Research Article

Influence of Exogenous Spermidine on to Peel Color Change and Browning Symptom of *Musa* (AAA group) cv. Kluai Hom Thong at Low Temperature Storage

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Received February 24, 2014; Accepted March 10, 2015

Abstract

Musa (AAA Group) cv. Kluai Hom Thong is popular and widely consumed fruits in Thailand and overseas. This experiment showed an influence of spermidine (SPD) on change of peel color and browning in banana. Ripe bananas were soaked with 0, 0.2, 0.5 and 2.0 mM and then stored at 10 ºC, 85±5 %RH. Bananas, treated with 0.5 and 2.0 mM SPD, showed alteration of biochemical changes such as color, ripping and enzymatic browning reaction. Biochemical changes included chlorophyll, carotenoid, phenolic compounds, as well as polyphenol oxidase and peroxidase activities which increased during storage. Bananas, treated with SPD at 0.5 and 2.0 mM, exhibited a reduction in an accumulation of phenolic compounds, polyphenol oxidase and peroxidase activities, comparing with the non-treated SPD samples.

***Key Words*:** Banana cv. Kluai Hom Thong, Spermidine, Phenol, Enzymatic Browning Reaction and Chlorophyll

Introduction

Polyamines (PAs) are low molecular weight compounds which present in all living organisms (Cohen, 1998). They are essential for growth and development in both prokaryotes and eukaryotes. The PAs in plants are divided into three types, including putrescine (PUT), spermidine (SPD) and spermine (SPM) (Flores et al., 1989). Several studies have shown that growth and development positively correlated with the concentration of either total PAs or one of the three main PAs (Biasi et al., 1988; Kushad, 1998; Ziosi et al., 2003; Singh and Malik, 2004). Furthermore, it has been reported that alteration of PAs correlated with various abiotic stress conditions, and stress-induced PAs accumulation is important for plants to respond to abiotic stresses.

PAs are used in postharvest handling as it has an effect in decreasing plant stress. For example, it had been reported that exogenous free PAs reduced softening and ripening of Mangoes (*Mangifera indica* L.) (Singh and Malik, 2004). Although, there was a study which indicated that PAs inhibited chilling injury (CI) in cucumber (*Cucumis sativus* L.) (Zhang et al., 2009), the mechanism in which PA affected CI has not been elucidated. Treating cold-sensitive produces with hot water may mitigate and reduce the chilling symptoms (Rodov et al., 1995; Gonza´lez-Aguilar, et al., 2000).

The catalytic action of enzymatic browning has an enormous impact of the quality of several fruits and results in alteration of color, flavor, texture and nutritional value (Vamos-Vigyazo, 1981). For export, banana fruits have been stored in the container at an optimum temperature at 13ºC. During storage and transportation, banana fruits have changed their skin color as a result of enzymatic reaction. The biochemical pathway of browning reaction during storage at a low temperature of bananas occurred and caused internal cell damage which induced the change in peel color by the action of polyphenoloxidase (PPO) and peroxidase (POD) (Mayer, 1987).

Several studies have shown that PPO activity increased during ripening, senescence or stress condition when the membrane was damaged and the relationship between POD and browning of fruits had been widely reported. This study aimed to investigate the influence of exogenous SPD - one of PAs - on biochemical change of banana during low temperature storage. Peel color, chlorophyll, carotenoid contents and phenolic compound as well as activity of polyphenol oxidase and peroxidase were assessed.

Materials and Methods

Plant material

Musa AAA (Group) cv. Kluai Hom Thong - harvested at 70% maturity - was purchased from Thayang Agricultural Cooperative Ltd., Phetchaburi province. They were immediately transported to the laboratory, Faculty of Animal Sciences and Agricultural Technology, Silpakorn University. Bananas were washed with 100 ppm hydrochloride solution for 5 minutes to suppress fruit rot disease and were subsequently immersed in 200 ppm Ethephon® for 10 minutes. They were then air-dried at an ambient temperature and stored at room temperature for 3 days. The ripe bananas were soaked with 0, 0.5 and 0.20 mM spermidine solution (SPD) for 5 minutes and stored at 10 °C. The fruits were randomly chosen from each treatment at 0, 6, 9 and 12 day to determine their biological changes.

Peel color

Peel color change during storage were measured by colorimeter (Model MiniScan EZ, HuterLab). Hunter L^*, a^* and b^* values were assessed. All color measurements were taken under the condition of standard illuminant D65.

Chlorophyll and carotenoid contents

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

Total phenolic compounds

Total phenolic compound was determined using the modified method of Ketsa and Atantee (1998). One grams of banana peel were homogenized with 12 ml of 80% ethanol for 1 minute. The homogenized mixture was centrifuged at 4,400 rpm for 20 minutes. The supernatant liquid at 1 ml. was mixed with 10% folin-ciocalteu reagent at 4 ml. and 7.5% sodium carbonate at 5 ml. and then allowed to settle for 30 minutes. The absorbance of the sample solution was measured by a spectrophotometer (Model Libra S22, Biochrom) at 765 nm. Total phenolic concentration was determined based on a standard curve of gallic acid.

Polyphenol oxidase and peroxidase extraction and enzyme activities

PPO and POD extraction was carried out at 4 °C and the activities were measured using the modified method from De Oliveira Lima et al. (1999). One gram of pulp was extracted into 10 ml of 0.05M sodium phosphate buffer pH 6.5 and then supernatant was used for measurement of PPO and POD activities as described by Flurkey and Jen (1978) with some modifications. The result was compared with the enzyme activity standard according to Bradford (1976).

Statistical analysis

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

Results and discussion

Relation between peel color and pigment as chlorophyll and carotenoids content

Bananas were sensitivity to low temperature which caused chilling injury (CI), leading to peel color change (from green to brown) as a result of the malfunction in membrane permeability. In this experiment, bananas were stored at a low temperature (at 10°C), resulting to CI. The result showed that treated-banana with 2.0 mM SPD had both reduced CI symptom and delayed change in peel color, compared with the non-treated-banana.

Change in peel color and fruit ripening was showed in Fig 1. L*, a* and b* were widely used to evaluate the browning symptom on fruits, with each value representing lightness, red-green and yellow-blue, respectively. The decrease in L* (lightness) value in non-treated-banana with browning symptom was observed at 12 days. The a* (red-green) values in banana treated with 2.0 mM SPD was lower than that of other treatments, indicating that SPD may play a role in delaying the occurrence of browning symptom. However, there was no significant difference in b* (yellow-blue) value between each treatment.

The L* and a* values of the peel color directly correlated with pigment color of chlorophyll and carotenoid contents (Fig 2). The change of pigment color has been considered as an indicator of physiological development which can be used to evaluate the fruit quality after harvesting. In this study, the chlorophyll content in banana treated with 2.0mM SPD was highest and carotenoid content was lowest, which was correspondent to the L* and a* values. The high chlorophyll content and low carotenoid contents indicated that fruit ripening and CI had been delayed. Hence, the change of peel color, chlorophyll and carotenoid contents indicated that SPD was effective in delaying fruit ripening during storage. It is possible that PAs have affected the ethylene synthesis. It had been reported that PAs could delay senescence in plant tissues by inhibiting ACC synthesis (Mattoo and Handa, 2008; Chen et al., 2013).

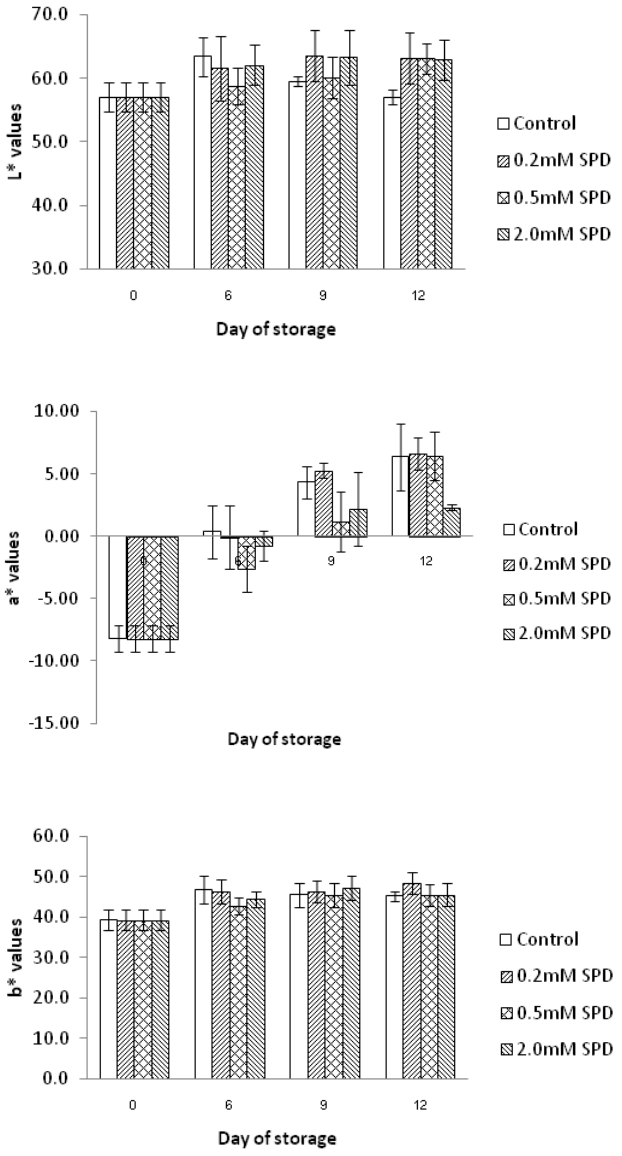

Figure 1 L*, a* and b values of peel color of bananasi, which were dipped with 0, 0.2, 0.5 and 0.2 mM SPD. Each data point represent the mean of four experiment replications

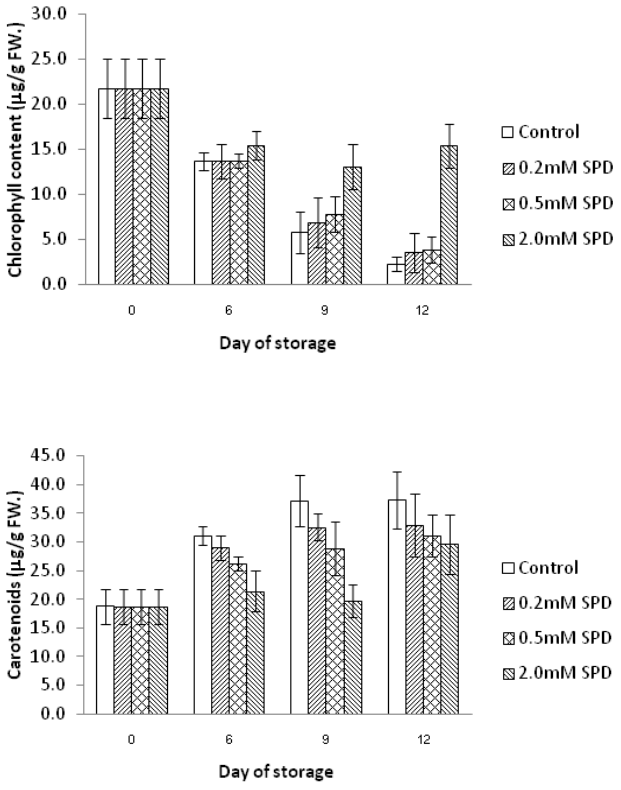

Figure 2 Chlorophyll and carotenoid contents of banana peels which were dipped with 0, 0.2, 0.5 and 0.2 mM SPD. Each data point represented the mean of four experiment replications

Relation between phenolic compounds and enzyme activity on browning symptom

Phenolic compound change in banana peel occurred during the development of browning symptom (Figure 3). The phenolic compounds interacted with oxygen in the atmosphere, catalyzing by polyphenol oxidase (PPO) and peroxidase (POD) and producing the complex browning polymer end-product. This enzymatic browning reaction caused deterioration not only in the peel color but also in the flavor and the nutritional quality (Walker and Ferrar, 1998). In this experiment, bananas were stored at 10 ^{o}C, initiating chilling injury and causing change in peel color. Consequently, the increase of phenolic compound and browning symptom of banana were detected. The suitable temperature during storage and transportation has been reported to be above 13^{o}C (Kerbel, 2004).

Phenolic compounds in the non-treated-bananas increased rapidly until the final day of storage. The phenolic compounds of all SPD-treated-bananas showed that bananas treated with 0.5 mM SPD had the lowest

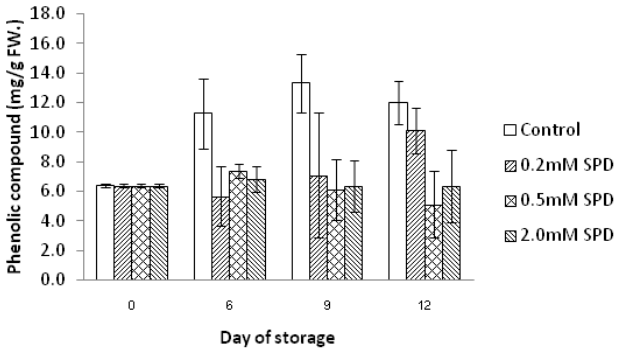

Figure 3 Phenolic compounds (mg/g.FW) of banana peels which were dipped with 0, 0.2, 0.5 and 0.2 mM SPD. Each data point represents the mean of four experiment replications

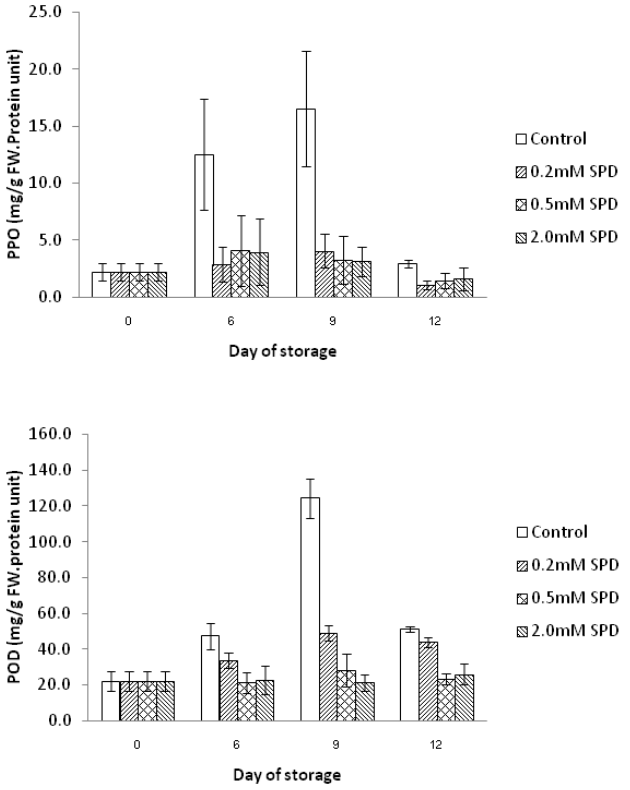

Figure 4 Enzymatic activity of banana peels, including PPO and POD activities (mg/g.FW), which were dipped with 0, 0.2, 0.5 and 0.2 mM SPD. Each data point represents the mean of four experiment replications

content of phenolic compounds, compared with the non-treated-bananas, indicating that SPD may delay the production of phenolic compounds in banana tissues.

In this study, PPO and POD activities were found to increase during storage, corresponding with the increase in the phenolic compounds. The increased PPO and POD activities affected the accumulation of the phenolic compounds in banana peel (Figure 4). The non-treated bananas were shown to possess the rapid increase in PPO and POD activities until 9 days, and these activities decreased until the final day of storage. After 9 days, the levels of PPO and POD activities in control treatment significantly reduced 3.0- and 2.5-fold, respectively.

The activity of PPO and POD in the 0.5mM SPD-treated bananas was lowest, with significant difference ($P \leq 0.01$) (Figure 4A and 4B). This indicated that SPD may play a role in inhibiting the browning reaction enzyme activity. Several studies have shown that PAs reduced chilling injury and browning symptom in fruits and vegetable such as pepper fruit (Gonzalez-Aguilar, et al., 2000), mango (Malik and Singh, 2005), peach (Liu et al., 2006) and cucumber (Zhang, et al., 2009). PAs may enhance plant tolerance to cold stress by stimulating the activity of several antioxidants such as catalyses (CAT), superoxide dismutase (SOD) and ascorbate peroxidase (APX). The increase in the antioxidant agents may reduce reactive oxygen species (ROS) – an agent initiating cell damage.

Conclusion

Consumers are sensitive to abnormally in shape and color of horticultural produces. The change of peel color and browning symptom in banana can be used to indentify senescence and fruit ripening during storage. This study found that 2.0 mM SPD was effective in both delaying a change in peel color of banana and inhibiting the degradation of chlorophyll. Additionally, treating bananas with 0.5 mM and 2.0 mM SPD reduced the browning

symptom in storage at low temperature. These bananas also possessed lower phenolic compounds and had lower activity of PPO and POD, compared with non-treated bananas.

Acknowledgement

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.

References

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

Biasi, R., Bagni, N., and Costa, G. (1988) Endogenous polyamines in apple and their relationship to fruit set and fruit growth. *Physiologia Plantarum* 73: 201-205.

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

Chen, T., Xu, Y., Wang, J., Wang, Z., Yang, J., and Zhang, J. (2013) Polyamines and ethylene interact in rice grains in response to soil drying during grain filling. *Journal of Experimental Botany* 64(8): 2523-2538.

Cohen, S. S. (1998) *A guide to the polyamines*, Oxford University Press, New York.

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.

Flores, H. E., Protacio, C. M., and Signs, M. W. (1989) Primary and secondary metabolism of polyamines in plant. In *Recent Advance in Phytochemistry* (Poulton, J.E., Romeo, J.T. and Conn, E.E., eds.) pp. 329-393. Plant Nitrogen Metabolism. Pienum Press, New York.

Flurkey, W. H. and Jen, J. J. (1978) Peroxidase and pholyphenol oxidase activities in developing peaches. *Journal of Food Science* 48: 403-405.

Gonzalez-Aguilar, G. A., Gayosso, L., Cruza, R., Fortiz, J., Baez, 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: 19–26.

Kerbel, E. (2004) Banana and Plaintain, Agriculture Handbook Number 66, The Commercial Storage of Fruits, Vegetables, and Florist and Nursery Stocks, [Online URL: www.ba.ars.usda.gov/hb66/contents.htm] accessed on April, 2004.

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.

Kusano, T., Berberich, T., Tateda, C., and Takahashi, Y. (2008) Polyamines: essential factor s for growth and survival. *Planta* 228: 367-381.

Kushad, M. M. (1998) Changes in polyamine levels in relationship to the double-sigmoidal growth curve of peaches. *Journal of the American Society for Horticultural Science* 129: 950-955.

Liu, J., Nada, K., Pang, X., Honda, C., Kitashiba, H., and Moriguchi, T. (2006) Role of polyamines in peach fruit development and storage. *Tree Physiology* 26: 791-798.

Malik, A. U. and Singh, Z. (2005) Pre-storage application of polyamines improves shelf life and fruit quality of mango. *The Journal of Horticultural Science and Biotechnology* 80: 363-369.

Mattoo, A. K. and Handa, A. K. (2008) Higher polyamines restore and enhance metabolic memory in ripening fruit. *Plant Science* 174: 386–393.

Mayer, A. M. (1987) Polyphenol oxidases in plants-recent progress. *Phytochemistry* 26: 11-20.

Rodov, V., Ben-Yehoshua, S., Albagli, R., and Fang, D. Q. (1995) Reducing chilling Injury and decay of stored citrus fruit by hot water dips. *Postharvest Biology and Technology* 5: 119–127.

Singh, Z. and Malik, A. U. (2004) Endogenous Free Polyamines of mangos in relation to development and ripening. *Journal of the American Society for Horticultural Science* 129(3): 280-286.

Vamos-Vigyazo, L. (1981) Polyphenol oxidase and peroxidase in fruits and vegetables. *Critical Reviews in Food Science and Nutrition* 15:49-127.

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

Zhang, W., Jiang, B., Li, 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.

Ziosi, V., Scaramagli, S., Bregoli, A. M., Biondi, S., and Torrigiani, P. (2003) Peach (*Pruns persica* L.) fruit growth and ripening: transcript levels and activity of polyamine biosynthetic enzymes in the mesocarp. *Journal of Plant Physiology* 160: 1109-1115.