$ -Control,  $T_2$ -0.50mM L<sup>-1</sup> Spermine, T<sub>3</sub>-1.0mM L<sup>-1</sup> Spermine,  $T_{4}$ - 1.5mM L<sup>-1</sup> Spermine,  $T_{5}$ - 0.50 mM  $L^{-1}$ Spermidine, $T_{6}$ - 1 mM  $L^{-1}$ Spermidine, $T_{7}$ -1.5mM L<sup>-1</sup> Spermidine, T<sub>8</sub>- 2 mM L<sup>-1</sup> Putrescine,  $T_{9}$ - 3 mM L<sup>-1</sup>Putrescine,  $T_{10}$ - 4 mM L<sup>-1</sup>Putrescine.

Papaya fruits required for the experiment were procured from the papaya orchard located in the out skirts of Bagalkot situated at 15 km away from the experimental department. The fruits were carefully chosen from the orchard and manually harvested and gathered in the field. The fruits were selected based on the appearance of one or two yellow streaks on the surface of the fruits. After selection, individual fruits were wrapped with paper and placed in plastic crate and brought to laboratory in a small vehicle. In laboratory, fruits were uncovered with papers and immediately, washed with water containing 0.2 percent sodium hypochlorite and air dried under electrical fan. The air dried fruits were dipped in the putrescine at 2, 3, 4 milli molar, spermine and spermidine at 0.5, 1, 1.5 milli molar and one with distilled water which served as control.

Preparation of different concentration of putrescine, spermine and spermidine:

2 milli molar putresine: 322.1 mg putrescine was dissolved in 1 L distilled water

3 milli molar putresine: 483.2 mg putrescine was dissolved in 1 L distilled water

4 milli molar putresine: 644.3 mg putrescine was dissolved in 1 L distilled water

0.5 milli molar spermine: 101.1 mg spermine was dissolved in 1 L distilled water

1.0 milli molar spermine: 202.3 mg spermine was dissolved in 1 L distilled water

1.5 milli molar spermine: 303.5 mg spermine was dissolved in 1 L distilled water

0.5 milli molar spermidine: 72.6 mg spermidine was dissolved in 1 L distilled water

1.0 milli molar spermidine: 145.2 mg spermidine was dissolved in 1 L distilled water

1.5 milli molar spermidine: 217.8 mg spermidine was dissolved in1 L distilled water

The fruits were dipped in putrescine, spermine and spermidine for 5min, fruits were taken out from the solutions and air dried for 5 min under electrical fan. Then the individual fruits were placed in plastic crates by maintaining 10 fruits per replication. The crates were placed in ambient and cold storage for further studies.

## **2.2 Parameters Studied**

#### **2.2.1 Physiological loss in weight (%)**

Fruits from each replication were taken to record the physiological loss in weight (PLW). The weight of the fruits was recorded using electronic weighing balance before storage. Thereafter, the weights were recorded regularly during storage and the PLW was calculated with the following formula and expressed as per cent physiological loss in weight.

Physiological loss in weight  $(\%)$  = (Initial weight  $(g)$  – Final weight  $(g)$  / Initial weight  $(g)$ ) x 100

#### **2.2.2 Rate of respiration (ml CO2/kg/h)**

The rate of respiration was measured by static head space method using gasanalyzer (PBI, DANSENSOR, CHECKMATE 2) and expressed as ml  $CO_2$ kg<sup>-1</sup>h<sup>-1</sup>. Forthis, papaya fruits were trapped in 3 litre airtight containers having twisttop lid fitted with a silicone rubber septum at the centre of the lid. The containers were kept for 1 h for accumulation of respiratory gases at the headspace. After specified time, the head space gas was sucked to the sensor of the analyzer through the hypodermic hollow needle and the displayed value of evolution rate of  $CO<sub>2</sub>concentration$  (%) was recorded. Rate of respiration was calculated on the basis of rate of evolution of  $CO<sub>2</sub>$  from the fruit per unit weight per unit time using the following formula.

Rate of respiration (ml  $CO<sub>2</sub>/kq/h$ ) =  $(CO<sub>2</sub>)$ concentration (%) x Head space / 100 x Weight of the fruit (Kg) x Time (h))

#### **2.2.3 Fruit firmness (N)**

Fruit firmness was determined using texture analyzer. Firmness evaluation was carried out by taking whole fruit with skin and penetrating it with a 2 mm diameter cylindrical needle. Three measurements were performed and values of the samples were averaged. Firmness is evaluated using a TAXT plus Texture Analyser (Make: Stable Micro System, Model: Texture Export Version 1.22). The force with which the sample gets penetrate was recorded in graph and the peak value in the graph was taken as the texture value in terms of Newton force (N).

#### **2.2.4 Pulp and peel Colour (***L\*, a\*, b\****)**

Colour of the samples was measured using Lovibond colour meter (Model: Lovibond  $RT<sub>3</sub>00$ , Portable Spectrometer, the Tintometer Limited, Salisbury, UK) fitted with 8 mm diameter aperture. The instrument was calibrated using black and white tiles provided. Colour was expressed in Lovibond units *L\**  (lightness/darkness), *a\** (redness/greenness), *b\** (yellowness/blueness). Papaya sample was placed across the aperture of the colour meter. Three measurements were performed and values of the samples were averaged.

The colour of the papaya peel and pulp in terms of luminance (*L*\*), green or red colour (*a*\*) and blue or yellow colour (*b*\*) values were determined using a colorimeter. *L*\* measures lightness and varies from 100 for perfectly reflective white to zero for perfectly absorptive black; *a*\* measures redness when positive, gray when zero and greenness when negative; and *b*\* measures yellowness when positive, gray when zero and blueness when negative. Papaya fruits colour of skin was measured at three different points of each fruit and the values of samples were averaged.

#### **2.2.5 Fruit disease index (FDI)**

The fruit disease index was measured by visual inspection during storage. For the deterioration grade, the peel hydration, damage by mechanical and/or caused by fungi was considered based on the scale. 0-5 scale is used i.e.,  $0 -$  No lesions,  $1 - 5\%$  to  $\leq 15\%$  lesions,  $2 -$ ≥15% to  $\leq$  25 % lesions, 3 - ≥25% to  $\leq$  50 % lesions, 4 - ≥50% to ≤ 75 % lesions,5 - ≥75% to 100 % lesions. PDI was calculated with the following formula and expressed as per cent disease index [11].

FDI  $(\%)$  = (Sum of all disease rating / Total number of rating x Maximum disease grade) x 100

#### **2.2.6 Shelf life**

The number of days of the ripe fruits in edible condition was taken as the shelf-life or keeping quality of ripe fruits.

#### **2.3 Statistical Analysis**

The data of experiment was analyzed as applicable to completely randomized design (CRD). Statistical analyses of experiments were performed using Web Agri Stat Package (WASP) Version 2. The level of significance used in 'F' and't' was p=0.01 and p=0.05 for some parameters. Critical difference values were calculated whenever F-test was found significant.

## **3. RESULTS AND DISCUSSION**

#### **3.1 Physiological Loss in Weight (%)**

Physiological loss in weight is one of the important economic parameter that decides the shelf life even if fruit is free from physical and microbial abuse [12,13]. They appear shriveled, shrunk, misshaped, lose its crispness, flavour, turgour and other organoleptic qualities. The physiological weight loss of papaya fruits was observed to increase with ripening process. There was a significant difference among the treatments as affected by the postharvest application of polyamines [14,15].

Among the various postharvest treatments, the control  $(T_1)$  fruits lost maximum weight of 26.53 per cent during 9 days of storage. While fruits treated with putrescine @ 4mM, lost minimum weight of 21.23 per cent during 9 days under ambient conditions (Table1). The physiological loss in weight results mainly by the respiration and transpiration losses during the metabolic processes of fruits coupled with atmospheric storage conditions in terms of low relative humidity triggers the pressure difference between fruits and surrounding storage conditions [16,17].

It is known that polyamines have a unique antisenescence property due to their lower molecular weight and poly cationic structure, which lowers the maturation process, thereby lower the respiration rate and enzymatic activities responsible for the cell wall degradation [18, 19, 20]. The lower weight loss in putrescine treated fruits could be attributed to stabilization and consolidation of both cell integrity and the permeability of the tissues as it forms linkage with cell membranes and preserves waxes of cuticle layer thereby delay the removal of epicuticular waxes, which play a very important role in water exchange through the skin. Further, increase in PLW with the increase in storage period may be due to increase in moisture loss from the fruits. In several studies, researchers have reported that, there is always increase in PLW in different fruits with the increase in storage period [18, 19, 20].The results of present experiment is justified by [21] in mango cv. Dashehari, Shiri et al. [22] in grape cv. Shahroudi and Midehghan (2007) in pomegranate.

# **3.2 Respiration Rate (ml CO<sup>2</sup> kg-1 h -1 )**

The respiration rate of papaya fruits increased initially and decline later in all the treatments. It was observed that, a significant difference among the treatments with respect to respiration rate during storage. The maximum respiration rate was recorded in control (30.05ml  $CO<sub>2</sub>/kg/hr$ ) and minimum respiration rate was recorded in putrescine  $@$  4mM (22.33 ml  $CO_2/kg/hr$ ) under<br>ambient condition (Table 1.) because ambient condition (Table 1.) polyamines could delay ripening of fruits, probably through inhibition of ethylene biosynthesis or its action. Such influence of polyamines on respiration rate may be ascribed to strong anti-senescence properties of polyamines [23].

It is well known that, any factor increasing ethylene production or activity leads to increase in respiration rate and any factor increasing respiration rate leads to increase in ethylene production and activity [24]. It has been demonstrated that, polyamines in a concentration dependent manner effectively reduces the respiration in plants and harvested fruits [25, 26, 27]. Decrease in fruit metabolic activities results in a decrease in fruit water loss and carbohydrate depletion rate and consequently and effectively delays fruit<br>senescence process [24]. The minimum senescence process [24]. respiration rate in putrescine treated fruits is mainly due to its anti-senescence properties, inhibition of ethylene bio-synthesis or reduced rate of metabolism and favourable water activity [28, 29]. The results are in conformity with report of Barbang et al. [30] in banana; Barman et al. [28] in pomegranate and Malik et al. [31] in mango.

## **3.3 Firmness (N)**

The firmness of the fruit tissue at harvest is mainly due to the physical properties of the individual cell walls and the middle lamella, which contains the cementing pectic material. As the fruit approaches ripening, the firmness decreases with the increase in storage period, primarily because there might have been progressive increase in fruit softening due to increased activities of lipoxygenase (LOX), polygalacturonase (PG) and pectinmethylesterase (PME) enzymes, rendering much softer with increase in storage period [32].

The control fruits recorded lowest firmness of 0.87 N during 9 days of storage. While, fruits treated with putrescine @ 4mM showed highest firmness of 4.62 N during 9 days of storage under ambient conditions (Table 1).

The soluble pectin is much higher where higher temperature or no  $CO<sub>2</sub>$  are involved. The rate of pectin degradation is affected by both time and conditions of storage by Salunkhe et al. [33]. Santos et al. [34] observed the existence of a relation between mass loss and fruit firmness, that is, whenever there is an increase in percentage of mass loss, there is also a reduction in firmness. In the present study, there was a significant decrease in firmness of papaya fruits as the storage period increases and fruits started ripening at faster rate under room temperature. These results were in conformation with the results reported by Reboucas et al. [35]; Correa et al. [36]; Bron and Jacomino [37]; Hendriod et al. [38] in papaya.

Fruit firmness is one of the most crucial factors in determining the postharvest quality of fruits [39]. In the present study, softening of papaya fruits was remarkably delayed with polyamines treatment during storage period.

The effect of polyamines could be due to modification of genes involved in ethylene biosynthesis, ethylene perception, alteration of cell wall associated enzymes and polyamine conjugation [40]. Valero et al. [9] reported that the effect of polyamine on maintaining fruit firmness is thought to be a result of their cross linkage to the carboxylic group of the pectin

substance in the cell wall, resulting in rigidification. The bindings between polyamines and pectin also inhibit the activity of cell wall degrading enzymes, such as pectinase, pectinmethylesterase and polygalacturonase and reduced fruit softening during storage. The effect of putrescine on fruit firmness loss has been reported in wide range of fruits such as mango cv. Dashehari [21], blueberry [41] and Cavendish banana [30].

## **3.4 Instrumental Colour Values (***L\*, a\*,b\*)*

In the present study, colour values of both pulp and peel increased significantly throughout the storage period irrespective to the treatments. The increase in color development was probably due to its effects on stimulating the activity of some enzymes that are responsible for ripening of fruits [42].

The colour changes in papaya fruit peel was remarkably delayed with putrescine @ 4Mm treatment during ambientstorage. The highest *L\** values were recorded in control  $T_1$  (61.35) followed by  $T_5$  (57.52). Whereas lowest  $L^*$  value was in T<sub>10</sub> (53.28) and T<sub>7</sub>(56.68) at 9<sup>th</sup> day under ambient storage (Table 2). This might be due to the chlorophyll degradation which subsequently reveals the yellow carotenoid pigments [43, 44].

The change in pulp colour of papaya fruits is significantly affected in response to polyamine treatments. Under ambient condition, consistently the maximum *L\** value was recorded in control fruits (64.91) and minimum *L\** value was registered in  $T_{10}$  (putrescine @ 4mM) (59.97) (Table 3). This might be due to the synthesis of yellow carotenoid pigments upon ripening [43].

The colour changes in papaya fruit peel was remarkably delayed with putrescine @ 4mMtreatment during ambientstorage. The highest a\* values were recorded in control T<sub>1</sub> (19.61) followed by  $T_5$  (15.91). Whereas, lowesta<sup>\*</sup> value was in T<sub>10</sub> (13.77) and T<sub>7</sub>(14.85) at  $9<sup>th</sup>$  day under ambient storage (Table 2). This indicating that the pathways for chlorophyll degradation were affected by polyamine treatment [45].

The change in pulp colour of papaya fruits is significantly affected in response to polyamine treatments. Under ambient condition, consistently the maximum *a\** value was recorded in control fruits (34.69) and minimum *a\** value was registered in  $T_{10}$  (putrescine @ 4mM) with

(29.89) (Table 3). This indicating that the pathways for carotenoid synthesis were affected by polyamine treatment [5].

The colour parameter *b\**has been described as best to reflect the colour changes in fruit tissue during fruit ripening (Martinez et al.*,*2002). Thecolour changes in papaya fruit peel was remarkably delayed with putrescine@ 4mMtreatment during ambient storage. The highest  $b^*$  values were recorded in control  $T_1$ (50.83) followed by  $T_5$  (43.29). Whereas, lowest*b*<sup>\*</sup> value was in  $T_{10}$  (37.56) and  $T_7$  (42.29) at  $9<sup>th</sup>$  day under ambient storage (Table 2). The peel degreening is influenced by ethylene produced by the pulp [46]. In the present work the polyamines inhibit the ethylene production and hence disrupted the degreening process.

The change in pulp colour of papaya fruits is significantly affected in response to polyamine treatments. Under ambient condition, consistently the maximum *b\** value was recorded in control fruits (47.72) and minimum *b\** value was registered in  $T_{10}$  (putrescine @ 4mM) (42.24) (Table 3). Carotenoids synthesis in pulp is influenced by ethylene production [46]. As polyamines inhibit the ethylene production hence disrupted the colour synthesis in pulp.Variation of pulpcolour in papaya as a reference of maturation stage is also explained by Fonseca et al. [41] and Sancho et al. [47].

Polyamines coating resulted in slow rate of respiration and reduced ethylene production, leading to a modified internal atmosphere of the fruit. This, inturn delayed the ripening and senescence of the fruit, resulting in reduced colour change. Similar results have been reported in papaya [48], tomato [49] and bell pepper [50, 51].

## **3.5 Fruit Disease Index (FDI)**

The Fruit disease index (FDI) of control fruits rapidly increased and reached the maximum of 73.76 per cent at 9 days of storage. The FDI of papaya fruits treated with  $T_{10}$  (putrescine  $\omega$ 4mM) showed the lowest value of 19.32 per cent in 9 days under ambient storage (Table 4). The prevention of decay or slow rate of decay in polyamine treated papaya fruits due to the antisenescent property of the polyamines, which might have retarded the maturation process of the treated fruits [52].Postharvest application of polyamines may provide a useful means of controlling postharvest decay there by extending the storage life [23].

Reduced spoilage can be attributed to a decrease in the microbial activity of fruits [53]. Polyamines conjugated to phenolic compounds and hydroxycinamic acid amides have been shown to accumulate in cells in interactions between plants and a variety of pathogens [54]. Thus, putrescine treated fruits had less fungal infection than untreated ones. Similar findings were also observed by Mirdehghanet al.*,* [53] in pistachio nut and Mirdehghan et al.*,* [55] in grape.

## **3.6 Shelf Life (Days)**

Shelf life of papaya fruits is interconnected with fruit disease index parameter. The changes were observed in shelf life of papaya cv. Red Lady fruits, which was influenced by postharvest application of polyamines under ambient storage condition.

Significantly maximum shelf-life was observed in the fruits treated with putrescine @ 4Mm (9 days) and minimum shelf-life was recorded in the control fruits (4.66 days) under ambient storage (Table 4.). The maximum shelf-life of papaya is obtained by slow ripening [56]. The shelf-life of papaya increased with the exogenous application of polyamines by retarding fruit softening, weight loss, inhibiting in respiration rate, colour development and delaying ripening process without affecting organoleptic properties of the fruit. In many plant system, leaf and fruit ageing and senescence is correlated with a decrease in polyamine levels. The exogenous application of polyamines often delays or prevents progression of senescence [57]. The honey dew melon fruits treated with exogenous putrescine reduced membrane peroxidation indicated by lower production of malondialdehyde, decreased lipoxygenase and phospholipase-D activities and decreased petrubation of plasma membrane [58]. This antisenescence property of polyamines is the main reason to improve the shelf-life of fruits under storage. Since, these compounds affect ethylene production and expression of genes involved in ethylene bio-synthesis, this hormone is likely to influence the mode of action by which these polyamines affect shelf-life [59].

The polyamines has been reported to improve the shelf-life of fruits as reported by Dibble et al. [60] in tomato, Mirdehghanet al. [61] in pomegranate, Khoshroshahi et al. [62] in strawberry, Jawandha et al. [63] in mango, Shiri et al. [22] in grapes and in papaya [64].



**Table 1. Effect of different concentrations of polyamines on Physiological loss in weight (PLW) (%), Respiration rate (ml CO2/kg/h), Firmness (N) of papaya cv. Red Lady fruits during ambient storage**

**Table 2. Effect of different concentrations of polyamines oncolour (***L\****), colour (***a\****), colour (***b\****) values of papaya peel cv. Red Lady fruits during ambient storage**





#### **Table 3. Effect of different concentrations of polyamines oncolour (***L\****), colour (***a\****), colour (***b\****) values of papaya pulp cv. Red Lady fruits during ambient storage**

**Table 4. Effect of different concentrations of polyamines on fruit disease index (%) and shelf life (days) of papaya cv. Red Lady fruits during ambient storage**



## **4. CONCLUSION**

Results revealed that post-harvest application of polyamines influence on physico-physiological characteristics and shelf life of papaya fruit and there byover all quality improvement during ambient storage. The fruits treated with putrescine  $@$  4mM ( $T_{10}$ ) recorded significantly minimum physiological loss in weight, respiration rate, pulp and peel Colour changes, fruit disease index and maximum fruit firmness and shelf life. Hence, it is confirmed from the study that Putrescine at 4 mM  $(T_{10})$  was found to be effective in delaying the ripening of papaya fruits.

## **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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