Research Article

Role of Exogenous Putrescine and Spermine Applications for Improving Fruit Quality of Banana cv. Hom Thong at Low Temperature Storage

Phrutiya Nilpraprucк*, Pimjai Meetum, and Chanika Chanthasa

Faculty of Animal Sciences and Agricultural Technology, Silpakorn University, Phetchaburi IT Campus, Sampraya, Cha-am, Phetchaburi, 76120 Thailand

**Corresponding author. Email address: nilpraprucк_p@silpakorn.edu*

Received September 14, 2016; Accepted December 13, 2017

Abstract

Putrescine (PUT) and spermine (SPM) are polyamines (PAs) which attribute to an intermediate for plant growth regulators. Moreover, there is an evidence that PAs are associated with the reduction of biotic and abiotic stresses in plant cell. To determine the effect of PUT and SPM on qualities of *Musa* cv. Hom Thong, mature bananas were immersed with 0.5 mM PUT or SPM for 5 min and stored at 10°C. The result indicated that PAs improved postharvest qualities and reduced browning symptom compared to untreated fruit. PUT and SPM also reduced weight loss and ion leakage. Additionally, the diminution in phenolic compounds, polyphenol oxidase and peroxidase activities indicated a reduction in browning symptom. Both PUT and SPM also affected fruit ripening. The treatments delayed the increase of total sugar and chlorophyll degradation indicating by the lower chlorophyll content, a* and b* values. SPM showed higher efficiency in improving qualities of banana cv. 'Hom Thong' than PUT.

***Key Words*:** Banana fruit; Putrescine; Spermine; Enzymatic browning; Postharvest quality

Introduction

Banana fruit is one of the most popular fruit worldwide. The major problem in banana export and storage is a rapid ripening and browning leading to a short shelf-life. There are several methods that have been used to inhibit the physiological change; one of these method is a low temperature storage (Facundo et al., 2015; Jing et al., 2004). However, banana is highly sensitive to temperature below 13°C which induces chilling injury (CI). The CI symptom induces peel browning, pitting and softening, which has a direct effect on consumer acceptance and market demand (Jiang et al., 2004).

The polyamines (PAs), mainly putrescine (PUT), spermidine (SPD) and spermine (SPM) are polycationic compound that are represented in association with the plant growth and developmental processes such as cell division, fruit ripening and senescence (Pandey et al., 2000; Theiss et al., 2002). PAs are not only involved in plant growth and development, but also play important roles in increasing the plant tolerance to biotic and abiotic stresses (Bouchereau et al., 1999; Gill and Tuteja, 2010; Hussaina et al., 2011). There have been reports indicated that the augmentation of the free PAs level under stress condition correlated with increasing the defense response of plants (Roy and Wu, 2005; Liu et al., 2015; Zhang et al., 2015). PAs application in postharvest fruit has been shown to effect shelf life and quality of various fruit such as litchi (Jiang and Chen, 1995), pepper (Gonza´lez-Aguilar, 2000), plum (Serrano et al., 2003), pomegranate (Mirdehghan et al., 2007) and zucchini (Palma et al., 2014). Research studies showed that PAs can increase stress tolerance during cold storage and retard shelf life of fruit as a result from an induction of antioxidant system. Additionally, the studies indicated that PAs level in fruit is involved in physiological changes such as chlorophyll degradation,

ISSN 1905-9159 (Print), ISSN 2586-842x (Online)

ethylene production, fruit ripening and enzymatic browning reaction (Cohen et al., 1979; Morilla et al., 1996; Jhalegar et al., 2012; Nilprapruck et al., 2016). Hence, the objective of this study was to investigate the role of PUT and SPM on maintaining quality and reduce browning symptom in *Musa* AAA group cv. 'Hom Thongat low temperature.

Materials and Methods

Plant material and treatment

Banana fruits (*Musa* (AAA group) cv. 'Hom Thong') at 70% maturity stage from Thayang Agricultural Cooperative Ltd., Phetchaburi province were used as material. Banana fruits of uniform size and shape were selected, each hand comprised 10-12 banana fingers. The fruits were washed with 100 ppm hypochlorite solution for 5 min to suppress fruit rot disease and then immersed in 200 ppm ethephon (Superior Chemical Industry, Thailand) for 10 min, and were air-dried at an ambient temperature. The fruits were stored at room temperature for 3 days to stimulate fruit ripening.

The selected ripe fruits (360 fruits, unique color) were randomly divided into 3 groups. The first group was immersed in distilled water (control) and the second and third groups were received an exogenous application of 0.5 mM putrescine (PUT) (≥ 98.5% Sigma, USA) and spermine (SPM) (≥ 97% Sigma, USA) for 5 min and were stored at 10 °C. The banana fruits were taken at 0, 3, 6, 9 and 12 days for quality assessment.

Weight loss

Each banana was weighed to calculate percentage of weight loss with respect to the initial weight as followed:

$$\text{Weight loss (\%)} = \left(\frac{\text{Initial weight-Final fresh weight}}{\text{Initial fresh weigh}}\right) \times 100$$

Electrolyte leakage

Electrolyte leakage was determined based on the method of McCollum and McDonald (1991). Twelve tissue samples in peel were placed with 50 ml of 0.4 M mannitol and incubated at 25 °C for 3 hours (data A). The Electrical conductivity of the solution was measured at room temperature using a conductivity meter (Model EcoScan CON6, EUTECH). Total electrolyte was determined on the same samples after they were autoclaved for 30 min at 121 °C and cooled to room temperature (data B). Leakage data were expressed as a percentage of total electrolyte reading.

$$\text{Total electrolyte leakage (\%)} = \frac{B}{A} \times 100$$

Total phenolic compounds of banana peel

To determine total phenolic compounds, 1 g of frozen peel was ground in a mortar with liquid nitrogen and extracted with 12 ml of 80% ethanol, then centrifuged at 4,400 rpm for 20 min at 4°C. The supernatant was used to assay total phenolic compounds according to Ketsa and Atantee (1998). Reaction mixtures contained 4 ml of 10% Folin-Ciocalteu reagent and 5 ml of 7.5% sodium carbonate. The absorbance was measured by a spectrophotometer (Model Libra S22, Biochrom, USA) at 765 nm. Total phenolic compounds were determined based on a standard curve of gallic acid.

Polyphenol oxidase and peroxidase assay

PPO and POD extraction was carried out at 4°C and the activity was measured using the modified method of De Oliveira Lima et al. (1999). Peel tissue (1.0g) were homogenized in a cooled mortar using a pestle with 10 ml of 0.05M sodium phosphate buffer (pH 6.5). The homogenate was centrifuged at 4,400 rpm for 30 min at 4°C. The supernatant was thoroughly used as enzyme extracts for assaying PPO and POD activity (Flurkey and Jen, 1978). The specific activity of enzyme is expressed as unit mg protein^{-1}.

Protein content in the enzyme extracts were determined following the method of Bradford (1976). The reaction mixture used in assay consisted of enzyme extracts and Braford reagent. The absorbance at 595 nm was measured using spectrophotometer (Model Libra S22, Biochrom, USA). The protein content was calculated using bovine serum albumin as a standard and expressed as mg protein ml^{-1} of enzyme extract.

Total sugar content

One gram of pulp was extracted with 20 ml of 50% ethanol at 60 °C for 2 h. One milliliter of the supernatant

was mixed with 0.5 ml of 0.1N hydrochloric acid and boiled for 15 min. This mixture was then mixed with 0.5 ml of 0.1N sodium hydroxide. One milliliter of the supernatant liquid was then taken for quantifying the total sugar using Somogy-Nelson's method (Hodge and Hofreiter, 1962). The absorbance of Somogy-Nelson's method was measured by a spectrophotometer (Model Libra S22, Biochrom, USA) at 520 nm. The total sugar content was calculated using glucose as a standard and expressed as mg. $100g^{-1}$.

Color measurement

The development of peel color was measured by color meter (Model MiniScan EZ, HuterLab, Germany), the L^*, a^* and b^* values were assessed. All color measurements were taken under the condition of standard illuminant D65.

Chlorophyll and carotenoid content

Chlorophyll and carotenoid content were determined using the method of Arnon (1949). One gram of peel was extracted with 80% acetone and then filtered through filter paper. The absorbance of the extract was measured by a spectrophotometer (Model Libra S22, Biochrom) at 480, 645 and 663 nm. The chlorophyll and carotenoid content was determined by using the following formulas:

Total chlorophyll (mg/l) = 20.2 (A645) + 8.02 (A663)

Carotenoids (mg/l) = A480 + (0.114 × A663) - (0.638 × A645)

Statistical analysis

The completely randomized design (CRD) was used throughout the whole experiment with three replications. The data were analyzed with one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests. Differences at $P \leq 0.01$ were considered as statistically significant.

Result and Discussion

Effect of PAs on weight loss and electrolyte leakage

Weight loss is the primary quality attribute measured in fruits that relates to texture and associated with cellular breakdown, loss of membrane integrity. In this experiment, the weight loss in SPM-treated banana was significantly lower than PUT-treated banana and non-treated bananas, respectively (Figure 1). The increase in weight loss seems to be also correlated with higher electrolyte leakage (Figure 2). Banana is susceptible to low temperature at a long time storage resulting in stress, which is an important cause of membrane cell damage so called chilling injury (CI). Change of membrane permeability can be investigated by electrolyte leakage. This experiment indicated that non-treated banana showed significantly higher electrolyte leakage than SPM-treated fruit. There are several reports indicated that exogenous PAs treatment can inhibit CI during storage by increasing antioxidation group in grape, apricot and cucumber (Champa et al., 2014; Kousheshsaba et al., 2012; Zhang et al., 2009).

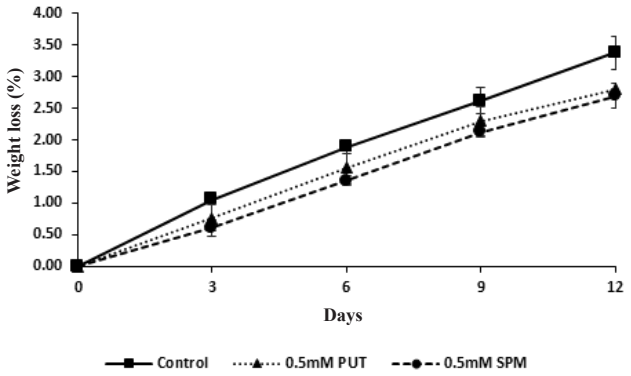

Figure 1 Change of weight loss (%) in bananas which had been immersed with water, 0.5mM PUT and 0.5mM SPM and stored at 10 °C. Each data point is the mean ±SE of three replicate samples.

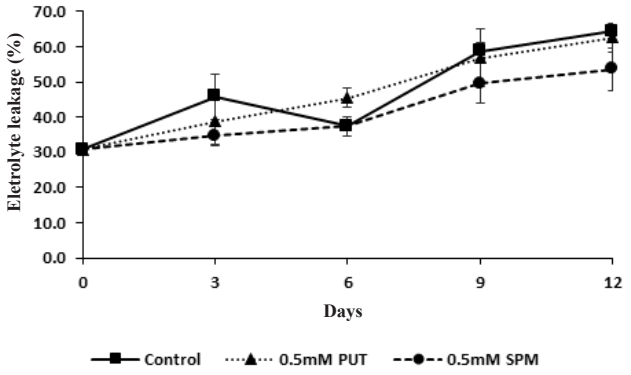

Figure 2 Change of electrolyte leakage (%) in bananas which had been immersed with water, 0.5 mM PUT and 0.5 mM SPM and stored at 10°C. Each data point is the mean ±SE of three replicate samples.

Generally, a common CI symptom in bananas appears more quickly and severer at temperature below 12°C, leading to pitting and discoloration of peel (Wills et al., 1998; Jiang et al., 2004). According to this experiment, non-treated banana showed the CI symptom on peel while PUT-treated banana and SPM-treated banana reduced the degree of CI symptom. Moreover, it indicated that the increase in CI symptom related to increase in electrolyte leakage.

Effect of PAs on phenolic compounds, PPO and POD activities

The amount of phenolic compounds in non-treated fruit increased as storage period increased, while the PAs treated fruits resulted in only slight increase (Figure 3). Phenolic compounds in non-treated banana was initially 6.38 ± 0.51 mg.g^{-1} and increased gradually during storage, reaching a maximum of 12.00 ± 0.73 mg.g^{-1} on final day of storage. It is well known that a main cause of browning symptom derived from oxidation of phenolic substrates by PPO. Hence, the accumulation of phenolic compounds has been proposed to play a role in particular browning symptom in various fruits (Mayer and Harel, 1979; Vaughn et al., 1988; Walker and Ferrar, 1998). The occurrence of phenolic compound in different treatments was statistically different. SPM-treated banana efficiently inhibited increasing phenolic compounds. It could be that the low accumulation of phenolic compounds in the peel correlated with decreasing browning symptom. In this case of study, it can suggest that PUT and SPM treatments can inhibit browning symptom by correlation with reduce in phenolic compounds.

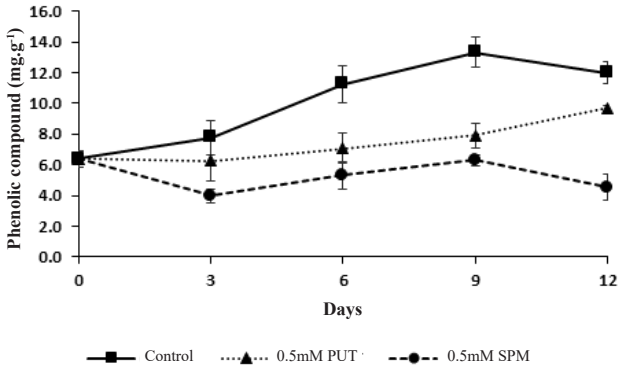

Figure 3 Change of phenolic compounds in bananas which had been immersed with water, 0.5 mM PUT and 0.5 mM PM and stored at 10 °C. Each data point is the mean ±SE of three replicate samples.

The expression of enzyme activity such as PPO (Figure 4A) and POD (Figure 4B) in banana was significantly different, the highest PPO and POD activity in non-treated banana increased in day 9 of storage, after that they decreased rapidly while PUT and SPM treatments were relatively constant. The activity of PPO in SPM-treated banana was obviously lower than PUT-treated and non-treated bananas. PPO is considered to be the key enzyme in tissue browning of cold-damaged fruit. There had been suggested that higher activity of PPO can indicate a severe browning symptom in cell (Arpita et al., 2010; Ding and Ling, 2014). Hence, this study indicated that PAs can reduce browning symptom which associated with the reduce of phenolic compounds and PPO activity.

The POD can be considered a stress enzyme stimulated by low temperatures (El-Hilari et al., 2003). POD activity in PUT-treated banana and SPM-treated banana were relatively stable all time of storage, whereas non-treated banana had an increasing activity in day 9. The result indicated that the application of PUT and SPM was noticed parallel to reduce in POD activities during storage compared to non-treated banana.

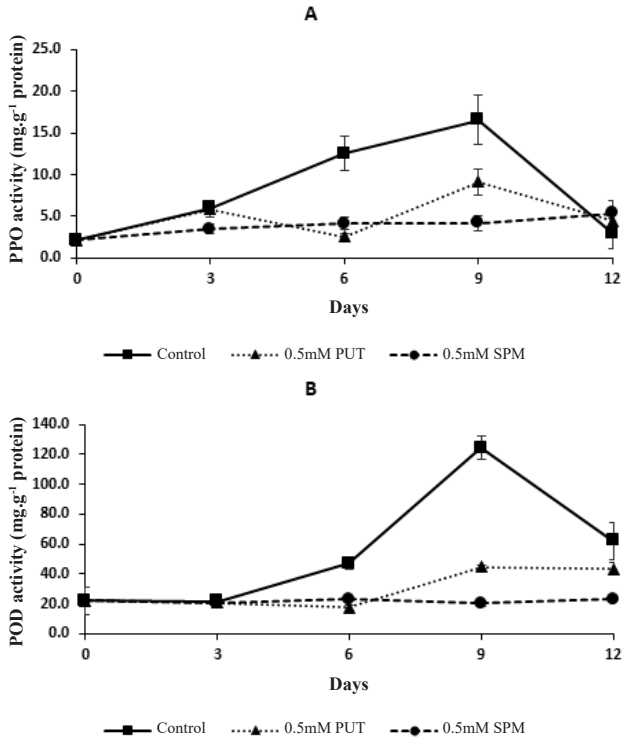

Figure 4 Change of PPO (A) and POD (B) activity in bananas which had been immersed with water, 0.5 mM PUT and 0.5 mM SPM and stored at 10°C. Each data point is the mean ±SE of three replicate samples.

Effect of PAs on total sugar, peel color, chlorophyll and carotenoid content

The result in Figure 5 illustrates the change of total sugar in pulp during storage. PAs can reduce a significant increase in total sugar. These PUT-treated banana appears to be less susceptible to total sugar than SPM-treated banana. Usually, the increase of total sugar associated with ripening bananas. It has been postulated that PAs, which reduce total sugar in fruits, may participate in ethylene biosynthesis. Because ethylene and PAs biosynthesis share the common precursor S-adenosylmethionine, it exerts opposite effects with respect to fruit ripening. Furthermore, it has been suggested that the balance between polyamines and ethylene controls fruit ripening (Paksasorn et al. 1995, Pandey et al. 2000, Franco-Mora et al. 2005).

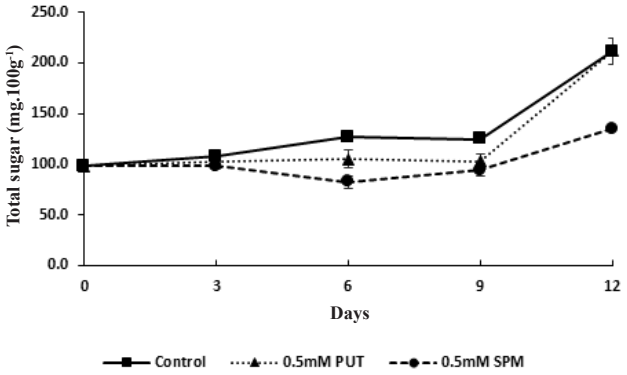

Figure 5 Change of total sugar in bananas which had been immersed with water, 0.5mM PUT and 0.5mM SPM and stored at 10 °C. Each data point is the mean ±SE of three replicate samples.

The development of peel color can be used to investigate fruit ripening, which proposed a change of L^*, a^* and b^* values (Figure 6). The L^* value in non-treated banana was significantly higher than SPM-treated banana on day 6 and was lower than SPM-treated banana on day 12. Additionally, the a^* value range from a negative value is green and a positive value is red, and the b^* value range from a negative value is blue and a positive value is yellow. The non-treated banana was higher b^* value than PUT and SPM treatment associated with ripening and browning during storage.

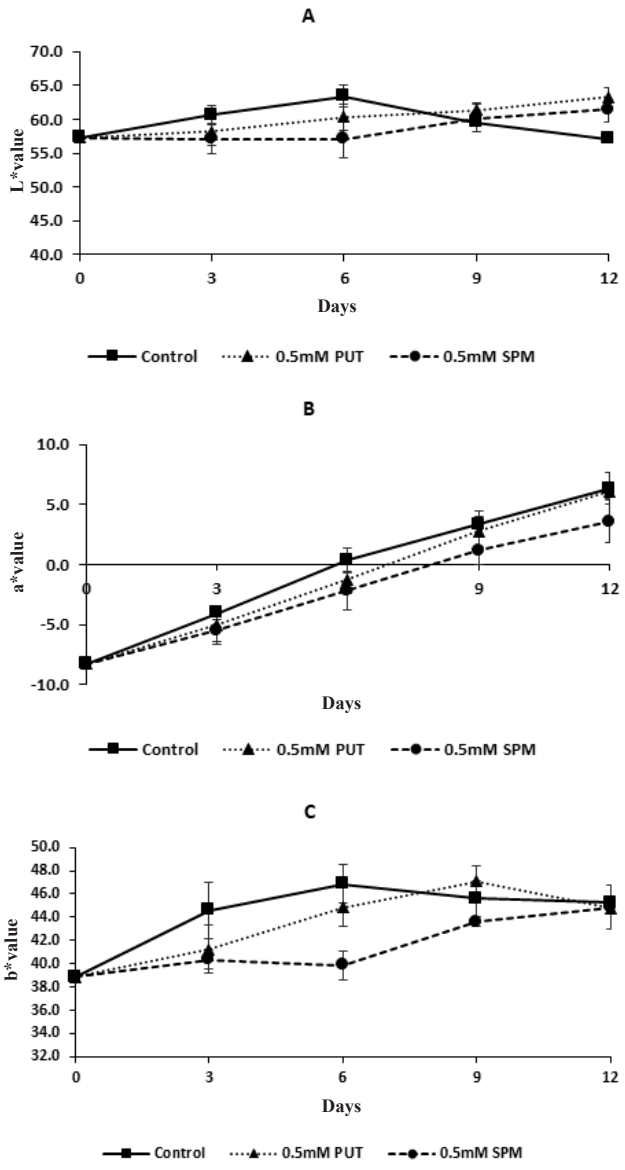

Figure 6 Change of L* (A) a* (B) and b* (C) value in bananas which had been immersed with water, 0.5mM PUT and 0.5mM SPM and stored at 10 °C. Each data point is the mean ±SE of three replicate samples.

To investigate a relation between ripening fruit and chlorophyll degradation, changes of chlorophyll and carotenoid contents were measured during storage (Figure 7). The decrease of chlorophyll content was involved in the increase of carotenoid content. PAs had significantly effective in inhibition of chlorophyll degradation and carotenoid biosynthesis. According to several researches, PAs can prevent chlorophyll loss in thylakoid membranes

by stabilizing photosystem complex and retarding the loss of apoprotein of the light-harvesting chlorophyll a/b-protein complex of photosystem II from thylakoid membranes (Cohen et al., 1979; Cheng et al., 1984; Legocka and Zajchert, 1999; Sen et al., 2014).

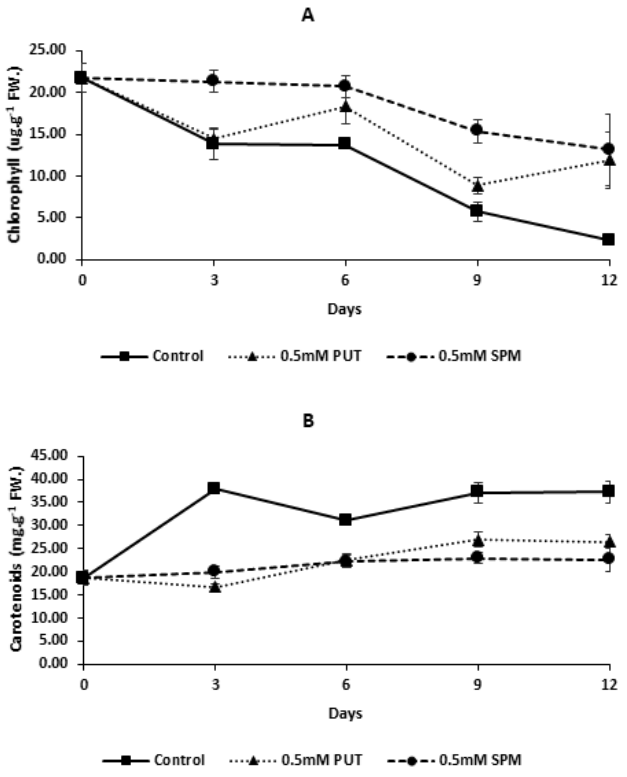

Figure 7 Change of chlorophyll (A) and carotenoid (B) content in bananas which had been immersed with water, 0.5mM PUT and 0.5mM SPM and stored at 10 °C. Each data point is the mean ±SE of three replicate samples.

Conclusion

The results of the present experiment on the effect of PUT and SPM treatment on improving quality of banana at low temperature storage, indicated that PUT and SPM treatment extended the shelf life, delayed the ripening fruit and inhibited the browning symptom. SPM showed higher efficacy than PUT. The possibility that the process of exogenous PUT and SPM can reduced chlorophyll degradation and phenolic biosynthesis.

Acknowledgements

I wish to express thanks to scientist of Faculty of Animal Sciences and Agricultural Technology for supporting. This research was funded by the Silpakorn University Research and Development Institute (SURDI), Silpakorn University, Thailand.

Reference

Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts: Polyphenol oxidase in *Beta vulgaris*. *Plant Physiology*, *24*, 1-15.

Arpita, S., Subroto, D., Pinaki, B., and Bidyut, B. (2010). Inhibition of polyphenol oxidase in banana, apple and mushroom by using different anti-browning agents under different conditions. *International Journal of Chemical Sciences*, *8*, S550-S558.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*, 248–254.

Bouchereau, A., Aziz, A., Larher, F., and Martin-Tanguy, J. (1999). Polyamines and environmental challenges: recent development. *Plant Science*, 140, 103–125.

Champa, W. A. H., Gill, M. I. S., Mahajanb, B. V. C., and Arora, N. K. (2014). Postharvest treatment of polyamines maintains quality and extends shelf-life of table grapes (*Vitisvinifera* L.) cv. Flame Seedless. *Postharvest Biology and Technology*, *91*, 57–63.

Cheng, S. H., Shyr, Y. Y., and Kao, C. H. (1984). Senescence of rice leaves XII. Effect of 1, 3 diaminopropane, spermidine and spermine. *Botanical Bulletin of Academia Sinica*, *25*, 191-196.

Cohen, A. S., Popovic, R., and Zalik, S. (1979). Effects of polyamines on chlorophyll and protein content, photochemical activity, and chloroplast ultrastructure of barley leaf discs during senescence. *Plant Physiology*, *64*, 717–720.

De Oliveira Lima, L. C., Chitara, A. B., Chitara, M. I. F., and Silva, E. B. (1999). Enzymatic activities changes in spongy tissue: A physiological ripening disorder of 'Tommy Atkins' mango. *ISHS Acta Horticulturae*, *485*, 255-285.

Ding, P., and Ling, Y. S. (2014). Browning assessment methods and polyphenol oxidase in UV-C irradiated Berangan banana fruit. *International Food Research Journal*, *21*(4), 1667-1674.

El-Hilari, F., Ail-Oubahou, A., Remah, A., and Akhayat, O. (2003). Chilling injury and peroxidase activity change in "Fortune" mandarin fruit during low temperature storage. *Bulgarian Journal of Plant Physiology*, *29*, 44-54.

Facundoa, H. V. V., Gurak, P. D., Mercadante, A. Z., Lajolo, F. M., and Cordenunsi, B. R. (2015). Storage at low temperature differentially affects the colour and carotenoid composition of two cultivars of banana. *Food Chemistry*, *170*, 102–109.

Flurkey, W. H. and Jen, J. J. (1978). Peroxidase and polyphenoloxidase actives in developing peaches. *Journal of Food Science*, *43*, 1826-1831.

Franco-Mora, O., Tanabe, K., Itai, A., Tamaru, F., and Itamura, H. (2005). Relationship between endogenous free polyamine content and ethylene evolution during fruit growth and ripening of Japanese pear (*Pyrus pyrifolia Nakai*). *Journal of the Japanese Society for Horticultural Science*, *74*(3), 221-227.

Gill, S. S., and Tuteja, N. (2010). Polyamines and abiotic stress tolerance in plants. *Plant Signaling and Behavior*, *5*(1), 26–33.

González-Aguilar, G. A., Gayosso, L., Cruz, R., Fortiz, J., Báez, R., and Wang, C. Y. (2000). Polyamines induced by hot water treatments reduce chilling injury and decay in pepper fruit. *Postharvest Biology and Technology*, *18*(1), 19-26.

Hodge, J. E. and Hofreiter, B. T. (1962). Carbohydrates. *In* Methods in Carbohydrate Chemistry (Whistler, R. L. and Miller, J. N. B. eds.). pp. 17-22. Academic Press, New York.

Hussaina, S. S., Alib, M., Ahmadc, M., and Siddique, K. H. M. (2011). Polyamines: Natural and engineered abiotic and biotic stress tolerance in plants. *Biotechnology Advances*, 29(3), 300-311.

Jhalegar, Md. J., Sharma, R. R., Pal, R. K., and Rana, V. (2012). Effect of postharvest treatments with polyamines on physiological and biochemical attributes of kiwifruit (*Actinidia deliciosa*) cv. Allison. *Fruits*, *67*, 13–22.

Jiang, Y. M., and Chen, F. (1995). A study on polyamine change and browning of fruit during cold storage of litchi (*Litchi chinensis Sonn*.). *Postharvest Biology and Technology*, *5*(3), 245-250.

Jiang, Y. M., Joyce, D. C., Jiang, W. B., and Lu, W. J. (2004). Effects of chilling temperatures on ethylene binding by banana fruit. *Plant Growth Regulation*, *43*, 109-115.

Ketsa, S., and Atantee, S. (1998). Phenolics, lignin, peroxidase activity, and increased firmness of damaged pericarp of mangosteen fruit after impact. *Postharvest Biology and Technology*, *14*, 117-124.

Kousheshsaba, M., Arzani, K., and Barzegar, M. (2012). Postharvest polyamine application alleviates chilling Injury and affects apricot storage ability. *Journal of Agricultural and Food Chemistry*, *60*, 8947−8953.

Legocka, J., and Zajchert, I. (1999). Role of spermidine in the stabilization of the apoprotein of the light-harvesting chlorophyll a/b-protein complex of photosystem II during leaf senescence process. *Acta Physiologiae Plantarum*, 21, 127–132.

Liu, J. H., Wang, W., Wu, H., Gong, X., and Moriguchi, T. (2015). Polyamines function in stress tolerance: from synthesis to regulation. *Frontiers in Plant Science*, *6*, 1-10.

Mayer, A. M., and Harel, E. (1979). Polyphenol oxidases in plants. *Phytochemistry*, *18*, 193-214.

McCollum, T. G., and McDonald, R. E. (1991). Electrolyte leakage, respiration and ethylene production as indices of chilling injury in grapefruit. *HortScience*, *26*, 1191–1 192.

Mirdehghan, S. H., Rahemi, M., Castillo, S., Martínez-Romero, D., Serrano, M., and Valero, D. (2007). Pre-storage application of polyamines by pressure or immersion improves shelf-life of pomegranate stored at chilling temperature by increasing endogenous polyamine levels. *Postharvest Biology and Technology*, *44*(1), 26-33.

Morilla, A., Garcia, J.,, and Albi, M. A. (1996). Free polyamine contents and decarboxylase activities during tomato development and ripening. *Journal of Agricultural and Food Chemistry*, *44*, 2608-2611.

Nilprapruck, P., Meetum, P., and Chanthasa, C. (2016). Influence of exogenous spermidine on to peel color change and browning symptom of *Musa* (AAA group) cv. Kluai Hom Thong at low temperature storage. *Silpakorn University Science and Technology Journal*, *10*(1), 38-42.

Paksasorn, A., Hayasaka, T., Matsui, H., Ohara, H., and Hirata, N. (1995). Relationship of polyamine content to ACC content and ethylene evolution in Japanese apricot fruit. *Journal of the Japanese Society for Horticultural Science*, *63*, 761–766.

Palma, F., Carvajal, F., Jamilena, M., and Garrido, D. (2014). Contribution of polyamines and other related metabolites to the maintenance of zucchini fruit quality during cold storage. *Plant Physiology and Biochemistry, 82*, 161-171.

Pandey, S., Ranade, S. A., Nagar, P. K., and Kumar, N. (2000). Role of polyamines and ethylene as modulators of plant senescence. *Journal of Biosciences*, *25*(3), 291-299.

Roy, P., Niyogi, K., Sengupta, D. N., and Ghosh, B. (2005). Spermidine treatment to rice seedlings recovers salinity stress-induced damage of plasma membrane and PM-bound H+-ATPase in salt-tolerant and salt-sensitive rice cultivars. *Plant Science*, *168*, 583–591.

Sen, G., Eryilmaz, I. E., and Ozakca, D. (2014). The effect of aluminium-stress and exogenous spermidine on chlorophyll degradation, glutathione reductase activity and the photosystem II D1 protein gene (psbA) transcript level in lichen Xanthoriaparietina. *Phytochemistry*, *98*, 54-59.

Serrano, M., Martinez-Romero, D., Guillén, F., and Valero, D. (2003). Effects of exogenous putrescine on improving shelf life of four plum cultivars. *Postharvest Biology and Technology*, *30*(3), 259-271.

Theiss, C., Bohley, P., and Voigt, J. (2002). Regulation by polyamines of ornithine decarboxylase activity and cell division in the unicellular green alga Chlamydomonas reinhardtii. *Plant Physiology*, *128*(4), 1470–1479.

Vaughn, K. C., Lax, A. R., and Andduke, S. O. (1988). Polyphenoloxidase: the chloropIast oxidase with no established function. *Physiology of Plants*, *72*, 659-665.

Walker, J. R. L., and Ferrar, P. H. (1998). Diphenol oxidases, enzyme-catalysed browning and plant disease resistance. *Biotechnology and Genetic Engineering Reviews*, 15, 457-498.

Wills, R., Mcglasson, B., Graham, D., and Joyce, D. (1998). Effects of temperature. In: Postharvest, 4th ed., UNSW Press, Australia.

Zhang, W., Jiang, B., Lia, W., Song, H., Yu, Y., and Chen, J. (2009). Polyamines enhance chilling tolerance of cucumber (*Cucumis sativus* L.) through modulating antioxidative system. *Scientia Horticulturae*, *122*, 200–208.

Zhang, Y., Zhang, H., Zou, Z.R., Liu, Y., and Hu, X. H. (2015). Deciphering the protective role of spermidine against saline-alkaline stress at physiological and proteomic levels in tomato. *Phytochemistry*, *110*, 13–21.