POLYAMINE ACCUMULATION AND RESPONSES OF MANGO FRUIT TO POSTHARVEST POLYAMINE APPLICATION

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น.ส.บุษรินทร์ วรรณบุษปวิช

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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มะม่วงเป็นผลไม้เขตร้อนที่ได้รับความนิยม และมีมูลค่าทางตลาดทั้งในประเทศและต่างประเทศสูง มะม่วงเป็นผลไม้ประเภท climacteric ซึ่งมีการผลิตเอทิลีนเพิ่มขึ้นเมื่อผลเริ่มสุก ทำให้มะม่วงสุกเร็วขึ้นและมีอายุหลังการเก็บเกี่ยวสั้น มีรายงานว่าพอลิเอมีนมีความสัมพันธ์กับการสุกของผลไม้ การทดลองนี้ได้ติดตามการเปลี่ยนแปลงการสะสมพอลิเอมีนในมะม่วงสองพันธุ์ คือ มะม่วงน้ำดอกไม้เบอร์ 4 และมะม่วงน้ำดอกไม้สีทอง ขณะที่เข้าสู่ระยะสุกที่อุณหภูมิ 25 องศาเซลเซียสหลังเก็บเกี่ยว พบว่า มะม่วงน้ำดอกไม้เบอร์ 4 ผลิตเอทิลีน อัตราการหายใจ ของแข็งที่ละลายในน้ำสูงกว่า และมีปริมาณกรดที่น้อยกว่ามะม่วงน้ำดอกไม้สีทอง ระหว่างการสุกของผลไม้ ปริมาณพิวเทรสซีนลดลงและไม่มีความแตกต่างอย่างมีนัยสำคัญทางสถิติระหว่างมะม่วงสองสายพันธุ์ ปริมาณสเปอร์มิดีนและสเปอร์มีนเพิ่มขึ้นต่อเนื่อง และมีปริมาณในมะม่วงน้ำดอกไม้สีทองมากกว่าในมะม่วงน้ำดอกไม้เบอร์ 4 นอกจากนี้ มะม่วงน้ำดอกไม้เบอร์ 4 ซึ่งมีการผลิตเอทิลีนมากกว่าและมีระดับพอลิเอมีนต่ำกว่ามะม่วงน้ำดอกไม้สีทอง แสดงคุณภาพทางการตลาดดีกว่ามะม่วงน้ำดอกไม้สีทอง ซึ่งแสดงการผลิตเอทิลีนที่ต่ำกว่า ผลการทดลองแสดงให้เห็นว่าพอลิเอมีน โดยเฉพาะสเปอร์มิดีนมีบทบาทในการคงสภาพและควบคุมการสุกของผลมะม่วง และการเพิ่มขึ้นของปริมาณพอลิเอมีนอาจจะเกี่ยวข้องกับการชะลอการสุกของมะม่วงผ่านการลดอัตราการผลิตเอทิลีน การประยุกต์ใช้พอลิเอมีนเป็นวิธีการที่ปลอดภัยและมีประสิทธิภาพต่อการปรับปรุงคุณภาพและคงอายุการเก็บรักษาในมะม่วง ทดลองจุ่มมะม่วงน้ำดอกไม้เบอร์ 4 ในพิวเทรสซีน ที่ความเข้มข้น 0 1 2 และ 4 มิลลิโมลาร์ เป็นเวลา 20 นาที และเก็บรักษาไว้ที่ 14 องศาเซลเซียส เป็นเวลา 9 วัน และย้ายมาเก็บรักษาที่อุณหภูมิ 25 องศาเซลเซียส เป็นเวลาอีก 9 วัน บันทึกการเปลี่ยนแปลงทางสรีรวิทยาและชีวเคมีของผลในวันที่ 0 9 12 15 และ 18 พบว่า การใช้พิวเทรสซีน 2 มิลลิโมลาร์หลังการเก็บเกี่ยวเป็นวิธีที่มีประสิทธิภาพสูงสุดในการลดการผลิตเอทิลีน อัตราการหายใจ การสูญเสียน้ำหนัก ปริมาณของแข็งที่ละลายน้ำ และเพิ่มปริมาณกรดของผลมะม่วง นอกจากนี้ การใช้พิวเทรสซีน 2 มิลลิโมลาร์ช่วยเพิ่มความแน่นเนื้อ ลดปริมาณเพกทินที่ละลายน้ำ การทำงานของเอนไซม์พอลิกาแลกทูโรเนส และเพกทินเมทิลเอสเตอร์เลส ระหว่างการเก็บรักษา นอกจากนี้ พบว่า การประยุกต์ใช้พิวเทรสซีน 2 มิลลิโมลาร์ ทำให้การทำงานของเอนไซม์ซุปเปอร์ออกไซด์ดิสมิวเทส คะตะเลส และกัวไอเอคอลเปอร์ออกซิเดส แอสคอร์เบทเปอร์ออกซิเดส กลูตาไทโอน รีดักเทส และความสามารถในการต้านอนุมูลอิสระ ที่วิเคราะห์ด้วยวิธี DPPH radical scavenging และ FRAP ระหว่างการเก็บรักษา เพิ่มขึ้น การผลิตเอทิลีนที่ลดลงมีความสัมพันธ์กับการเพิ่มขึ้นของปริมาณพอลิเอมีนภายในผลหลังการใช้พิวเทรสซีน ผลการทดลองแสดงให้เห็นว่าการใช้พิวเทรสซีน 2 มิลลิโมลาร์หลังเก็บเกี่ยวเป็นวิธีที่ช่วยยืดอายุการเก็บรักษาและคงคุณภาพของมะม่วงน้ำดอกไม้เบอร์ 4 หลังการเก็บเกี่ยว

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Bussarin Wonnabussapawich : POLYAMINE ACCUMULATION AND RESPONSES OF MANGO FRUIT TO POSTHARVEST POLYAMINE APPLICATION. Advisor: Asst. Prof. Kanogwan Seraypheap, Ph.D. Co-advisor: Asst. Prof. Saowarath Jantaro, Ph.D.

Mango (*Mangifera indicia* L.) is a popular tropical fruit which has a great value in both domestic and international markets. Mango is a climacteric fruit which ethylene production increases dramatically, thus leading to a quick ripening process and a short postharvest life. Polyamines (PAs) have been reported to be involved in fruit ripening. Changes in endogenous free polyamines accumulation were measured in two mango cultivars; 'Nam Dok Mai No.4' and 'Nam Dok Mai Si Tong'. Mangoes were allowed to ripen at 25±1 ºC after harvest. It was found that 'Nam Dok Mai No.4' mango exhibited higher ethylene production, respiration rate, soluble solid contents and lower titratable acidity than 'Nam Dok Mai Si Tong' mango. During fruit ripening, Putrescine (PUT) content decreased and displayed no significant differences between both cultivars. SPD and SPM contents constitutionally increased and were higher in 'Nam Dok Mai Si Tong' mango than 'Nam Dok Mai No.4' mango. In addition, 'Nam Dok Mai No.4' mango, which had a higher ethylene production and a lower level of PAs showed greater market qualities than 'Nam Dok Mai Si Tong', which displayed a lower ethylene production. These results suggested that polyamines, especially spermidine (SPD) played a rejuvenating role and regulated mango fruit ripening. The increase in PAs contents may be related to a delay of mango ripening through the reduction of ethylene production. Application of polyamines after harvest also represents a safe and potentially effective method for improving quality and maintaining shelf life of mango fruit. 'Nam Dok Mai NO.4' mango was immerged in 0, 1, 2 and 4 mmol/L PUT for 20 minutes and stored at 14 ℃ for 9 days, then transferred to 25 ℃ for another 9 days. The physiological and chemical changes of treated fruit were measured on days 0, 9, 12, 15, and 18. The results showed the most effective treatment was 2 mmol/L PUT, which significantly reduced ethylene production, respiration rate, weight loss, total soluble solids, and increased titratable acidity. In addition, 2 mmol/L PUT treatment increased fruit firmness, reduced soluble pectin content, polygacturonase and pectin methyl esterase activities during storage period. Moreover, our results revealed that the exogenous application of 2 mmol/L PUT increased superoxide dismutase, catalase, guaiacol peroxidase, ascorbate peroxidase and glutathione reductase activities and total antioxidant activities by DPPH radical scavenging and FRAP during storage. The reduction of ethylene production was correlated with the increase of endogenous PAs after exogenous application of PUT. These findings suggested that postharvest exogenous application of 2 mmol/L PUT can be used as an effective method for prolonging storage life and maintaining the quality of 'Nam Dok Mai No.4' mango after harvest.

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CHAPTER I
INTRODUCTION

Mango (*Mangifera indica* L.) is one of the most popular fruits in both domestic and international markets due to its attractive aroma, good taste and nutritional properties (Ding et al., 2007). Among mango cultivars, 'Nam Dok Mai No.4' and 'Nam Dok Mai Si Tong' mangoes are the most popular cultivars with sweet and juicy pulp and golden yellow skin that attract consumers. However, mango is a climacteric fruit which ripens quickly and has a short postharvest life. There are many methods for extending shelf life of mango fruit such as modified atmosphere packaging (Kumpoun and Uthaibutra, 2010), hot water treatment (Yimyong et al., 2011) and application of polyamines (PAs) (Razzaq et al., 2014; Jongsri et al., 2017). Among those methods, application of polyamines represents a safe and potentially effective method for maintaining the storage life and quality of many fruits.

PAs are organic metabolites having low molecular weight polycations that are presented in almost all living organisms (Kusano et al., 2008). In plants, PAs functions are associated with many metabolic processes including flower development, fruit maturation, fruit softening, fruit ripening, and fruit senescence (Gill and Tuteja, 2010). Putrescine (PUT) is synthesized from ornithine and arginine through ornithine decarboxylase (ODC) and arginine decarboxylase (ADC), respectively. PUT is converted to spermidine (SPD) and spermine (SPM) by the addition of two aminopropyl residues from decarboxylated *S*-adenosylmethionine which is derived from *S*-adenosylmethionine (SAM) (Alcázar et al., 2010).

Changes in endogenous PAs level have been reported in the ripening process, which depends on cultivar, tissue type and stage of (Malik and Singh, 2004). The decrease of the endogenous PAs was found during ripening in various fruits, such as peach (Liu et al., 2006a) and tomato (Morilla et al., 1996). On the contrary, in mandarin and rambutans, PAs increased during fruit ripening (Nathan et al., 1984; Kondo et al., 2001). Although PAs utilize a common substrate with ethylene, there was an evidence indicated that ethylene and PAs biosynthesis did not compete with each other due to the concurrent increase in both PAs and ethylene levels during mango fruit ripening (Malik (Malik and Singh, 2004). The ethylene biosynthetic precursor is regulated under a crucial balance between PAs and ethylene biosynthesis (Van de Poel et al., 2013). Moreover, overexpression of *ODC* gene increased the biosynthesis of PUT, SPD, and SPM and inhibited ethylene production which led to increase quality of tomato fruit (Gupta et al., 2013). Although, PAs and ethylene create opposite effects in fruit ripening, they fountionally balance each other to control ripening process. However, the mechanism of polyamines biosynthesis during maturation and ripening of 'Nam Dok Mai No.4' and 'Nam Dok Mai Si Tong' are not yet known.

Ripening of mango fruit exhibits various changes of qualitative and nutritional properties including color, textural softening, sugar contents and taste (Singh et al., 2013). Putrescine is the main polyamine and is closely linked with fruit ripening (Dibble (Dibble et al., 1988). Recently, exogenous putrescine was found to inhibit ethylene production and delay fruit ripening. In Hayward kiwi, 1 mM exogenous putrescine repressed ethylene production, reduced respiration and maintained fruit firmness (Petkou et al., 2003). In two apricot cultivars, 'Lasgerdi' and 'Shadodi', 4

mM putrescine treatment significantly reduced weight loss and maintained firmness (Davarynejad et al., 2013). In addition, ‘Langra’ mango fruit treated with 2 mM putrescine maintained the best quality with a good blend of total soluble solids and acidity (Jawandha et al., 2012).

Ripening of mango fruit is developmentally regulated by a program associated with modification of cell wall and texture, which eventually helps in limiting the shelf-life of fruit (Singh et al., 2013). Biochemical studies showed that changes and rearrangements of the cell wall structure occurred in pectin, hemicellulose and cellulose (Seymour et al., 1990). Modification of cell wall polysaccharides involves in the coordinate action of various cell wall degrading enzymes, such as polygalacturonase (PG), pectin methylesterase (PME), pectate lyase (PL), β-galactosidase (β-Gal) and β-1,3 glucanase (Glu) (Hadfield and Bennett, 1998). The effects of polyamines are involved in maintaining cell membrane stability. Exogenous polyamines have been revealed to inhibit polygalacturonase and decline fruit softening ‘Golden Delicious’ apple (Wang and Kramer, 1990). ‘Allison’ kiwi treated with polyamines exhibited the delayed fruit softening by inhibiting activities of polygalacturonase and lipoxygenase (Jhalegar et al., 2012).

During fruit ripening, excessive production and accumulation of reactive oxygen species (ROSs) such as superoxide anion, hydroxyl radical and hydrogen peroxide. The oxidative damage which leads to the reduction of the ability of the antioxidant system to eliminate these ROSs. Without the scavenging activity, ROSs rapidly react with various molecules, including DNA and proteins resulted in membrane lipid peroxidation which leads to cell damage or cell death (Blokhina et al., 2003). The formation of ROSs is scavenged by the stimulation of antioxidant defense

enzymes such as superoxide dismutase, catalase, ascorbate peroxidase and glutathione peroxidase (Apel and Hirt, 2004). Recently, polyamine treatment demonstrated the increased antioxidant enzyme activities in ‘Angelino’ plum (Khan et al., 2008). Moreover, polyamine treatment increased catalase and superoxide dismutase activities in two commercial ‘Bagheri’ and ‘Asgarabadi’ apricots (Koushesh et al., 2012).

However, to our knowledge, the polyamines regulation has not yet been reported in ‘Nam Dok Mai No.4’ and ‘Nam Dok Mai Si Tong’ mango during fruit ripening, which is essential to maximize the application of postharvest exogenous polyamine treatments. Therefore, the role of polyamines in mango fruit ripening was investigated. Furthermore, physiological and biochemical responses of ‘Nam Dok Mai No.4’ mango to exogenous polyamine treatments were determined in terms of fruit ripening, cell wall enzyme and antioxidant enzyme metabolisms.

Objectives:

1. To determine the accumulation of polyamines in ‘Nam Dok Mai No.4’ and ‘Nam Dok Mai Si Tong’ mango fruits during fruit maturation and ripening

2. To investigate the physiological and biochemical responses of ‘Nam Dok Mai No.4’ mango during postharvest storage by polyamine application

Expecting benefits:

1. This research will lead us to gain the understanding of endogenous polyamines’role on mango fruit ripening process.

2. Exogenous polyamines application is an alternative advantage for commercial use in postharvest quality storage of mango fruit.

Content of the thesis:

1. Literature reviews

2. Investigate the accumulation of endogenous polyamines and polyamine biosynthesis enzyme activities in relation to mango fruit ripening

3. Investigate the physiological and biochemical responses of mango fruit to exogenous putrescine application

4. Results and discussion

5. Conclusion

CHAPTER II

LITERATURE REVEIWS

2.1 Mango

Mango (*Mangifera indica* L.) is an important tropical fruit having heavy demand in the World market because of its decent flavor, attractive aroma, great taste and nutritional value (Masibo and He, 2008). Mango belongs to *Anacardiaceae*, and is considered as a drupe type of fruit which the pericarp is typically divided into three distinct layers. The epicarp (also known as exocarp) is a thin outer skin while the mesocarp is an edible fleshy middle layer and the endocarp is an inner hard shell surrounding the ovary (Jacobi et al., 2001). At the present time, mango is one of the most popular fruits in both domestic and international markets. The leading mango producing countries are India, China, Thailand, Mexico, Indonesia, Pakistan, Brazil, Egypt, Bangladesh and Nigeria based on FAO statistics for the year 2016 (Fig. 1). Moreover, there are data of top net production value of mango, mangosteens and guavas as shown in Fig. 2, which Thailand is one of top five countries in producing mango (Food and Agriculture Organization Corporate Statistical Database (FAOSTAT). 2016).

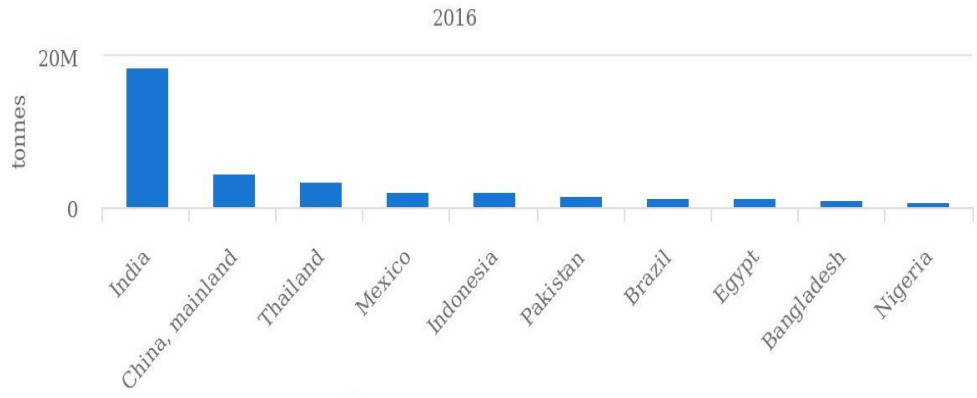


Figure 1 Top 10 country production of mango (FAOSTAT, 2016)

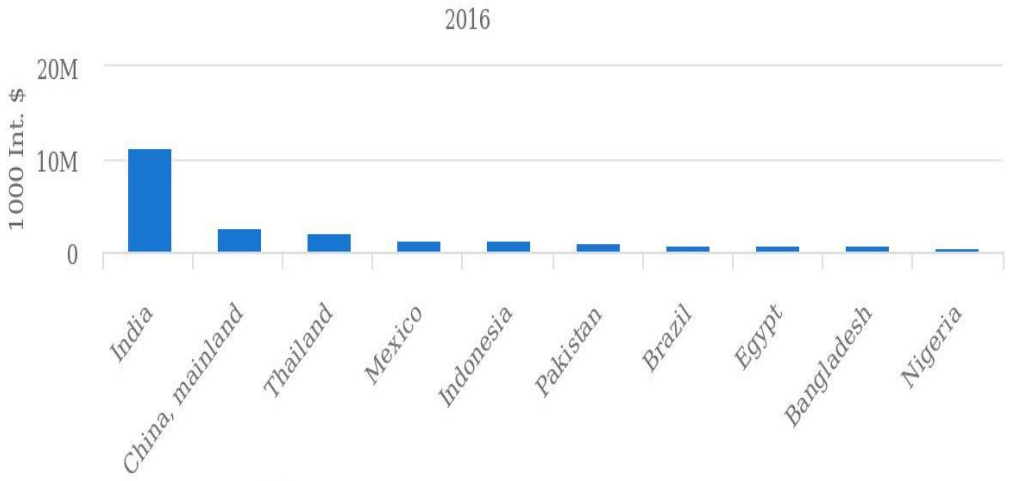


Figure 2 Top net production value of mango (FAOSTAT, 2016)

Among mango cultivars, ‘Nam Dok Mai No.4’ and ‘Nam Dok Mai Si Tong’ mangoes are the most popular cultivars with sweet juice and golden yellow skin which flaverable to most consumers. For ‘Nam Dok Mai No.4’ mango, peel has green-yelllow color at unripe stage and its changes to yellow color at ripe stage. Propagation of ‘Nam Dok Mai No.4’ mango by grafting from ‘Nam Dok Mai Tawai’ mango. Moreover, ‘Nam Dok Mai No.4’ mango is sweeter but is more susceptible to the anthracnose disease than ‘Nam Dok Mai Si Tong’ mango. On the other hand, the peel color of ‘Nam Dok Mai Si Tong’ mango changes from yellow color to a draker yellow color at ripen stage. ‘Nam Dok Mai Si Tong’ mango was the mutated cultivar of ‘Nam Dok Mai Phra Pradaeng’ mango. The peel of ‘Nam Dok Mai Si Tong’ is thicker than ‘Nam Dok Mai No.4’ mango led to more tolerance to the anthracnose disease (Manoo and Tavatchai, 2013). In 2017, Thailand exported fresh mango fruit of about 33,379,862 kilograms, which accounted for 1,672,989,317 Baht (Office of Agricultural Economics., 2017). It is well known that mango is a climacteric fruit which ripens quickly and exhibits high respiration rate, and high ethylene production (Mahto and Das, 2013). The changes occurred during mango fruit ripening cause loss

of weight, fruit softening and fruit quality degradtion after harvest and lead to a short postharvest life (Ding et al., 2007). In addition, anthracnose is the main disease occurred in mango after posharvest caused by *Colletotrichum gloesporioides*. Its infection was affected when fruit is still intact and immature and later attack mature fruit. Generally, the disease symptom occurs at ripening stage (Dodd et al., 1997). Moreover, fruit flies of the family Tephritidae are the major insects that infect mango concern with a quarantine risk in importing markets. These problems obviously lead to postharvest losses during storage, transport and market period (Mitra and Baldwin, 1997). There are many methods for ipreventing those losses and extending shelf life of mango.

2.2. Metabolisms involving in mango quality after harvest

2.2.1 Fruit ripening

Generally, fruits are divided into climacteric and non-climacteric groups based on their respiration rate and ethylene production during fruit ripening. The climacteric fruits such as apple, banana, mango, pear, tomato and other fruits show dramatic increase in respiration rate and ethylene production during ripening, then they declines (Gamage and Rahman, 1999). Non-climacteric fruits for example, citrus, grape, strawberry, etc., do not show a dramatic increase in respiration rate and ethylene production during ripening. Ethylene is the major factor involved in mango fruit ripening via coordinating the expression of many genes that are responsible for chlorophyll degradation, carotenoid synthesis, starch degradation, sugar synthesis and cell-wall modulation (Theologis et al., 1993). Ethylene controls fruit ripening since its biosynthesis, perception by receptors, signal transduction, and regulation of target

gene expression (Bouzayen et al., 2010). Methionine is a precursor for ethylene synthesis, it starts by converting methionine to S-adenosyl methionine (SAM) through methionine adenosyltransferase. Afterward, SAM changes to 1- aminocyclopropane-1-carboxylic acid (ACC) via the action of 1-aminocyclopropane-1-carboxylate synthase (ACC synthase, ACS). Then, ACC is oxidized to ethylene by 1-aminocyclopropane-1-carboxylate oxidase (ACC oxidase, ACO) (Yang and Hoffman, 1984; Kende, 1993) (Fig. 3). The changes of physiological process in fruit usually occur at the latest stage of fruit development and the earliest stage of senescence (Asif and Nath, 2005). During fruit ripening, the tranformation of physiology and biochemisty of fruit resulted in a unique texture, aroma, color, organic acid, sugars and flavor of each fruit (Goulao and Oliveira, 2008). Normally, fruit ripening is started when the etlylene production increases and reaches the threshold level, and the fruit will be at maturity stage. Under the stress condition, for instance, water limitation, physical damages during handling and pathogen infection period, fruit may commit to earlier ripening stage (Thompson, 2008).

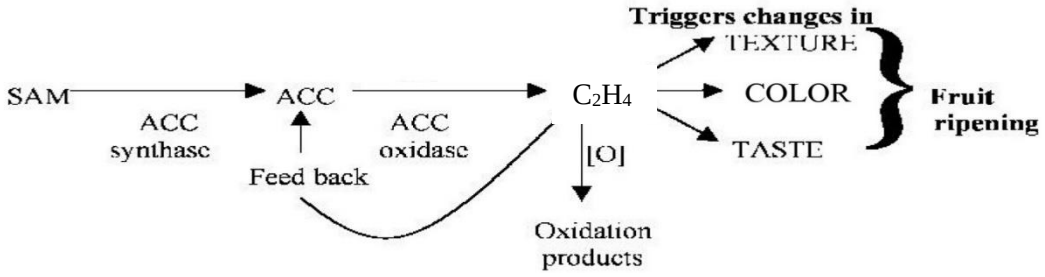


Figure 3 Pathway of ethylene biosynthesis (Prasanna et al., 2007).

Peel color is an important characteristic which is easy to observe during fruit ripening. Color changes in mango are due to chlorophyll degradation and appearance of carotenoid pigments (Lakshminarayana, 1980; Parikh et al., 1990; Lizada, 1993). Changes in peel color during mango ripening which varied among mango cultivars depend on the cultivar and pigment accumulation. Peel color of mango fruit changes from green to yellow, sometimes reddish, orange-yellow or yellowish. Carotene and xanthophylls are the main pigments in yellow cultivars, while anthocyanins appear in the peel of some cultivars (Proctor and Creasy, 1969).

Flavor is one of the factors that reported with the fruit taste, texture and aroma during ripening process. The increase in soluble solids and volatile compounds and a decrease in acidity are observed during ripening. Taste development is because of an increase in sweetness, which is mainly due to the hydrolysis of polysaccharides (especially starch) to sugar (Pantastico, 1975). In addition, the decrease in acidity is associated with the concentration of organic acid that many acids are used as substrates in the Krebs cycle (respiration process) during mango ripening, thus high respiration rate contributes to a reduction in the acid content (Seymour et al., 1990; Khosroshahi et al., 2007). The sweetness is established via the concentrations of the main sugars such as fructose, sucrose, and glucose. On the other hand, the sourness or acidity is determined by the level of the main organic acids, including citric acid, malic acid, tartaric acid, aspartic acid and glutamic acid. However, there are many parameters that affect sugar and acid concentrations, for example cultivar (Kapse et al., 1989; Kundu and Ghosh, 1992) stage of maturity at harvest (Morga et al., 1979; Tandon and Kalra, 1983), postharvest treatments (Kumar et al., 1993), and storage conditions (Vazquez-Salinas and Lakshminarayana, 1985).

2.2.1 Fruit softening

Fruit ripening is correlated with the increase in cell wall degrading enzymes. During ripening, the texture change is associated with fruit softening and changes of various metabolic metabolisms, such as loss of cell wall turgidity, breakdown of starch to sugars, and modification of cell wall structure (Goulao and Oliveira, 2008). However, the modification of cell wall is considred to be a dominant factor for the changes of fruit texture (Fischer and Bennett, 1991; Hadfield and Bennett, 1998).

Cell wall displays complex network structure which supports structural strength in the plant body. The major classes of cell wall polysaccharides include pectins, celluloses, and hemicelluloses (Prasanna et al., 2007) (Fig. 4). Generally, pectin is the major class of polysaccharides within the cell wall matrix and the middle lamella between the cell walls of plant (Willats et al., 2001). It has been reported that the changes of cell wall related to fruit softening and take place in pectin, hemicelluloses, and cellulose (Seymour et al., 1990). Fruit softening is characterized by structural changing in pectin matrix which shows the convertion of water-insoluble pectin to water-soluble pectin via cell wall degrading enzyme. Thus, this event can lead to the loosening of cell wall structure and textural changes resulting in the decline of fruit firmness (Manrique and Lajolo, 2004; Yashoda et al., 2005). Fruit softening can be varied which may relate to fruit cultivar (Selvaraj et al., 1989).

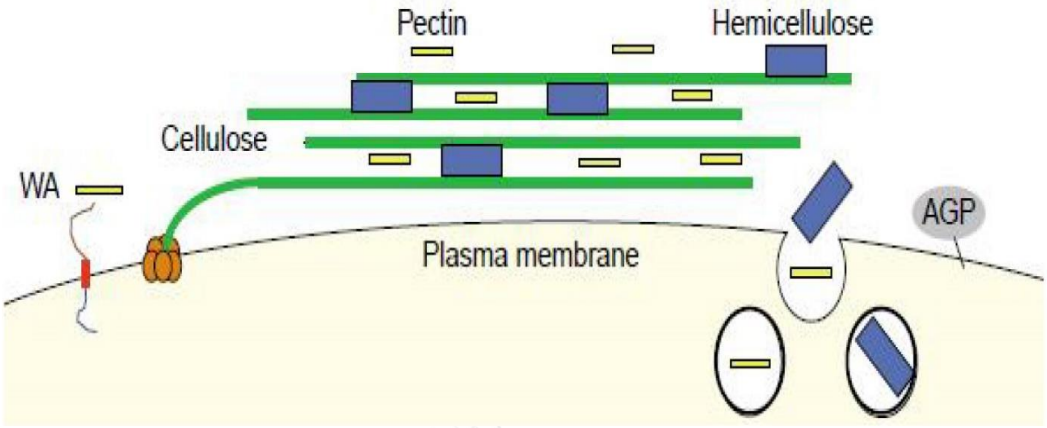


Figure 4 Cell wall composition and structure (Kohorn, 2001).

The decline of fruit firmness during ripening is involved with the actions of cell wall degrading enzymes. Among cell wall modifying enzymes, pectin degrading enzymes are mainly related to the loss of fruit firmness. Polygalacturonase (PG) is a pectin degrading enzyme catalyzing the hydrolysis of the α-1,4-glycosidic bonds between the galacturonic acid residues in galacturonans (Prasanna et al., 2007) (Fig. 5). Fruit softening affected by PG activity has been found in several plants. PG is the dominant enzyme that reacts to fruit ripening. The increase in PG activity led to high concentration of soluble pectin and an increased fruit softening (Fischer and Bennett, 1991). In tomato, the PG activity correlated to ethylene induction (Sitrit and Bennett, 1998). Moreover, the increase in PG activity was also reported in capsicum (Priyasethu et al., 1996), and banana (Prabha and Bhagyalakshmi, 1998).

Pectin methyl esterase (PME) is an enzyme that acts mainly in the hydrolysis of methyl esters from the C6 position of galacturonic acid residues contributed to deesterification of pectin (Sozzi, 2004) (Fig 2.5). Previous report showed that PME correlated to pectin degradation by lowering the degree of pectin methoxylation, leading to polygalacturonan available for degradation by PG (Koch and Nevins,

1989). It has been reported that PME activity related to many metabolic processes, such as cell wall maturation, abscission and pathogen infection (Blumer et al., 2000). Besides, PME activity was observed in many fruits, for instance avocado, mango, papaya, banana, and pear during ripening (Goulao and Oliveira, 2008). The PME activity of 'Tainong' mango dropped significantly during mango ripening (Zhang et al., 2012). Level of PME activity decreased in ripening stage of 'Kent' mango (Islas-Osuna et al., 2010).

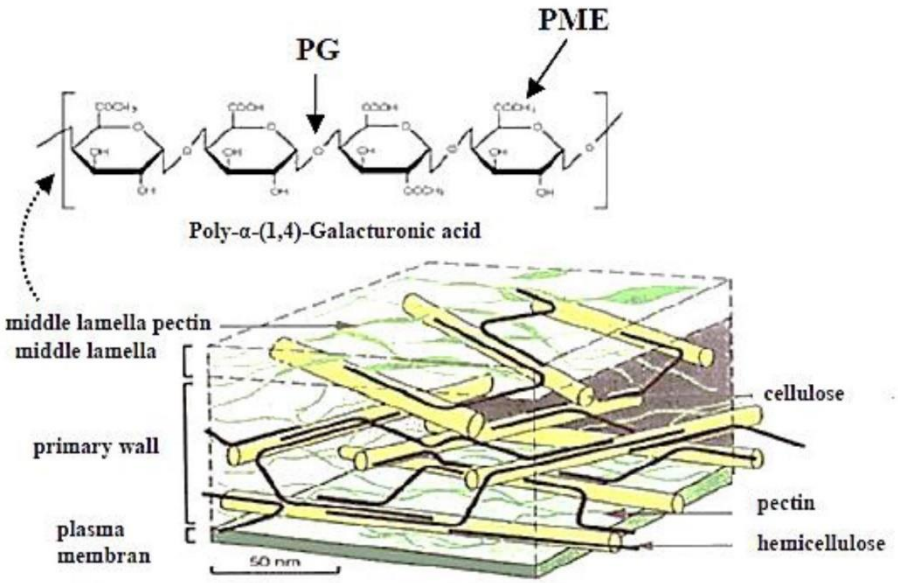


Figure 5 Function site of polygalacturonase (PG) and pectin methyl esterase (PME) react with cell wall components (International pectin producers association (IPPA). 2018).

2.3 Postharvest management of mango fruit

Generally, the shelf life of mango fruit does not exceed 2-3 weeks because of the physiological degradation and pathogen infection. Thus, various methods have been used to extend shelf life of mango, decrease losses, and inhibit ethylene production and respiration rate. These include low temperature, modified and controlled atmosphere storage, heat treatment and application of polyamines.

2.3.1 Low temperature storage

Low temperature storage is a basic method for extending the postharvest life of many kinds of fruits. Storage of fruits in a cold room can decrease metabolic activity, reduce weigh loss, reduce disease and delay ripening. However, the concept of storage is to prolong their posthavest life via storing them in proper temperature for maintaining the fruit quality. Mango is a tropical fruit which is sensitive to chilling stress when kept below a critical minimum temperature. Moreover, mango exhibits high susceptibility to disease and injury, therefore, it is needed to control the temperature during the storage. It has been reported that mango is susceptible to chilling injury (CI) when stored at temperature lower than 13 ℃ (Nair and Singh, 2003; Suresh Nair et al., 2004). The symptoms of CI in mango fruits include dark coloration of the skin, prominence of lenticels, uneven ripening, and development of off flavor (Nair et al., 2000). ‘Okrong’ mango fruit can be safely stored at 12 ℃ for up to 25 days (Phakawatmongkol et al., 2004). However, a typical aroma and flavor of ‘Alphonso’ mangoes were retarded during ripening storage at 10 ℃ for 30 days (Thomas and Oke, 1983). ‘Kensington Pride’ mango stored at 5-15 ℃ resulted in a reduction of total aroma volatile compounds in ripe fruit (Nair and Singh, 2003). Besides, mango fruit remained green at 28 ℃ or higher than 28 ℃, while mango did

not ripen when stored at 33 ℃ or higher temperature during storage (Tasneem, 2004; Yahia, 2005).

2.3.2 Heat treatment

Heat treatment is often used for prolonging postharvest life of fruit in many ways, such as hot water treatment, hot water brushing, vapor heat, and hot air (Lurie, 1998). Hot water treatment (HWT) is mainly applied in various fruits and also in mango fruit because it is cheaper than other treatments (Aveno and Orden, 2004). However, there are many factors affecting on heat treatment based on species, cultivars, fruit size, morphological and physiological ages, and growing conditions (Paull and Chen, 2000; Jacobi et al., 2001). Aveno and Orden (2004) reported that mango treated with HWT at 52-55 °C for 10 min showed a decline of anthracnose disease and stem-end rot. Moreover, HWT improved the qualities of fruit and led to a higher marketing value (Aveno and Orden, 2004). Vapor heat treatment, as high humidity heating, is a method of heating air with high moisture content and passing the air stream through the fruit to kill insect eggs and larvae (Jacobi et al., 2001). The heat from fruit surface was transferred toward the fruit pulp (Jordan, 1993).

The protocols of vapor heat treatment are widely accepted for mango in commercial, in order to improve mango qualities in many countries, for example, Thailand, Philippines, the United States and Australia (Sunagawa et al., 1987). In Philippines, ‘Carabao’ mango was heated at 46 °C for 10 min to improve the quality (Mendoza et al., 1972). ‘Kensington’ mango was heat at 47 °C for 15 min in Australia (Heather et al., 1997). Forced hot air heating was conducted by passing hot air to fruit with controlled temperature. Forced hot air heating maintained dryness of fruit peel and showed relative humidity about 30% (Hallman and Armstrong, 1994). Forced hot

air heating method was used in the United States in order to improve the mango quality (Mangan and Ingle, 1992). In addition, forced hot air heating method reported to be used with other fruit such as papaya in Hawaii (Armstrong, 1996).

2.3.3 Modified and controlled atmospheres

Modified atmosphere and controlled atmosphere storages are an alternative method to retard the storage life, maintain quality and enable long distance export of fruits. Modified atmosphere and controlled atmosphere methods involve in an atmosphere comprised of higher CO_2 and lower O_2 than common air, at optimum temperature and relative humidity leading to a delay of fruit ripening by inhibiting ethylene production, and the deteriorative processes. Thus, these methods maintained peel and pulp color, flavor and texture (fruit firmness), and induced oxidative process for pathogens resistance (Prusky and Keen, 1993). Kim et al. (2007) reported that a controlled atmosphere (3% O_2 and 10% CO_2) of 'Tommy Atkins' mango could reduce the disease in 'Tommy Atkins' mango. Many researchers attempted to optimize the composition, the suitable atmosphere compositions of controlled atmosphere (O_2 concentration at 3-5% and CO_2 concentration at 5-10%) for extending storage life of mango fruit (Yahia, 2009). Using microperforated bag with 1-methylcyclopropene (1-MCP) reduced weight loss, increased fruit firmness by reducing pectin esterase (PE) and polygalacturonase (PG) activities in 'Manila' mango (Vázquez-Celestino et al., 2016). However, modified atmosphere and controlled atmosphere methods could exhibit the anaerobic respiration leading to the fermentation. Application of chitosan at 1.5% and 2% presented discoloration of mango skin, unripe fruit and off flavor due to the formation of acetaldehyde and ethanol in 'Nam Dok Mai' mango (Jitareerat et al., 2007).

2.3.4 Polyamines treatment

Polyamines (PAs) are organic metabolites having a low molecular weight and widely discovered in almost all living organisms (Kusano et al., 2008). A major number of PAs are present in the plant, including putrescine (a diamine), spermidine (Spd, a triamine) and spermine (Spm, a tetramine) (Liu et al., 2006b) (Fig. 6). In plants, PAs are associated with many metabolic processes including flower development, fruit maturation, fruit softening, fruit ripening, and fruit senescence (Gill and Tuteja, 2010). PAs can be extracted from various organisms, such as leaves and stems of corn, oat, and radish, or from microbial sources such as *Saccharomyces cerevisiae* and *Candida utilis* (Asrey et al., 2008).

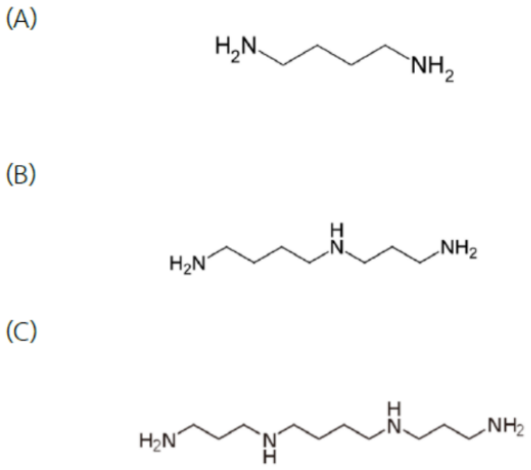


Figure 6 Structures of putresine (A), spermidine (B) and spermine (C) (Nishikawa et al., 2012).

2.3.4.1 Biosynthesis of polyamines

Higher plants synthesize PAs in two different pathways (Fig. 7). In the first pathway, putrescine (PUT) can be synthesized from ornithine in a single reaction catalyzed by ornithine decarboxylase (ODC). In the second pathway, arginine was catalyzed by arginine decarboxylase (ADC) with two intermediate products, agmatine and N-carbamoyl putrescine, which is finally converted into PUT. PUT is converted to spermidine (SPD) and spermine (SPM) by the addition of two aminopropyl residues from decarboxylated S-adenosylmethionine which is derived from S-adenosylmethionine (SAM) (Alcázar et al., 2010).

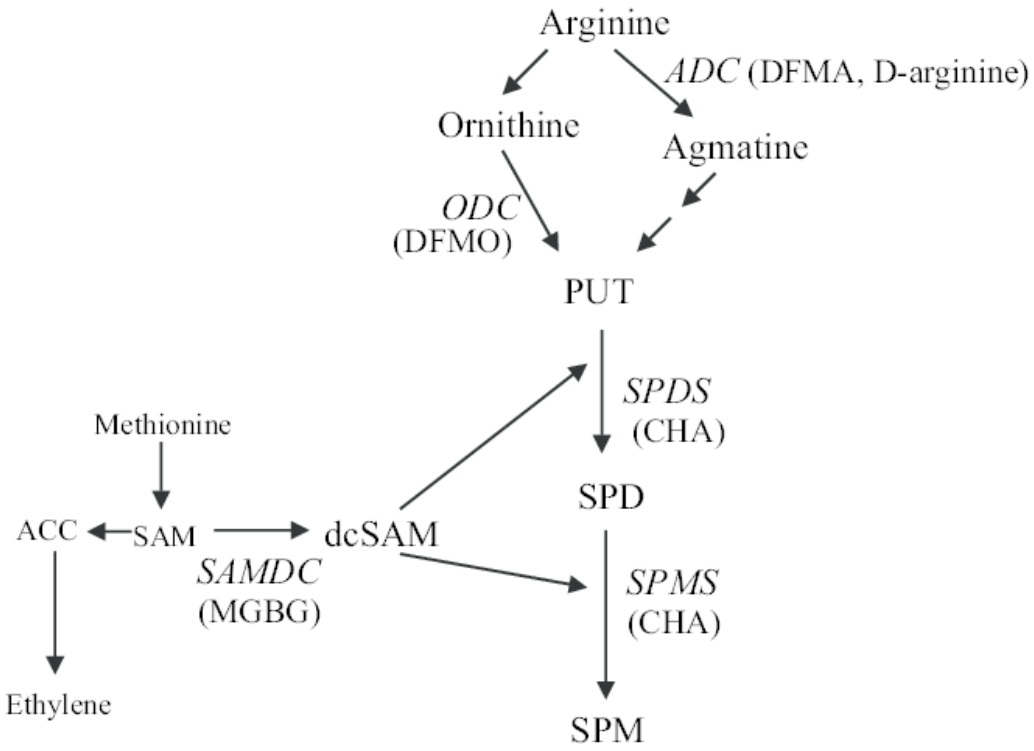


Figure 7 Polyamine biosynthesis and interconnection with ethylene biosynthesis (Liu et al., 2006b).

2.3.4.1 Role of polyamines in fruit ripening

Fruit ripening which represents the terminal phase of plant development involves changes in both biochemical and physiological characteristics, including color, texture, flavor, aroma, nutritional properties, and plant hormones (Giovannoni, 2004). Climacteric fruit ripening, such as mango, banana, and tomato is known to be controlled by ethylene (Alexander and Grierson, 2002). However, polyamines (PAs) have been reported to be involved in fruit ripening of climacteric and non-climacteric fruits (Bregoli et al., 2002; Agudelo-Romero et al., 2013) along with a transient expression of polyamine synthetic genes (Agudelo-Romero et al., 2013).

The level of endogenous PAs has been reported to change in the ripening process, which depends on cultivar, tissue type and stage of development (Malik and Singh, 2004). In general, the endogenous PAs decreased during ripening in various fruits such as peach (Liu et al., 2006a), grape (Fortes et al., 2011), apple (Biasi et al., 1988) and tomato (Morilla et al., 1996). On the contrary, in strawberry (Guo et al., 2018), mandarin (Nathan et al., 1984), and rambutan (Kondo et al., 2001), PAs were found to increase during fruit ripening. PAs biosynthetic pathway shows a common substrate with ethylene. However, there was an evidence that ethylene and PAs biosynthesis did not compete for the substrate, since the concurrent increase in PAs and ethylene was observed during mango fruit ripening (Malik and Singh, 2004). Nevertheless, the ethylene biosynthesis precursor can still be a critical balance between PAs and ethylene biosynthesis (Van de Poel et al., 2013). The level of *ADC1*, *ADC2*, and *ODC1* genes display induction during the change of color stage and then decreased at the red ripe stage in tomato (Tsaniklidis et al., 2016). The up-regulated *ADC* gene was observed, while *ODC* genes were not expressed during ripening in grape fruit (Agudelo-Romero et

al., 2013). Besides, overexpression of *ODC* gene resulted in the stimulation of PUT, SPD, and SPM and inhibition of ethylene (Gupta et al., 2013). Thus, PAs and ethylene generate opposite effects in fruit ripening, and may balance each other to control ripening process.

2.3.4.2 Effect of exogenous polyamines on fruit quality

Previous reports in many fruits found that exogenous polyamines could maintain fruit quality during storage period. The exogenous application of 1 mmol/L PUT has been reported to maintain quality, delay color changes, reduce weight loss, and inhibit ethylene production as well as decrease respiration rate in apricots (*Prunus armeniaca* L. cv Mauricio) during the storage period (Martinez-Romero et al., 2002). PAs reduced weight loss, delayed fruit color change and improved fruit quality of 'Kensington Pride' mango (Malik et al., 2005). Serrano et al. (2003) presented that application of 1 mmol/L PUT improved storage life, maintained fruit firmness, lowered soluble solids concentration, and delayed color changes in four plum cultivars, including 'Golden Japan', 'Black Diamond', 'Black Star', and 'Santa Rosa'. Barman et al. (2011) studied the effect of PUT combination with carnauba wax pretreatments on postharvest quality of pomegranate and observed the inhibited respiration and ethylene evolution rate, increased firmness, decreased soluble solids and increased titratable acidity in the pomegranate treated fruit.

2.3.4.3 Effect of exogenous polyamines on respiration rate

Respiration is a continuous process in living systems. The losses after postharvest occur because of continued respiration, transpiration, and the metabolic activities that

polysaccharides change to energy in the occurrence of oxygen (Siddiqui, 2015). In addition, the increase of respiration rate is due to ethylene production, fruit senescence and expansion of a fungal. Therefore, the reduction of respiration rate could lead to delay fruit softening as well as improve the fruit quality after harvest. During storage, ‘Allison’ kiwi treated with PAs showed a reduced respiration rate (Jhalegar et al., 2012). The 0.5 mmol/L PUT was also reported to slow down the respiration rate in‘Samar Bahisht Chaunsa’ mango when stored at 32 ± 2 °C and at 11 ± 1 °C (Razzaq et al., 2014). A PUT treatment could delay the ripening process by inhibiting respiration rate in ‘Angelino’ plum (Khan et al., 2008). Barman et al. (2011) reported that a combined PUT with carnauba wax in pomegranates stored at low temperature showed delayed respiration rate and improved quality.

2.3.4.3 Effect of exogenous polyamines on ethylene production

Ethylene is a gaseous plant hormone that influences fruit ripening process. The postharvest life of the fruit involved in the increase of ethylene concentration which leads to senescence. The biosynthesis of PAs and ethylene share a common precursor, which is SAM but have antagonistic effects during fruit ripening and senescence (Nichols et al., 1983). Several researchers have reported that PAs act as anti-ethylene agents. The application of PUT to ‘Liberty’ tomato has been reported to be more effective due to low ethylene production and improve the storage life (Saftner and Baldi, 1990). In addition, PUT treatment inhibited ethylene biosynthesis via inhibiting ACC-synthase activity and ACC synthesis (Lee et al., 1997). The 2 mmol/L PUT treatment reduced ethylene biosynthesis in ‘Selva’ strawberry (Khosroshahi et al., 2007). Two mmol/L PUT significantly reduced ethylene production in ‘Samar

Bahisht Chaunsa' mango throughout the storage period (Razzaq et al., 2014). The exogenous application of 1 mmol/L PUT and SPD also inhibited ethylene production and enhances shelf life in 'Bagheri' and Asgarbadi' apricots (Saba et al., 2012).

2.3.4.3 Effect of exogenous polyamines on weight loss

Weight loss during storage period is an important parameter to determine the quality of fruit. PAs reduce the weight loss because of inhibition of respiration rate and ethylene production during handling and storage. Valero et al. (1998b) assessed the effects of PUT and found that PUT significantly reduced weight loss with decreased ethylene production and respiration rate. Davarynejad et al. (2013) assessed the effect of PUT on quality of two apricot cultivars 'Lasgerdi' and 'Shahrodi' and found that fruit treated with 4 mmol/L PUT could reduce the weight loss, whereas the maximum physiological loss in weight was in untreated fruit. Application of 1 mmol/L PUT significantly reduced weight loss in 'Kensingtion Pride' mango (Malik et al., 2005). The 1 mmol/L PUT-treated apricot exhibited lower weight loss than the control fruit during storage (Martinez-Romero et al., 2002). Barman et al. (2011) found that a combination between 2 mmol/L PUT and carnauba wax (1:10) treatment was the most effective method in reducing physiological weight loss of 'Mridula' Pomegranate during storage.

2.3.4.4 Effect of exogenous polyamines on fruit firmness

Fruit firmness is the major limiting factor influences the marketing and storage life of fruit. Firmness is due to presence of pectic substances in the cell wall of plant. During fruit ripening, changes of cell wall degrading enzymes such as polygalacturonase (PG), pectin methyeaterase (PME), pectin esterase, and cellulase resulted in fruit

softening (Mitcham et al., 1991). The exogenous PUT (4 mmol/L) application enhanced the storage life of ‘Santa Rosa’ plum by maintaining fruit firmness (Davarynejad et al., 2015). The application of 0.5 mmol/L PUT and 0.5 mmol/L SPD of ‘Flame Seedless’ table grapes .reduces the fruit softness during storage (Champa et al., 2014). PAs reduced the softening of fruits due to the binding between PAs and pectin substance and blocked the entry of cell wall-degrading enzymes, such as pectin esterase, polygalacturonase (PG), and pectin methyeaterase (PME) (Valero et al., 2002).

2.3.4.5 Effect of exogenous polyamines on soluble solid contents (SSC) and titratable acidity (TA)

SSC is an important parameter influenced their acceptability among consumers. The SSC is continuously increased because of the hydrolysis of polysaccharides during the storage period (Mahto and Das, 2013). TA contents decreased, which is involved in the concentration of organic acid. The respiration appears to provoke consumption of organic acids (Khosroshahi et al., 2007). The application of PUT significantly reduced the SSC in two apricot cultivars, ‘Lasgerdi’ and ‘Shahrodi’ (Davarynejad et al., 2013). Other researchers reported that PUT-treated plum fruit exhibited lower SSC, higher TA and delayed respiration rate when compared with the control fruit (Khan et al., 2008). Effects of application PAs on SSC and TA have also been reported in different fruits, such as pomegranate (Mirdehghan et al., 2007) and apricot (Martinez-Romero et al., 2002), mango (Jawandha et al., 2012), and strawberry (Zafari et al., 2015).

2.3.4.6 Effect of exogenous polyamines on reactive oxygen species and antioxidant activities

The production and accumulation of reactive oxygen species (ROSs) progressively increase during fruit ripening process. Plants are damaged usually because of an imbalance between the production and elimination of ROSs. Thus, ripening can be considered a stressful process because of an increased level of ROSs (Scandalios, 1993; Jimenez et al., 2002). Oxidative stress happens when the level of ROSs increases, for example, the superoxide radical, hydrogen peroxide and the hydroxyl radical (Vierling and Kimpel, 1992). The sources of ROSs involve in normal metabolism, including photosynthesis and respiration, as well as the pathways enhanced during abiotic stresses (Mittler, 2002). Generally, the antioxidant defense system includes enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), peroxidase(POX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), guaiacol peroxidase (GPOX), and glutathione reductase (GR). The oxidative stress can also be scavenged by non-enzymatic antioxidants, including ascorbic acid, beta-carotene and glutathione (Gill and Tuteja, 2010). It has been reported that zucchini fruit treated with 1 mmol/L PUT had higher APX and GR activities in respect to control in zucchini fruits (Palma et al., 2016). Saba et al. (2012) reported that the application of 1 mmol/L PUT and SPM increased the activities of CAT, SOD, and POX in both ‘Bagheri’ and Asgarbadi’ apricot. ‘Samar Bahisht Chaunsa’ mango with PUT treatment showed an increase in CAT, SOD, and POX during storage (Razzaq et al., 2014). It can be inferred that the effect of PAs on antioxidant enzymes caused PAs to act as ROSs scavengers or binding to antioxidant enzymes molecules to scavenge free radical (Duan et al., 2008; Groppa and Benavides, 2008).

Antioxidant activity is a crucial nutrition and play vital roles to maintain fruit quality during postharvest storage. Fruits are rich in antioxidant, which is an importance to the consumer's health by given resistant against many diseases. Thus, it is necessary to preserve antioxidant activity during storage without hampering fruit quality. PAs have been reported to maintain significant antioxidant capacity in 'Allison' kiwi fruit during storage (Jhalegar et al., 2012). Shiri et al. (2013) reported that the application of PUT maintained the antioxidant activity in 'Shahroud' grape. Davarynejad et al. (2015) found that the 'Santa Rosa' plum fruit treated with 4 mmol/L putrescine enhanced the total antioxidant during storage period. These antioxidants were well conserved by putrescine, which are basically anti-senescence agents (Seiler and Raul, 2005).

2.3.4.7 Effect of exogenous polyamines on postharvest diseases

PAs metabolism has altered responses to biotic stress and undergo profound changes in plants interacting with fungal and pathogens. It was found that 'Selva' strawberry treated with PUT showed less fungal infection symptoms when compared with control fruit and maintained the fruits with a very good appearance (Khosroshahi et al., 2007) The increase in PAs content have been presented in leaves of barley infected with the brown rust (*Puccinia hordei*) and powdery mildew fungi (*Blumeria graminis* f. sp. *hordei*) (Greenland and Lewis, 1984; Walters and Wylie, 1986). The chitosan combined with spermidine reduced the severity of anthracnose of 'Nam Dok Mai' mango (Jongsri et al., 2017). In addition, the application of PAs of pomegranates reduced the severity of husk scald (Mirdehghan et al., 2007). It can suggest that exogenous putrescine may have an anti-pathogenic function in fruits.

CHAPTER III

MATERIALS AND METHODS

3.1 Materials

3.1.1 Plant materials

Two mango cultivars (*Mangifera indica* L.), cv. ‘Nam Dok Mai No.4’ and cv. ‘Nam Dok Mai Si Tong’, were obtained from Saichon Commercial Orchard, Nakhon Ratchasima province at commercial maturity (90-100 days after fruit set). Mango fruit were selected for uniformity of size (350-450 g), color and as well as lack of noticeable defects.

3.2. Instruments

3.2.1 Equipments for PUT treatment and storage

- Phytotron room
- Beaker
- Cylinder
- Balance (PG503-S, Mettler Toledo, USA)
- Plastic basket
- Plastic tray
- Timer

3.2.2 Equipments for sample collecting

- Knife and cutting board
- Aluminium foil
- Liquid nitrogen
- Balance (U6100S, Sartorius Universal, USA)
- Balance (ML204, Mettler Toledo, USA)
- Balance (PG503-S, Mettler Toledo, USA)
- Balance (AG2885, Mettler Toledo, USA)
- Deep freezer -80 ℃ (New Brunswick Scientific, Belgium)

3.2.3 Equipments for studying some physiological changes

- Balance: U6100S (Sartorius Universal, USA)
- Penetrometer (Hardness tester FHM-1, Takemura, Japan)
- Colorimeter (Color Reader CR-10, KONICA MINOLTA SENSING, INC., Japan)
- Refractometer (N-1E, Japan)
- Gas chromatograph (GC 7890B, Agilent Technologies, Inc., USA).
- Glass jar 2.4 L
- Syringe 10 mL

3.2.4 Equipments for measuring biochemical responses, the accumulation of endogenous polyamines and activities of the enzymes that involve in polyamine synthesis of mango fruit ripening

- Refractometer (N-1E, Japan)
- Knife and cutting board
- Balance (ML204, Mettler Toledo, USA)
- Microcentrifuge (Sorvall Biofuge Pico, Germany)
- Micropipette: (Lite XLS Rainin, Mettler Toledo, USA France)
- Pipette tip
- Mortars and pestles
- Cylinder
- Beaker
- Flasks
- Spatulas
- Freezer -20 ℃ (Sanyo Electric Co., Ltd, Japan,)
- Refrigerated centrifuge (Universal 320R, Hettich, Germany)
- Refrigerated centrifuge (Universal 32R, Hettich, Germany)
- Vortex mixture (Vortex-Genie 2, Scientific Industries, Inc., USA)
- Proline prospenser bottle top dispenser (BIOHIT PROLINE®Finland)

- Eppendorf tubes

- Timer

- Autoclave: TC-459 (Taichung, Taiwan)

- Liquid nitrogen container

- Hand sprayers for ethanol

- Forceps

- pH meter (S220 Seven Compact, Mettler Toledo, USA)

- Cellulose acetate membrane (0.45 µm)

- Septum

- Syringe 10 mL

- Tube for HPLC analysis

- High-performance liquid chromatograph (HPLC, shimadzu CT-10AS VP, Japan)

3.2.5 Equipments for analysis of fruit softening enzyme activities

- Digital water bath (DAIHAN LABTECH CO., LTD)

- Incubator (memmert, BE600)

- Vortex mixture (Vortex-Genie 2, Scientific Industries, Inc., USA)

- Microplate readers (SpectraMax M3, Molecular Devices, Inc., USA)

- Micropipette: Pipet-Lite XLS (Rainin, Mettler Toledo, USA France)

- 96 well plate

- Hotplate stirrer

- Shaker

- Centrifuge (Universal 32R Hettich, Germany)

- Test tube

3.2.6 Equipments for analysis of activities of antioxidant enzymes

- Mortars and pestles

- Spatula

- Liquid nitrogen container

- Refrigerated centrifuge (Universal 32R, Hettich, Germany)

- Microplate readers (SpectraMax M3, Molecular Devices, Inc., USA)

- Micropipette: Pipet-Lite XLS (Rainin, Mettler Toledo, USA France)

3.3 Chemicals and reagents

3.3.1 Chemicals for preparing PUT solution

- Distilled water

- Tween® 80

- Putrescine

3.3.2 Chemicals for measuring titratable acidity (TA)

- Distilled water

- Sodium hydroxide

- Phenolphthalein

3.3.3 Chemicals for measuring polyamines content

- Perchloric acid

- 1, 8 diaminooctane

- Sodium hydroxide

- Benzoyl chloride

- Sodium chloride

- Diethyl ether

- Absolute methanol

- Putrescine

- Spermidine

- Spermine

3.3.4 Chemicals for ADC and ODC enzyme extraction and analysis

- Potassium phosphate buffer

- Phenylmethylsulfonyl fluoride (PMSF)

- Dithiothreitol (DTT)

- Ascorbic acid

- Pyridoxal phosphate (PLP)

- Ethylenediaminetetraacetic acid (EDTA)

- Polyvinylpyrrolidone (PVPP)

- Tris-HCl buffer

- L-arginine

- L-ornithine

- Perchloric acid

- 1,8 diaminooctane

- Sodium hydroxide

- Benzoyl chloride

- Sodium chloride

- Diethyl ether

- Absolute methanol

- Putrescine

- Spermidine

- Spermine

3.3.5 Chemicals for extraction of fruit softening enzymes

- Tris-HCl (pH 7.0)

- Ethylenediaminetetraacetic acid (EDTA)

- Triton x-100

- Cysteine-HCl

3.3.6 Chemicals for PG activity assay

- Polygalacturonic acid

- Sodium acetate

- Dinitrosalicylic acid (DNS) reagent

- Galacturonic acid

3.3.7 Chemicals for PME activity assay

- Citrus pectin

- Bromothymol blue

- Potassium phosphate buffer

3.3.8 Chemicals for measuring soluble pectin content

- Absolute ethanol

- Sodium hydroxide

- Sodium tetraborate

- Sulfuric acid

- M-hydroxydiphenyl

- D-galacturonic acid

3.3.9 Chemicals for antioxidant enzyme extraction

- Potassium phosphate buffer (pH 7.0)

- Polyvinylpyrrolidone (PVPP)

- Dithiothreitol (DTT)

- Phenylmethylsulfonyl fluoride (PMSF)

3.3.10 Chemicals for SOD activity assay

- Potassium phosphate buffer (pH 7.8)

- Ethylenediaminetetraacetic acid (EDTA)

- cytochrome C

- Xanthine

3.3.11 Chemicals for CAT activity assay

- Potassium phosphate buffer (pH 7.0)

- Hydrogen peroxide

3.3.12 Chemicals for GPOX activity assay

- Potassium phosphate buffer (pH 6.0)

- Hydrogen peroxide

- Guaiacol

3.3.13 Chemicals for APX activity assay

- Potassium phosphate buffer (pH 7.0)

- Hydrogen peroxide

- Ethylenediaminetetraacetic acid (EDTA)

- Ascorbic acid

3.3.14 Chemicals for GR activity assay

- Potassium phosphate buffer (pH 7.5)
- Ethylenediaminetetraacetic acid (EDTA)
- 5, 5-dithiobis-2-nitrobenzoic acid (DTNB)
- Dihydronicotinamide adenine dinucleotide phosphate (NADPH)
- Glutathione disulfide (GSSG)

3.3.15 Chemicals for H_2O_2content analysis

- Phosphate buffer (pH 6.5)
- Hydroxylamine
- Titanium sulphate
- Sulfuric acid

3.3.16 Chemicals for DPPH free radical scavenging assay

- 1, 1-diphenyl-2-picrylhydrazyl (DPPH)
- Ethanol

3.3.17 Chemicals for ferric reducing antioxidant power assay

- Acetate buffer, pH 3.6
- 2,4,6-tris (1-pyridy)-5-triazine (TPTZ)
- Hydrochloric acid
- Iron trichloride ($FeCl_3$)
- Iron (II) sulfate heptahydrate ($FeSO_4$. $7H_2O$)

3.4 Methods

3.4.1 Determinations of endogenous polyamine accumulation and polyamine biosynthetic enzyme activities in relation to mango fruit ripening

'Nam Dok Mai No.4' and 'Nam Dok Mai Si Tong' mangoes were collected during their commercial maturity (90-100 days after fruit set) and immediately transported to the laboratory. All mango fruit were cleaned with tap water and air dried. Thereafter, the fruit were allowed to ripen at 25 ± 1 ºC with 65% ± 5% relative humidity for 12 days in the dark. The experiments were performed by means of completely randomized design with 4 independent replications and each replication consists of at least 2 samples. Fruit were analyzed on days 0, 3, 6, 9 and 12. The whole fruit was used to determine in weight loss, color change, ethylene production, and respiration rate. The mesocarp was collected, and frozen at -80 ºC until the analyses of soluble solids content (SSC), titratable acidity (TA), polyamine contents, and enzyme activities were performed.

3.4.1.1 Weight loss

Weight loss of each fruit was evaluated after 3, 6, 9 and 12 days of storage. Weight loss was calculated by subtracting final weight from initial weight of fruit. The weight loss was determined using formula as described by AOAC (1980).

$$\text{Weight loss (\%)} = \frac{\text{(weight at the time of harvest - weight after storage)}}{\text{weight at the time of harvest}} \times 100$$

3.4.1.2 Firmness

Fruit firmness determination was carried out by using a penetrometer (Hardness tester FHM-1, Takemura, Japan). The measurement of the force was made

on 3 equatorial regions of fruit, including blossom end, middle, and stem end of the fruit. Results were reported in Newtons (N).

3.4.1.3 Peel color changes

Color of mango was measured using colorimeter (Konica Minolta Sensing, Inc., Japan). Color was expressed as L*, a, b*, C, and H° parameters as an average from three points (blossom end, middle and stem end) for each fruit.

3.4.1.4 Soluble solid contents (SSC)

The soluble solids content of mango was determined using a hand-held refractometer (N-1E, Japan) and SSC was expressed as °Brix.

3.4.1.5 Titratable acidity (TA)

Titratable acidity (TA) analysis was determined using titration method AOAC (1980). Ten g of sliced mango was added in 100 mL of distilled water and filtered. Then, the mixture was evaluated using phenolphthalein solution as indicator and titrating against with 0.1 mol/L NaOH. The results were expressed as percentage of citric acid following the equation:

$$\%TA = \frac{NaOH\ (mL) \times 0.1\ NaOH\ (mol/L) \times 0.07}{10\ g} \times 100$$

3.4.1.6 Respiration rate and ethylene production

Respiration rate and ethylene production were measured after recording fruit weight by placing mango fruit in a sealed jar (2.4 L) for 1 h at 25 ºC. Headspace gas sample (1mL) was withdrawn using a syringe and injected into a gas chromatograph (GC 7890B, Agilent Technologies, Inc., USA). The temperature of the column was 60 ºC and 75 ºC for carbon dioxide and ethylene, respectively. Helium was utilized as a carrier gas. Results of respiration rate were measured as carbon dioxide released per kg of fruit per

hour and shown as mg CO_2 $kg^{-1}h^{-1}$. Ethylene production was determined and calculated as µL $kg^{-1}h^{-1}$.

3.4.1.7 Polyamine contents

Polyamine contents were analyzed according to the method of Flores and Galston (1982). Extraction of polyamine from the mesocarp (1 g) was ground in liquid nitrogen to powder with 10 mL of 5% cold perchloric acid, incubated at 4 °C for 1 h and 1,8 diaminooctane (0.5 mmol/L) was used as internal standard. After extraction, the homogenate was centrifuged at 10000×g for 15 min at 4 °C and supernatant (500 µL) was further used to determine polyamine by benzoylation. Sequential addition of 1 mL of 2 NaOH and 10 µL of benzoyl chloride were carried out into the sample. Sample tubes were vortexed for 30 s and then incubated for 20 minutes at room temperature (25 °C). After that, the sample tube was mixed with saturated sodium chloride (2 mL) and shaken for 30 s to terminate the reaction. Then, the benzoylated polyamines were extracted with diethyl ether (2 mL) and were centrifuged at 10000×g for 5 min at 4 °C. The diethyl ether phase (1 mL) was transferred into the new tube, and evaporated samples to dryness. The samples were dissolved in 1 mL of methanol and filtered with a 0.45 µm cellulose acetate membrane. Finally, the filtered solution (30 µL) was injected into high-performance liquid chromatograph (HPLC, shimadzu CT-10AS VP, Japan), using the C18 column (Inertril ODS-3, 4.6×150 mm, 5 µm; GL Science Inc., Japan) at 40 °C with UV detector at 254 nm. The solvent flow rate was 1.0 mL/min and the mobile phase was methanol: water (volume : volume : 60: 40) for 35 min. The PAs contents were expressed as nmol g^{-1} fresh weight.

3.4.1.8 ADC and ODC activities

ADC and ODC activities were measured following the method of Birecka et al. (1985). Mango pulp tissue (1 g) was homogenized in 5 mL of potassium phosphate buffer (100 mmol/L, pH 8.0) containing 0.1 mmol/L phenylmethylsulfonyl fluoride (PMSF), 5 mmol/L dithiothreitol (DTT), 25 mmol/L ascorbic acid, 1 mmol/L pyridoxal phosphate (PLP), 5 mmol/L ethylenediaminetetraacetic acid (EDTA), and 0.1% polyvinlypyrrolidone (PVPP). The homogenate was centrifuged at 4 °C and 10,000×g for 15 min and the supernatant was collected for further evaluation of ADC and ODC activities.

The sample mixtures contained 100 mmol/L Tris-HCl buffer (pH 7.5), 5 mmol/L EDTA, 5 mmol/L DTT, 50 mmol/L PLP, and 300 µL extracted protein solution. After vortexing for 10 s and incubated for 2 min at 37 °C, the ADC and ODC activities were treated with L-arginine (50 mmol/L) and L-ornithine (50 mmol/L), respectively, and incubated at 37 °C for 60 min. Then, the reaction mixture was centrifuged at 10000×g for 15 min. The supernatant fraction (500 µL) was mixed with 1 mL of 2 NaOH and 10 µL of benzoyl chloride. The samples were vortexed for 30 s, and incubated for 20 min at room temperature, and saturated sodium chloride (2 mL) was added in order to stop the reaction. The solution was centrifuged at 10,000×g for 15 min, 2 mL of diethyl ether was added. One mL of the diethyl ether phase was collected and evaporated to dryness. Lastly, the samples were dissolved in 1 mL of methanol and 30 µL of the sample was injected into the HPLC. The ADC and ODC activities were measured by analyzing the putrescine production by HPLC (The HPLC conditions were the same as defined in 3.4.1.7). The results were expressed as nmol putrescine g^{-1} fresh weight.

3.4.1.9 Protein contents

The protein concentration was determined by following the Bradford (1976) method. The reaction samples consisted of 50 µL Bradford dye reagent (Biorad), 100 µL of distilled water, and 50 µL of enzyme extract. Then, the samples were incubated for 5 min at room temperature. The absorbance was read at 595 nm and using bovine serum albumin as the standard.

3.4.1.10 Statistical analysis

Data were subjected to statistical analysis of variance using the IBM SPSS Statistics 22 (IBM SPSS, USA). Comparisons by means of different time point using the independent sample t-test at a significance level of 0.05 and the experimental data were expressed as mean ± standard errors (SE).

3.4.2 Determination of the physiological and biochemical responses of mango fruit to exogenous putrescine application

Mangoes (*Mangifera indica* L. cv. 'Nam Dok Mai No.4') grown at Saichon Commercial Orchard, Nakhon Ratchasima province were harvested during their commercial maturity (90-100 days after fruit set) on June 2016 and immediately transported to the laboratory within 4 h. Mangoes with uniformity of size and color, disease-free were selected for the experiment. The experiment was designed with a CRD, including 4 replications and each replication consists of at least 2 samples. Thirty-two mangoes were selected to measure physiological quality before treatments (day 0). Afterward, fruits were immersed in various solutions for 20 min. All solution contained tween-20 (0.2% v/v) to improve the absorption of the polyamine and the treatment was performed as described below:

Treatment 1: distilled water (control)

Treatment 2: 1 mmol/L putrescine

Treatment 3: 2 mmol/L putrescine

Treatment 4: 4 mmol/L putrescine

After treatments, mangoes were led to dryness before storage. To study the responses of putrescine application treated mango at low temperature during a commercial transportation, all of the control and treated fruits were stored in a control room at 14 °C with a relative humidity of 90 ± 5% for 9 days, and fruits were transferred to 25±1 °C with a relative humidity of 65±5%. Then, fruits were randomly sampled at 3, 6, and 9 days of shelf life at 25 °C±1 °C (room temperature). The mesocarp was collected, frozen in liquid nitrogen, and stored at -80 ºC for further physiological and chemical analysis.

3.4.2.1 Weight loss

Weight loss of each fruit was analyzed as previously described (3.4.1.1)

3.4.2.2 Firmness

Fruit firmness determination was measured by a penetrometer (Hardness tester FHM-1, Takemura, Japan) and calculated as previously described (3.4.1.2)

3.4.2.3 Peel color changes

Color changes were determined using colorimeter (Konica Minolta Sensing, Inc., Japan) and analyzed as previously described (3.4.1.3)

3.4.2.4 Soluble solids content (SSC)

The SSC was measured by a hand-held refractometer (N-1E, Japan), and SSC was expressed as °Brix.

3.4.2.5 Titratable acidity (TA)

Titratable acidity (TA) analysis was determined using titration method and calculated as previously described in 3.4.1.5

3.4.2.6 Respiration rate and ethylene production

Respiration rate and ethylene production were measured by GC (GC 7890B, Agilent Technologies, Inc., USA) as previously described (3.4.1.6).

3.4.2.7 Soluble pectin content

Soluble pectin content in the pulp was extracted and analyzed following Robertson (1979). One g of sample was homogenized in 95% (v/v) ethanol, boiled at 85 ºC for 10 min, shaken and centrifuged at 20 °C at 10,000×g for 15 min. After that, insoluble fraction was added with 63% (v/v) ethanol, boiled at 85 ºC for 10 min and shaken. The insoluble was centrifuged at 20 °C at 10,000×g for 15 min. Then, the homogenate was filled with 6 mL of distilled water and blew for 10 min, centrifuged at 20 °C and 10,000×g for 15 min. The supernatant 5 mL was added with 0.5 mL of 1 N NaOH and 4.5 mL of distilled water. Finally, the supernatant was incubated at room temperature for 15 min before analysis.

For soluble pectin determination, 100 µL of supernatant were added with 900 µL of distilled water on ice. The mixture was mixed with 5 mL of 0.0125 mmol/L $Na_2B_4O_7$ (in conc. Sulfuric acid). To stop the reaction, the sample was boiled at 100 °C for 10 min and transferred to an ice bath. The mixture was added with 100 µL of 0.15% m-hydroxydiphenyl (in 0.5% NaOH), shaken and mixed for 15 min. The absorbance was read at a wavelength 520 nm, using D-galacturonic acid as standard.

3.4.2.8 Enzyme extraction and cell wall-degrading enzymes analysis

Mango pulp (1 g) was homogenized in 20 mmol/L Tris-HCl (pH 7.0), 0.02 mol/L EDTA, 0.05% triton X-100 and 0.02 mol/L cysteine-HCl. Afterward, homogenate was centrifuged at 10,000 x g for 15 min at 4 ºC. The supernatant was collected for PG and PME activities.

3.4.2.8.1 Polygalacturonase (PG) activity

PG activity was evaluated as described by Pathak and Sanwal (1998). The reaction mixture containing 500 µL of the enzyme extract, 300 µL of 1% polygalacturonic acid (PGA, 50 mmol/L sodium acetate pH 4.5) and 200 µL of 0.2 mol/L sodium acetate buffer pH 4.5. The mixture was incubated at 37 ºC for 1 h. The activity was determined by measuring the amount of reducing sugar released from polygalacturonic acid (Miller, 1959), 0.5 ml of dinitrosalicylic acid (DNS) was added to the reaction mixture. Then, the sample was boiled for 5 min and cooled at room temperature. The sample was measured at 520 nm, PG activity was shown as nmol of galacturonic acid per min per milligram of protein.

3.4.2.8.2 Pectin methylesterase (PME) activity

PME activity was measured following Hagerman and Austin (1986). The sample containing 400 µL of 0.02% (w/v) of bromothymol blue (0.003 mol/L potassium phosphate buffer pH 7.5), 2 ml of 0.5 % (w/v) citrus pectin, 50 µL of water and 50 µL of enzyme extract. The supernatant was used to measure the absorbance at 620 nm. One unit of PME activity was calculated as nmol of methyl ester hydrolyzed per min per milligram of protein.

3.4.2.9 Enzyme extraction and antioxidant enzyme activity analysis

Fruit extract for determination of SOD, CAT, GPOX, APX, and GR activity were prepared by grinding 1 g pulp tissue with 50 mmol/L potassium phosphate buffer (pH 7.0) containing 4% (w/v) polyvinylpyrrolidone, 4 mmol/L dithiothreitol, and 1 mmol/L phenylmethylsulfonyl. The homogenate was centrifuged at 10,000 x g for 15 min at 4 °C. The supernatant was used for analysis of antioxidant enzyme activities and protein content.

3.4.2.9.1 SOD activity assay

SOD activity was analyzed as described by McCord and Fridovich (1969). The mixture of 200 µL contained 50 mmol/L potassium phosphate buffer (pH 7.8), 0.02 mmol/L cytochrome C, 0.2 mmol/L EDTA (pH 8.0), and 20 µL of the enzyme extract. Then, 0.1 mmol/L Xanthine was added to the mixture sample. SOD activity was determined by its 50% inhibition of the rate of reduction and monitored at a wavelength 290 nm. The results were shown as units of enzyme per milligram of protein.

3.4.2.9.2 CAT activity assay

The activity of CAT was analyzed according to Beers and Sizer (1952). The reaction mixture for CAT activity assay contained 50 mmol/L potassium phosphate buffer (pH 7.0), 10 mmol/L hydrogen peroxide, and 20 µL of the enzyme extract. The absorbance at 240 nm was recorded. CAT activity was calculated and presented as units of enzyme per milligram of protein.

3.4.2.9.3 GPOX activity assay

The activity of GPOX was analyzed by following MacAdam et al. (1992). Twenty µL of the pulp enzyme extract was added to 50 mmol/L potassium

phosphate buffer (pH 6.0), 0.2 mmol/L hydrogen peroxide and 2.5 mmol/L guaiacol. The mixture samples were monitored for the absorbance at 470 nm and the data were calculated and expressed in units of enzyme per milligram of protein.

3.4.2.9.4 APX activity assay

APX activity was determined according to Nakano and Asada (1981). The 200 μL reaction mixture contained 20 μL of the pulp enzyme extract, 50 mmol/L potassium phosphate buffer (pH 7.0), 10 mmol/L hydrogen peroxide, 1 mmol/L EDTA (pH 8.0), 0.5 mmol/L ascorbic acid. The APX activity was determined as the decrease in absorbance at 290 nm and expressed as units of enzyme per milligram of protein.

3.4.2.9.5 GR activity assay

GR activity was analyzed using the method of Smith et al. (1988). The GR reaction solution contained 100 mmol/L potassium phosphate buffer (pH 7.5), 0.5 mmol/L EDTA (pH 8.0), 0.75 mmol/L 5,5′ dithiobis (2-nitrobenzoic acid), 0.1 mmol/L NADPH, 0.5 mmol/L GSSG, and 20 μL of pulp extract in a final volume of 200 μL. Changes in the increase of the absorbance at 290 nm were determined. The GR activity was defined as units of enzyme per milligram of protein.

3.4.2.10 Hydrogen peroxide content

The hydrogen peroxide content was determined as described by Jana and Choudhuri (1982). To determine the hydrogen peroxide content, an enzyme extract was prepared by homogenizing 1 g of mesocarp tissue in 100 mmol/L potassium phosphate buffer (pH 6.5) and 1 mmol/L hydroxylamine at 0 °C. The homogenate was then centrifuged at 10,000g for 15 min at 4 °C, and the supernatant was used for the hydrogen peroxide content. The assay was carried out in a mixture consisting of 600 μL

supernatant and 90 µL of 0.3% titanium sulphate in 20% H_2SO_4 were freshly prepared before use. The reaction mixture was centrifuged at 10,000g for 10 min. The amount of H_2O_2 were measured at 410 nm and express as µmol/g FW.

3.4.2.11 DPPH free radical scavenging assay

The DPPH assay was performed based on the methods of Choi et al. (2006). Mango pulp (1 g) was homogenized in 10 mL of 80% ethanol. Then, homogenate was centrifuged at 10,000g for 15 min at 4 °C. Clear supernatant was used for the analysis. The activity was analyzed in a reaction mixture containing 300 µL of 80% ethanol, 400 µL of 0.2 mmol/L DPPH solution and 100 µL of fruit extract and incubated in the dark for 10 min. The absorbance was measured at a wavelength of 515 nm. An ethanol without DPPH radical was used as the control in the experiment. Results were expressed as a percentage of inhibition of the DPPH radical and calculated from the following equation:

$$\% \text{ Inhibition} = \frac{(1 - \text{Absorbance (sample)}) \times 100}{\text{Absorbance (control)}}$$

3.4.2.12 Ferric reducing antioxidant power assay (FRAP assay)

FRAP assay was conducted using the method as described by Benzie and Strain (1999). To prepare the FRAP solution, mixing 25 mL of 0.3 mol/L acetate buffer (pH 3.6), 2.5 mL of 10 mmol/L 2,4,6-tris (1-pyridy)-5-triazine (TPTZ) solution in 40 mmol/L HCl, and 20 mmol/L $FeCl_3.6H_2O$. The reaction mixtures contained 3000 µL of FRAP solution and 150 µL of fruit extracts. After incubation for 30 minutes in a dark condition. The mixtures were measured the absorbance at 593 nm and expressed as micromoles of ferrous equivalent Fe (II) per gram fresh weight.

3.4.2.13 Polyamine contents

Polyamine contents including PUT, SPD and SPM were analyzed as previously described (3.4.1.7).

3.4.2.14 ADC and ODC activities

ADC and ODC activities were measured as previously described in 3.4.1.8

3.4.2.15 Protein contents

The protein concentration in each sample was estimated as previously described (3.4.1.9)

3.4.2.16 Statistical analysis

The results were shown as means ± S.E. The data were statistically analyzed by one-way analysis of variance (ANOVA). The means were compared by Duncan's Multiple Range Test at a significant level of 0.05 ($p \leq 0.05$) using the IBM SPSS Statistics 22 (IBM SPSS, USA).

CHAPTER IV

RESULTS

4.1 The accumulation of endogenous polyamines and polyamine biosynthetic enzyme activities in relation to mango fruit ripening

4.1.1 Weight loss and firmness evaluations

During mango fruit ripening, percentage of weight loss in ‘Nam Dok Mai No.4’ and ‘Nam Dok Mai Si Tong’ mangoes gradually increased throughout the storage period. It was found that there was no significant difference in weight loss between ‘Nam Dok Mai No.4’ and ‘Nam Dok Mai Si Tong’ mangoes during the whole storage period (Table. 1). Moreover, mango fruit exhibited a decrease in firmness throughout storage time in both cultivars. However, no significant difference in the fruit firmness was observed in both mango cultivars during the storage period (Table. 1).

4.1.2 SSC and TA evaluations

SSC in both mango cultivars were shown in table 1. The SSC continued to increase constantly until the end of storage period from 8.3±0.18 to 17.50±0.32 °Brix for ‘Nam Dok Mai No.4’ and from 7.8±0.13 to 16.90±0.23 °Brix for ‘Nam Dok Mai Si Tong’. The finding in this research showed that TA content decreased constantly with the storage period. TA content of ‘Nam Dok Mai Si Tong’ was significantly higher than ‘Nam Dok Mai No.4’ throughout the storage time (Table. 1).

Table 1: Changes in weight loss, firmness, SSC and TA of 'Nam Dok Mai No.4' and 'Nam Dok Mai Si Tong' mangoes during 12 days of storage period at 25 °C

Time (Days)	Mango cultivar	Weight loss (%)	Firmness (N)	SSC (°Brix)	TA (%)
0	No.4	0	8.56 ± 0.7 ns	8.30 ± 0.18 a	2.05 ± 0.08 b
	Si Tong	0	8.57 ± 0.06 ns	7.80 ± 0.13 b	2.25 ± 0.07 a
3	No.4	1.60 ± 0.09 ns	8.31 ± 0.06 ns	9.64± 0.21 a	1.49 ± 0.06 b
	Si Tong	1.47 ± 0.04 ns	8.46 ± 0.05 ns	8.73± 0.07 b	1.68 ± 0.06 a
6	No.4	4.48 ± 0.27 ns	8.14 ± 0.07 ns	12.89 ± 0.11 a	1.01 ± 0.06 b
	Si Tong	4.20 ± 0.08 ns	8.26 ± 0.07 ns	9.79 ± 0.10 b	1.36 ± 0.04 a
9	No.4	7.15 ± 0.47 ns	7.49 ± 0.04 ns	15.22 ± 0.28 a	0.50 ± 0.03 b
	Si Tong	7.09 ± 0.16 ns	7.46 ± 0.13 ns	14.70 ± 0.27 b	0.97 ± 0.04 a
12	No.4	12.01 ± 0.49 ns	5.92 ± 0.08 ns	17.50 ± 0.32 a	0.10 ± 0.01 b
	Si Tong	12.11 ± 0.24 ns	5.91 ± 0.08 ns	16.25 ± 0.23 b	0.18 ± 0.01 a

For each column, different letter for the same storage period were significantly different at $p \leq 0.05$ according to t-tests while ns showed no significance. Data are the means ± SE.

4.1.3 Peel color evaluation

The peel color of ‘Nam Dok Mai No.4’ and ‘Nam Dok Mai Si Tong’ slightly changed during the storage period. The peel color of ‘Nam Dok Mai No.4’ mango changed from green color to yellow. However, peel color of ‘Nam Dok Mai Si Tong’ changed from light yellow color to dark yellow (Fig. 8). The L* value of ‘Nam Dok Mai No.4’ increased from day 0 until the end of storage time while ‘Nam Dok Mai Si Tong’ was found to decline throughout the shelf life period (Table 2). The a* value ranged from the negative which was a green color to the positive which was a red color. The results demonstrated that there was a gradual decrease in the a* value of ‘Nam Dok Mai No.4’, while increasing for ‘Nam Dok Mai Si Tong’ during ripening at ambient temperature (Table 2). The b* value ranged from the negative which was a blue color to the positive which was yellow. The b* value of peel mango revealed a gradual increase during the ripening process in both cultivars. However, the b* value in ‘Nam Dok Mai No.4’ was higher than that of ‘Nam Dok Mai Si Tong’ on days 0, 3, and 6 of the storage time and there were no significant differences on days 9 and 12 (Table 2). In addition, C* value showed a slight increase throughout the storage time in both mango cultivars. C* value of ‘Nam Dok Mai No.4’ was significantly higher than ‘Nam Dok Mai Si Tong’ ($P<0.05$) on days 0, 3, 6 and 9 (Table 2). During the storage period, H° value demonstrated a steady decline, when storage time increased. ‘Nam Dok Mai No.4’ exhibited a higher H° value than ‘Nam Dok Mai Si Tong’ during ripening at room temperature ($P<0.05$) (Table 2).

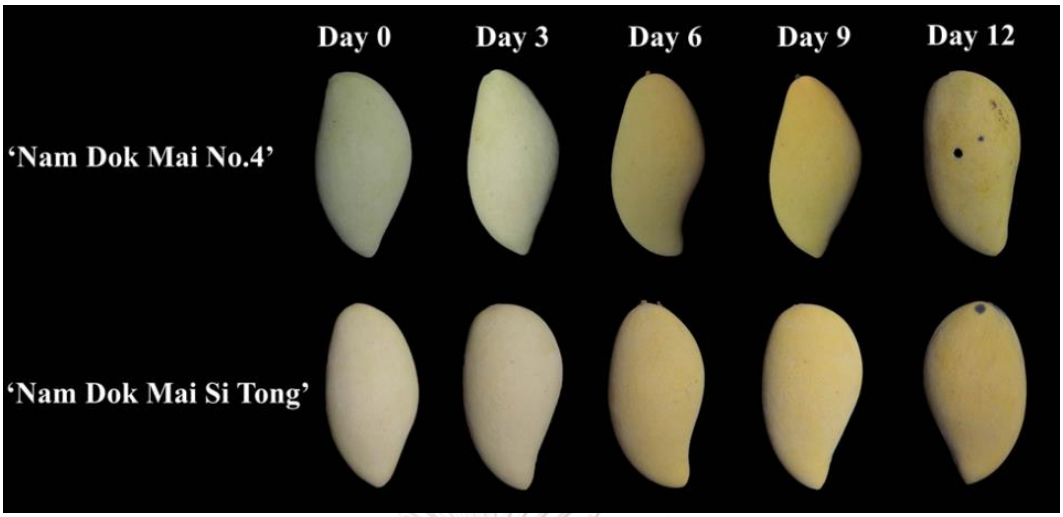


Figure 8: Peel color change of 'Nam Dok Mai No.4' and 'Nam Dok Mai Si Tong' mangoes during storage at 25 ºC for 12 days.

Table 2: Changes in color of 'Nam Dok Mai No.4' and 'Nam Dok Mai Si Tong' mangoes during for 12 days of storage at 25 °C

Storage periods	Cultivar	L* value	a* value	b* value	C* value	H° value
0	No.4	69.79 ± 0.30 b	12.20 ± 0.53 a	34.65 ± 0.58 b	37.31 ± 0.61 a	109.13 ± 0.80 a
	SiTong	78.05 ± 0.50 a	1.10 ± 0.18 b	29.80 ± 0.54 a	30.08 ± 0.59 b	96.61 ± 0.54 b
3	No.4	71.60 ± 0.50 b	11.83 ± 0.62 a	35.18 ± 0.50 b	37.53 ± 0.45 a	108.53 ± 0.46 a
	SiTong	77.35 ± 0.50 a	2.53 ± 0.42 b	30.75 ± 0.92 a	30.05 ± 0.59 b	96.10 ± 0.52 b
6	No.4	72.48 ± 0.30 b	10.43 ± 0.38 a	35.48 ± 0.44 b	38.26 ± 0.24 a	106.91 ± 0.74 a
	SiTong	75.38 ± 0.27 a	3.20 ± 0.31 b	30.09 ± 0.54 a	32.25 ± 0.26 b	94.93 ± 0.64 b
9	No.4	73.54 ± 0.40 a	6.64 ± 0.50 a	37.75 ± 1.01 ns	39.58 ± 0.20 a	99.30 ± 1.03 a
	SiTong	71.70 ± 0.72 b	4.60 ± 0.18 b	37.23 ± 0.73 ns	37.31 ± 0.47 b	88.59 ± 0.28 b
12	No.4	74.44 ± 0.29 a	3.51 ± 0.64 b	45.61 ± 0.77 ns	46.20 ± 0.71 ns	86.79 ± 0.86 a
	SiTong	71.23 ± 0.48 b	7.18 ± 0.38 a	47.11 ± 0.44 ns	47.83 ± 0.43 ns	81.33 ± 0.47 b

For each column, different letter for the same storage period were significantly different at p = 0.05 according to T-tests, while ns showed no significance. Data are the means ± standard errors(n=4)

4.1.4 Ethylene production

The results showed that ethylene production in both mango cultivars gradually decreased after reaching its maximum production on day 6. The decline in ethylene production was observed until day 12. ‘Nam Dok Mai Si Tong’ mango showed significantly lower ethylene production than ‘Nam Dok Mai No.4’ mango (Fig. 9).

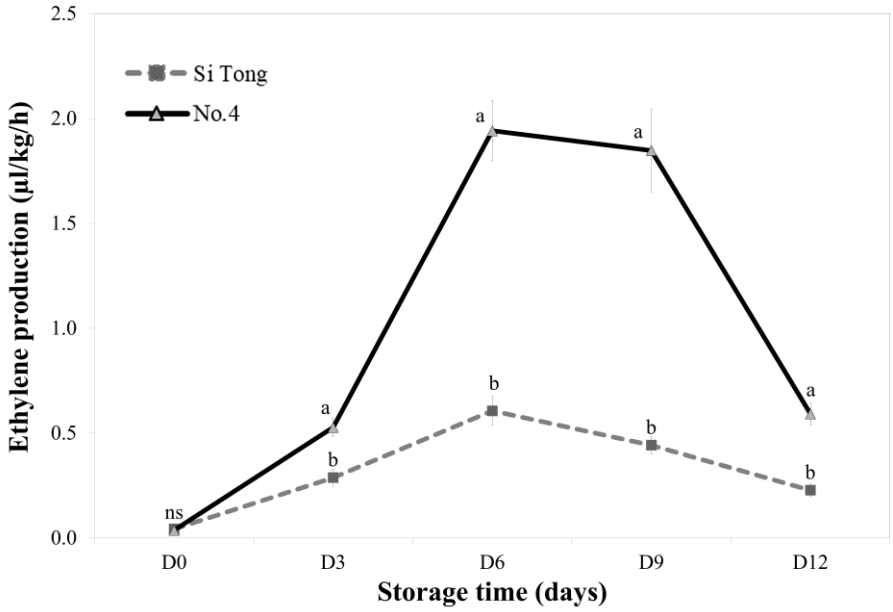


Figure 9: Ethylene production of ‘Nam Dok Mai No.4’ and ‘Nam Dok Mai Si Tong’ mangoes during 12 days of storage period at 25±1 ºC. Different letters indicate significant differences among cultivars ($p \leq 0.05$).

4.1.5 Respiration rate

The respiration rate of both mango cultivars steadily increased followed by the decrease until the end of storage. Our research found that ‘Nam Dok Mai Si Tong’ mango had a lower respiration rate when compared with ‘Nam Dok Mai No.4’ mango throughout the storage time (Fig. 10).

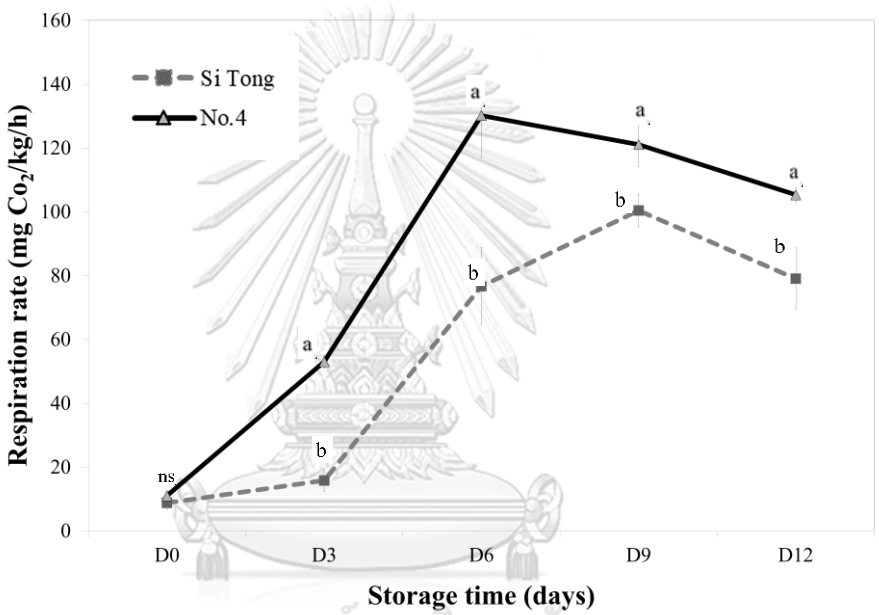


Figure 10: Respiration rate of ‘Nam Dok Mai No.4’ and ‘Nam Dok Mai Si Tong’ mangoes during 12 days storage period at 25±1 ºC. Different letters indicate significant differences among cultivars ($p \leq 0.05$).

4.1.6 ADC and ODC activities

It was found that ADC activity showed a gradual increase from day 0 to day 9, then slightly decreased at the end of storage period (Fig 11A). ODC activity exhibited a steady increase until day 9, then decreased slightly for the rest of storage time. In addition, 'Nam Dok Mai Si Tong' mango exhibited a higher level of ADC and ODC activities than 'Nam Dok Mai No.4' mango (Fig 11B).

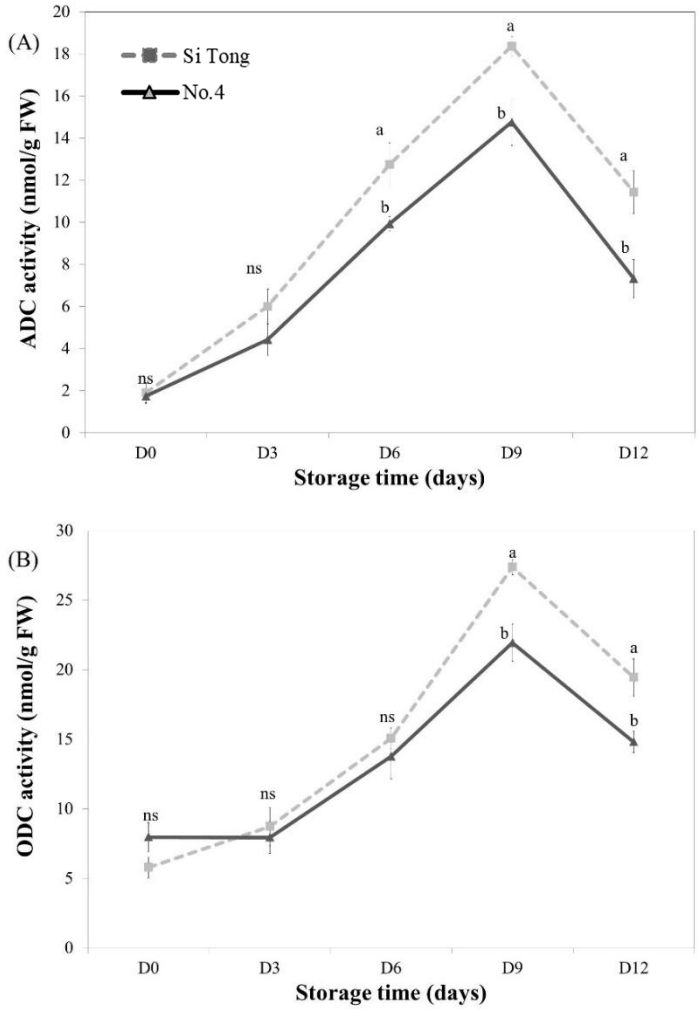


Figure 11: ADC (A) and ODC activities (B) of 'Nam Dok Mai No.4' and 'Nam Dok Mai Si Tong' mangoes during 12 days of storage at 25±1 ºC. Different letters indicate significant differences among cultivars ($p \leq 0.05$).

4.1.7 PAs contents during ripening

PAs contents of ‘Nam Dok Mai No.4’ and ‘Nam Dok Mai Si Tong’ mangoes were shown in Fig 12. During mango fruit ripening, the results showed a similar trend of PAs contents in both mango cultivars (Fig 12). Our results found that PUT contents decreased slightly during storage, however PUT contents showed no significant differences in both cultivars (Fig 12A). For SPD content, the SPD content increased gradually with storage time in both cultivars. The SPD content of ‘Nam Dok Mai Si Tong’ mango was statistically higher than ‘Nam Dok Mai No.4’ mango along this study (Fig 12B). SPM content was low during storage time and increased slightly during the whole storage period. ‘Nam Dok Mai Si Tong’ mango had higher of SPM contents when compared with ‘Nam Dok Mai No.4’ mango (Fig 12C).

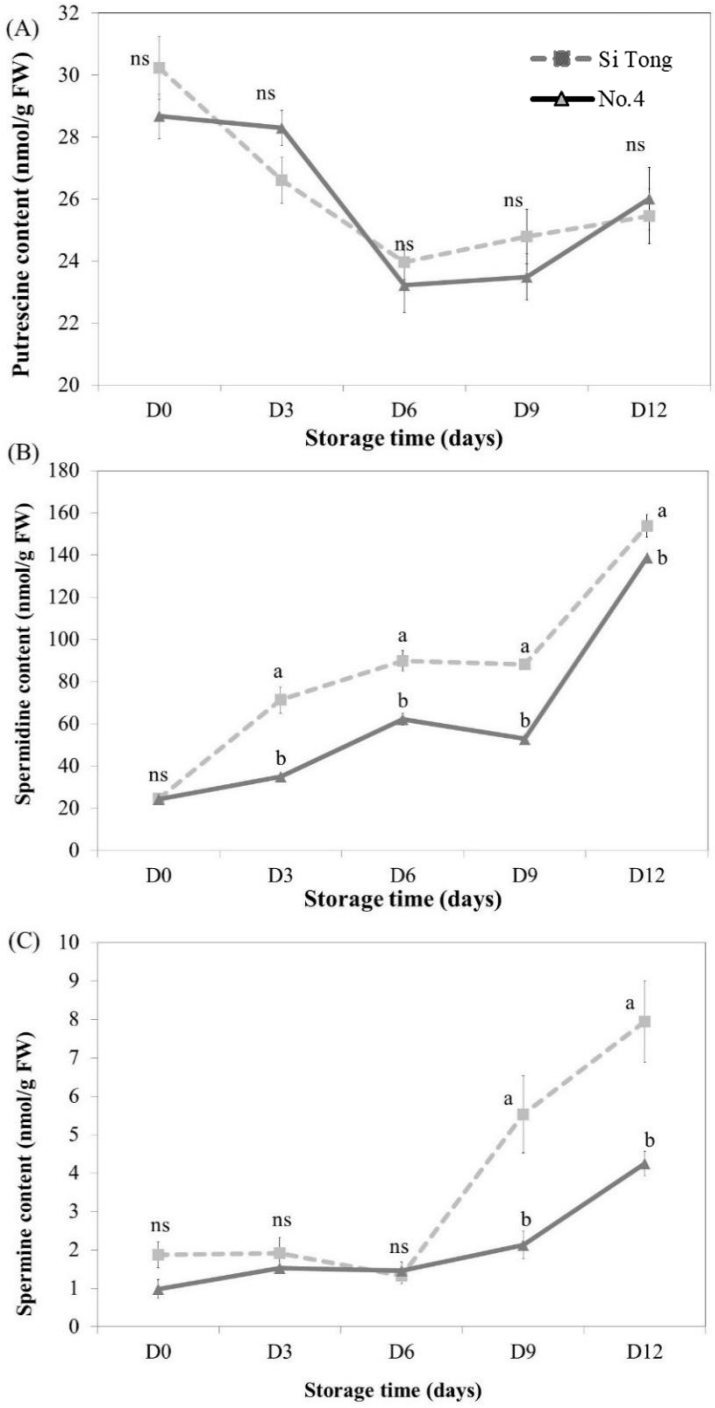


Figure 12: The PUT contents (A), SPD contents (B), and SPM contents (C) of 'Nam Dok Mai No.4' and 'Nam Dok Mai Si Tong' mangoes during 12 days of storage at 25±1 ºC. Different letters indicate significant differences among cultivars ($p \leq 0.05$).

4.2 Physiological and biochemical responses of mango fruit to exogenous putrescine application

4.2.1 Effect of exogenous PUT on weight loss and fruit firmness

An increase in percentage of weight loss was observed in all treatments during storage (Table 3). The lowest weight loss was observed in 2 mmol/l PUT treated fruit compared to other treatments after day 9 until the end of storage period. There was no significance difference in percentage of weight loss between 1 mmol/l PUT, 4 mmol/l PUT, and control treatments. Fruit firmness of stored fruit has been found to decrease with advancement of storage period in all treatments. Although, no significant difference was found among treatments during the first 9 days of storage at low temperature, after day 9 until the end of storage, 2 mmol/l PUT treated fruits displayed the highest level of fruit firmness among other treatments.

4.2.2 Effect of exogenous PUT on SSC and TA

During the storage period, all treatments showed a consistent increase in SSC with the prolongation of storage duration (Table 3). On day 12, 15 and 18, the lowest of SSC was recorded in 2 mmol/l PUT treated fruits when compared with 1 mmol/l, 4 mmol/l, and control fruit. Our results revealed that TA content was found to decrease constantly in all treatments. There were no significant differences over the first nine days. During day 12 to the end of storage period, 2 mmol/l PUT treated fruits had a higher TA than other treatments.

Table 3: Effect of exogenous PUT on weight loss, firmness, SSC and TA of 'Nam Dok Mai No.4' during storage period at 14 ºC for 9 days and shelf life at 25 ºC for another 9 days.

Storage time(day)	Treatment	Weight loss (%)	Firmness (N)	SSC (°Brix)	TA (%)
0	Control	0	8.77 ± 0.07 ns	9.28 ± 0.23 ns	2.39 ± 0.06 ns
	1 mM PUT	0	8.75 ± 0.06 ns	9.38 ± 0.20 ns	2.28 ± 0.10 ns
	2 mM PUT	0	8.84 ± 0.05 ns	9.38 ± 0.21 ns	2.45 ± 0.02 ns
	4 mM PUT	0	8.78 ± 0.05 ns	9.16 ± 0.18 ns	2.38 ± 0.08 ns
9	Control	4.65 ± 0.22 a	8.72 ± 0.04 ns	13.49± 0.34 ns	1.40 ± 0.04 ns
	1 mM PUT	4.52 ± 0.25 a	8.73 ± 0.05 ns	13.57± 0.32 ns	1.45 ± 0.06 ns
	2 mM PUT	3.73 ± 0.20 b	8.73 ± 0.05 ns	13.33± 0.47 ns	1.48 ± 0.05 ns
	4 mM PUT	4.36 ± 0.17 a	8.70 ± 0.04 ns	13.33± 0.72 ns	1.35 ± 0.03 ns
12	Control	5.99± 0.26 a	8.72 ± 0.22 ab	16.91 ± 0.09 a	1.12 ± 0.09 b
	1 mM PUT	5.81± 0.35 a	8.81 ± 0.25 ab	16.80 ± 0.31 a	1.24 ± 0.07 b
	2 mM PUT	4.84± 0.22 b	8.11 ± 0.08 a	14.76 ± 0.58 b	1.46 ± 0.02 a
	4 mM PUT	5.65± 0.26 a	7.27 ± 0.20 b	17.79 ± 0.22 a	1.07 ± 0.06 b
15	Control	7.78 ± 0.30 a	6.49 ± 0.13 bc	17.75 ± 0.19 a	0.18 ± 0.02 c
	1 mM PUT	7.46 ± 0.39 a	6.78 ± 0.07 ab	16.23 ± 0.23 b	0.26 ± 0.02 b
	2 mM PUT	6.10 ± 0.41 b	7.00 ± 0.07 a	15.58 ± 0.19 c	0.35 ± 0.01 a
	4 mM PUT	7.32 ± 0.31 a	6.30 ± 0.22 c	17.23 ± 0.15 a	0.16 ± 0.02 c
18	Control	9.93 ± 0.30 a	5.69 ± 0.15 b	17.75 ± 0.19 a	0.12 ± 0.01 b
	1 mM PUT	9.61 ± 0.45 a	5.60 ± 0.13 b	17.76 ± 0.31 a	0.12 ± 0.01 b
	2 mM PUT	8.49 ± 0.27 b	6.21 ± 0.08 a	16.53 ± 0.36 b	0.17 ± 0.00 a
	4 mM PUT	9.57 ± 0.37 a	5.77 ± 0.11 b	17.70 ± 0.21 a	0.11 ± 0.01 b

Means in a column followed by a different letter for the same storage period were significantly at $p \leq 0.05$ by Duncan's Multiple Range Test. ns, not significant. Data are accompanied by standard errors of the means (n=4).

4.2.3 Effect of exogenous PUT on peel color changes

All mango fruits showed a progressive increased of the L value during storage (Table 4). Fruit treated with 2 mmol/l PUT had a significant delay ($p \leq 0.05$) in peel color change on days 12, 15 and 18. Fruit treated with 2 mmol/l PUT showed the lowest L value, which was followed by 1 mmol/l, 4 mmol/l, and control fruit. Hue angles (H° value) significantly decreased for all fruit during storage. The 2 mmol/l PUT fruits showed the highest of the hue angles value followed by 1 mmol/l and no significant difference was found between 4 mmol/l, and control fruit. During mango ripening, the a* value of control and mango treated with PUT was rapidly declined after moving to room temperature, but 2 mmol/l PUT showed the highest of the a* value on days 12, 15 and 18. The changes of b* value were different between treated and control fruits. The b* value increased during storage period; and 2 mmol/l PUT treatment had lower b* value than other treatment. C* value increased in all fruit during storage. The least decrease was recorded in 2 mmol/l PUT treated fruit after transferring the mango to 25 ºC for another 9 days, followed by 1 mmol/l treated, 4 mmol/l treated, and control fruits.

Table 4: Effect of exogenous PUT on peel color changes of 'Nam Dok Mai No.4' during storage period at 14 ºC for 9 days and shelf life at 25 ºC for another 9 days.

Storage time(day)	Treatment	L* value	a* value	b* value	C* value	H° value
0	Control	68.34±0.71ns	18.07±0.08ns	30.86±0.22ns	34.70±0.12ns	108.11±0.40ns
	1 mM PUT	68.88±0.42ns	18.02±0.1ns	30.45±0.17ns	34.82±0.16ns	108.55±0.37ns
	2 mM PUT	68.58±0.70ns	18.14±0.11ns	30.60±0.17ns	34.30±0.35ns	108.09±0.40ns
	4 mM PUT	68.83±0.61ns	18.00±0.16ns	30.76±0.19ns	34.68±0.28ns	108.25±0.32ns
9	Control	69.70±0.26ns	16.53±0.26ns	31.02±0.23ns	36.72±0.24ns	106.11±0.22ns
	1 mM PUT	68.53±0.42ns	15.84±0.23ns	31.35±0.31ns	36.47±0.26ns	106.39±0.32ns
	2 mM PUT	68.85±0.65ns	15.91±0.26ns	31.16±0.26ns	36.07±0.16ns	106.96±0.39ns
	4 mM PUT	68.04±0.42ns	15.73±0.20ns	31.13±0.41ns	36.67±0.30ns	106.25±0.44ns
12	Control	74.41±0.54 a	10.43±0.10 d	41.10±0.10 a	42.79±0.21 a	98.26±0.29 c
	1 mM PUT	71.15±0.42 b	11.60±0.18 b	37.70±0.13 c	38.74±0.14 b	100.05±0.35 b
	2 mM PUT	69.17±0.32 c	12.95±0.08 a	36.13±0.11 d	37.25±0.21 c	103.47±0.34 a
	4 mM PUT	73.87±0.42 a	10.90±0.09 c	40.05±0.15 b	42.38±0.21 a	98.80±0.25 c
15	Control	76.28±0.61 a	6.65±0.20 d	43.06±0.56 a	44.90±0.40 a	92.89±0.46 c
	1 mM PUT	72.72±0.22 b	8.19±0.10 b	40.64±0.18 b	42.90±0.21 b	95.55±0.33 b
	2 mM PUT	70.46±0.25 c	10.27±0.14 a	38.57±0.27 c	39.75±0.15 c	98.51±0.35 a
	4 mM PUT	75.40±0.37 a	7.37±0.16 c	42.60±0.23 a	44.32±0.38 a	93.69±0.36 c
18	Control	76.68±0.49 a	3.14±0.07 c	46.95±0.19 a	47.25±0.11 a	87.83±0.33 c
	1 mM PUT	73.64±0.30 b	4.90±0.05 b	44.85±0.23 b	45.47±0.30 b	91.25±1.25 b
	2 mM PUT	71.76±0.18 c	6.27±0.15 a	42.22±0.10 c	43.85±0.20 c	93.38±0.24 a
	4 mM PUT	76.61±0.48 a	3.43±0.15 c	45.31±0.30 a	46.60±0.31 a	89.29±0.26 c

Means in a column followed by a different letter for the same storage period were significantly at $p \leq 0.05$ by Duncan's Multiple Range Test. ns, not significant. Data are accompanied by standard errors of the means (n=4).

4.2.4 Effect of exogenous PUT on respiration rate

Figure 13 showed that respiration rate increased, reached its maximum on day 15 and then decreased at the end of storage period. Fruit treated with PUT showed significant lower respiration rate than control fruit. Fruit treated with 2 mmol/L PUT displayed the lowest respiration rate compared to other treatments.

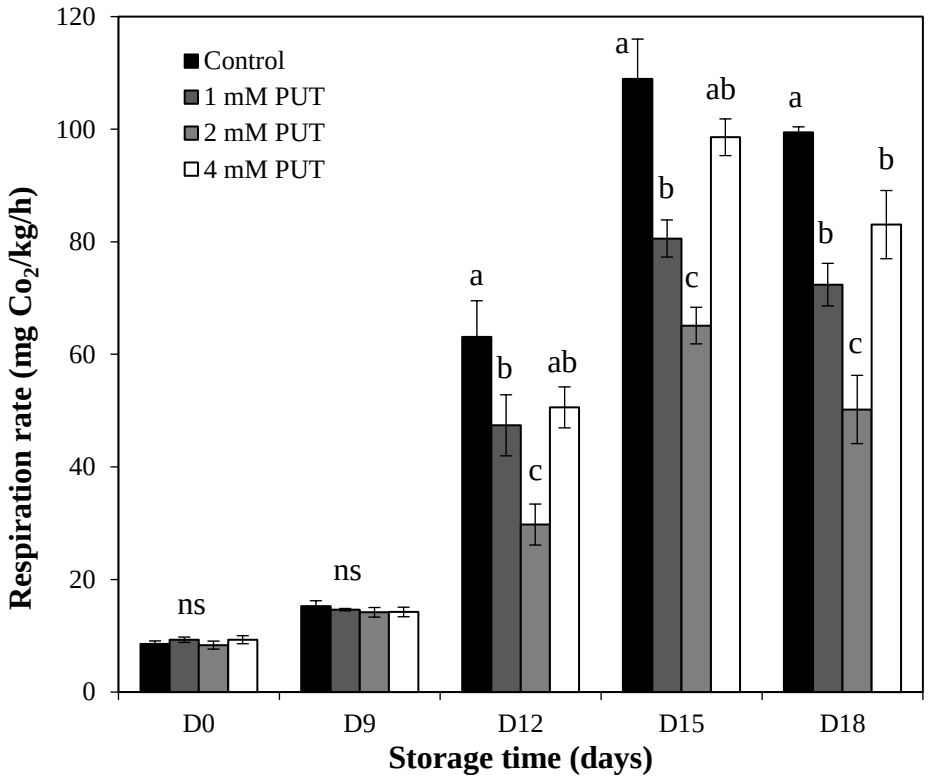


Figure 13: Respiration rate in 'Nam Dok Mai No.4' mango fruit treated with exogenous PUT during storage period at 14 ºC for 9 days and shelf life at 25 ºC for another 9 days. Vertical bars represent mean ± SE of four biological replicates for each treatment.

4.2.5 Effect of exogenous PUT on ethylene production

PUT treatment significantly decreased the rate of ethylene production on day 12 to day 18 compared to control treatment (Fig. 14). There was no significant difference in ethylene production in all treatments during storage at 14 ºC for the first 9 days. However, marked difference was recorded in ethylene production after transferred to 25 ºC. The highest ethylene production was found under control and the lowest level was recorded under 2 mmol/L PUT treatment.

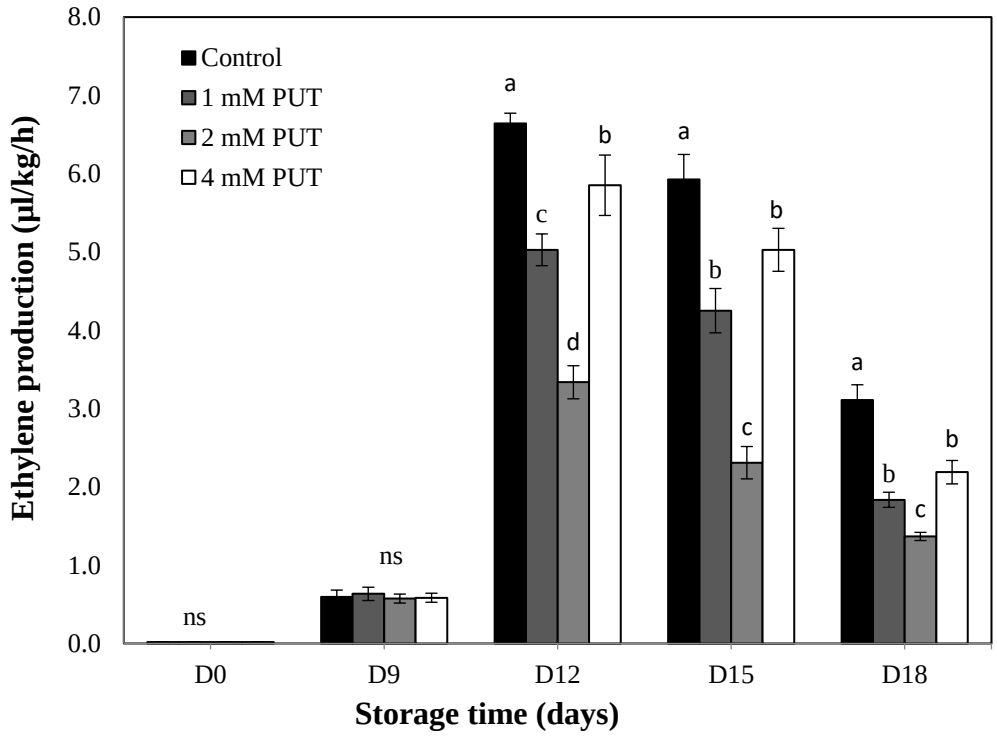


Figure 14: Ethylene production in 'Nam Dok Mai No.4' mango fruit treated with exogenous PUT during storage period at 14 ºC for 9 days and shelf life at 25 ºC for another 9 days. Vertical bars represent mean ± SE of four biological replicates for each treatment.

4.2.6 Effect of exogenous PUT on soluble pectin content

Changes in soluble pectin content of ‘Nam Dok Mai No.4’ mango fruit treated with PUT were presented in Figure 15. All treatments showed consistent increased in the soluble pectin content during storage period. The soluble pectin content in 2 mmol/L PUT treated fruit was the lowest when compared with other treatments on days 9, 12, 15 and 18. The highest soluble pectin content was observed in control fruit after day 9 until the end of the storage.

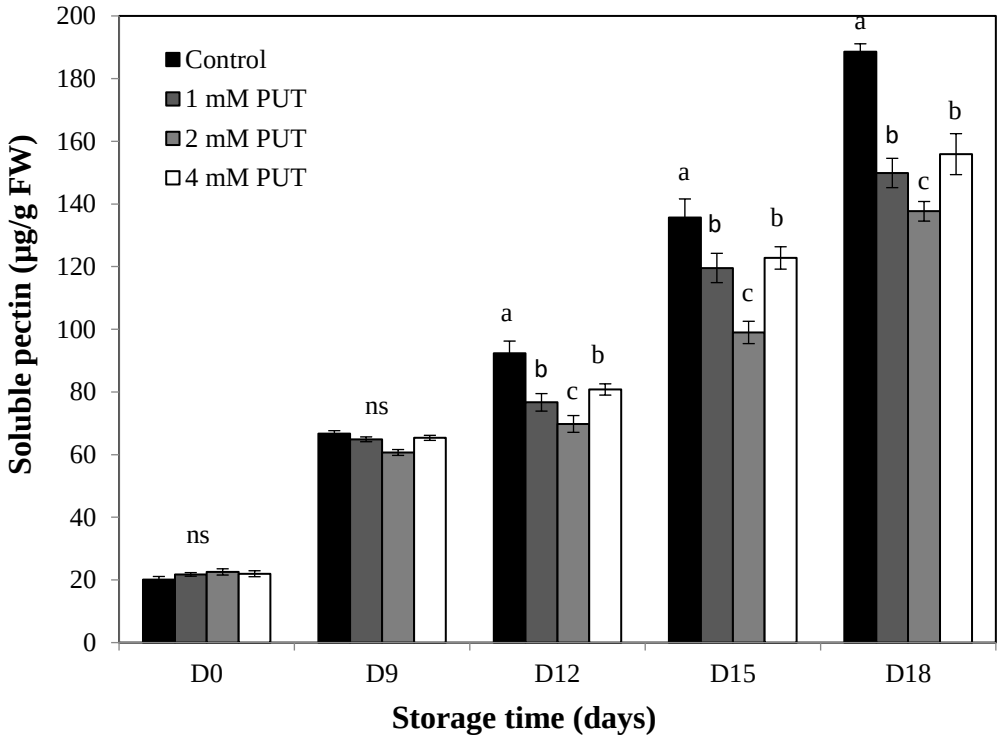


Figure 15: Soluble pectin content in ‘Nam Dok Mai No.4’ mango fruit treated with exogenous PUT during storage period at 14 ºC for 9 days and shelf life at 25 ºC for another 9 days. Vertical bars represent mean ± SE of four biological replicates for each treatment.

4.2.7 Effect of exogenous PUT on polygalacturonase (PG) activity

Our results showed that PG activities of all treatments increased progressively during storage (Fig. 16). Two mmol/L PUT treatment exhibited a lower PG activity when compared to other treatments on day 9 until the end of storage. On the other hand, control treatment showed the highest PG activity on day 9, 12, 15 and 18.

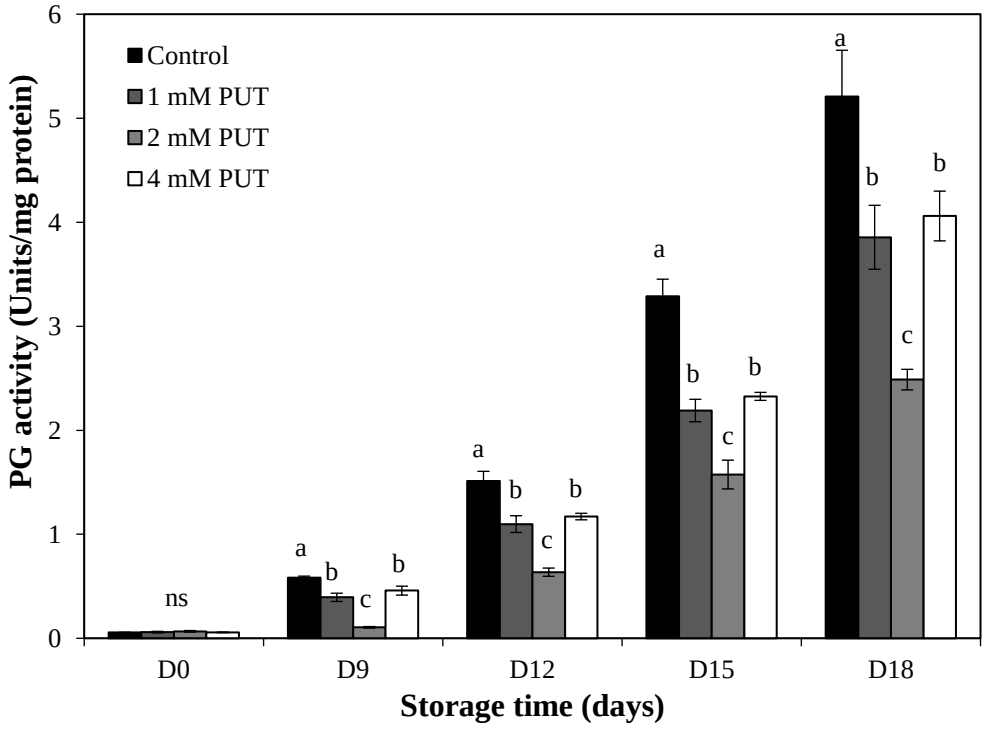


Figure 16: PG activity in 'Nam Dok Mai No.4' mango fruit treated with exogenous PUT during storage period at 14 ºC for 9 days and shelf life at 25 ºC for another 9 days. Vertical bars represent mean ± SE of four biological replicates for each treatment.

4.2.8 Effect of exogenous PUT on pectin methylesterase (PME) activity

PME activity of all treatments showed a decreasing trend along 18 days (Fig. 17). PUT clearly affected the PME activity of mango. Two mmol/L PUT treated fruit showed a significantly lower PME activity than other treatment is from day 9 to day 18. However, PME activity showed no significant difference between 1 mmol/L and 4 mmol/L treated fruit. Among all treatments, the highest of PME activity were found in control fruit during storage at room temperature.

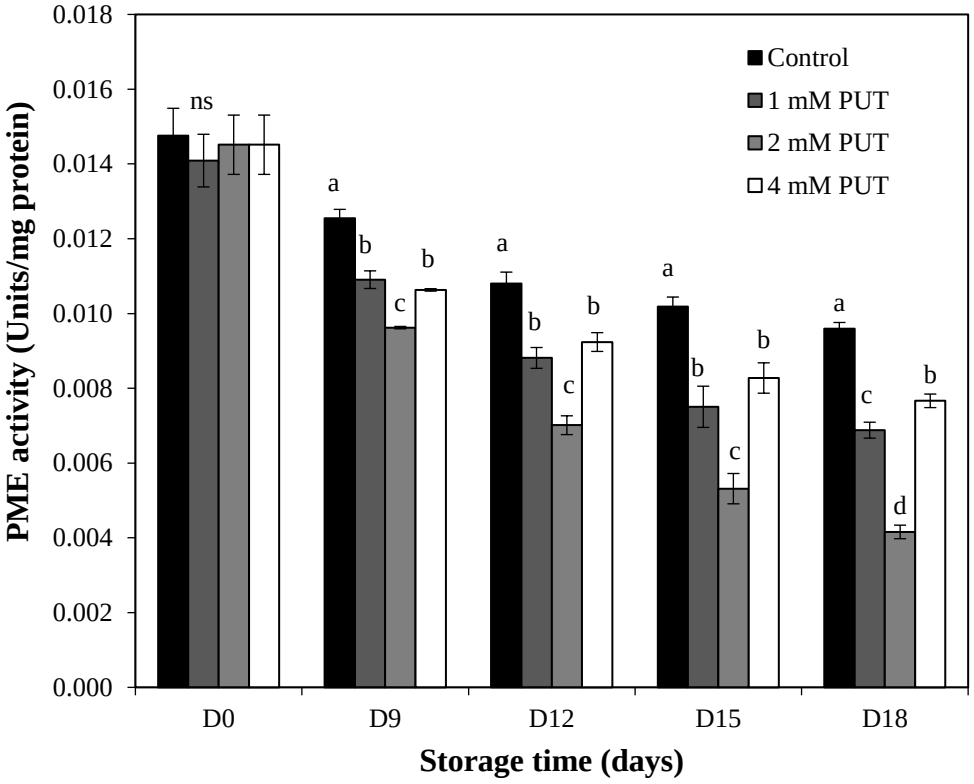


Figure 17: PME activity in 'Nam Dok Mai No.4' mango fruit treated with exogenous PUT during storage period at 14 ºC for 9 days and shelf life at 25 ºC for another 9 days. Vertical bars represent mean ± SE of four biological replicates for each treatment.

4.2.9 Effect of exogenous PUT on SOD activity

Progressively decreasing SOD activity was observed during storage (Fig. 18). Although SOD activity in all treatment decreased during storage, SOD activity in 2 mmol/l PUT treated fruit was higher than other treatments. Control fruit presented lower SOD activity throughout the storage period.

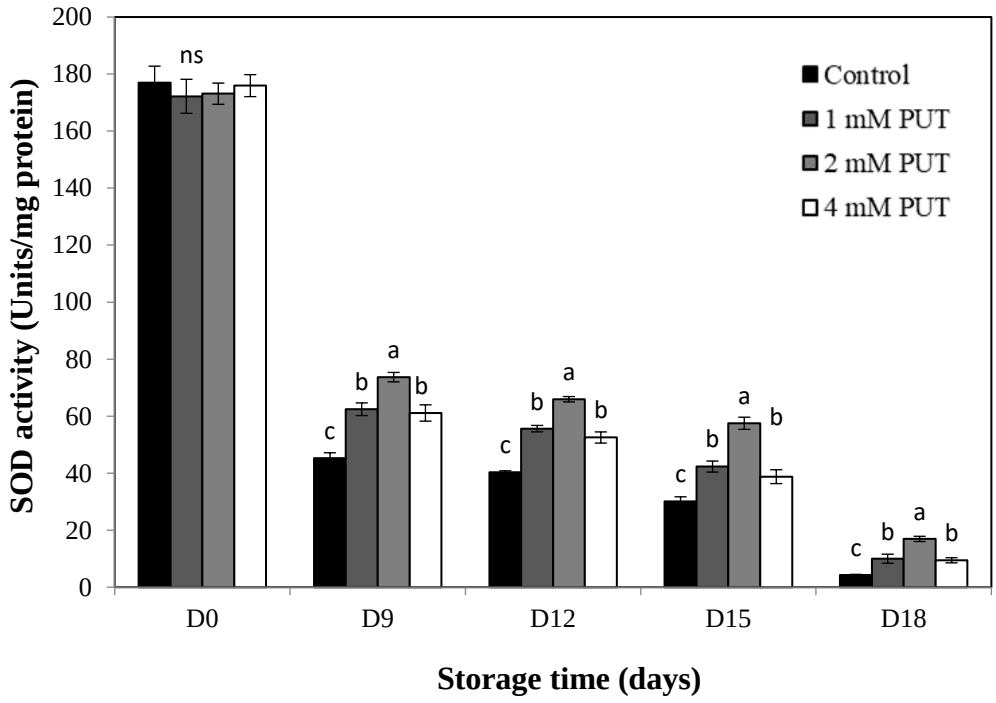


Figure 18: SOD activity in 'Nam Dok Mai No.4' mango fruit treated with exogenous PUT during storage period at 14 ºC for 9 days and shelf life at 25 ºC for another 9 days. Vertical bars represent mean ± SE of four biological replicates for each treatment.

4.2.10 Effect of exogenous PUT on CAT activity

CAT activities in control and PUT treated fruit increased rapidly on day 12 and reached a maximum on day 15 (Fig. 19). There was a noted decrease of CAT activity at the end of storage. Two mmol/l PUT treated fruit had the highest CAT activity among other treatments throughout the experiment. CAT activity in control fruit showed the lowest level compared with other treatments on day 18.

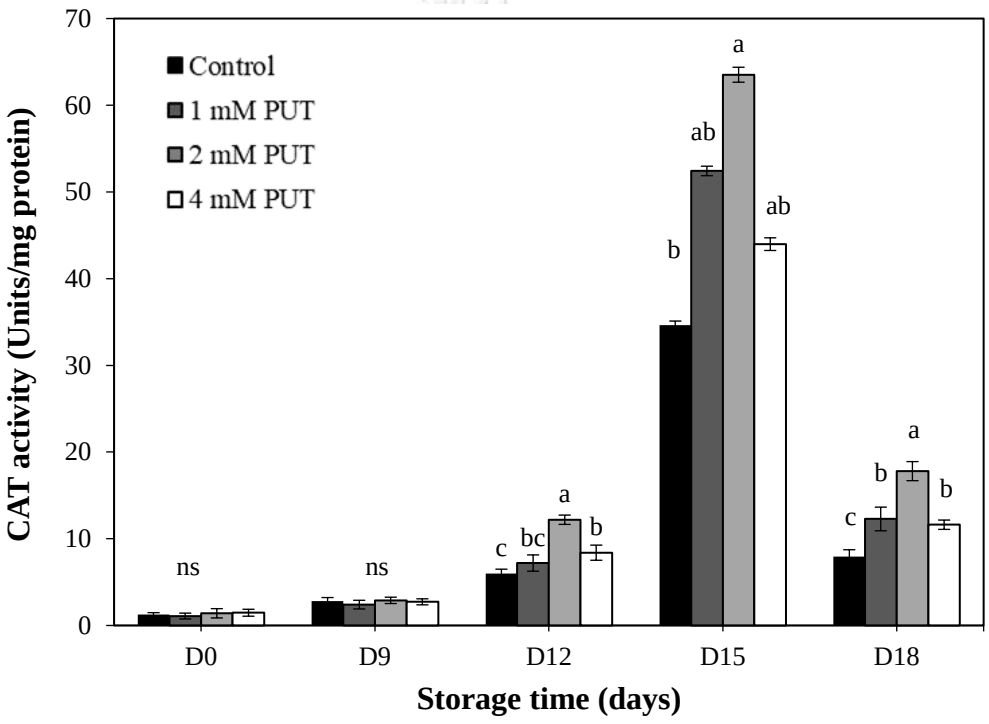


Figure 19: CAT activity in 'Nam Dok Mai No.4' mango fruit treated with exogenous PUT during storage period at 14 ºC for 9 days and shelf life at 25 ºC for another 9 days. Vertical bars represent mean ± SE of four biological replicates for each treatment.

4.2.11 Effect of exogenous PUT on GPOX activity

GPOX activity of mango fruit displayed an increase up to day 15, and then gradually declined on day 18 (Fig. 20). PUT treatment at the concentration of 2 mmol/l showed a higher GPOX activity in comparison to 1 mmol/l and 4 mmol/l. One mmol/l and 4 mmol/l PUT treatments showed no significant differences between them during the storage period while, control treatment had the lowest GPOX activity.

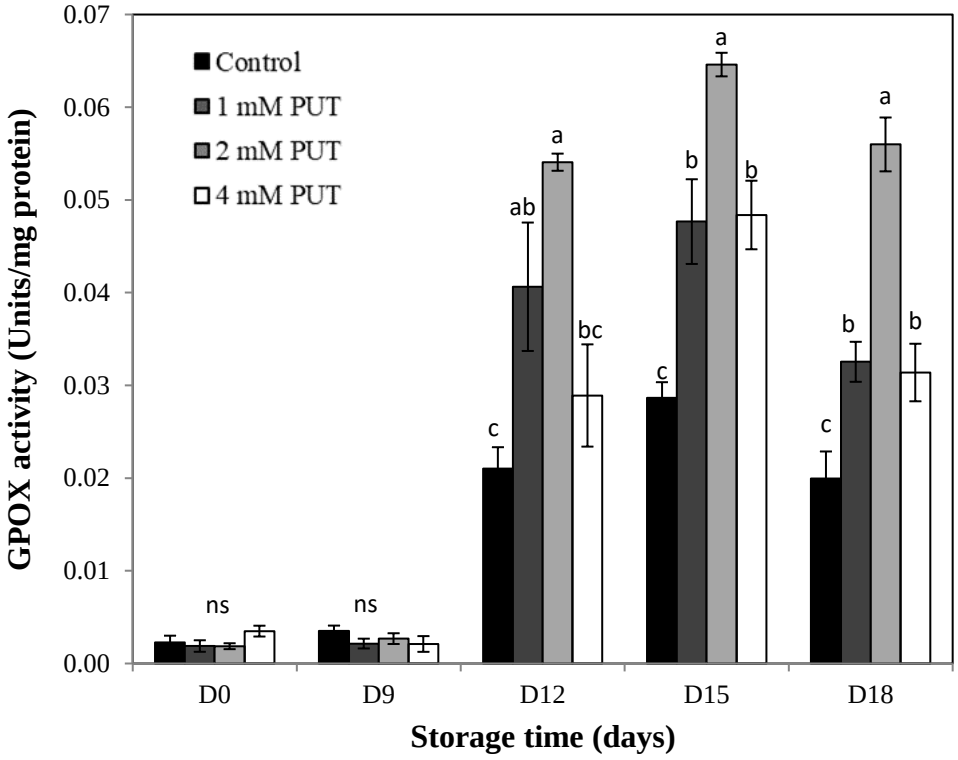


Figure 20: GPOX activity in 'Nam Dok Mai No.4' mango fruit treated with exogenous PUT during storage period at 14 ºC for 9 days and shelf life at 25 ºC for another 9 days. Vertical bars represent mean ± SE of four biological replicates for each treatment.

4.2.12 Effect of exogenous PUT on APX activity

APX activity constantly increased until day 15 and sustained until a decline was observed on day 18 (Fig. 21). The 2 mmol/l PUT treated samples displayed significantly higher APX activity than 1 mmol/l and 4 mmol/l treated fruit, which showed no significant difference between 1 mmol/l and 4 mmol/l on days 15 and 18. At the end of experiment, control fruit had the lowest APX activity compared with other treatments.

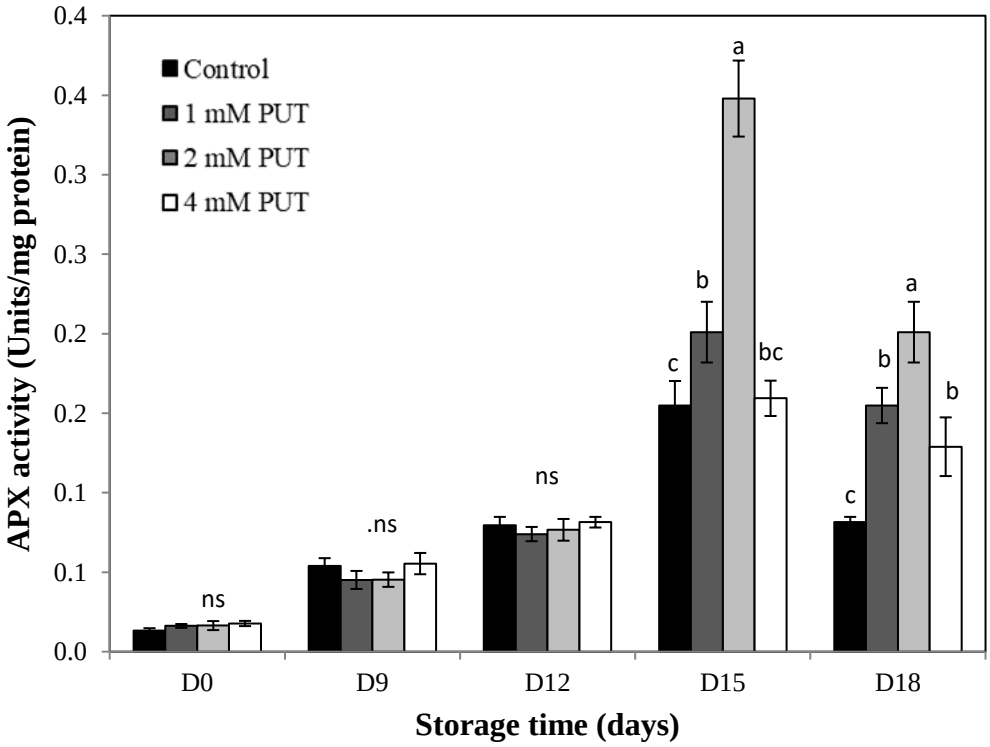


Figure 21: APX activity in 'Nam Dok Mai No.4' mango fruit treated with exogenous PUT during storage period at 14 ºC for 9 days and shelf life at 25 ºC for another 9 days. Vertical bars represent mean ± SE of four biological replicates for each treatment.

4.2.13 Effect of exogenous PUT on GR activity

Despite mango treated with PUT or control, GR activity showed a steady increase from the day 0 to day15 and it decreased at the end of storage period (Fig. 22). Two mmol/l PUT treatment maintained higher GR activity when compared with other treatments on days 12, 15 and 18. The lowest GR activity was found in control fruit after removal from low temperature.

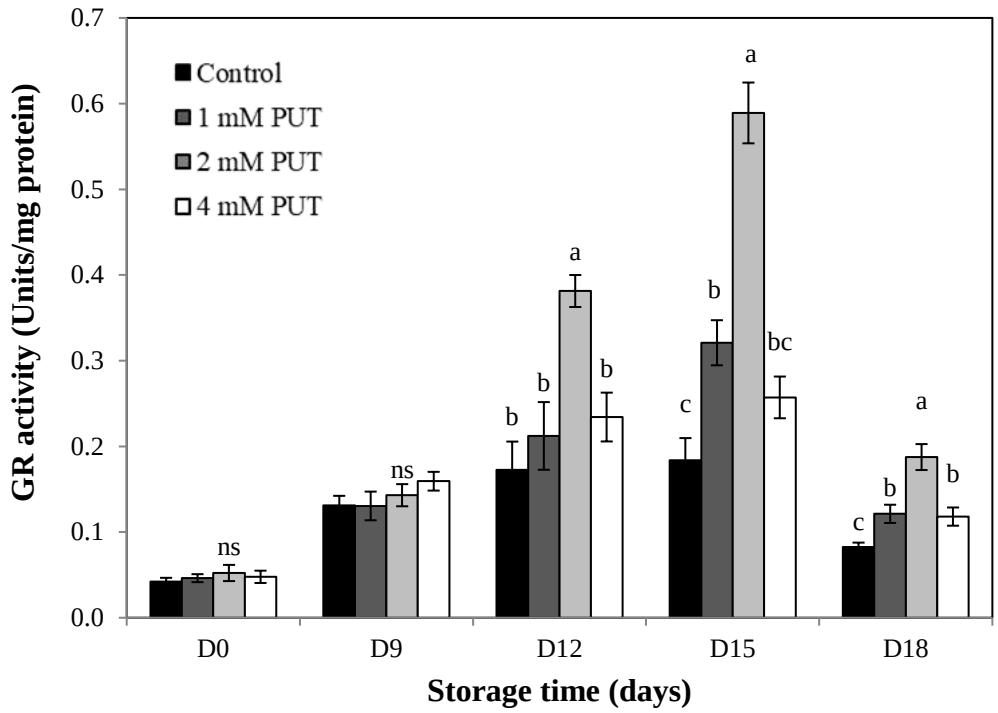


Figure 22: GR activity in 'Nam Dok Mai No.4' mango fruit treated with exogenous PUT during storage period at 14 ºC for 9 days and shelf life at 25 ºC for another 9 days. Vertical bars represent mean ± SE of four biological replicates for each treatment.

4.2.14 Effect of exogenous PUT on H_2O_2 activity

Our results found that H_2O_2 content increased gradually with the increased of storage time (Fig. 23). The lowest H_2O_2 content was displayed by 2 mmol/l PUT treatment, whereas, the highest content was shown in control treatment from day 0 until day 18. However, there was no significant differences in H_2O_2 content among the 1 mmol/l and 4 mmol/l treated fruit after stored at room temperature.

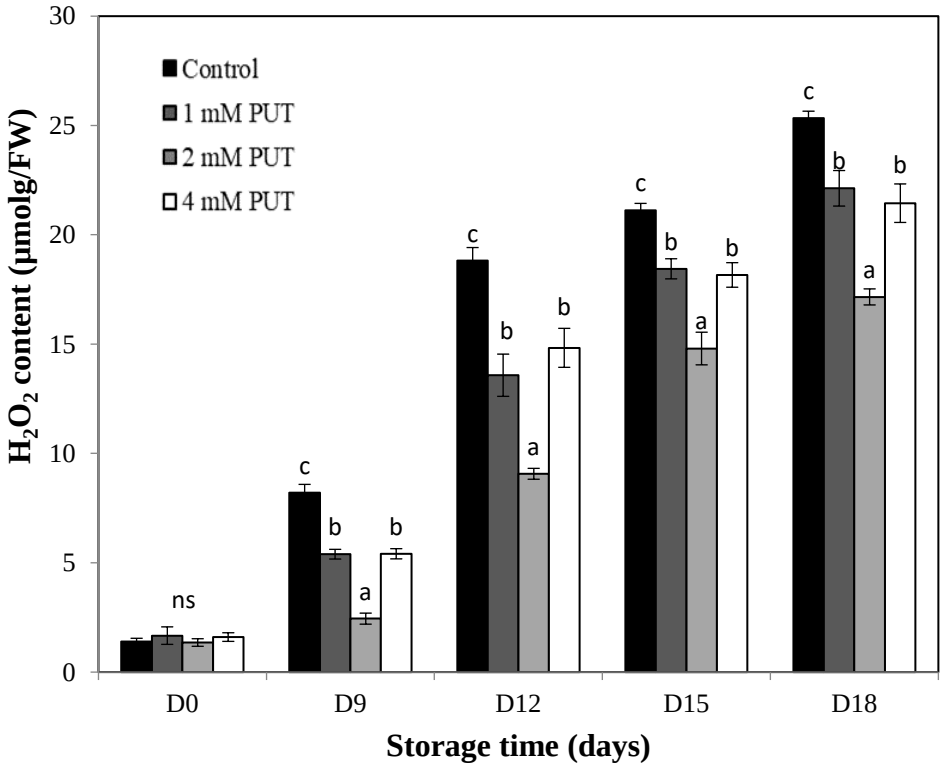


Figure 23: H_2O_2 content in 'Nam Dok Mai No.4' mango fruit treated with exogenous PUT during storage period at 14 ºC for 9 days and shelf life at 25 ºC for another 9 days. Vertical bars represent mean ± SE of four biological replicates for each treatment.

4.2.15 Effect of exogenous PUT on DPPH free radical scavenging capacity

The antioxidant capacity was measured by DPPH free radical scavenging assay (Fig. 24). As the results, total antioxidant capacity slightly increased for all treatments until day 15, which was followed by a decrease at the end of storage time. The total antioxidant content was significantly ($p < 0.05$) affected by PUT treatment. Among the PUT treatments and the control, 2 mmol/l PUT treated fruit had the highest total antioxidant content. The lowest total antioxidant content was observed in control fruit.

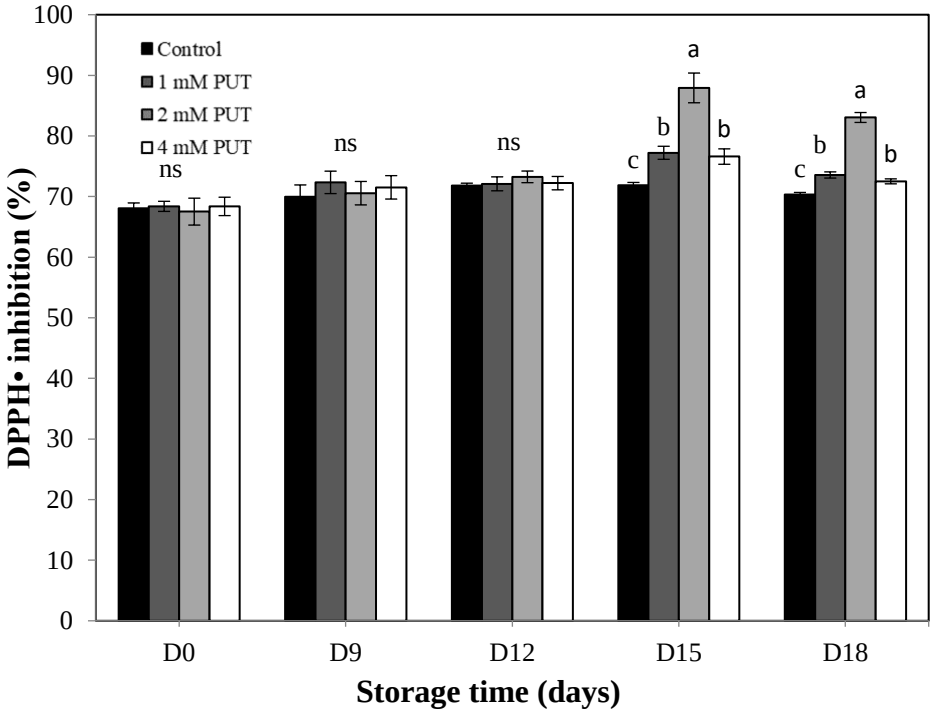


Figure 24: Content of DPPH in 'Nam Dok Mai No.4' mango fruit treated with exogenous PUT during storage period at 14 ºC for 9 days and shelf life at 25 ºC for another 9 days. Vertical bars represent mean ± SE of four biological replicates for each treatment.

4.2.16 Effect of exogenous PUT on FRAP assay

Total antioxidant level slightly increased for all treatments until day 15 during storage period, and thereafter, total antioxidant level exhibited a slight reduction on day 18 (Fig. 25). As a result, fruit treated with 2 mmol/l PUT exhibited the highest total antioxidant content when compared to other treatments on day 12, 15 and 18. The control fruit had the lowest of total antioxidant from day 12 until the end of the storage time.

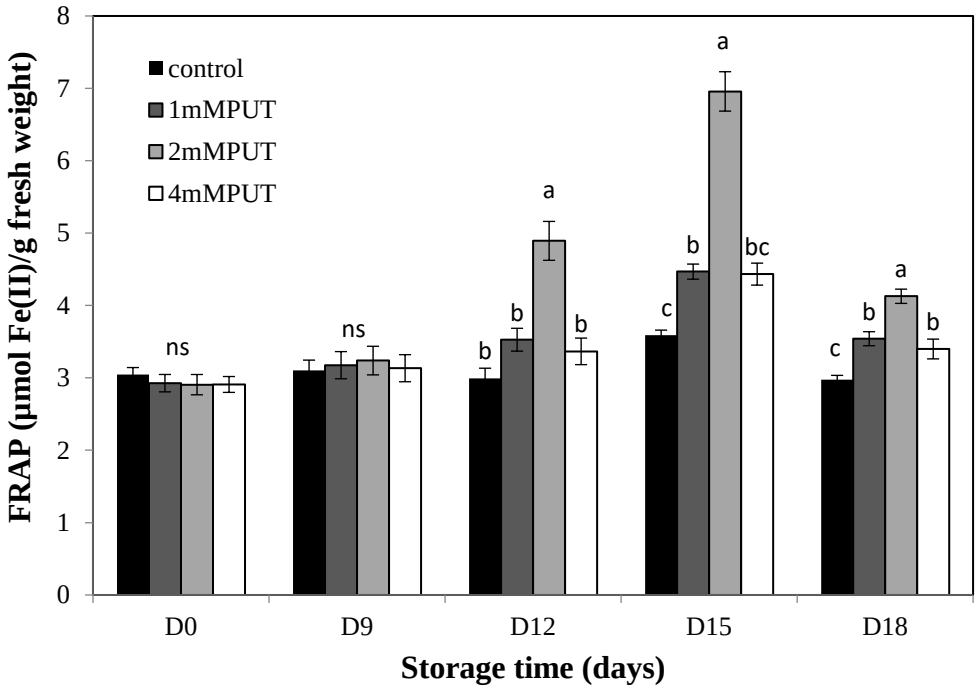


Figure 25: Content of FRAP in 'Nam Dok Mai No.4' mango fruit treated with exogenous PUT during storage period at 14 ºC for 9 days and shelf life at 25 ºC for another 9 days. Vertical bars represent mean ± SE of four biological replicates for each treatment.

4.2.17 Effects of exogenous PUT on ADC and ODC activities

ADC activity rose noticeably on day 9 and reached its maximum on day 15 (Fig. 26). The activity gradually decreased in mango fruit on day 18. The highest ADC activity was found in 4 mmol/L PUT treated fruits followed by 2 mmol/L and1 mmol/L while control fruit had the lowest ADC activity. Similarly, ODC activity showed a steady increase upon removal from low temperature storage but it began to decrease at the end of the storage period. Mango fruit treated with 4 mmol/L PUT had significant highest ODC activity when compared with other treatments.

4.2.18 Effect of exogenous PUT on PAs contents

Endogenous PUT content decreased constantly throughout the storage period in all treatments (Fig. 27). Following removal from low temperature storage, 4 mmol/L PUT treated fruits had the highest endogenous PUT content followed by 2 mmol/L PUT, 1 mmol/L, and control fruit respectively. On the other hand, endogenous SPD content was continuously increased during day 0 to day 18. The 2 mmol/L PUT treated fruit showed the highest SPD content when compared with other treatments on days 9, 12, 15 and 18. For SPM content, SPD content showed a slight increase in all treatments during storage time. Mango fruits treated with 2 mmol/L PUT had significant higher SPM content compared with other treatments on days 15 and 18.

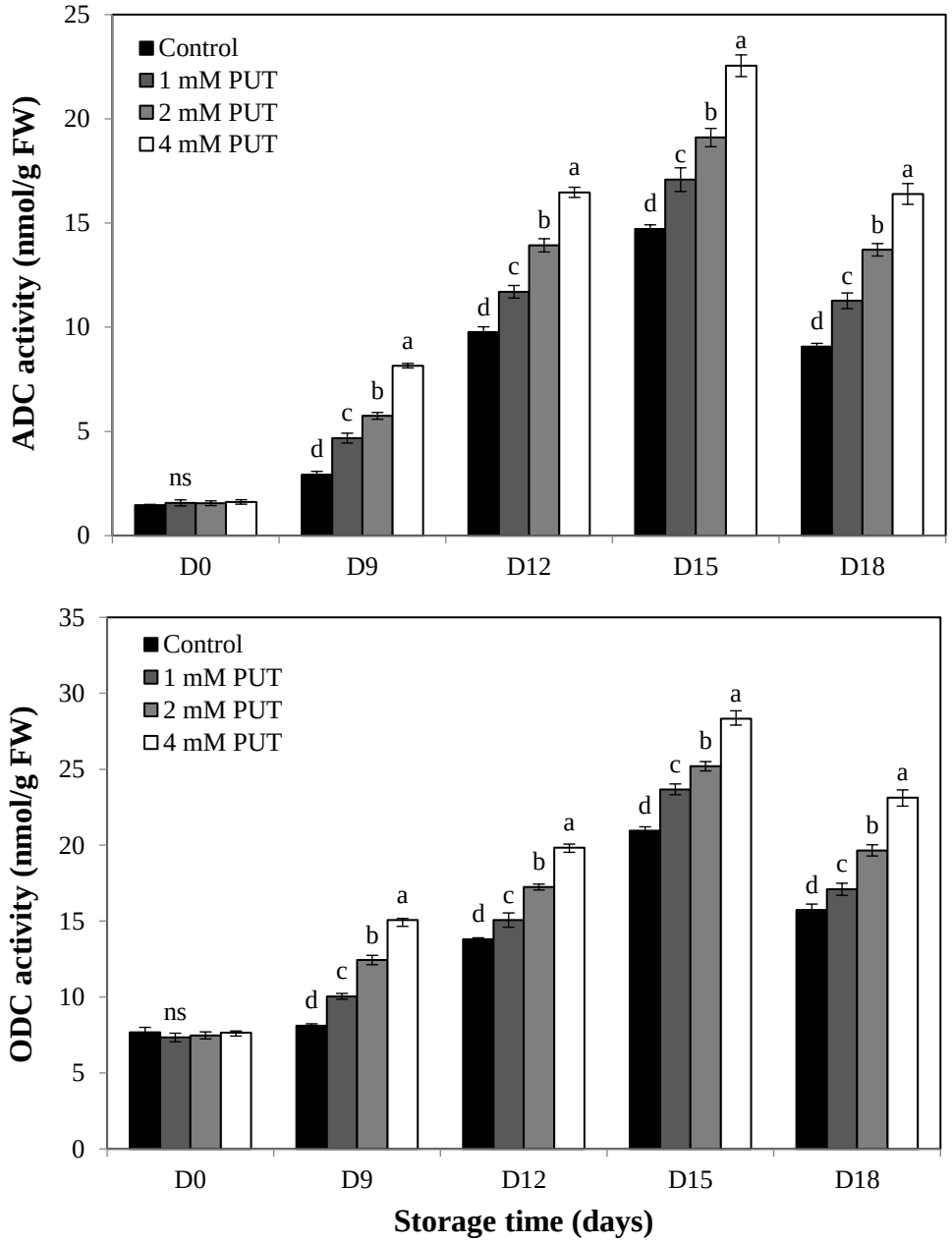


Figure 26: ADC and ODC activities of 'Nam Dok Mai No.4' mango fruit treated with exogenous PUT during storage period at 14 ºC for 9 days and shelf life at 25 ºC for another 9 days. Vertical bars represent mean ± SE of four biological replicates for each treatment

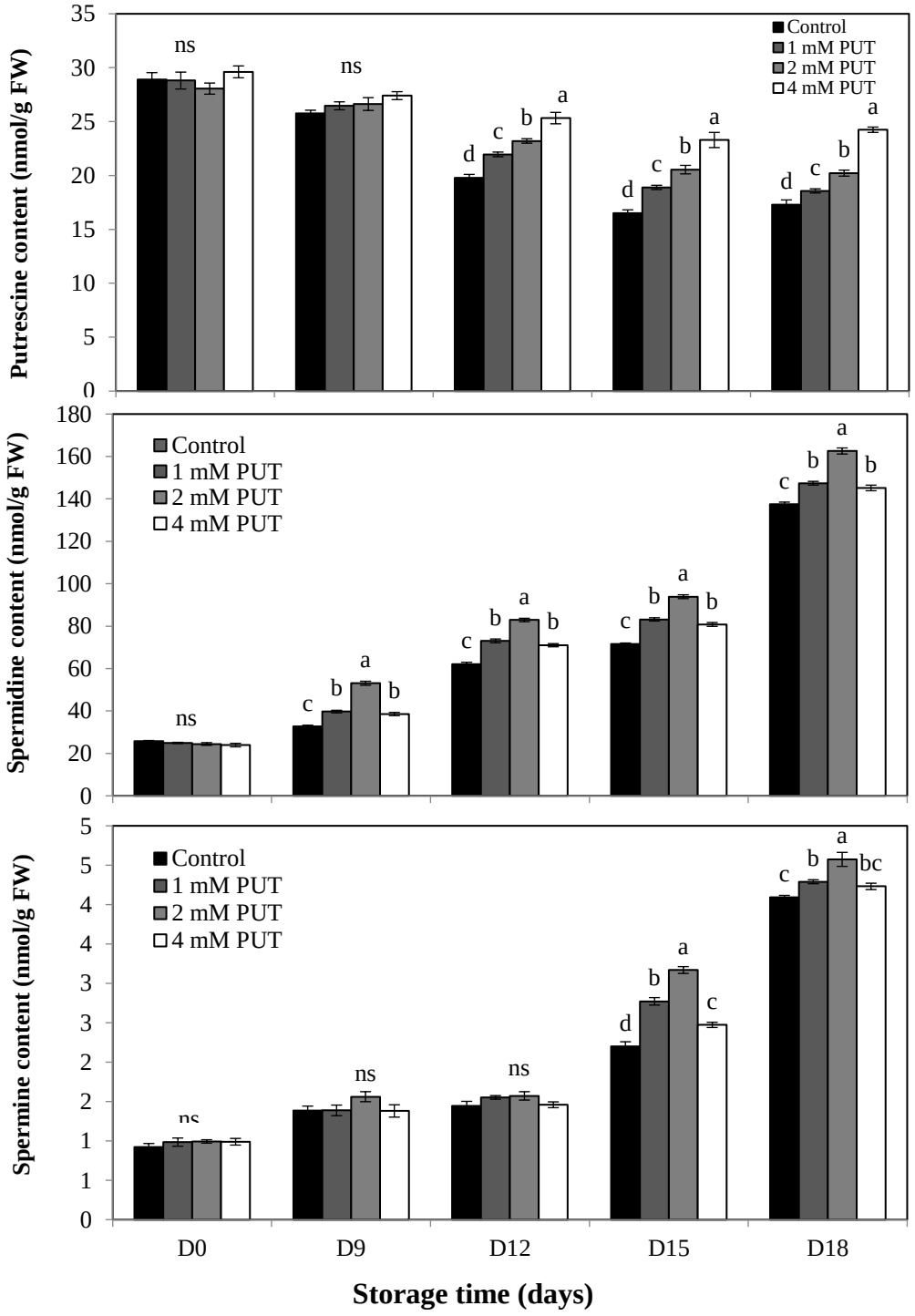


Figure 27: PUT, SPD and SPM contents of 'Nam Dok Mai No.4' mango fruit treated with exogenous PUT during storage period at 14 ºC for 9 days and shelf life at 25 ºC for another 9 days. Vertical bars represent mean ± SE of four biological replicates for each treatment.

CHAPTER V
DISCUSSION

5.1 Changes in weight loss, firmness, SSC, TA, and color of 'Nam Dok Mai No.4' and 'Nam Dok Mai Si Tong' mangoes during storage

The physical appearance of mango is an important feature to determine the loss of postharvest shelf life and quality of mango fruit. Moreover, weight loss and fruit firmness also exhibit changes throughout the ripening stage which are also essential characteristics after harvest. Our results revealed that weight loss of mango fruits increased while fruit firmness rapidly declined during 12 days of storage at 25 ºC in both cultivars as expected (Table 1). No significant differences in weight loss and fruit firmness were observed between 'Nam Dok Mai No.4' and 'Nam Dok Mai Si Tong' mangoes until the end of the storage period. Loss of weight in fruit during the ripening process is caused by water vapor exchange between the internal and external atmosphere, transpiration rate, and respiration metabolism of fruit (Zhu et al., 2008). Fruit softening is a result of cell wall digestion by cell wall-degrading enzymes such as cellulase, pectin esterase, polygalacturonase, and pectin methyeaterase (Yashoda et al., 2007). Previous fruit ripening researches reported weight loss, and fruit firmness reduction during the ripening process in many fruits (Boudhrioua et al., 2002; Khan and Singh, 2010). It has been shown that 'Chok Anan' mango had higher fruit firmness than 'Nam Dok Mai' mango at most of the development stages but not at the ripe stage (Rimkeeree and Charoenrein, 2014). Kader (1992) revealed that the difference of weight loss among fruits could be due to the variability among species, varieties, ripening stage and storage condition.

SSC and TA contents are also considered to be very crucial aspects for postharvest quality of tropical fruit. The increase in SSC during ripening is due to the conversion of carbohydrates into simple sugar (Khosroshahi et al., 2007) which is associated with the sweetness of mango while the decrease in TA is associated with the concentration of organic acid that are used as substrates for respiration during development process (Khosroshahi et al., 2007). SSC content in 'Nam Dok Mai No.4.' mango was found to be significantly higher than that of 'Nam Dok Mai Si Tong' mango. In contrast, 'Nam Dok Mai Si Tong' mango showed higher TA content throughout the storage time. The results in this experiment revealed that ' Nam Dok Mai No.4 ' is the sweeter cultivar because of the high accumulation of sugar and low content of TA. It could be suggested that this is one of the physiological variations between these two cultivars. Difference in sweetness aspect among mango cultivars was reported previously as the finding of Nambi et al. (2015) stated that the rate of change of TA and SSC was observed to be significantly higher in 'Alphonso' mango than 'Banganapalli' mango when kept in chamber at 20 ºC. Elsheshetawy et al. (2016) confirmed a variation of SSC and TA contents of six popular Egyptian mango cultivars. The similarly patterns of SSC and TA for 'Ataulfo' mango (Palafox-Carlos et al., 2012) and 'Haden' (Nassur et al., 2015) were also presented. Kaur et al. (2014) reported that the variation of TA and SSC between cultivars is directly related to the genotype, climate condition, growing condition and the ripen stage at the time of analysis.

During mango ripening, changes in peel color during ripening is due to chlorophyll degradation. The presence of yellow peel of mango represents carotenoid pigments which varied among mango cultivars (Ornelas-Paz et al., 2008). Our results showed that L* value indicated the lightness of 'Nam Dok Mai No.4' mango peel

increased from day 0 to day 12. On the other hand, the lightness of ‘Nam Dok Mai Si Tong’ mango peel was found to reduce with storage time. Meanwhile, a* value ranged from the negative which was green to the positive which was red. It was found that there was a gradual decrease in a* value of ‘Nam Dok Mai No.4’ mango peel while there was an increase for ‘Nam Dok Mai Si Tong’ mango peel throughout the ripening period. In both cultivars of mangoes, b* value which ranged from the negative to the positive representing the yellowness of peel mango exhibited a gradual increase from day 0 to day 12. C* value of ‘Nam Dok Mai No.4’ mango peel was significantly greater than ‘Nam Dok Mai Si Tong’ mango peel on days 0, 3, 6 and 9. ‘Nam Dok Mai No.4’ mango peel exhibited a higher of H° value than ‘Nam Dok Mai Si Tong’ mango peel during ripening. These results are in concordance with the finding of other mango cultivars (Palafox-Carlos et al., 2012; Ibarra-Garza et al., 2015). Our results confirmed significant differences in peel color between these 2 cultivars of mangoes.

5.2 Changes in respiration rate and ethylene production of ‘Nam Dok Mai No.4’ and ‘Nam Dok Mai Si Tong’ mangoes during storage at room temperature

Ripening of mango fruit is triggered via the increase of ethylene production and respiration rate (Venkatesan and Tamilmani, 2011). Both cultivars of mangoes exhibited different levels of ethylene production and respiration rate despite being climacteric fruit and closely related cultivars. Our results observed that ethylene production and respiration rate in both cultivars became higher and peaked on day 6, and then declined after ripening. Both parameters were found significantly greater in ‘Nam Dok Mai No.4’ mango when compared to ‘Nam Dok Mai Si Tong’ mango. These results indicated that ethylene production and respiration rate depend upon the mango cultivar and correlate

with SSC in both cultivars. It has been well established that ethylene production and respiration rate vary among cultivars and under different climacteric condition (Singh et al., 2013). Previous studies have shown that there was significant difference in ethylene production and respiration rate of 'Chokanan', 'Golden phoenix' and 'Water lily' mangoes (Lawson et al., 2019). The ethylene production and respiration rate patterns of our results are in concordance with 'Ataulfo' mango (Palafox-Carlos et al., 2012) and 'Cogshall' mango (Nordey et al., 2016).

5.3 Changes in ADC and ODC activities of 'Nam Dok Mai No.4' and 'Nam Dok Mai Si Tong' mangoes during storage at room temperature

It is well known that ADC and ODC are enzymes that play critical roles in PUT synthesis. Our results indicate that both enzyme activities showed a gradual increase until day 9 of storage and then a slight decrease at the end of storage period. Furthermore, 'Nam Dok Mai Si Tong' mango exhibited a higher level of ADC and ODC activities than 'Nam Dok Mai No. 4' mango after 3 days of storage time. This indicates that endogenous production of polyamines is vital in mango fruit ripening and may vary among cultivars. The existence of endogenous polyamines is required during fruit ripening. Similar results were observed in tomato (Tsaniklidis et al., 2016) which the levels of *ADC1*, *ADC2*, and *ODC1* genes increased during the change of color stage and then decreased at the red ripe stage. However, in grape fruit, the up-regulated *ADC* gene was observed whereas, *ODC* genes were not differentially expressed during ripening (Agudelo-Romero et al., 2013). The role of both *ADC* and *ODC* enzymes may depend on plant species (Palma et al., 2014).

5.4 Changes in PAs contents of ‘Nam Dok Mai No.4’ and ‘Nam Dok Mai Si Tong’ mangoes during storage at room temperature

PUT, SPD and SPM are the major polyamines found in higher plants at different maturity stage because PAs are associated with many metabolic processes, for example, flower development, fruit production and development (Gill and Tuteja, 2010). The role of PAs during mango fruit ripening may involve with softening and other climacteric characteristics. PUT, SPD, and SPM contents were analyzed during storage of mango fruit. Our results found similar characteristics of PAs contents in both cultivars during mango ripening. PUT contents showed similar contents in both cultivars although significant differences in ADC and ODC activities were observed between these two mango cultivars as mentioned above. On the other hand, SPD content in ‘Nam Dok Mai Si Tong’ mango unveiled higher content during ripening period while SPM contents was low. ‘Nam Dok Mai Si Tong’ mango displayed greater content of SPM. This finding suggests that PAs, especially SPD could be a key polyamine in mango ripening process. Higher SPD contents were also reported in ‘Tommy’ mango (Santiago-Silva et al., 2011) and oil palm fruit (Teh et al., 2014). Moreover, the concentration of PAs was found to increase during development of non-climacteric fruit such as strawberry (Guo et al., 2018), mandarin (Nathan et al., 1984), and rambutans (Kondo et al., 2001). In contrast, PAs contents declined following maturation period in peach (Liu et al., 2006a), grape (Fortes et al., 2011), and apple (Biasi et al., 1988).

The concrete mechanism describing a relationship between PAs biosynthesis and ethylene production is still under investigation. Our study discovered that ethylene production during mango fruit ripening increased at the same time as the rise of PUT biosynthesis enzyme activities, SPD, and SPM contents. It should be noted that ‘Nam

Dok Mai No.4' mango which had higher ethylene production had a lower level of SPD and SPM than 'Nam Dok Mai Si Tong' mango, which had the lower ethylene production. Similar results have also been reported that the maximum ethylene production in plum fruit was inversely correlated to PAs contents, as can be seen with 'Black Star' cultivar that had the highest ethylene production and had lowest PAs contents (Serrano et al., 2003). Based on our results, it can be concluded that endogenous SPD and SPM may have an inhibitory role on ethylene production in these two mango cultivars as was observed in plums. Likewise, several reports indicated that the balance between PAs biosynthesis and ethylene production controls fruit ripening (Pandey et al., 2000; Franco-Mora, 2005). Thus, the increase in PAs contents may be involved in delaying fruit ripening through the reduction of ethylene production.

5.5 Effects of exogenous PUT on weight loss and fruit firmness

Weight loss of mango fruit is mainly because of an evaporation of water from the peel of the fruit. Our experiment exhibited that application of exogenous 2 mmol/L PUT was able to slow down the rate of weight loss. Based on our results, it is indicated that PUT inhibited respiration rate and ethylene production leading to a delay in weight loss of mango fruit during storage. Similar results were observed by Valero et al. (1998a) reporting that PUT delayed weight loss by inhibiting respiration rate and ethylene production. These results are consistent with 4 mmol/L PUT treatment in two apricot cultivars 'Lasgerdi' and 'Shahrodi' which showed that PUT significantly reduced the weight loss in apricot fruit stored at 4 °C with 95% RH for 20 days (Davarynejad et al., 2013). In addition, application of exogenous 1 mmol/L PUT led to a significant reduction in weight loss in 'Kensingtion Pride' mango (Malik et al., 2005). 'Mauricio'

apricot treated with 1 mmol/L PUT exhibited reduced ethylene emission, respiration rate and physiological loss in weight in apricot fruits stored at 10 °C for 6 days (Martinez-Romero et al., 2002). Moreover, combination between 2 mmol/L PUT and carnauba wax (1:10) treatment delayed respiration rate, ethylene production and reduced weight loss of 'Mridula' Pomegranate (Barman et al., 2011).

Fruit firmness is the major limiting factor for marketing and storage life of mango fruit, which rapidly decreased with the advancement of storage period. Fruit softening during the ripening process is the result of the changes of cell wall degrading enzymes such as polygalacturonase (PG), pectin methyeaterase (PME), pectin esterase, and cellulase (Mitcham et al., 1991). Our finding exhibited that application of exogenous 2 mmol/l PUT significantly maintained fruit firmness during storage period. These results are in accordance with the result of Davarynejad et al. (2015) who reported that 4 mmol/L putrescine enhanced fruit firmness of 'Santa Rosa' plum stored at 4 °C with 95 % RH for 25 days. The use of 1 mmol/L PUT treatment also displayed a high retention of fruit firmness in 'Mauricio' apricot kept at 10 °C for 6 days (Martinez-Romero et al., 2002). Barman et al. (2011) reported higher pomegranate firmness retention by coating with 2 mmol/L PUT combination with carnauba wax at 3 °C with a RH of 90±5% for 60 days. Moreover, the application of 0.5 mmol/L PUT and 0.5 mmol/L SPD effectively retained fruit firmness of table grapes cv. Flame Seedless stored at 3-4 °C, and 90–95% RH for 75 days (Champa et al., 2014). The effect of polyamines on maintaining fruit firmness is due to their capacity in cross-linking to the carboxyl group of the pectin substances in the cell wall, resulting in rigidification (Abbott et al., 1989). This binding between PAs and pectin substance also block the entry of cell wall-degrading enzymes,

such as pectin esterase, polygalacturonase (PG), and pectin methyeaterase (PME) (Valero et al., 2002).

5.6 Effects of exogenous PUT on SSC and TA

The SSC and TA contents play a vital role in maintaining the quality of fruit. During storage period, the concentration of SSC continuously increases because of the hydrolysis of polysaccharides (Mahto and Das, 2013). On the other hand, TA content declines which is involved in the concentration of organic acid. The respiration appears to provoke consumption of organic acids and reduce TA of the fruits (Khosroshahi et al., 2007). The SSC and TA contents were significantly affected by the exogenous PUT treatment in our present experiment. Our finding showed that mango treated with 2 mmol/L PUT displayed the lowest SSC and the highest TA content during storage. The reduction of respiration rate and ethylene production can be attributed to effect of PUT, thus delaying the mango fruit ripening process. Similar results have also been reported in two apricot 'Lasgerdi' and 'Shahrodi' treated with 1, 2, 3 and 4 mmol/L PUT and stored at 4°C and 95% RH for 20 days which showed a lower SSC and higher TA when compared with control fruit (Davarynejad et al., 2013). Likewise, in 'Angelino' plum, PUT treated fruit showed lower SSC content, higher TA and delayed respiration rate compared to control fruit (Khan et al., 2008). Climacteric and non-climacteric fruits also exhibited a similar effect, including pomegranate (Mirdehghan et al., 2007), apricot (Martinez-Romero et al., 2002), mango (Jawandha et al., 2012), and strawberry (Zafari et al., 2015).

5.7 Effect of exogenous PUT on peel color changes

During ripening, the changes in peel color are because of the degradation of the chlorophyll pigments and increased synthesis of carotenoids which are involved in the climacteric increased in respiration rate via the action of ethylene (Wang et al., 1971; Saltveit, 1999). In our experiment, application of 2 mmol/l PUT could delay peel color change during 9 days of shelf life at 25 °C. Polyamines have been reported to reduce hydrolytic activities acting on chloroplast thylakoid membranes (Lester, 2000). A similar effect has been observed previously which 0.5 mmol/l PUT effectively maintained peel color (L*, C*, h◦) of table grapes for 75 days (Champa et al., 2014). Martinez-Romero et al. (2002) reported that PUT inhibited the peel color changes in apricot. From the previous work, the effect of PUT delayed peel color changes by reducing senescence process in a lemon (Valero et al., 1998b) and pomegranate (Barman et al., 2011).

5.8 Effect of exogenous PUT on respiration rate

Respiration during mango fruit ripening plays a crucial role related to the physiological process until the senescence of fruit and also is involved in commercial quality loss after fruit harvest. In our study, 2 mmol/L PUT treated fruits showed the lowest respiration rate when compared to other treatments. Thus, 2 mmol/L PUT treated fruits ripened slower than other treatments. The reduction in respiration rate with exogenous PUT application has also been reported in previous studies in numerous fruit. Exogenous application of polyamines on ‘Allison’ kiwi fruit was shown to significantly reduce the respiration rate (Jhalegar et al., 2012). The reduction in respiration rate of ‘Samar Bahisht Chaunsa’ mango was observed when treated with 0.5 mmol/L PUT and kept at 32±2 °C and at 11±1 °C (Razzaq et al., 2014).

Similarly, results were noticed when PUT treatment resulted in inhibiting respiration rate of ‘Angelino’ plum led to improve on fruit quality when stored at 0±1°C (Khan et al., 2008). The respiration rate decreased during the mango storage period led to a delay of fruit ripening which matches with our previous results that 2 mmol/L PUT slowed down physiological changes of mango fruit, including weight loss, fruit hardness, peel color, soluble solid content, and titratable acidity of mango fruit. Based on the present results, it could be concluded that PUT delayed respiration rate, and thus, delaying postharvest climacteric phenomenon resulting in the prolonged shelf life of mango fruit. However, it has been reported that high concentration of PUT caused chemical injury and higher respiration rate, water vapor and increased weight loss (Woods, 1990). In another study, higher PAs concentration induced the development of small black spots in ‘Red Delicious’ and ‘McIntosh’ apples (Kramer et al., 1991). Shiri et al. (2013) found that application of high PUT (2 mmol/L PUT) caused an injury to Table grape cv. Shahroudi.

5.9 Effect of exogenous PUT on ethylene production

Ethylene production of ‘Nam Dok Mai No.4’ mango was suppressed when mangoes were treated with 2 mmol/L PUT after being removed from low temperature. Anti-ethylene production function of PAs can be as PAs and ethylene use the common substrates, which is S-adenosyl methionine (SAM) for their biosynthesis (Nichols et al., 1983). Therefore, application of PUT inhibits ethylene biosynthesis via inhibiting ACC-synthase activity and ACC synthesis (Lee et al., 1997). Drolet et al. (1986) reported that PAs are able to block ACC-oxidase activity by elimination of superoxide free radical, which is crucial to convert ACC to ethylene

leading to reduced ethylene synthesis. Previous study showed that the increased in PUT production with declined ethylene production of ‘Liberty’ tomato during ripening leading to improve the storage life (Saftner and Baldi, 1990). It has been confirmed that 2 mmol/L PUT application resulted in delaying ethylene production and improvement of fruit quality in ‘Selva’ strawberry (Khosroshahi et al., 2007). Similar findings were obtained in ‘Samar Bahisht Chaunsa’ mango that 2 mmol/L PUT decreased ethylene production and enhanced fruit quality (Razzaq et al., 2014). Moreover, the application of 1 mmol/L PUT and SPD delayed ethylene production in ‘Bagheri’ and Asgarbadi’ apricots stored at 1 °C for 21 days (Saba et al., 2012).

5.10 Effect of exogenous PUT on soluble pectin content

Pectin is an important component of the primary cell wall and middle lamella, which are degraded by cell wall-degrading enzymes during fruit ripening, leading to loss of fruit firmness (Posé et al., 2015). Fruit firmness is considered to be the major factor for marketing and quality of mango fruit. Previous studies reported that water-insoluble pectin is converted to water soluble, which is always increased during ripening period (Yashoda et al., 2005). Our results showed that the soluble pectin content increased progressively in all treatment during storage period. However, the lowest level of soluble pectic was observed in 2 mmol/L PUT treated fruit compared with 1 mmol/L, 4 mmol/L, and control fruit on days 9, 12, 15 and 18. Our present study indicated that polyamines could control the esterification of pectin within the cell wall. The effect of PAs can be attributed to their capacity to bind to the –COO−group of the pectic substances in the cell wall, resulting in rigidification. This binding makes pectin less accessible to cell wall-degrading enzymes such as pectin

methylesterase (PME) and polygalacturonase (PG), leading to a delay the reduction of fruit firmness (Valero et al., 2002). These results are in concordance with the finding of Jongsri et al. (2017) which reported that the combination of 1% CTS and 0.1 ppm SPD inhibited the increase in soluble pectin content and maintained fruit firmness of ‘Nam Dok Mai No.4’ mongo. In accordance with our results above, exogenous PUT application resulted in higher firmness of ‘Nam Dok Mai No. 4’ mango than other treatments. Similar results were also observed in plums (Davarynejad et al., 2015), apricot (Martinez-Romero et al., 2002) and pomegranates (Barman et al., 2011).

5.11 Effects of exogenous PUT on polygalacturonase (PG) and pectin methylesterase (PME) activities

During fruit softening, PG and PME are two key enzymes, which are responsible for pectin degradation during fruit ripening, leading to loss of cell wall structure. PG is capable of catalyzing the hydrolysis of the glycosidic bonds in galacturonans (Prasanna et al., 2007), and PME is related to pectin degradation via catalyzing the hydrolysis of methyl esters from galacturonic acid residues, making polygalacturonan available for degradation by PG which lead to de-esterification of pectin (Koch and Nevins, 1989). The results presented here indicated that the PG activity in all treatment steadily increased throughout the storage period. Upon removal from cold storage to room temperature, 2 mmol/L PUT treated fruit showed a lower PG activity when compared to other treatments on day 9, 12, 15 and 18. Moreover, 2 mmol/L PUT greatly reduced PME activity to the lowest activities during storage at room temperature. In addition, our results found that PUT inhibited PG and PME activities and could control the esterification of pectin within the cell

wall leading to maintained fruit firmness. Thus, PUT interacted with carboxyl group of pectic substrate which created the binding that blocked the access of PG and PME activities and resulted in reduced rate of softening during storage period (Valero et al., 2002). Similarly, 2 mmol/L PUT treatment reduced activities of PG and PE in 'Samar Bahisht Chaunsa' mango when applied at 32±3 ºC for 7 days and at 11±1 ºC for 28 days (Razzaq et al., 2014). Our results are in concordance with Champa et al. (2014) who showed that a lower concentration of 0.5 mmol/L PUT and SPD inhibited PME activity leading to maintained fruit firmness in table grapes fruit during storage at 3-4 ºC for 75 days. On the other hand, higher concentration of PAs resulted in a higher PME activity, and showed a harmful effect on most of the quality parameters.

5.12 Effect of exogenous PUT on antioxidant enzymes activities

The production and accumulation of ROSs, including hydrogen peroxide and superoxide anion progressively increase during fruit ripening process. Therefore, ripening can be considered a stressful process with a continuous increase in antioxidant (Jimenez et al., 2002). The oxidative stress of lipid membrane, nucleic acid, and proteins is caused by generation of ROSs (Scandalios, 1993). Generally, there are two types of the mechanisms for scavenging ROSs in plant. The first one, the damage caused from ROSs could be scavenged by plant enzymatic defenses, for instance SOD, CAT, APX, POX, MDHAR, DHAR, GPOX, and GR. For another system, the oxidative stress was scavenged by non-enzymatic antioxidants, including, ascorbic acid, beta-carotenoid and glutathione (Gill and Tuteja, 2010). Previous studies reported that PAs might be associated with the antioxidant system in plants (Verma and Mishra, 2005). This research is an attempt to understand the mechanism

by which polyamines increase the antioxidant in mango fruit. ROSs have been reported to be stimulated during plant senescence, fruit ripening, and fruit storage, which are characterized by a breakdown of the cell wall, membrane disruption, and cellular decompartmentation (Kumar et al., 2011). The superoxide radicals are converted into H_2O_2 by SOD activity, afterword CAT and POX disintegrated it into H_2O and O_2 (Gill and Tuteja, 2010). APX is thought to play the most essential role in scavenging ROSs, catalyzing the reduction of H_2O_2 to water using the reducing capability of ascorbate (Noctor and Foyer, 1998). Increased GR may also maintain a high ratio of GSH/GSSG which is required for the regulation of ascorbate threshold level (Foyer and Halliwell, 1976). Oxidative stress is associated with reduced antioxidant enzyme activities, leading to a short postharvest life of fruit. The accumulation of ROSs contributed to higher membrane deterioration and loss of tissue structure (Kumar et al., 2011). Our results found that the 2 mmol/L PUT treatment effectively induced activities of antioxidant enzyme activities, for instance SOD, CAT, GPOX, APX and GR contributed to improved fruit quality. It can be conclude that the effect of PAs on antioxidant enzymes caused PAs may act as ROS scavengers or binding to antioxidant enzymes molecules to scavenge free radical (Duan et al., 2008; Groppa and Benavides, 2008). These similar results have been reported by Palma et al. (2016) when zucchini fruits were treated with 1 mmol/L PUT and resulted in higher APX and GR activities respect to control. In agreement with our finding, previous studies have shown that 1 mmol/L PUT and SPM significantly increased the activities of CAT, SOD, and POX in both ‘Bagheri’ and Asgarbadi’ apricot (Saba et al., 2012). Besides, Razzaq et al. (2014) showed an increase in the

CAT, SOD, and POX activities of PUT treated fruit in ‘Samar Bahisht Chaunsa’ mango fruit during the storage period.

5.13 Effect of exogenous PUT on hydrogen peroxide

It has been suggested that an excess of hydrogen peroxide is considered an oxidative stress parameter during ripening. In our experiment, it was found that the application of 2 mmol/L was the one with the highest reduction in H_2O_2 content in mango fruit. A considerably similar reaction was observed in ‘Sinatra’ zucchini fruit treated with 1 mmol/L PUT that had the highest influence in the reduction of H_2O_2 content kept at 4 ºC in 85-90% RH for 14 days (Palma et al., 2015).

5.14 Effect of exogenous PUT on total antioxidant capacity

The determination of an antioxidant capacity is one of the access that expresses the nonenzymatic antioxidant system and biological properties of fruit which are an important parameter to maintain fruit quality. The data indicated that 2 mmol/L PUT treatment was effective in maintaining the total antioxidant contents of ‘Nam Dok Mai No.4’ mango assayed by both FRAP and DPPH methods. These antioxidants were well conserved by PUT, which are basically anti-senescence agents (Seiler and Raul, 2005). Moreover, application of 2 mmol/L PUT also maintained total antioxidant property by enhancing the SOD, CAT, GPOX, APX, and GR as shown in our data. Our results are in concordance with many reports, which described as polyamine associated with maintained antioxidant in several fruits. In ‘Santa Rosa’ plum, 4 mmol/L PUT treatment enhances the total antioxidant during storage period (Davarynejad et al., 2015). Jhalegar et al. (2012) reported that polyamines induced the

total antioxidant in ‘Allison’ kiwi fruit. These results are in agreement with the results obtained by Shiri et al. (2013), who found that application of exogenous PUT had higher the total antioxidant than control fruit in ‘Shahroud’ grape.

5.15 Effects of exogenous PUT on ADC and ODC activities

It is well known that the biosynthesis of PA is initiated from arginine and ornithine which are the two main amino acid that converted to PUT in 2 routes. The first route, ornithine is catalyzed to PUT by ODC. The second route, decarboxylation of arginine is controlled by ADC via agmatine and N-carbamoylputrescine intermediates which are converted to PUT (Alcázar et al., 2010). Our results found that 4 mmol/L PUT treatment resulted in the highest ADC and ODC activities followed by 2 mmol/L, 1 mmol/L and control, respectively. In agreement with our research, Pandey et al. (2015) demonstrated that transgenic tomato plant overexpressing the mouse ODC gene produced fruit with higher PUT, SPD and SPM contents when compared with fruit from untransformed plants, and showed inhibited ethylene production and respiration rate.

5.16 Effect of exogenous PUT on PAs contents

It is well established that there is a central role for the common substrate, S-adenosylmethionine (SAM), in ethylene and PAs biosynthesis pathway. The reduction in ethylene production could be a result of substrate competition with PAs biosynthesis leading to a delay in fruit ripening. Our study showed that 2 mmol/L PUT treatment increased PUT, SPD and SPM content during storage period resulting in a reduction of ethylene production. On the other hand, 4 mmol/L PUT treatment

induced higher PUT content than 2 mmol/L PUT treatment but reduced SPD and SPM contents and also showed higher ethylene production. Based on our results, it can be suggested that high concentration exogenous PUT applied led to an increase of endogenous PUT production, however PUT might not be converted to SPD. High content of PUT could be toxic in fruit tissue and resulted in higher ethylene production. Serrano et al. (2003) that PUT treated plum showed higher PUT and SPD content and a delay in ethylene production in 'Golden Japan', 'Black Diamond', 'Black Star' and 'Santa Rosa' plum treated with 1 mmol/L PUT, and stored at 20 ºC in the dark and 90%RH. Also, according to Valero et al. (1998a), lemon infiltrated with 1 mmol/L PUT exhibited an active metabolism from PUT to SPD in the biosynthesis pathway, but could not be detected significant level of SPM which increased fruit firmness and lowered weigh loss. In addition, Kramer et al. (1991) reported that PAs infiltration increased the endogenous levels of PUT, SPD and SPM in treated apples fruit. However, it has been reported that high concentration of PUT could cause chemical injury, higher respiration rate, water vapor and increased weight loss (Woods, 1990). In addition, Champa et al. (2014) revealed that higher concentration of PUT and SPD treatments had a harmful effect on most of the fruit quality parameters such as weight loss, decay incidence, rachis browning and organoleptic properties.

CHAPTER VI

CONCLUSION

6.1 The accumulation of endogenous polyamines and polyamine biosynthesis enzyme activities in relation to mango fruit ripening

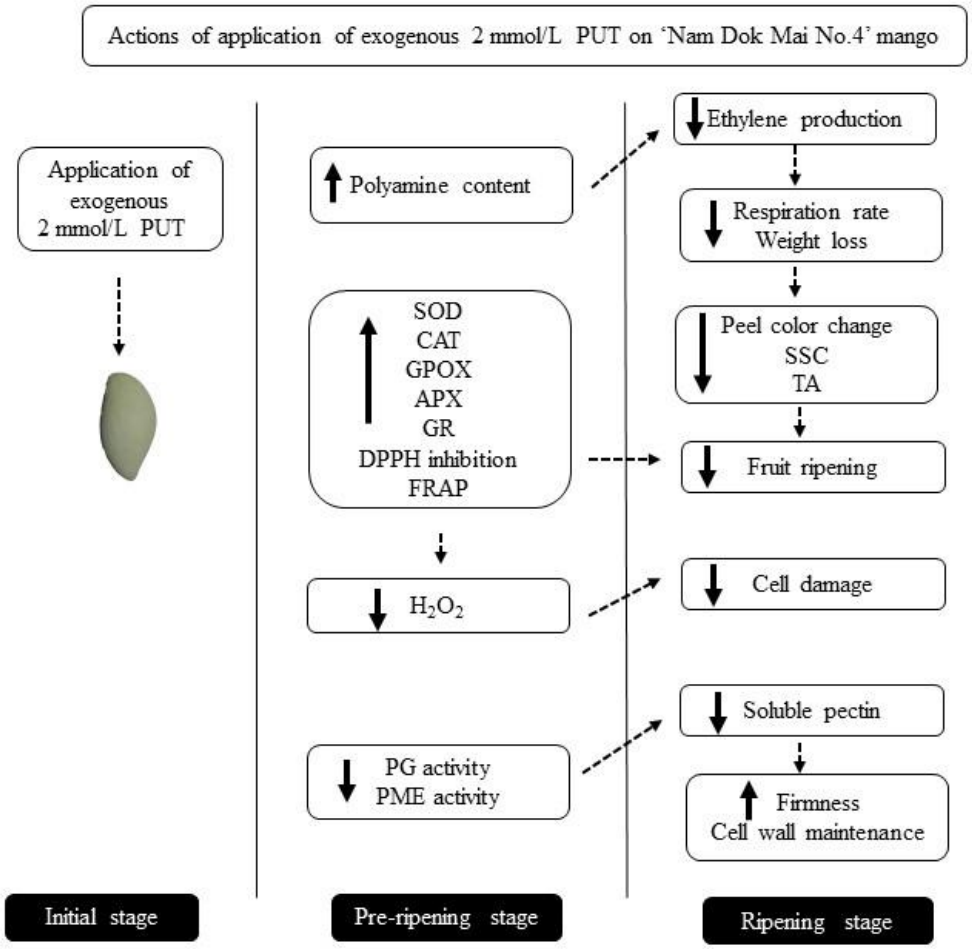
The investigation of the accumulation of endogenous polyamines and polyamine biosynthesis enzyme activities in relation to ‘Nam Dok Mai No.4’ and ‘Nam Dok Mai Si Tong’ mango fruit ripening throughout the storage period demonstrated that the physiological changes, including weight loss, firmness, SSC, TA and peel color depend upon the cultivar of mango. During mango fruit ripening, the PUT contents decreased ,whereas SPD and SPM contents increased. The highest PAs was observed in SPD contents. In addition, ‘Nam Dok Mai No.4’ mango, which had higher ethylene production had lower levels of ADC and ODC activities and SPD and SPM contents than ‘Nam Dok Mai Si Tong’ mango, which had lower ethylene production. The findings suggest that endogenous PAs, especially SPD plays an important role in mango fruit ripening through inhibition of ethylene production. Thus, PAs have an inhibitory role on ethylene production in these two mango cultivars. The increased in PAs contents may be involved in delaying fruit ripening through the reduction of ethylene production.

6.2 The physiological and biochemical responses of mango fruit to exogenous putrescine application

The investigation of the physiological and biochemical responses of mango fruit to exogenous PUT application demonstrated that exogenous application of 2 mmol/L PUT was an effective approach to retard fruit softening, improve postharvest quality and

antioxidant activities of ‘Nam Dok Mai No.4′ mango during storage period at 14 °C for 9 days at 25 ± 1 °C. The reduction of ethylene production was being enhanced after exogenous application of PUT. Higher fruit firmness, lower soluble solids concentration, higher titratable acidity, reduced weight loss, delayed color changes and reduced respiration rate were observed in 2 mmol/L PUT treated fruit during storage period.

Moreover, the exogenous application of 2 mmol/L PUT reduced soluble pectic content, and PG and PME activities leading to delaying fruit softening by inhibiting the activities of cell wall-degrading enzymes and improving postharvest quality. Positive effects of exogenous application of 2 mmol/L PUT were confirmed from its ability to reduce hydrogen peroxide content and maintain high SOD, CAT, GPOX, APX, and GR enzyme activities and total antioxidant contents of fruit during storage. The lower ethylene production was associated with the increase of endogenous PAs and the reduction of ethylene production after exogenous application of PUT. Thus, competition between ethylene and polyamine biosynthesis could be suggested from the results obtained with ‘Nam Dok Mai No.4′ mango. The outcomes reveal that the delaying of mango fruit ripening after exogenous application of PUT is encouraging for manufacturers and can be economically advantageous as mango can potentially reach a wider market.


Actions of application of exogenous 2 mmol/L PUT on 'Nam Dok Mai No.4' mango
Application of exogenous 2 mmol/L PUT
Polyamine content
SOD
CAT
GPOX
APX
GR
DPPH inhibition
FRAP
H_2O_2
PG activity
PME activity
Ethylene production
Respiration rate
Weight loss
Peel color change
SSC
TA
Fruit ripening
Cell damage
Soluble pectin
Firmness
Cell wall maintenance
Initial stage
Pre-ripening stage
Ripening stage

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APPENDIX

1. Chemical solutions

1.1 Enzyme extraction

50 mM Phosphate buffer (pH 6.5)	100 mL
1% PVPP (Add before use)	100 g
0.1%DTT	0.1 g
100 mM PMSF	1 mL

1.2 1 M Tris-HCl pH 7.0, 7.5

Tris base	60.57 g

Dissolve Tris base in water and adjust pH to 7.0, 7.5 with HCl, after bring total volume to 500 mL.

1.3 0.5 M EDTA pH 8.0

Na_2EDTA	186.1 g
NaOH pellet	20 g

Dissolve Na_2EDTA in water and adjust pH to 8.0 with NaOH, after bring total volume to 1,000 mL.

1.4. DNS reagent

DNS	10 g
NaOH	16 g
Na-K tartrate	300 g
Water	1,000 mL

Store in dark bottle.

1.5 Enzyme extraction buffer

Tris-HCl (pH 7.0)	100 ml
0.02 mol/L EDTA	7.44 g
0.05% Triton X-100	10 g
0.02 M Cysteine-HCl	2.423 g
1 mM PMSF (Add before use)	0.1742 g

Total volume 1,000 mL

2. Respiration rate

Percentage of CO_2 were determined by Gas Chromatography.

Calculate	Air 100 mL	have CO_2	A	mL
	Air (in jar) 2,400 mL	have CO_2	$\frac{Ax2,400}{100}$	mL
	Mango B kg.	have CO_2	$\frac{Ax2,400}{100}$	mL
	Mango 1 Kg	have CO_2	$\frac{Ax2,400}{100B}$	mL

Formula	PV=nRT (Boyle 's law; at 25ºC)			
	CO_2 24,453 mL	weight	44000	mg.
	CO_2 $\frac{Ax2,400}{100B}$ mL	weight	$\frac{(44000)(Ax2,400)}{(24,453)(100B)}$	mg.

3. units / mg protein

$$= \frac{(\Delta A_{sample}/min - \Delta A_{reference}/min) \times (vol\ of\ reaction) \times (dilution\ factor)}{(e^{mM}) \times (vol\ of\ crude\ extract) \times (path\ length\ in\ cm) \times (mg\ protein/\ ml\ crude\ extract)}$$

e^{mM} = milimolar extinction coefficient ($mM^{-1}cm^{-1}$)

catalase (CAT); extinction coefficient = 0.0436 $mM^{-1}cm^{-1}$

guaiacol peroxidase (GPX)); extinction coefficient = 26.6 $mM^{-1}cm^{-1}$

ascorbate peroxidase (APX); extinction coefficient = 2.8 $mM^{-1}cm^{-1}$

glutathione reductase (GR)); extinction coefficient = 14.15 $mM^{-1}cm^{-1}$

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