CAROTENOID METABOLISM AND RELATED GENE EXPRESSION DURING DEVELOPMENT AND MATURATION IN PUMMELO FRUIT

PAEMIKA PROMKAEW

Graduate School of Horticulture
CHIBA UNIVERSITY

March 2020

千葉大学学位申請論文

CAROTENOID METABOLISM AND RELATED GENE EXPRESSION DURING DEVELOPMENT AND MATURATION IN PUMMELO FRUIT

PAEMIKA PROMKAEW

Graduate School of Horticulture
CHIBA UNIVERSITY

March 2020

CONTENTS

PAGE

CONTENTS i
ABSTRACT (ENGLISH) v
LIST OF TABLES vii
LIST OF FIGURES viii

CHAPTER 1 INTRODUCTION **1**
1.1 Motivation 1
1.2 Objectives 3
1.3 Scopes 3

CHAPTER 2 LITERATURE REVIEW **4**
2.1 'Tubtim siam' pummelo 4
2.1.1 Fruit production 4
2.1.2 Fruit morphology and Characteristics 5
2.2 Carotenoid accumulation in plants 6
2.2.1 General carotenoid accumulation in plants 6
2.2.2 Carotenoid biosynthesis in pants 7
2.2.3 Carotenoid in citrus fruit 9
2.2.4 Accumulation of lycopene in citrus fruit 11
2.2.5 Carotenoid in Pummelo 11
2.3 Ethylene 13
2.3.1 The regulation of ethylene biosynthesis during fruit ripening 13
2.4 Postharvest change in citrus fruit and the role of ethylene 15
2.4.1 Effect of ethylene on citrus fruit 15
2.4.2 Effect of ethylene on physicochemical changes 16
2.4.2.1 Water loss 16
2.4.2.2 Peel color changes 17
2.4.2.3 Pulp color changes 17

PAGE

2.4.2.4 Sugar content 18

2.4.2.5 Total ascorbic acid content 18

2.5 Controlling Ethylene Action 19

2.6 Aminoethoxyvinyl glycine (AVG) 19

2.6.1 AVG effect on fruit 20

2.7 1-methyl cyclopropene (1-MCP) 20

2.7.1 1-MCP effect on fruits 21

2.7.2 1-MCP microbubbles 23

2.7.3 Micro and Nano bubbles2 23

CHAPTER 3 CAROTENOIDS ACCUMULATION AND CAROTENOIDS BIOSYNTHESIS GENE EXPRESSION DURING FRUIT DEVELOPMENT IN PULP OF 'TUBTIM-SIAM' PUMMELO FRUIT **25**

3.1 Introduction 25

3.2 Materials and Methods 25

3.2.1 Plant materials and experimental treatments 26

3.2.2 Pulp color analyses 26

3.2.3 The lycopene, β-carotenoid and total carotenoid analyses 27

3.2.4 Total RNA Extraction 27

3.3 Statistical analysis 27

3.4 Results 28

3.4.1 Effect of tree age on concentrations of lycopene, β-carotene, total carotenoid content and a* value in fruit pulp 30

3.4.2 Effect of tree age on color change in fruit pulp 32

3.4.3 Carotenoid profile and expression of caroteneoid biosynthesis genes analyses 33

3.5 Discussion 35

3.6 Conclusions 37

PAGE

CHAPTER 4 LYCOPENE SYNTHESIS AND RELATED GENE EXPRESSION IN PUMMELO PULP INCREASED IN SHADE-GROWN FRUIT **38**

4.1 Introduction 38

4.2 Materials and Methods 41

4.2.1 Fruit sample and treatments 41

4.2.2 Analyses of sugar, carotenoid, flavonoid, ascorbic acid, chlorophyll, antioxidant activity and ABA 41

4.2.3 Total RNA extraction 43

4.2.4 Quantitative real-time PCR analysis 43

4.3 Statistical analysis 45

4.4 Results 45

4.4.1 Effect of bagging on change in temperature 45

4.4.2 Effects of bagging treatment on chlorophyll, carotenoid, sugar, flavonoid, ascorbic acid, antioxidant activity, and carotenoid biosynthesis gene expression 45

4.5 Discussion 52

4.6 Conclusion 53

CHAPTER 5 APPLICATION OF AVG OR 1-MCP-MBS ON POSTHARVEST QUALITY OF PUMMELO CV. 'TUBTIM SIAM' (CITRUS MAXIMA BURM.) **54**

5.1 Introduction 54

5.2 Materials and Methods 57

5.2.1 Fruit sample and treatments 57

5.2.2 Physiological analysis 58

5.2.3 Chemical Analysis 59

5.3 Statistical analysis 59

5.4 Results and discussion 59

5.4.1 Effects of AVG and 1-MCP-MBs on respiration rate, ethylene production and weight loss. 59

PAGE

5.4.2 Effects of endogenous ethylene production on senescence occurrence in Tabtim Siam pummelo fruit treated with AVG and 1-MCP-MBs. 61

5.4.3 Effects of AVG and 1-MCP-MBs on quality of biochemical composition of fruit. 63

5.5 Conclusions 65

CHAPTER 6 GENERAL DISCUSSION **66**

REFERENCES **69**

ACKNOWLEDGEMENTS **88**

LIST OF PUBLICATION AND PRESENTATION **89**

ABSTRACT

Red pulp color of 'Tubtim siam' pummelo (*Citrus maxima* (Burm.) Merr.) is a distinct specific characteristic, which is the preferred in the international market. This present study aimed to determine (1) the influence of tree age on carotenoid accumulation during fruit development, and (2) the carotenoid profile and related biosynthesis gene expression in 'Tubtim siam' Pummelo fruit. Fruit were selected randomly from all directions of the tree at different stages of fruit development during the months of June, that is 3 months after fruit setting (3MAFS) to October (7MAFS). The carotenoid profile in the fruit pulp observed include lycopene, β-carotene, zeaxanthin and lutein. From breaker stage to commercial harvesting time, the 8-year old tree fruit observed a significantly higher ration of lycopene, β-carotene, total carotenoid concentrations and higher a* value compared to fruit in 12-year old tree. The expression of carotenoid biosynthesis genes profile in pulp of 8-year old tree fruit revealed the up-regulation of *CsPSY*, *CsZDS* and *CsZEP* gene expression during development and down-regulation of *CsβLCY*, *CsβCHX* and *CsεCHX* gene expression after breaker stage (5MAFS); that appeared to be a key factor triggering lycopene accumulation because these were the key genes expressed during fruit development. Moreover, the effects of light conditions on lycopene synthesis and the expression of related genes in the pulp of 'Tubtim siam' pummelo (*Citrus maxima* Burm.) were investigated. Glucose, ascorbic acid, and flavonoid concentrations and DPPH scavenging activity were decreased in fruit covered with bags while still on the tree (0.01 $\mu mol.m^{-2}.s^{-1}$ photosynthetic photon flux density (PPFD)) compared to the untreated control (596.7 $\mu mol.m^{-2}.s^{-1}$ PPFD). The bagging treatment significantly decreased the temperatures on the surface in the bag. In addition, the bagging treatment decreased abscisic acid (ABA) concentrations in the peel and pulp. On the other hand, the bagging treatment increased lycopene concentrations, upregulated phytoene synthase (*CsPSY*) and ζ-carotene desaturase (*CsZDS*) gene expressions; downregulated chromoplast-specific lycopene cyclase (*CsβLCY*), β-carotene hydroxylase (*CsβCHX*), and ε-ring hydroxylase (*CsεCHX*); and decreased 9-cis-epoxycarotenoid dioxygenase (*CsNCED1*) gene expressions in the pulp. It is possible that maintaining a temperature of around 25°C in fruit covered with bags may increase the lycopene concentration in the pulp with the upregulation of *CsPSY* and *CsZDS* and the downregulation of *CsβLCY*, *CsβCHX*, *CsεCHX*, and *CsNCED1* gene expressions in the pulp. Additionally, degradation of green peel and change in the red-ruby pulp color are two main deteriorative postharvest quality

parameters caused by exogeneous ethylene. The aim of this study was to evaluate the effectiveness of Aminoethoxyvinylglycine (AVG) and 1-metthylcyclopropene-microbubbles (1-MCP-MBs) on maintaining postharvest quality of 'Tubtim siam' pummelo through dipping in AVG and 1-MCP-MBs at 500 and 5 ppm, respectively. The control was fruit without any treatment. The fruits were stored at room temperature (25±2 ˚C) for 21 days. Fruit treated with AVG resulted in a remarkably reduced respiration rate and ethylene production compared to other treatments. Moreover, delaying of peel yellowing was indicated by significantly higher total chlorophyll contents, hue angle values of peel and delayed degradation of red-ruby pulp color were also observed in 1-MCP-MBs treated fruit. On the other hand, application of AVG and 1-MCP-MBs retarded a reduction of weight loss compared to the control. Chemical composition in pulp including; total carotenoid, beta-carotenoid, lycopene, vitamin C, DPPH free radical scavenging activity and total phenolic content were maintained and were significantly different when compared with the control treatment. In conclusion, AVG and 1-MCP-MBs treatments could maintain both external and internal postharvest quality of 'Tubtim siam' pummelo fruit.

LIST OF TABLES

TABLE		PAGE
2.1	Carotenoid content and composition in the peel and pulp of mature fruits of selected pummelo (*Citrus maxima* (Burm.) Merr.) cultivars.	12
3.1	Primer used for real-time RT-PCR.	29
4.1	Primer sequences for the quantification of transcripts by RT-PCR.	43

LIST OF FIGURES

FIGURE		PAGE
2.1	The morphology of 'Tubtim-siam' pummelo fruit at immature green stage (3 months after fruit set (3 MAFS)) (A) and at commercial harvesting time (7 MAFS) (B).	5
2.2	The morphology of the segment containing "juice sac" of 'Tubtim-siam' pummelo fruit pulp at immature green stage (3 months after fruit set (3 MAFS)) (A) and at commercial harvesting time (7 MAFS) (B).	6
2.3	Chemical structure of lineal (phytoene), bicyclic (carotene) and epoxy carotenoids (violaxanthin).	7
2.4	Carotenoid biosynthetic pathway in citrus. GGPP, geranylgeranyl diphosphate; PSY, phytoene synthase; PDS, phytoene desaturase; ZDS, ζ-carotene desaturase; Ɛ-LCY, lycopene Ɛ-cyclase; β-LCY, lycopene β-cyclase; β-CHX, β-carotene hydroxylase; Ɛ-CHX, Ɛ-carotene hydroxylase; ZEP, zeaxanthin epoxidase.	8
2.5	Chemical structure of specific citrus apocarotenoids and apocarotenals.	10
2.6	Differential expression of ACS and ACO genes associated with system 1 and system 2 ethylene synthesis during fruit development and ripening in tomato. Au-to inhibition of ethylene synthesis during system 1 ethylene production is mediated by a reduction in LeACS1A and 6 expression.	14

FIGURE		PAGE

2.7	Autocatalytic ethylene synthesis at the onset of fruit ripening is mediated through ethylene-stimulate dexpression of LeACS2 and 4 and LeACO1 and 4.	22
2.8	Binding of ethylene molecule with the receptor "unlocks" the receptor and leads to a chemical reaction in the plant tissue; Blankenship, diagram by Jenny Bower, Dept. of Pomology, UC Davis (a).	22
2.9	When 1-methylcyclopropene (1-MCP) binds to the ethylene receptor, it does not "unlock" the receptor and remains locked to the receptor preventing the binding of ethylene and the chemical reaction does not occur; Blankenship, diagram by Jenny Bower, Dept. of Pomology, UC Davis (b).	24
3.1	Effect of tree age on accumulation of carotenoid composition namely lycopene (A), β-carotene (B), total carotenoid(C) and a* value (D) in pulp of 'Tubtim siam' pummelo during fruit development. Each value represents the means ±SE and * or ** shows significant difference by *t*-test at $P \leq 0.05$ or $P \leq 0.01$ on each date, respectively.	31
3.2	The pulp coloration in 'Tubtim siam' pummelo fruit (The number in the parenthesis shows month after fruit set; MAFS).	32

FIGURE		PAGE
3.3	Chromatograms (450 nm) of the saponified samples. Pigments of pulp of 'Tubtim-Siam' pummelo were investigated to elucidate the carotenoid profile (3A) and the accumulation of main carotenoids content (3B) at commercial harvesting time using LC/MSD.	32
3.4	The expression of carotenoid biosynthesis genes profile in pulp of 'Tubtim-Siam' pummelo during fruit development. Fruit were harvest in June (3 MAFS), July (4 MAFS), August (5 MAFS), September (6 MAFS) and October (7 MAFS). The genes analyzed were phytoene synthase (*CsPSY*), ζ-carotene desaturase (*CsZDS*), lycopene β-cyclase (*CsβLCY*), β-carotene hydroxylase (*CsβCHX*), Ɛ-carotene hydroxylase (*CsƐCHX*) and zeaxanthin epoxidase (*CsZEP*).	34
4.1	Schematic diagram of the carotenoid biosynthesis pathway in plants. Genes analyzed in this study appear in boldface. GGPP, geranylgeranyl diphosphate; PSY, phytoene synthase; PDS, phytoene desaturase; ZDS, ζ-carotene desaturase; Ɛ-LCY, lycopene Ɛ-cyclase; β-LCY, lycopene β-cyclase; β-CHX, β-carotene hydroxylase; Ɛ-CHX, Ɛ-carotene hydroxylase; ZEP, zeaxanthin epoxidase; VDE, violaxanthin de-epoxidase; NSY, neoxanthin synthase; NCED, nine-cis-epoxycarotenoid dioxygenase; ABA, abscisic acid.	39

FIGURE		PAGE

4.2	The temperature inside the bag (the number of months after fruit set is shown in parentheses) during fruit development from 3 months after fruit set (June) to harvest at 7 months after fruit set (October). Each value represents the means ±SE, and * or ** shows a significant difference by *t*-test at $P \leq 0.05$ or $P \leq 0.01$ on each date, respectively.	44
4.3	Effects of bagging on chlorophyll (A) and carotenoid concentration (B) in the peel (the number of months after fruit set is shown in parentheses). Each value represents the means ± SE, and * or ** shows a significant difference by *t*-test at $P \leq 0.05$ or $P \leq 0.01$ on each date, respectively.	45
4.4	The pulp coloration in 'Tubtim-Siam' pummelo fruit (the number of months after fruit set is shown in parentheses).	46
4.5	Effects of bagging on fructose (A), sucrose (B), glucose (C), inositol (D), and total sugar (E) concentrations in the pulp of 'Tubtim Siam' pummelo fruit (the number of months after fruit set is shown in parentheses). Each value represents the means ±SE, and * or ** shows a significant difference by t-test at $P \leq 0.05$ or $P \leq 0.01$ on each date, respectively.	47
4.6	Effects of bagging on the concentrations of lycopene (A), β-carotene (B), total carotenoid (C), flavonoid (D), ascorbic acid (E), and DPPH scavenging activity (F) in the pulp (the number of months after fruit set is shown in parentheses). Each value represents the means ±SE, and * or ** shows a significant difference by *t*-test at $P \leq 0.05$ or $P \leq 0.01$ on each date, respectively.	48

FIGURE **PAGE**

4.7 Effects of bagging on the expression of carotenoid biosynthesis genes in pulp and ABA concentrations in pulp and peel (the number of months after fruit sets is shown in parentheses). Each value represents the means ±SE, and * or ** shows a significant difference by *t*-test at $P \leq 0.05$ or $P \leq 0.01$ on each date, respectively. 50

5.1 Schematic diagram of 1-MCP micro bubble generation system. 57

5.2 Changes in respiration rate (A), ethylene production rate (B) and weight loss (C) of 'Tubtim-Siam' pummelo fruit. Fruit were treated with 1-MCP-MBs and AVG at 5 ppm and 500 ppm concentration respectively. The control was fruit without treatment. The fruit were stored at room temperature (25±2 ˚C) for 21days. Each point represents the means ±SE, and * or ** shows a significant difference between means at each storage time (Duncan, $P<0.05$) 60

5.3 Changes in total chlorophyll content of peel (A), hue angle value of peel (B) and hue angle value of pulp (C) of 'Tubtim-Siam' pummelo fruit. (Hue angle ho=tan-1(b*/a*) represents different color, 0° = red-purple, 45° = orange, 90° = yellow, 180° =bluish green and 270° =blue.) Fruit were treated with 1-MCP-MBs and AVG at 5 ppm and 500 ppm concentration respectively. The control was fruit without treatment. The fruit were stored at room temperature (25±2 ˚C) for 21day. Each point represents the means ±SE, and * or ** shows a significant difference between means at each storage time (Duncan, $P<0.05$) 61

FIGURE		PAGE
5.4	Changes in total carotenoid content (A), total lycopene content (B) and total beta-carotene content (C) of 'Tubtim siam' pummelo fruit. Fruit were treated with 1-MCP-MBs and AVG at 5 ppm and 500 ppm concentration respectively. The control was fruit without treatment. The fruit were stored at room temperature (25±2˚C) for 21days. Each point represents the means ±SE, and * or ** shows a significant difference between means at each storage time (Duncan, P<0.05)	63
5.5	Changes in total phenolic content (A), total ascorbic content (B) and DPPH scavenging activity (C) of 'Tubtim siam' pummelo fruit. Fruit were treated withed 1-MCP-MBs and AVG at 5 ppm and 500 ppm concentration respectively. The control was fruit without treatment. The fruit were stored at room temperature (25±2˚C) for 21days. Each point represents the means ±SE, and * or ** shows a significant difference between means at each storage time (Duncan, P<0.05)	64

CHAPTER 1
INTRODUCTION

1.1 Motivation

'Tubtim-Siam' pummelo is the geographical indications (GI) product in Pak Panang Basin, Pak Panang District, Thailand and a popular new pummelo cultivar in the premium fresh-fruit marketplace. The external appearances of 'Tubtim-Siam'pummelo fruit have the green colour and cover with soft hair, the internal appearances of 'Tubtim-Siam'pummelo thin light pink peel with tight row of small dark pink to red pummelo fresh, juicy with a sour - sweet taste (Kaewtubtim and Issarakraisila, 2011). All of production for domestic consumption and exporting. Nowadays, the price of 'Tubtim-Siam' pummelo from the hand of the farmer is 150-250 bath/fruit, the farmer's orchard expands to plantation increasing continuously for commercial purpose.

The major problem of 'Tubtim-Siam' pummelo in the production area is the climate variability, which is induced by global warming effects, has become a global concern as it may have many consequences on the various system and sectors that may threaten human wellbeing (IPCC,2001). Climate change has a major impact on the phonological cycle and agricultural productivity (Solomon and Shugart, 1993). The effected of climate variation make of the 'Tubtim-Siam' pummelo had a fruit set 3 times around the year, a quality of fruit especially the red pulp color at each period of the year is not stable. Nowadays, only a limited information on the climate variability of 'Tubtim-Siam' pummelo production in Thailand has been published based on fruit growth and quality. The farmer they lack data on the effects of the climate variation to temperature, light, tree age and fruit age on fruit development and fruit quality especially in red pulp color for making a decision by harvesting time of the 'Tubtim-Siam' pummelo.

In 'Tubtim-Siam' pummelo fruit for example, the red pulp color influences consumer acceptance and this may be related to the presence of a specific pigment in the pulp. Carotenoids are essential to human health; carotenoids with a β-ring end group are precursors of vitamin A and act as antioxidants which reduce a risk of chronic diseases (Rao and Rao, 2007). However, red color development is the main factor affecting quality and market acceptance for the 'Tubtim-Siam' pummelo fruit. Several reports have been documented on pre-harvest factors which affect production and quality of fruit trees

(Duran-Zuazo et al., 2004; Dayal et al., 2016 and Haldankar et al., 2015). Among these factors, tree age plays an important role in fruit quality (Hearn, 1993, khalid et. Al, 2012 and Nanakhon and Chalumpak, 2016). Being a geographical indicator fruit in Thailand, this study attempted to find out (1) the influence of tree age on carotenoid accumulation during development, and (2) the carotenoid profile and related biosynthesis gene expression in 'Tubtim-Siam' pummelo fruit.

Light is one of the most crucial environmental factors in carotenoid accumulation in plant tissues (Pizarro and Stange, 2009). Light has been shown to induce carotenoid accumulation and carotenoid biosynthetic genes in tomato fruit (*Solanum lycopersicum*) (Schofield and Paliyath, 2005, Azari et al., 2010). In contrast, darkness induced carotenoid accumulation in carrot roots (*Daucus carota subsp. sativus*) (Fuentes et al., 2012, Rodriguez-Concepcion and Stange, 2013). The accumulation of carotenoids in darkness correlated with upregulated activity of PSY, the first committed enzyme of carotenogenesis) and with the induction of PSY gene expression in cotyledons in Arabidopsis seedlings (*Arabidopsis thaliana*) (Villalón et al., 2009). These reports suggest that the effects of environmental conditions on carotenoid synthesis may vary among plant species. This study investigated the effects of bagging-induced reductions in light conditions on lycopene production and related gene expression, antioxidant capacity, and sugar composition in pummelo.

However, Degradation of green peel color, red-ruby pulp color and biochemical compound are postharvest problem of 'Tubtim-Siam' pummelo may cause by ethylene. Non-climacteric fruits are also reported to respond to the exogenous and endogenous of ethylene. But, plant bioregulators such as 1-methylcyclopropene (1-MCP) and Aminoethoxyvinylglycine (AVG) are commonly used in postharvest pre-storage treatments for mandarins (Asrey, 2012) and 'Kinnow' mandarin (Tavallali and Moghadam, 2015). However, using 1-MCP as fumigation technique may not be practical on a commercial scale because of high investment costs for airtight systems, take for a long time to fumigate. Therefore So, 1-MCP-MBs application were used for delay ethylene production cause by maintain the postharvest quality change in 'Tubtim-siam' pummelo., the aim of this study was to evaluate the effectiveness of AVG and 1MCP-MBb on maintaining postharvest quality of 'Tubtim-siam' pummelo through dipping in AVG and 1-MCP-MBs.

1.2 Objectives

1) To investigate the influence of tree age on carotenoid accumulation during development in 'Tubtim-siam' pummelo fruit.
2) To investigate the carotenoid profile and related biosynthesis gene expression in 'Tubtim-siam' pummelo fruit.
3) To investigate the effects of bagging-induced reductions in light conditions on lycopene production and related gene expression, antioxidant capacity, and sugar composition in pummelo.
4) To investigate the effectiveness of AVG and 1MCP-MBb on maintaining postharvest quality of 'Tubtim-siam' pummelo through dipping in AVG and 1-MCP-MBs.

1.3 Scopes

1) The carotenoid profile in pulp of 'Tubtim-siam' pummelo fruit at commercial harvesting stage (7 MAFS)
2) The expression of carotenoid biosynthesis genes profile in pulp of 8-year old tree fruit
3) Study of 8 and 12year old tree fruit on carotenoid biosynthesis related genes including *CsPSY*, *CsZDS*, *CsβLCY*, *CsβCHX*, *CsƐCHX* and *CsZEP* in 'Tubtim-siam' pummelo
4) Bagging conditions on lycopene production and related gene expression, antioxidant capacity, and sugar composition in pummelo.
5) Study of postharvest quality of 'Tubtim-siam' pummelo through dipping in AVG and 1-MCP-MBs at 500 and 5 ppm.

CHAPTER 2

LITERATURE REVIEW

2.1 'Tubtim-siam' pummelo

2.1.1 Fruit production

Pummelo is grown in many Asian countries including China, Japan, India, Fiji, Malaysia, Vietnam and Thailand. It is also grown in the Caribbean and the United States (California and Florida). In Thailand, pummelo is a commercial fruit produced for both local consumption and the export market. Pummelo is a less perishable fruit and therefore can be kept for a long time for long distant transport. Meanwhile, growing trends of international market have also increased demand, especially in China, Hong Kong, and Cambodia. In 2010 Thailand was a major producer of a variety of high-quality pummelo to serve consumer demand for both domestic and export markets. However, pummelo export from Thailand constituted only 4% of the total production (Office of Agricultural Economic, 2015), although Athipanyakul and Chancharat (2014) reported that Thailand is a major global producer and exporter of pummelo to China, Hong Kong, Canada, Singapore, Vietnam, Myanmar, Cambodia and the Netherlands. During 2011-2013, volume and value of pummelo exported to China was approximately 6.5 million tons, valued at 98.5 million baht. Exports to Hong Kong amounted to approximately 4.6 million tons, with a value of 36.2 million baht. The Office of Agricultural Economics (2015) concluded that pummelo cultivation area had increased from 28,898.2 hectares to 32,499.6 hectares during the period 2003-2009, alongside production increases of 7,313 tons to 11,218 tons during 2004-2008. These increases are directly related to increasing international market trends.

'Tubtim-siam' pummelo is an economically important tropical fruit in Thailand and is ranked as the sixth minor economic fruit crop in Thailand (FAO, 2012). Recently, the demand for this fruit has gradually increased in both domestic and international markets, especially in China, Taiwan, Malaysia, Singapore and Brunei (Na Nakorn and Chalumpak, 2016). It has become more popular in fruit market because of the distinct characteristics such as red ruby pulp, fibreless, delicious taste, juicy flesh, and sweet aroma (Kaewtubtim and Issarakraisila, 2011) Additionally, the red ruby colored pulp is

due to various dietary antioxidants and Phyto-compounds such as, ascorbic acid, α-tocopherol, phenolic, flavonoids and carotenoid (Kaewsuksaeng, 2015 and Promkaew et al., 2019).

2.1.2 Fruit morphology and Characteristics

'Tubtim-siam' pummelo fruits are the biggest of the fruit in premium grade, with one piece weighing as much as 2 kg (for a Jumbo size), large to very large (15–20 cm in diameter or even larger), They are pear-shaped and the stomata on the skin are noticeable. The rind is thick to very thick (3 to 4 cm) cover with soft hair, green- to yellow-colored surface when mature. The succulent interior pulp of each fruit is divided into only 9 to 12 segments. The internal appearances of fruit thin light pink peel with tight row of small dark pink to red pummelo pulp, juicy with a sour - sweet taste. The soft pulp clings together and is finely detailed. Flesh is firm and crisp, juice vesicles are separable, and core is open and hollow. Fruit is seeded to seedless and flavor is mild to strong (Kaewtubtim and Issarakraisila, 2011; Milind and Ladaniya, 2010)

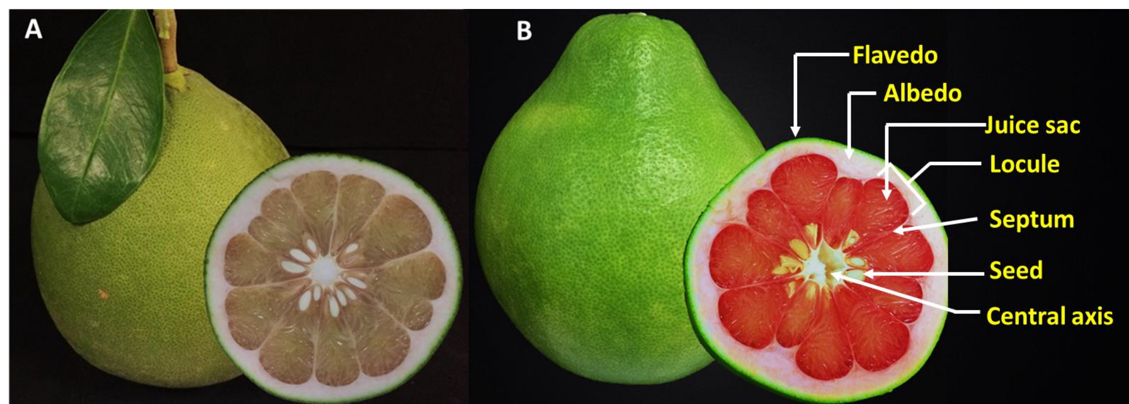


Fig. 2.1 The morphology of 'Tubtim-siam' pummelo fruit at immature green stage (3 months after fruit set (3 MAFS)) (A) and at commercial harvesting time (7 MAFS) (B).

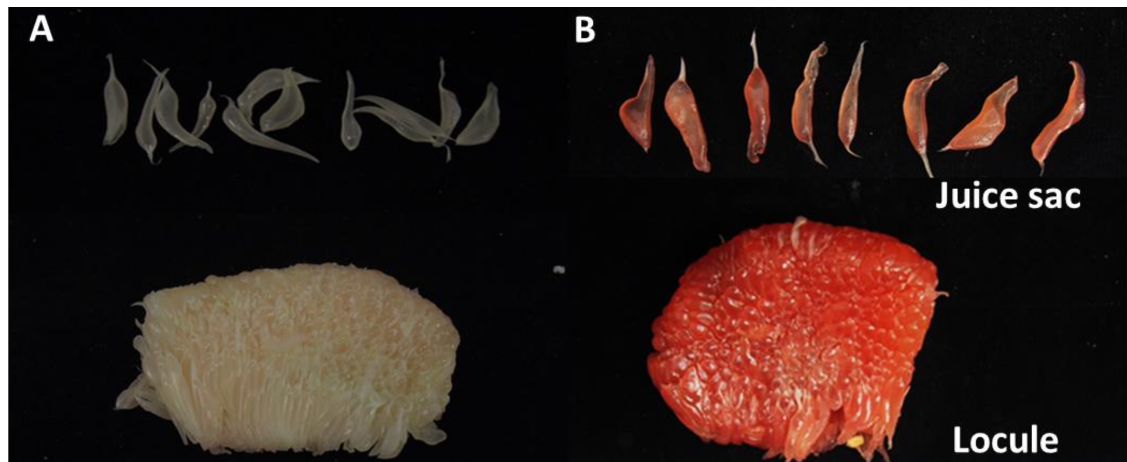


Fig. 2.2 The morphology of the segment containing "juice sac" of 'Tubtim-siam' pummelo fruit pulp at immature green stage (3 months after fruit set (3 MAFS)) (A) and at commercial harvesting time (7 MAFS) (B).

2.2 Carotenoid accumulation in plants

2.2.1 General carotenoid accumulation in plants

Carotenoids were first described as isoprenoid compounds at the beginning of the 20th century (Gross, 1987). Their basic structure is a C_{40} backbone skeleton, formed by tail to tail linkage of two geranylgeranyl diphosphate molecules. This skeleton can be modified by hydrogenation, dehydrogenation, cyclization, shortening or extension of the chain, isomerization, or by the addition of oxygen or other functional groups. Hydro carbonated carotenoids are known as carotenes, while carotenoids that contain one or more oxygen atoms are referred to as xanthophylls. The most common oxygenated groups are hydroxy- and epoxy-, but aldehyde, carboxy, carbomethoxy and methoxy groups have been also described in carotenoids (Gross, 1987). The structure of a linear, a bicyclic and an oxygenated carotenoid, corresponding to lycopene, β-carotene and violaxantin, respectively, are shown in Fig. 2.3.

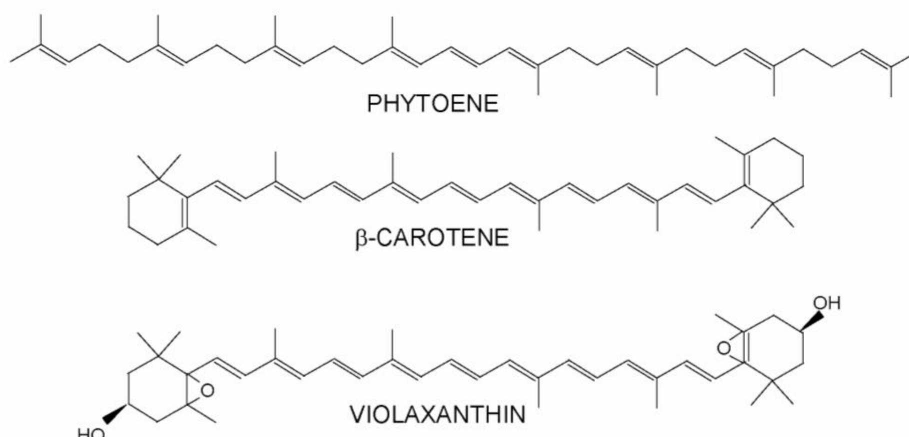


Fig. 2.3 Chemical structure of lineal (phytoene), bicyclic (β-carotene) and epoxy carotenoids (violaxanthin).

2.2.2 Carotenoid biosynthesis in plants

The pathway of carotenoid biosynthesis in plants is illustrated in Figure1 (Cunningham and Gantt, 1998; Ronen et al., 1999). The first committed step in carotenoid biosynthesis is the head-to-head condensation of two molecules of geranylgeranyl pyrophosphate (C20; GGPP) to form colorless phytoene (C40) catalyzed by phytoene synthase (PSY). Phytoene desaturase (PDS) and ζ-carotene desaturase (ZDS) introduce four double bonds into phytoene to yield lycopene via phytofluene, ζ-carotene, and neurosporene. Cyclization of lycopene is a crucial branching point in this pathway, yielding α -carotene with one ε-ring and one β-ring, and β-carotene with two β-rings, in which two cyclases, namely, lycopene β-cyclase (LCYb) and lycopene ε-cyclase (LCYe), are responsible for these reactions (Cunningham et al., 1996). α-Carotene is converted to lutein by sequential hydroxylation, which is catalyzed by ε-ring hydroxylase and β-ring hydroxylase (HYb), respectively. β-Carotene is converted to zeaxanthin via β-cryptoxanthin by two-step hydroxylation, which is catalyzed by HYb. Furthermore, zeaxanthin is converted to violaxanthin via antheraxanthin by zeaxanthin epoxidase (ZEP) (Fig. 2.4).

Recently, genes encoding enzymes for the main steps of carotenoid metabolism have been isolated and their expression has been characterized in plants (Alquézar et al., 2009; Kato et al., 2004, 2006; Kita et al., 2007). During fruit ripening, transcriptional regulation of

carotenoid genes appears to be the major mechanism by which the biosynthesis and accumulation of specific carotenoids are regulated.

Carotenoid biosynthesis and its regulation have been studied in various plant species, such as Arabidopsis (Park et al., 2002; Pogson et al., 1996) and tomato (Fraser et al., 1994; Giuliano et al., 1993; Isaacson et al., 2002; Ronen et al., 1999). Bramley (2002) reviewed carotenoid biosynthesis and regulation during ripening and development in tomato fruit. During tomato fruit ripening, the expression of PSY and PDS increased (Fraser et al., 1994; Giuliano et al., 1993; Isaacson et al., 2002; Ronen et al., 1999), whereas the expressions of both LCYb and LCYe disappeared (Pecker et al., 1996; Ronen et al., 1999), leading to marked accumulation of lycopene.

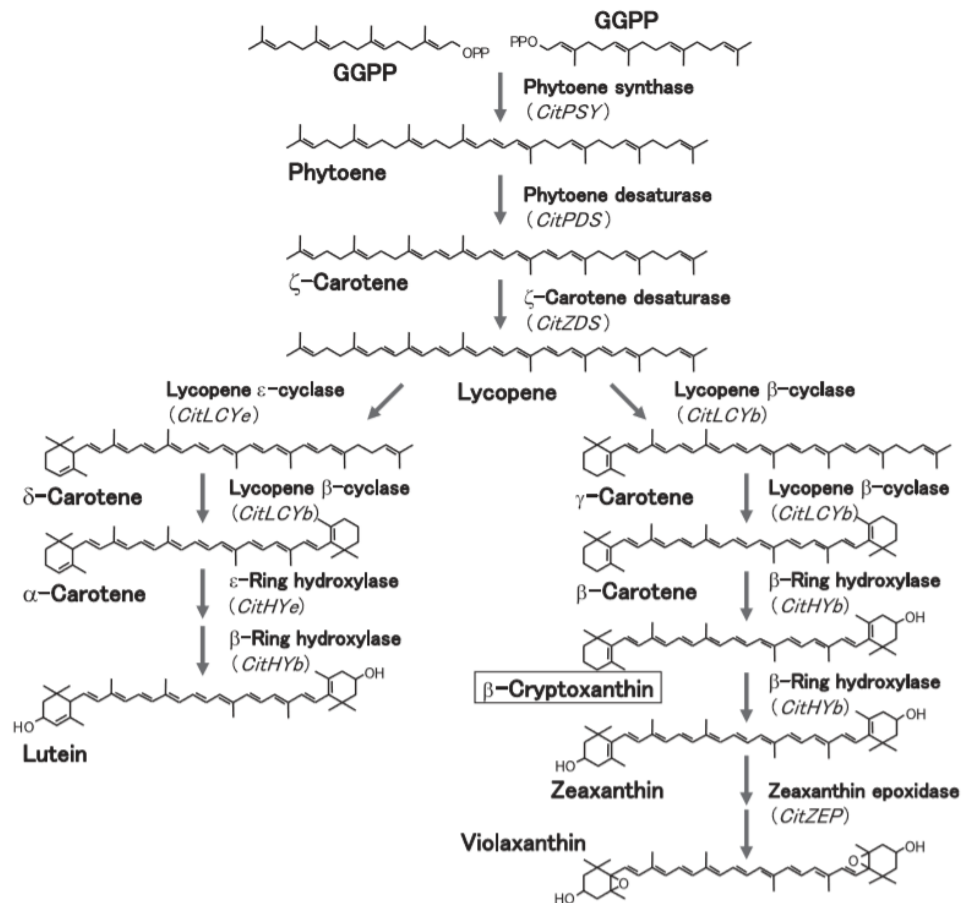


Fig. 2.4 Carotenoid biosynthetic pathway in citrus. GGPP, geranylgeranyl diphosphate; PSY, phytoene synthase; PDS, phytoene desaturase; ZDS, ζ-carotene desaturase; Ɛ-LCY, lycopene Ɛ-cyclase; β-LCY, lycopene β-cyclase; β-CHX, β-carotene hydroxylase; Ɛ-CHX, Ɛ-carotene hydroxylase; ZEP, zeaxanthin epoxidase.

2.2.3 Carotenoid in citrus fruit

Citrus fruits are, in general, a complex source of carotenoids in which up to 110 different carotenes and xanthophylls have been reported, although many of them may be isomers (Stewart and Wheaton, 1973). These pigments are responsible for the external and internal coloration of fruit of most citrus species. In the commercialization of citrus fruits for fresh consumption, the external color is probably the first perception of the consumer and a critical factor for their acceptance. In the juice, the color is also an important attribute of quality. An interesting genetic feature of mature citrus fruit is the diversity of peel color which varies from the typical bright yellow of lemon, grapefruit or pummelo, to the characteristic orange of mandarins and oranges, or the red color of some grapefruit or pummelos.

Results collected from the decades of 1970-80 mainly provided correlations between peel or pulp color and carotenoid content and composition (Gross et al., 1972; Stewart, 1977a, 1977b, 1980). From these results it become evident that color in citrus fruits is highly related to total content of carotenoids but more importantly to specific carotenoids, that although may be at low concentrations have an important contribution in peel or juice color. More recently, nutritional values of citrus fruit carotenoids have been also evaluated (Dhuique Mayer et al., 2005; Meléndez Martínez et al., 2005).

The diversity of citrus fruits carotenoids and the uncertain genetic origin of main cultivated species and varieties made unattainable the understanding of the phylogenetic background regulating carotenoid content and composition. Exhaustive comparative analysis of carotenoid accumulation in fruit of different species and mutants, and their potential association with changes in gene expression are now generating new insights into this important metabolic pathway in citrus. Based on the carotenoid composition in the peel and juice sacs of 39 citrus cultivars during fruit ripening. Matsumoto et al. (2007) have established five clusters; carotenoid poor, phytoene-abundant, violaxanthin-abundant, violaxanthin- and β-cryptoxanthin-abundant, and phytoene-, violaxanthin-, and β-cryptoxanthin-abundant, respectively. This classification was identical for juice sacs with the exception of phytoene-abundant cluster, which was not detected in that tissue. It is interesting the fact that for most varieties, the carotenoid profile was qualitatively similar in peel and pulp (Matsumoto et al., 2007). Other authors suggest that cis-violaxanthin and β-cryptoxanthin content were strong determinants for the classification

of 25 citrus genotypes (Fanciullino et al., 2006). Different C_{30} citrus-specific carotenoids, referred to as apocarotenoids (Fig. 2.5), were also present in the peel and pulp of the fruit. One of the most abundant apocarotenoids β-citraurin, which is only present in the fruit peel and provides the intense orange reddish coloration to some oranges, mandarins or hybrids (Stewart and Wheaton, 1972; Stewart and Wheaton, 1973b). In addition, other apocarotenoids, with longer (β-apo-2´carotenal) or shorter (β-apo-14´-carotenal) chain have been described. The biosynthetic origin of these aporacotenoids is still uncertain (Oberholster et al., 2001).

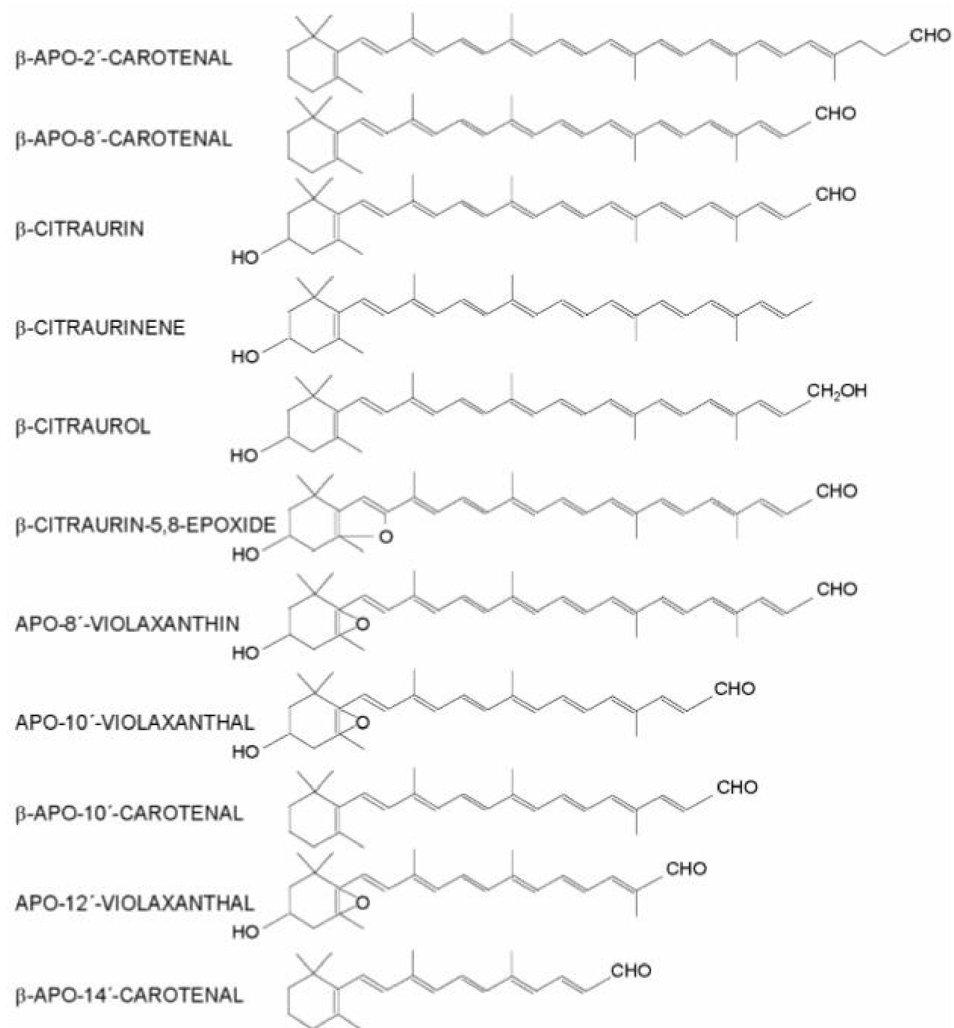


Fig. 2.5 Chemical structure of specific citrus apocarotenoids and apocarotenals.

2.2.4 Accumulation of lycopene in citrus fruit

Accumulation of lycopene in citrus fruit is of particular interest because it is an unusual feature restricted to only a few species such as grapefruit (*Citrus paradisi*), pummelo (*Citrus grandis*) and the flesh of some orange (*Citrus sinensis*) mutants (Liu et al, 2007, Alquézar et al., 2008, 2013). Recent studies indicate that differential mechanisms may be operating in lycopene-accumulating mutants (Alquézar et al., 2009, Pan et al., 2009, Xu et al, 2010, Mendes et al., 2011, Costa et al., 2012). In the red 'Hong Anliu' orange, a higher expression of PSY and ZDS genes and lower of βLCYs compared to the parental variety appears to be a key factor triggering lycopene accumulation (Xu et al., 2010). Similarly, in the red grapefruits 'Star Ruby'(SR) and 'Flame', the presence of lycopene seems to be related to a lower expression level of the fruit-specific βLCY2 gene (Alquézar et al., 2009, 2013, Mendes et al., 2011) while in Cara Cara orange it has been associated with an enhanced expression of the genes of the methyl-D-erythritol-4-phosphate (MEP) pathway (Alquézar et al., 2008), the main route supplying precursors for carotenoid biosynthesis. The flux into the MEP pathway is controlled by two key enzymes: the 1-deoxy-D-xylulose-5-phosphate synthase (DXS), located upstream, and the hydroxy methyl butenyl diphosphate reductase (HDR), downstream in the pathway, constituting two regulatory steps that directly influence carotenoid accumulation (Botella-Pavía et al., 2004, Peng et al., 2013). At the end of the MEP pathway, the formation of the key precursor geranyl geranyl pyrophosphate (GGPP), which constitutes a key branch point in isoprenoid biosynthesis in plants, is catalyzed by the enzymes GGPP synthases (GGPPS), a complex family of different isoforms that vary in expression, localization and activity (Beck et al., 2013). Most GGPP synthases have been shown to be localized in plastids and are directly related to carotenoid accumulation in vitro (Thabetet al., 2012). Their gene transcript levels and enzymatic activity were strongly induced during color change in ripening pepper fruits (Kuntz et al., 1992).

2.2.5 Carotenoid in pummelo

Pummelo or shaddock (*Citrus maxima* (Burm.) Merr.) is considered one of the three true species of the genus citrus. The fruit surpasses all other citrus fruit in size and, depending on the varieties, shows distinct morphological characteristics: as seedy or seedless, yellow,

red and colorless (white). Carotenoid content and composition in these fruits had received little attention. Table 2.1 summarizes results from studies conducted in ripe pummelo fruit of white and red-fleshed varieties. Flavedo and pulp of most white pummelo fruits accumulated almost exclusively linear (phytoene, phytofluene and lycopene) and bicyclic (β-carotene) carotenes, explaining the low coloration of these tissues. Carotenoid content in the flavedo ranged between 5 and 15 μg/g FW, while in the pulp it is almost zero. Two main features distinguish red pummelo fruit: the presence of lycopene, even at low concentrations but conferring the characteristic color, and that the carotenoid content is similar in both peel and pulp tissues (Table 2.1). Contrary to the pattern of carotenoid accumulation during maturation of colored Citrus fruits, in pummelo the maximum carotenoid content was detected in green fruits and decreased thereafter. In the pulp, carotenoid content is very low and constant throughout the ripening process (Gross and Timberg, 1983; Matsumoto et al., 2007).

Table. 2.1 Carotenoid content and composition in the peel and pulp of mature fruits of selected pummelo (*Citrus maxima* (Burm.) Merr.) cultivars.

Cultivar	μg/g FW	Carotenoids	Reference
Chandler			
Peel	19.3	32% lutein 22% violaxanthin	Gross 1987
Pulp	13.2	90% lycopene 5% β-carotene	Gross 1987
Chuzhou Early Red			
Peel	4.8	31% phytoene 25% phytofluene	Xu *et al*. 2006a
Pulp	5.3	51% lycopene 20% β-carotene	Xu *et al*. 2006a
Goliath			
Peel	5.0	67% phytofluene	Gross and Timberg 1983
Pulp	0.4	23% lutein 19% phytofluene 12% β-carotene	Gross and Timberg 1983
Yuhuan			
Pulp	traces	35% phytoene 35% lutein	Xu *et al*. 2006a

2.3 Ethylene

Ethylene (C_2H_4) is a simple, naturally occurring organic molecule that is a colorless gas at biological temperatures. Moreover, is the only plant hormone in gaseous form and is active at very low concentrations, in the range of ppm and ppb, readily diffusing from sites of production. C_2H_4 is synthesized by plants in small amounts and appears to coordinate growth and development (Saltveit et al., 2006). Depending on the desired effect, C_2H_4 has both beneficial and unfavorable effects on harvested fruits, vegetables, and ornamentals. For instance, C_2H_4 is used to promote ripening of bananas and tomatoes; citrus degreening; and synthesis of pigments in apples. Yet the same changes are unwanted when C_2H_4 promotes fruit over-ripening, yellowing of green vegetables, development of brown spot lesions in lettuce, and senescence of flowers (Saltveit et al., 1999).

2.3.1 The regulation of ethylene biosynthesis during fruit ripening

Fruits have classically been categorized based upon their abilities to undergo a program of enhanced ethylene production and an associated increase in respiration rate at the onset of ripening. Fruits that undergo this transition are referred to as climacteric and include tomato, apple, peach, and banana, whereas fruits that do not produce elevated levels of ethylene are known as no climacteric and include citrus, grape, and strawberry. However, these distinctions are not absolute, as closely related melon and capsicum species can be both climacteric and non-climacteric and some so-called non-climacteric fruits display enhanced ripening phenotypes in response to exogenous ethylene. Nevertheless, increased ethylene synthesis at the onset of ripening is required for the normal ripening of many fruits.

Two systems of ethylene production have been defined in plants. System 1 functions during normal growth and development and during stress responses, whereas system 2 operates during floral senescence and fruit ripening. System 1 is autoinhibitory, such that exogenous ethylene inhibits synthesis, and inhibitors of ethylene action can stimulate ethylene production (Fig. 6). In contrast, system 2 is stimulated by ethylene and is therefore autocatalytic, and inhibitors of ethylene action inhibit ethylene production (McMurchie et al., 1972).

The biochemical features of the ethylene biosynthesis pathway in higher plants are well defined and have been reviewed previously (Bleecker and Kende, 2000). Briefly, ethylene is synthesized from methionine in three steps: (1) conversion of methionine to S-adenosyl-L-methionine (SAM) catalyzed by the enzyme SAM synthetase, (2) formation of 1-aminocyclopropane-1-carboxylic acid (ACC) from SAM via ACC synthase (ACS) activity, and (3) the conversion of ACC to ethylene, which is catalyzed by ACC oxidase (ACO). The formation of ACC also leads to the production of 5¢-methylthioadenosine (MTA), which is recycled via the methionine cycle to yield a new molecule of methionine. Increased respiration provides the ATP required for the methionine cycle and can lead to high rates of ethylene production without high levels of intracellular methionine. SAM is an important methyl donor and is involved in multiple aspects of cellular metabolism. Consequently, the two committed steps in the synthesis of ethylene are the formation of ACC and its conversion to ethylene. The genes encoding ACS and ACO have thus been studied in more detail than other enzymes in the pathway, although there is evidence that several other genes involved in methionine synthesis and the methionine salvage pathway are differentially expressed during ripening and in response to ethylene (Alba et al., 2005; Zegzouti and et al., 1999).

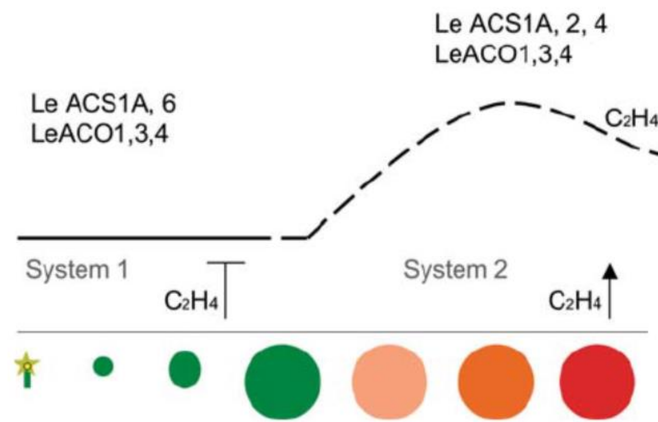


Fig. 2.6 Differential expression of ACS and ACO genes associated with system 1 and system 2 ethylene synthesis during fruit development and ripening in tomato. Au-to inhibition of ethylene synthesis during system 1 ethylene production is mediated by a reduction in LeACS1A and 6 expression. Autocatalytic ethylene synthesis at the onset of fruit ripening is mediated through ethylene-stimulated expression of LeACS2 and 4 and LeACO1 and 4.

2.4 Postharvest change in citrus fruit and the role of ethylene

2.4.1 Effect of ethylene on citrus fruit

Citrus is generally characterized as less perishable fruit in comparison to loquat, lychee, fresh fig and mango (Kader and Arpaia, 2002). Fruits are usually morphologically classified into different groups, such as silique (e.g. Arabidopsis), pome (e.g. apple), berry (e.g. tomato), and hesperidium (e.g. sweet orange). Citrus can be classified into two classes in commercial post-harvest practices: tight-skin (hard-peel) citrus (such as sweet orange and pummelo) and loose-skin (easy-peel) citrus (such as satsuma mandarin and ponkan mandarin). The two classes have different degrees of tightness in flesh-rind anatomic structure and different storage characteristics, as tight-skin citrus fruit has a longer storage life than loose-skin ones.

Fruit ripening and senescence are inevitable and irreversible processes in plant lifecycle and the underlying mechanisms are unique among different fruit types. According to the amount of ethylene biosynthesis and its signal transduction, fruits are physiologically classified into climacteric fruit (e.g. tomato, apple and banana) and non-climacteric fruit (e.g. citrus, strawberry and grape). After harvest, citrus fruit remains animate and active.

In climacteric fruit, ethylene plays a key role in governing physiological and biochemical changes that occur during ripening, including color break, softening, and accumulation of sugars, acids, aroma volatiles, vitamins, etc. (Lelievre et al., 1997; Barry and Giovanoni, 2007). In contrast, citrus fruit are non-climacteric, i.e., their natural ripening is not accompanied by rises in respiration and ethylene production rates (Eaks, 1970).

Investigations on in planta levels of CO_2 and ethylene of fruits during storage supported the role and involvement of changes in the rate of respiration and ethylene production by presence of a characteristic rise in CO_2 levels and a burst in ethylene production in some non-climacteric fruits (Vijay Paul et al., 2012) such as strawberry (Trainotti et al., 2005; Cancel and Larsen, 2002; Iannetta et al., 2006), grapes (Chervin et al., 2004) and citrus (Stewart and Wheaton 1972; Purvis and Barmore 1981; Goldschmidt et al., 1993; Goldschmidt 1997; Katz et al., 2004). However, exposure to exogenous ethylene has been shown to stimulate various ripening related processes, such as destruction of the green chlorophyll pigments and accumulation of orange/yellow carotenoids, in citrus peel tissue (Stewart and Wheaton, 1972; Barmore, 1975; Purvis and Barmore, 1981; Rodrigo

and Zacarias, 2007) Nevertheless, despite widespread knowledge of the effect of ethylene on peel color development, it is not yet known whether exogenous ethylene regulates other biochemical changes associated with internal ripening of citrus fruit, as it does in climacteric fruit (Goldschmidt, 1998). The common dogma is that, in contrast to its effects on peel color change, ethylene has only relatively minor effects on ripening processes in citrus flesh, but this has never yet been examined systematically. On the contrary, several lines of evidence suggest that ethylene may regulate various processes related to internal ripening. First, it is well known that exposure to ethylene accelerates respiration and ethylene-production rates of citrus fruit, and these rates are indicators of activation of biochemical changes, such as breakdown of sugars and acids that serve as respiratory substrates (Aharoni, 1968; Vines et al., 1968; Eaks, 1970). Second, previous studies have shown that ethylene degreening affects various metabolic pathways in citrus flesh. For example, ethylene degreening decreased acidity levels in 'Mosambi' oranges (Ladaniya and Singh, 2001), increased production of aroma volatiles in green lemons (Norman and Craft, 1968) and slightly affected accumulation and composition of carotenoid pigments in the flesh of 'satsuma' mandarins (Matsumoto et al., 2009). Third, it has been reported that presence of ethylene in storage rooms results in loss of desired flavor, and enhanced accumulation of off-flavors in oranges, whereas removal of ethylene from storage rooms improves overall fruit quality (McGlasson and Eaks, 1972; Testoni et al., 1992).

2.4.2 Effect of ethylene on physicochemical changes

2.4.2.1 Water loss

As a plant organ, the peel or skin of fruits and vegetables plays an important role in gas exchange between the product and the surrounding environment. The skin allows the fruit to maintain a high-water content despite a low relative humidity in the air surrounding the produce. This protection against dehydration is particularly important after harvest. Fruit water loss occurs through the stomata, lenticels, cuticle and epicuticular wax platelets, as well as through the calyx, pedicel or floral ends. Fruit water loss accounts as a major source of loss in dragon fruit during storage (Kammapana, 2014). Temperature and humidity are the environmental factor that have the strongest influence on fruit

quality. The effect of humidity on fruit quality varies among crops. In addition, the rate of water loss differs among species (Blasiak, and Musgrave, 2002) Some of the fruit factors that affect transpiration in fruit are its surface area per volume or surface area per mass ratio (Díaz-Pérez et al., 20017), the surface structure of the fruit, including the number and size of stomata and lenticels, and the thickness and composition of the cuticle (Lownds et al., 1993). Studies regarding ethylene involvement in weight-loss related metabolisms, are usually compared against treatments inhibiting ethylene biosynthesis or action as low oxygen-controlled atmospheres, 1-MCP treatments or silver application to flowers (Yahia, 1991 and Baritelle, 2001)

2.4.2.2 Peel color changes

In leafy vegetables and green fruits, one of the main factors affecting quality reduction is the loss of green color. This loss of green color and a consequent yellowing of the leaf, peel is associated with the synthesis of pigments such as carotenoids and the breakdown of chlorophyll by chlorophyll degrading enzymes. Treatment with C_2H_4 accelerates chlorophyll degradation and the appearance of yellow or orange colors, similar to what occurs in bananas where C_2H_4 stimulates chlorophyll loss and the appearance of yellow color (Saltveit, 1999) and in broccoli (Tian, 1994). Pigment changes in peel of the most citrus cultivars consist of breakdown of chlorophyll and buildup of carotenoids, both of which are enhanced by ethylene and can delayed by plant bioregulators (Barmor, 1975; Shimokawa et al., 1978; Hirschfeld and Goldschmidt, 1983). Additionally, exposure to exogenous ethylene has been shown to stimulate various ripening related processes, such as destruction of the green chlorophyll pigments and accumulation of orange/yellow carotenoids, in citrus fruit (Stewart and Wheaton, 1972; Barmore, 1975; Purvis and Barmore, 1981; Rodrigo and Zacarias, 2007).

2.4.2.3 Pulp color changes

Pigment changes in pulp of citrus cultivars is related to oxidative degradation of carotenoids has led to cis-trans isomerization and formation of carotenoid epoxides (Mordi et al., 1993; Wacheä et al., 2003). Carotenoids act as antioxidants against lipid

peroxidation by quenching singlet oxygen and trapping free peroxyl radicals (Palozza and Krinsky, 1991). Investigations have shown that singlet oxygen quenching ability of the carotenoids depends on their structural differences, such as number of conjugated double bonds, end groups (acyclic or cyclic), and substituent functional groups in the rings (Stahl and Sies, 1996; Hirayama et al., 1994). As lycopene with a lesser extent of beta-carotene are the major pigments in red grapefruit cultivars (Curl and Bailey, 1957; Khan and MacKinney, 1953; Rouseff et al., 1992) Di Mascio et al. (1989) reported that the singlet oxygen quenching capacity of the carotenes was as follows: lycopene > alfa-carotene > beta-carotene. Esterification of carotenoids with fatty acids occur during fruit ripening and post-harvesting of the fruit may induce ripening process by endogenous ethylene, which play affect the color intensity (Minguez and Mendez, 1994). The physiological and chemical changes associated with fruit ripening can be halted or delayed by inhibiting ethylene perception, even when the fruit has reached advanced stages of ripening (Hoeberichts et al., 2002).

2.4.2.4 Sugar content

Carbohydrates are the most abundant food component from plants and the content in fruit varies in a general range between 10 and 25%. The carbohydrate content is directly related to texture, taste and food value of fresh fruit. The respiratory metabolism uses carbohydrates as fuel for other plant processes including ripening and senescence. Ripening is stimulated by C_2H_4 in climacteric and some non-climacteric fruits. Although depending on the marketing chain stage of the commodity this can be a negative effect. Consumption and transformation of carbohydrates are C_2H_4 dependent.

2.4.2.5 Total ascorbic acid content

Vitamin C is one of the most important nutritional quality factors in many horticultural crops. The content of vitamin C in fruits and vegetables can be influenced by various factors such as genotypic differences, pre harvest climatic conditions and cultural practices, maturity and harvesting methods and postharvest handling procedures. Conditions favorable to water loss after harvest result in a rapid loss of vitamin C, in

regard of C_2H_4 effect, vitamin C loss are associated with a shorter life of the fresh produce instead of a direct effect on its concentration. Furthermore, some chilling sensitive crops showed more losses in vitamin C at lower temperatures. Handling temperature after harvest is the most important factor to maintain vitamin C in fruits and vegetables; losses are accelerated at higher temperatures and with longer storage periods.

2.5 Controlling Ethylene Action

Three ways to control the action of C_2H_4 in plants. The first is to prevent the plant from being exposed to biologically active levels of C_2H_4, by controlling the produce surrounding and even inhibiting C_2H_4 synthesis. The second is to prevent the plant tissue from perceiving the C_2H_4 that is in its surrounding atmosphere using inhibitors of C_2H_4 perception: CO_2, silver (for example, silver thiosulfate), aminoethoxyvinyl glycine (AVG) and 1-methylcyclopropene (1-MCP) are commonly used in postharvest pre-storage treatments for mandarins (Asrey, 2012) and ‘Kinnow' mandarin (Tavallali and Moghadam, 2015). The third is to prevent the plant from responding to the perceived C_2H_4, which can be achieved by controlled atmospheres and low temperatures among the most common technologies used.

2.6 Aminoethoxyvinylglycine (AVG)

AVG (a water-soluble powder) is commercially sold under the name of ReTain®. It is a human and environmentally friendly organic product registered use for apples, pears, peaches, plums, mandarins and nectarines in several countries (Greene and Schupp, 2004; Rath and Prentice, 2004). AVG is frequently used as a specific inhibitor of ethylene biosynthesis to determine the effects of ethylene on plant growth, development, and response to stress (Abeles et al., 1992). AVG has been used to study the participation of ethylene synthesis in fruit ripening (Clayton et al., 2000; Wang and Dilley, 2001) and response to chilling stress (Hong and Gross, 2000). Effective tissue concentrations of AVG are hard to determine from published studies since the methods of application include foliar sprays (124 g ha^{-1}), soil drenches (5–20 μM), and sprays or dips of excised tissue (1 g L^{-1})

2.6.1 AVG effect on fruit

AVG inhibits the synthesis of ethylene at the level of the aminocyclopropane carboxylic acid synthase enzyme (ACS), responsible for the conversion of S-adenosylmetionine to 1-aminocyclopropane-1-carboxylic acid (ACC), the latter an immediate precursor of ethylene (Adams and Yang, 1979). ACC synthase is considered a key enzyme in the biosynthesis of ethylene (Kende, 1993). Autio and Bramgage (1982) observed that AVG treatments delayed ripening and harvest, increased fruit firmness and prolonged storage life of fruit. Pre-harvest treatment of fruits with AVG decreases ethylene production, delays fruit maturity, and allows fruit to ripen more slowly (Bregoli, 2002; Torrigiani, 2004; Cline, 2006). However, the effect is timing or cultivar dependent (Byers, 1997; Belding and Lokaj, 2002). Furthermore, it is difficult to directly evaluate the shelf-life of AVG-treated fruit, because AVG affects fruit maturity, which in turn influences shelf-life. Postharvest application of AVG significantly suppresses ethylene production and reduces fruit ripening and therefore postharvest rotting (Byers, 1997; Garner, 2001).

2.7 1-methyl cyclopropene (1-MCP)

1-Methylcyclopropene (1-MCP) is a cyclopropene which is a naturally occurring substance whose molecular weight is 54.09g/mol. It is a volatile gas at normal temperature, when 1-MCP inhibited ethylene receptor that affects a lot to fruit quality and fruits responded very well. It is used in commercial as a preservative fruit quality. At the recent, the acceptance of 1-MCP which are using more than 34 countries including Australia, Canada, UK, Israel, the European Union and the United States, etc. (Watkins, 2016)

1-MCP for its ethylene antagonist role is widely used on different horticultural produce, able to extend postharvest storage live and prevent spoilage; it has been commercially used in dozens of countries worldwide and registered for application to at least 18 distinct climacteric crops (Li, 2016). However, in the recent years, the amount of studies supporting positive results by treating non-climacteric commodities like dragon fruit have gained importance; although its exact mode of action inhibiting ethylene-related processes is not yet well understood (Merchante et al., 2013 and Villarreal, 2010)

The ripening of fruits appears when ethylene binds with an ethylene receptor. It acts like a key which unlocks the receptor, schematic shown in Figure 2.2 and Figure 2.3 (Blankenship, 2001). Then, a chemicals signal is sent to the cell which created enzymes for metabolism and ripening of fruits (Deaquiz et al., 2014). 1-MCP bounds with receptor with an affinity 10 folds greater than ethylene blocking ethylene perception.

2.7.1 1-MCP effect on fruits

1-methylcyclopropene is widely used to retain quality and extend commercial life on climacteric fruits, however positive results have been obtained when treating some non-climacteric fruits as dragon fruit, inhibiting degreening and color changes, though its application has minor effects on internal fruit-quality parameters (Li, 2016). 1-MCP is usually delivered as a gas in sealed environments to prevent the1-MCP gas from being released and long time to use around 6 to 24 h depending on the commodity. In the case of bananas, the periods of fumigation are between 12 and 24 h at concentrations ranging from 5 to 1000 $nL \cdot L^{-1}$ and higher (Fernando, 2013). However, this fumigation technique may not be practical on a commercial scale because of high investment costs for airtight systems and other facilities. Recently, preparations of 1-MCP designed for use as aqueous solutions have been formulated, facilitating broader agricultural applications for this ethylene-action inhibitor. However, the results of ripening at attenuation have not always been beneficial because of the limitations of solubility of 1-MCP in water at standard pressure. The effect of 1-MCP on fruits could have negative effects such as: uneven ripening and induce chilling injury via ethylene inhibition (Biswas, 2014)

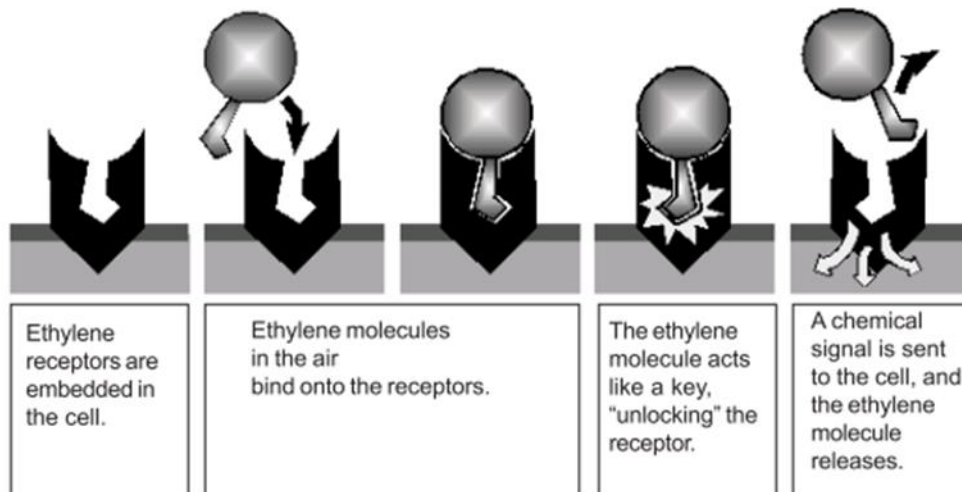


Fig. 2.7 Binding of ethylene molecule with the receptor "unlocks" the receptor and leads to a chemical reaction in the plant tissue; Blankenship, diagram by Jenny Bower, Dept. of Pomology, UC Davis (a).

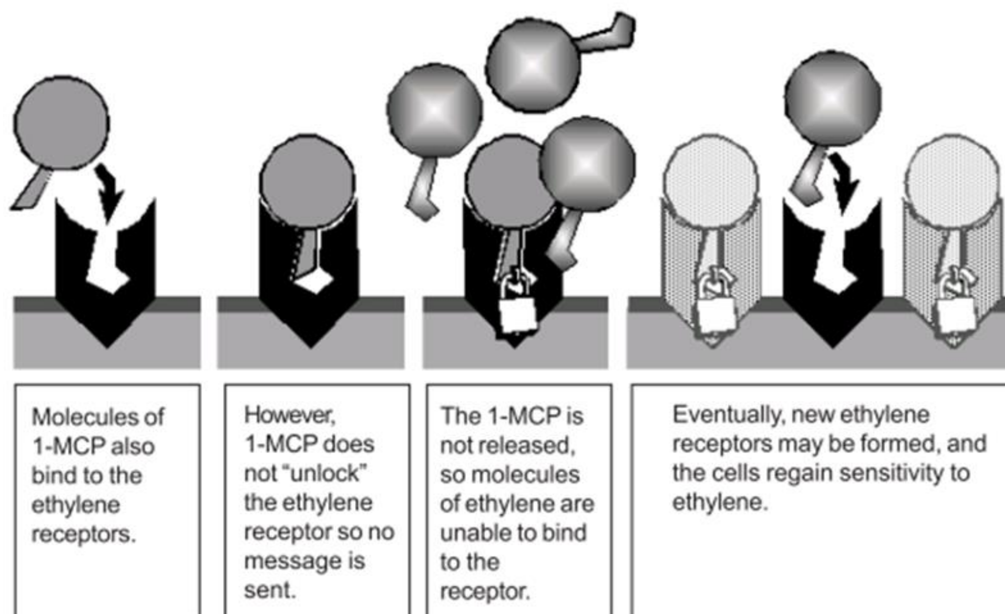


Fig. 2.8 When 1-methylcyclopropene (1-MCP) binds to the ethylene receptor, it does not "unlock" the receptor and remains locked to the receptor preventing the binding of ethylene and the chemical reaction does not occur; Blankenship, diagram by Jenny Bower, Dept. of Pomology, UC Davis (b).

2.7.2 1-MCP microbubbles

A process has been developed in which 1-MCP may be applied via immersion, but this is not used commercially, one (Manganaris, 2008) of the main reasons regards in the different legislation barriers needed in every country for each horticultural crop. Presenting a problem, the availability of the chemical limiting the technique (Watkins, 2016).

A novel technique for 1-MCP application has been proposed by Pongprasert and Srilaong (2015) via micro bubbles technology, using the commercially and widely permitted gaseous form of the chemical. One of the most significant characteristics of MBs is they shrink in water and ultimately collapse if they reside in water for a long time, easing the reach of ethylene receptors in the treated water-immersed commodity. In contrast, ordinary macro bubbles quickly rise and burst at the surface of water, failing to deliver 1-MCP to the ethylene receptor (Takahashi, 2007)

2.7.3 Micro and nano bubbles

Micro and nano bubbles are also termed as ultrafine or fine bubbles, having very small size with diameters from 10 to 50 µm and less than 200 nm, respectively (Takahashi, 2007). The ordinary bubbles have diameters greater than 50 µm. Gas composition is higher; thus, they go up and burst rapidly at the surface of aqueous solution. By the way it tends to be expanded by binding bubbles together. While micro bubble is smaller and eventually disappear due to the solubility of interior gases into the non-saturation solution through water-air interface (Matsumoto and Tanaka, 2008). And as a result, with high internal gas pressure of bubble is completely dissolved in aqueous solution. Especially fine bubbles have an extremely small size compared with others one. It is less than 50 µm, therefore it contains little gas that is one of reasons why it can be continuously existing in aqueous solution and keeps stable for a long period time. Fine bubbles have many undeniable advantages in various field such as agriculture, sewage treatment, postharvest technology and etc.

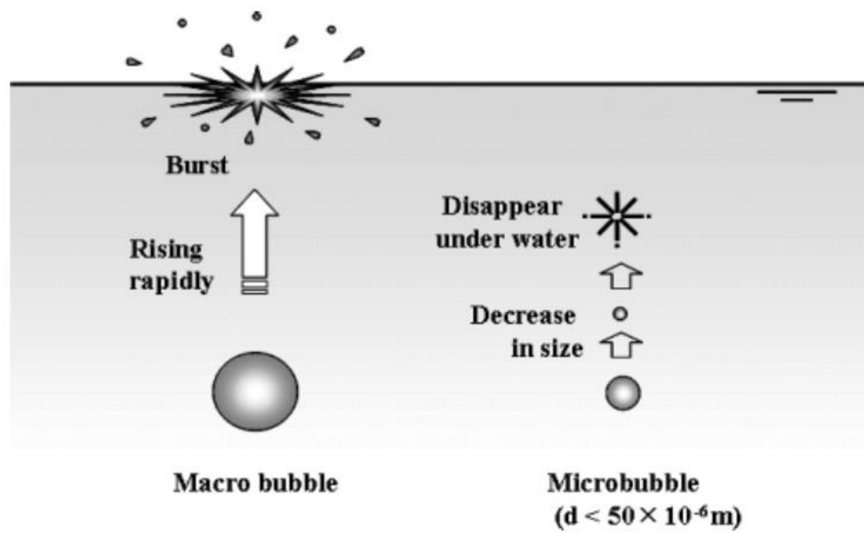


Fig.2.9 Microbubble behavior. The ordinary macro bubbles rise rapidly and burst at the surface of the water; (Takahashi et al., 2007)

CHAPTER 3

CAROTENOIDS ACCUMULATION AND CAROTENOIDS BIOSYNTHESIS GENE EXPRESSION DURING FRUIT DEVELOPMENT IN PULP OF 'TUBTIM-SIAM' PUMMELO FRUIT

3.1 Introduction

Plant pigments such as carotenoids play important components in fruit quality and their presence dictates peel and pulp color of tropical fruit. In 'Tubtim-Siam' pummelo fruit for example, the red pulp color influences consumer acceptance and this may be related to the presence of a specific pigment in the pulp. Carotenoids are essential to human health; carotenoids with a β-ring end group are precursors of vitamin A and act as antioxidants which reduce a risk of chronic diseases (Rao and Rao, 2007). Apart from health benefit, pummelo is an economically important tropical fruit in Thailand and is ranked as the sixth minor economic fruit crop (FAO, 2012). Recently, the demand for this fruit has gradually increased in both domestic and international markets, especially in China, Taiwan, Malaysia, Singapore and Brunei (Na Nakorn and Chalumpak, 2016). However, red color development is the main factor affecting quality and market acceptance for the 'Tubtim-Siam' pummelo fruit. Several reports have been documented on pre-harvest factors which affect production and quality of fruit trees (Duran-Zuazo et al., 2004; Dayal et al., 2016 and Haldankar et al., 2015). Among these factors, tree age plays an important role in fruit quality (Hearn, 1993, khalid et.al, 2012 and Nanakhon and Chalumpak, 2016). Being a geographical indicator fruit in Thailand, this study attempted to find out how tree age influences on red color accumulation in 'Tubtim-Siam' Pummelo fruit.

A plethora of information is available differentiating citrus species on the basis of carotenoid profile (Kato et al., 2004; Rodrigo et al., 2004; Fanciullino et al., 2006; Alquezar et al., 2009). Red coloration in particular, mostly exhibited in the pulp of some citrus is of our interest. Previous studies have reported that red coloration in oranges and pummelo was mostly due to anthocyanins such as Cyanidin-3-glucoside (Maccarone et al., 1983; Kaewsuksaeng and Sangwanangkul, 2015). To the contrary, several studies

have revealed that in star ruby grape fruit (Lado et al., 2015; Alquezar et al., 2013) and cara-cara orange mutants (Xu et al., 2006; Fanciullino et al., 2008 and Alquezar et al., 2008) red coloration is due to carotenoid pigment called lycopene rather than anthocyanins.

Recently, genes encoding for enzymes of the main steps of the carotenoid biosynthetic pathway have been identified and their expression studied in fruit tissues of different citrus species (Kato et al., 2004, Rodrigo et al., 2004 and Alos et al., 2006). The carotenoids synthesis process is initiated by the up-regulation of the phytoene synthase (PSY) and followed by an increment in of phytoene desaturase (PDS), ζ-carotene desaturase (ZDS), β-carotene hydroxylase (β-CHX) and the induction of the chromoplast-specific lycopene cyclase (βLCY) genes and lycopene ε-cyclase (ƐLCY) (Cunningham et al., 2002). α-Carotene is converted to lutein by sequential hydroxylation, which is catalyzed by ε-ring hydroxylase (ƐCHX) and β-ring hydroxylase (βCHX), respectively. β-Carotene is converted to zeaxanthin via β-cryptoxanthin by two-step hydroxylation, which is catalyzed by (βCHX). Furthermore, zeaxanthin is converted to violaxanthin via antheraxanthin by zeaxanthin epoxidase (ZEP) (Kato et al. 2004; Rodrigo et al. 2004 and Alquézar et al., 2013). To the best of our knowledge, no studies are available reporting about the carotenoid profile and gene expression in 'Tubtim siam' pummelo fruit. Therefore, our study sought to find out (1) the influence of tree age on carotenoid accumulation during development, and (2) the carotenoid profile and related biosynthesis gene expression in 'Tubtim-Siam' pummelo fruit.

3.2 Materials and Methods

3.2.1 Plant materials and experimental treatments

The experiment was carried out at a commercial orchard in Klongnoi sub-district, Pakpanang district, Nakhon Si Thammarat province, Thailand (8.35° N, 100.09° E). 'Tubtim-Siam' pummelo trees of 8 and 12-years old were selected from the same orchard, grafted on to local rootstock (sourced from a single mother plant). The fruit trees under investigation were subject to rigorous and appropriate cultural practices. Fruit were selected randomly from all directions and tagged after fruit setting. The fruit were

harvested at different stages of development in each month from June-October 2017 and 2018 (Peak harvesting time of 'Tubtim-Siam' pummelo); first harvest commenced 3 months after fruit setting (3 MAFS) and in mature green: 4 MAFS (July), breaker: 5 MAFS (August), after breaker: 6 MAFS (September) and full color or commercial harvesting time: 7 MAFS (October) stages. The experimental design was a completely randomized design (CRD) with three trees per treatment (8 and 12-year old fruit trees respectively), 5 fruit per tree were used at each harvesting time. Fruit pulp was separated and immediately frozen in liquid nitrogen, then stored at -80ºC until use. For gene expression was only analyzed in 8-year old tree fruit.

3.2.2 Pulp color analyses

Pulp surface color was measured with a colorimeter (Chromameter Model RC-400, Minolta Corp, Japan) and presented as a* value (negative a* value indicates greenness and positive for redness)

3.2.3 The lycopene, β-carotenoid and total carotenoid analyses

Carotenoid concentrations were measured according to Fish et al. (2002). 0.5 g of pulp was extracted with hexane, ethanol and acetone containing 0.05% butylated hydroxytoluene and determined at different absorbances at 445 nm for total carotenoid, 450 nm for beta carotene and 503 nm for lycopene, using a spectrophotometer (Shimadzu, UV-1800, Japan).

3.2.4 Total RNA Extraction

Total RNA was isolated from the pulp of fruit at each harvest date. Pulp of 100 mg was ground in liquid nitrogen, followed by thorough mixing with 1 ml trizol and transferred to a centrifuge tube. The mixture was centrifuged for 10 min at 12,000 ×g at 4°C. The upper aqueous phase was transferred into a new centrifuge tube and 240 µl of chloroform was added and thoroughly mixed on ice for 5 min, followed by centrifuging for 15 min at 12,000 ×g at 4°C. The upper aqueous phase was further transferred into another

centrifuge tube and 500 µl of isopropanol and 0.3 ml of a high-salt precipitation solution (2 M of sodium citrate and Sodium chloride) was added to each sample and the samples were left to precipitate for 10 min at room temperature. The mixture was then centrifuged for 10 min at 12,000 ×g and 4 °C. The upper aqueous phase was removed, and the remaining RNA pellet was dried. The RNA pellet was then washed using 1 ml of 75% ethanol and total RNA was precipitated by centrifuging for 5 min at 12,000 ×g at 4°C (This was performed twice). The RNA pellets were completely air dried for 5 min, thereafter, RNA was dissolved in 50 µl of RNase free water, and finally stored at -80°C.

3.3 Statistical analysis

The data were presented as mean values ± SE and the t-test at 5% level (SPSS Inc.; Chicago, IL, USA).

Table. 3.1 Primer used for real-time RT-PCR.

Gene	Forward/reverse primer (5′-3′)
CsPSY	GGTCGTCCATTTGATATGCTTG CCTAAGGTCCATCCTCATTCCT
CsZDS	CGATCCTTACATGCCCTTAC AGGTCCCTCACGGTACAAAG
CsβLCY	CCCATGTATGACCCATCAAAG TGGGAGATGGATCAATCGAG
CsβCHX	GGTGCTGGACTTGGCATTAC AGCGACTCTCCGGAAATAAG
CsƐCHX	CGGCACCAAGTATGCTAAAGG CAGCAGTTCCATTTAGAGGG
CsZEP	TTGGTTGATGGGATTTCTGG TCCCCAACCGCTTTAGCTAG
CsActin	ATCTGCTGGAAGGTGCTGAG CCAAGCAGCATGAAGATCAA
CsGAPDH	GTGTTTCTATGTAGAGGGTCTGAGTTT TCTAAGCACAACCCTGCATGAG
CsUBL5	CTCGAGCCGACAAGATTCG CCATGCCGTCGTGAATCTC
References	(Fanciullino et al., 2008 and Wu et al., 2014)

3.4 Results

3.4.1 Effect of tree age on concentrations of lycopene, β-carotene, total carotenoid content and a* value in fruit pulp

Carotenoid composition expressed as total lycopene, total β-carotene and total carotenoid concentrations is shown in Fig. 3.1A, B and C. All the carotenoid concentrations in the pulp slightly increased in the first two months of development; the lycopene and β-carotene concentrations in the pulp of 12-year old tree fruit showed a significantly higher value a month before breaker stage (Jul 4) compared with the 8-year old tree fruit however, at the same developmental time no significant difference was observed between fruit from two tree ages in relation to total carotenoid concentrations. After 4 months of development, all different carotenoid compositions in pulp of 8-year old tree fruit increased significantly during ripening and reached a maximum at 7 MAFS. Initially, before breaker stage (3 and 4 MAFS), the a* value in the pulp of 8-year old tree fruit showed a significantly lower a* value which was more negative. However, at breaker stage (5 MAFS) until commercial harvesting time (7 MAFS) a tremendous increase in the a* value was observed in 8-year old tree fruit compared to 12-year old tree fruit.

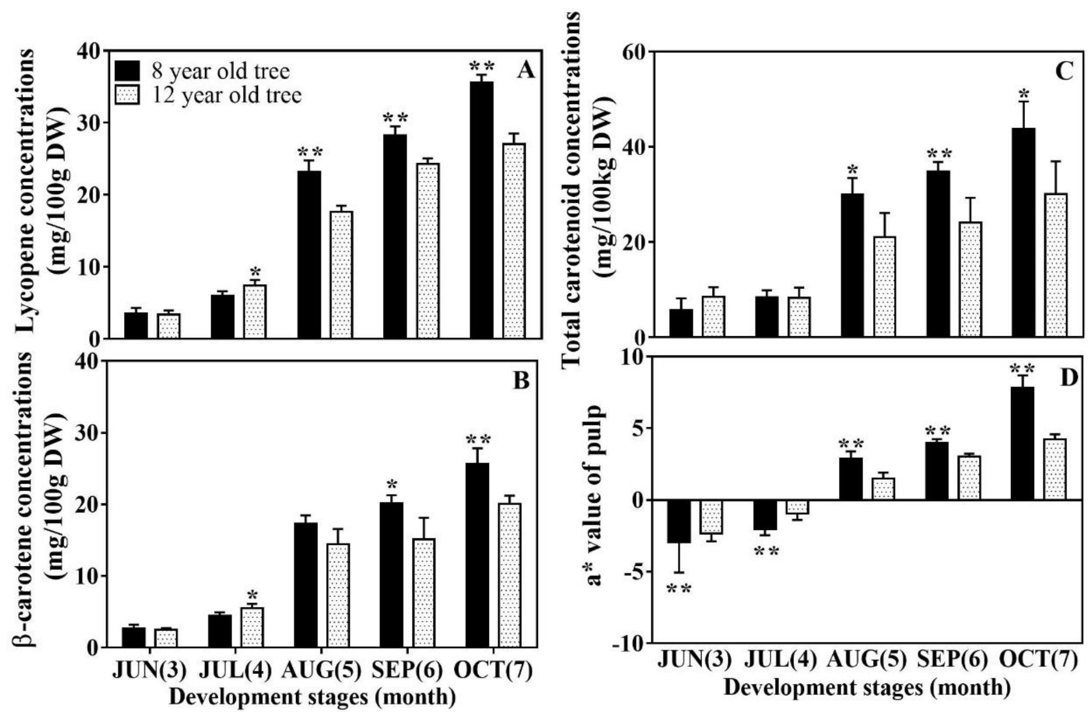


Fig. 3.1 Effect of tree age on accumulation of carotenoid composition namely lycopene (A), β-carotene (B), total carotenoid(C) and a* value (D) in pulp of 'Tubtim Siam' pummelo during fruit development. Each value represents the means ±SE and * or ** shows significant difference by t-test at $P \leq 0.05$ or $P \leq 0.01$ on each date, respectively.

3.4.2 Effect of tree age on color change in fruit pulp

The pulp of fruit from 12-year old tree showed a yellow to slight orange color at 3 MAFS unlike that from 8-year old tree, the pulp color remained yellow. Subsequently, at 4 MAFS the pulp color of fruit from 12-year old tree still observed more orange color compared that from 8-year old tree. However, at 5 MAFS the pulp color of fruit from 8-year old tree changed to more intense redder color which persisted until the commercial harvesting time (7 MAFS) (Fig. 3.2).

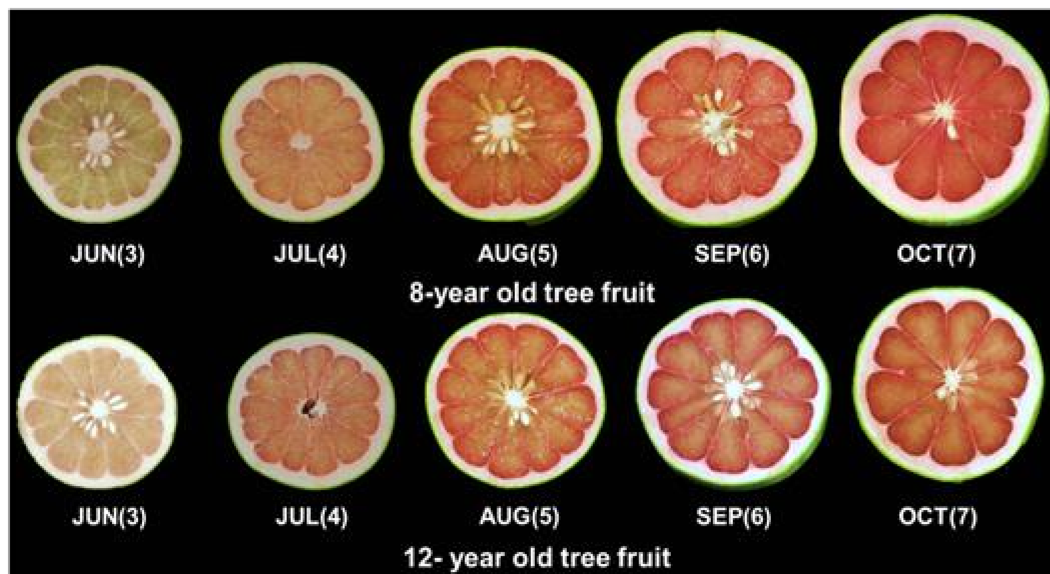
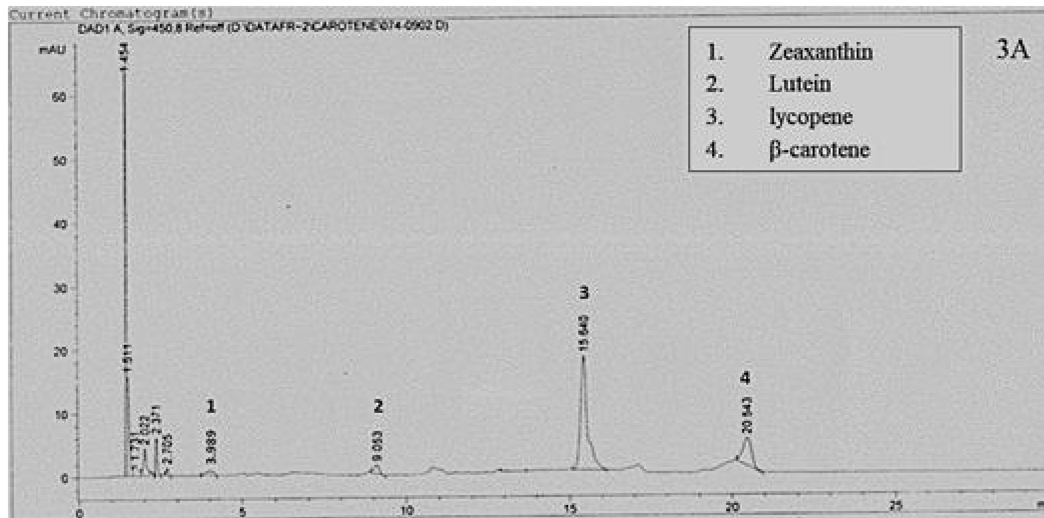


Fig. 3.2. The pulp coloration in 'Tubtim-Siam' pummelo fruit (The number in the parenthesis shows month after fruit set; MAFS)

3.4.3 Carotenoid profile and expression of caroteneoid biosynthesis genes analyses

Carotenoid profiles in pulp of 'Tubtim-Siam' pummelo were identified (Fig. 3.3A); two xanthophylls namely lutein (2), and zeaxanthin (1) accounted for 79.85 and 62.17 µg/100gFW, respectively and two linear carotenes namely β-carotene (4) accounted for 113.53 µg/100gFW and lycopene (3) was the most abundant pigment with 777.23 µg/100gFW (Fig. 3.3B).



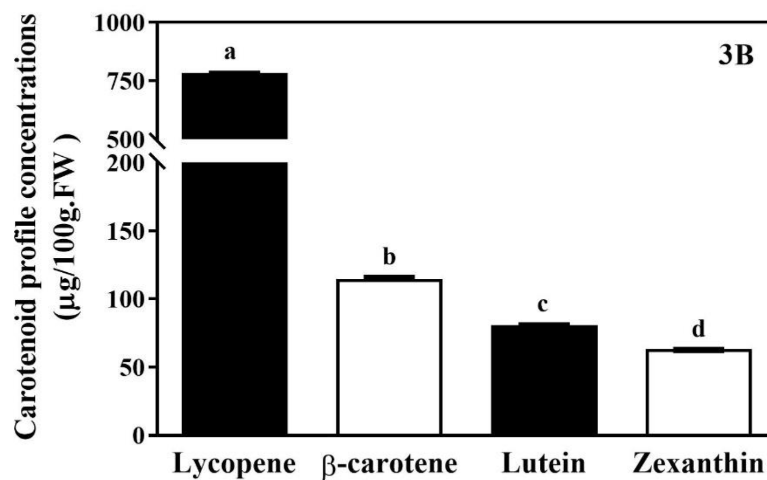


Fig. 3.3 Chromatograms (450 nm) of the saponified samples. Pigments of pulp of 'Tubtim siam' pummelo (*Citrus maxima* (Burm.) Merr.) were investigated to elucidate the carotenoid profile (3A) and the accumulation of main carotenoids content (3B) at commercial harvesting time using LC/MSD.

The expression of carotenoid biosynthesis genes profile in pulp of 'Tubtim siam' Pummelo during fruit development found that the first *CsPSY* transcripts gradually accumulated in first three months then markedly increased at 6 MAFS and then slightly declined during commercial harvesting time (7 MAFS). *CsZDS* gene expression showed a progressive accumulation from 3 to 6 MAFS then slightly declined at 7 MAFS. The relative expression of *CsβLCY* revealed three-fold increase 4 months during development, which peaked in the subsequent month. Thereafter, a tremendous decline observed in the 6 MAFS was maintained at the same level in the following month. The expression of *CsβCHX* was relatively the same in the 3 and 4 MAFS; however, it significantly increased in the 5 MAFS of which levels were almost the same in the 6 MAFS. Eventually, it drastically declines at commercial maturity stage (7 MAFS). *CsƐCHX* gene expression gradually increased from 3 to 5 MAFS then markedly declined in the last two months of development. *CsZEP* is the last gene expressed during carotenoid biosynthesis as its expression levels were similar in first two month of development stage, thereafter the expression progressively increased until commercial harvesting time (7 MAFS) (Fig. 3.4).

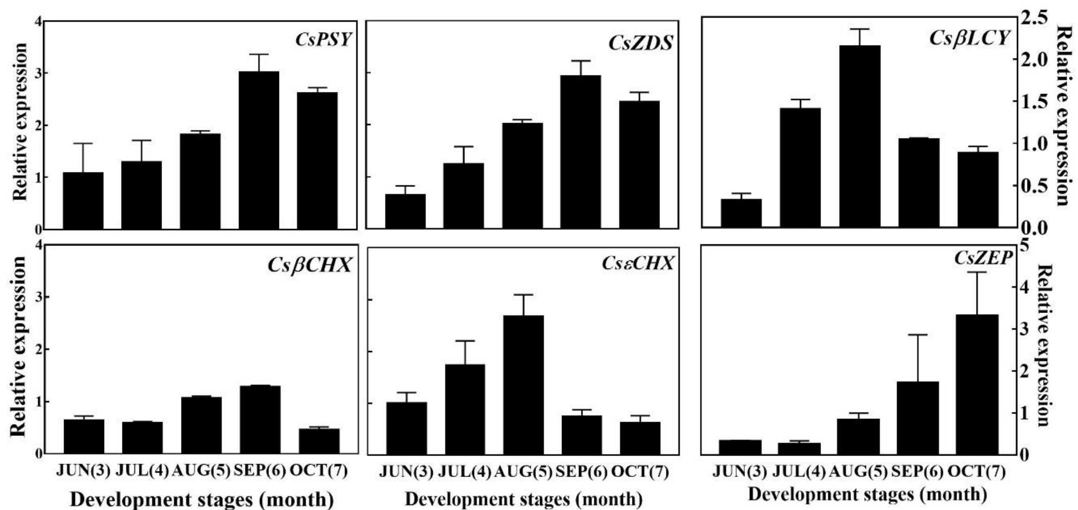


Fig. 3.4 The expression of carotenoid biosynthesis genes profile in pulp of 'Tubtim Siam' Pummelo during fruit development. Fruit were harvest in June (3 MAFS), July (4 MAFS), August (5 MAFS), September (6 MAFS) and October (7 MAFS). The genes analyzed were phytoene synthase (*CsPSY*), ζ-carotene desaturase (*CsZDS*), lycopene β-cyclase (*CsβLCY*), β-carotene hydroxylase (*CsβCHX*), Ɛ-carotene hydroxylase (*CsƐCHX*) and zeaxanthin epoxidase (*CsZEP*).

3.5 Discussion

Tree age is one of the critical factors in determining external and internal fruit quality of citrus species and this ultimately has effect on marketability and acceptability of the fruit. The results of our study indicated that tree age affected accumulation of main pigments contents in 'Tubtim-Siam' pummelo fruit pulp during development process. In the earlier stages of development, 12-year old tree fruit observed quick maturity changes (3 and 4 MAFS) indicated by a significantly higher a* value. However, the 8-year old tree fruit showed a markedly intense red color (higher a* value) later from the breaker stage (5 MAFS) until commercial harvesting time. To the contrary, in 'Kinnow' mandarin (Khalid et al., 2012) tree age did not have any effect on the orange color of the fruit. Additionally, in Amrapali Mango (*Mangifera indica*) fruit, the older tree fruit accumulated more carotenoid compared to the younger tree fruit (Kumar and Ram, 2018) which is in contrast with our finding. It is worth mentioning that carotenoid accumulation is likely related to the fruit color (Alquézar et al., 2013). Relatedly, according to our result we can suggest that the effect of tree age on carotenoid accumulation may be dependent on fruit tree cultivar.

Before beaker stage (4 MAFS), the contents of lycopene and β-carotene in 8-year old tree fruit were lower than 12-year old tree fruit. However, the lycopene, β-carotene and total carotenoids concentrations in pulp of 8-year old tree fruit significantly increased gradually from 5 MAFS and attained a maximum at 7 MAFS than those in 12-year old tree fruit. Fanciullino et al (2014) explained that sugar accumulation influences carotenoid metabolism during fruit development but indirectly. Relatedly in our result, we found that sugar concentrations in 8-year old tree fruit was higher than that in 12-year old tree fruit (data not showed). Thus, this may suggest that the sugar concentration in 8-year old tree fruit increased carotenoid concentration which eventually promoted fruit ripening

The carotenoid profile in our result observed the highest lycopene concentrations than other carotenoid compositions; this is related to the accumulation of lycopene in pulp of 'Tubtim siam' pummelo fruit which might have been involved in the expression of *CsPSY* and *CsZDS* genes which lie in upstream pathway during formation of lycopene. However, the gene expression of *CsβLCY*, *CsβCHX*, *CsεCHX* and *CsZEP* faded which control lycopene transformation to carotene. Carotenoid accumulation in red citrus fruit was

reported in Red 'Hong Anliu' orange that accumulated lycopene with higher expressions of *CsPSY* and *CsZDS* genes and with lower *CsβLCY* gene (Xu et al., 2006). Also, in grape fruit 'Star Ruby'(SR) and 'Flame' the lycopene accumulation was related to lower expression levels of *CsβLCY2* gene (Alquézar et al., 2013 and Mendes et al., 2011). Our result found that the accumulation of linear carotene was associated with the increase in expression of carotenoid biosynthetic genes evidenced by up-regulation of the *CsPSY*, *CsZDS* and *CsβLCY* genes during development. This increase in linear carotenes in the pulp was only partially mirrored by changes in the transcript accumulation of carotenoid biosynthetic genes. The most important changes in carotenoid composition occurred during breaker and after breaker stage, when *CsPSY* and *CsZDS* transcripts levels peaked at 6 MAFS then down-regulated at 7 MAFS; however, *CsβLCY* transcripts levels peaked at 5 MAFS then decreased in the following month (Fig. 4). This result is similar to that reported in other fruit like tomato (Gupta et al., 2014) and vegetative tissue of *A. thaliana* (Toledo-Ortiz et al., 2010). These results indicate that the activity of upstream steps in linear carotene might be sufficient to provide an important flux of intermediates into the pathway and therefore, an elevated lycopene production. Indeed, (Fig. 3A). However, the lycopene β-cyclase activity should not be enough to cyclize this carotene effectively, which is supported by the lower downstream products (xanthophylls) as revealed by a marked decline in *CsβCHX* and *CsƐCHX* genes expression at 7 MAFS (Fig.4). Relatedly, lower levels of lutein and zeaxanthin concentrations were also observed at commercial harvesting time. (Fig.3B). However, the expression of *CsZEP* progressively increased after 4 MAFS until 7 MAFS. Up-regulation of *CsZEP* transcripts may have been related to accumulation of ABA and has effect on level of carotenoid content (Lado et al., 2015 and Rodriguez, 2010). It may therefore, be suggested that the reduced increase in the transcript accumulation of *CsβLCY* could have contributed to enhanced lycopene content in 'Tubtim siam' pummelo.

3.6 Conclusions

Generally, the carotenoid profile in pulp of ‘Tubtim siam’ pummelo fruit at commercial harvesting stage (7 MAFS) include; lycopene, β-carotene, zeaxanthin and lutein. From breaker stage to commercial harvesting time, 8-year old tree fruit observed a significantly higher ration of lycopene, β-carotene, total carotenoid concentrations and higher a* value compare to fruit in 12-year old tree. The expression of carotenoid biosynthesis genes profile in pulp of 8-year old tree fruit revealed the up-regulation of *CsPSY*, *CsZDS* and *CsZEP* genes during development and down-regulation of *Csβ LCY*, *CsβCHX* and *CsƐCHX* genes after breaker stage. This may be related with the quality of red pulp color of ‘Tubtim siam’ pummelo which is contributed by a ratio among the main pigments which include, lycopene, β-carotene, lutein and zeaxanthin.

CHAPTER 4

LYCOPENE SYNTHESIS AND RELATED GENE EXPRESSION IN PUMMELO PULP INCREASED IN SHADE-GROWN FRUIT

4.1 Introduction

'Tubtim siam' pummelo is a geographical indication (GI) product with a ruby red pulp and a sweet aroma (Kaewtubtim and Issarakraisila, 2011). The pummelo peel is generally yellow or green when mature, depending on environmental factors (Porras et al., 1996). Carotenoids, which are isoprenoid-derived pigments, accumulate in the peel and pulp of citrus fruit (*rutaceae*) (Kato et al., 2004). Lycopene exhibits the highest physical quenching rate among carotenoids (Lindshield et al., 2007; Roldan-Gutierrez and De Castro, 2007). The higher expression levels of PSY and ZDS genes and the lower expression levels of βLCYs are key factors triggering lycopene accumulation in orange (Xu et al. 2010) (Fig. 4.1). In addition, lycopene is related to a lower expression level of the fruit-specific βLCY2 gene in red grapefruit (Alquézar et al., 2013; Mendes et al. 2011).

Lycopene accumulates in only a few species of citrus fruits, such as grapefruit (*Citrus paradisi*) and pummelo (*Citrus grandis*) (Liu et al., 2007; Alquézar et al., 2013). Lycopene synthesis is initiated by the upregulation of phytoene synthase (PSY) followed by increments of phytoene desaturase (PDS), ζ-carotene desaturase (ZDS), and β-carotene hydroxylase (β-CHX) and the induction of chromoplast-specific lycopene cyclase (βLCY) and lycopene ε-cyclase (ƐLCY) (Cunningham, 2002). α-Carotene is converted to lutein by sequential hydroxylation, which is catalyzed by ε-ring hydroxylase (ƐCHX) and β-ring hydroxylase (βCHX). β-Carotene is converted to zeaxanthin via β-cryptoxanthin by two-step hydroxylation, which is catalyzed by (βCHX). Furthermore, zeaxanthin is converted to violaxanthin via antheraxanthin by zeaxanthin epoxidase (ZEP) (Kato et al., 2004; Rodrigo et al., 2004 and Alquézar et al., 2013). The catabolism of some carotenoids influences carotenoid accumulation, such as that of cis-violaxanthin. The 9-cis-epoxycarotenoid dioxygenase (NCED), which catalyzes a limiting step in abscisic acid (ABA) biosynthesis, is involved in the regulation of carotenoid accumulation (Fig. 4.1). There are some reports on factors that affect peel color (Kato et al., 2004; Rodrigo et al., 2004 and Alquézar et al., 2013). However, few studies have

examined factors that affect carotenoid accumulation and related gene expression in pummelo pulp.

Light is one of the most crucial environmental factors in carotenoid accumulation in plant tissues (Pizarro and Stange, 2009). Light has been shown to induce carotenoid accumulation and carotenoid biosynthetic genes in tomato fruit (*Solanum lycopersicum*) (Schofield and Paliyath 2005; Azari et al., 2010). In contrast, darkness induced carotenoid accumulation in carrot roots (*Daucus carota subsp. sativus*) (Fuentes et al., 2012; Rodriguez-Concepcion and Stange 2013). The accumulation of carotenoids in darkness correlated with upregulated activity of PSY, the first committed enzyme of carotenogenesis) and with the induction of PSY gene expression in cotyledons in Arabidopsis seedlings (*Arabidopsis thaliana*) (Villalón et al., 2009). These reports suggest that the effects of environmental conditions on carotenoid synthesis may vary among plant species. This study investigated the effects of bagging-induced reductions in light conditions on lycopene production and related gene expression, antioxidant capacity, and sugar composition in pummelo pulp.

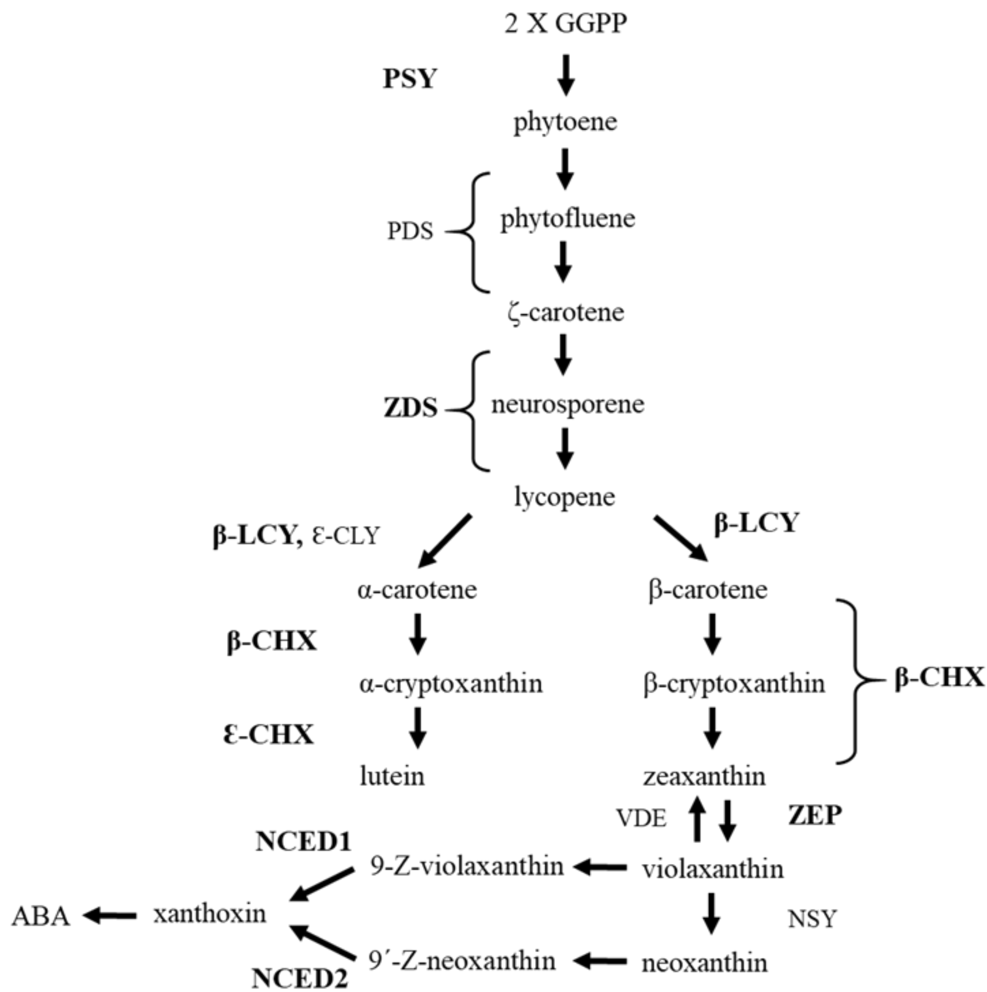


Fig. 4.1 Schematic diagram of the carotenoid biosynthesis pathway in plants. Genes analyzed in this study appear in boldface. *GGPP*, geranylgeranyl diphosphate; *PSY*, phytoene synthase; *PDS*, phytoene desaturase; *ZDS*, ζ-carotene desaturase; *Ɛ-LCY*, lycopene Ɛ-cyclase; *β-LCY*, lycopene β-cyclase; *β-CHX*, β-carotene hydroxylase; *Ɛ-CHX*, Ɛ-carotene hydroxylase; *ZEP*, zeaxanthin epoxidase; *VDE*, violaxanthin de-epoxidase; *NSY*, neoxanthin synthase; *NCED*, nine-*cis*-epoxycarotenoid dioxygenase; ABA, abscisic acid.

4.2 Materials and Methods

4.2.1 Fruit sample and treatments

The experiments were carried out in a commercial orchard in Thailand (lat. 8.34°N, long. 100.09°E) with an average *PPFD* of 1112 μmol/m^2/s and average rainfall of 2665 mm per year (data from 1943 to 2018). Eight-year-old trees of 'Tubtim siam' pummelo were selected randomly and used for the experiment in 2017 and 2018. Fruit from the outer part of the canopy from each of three trees were tagged at fruit set, after which half of the tagged fruit were covered with bagging paper (0.01 μmol/m^2/s of *PPFD*) while in an immature green stage (June: 3 months after fruit set (3 MAFS)). Three replicates of 5 fruit each (15 fruit per treatment) were sampled in June (3 MAFS) and in the mature green stage (4 MAFS, July), breaker stage (5 MAFS, August), after breaker stage (6 MAFS, September), and at full color (7 MAFS, October). Peels and pulp were immediately frozen in liquid nitrogen, ground to a fine powder, and stored at -80℃ until analysis.

4.2.2 Analyses of sugar, carotenoid, flavonoid, ascorbic acid, chlorophyll, antioxidant activity and ABA

The sugar concentrations were analyzed as reported by Kondo et al. (2014). A 1 g freeze-dried pulp sample (three replications) in 10 mL 80% (v/v) ethanol was boiled for 15 min, cooled, and then homogenized. The homogenate was filtered and evaporated. The residue was re-dissolved in 3 mL distilled water and analyzed using high-performance liquid chromatography (HPLC) (model L-6200; Hitachi, Tokyo, Japan) with a Shodex ODP2 HP-4E column (Showa Denko; Tokyo, Japan; 4.6 mm i.d. × 25 cm column). The column temperature was set at 30°C and the mobile phase flow rate was 1 mL/min (75% (v/v) acetonitrile). A refractive index detector was used to identify sugar components.

Carotenoid concentrations were measured according to Fish et al. (2002). Concentrations from the freeze-dried samples (0.5 g, three replications) were extracted with hexane, ethanol, and acetone containing 0.05% butylated hydroxytoluene and determined at different absorbances at 445 nm for total carotenoids, 450 nm for beta carotene, and 503 nm for lycopene using a spectrophotometer (UV-1800, Kyoto, Japan). Flavonoid concentrations were measured according to Zhishen et al. (1999). Concentrations from

the freeze-dried samples (0.5 g, three replications) were extracted with 100 mL ethanol for 1 h. After the addition of $NaNO_2$, $AlCl_3$, and NaOH, absorbance was measured at 510 nm using the spectrophotometer. Ascorbic acid concentrations (0.5 g of pulp, three replications) were measured according to the DNPH method (Kapur et al., 2012). Absorbance was measured at 540 nm. Chlorophyll concentrations were measured according to the method of Inskeep and Bloom (1985). Chlorophyll (0.5 g peel, three replications) was extracted with 20 mL of N, N-dimethylformamide. The concentrations were measured by absorbance of 647 and 664 nm.

Antioxidant activity was measured by the DPPH scavenging activity method according to Kondo et al. (2004). Pulp (0.5 g, three replications) was homogenized in 80% ethanol. After the homogenate was centrifuged for 10 min at 12,000 ×g at 4°C, 150 µL of the extract was added to 2.85 mL of a 0.2 mmol DPPH-methanolic solution. Absorbance was measured at 515 nm.

The ABA concentrations were analyzed according to Kondo et al. (2012). The samples, (1 g, three replications) were homogenized in a 20 mL solution of 80% (v/v) methanol with 0.1% L (+) ascorbic acid (Kanto Chemical, Tokyo, Japan) and 0.1% butylated hydroxytoluene (BHT; 2, 6-Di-tert-Butyl-p-cresol; Sigma-Aldrich, St. Louis, MO, USA) using 0.2 µg ABA-d6 as an intern al standard. The homogenate was filtered and the residue was washed with 20 mL of the initial methanol solution and then concentrated to an aqueous solution in vacuo. The pH was subsequently adjusted to 2.5 with 0.1 M phosphoric acid and extracted three times with 20 mL 100% (v/v) ethyl acetate to concentrate it to dryness, and finally dissolved in 1 mL of 25% acetonitrile containing 20 mM acetic acid. The solution was filtered and then subjected for 30 minutes to preparative HPLC; flow rate, 1.5 $mL.min^{-1}$; detection at 254 nm) equipped with an ODS-Mightysil RP-18 column (250 × 4.6 mm i.d.) eluted with a gradient of 25 to 50% acetonitrile containing 20 mM acetic acid, and finally held at 50% acetonitrile for 5 min. The fractions containing ABA were collected, dried in vacuo, and methylated using ethereal diazomethane for 10 min. The methyl ester of ABA was analyzed by gas chromatography-mass spectrometry selected ion monitoring (GC-MS-SIM; model QP5000; Shimadzu, Kyoto, Japan). The column temperature was a step gradient of 60 °C for 2 min, followed by 60–270 °C at 10 °C/min and 270 °C for 35 min. Ions were measured as ABA-d0 methylester/ABA-d6 methyl ester at m/z 190, 260, 194, and 264.

The ABA concentration was calculated from the ratio of peak areas for m/z 190 (d_0)/194 (d_6).

4.2.3 Total RNA extraction

Total RNA was isolated from the pulp at each sampling date. Each 100 mg pulp sample was ground in liquid nitrogen, thoroughly mixed with 1 mL trizol, and transferred to a centrifuge tube. The mixture was centrifuged for 10 min at 12,000 ×g at 4 °C. The upper aqueous phase was transferred into a new centrifuge tube, after which 240 µL of chloroform was added and thoroughly mixed on ice for 5 min, followed by centrifuging for 15 min at 12,000 ×g at 4 °C. The upper aqueous phase was further transferred into another centrifuge tube, and 500 µL of isopropanol and 0.3 mL of a high-salt precipitation solution (2 M of sodium citrate and sodium chloride) was added to each sample and left to precipitate for 10 min at room temperature. The mixture was then centrifuged for 10 min at 12,000 ×g at 4 °C. The upper aqueous phase was withdrawn, and the remaining RNA pellet was dried. The RNA pellet was then washed using 1 mL 75% ethanol, and total RNA was precipitated by centrifuging for 5 min at 12,000 ×g at 4°C (this was performed twice). Total RNA was treated with Recombinant DNase I (RNase-free) (Takara Bio, Inc., Otsu, Japan). The RNA pellets were completely air dried for 5 min, after which the RNA was dissolved in 50 µL of RNase-free water and finally stored at -80 °C.

4.2.4 Quantitative real-time PCR analysis

cDNA was synthesized from the extracted RNA using the iScriptTM Reverse Transcription Supermix for RT-qPCR (Bio-Rad Laboratories, Hercules, CA, USA). Quantitative real-time PCR was performed using a SYBR Green FAST ABI Prism qPCR Kit (Kapa Biosystems, Wilmington, MA, USA) according to the instruction manual. Gene-specific primers of *CsPSY*, *CsZDS*, *CsβLCY*, *CsβCHX*, *CsƐCHX*, *CsZEP* (Fanciullino et al., 2008), *CsNCED1*, and *CsNCED2* (Kato et al., 2004) for each gene were used for the PCR (Table 1). The cycling protocol consisted of 95 °C for 20 s, followed for 40 cycles of 95 °C for 3 s, 60 °C for 30 s and 95 °C for 15 s, followed by 40

cycles to construct a melting curve. The specificity of the reactions was verified by melting curve analysis at 60-95 °C after 40 cycles. Tree biological replicates were use during analysis. The transcript levels of target genes were estimated by the $2^{-\Delta\Delta CT}$ [2] algorithm, and these levels normalized against the transcript level of geometric averages of CsActin, CsGAPDH and CsUBL5 in each sample (Wu et al., 2014) (Table 4.1).

Table 4.1 Primer sequences for the quantification of transcripts by RT-PCR.

Gene	Forward/reverse primer (5′-3′)	References
CsPSY	GGTCGTCCATTTGATATGCTTG	
	CCTAAGGTCCATCCTCATTCCT	
CsZDS	CGATCCTTACATGCCCTTAC	
	AGGTCCCTCACGGTACAAAG	
CsβLCY	CCCATGTATGACCCATCAAAG	
	TGGGAGATGGATCAATCGAG	(Fanciullino et al.,2008)
CsƐCHX	GGTGCTGGACTTGGCATTAC	
	AGCGACTCTCCGGAAATAAG	
CsƐCHX	CGGCACCAAGTATGCTAAAGG	
	CAGCAGTTCCATTTAGAGGG	
CsZEP	TTGGTTGATGGGATTTCTGG	
	TCCCCAACCGCTTTAGCTAG	
CsNCED1	GGTGCCAACCCATTATTCGA	(Kato et al., 2006)
	GCCGTCACCGTCAAAGAAAT	
CsNCED2	CATTCAAGGCGTCTACGTCAGA	
	CGGCGACCGGTTCGT	
CsActin	ATCTGCTGGAAGGTGCTGAG	
	CCAAGCAGCATGAAGATCAA	
CsGAPDH	GTGTTTCTATGTAGAGGGTCTGAGTTT	
	TCTAAGCACAACCCTGCATGAG	(Wu et al.,2014)
CsUBL5	CTCGAGCCGACAAGATTCG	
	CCATGCCGTCGTGAATCTC	

4.3 Statistical analysis

The data were presented as mean values ± SE and evaluated by the t-test at the 1% or 5% level within the Statistical Program for Social Science 18 (SPSS, Chicago, IL, USA).

4.4 Results

4.4.1 Effect of bagging on change in temperature

In general, the temperature (average from 0600-1800 HR) on the fruit surface in the bag was significantly lower than that of the untreated control. The temperature on the fruit surface in the bag was around 25°C, and the temperature on the fruit surface of the untreated control was 35-40°C (Fig. 4.2).

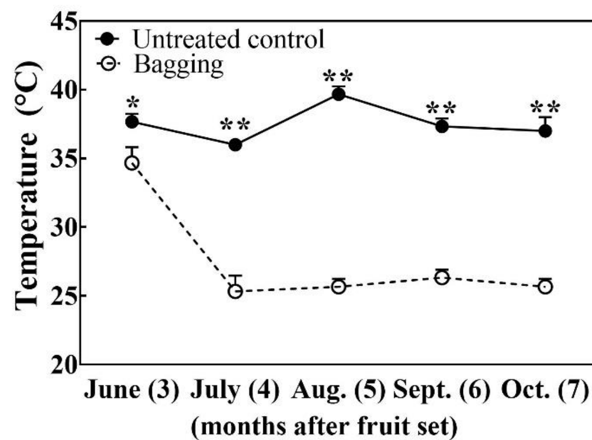


Fig. 4.2 The temperature inside the bag (the number of months after fruit set is shown in parentheses) during fruit development from 3 months after fruit set (June) to harvest at 7 months after fruit set (October). Each value represents the means ±SE, and * or ** shows a significant difference by *t*-test at $P \leq 0.05$ or $P \leq 0.01$ on each date, respectively.

4.4.2 Effects of bagging treatment on chlorophyll, carotenoid, sugar, flavonoid, ascorbic acid, antioxidant activity, and carotenoid biosynthesis gene expression

The concentrations of chlorophyll and carotenoid in the peel gradually declined after 3 MAFS (Fig. 4.3A and 4.3B). The fruit covered with bags showed significantly lower chlorophyll and carotenoid concentrations after 3 MAFS compared to the untreated control.

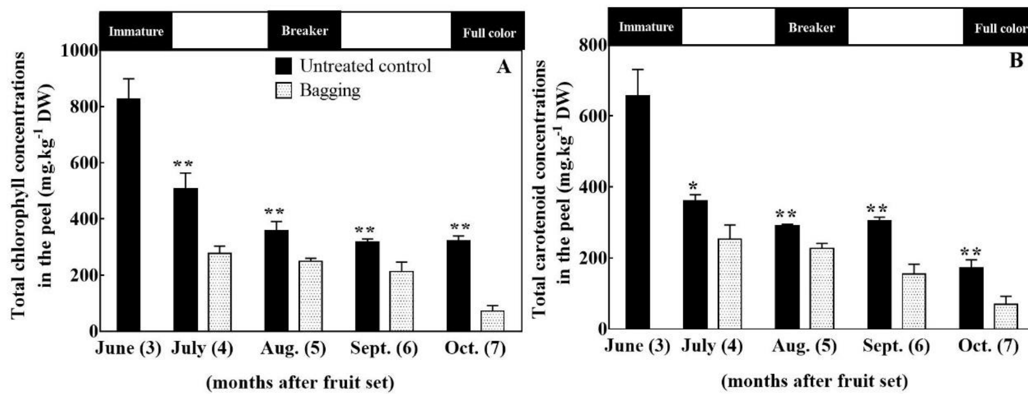


Fig. 4.3 Effects of bagging on chlorophyll (A) and carotenoid concentration (B) in the peel (the number of months after fruit set is shown in parentheses). Each value represents the means ± SE, and * or ** shows a significant difference by *t*-test at $P \leq 0.05$ or $P \leq 0.01$ on each date, respectively.

The pulp turned yellow after 3 MAFS in the fruit covered with bags and the untreated control fruit. The pulp of the fruit covered with bags was redder than that of the untreated control (Fig. 4.4).

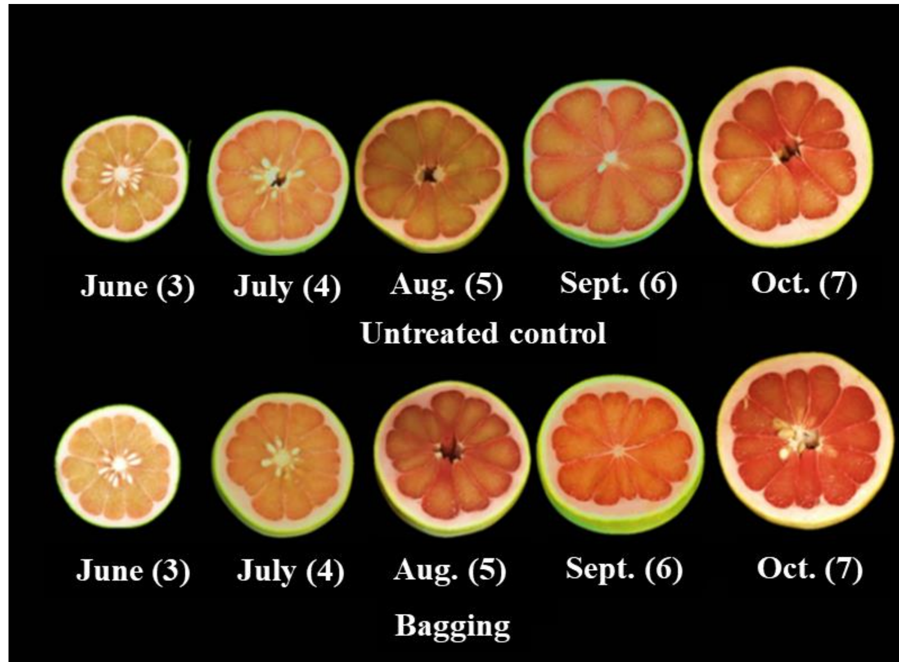


Fig. 4.4 The pulp coloration in 'Tubtim Siam' pummelo fruit (the number of months after fruit set is shown in parentheses).

The fructose concentrations after the breaker stage (5 MAFS) were significantly higher in the fruit covered with bags than in the untreated control. The total sugars, sucrose, and inositol concentrations at the breaker stage were also significantly higher in the fruit covered with bags than in the untreated control, although total sugar did not differ significantly between the two treatments at 7 MAFS (Fig.4.5A-4.5E).

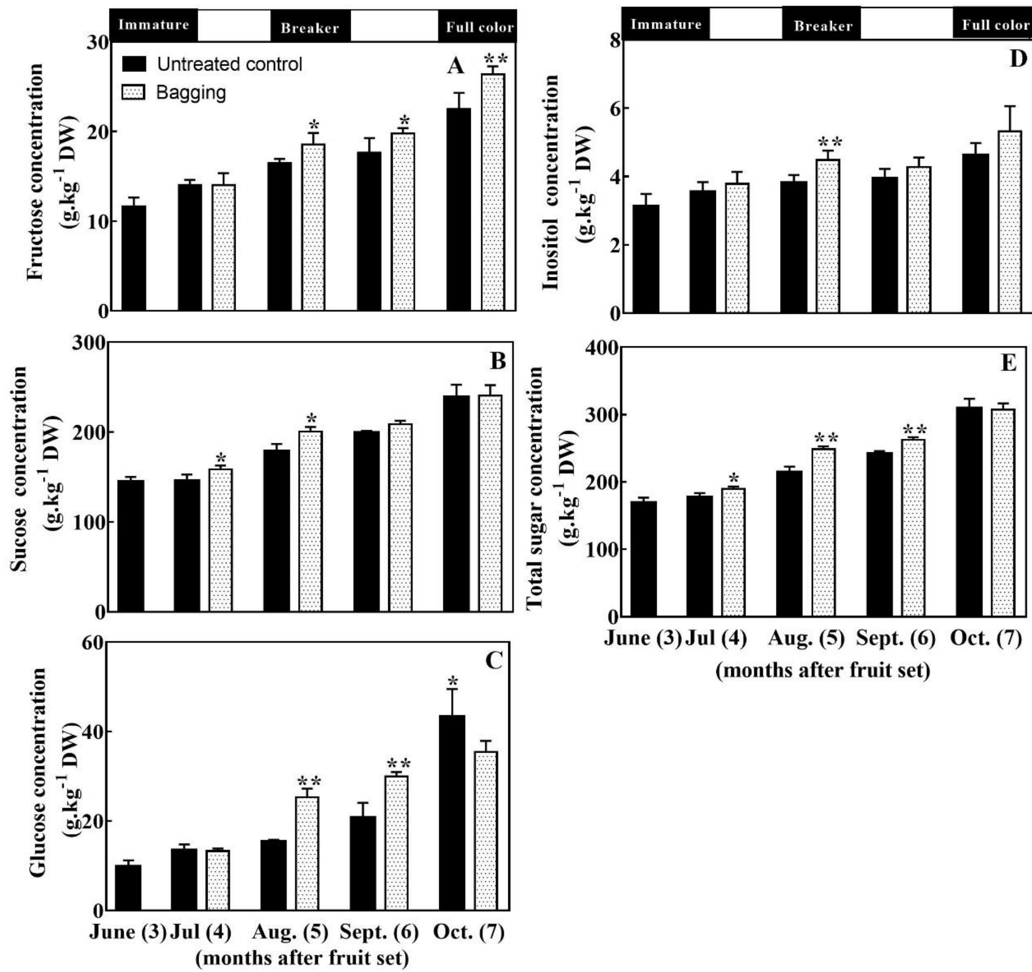


Fig. 4.5 Effects of bagging on fructose (A), sucrose (B), glucose (C), inositol (D), and total sugar (E) concentrations in the pulp of 'Tubtim Siam' pummelo fruit (the number of months after fruit set is shown in parentheses). Each value represents the means ±SE, and * or ** shows a significant difference by *t*-test at $P \leq 0.05$ or $P \leq 0.01$ on each date, respectively.

The lycopene and β-carotene concentrations in the fruit covered with bags were significantly lower than those in the untreated control fruit in July (4 MAFS). However, the lycopene, β-carotene, and total carotenoid concentrations in the fruit covered with bags were significantly increased after the breaker stage compared to the untreated control. (Fig. 4.6A, 4.6B, and 4.6C). The concentrations of flavonoid (Fig. 4.6D) and ascorbic acid (Fig. 4.6E) and the DPPH scavenging activity (Fig. 4.6F) were lower in the fruit covered with bags than in the untreated control fruit at 7 MAFS.

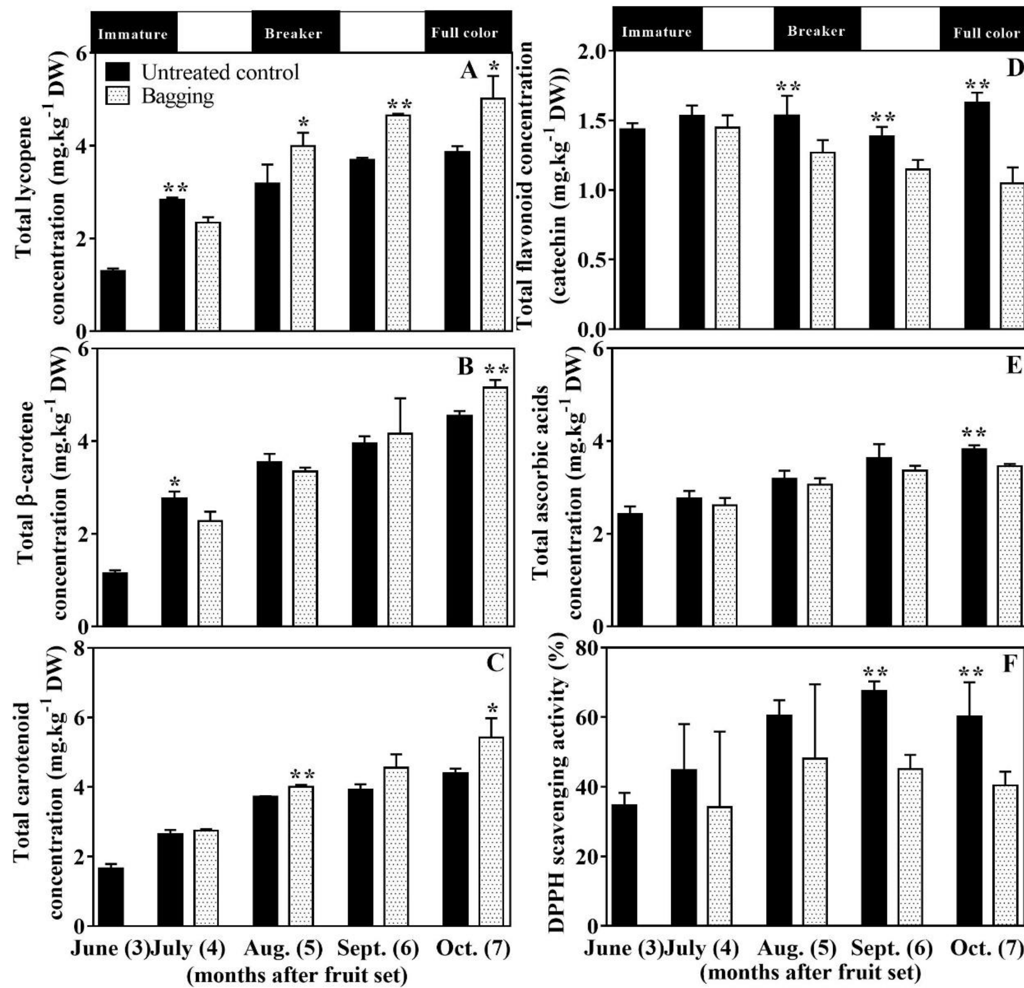


Fig. 4.6 Effects of bagging on the concentrations of lycopene (A), β-carotene (B), total carotenoid (C), flavonoid (D), ascorbic acid (E), and DPPH scavenging activity (F) in the pulp (the number of months after fruit set is shown in parentheses). Each value represents the means ±SE, and * or ** shows a significant difference by *t*-test at $P \leq 0.05$ or $P \leq 0.01$ on each date, respectively.

The *CsPSY* and *CsZDS* gene expression levels were significantly higher in the fruit covered with bags than in the untreated control fruit after the breaker stage. In contrast, the expression levels of *CsβLCY*, *CsβCHX*, and *CsεCHX* were lower in the fruit covered with bags than in the untreated control fruit after the immature stage. However, the expression of *CsβLCY* was significantly higher in the fruit covered with bags than in the untreated control fruit at 7 MAFS. In the fruit covered with bags, the expression of the *CsZEP* gene was significantly higher in the 4 and 5 MAFS but lower at 7 MAFS. *CsNCED1* gene expression levels were significantly lower in the fruit covered with bags than in the untreated control fruit. In both the peel and pulp, the ABA concentrations in the fruit covered with bags were significantly lower than in the untreated control fruit after the breaker stage (Fig. 4.7).

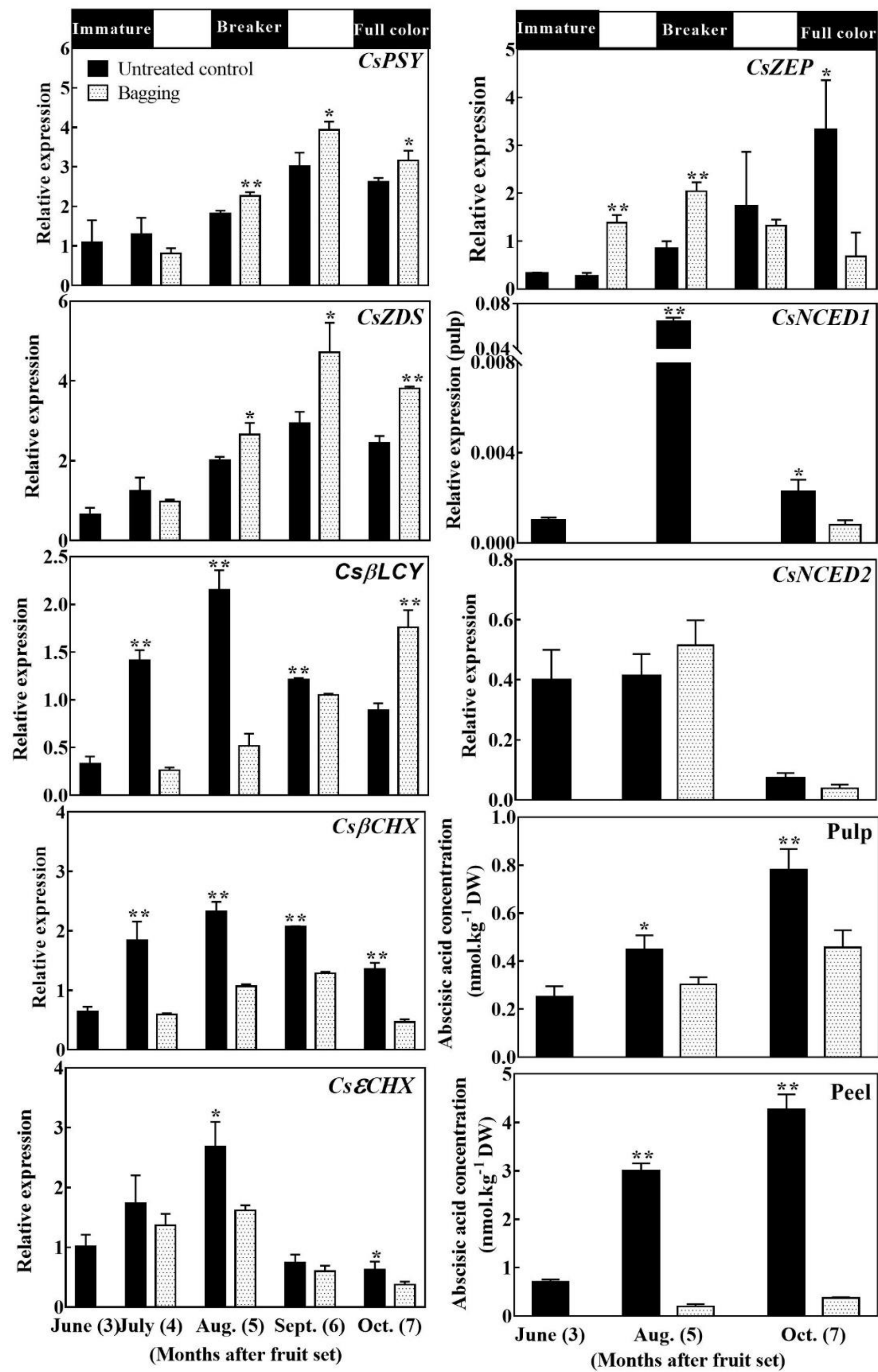


Fig. 4.7. Effects of bagging on the expression of carotenoid biosynthesis genes in pulp and ABA concentrations in pulp and peel (the number of months after fruit sets is shown in parentheses). Each value represents the means ±SE, and * or ** shows a significant difference by *t*-test at $P \leq 0.05$ or $P \leq 0.01$ on each date, respectively.

4.5 Discussion

It has been shown that bagging (0% light transmittance) decreased the flavonoid, carotenoid, and vitamin C contents, antioxidant capacity, and sugar concentrations in the pulp of loquat fruit (Eriobotrya japonica) (Huang et al., 2007). Our study also showed that the pummelo fruit covered with bags had lower glucose, ascorbic acid, flavonoid, and DPPH scavenging activity in the pulp and reduced chlorophyll in the peel than the untreated control fruit. In general, high temperature exhausts carbohydrates such as sugar in citrus (Holland et al., 2005). Noro et al. (1989) reported that the concentrations of fructose were higher in the fruit covered with bags than in the untreated control apples (Malus domestica). In addition, some studies have indicated that low temperatures during fruit maturation were associated with increased sugar levels in apples (Yamada et al., 1988) and tomatoes (Walker and Ho, 1977). Our study also revealed that the pulp of fruit covered with bags had higher fructose, sucrose, inositol, and total sugar concentrations at the breaker stage than the untreated control. The temperature around 25°C may therefore have contributed to the high sugar concentrations in the pummelo fruit covered with bags in our study.

Lycopene and β-carotene are the major pigments in red grapefruit cultivars (Lindshield et al., 2007; Roldan-Gutierrez and de Castro, 2007), and their concentrations have a significant relationship with color (Lee, 2001). Red orange (*Citrus sinensis*) accumulated lycopene with higher expression levels of *CsPSY* and *CsZDS* genes and with lower *CsβLCY* expression levels (Xu et al., 2006). Furthermore, lycopene seems to be related to lower expression levels of *βLCY2* gene in grapefruit (Alquézar et al., 2013). In general, light increased carotenoid levels in chromoplasts in tomato fruit (Liu et al., 2015). However, shade promoted chromoplast differentiation and carotenoid accumulation in grapefruit (Lado et al., 2015). In addition, dark conditions induced gene expression related to carotenoid synthesis by a putative repressor of photo morphogenesis that is encoded by the Y locus associated with carotenogenesis in carrot root (Iorizzo et al., 2016). Villalón et al. (2009) suggested that carotenoid biosynthesis was induced in dark conditions with the upregulation of phytoene synthase and the induction of *CsPSY* gene expression in cotyledons of arabidopsis. These reports support that the fruit covered with bags in our study showed high concentrations of lycopene and β-carotene concentration compared to the untreated control fruit with the upregulation of *CsPSY* and *CsZDS* and

the downregulation of *CsβLCY*, *CsβCHX*, and *CsεCHX* gene expression. In addition, our results showed that the increase of lycopene was associated with the decrease of ABA concentration and *CsNCED1* gene expression in the peel and pulp of the fruit covered with bags. In general, the increase of carotenoid is associated with the increase of ABA concentration in orange or white grapefruit (Alquézar et al., 2013). In additional, Lado et al. (2015) mentioned that dark conditions tended to decrease carotenoid concentrations in red grapefruit, resulting in a decreased endogenous ABA concentration. In our study, ABA and lycopene concentrations showed the opposite relationship. The results may show that another factor influenced lycopene concentrations in the fruit covered with bags. Some previous reports suggested that the ABA concentrations decreased at low temperature and rose at high temperature in mangosteen fruit (Garcinia mangostana) (Kondo and Jitratham, 2004). Hamauzu et al. (1995) also reported that tomato fruit changed from green to red during storage at 20°C and changed to yellow at 30°C. Our study suggests that a temperature around 25°C promoted lycopene synthesis, but that a temperature around 35-40°C inhibited synthesis in pummelo pulp.

4.6 Conclusion

The light conditions influenced chlorophyll in the peel and ascorbic acid, flavonoid, glucose concentrations and DPPH scavenging activity in the pulp. The bagging increased fructose, sucrose and inositol concentration in the pulp at breaker stage. Furthermore, it is possible that low temperature with bagging treatment increased lycopene concentration in the pulp with the upregulation of *CsPSY*, *CsZDS* and downregulation of *CsβLCY*, *CsβCHX*, *CsεCHX*, *CsNCED1* gene expressions in the pulp and decrease of ABA concentration in the peel and pulp.

CHAPTER 5

APPLICATION OF AVG OR 1-MCP-MBS ON POSTHARVEST QUALITY OF 'TUBTIM SIAM' PUMMELO (*CITRUS MAXIMA* (BURM.) Merr.)

5.1 Introduction

'Tubtim siam' pummelo (*Citrus maxima* (Burm) Merr.) is a geographical indentification (GI) product of Nakhon Si Thammarat province, Thailand. Pummelo is an economically important tropical fruit in Thailand and is ranked as the sixth minor economic fruit crop in Thailand (FAO, 2012). Recently, the demand for this fruit has gradually increased in both domestic and international markets, especially in China, Taiwan, Malaysia, Singapore and Brunei. It has become more popular in fruit market because of the distinct characteristics such as red ruby pulp, fibreless, delicious taste, juicy flesh, and sweet aroma (Kaewtubtim and Issarakraisila, 2011); Additionally, the red ruby colored pulp is due to various dietary antioxidants and Phyto-compounds such as, ascorbic acid, α-tocopherol, phenolic, flavonoids (Kaewsuksaeng, 2015) and carotenoid.

Carotenoid endow citrus fruit with nutritional function serve as precursors for vitamin A, which is essential to human and animal diets and as antioxidants, which play a role in reducing the risk of certain forms of cancer (Olson, 1989) and also play a key role in fruit color, the bright yellow, orange, and red colors provided by carotenoids accumulation in the chromoplasts of the peel and pulp of citrus fruit. The red color found in Red-flesh navel orange 'cara cara', red ruby star, red blood orange and red grapefruit cultivars is comparable to what we found in Tubtim siam pummelo display an even bright color, which make them appealing for direct eating (Lee, 2001; Curl and Bailey, 1957; Khan and Mac Kinney, 1953 and Rouseff et al., 1992). However, Degradation of green peel, red-ruby pulp color and biochemical compound are postharvest problem of Tubtim siam pummelo may cause by endogenous ethylene.

Non-climacteric fruits are also reported to respond to the exogenous and endogenous of ethylene. Investigations on in planta levels of CO_2 and ethylene of fruits during storage supported the role and involvement of changes in the rate of respiration and ethylene

production by presence of a characteristic rise in CO_2 levels and a burst in ethylene production in some non-climacteric fruits (Vijay Paul et al.,2012) such as strawberry (Trainotti et al.,2005; Cancel and Larsen, 2002; Iannetta et al., 2006) grapes (Chervin et al., 2004) and citrus (Stewart and Wheaton 1972; Purvis and Barmore 1981; Goldschmidt et al., 1993; Goldschmidt 1997; Katz et al., 2004). In citrus, the pigment changes in peel and pulp are the best visual markers of citrus fruit maturation.

Pigment changes in peel of the most citrus cultivars consist of breakdown of chlorophyll and buildup of carotenoids, both of which are enhanced by ethylene and can delayed by plant bioregulators (Barmor, 1975; Shimokawa et al., 1978; Hirschfeld and Goldschmidt, 1983). Pigment changes in pulp of citrus cultivars is related to oxidative degradation of carotenoids has led to cis-trans isomerization and formation of carotenoid epoxides (Mordi et al., 1993 and Wacheä et al., 2003). Carotenoids act as antioxidants against lipid peroxidation by quenching singlet oxygen and trapping free peroxyl radicals (Palozza and Krinsky 1991). Investigations have shown that singlet oxygen quenching ability of the carotenoids depends on their structural differences, such as number of conjugated double bonds, end groups (acyclic or cyclic), and substituent functional groups in the rings (Stahl and Sies 1996; Hirayama and others 1994). As lycopene with a lesser extent of beta-carotene are the major pigments in red grapefruit cultivars (Curl & Bailey 1957; Khan & MacKinney 1953; Rouseff et al. 1992) Di Mascio and others (1989) reported that the singlet oxygen quenching capacity of the carotenes was as follows: lycopene > alfa-carotene > beta-carotene. Esterification of carotenoids with fatty acids occur during fruit ripening and post-harvesting of the fruit may induce ripening process by endogenous ethylene, which play affect the color intensity (Minguez and Mendez,1994).
The physiological and chemical changes associated with fruit ripening can be halted or delayed by inhibiting ethylene perception, even when the fruit has reached advanced stages of ripening (Hoeberichts et al., 2002). Ethylene accelerates the above-mentioned changes, but plant bioregulators such as 1-methylcyclopropene (1-MCP) and aminoethoxyvinyl glycine (AVG) are commonly used in postharvest pre-storage treatments for mandarins (Asrey, 2012) and ‘Kinnow' mandarin (Tavallali and Moghadam, 2015).

1-MCP is an ethylene action inhibitor, prevents the ripening effects of ethylene in many climacteric and non-climacteric fruits (Blankenship and Dole, 2003), but its effects differ by species. 1-MCP significantly reduces ethylene production, delays degreening, weight loss and softening in citrus (Fan et al, 1999; Cin, 2006; Laamim, 2005 and Asrey, 2012). As with originally formulated and prior applications, 1-MCP is usually delivered as a gas in sealed environments to prevent the 1-MCP gas from being released. However, using 1-MCP as fumigation technique may not be practical on a commercial scale because of high investment costs for airtight systems, take for a long time to fumigate. In the case of climacteric fruits for example banana, the periods of fumigation are between 6 and 24 hours at concentrations ranging from 5 to 1000 nLL^{-1} and higher (Blankenship and Dole, 2003). And other facilities. 1-MCP is easily released as a gas when the powder is dissolved in water. Recently, preparation of 1-MCP designed for use as aqueous has been formulated, facilitating broader agricultural applications of this ethylene-action inhibitor (Elfving et al., 2007).

Microbuble (MB) technology has been used in many fields, including foam fractionation, food processing and purification processing of polluted water. One of the most significant characteristics of MBs are a highly efficient way of delivering dis solved gas into a solution. A crucial characteristic of MBs is that they are negatively changed on their surface (Takahashi, 2005) and have a high potential to be used for a variety of practical purpose (Pongprasert, and Srilalong, 2014). 1-MCP-MBb application were used for delay ethylene production cause by maintain the postharvest quality change in non-climacteric fruit such as Hom thong bananas (Pongprasert et al., 2012) and Khai bananas (Promkaew et al., 2015) in addition, also effect in non-climacteric fruit such as dragon fruit (Vera et al., 2017) and lime fruit (Tadmala, 2014).

AVG (a water-soluble powder) is commercially sold under the name of ReTain®. It is a human and environmentally friendly organic product registered use for apples, pears, peaches, plums, mandarins and nectarines in several countries (Greene and Schupp, 2004; Rath and Prentice, 2004). AVG inhibits the synthesis of ethylene at the level of the aminocyclopropane carboxylic acid synthase enzyme (ACS), responsible for the conversion of S-adenosylmetionine to 1-aminocyclopropane 1-carboxylic acid (ACC), the latter an immediate precursor of ethylene (Adams and Yang, 1979). ACC synthase is

considered a key enzyme in the biosynthesis of ethylene (Kende, 1993). Autio and Bramgage (1982) observed that AVG treatments delayed ripening and harvest, increased fruit firmness and prolonged storage life of fruit. Pre-harvest treatment of fruits with AVG decreases ethylene production, delays fruit maturity, and allows fruit to ripen more slowly (Bregoli, 2002; Torrigiani, 2004; Cline, 2006). However, the effect is timing or cultivar dependent (Byers, 1997; Belding and Lokaj, 2002). Furthermore, it is difficult to directly evaluate the shelf-life of AVG-treated fruit, because AVG affects fruit maturity, which in turn influences shelf-life. Postharvest application of AVG significantly suppresses ethylene production and reduces fruit ripening and therefore postharvest rotting (Byers, 1997; Garner, 2001). Therefore, the aim of this study was to evaluate the effectiveness of AVG and 1MCP-MBb on maintaining postharvest quality of Tubtim siam pummelo through dipping in AVG and 1-MCP-MBs.

5.2 Materials and Methods

5.2.1 Fruit sample and treatments

'Tubtim siam' pummelo at commercial harvesting time (about 210 days after fruit setting), were harvest and transported to the Postharvest Technology laboratory, School of Bioresources and Technology, King Mongkut's University of Technology Thonburi, Bangkhunthien Campus, Bangkok. Fruit samples were selected for uniformity of color, size and free from disease. Afterwards, fruit were cleaned with tap water and dried at room temperature.

A 1-MCP microbubble (1-MCP-MBs) generator system was constructed by the Department of Technology Thonburi (Bangkok, Thailand) according to the design (show in fig.5.1). Before starting the processes, 1-MCP (0.19% 1-MCP tablet, BioLene Co., Ltd., China) at doses corresponding to 5 ppm was fumigated in a 1.5 L closed chamber for 5 min. The pummelo fruit were put into 40 L of water, then water with 1-MCP-MBs were generated by a swiveling microbubble generator (56 mm diameter x 86 mm long; 5 and 13 mm diameter outlet and inlet, respectively; Model.BT-50; Thai Isekyu Co., Ltd., Bangkok, Thailand) (Pongprasert and Srilalong, 2014). Fig. 5.1 shows a schematic diagram of the experimental set up used in this study. For AVG treatment, fruit was applied by dipped with ReTain® (Valent Biosciences Corp., USA), a commercial product

containing 15% (w/w) AVG, at doses corresponding to 500 ppm for 5 min at room temperature (Palou and Crisosto, 2003). The control was fruit without treatment. The fruit were stored at room temperature (25±2 ˚C) and 85 ± 5% RH for 21 days. The fruit were analyzed on every 7 days to determine changes in fruit quality parameters. For each analysis period, 5 fruits were randomly selected and used for the fruit physiological and chemical characteristics.

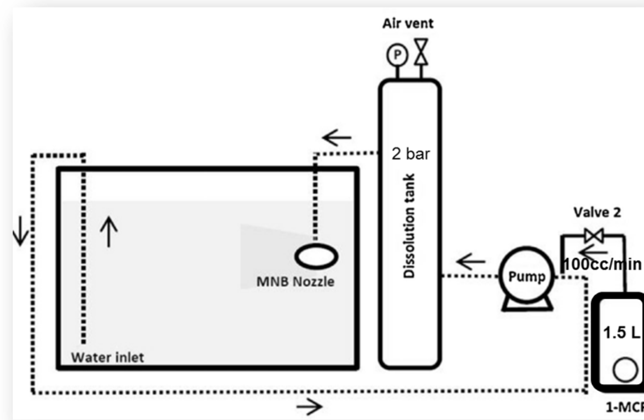


Fig. 5.1 Schematic diagram of 1-MCP micro bubble generation system.

5.2.2 Physiological analysis

Physiological changes, like change in respiration rate and ethylene production, weight loss and peel and pulp color (hue angle value) were evaluated in every 7day for 21day. For respiration rate and ethylene production were measured by placing each lot and replication of pummelo fruit in a 3.5 L air tight plastic chamber for 3 hours at (25 ± 2˚C). Gas sample (1 mL) was withdrawn with one milliliter plastic syringe and injected in the gas chromatography (GC–2014 B (Shimadzu, Japan)), installed with a flame ionization detector (FID) equipped with a 60/80 mesh Porapack-Q column. Nitrogen was used as a carrier gas with a flow rate of 35 mL/min and temperature of the injector and column maintained at 120 and 95°C respectively. For weight loss each sample was weighed to calculate the percentage of weight loss as follows: Weight loss (%) = ((Initial weight – Final weight) x 100)/Initial weight. For peel and pulp surface color, hue angle was measured with a colorimeter (Chromameter Model RC-400, Minolta Corp,).

5.2.3 Chemical Analysis

Chemical change, which include the change in total chlorophyll content, total carotenoid content, total beta-carotene content, total lycopene content, total phenolic content, total ascorbic content and DPPH free radical scavenging activity were evaluated in 7 days intervals. For total chlorophyll content of peel was determined using N, N-Dimethylformamide (Moran, 1982). Carotenoid content was measured using hexane, ethanol and acetone containing 0.05% butylated hydroxytoluene and determined at different absorbances at 445 nm for total carotenoid, 450 for beta carotene and 503 nm for lycopene (Fish et al., 2002). Total phenolic content was measured using the Folin–Ciocalteu method (Singleton, 1999). Total ascorbic content was measured according to the DNPH method (Kapur et al., 2012) and total antioxidant activity was measured by the DPPH method (Krings and Berger, 2001).

5.3 Statistical analysis

Experiment at design was completely randomized design. Data were analyzed by means of ANOVA. The data are presented as means ±SE. All data analysis was performed with SAS statistical software (SAS Institute Inc, 2006). The statistical analysis program procedure was used, and mean separation was analysis by least significant difference (Duncan, $P< 0.05$).

5.4 Results

5.4.1 Effects of AVG and 1-MCP-MBs on respiration rate, ethylene production and weight loss.

Respiration rate in all treatments markedly increased in 7 days after the treatment application but not significant difference was observed among treatments. After 7 storage days, the control treatment showed the highest respiration metabolism when compared to AVG and 1-MCP-MBs treatments, however AVG, treatment registered a significantly the low respiration rate until the end of the storage period (Fig. 5.2A). Ethylene production in all fruit increase slightly during the first week there after a peak of ethylene

production was observed at 14 days. Control treatment exhibited the highest increment of ethylene production at 14 days, followed by the AVG and 1-MCP-MBs treatments which significantly suppressed ethylene production with AVG treated observing the lowest treatment After the peak (14 days), ethylene production decreased as the storage proceeded in all treatments, however was not significant in AVG and 1-MCP-MBs treatments on the last day of storage (Fig. 5.2B). Ethylene accelerates ripening and senescence effects, but plant bioregulators such as 1-MCP and AVG have been developed to mitigate its effect. These results differ from other studies into the application of AVG and 1-MCP-MBs as an inhibitor to the biosynthesis of ethylene. The inhibitory effect on ethylene biosynthesis by AVG was reported in mandarins (Asrey, 2012), 'Kinnow' mandarin (Tavallali and Moghadam, 2015). AVG is an analog of rhizobiotoxine. This phytotoxin competitively inhibits the conversion of S-adenosylmethionine (SAM) to 1aminocyclopropane-1-carboxylic acid (ACC) in the synthesis of ethylene (Byers, 1997). 1-MCP is an ethylene action inhibitor by blocking access to the ethylene binding receptor, lowers action of maturation associated genes and enzymes (Sisler and Serek, 1997; Khan and Singh, 2007; Martinez, 2002) were affected on non-climacteric fruits, such as the strawberry (Tian et al., 2000) and the tangor cv. Murcote (Tavares et al., 2003), where a reduction in respiration rate was seen with the application of 1-MCP. Numerous research studies demonstrated that weight loss was associated with respiration processes and evaporation of water from the fruit (Amarante, 2001). In our study, after treatment of pummelo fruit, AVG showed a lowest of weight loss percentages from the first week to the last week of storage period (1.56% and 2% respectively) compere to other two treatments. However, 1-MCP-MBs also delayed weight loss in the last week of storage (2.5%) compare to control treatment (2.86%) (Fig. 5.2C). AVG seemed to be more effective than 1-MCP-MBs in controlling weight loss. Similar result with Tavallali and Moghadam (2015) work in 'Kinnow' mandarin found that AVG treatment showed positive effect to delayed weight loss than in 1-MCP treated. This result probably could be due to a protective role of AVG on fruit peel integrity which reduced water evaporation, gas exchange and decreased nutrient loss (Sigal-Escalada, 2006).

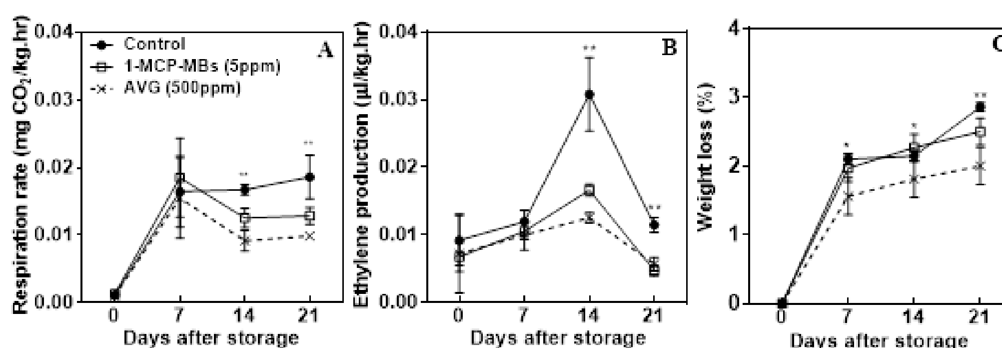


Fig. 5.2 Changes in respiration rate (A), ethylene production rate (B) and weight loss (C) of 'Tubtim siam' pummelo fruit. Fruit were treated with 1-MCP-MBs and AVG at 5ppm and 500ppm concentration respectively. The control was fruit without treatment. The fruit were stored at room temperature (25±2 ˚C) for 21day. Each point represents the means ±SE, and * or ** shows a significant difference between means at each storage time (Duncan, P<0.05)

5.4.2 Effects of endogenous ethylene production on senescence occurrence in 'Tabtim Siam' pummelo fruit treated with AVG and 1-MCP-MBs.

In most fruit, chlorophyll degradation occurs during development or in postharvest storage (Shemer et al., 2008), such as citrus (Jacob-Wilket al., 1999), tomato (Guyer et al., 2014), and banana (Pongprasert et al., 2012). 1-MCP treatment prevented or delayed chlorophyll degradation in a wide range of fruit specie. In orange cv. Pera, the degradation of chlorophyll and green peel change to yellow/orange was delayed by the application of 1-MCP (Golding et al., 1998). 1-MCP-MBs has also been reported to delay change in total chlorophyll content and hue angle value in Hom thong bananas (Pongprasert et al., 2012), Khai bananas (Promkaew et al., 2015), dragon fruit (Loor et al., 2017) and mature green lime fruit (Tadmala, N., 2014). However, to our knowledge AVG has no report in delaying chlorophyll degradation or change in green peel color in citrus fruit but has been report to effect in delaying postharvest change in respiration and ethylene production and softening in 'Kinnow' mandarin (Tavallali and Moghadam, 2015). Interesting in this study, we found that AVG and 1-MCP-MBs treatment delayed peel yellowing as indicated by significantly higher total chlorophyll contents and hue angle value of peel. The decrease was relatively slow from 0 day to 14 days of storage period and became

rapid after 14 days in the following 7days to the end of storage (Fig. 5.3). In contrast, the peel chlorophyll content of the control treatment observed a rapid decline from the initial time of storage to the end. Furthermore, the hue angle value of the same treatment was significantly lower in the last two weeks storage. Generally, both AVG and 1-MCP-MBs maintained a significantly high of the peel chlorophyll content and hue angle value on 14 and 21 days after storage (fig. 5.3A and 5.3B). The effectiveness of AVG and 1-MCP-MBs in inhibiting chlorophyll degradation confirmed by the reduction of total chlorophyll contents and hue angle value of pummelo fruit peel especially after one week of storage time. Exogenous or endogenous ethylene released from fruit flesh and core may accelerate chlorophyll degradation in fruit peel (Garcia-luis, Fornes,Guardiola, 1986; Jacob-Wilk, Holland, Goldschmidt, Riov ans Eyal, 1999; Porat et al., 199; Purvis and Barmore, 1981; Trebitsh et al., 1993). In this work, we observed that the ethylene production pattern in pummelo fruit was exactly matched the pattern of peel yellowing or decrease in total chlorophyll content and hue angle value. On the other hand, ethylene production was related to chlorophyll degradation in the second week of the storage period in which the peak of ethylene production appeared (Fig. 5.2B). Therefore, the AVG and 1-MCP massive reduction of endogenous ethylene could act as a signal to delayed enzyme and gene expression in chlorophyll catabolism such as PAO, CHL1 and RCCR in fruit peel (harpaz-Saad et al., 2007 and Jacob-Wilk et al., 1999)

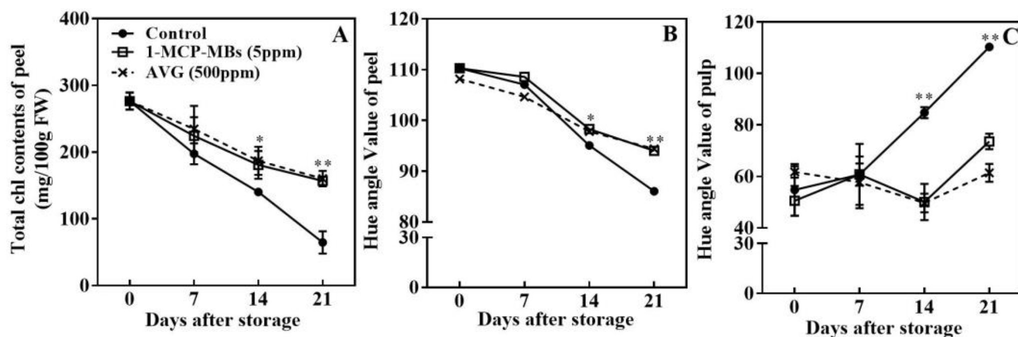


Fig. 5.3 showed changes in total chlorophyll content of peel (A), hue angle value of peel (B) and hue angle value of pulp (C) of 'Tubtim siam' pummelo fruit. (Hue angle ho=tan-1(b*/a*) represents different color, 0° = red-purple, 45° = orange, 90° = yellow, 180° =bluish green and 270° =blue.) Fruit were treated with 1-MCP-MBs and AVG at 5ppm and 500ppm concentration respectively. The control was fruit without treatment. The fruit were stored at room temperature (25±2 ˚C) for 21day. Each point represents the

means ±SE, and * or ** shows a significant difference between means at each storage time (Duncan, $P<0.05$)

5.4.3 Effects of AVG and 1-MCP-MBs on quality of biochemical composition of fruit.

Pulp quality determined by color is important when considering a postharvest application of AVG and 1-MCP-MBs. Equable red color was observed by H° angle value. Found that 1-MCP-MBs and AVG significantly delayed increased of H° angle value from 14 days to the last day of storage period (Fig. 5.3 C). The trends observed for pulp color were like those for total carotenoids content, total lycopene content and total beta-carotene content. 1-MCP-MBs and AVG treatment were delayed the reduction of total carotenoids, total lycopene and total beta-carotene contents from 0 day to the last day and significant effect was observed in 14 day and 21 days when compare to the control treatment. On the last day, the contents of total carotenoids, total lycopene and total beta-carotene in 1-MCP-Mbs treatment showed 12.77, 15.26 and 9.91 mg/100g, AVG treatment were 11.61,16.87 and 12.32 mg/100g and control treatment were 5.28, 6.79 and 5.94 mg/100g of each respectively. Changes in carotenoid levels during storage depend on the ripening process factor, the length of storage time light, temperature and high relative humidity. In this study fruit were storage at room temperature (25±2˚C), 85 ± 5% relative humidity, ethylene is the main factor to inhibit increasing respiration processes and evaporation of water from the fruit (Amarante, 2001). Increased of relative humidity may cause to reduction of carotenoid and carotenoid intensity. Furthermore, Minguez and Mendez,1993 report that maybe O_2 could be resulting to degradation of the structure, allowing carotenoids to oxidise by oxidation of the unsaturated fatty acids that form part of the lipid components of the membranes. This reaction is catalyzed by lipoxygenase during ripening and post-harvesting of the fruit (Minguez-Mosquera, M.I.,1990). Oxidative degradation of carotenoids also leads to cis-trans isomerization and formation of carotenoid epoxides (Mordi et al., 1993 and Wacheä et al., 2003). This is support by Henry et al. (2000) report that lycopene and beta-carotene degradation during hydroperoxide by Oxygen. 1-MCP-MBs and AVG are plant bioregulators reported the inhibitory effect on ethylene (Greene and Schupp, 2004; Rath and Prentice, 2004 and Blankenship and Dole, 2003). Byers (1997) reported biosynthesis and consequent suppression of ethylene production by various plant tissues. In agreement with the above,

this present study found that AVG and 1-MCP-MBs delayed the reduction in contents of total carotenoids total lycopene and total beta-carotene including delayed increased hue angle value of pulp. 1-MCP-MBs and AVG treatment significantly maintained total phenolic content with slight reduction observed in the last day of storage. Compare to the control treatment that showed remarkable decline throughout the storage period (Fig. 5.5A), similarly the content of total ascorbic acids registered significantly higher content in both AVG and 1-MCP-MBs treatment after the first week of storage compare to the control (Fig. 5.5B). The antioxidant activity delineated by DPPH scavenging activity observed significantly higher percentage in both AVG and 1-MCP-MBs treatment in most days during storage. Meanwhile, the control on the other hand showed lower DPPH scavenging activity. Natural substances with known antioxidant potential are phenolic compounds such as flavonoids vitamins A, C, E and carotenoids (Ara and Nur 2009, Arnao et al. 2001). Ethylene has a correlation with production of reactive oxygen species in fruits and vegetable during storage (Larrigudiere et al., 2004). This suggest that the ethylene inhibitory action of 1-MCP and AVG is involved in suppression of the generation of free radicals of plant tissues through the inhibition of ethylene. Thus, in agreement with what this study found.

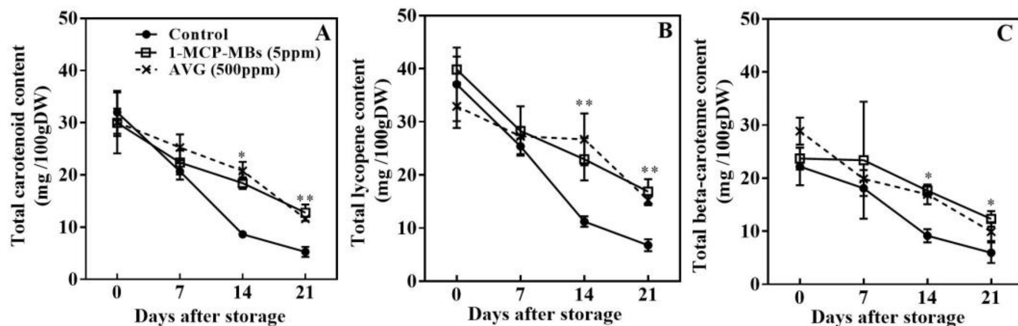


Fig. 5.4 showed changes in total carotenoid content (A), total lycopene content (B) and total beta-carotene content (C) of ‘Tubtim siam’ pummelo fruit. Fruit were treated with 1-MCP-MBs and AVG at 5ppm and 500ppm concentration respectively. The control was fruit without treatment. The fruit were stored at room temperature (25±2˚C) for 21day. Each point represents the means ±SE, and * or ** shows a significant difference between means at each storage time (Duncan, P<0.05)

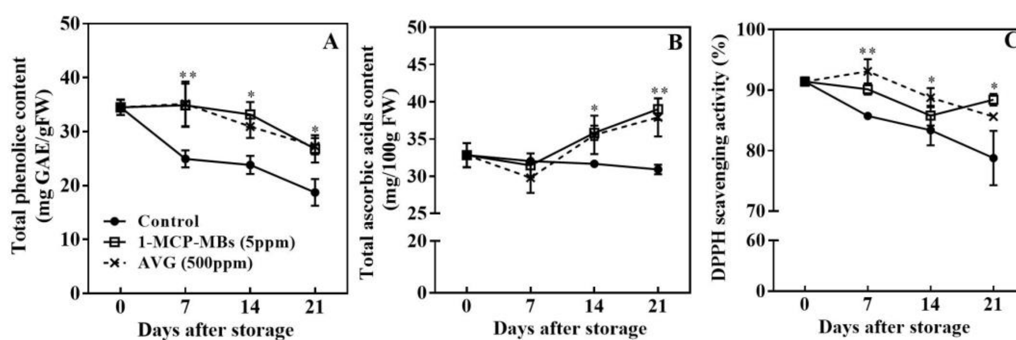


Fig. 5.5 showed changes in total phenolic content (A), total ascorbic content (B) and DPPH scavenging activity (C) of Tubtim siam pummelo fruit. Fruit were treated withed 1-MCP-MBs and AVG at 5ppm and 500ppm concentration respectively. The control was fruit without treatment. The fruit were stored at room temperature (25±2˚C) for 21day. Each point represents the means ±SE, and * or ** shows a significant difference between means at each storage time (Duncan, P<0.05)

5.5 Conclusions

This study clearly indicates that fruit treated with AVG resulted in a remarkably reduced respiration rate and ethylene production compared to other treatments. Consequently, delayed peel yellowing as indicated by significantly higher total chlorophyll contents, hue angle value of peel and delayed degradation of red-ruby pulp color was also observed in 1-MCP-MBs treated fruit. On the other hand, application of AVG and 1-MCP-MBs retarded a reduction of weight loss compared to the control. Chemical composition in pulp including; total carotenoid, beta-carotenoid, lycopene, vitamin C, DPPH free radical scavenging activity and total phenolic content were maintained and were significantly different when compared with the control treatment. In conclusion, AVG and 1-MCP-MBs treatments maintained both external and internal postharvest quality of ‘Tubtim siam’ pummelo.

CHAPTER 6

GENERAL DISCUSSION

Tree age is one of the critical factors in determining external and internal fruit quality of citrus species and this ultimately has effect on marketability and acceptability of the fruit. The results of our study indicated that tree age affected accumulation of main pigments contents in 'Tubtim siam' pummelo fruit pulp during development process.

The 8-year old tree fruit showed a markedly intense red color (higher a* value) later from the breaker stage (5 MAFS) until commercial harvesting time. To the contrary, in 'Kinnow' mandarin (Khalid et al., 2012) tree age did not have any effect on the orange color of the fruit. Additionally, in Amrapali Mango (*Mangifera indica*) fruit, the older tree fruit accumulated more carotenoid compared to the younger tree fruit (Kumar and Ram, 2018) which is in contrast with our finding. It is worth mentioning that carotenoid accumulation is likely related to the fruit color (Alquézar et al., 2013). Relatedly, according to our result we can suggest that the effect of tree age on carotenoid accumulation may be dependent on fruit tree cultivar. the lycopene, β-carotene and total carotenoids concentrations in pulp of 8-year old tree fruit significantly increased gradually from 5 MAFS and attained a maximum at 7 MAFS than those in 12-year old tree fruit. Fanciullino et al (2014) explained that sugar accumulation influences carotenoid metabolism during fruit development but indirectly. Relatedly in our result, we found that sugar concentrations in 8-year old tree fruit was higher than that in 12-year old tree fruit (data not showed). Thus, this may suggest that the sugar concentration in 8-year old tree fruit increased carotenoid concentration which eventually promoted fruit ripening

The carotenoid profile in the fruit pulp observed include lycopene, β-carotene, zeaxanthin and lutein. The expression of carotenoid biosynthesis genes profile in pulp of 8-year old tree fruit revealed the up-regulation of *CsPSY*, *CsZDS* and *CsZEP* gene expression during development and down-regulation of *CsβLCY*, *CsβCHX* and *CsεCHX* gene expression after breaker stage (5 MAFS). Additionally, the carotenoid profile in our result observed the highest lycopene concentrations than other carotenoid compositions; this is related to the accumulation of lycopene in pulp of 'Tubtim siam' pummelo fruit which might have been involved in the expression of *CsPSY* and *CsZDS* genes which lie in upstream

pathway during formation of lycopene. However, the gene expression of *CsβLCY*, *CsβCHX*, *CsƐCHX* and *CsZEP* faded which control lycopene transformation to carotene. that appeared to be a key factor triggering lycopene accumulation because these were the key genes expressed during fruit development.

Bagging decreased the flavonoid, carotenoid, and vitamin C contents, antioxidant capacity, and sugar concentrations in the pulp of loquat fruit (Eriobotrya japonica) (Huang et al., 2007). Our study also showed that the pummelo fruit covered with bags had lower glucose, ascorbic acid, flavonoid, and DPPH scavenging activity in the pulp and reduced chlorophyll in the peel than the untreated control fruit. However, our study also revealed that the pulp of fruit covered with bags had higher fructose, sucrose, inositol, and total sugar concentrations at the breaker stage than the untreated control. The temperature around 25°C may therefore have contributed to the high sugar concentrations in the pummelo fruit covered with bags in our study.

In general, light increased carotenoid levels in chromoplasts in tomato fruit (Liu et al., 2015). However, shade promoted chromoplast differentiation and carotenoid accumulation in grapefruit (Lado et al., 2015). Villalón et al. (2009) suggested that carotenoid biosynthesis was induced in dark conditions with the upregulation of phytoene synthase and the induction of *CsPSY* gene expression in cotyledons of arabidopsis. These reports support that the fruit covered with bags in our study showed high concentrations of lycopene and β-carotene concentration compared to the untreated control fruit with the upregulation of *CsPSY* and *CsZDS* and the downregulation of *CsβLCY*, *CsβCHX*, and *CsƐCHX* gene expression. In addition, our results showed that the increase of lycopene was associated with the decrease of ABA concentration and *CsNCED1* gene expression in the peel and pulp of the fruit covered with bags. The results may show that another factor influenced lycopene concentrations in the fruit covered with bags. Some previous reports suggested that the ABA concentrations decreased at low temperature and rose at high temperature in mangosteen fruit (*Garcinia mangostana*) (Kondo and Jitratham, 2004). Hamauzu et al. (1995) also reported that tomato fruit changed from green to red during storage at 20°C and changed to yellow at 30°C. Our study suggests that a temperature around 25°C promoted lycopene synthesis, but that a temperature around 35-40°C inhibited synthesis in pummelo pulp.

Degradation of green peel and change in the red-ruby pulp color are two main deteriorative postharvest quality parameters caused by exogeneous ethylene. The aim of this study was to evaluate the effectiveness of AVG and 1-MCP-microbubbles (MBs) on maintaining postharvest quality of 'Tubtim siam' pummelo through dipping in AVG and 1-MCP-MBs at 500 and 5 ppm, respectively. Fruit treated with AVG resulted in a remarkably reduced respiration rate and ethylene production compared to other treatments. Because of AVG and 1-MCP is an ethylene action inhibitor by blocking access to the ethylene binding receptor, lowers action of maturation associated genes and enzymes (Sisler and Serek, 1997; Khan and Singh, 2007 and Martinez, 2002) on non-climacteric fruits, such as the strawberry (Tian et al., 2000) and the tangor cv. Murcote (Tavares et al., 2003) Moreover, delaying of peel yellowing was indicated by significantly higher total chlorophyll contents, hue angle values of peel and delayed degradation of red-ruby pulp color were also observed in 1-MCP-MBs treated fruit. the AVG and 1-MCP massive reduction of endogenous ethylene could act as a signal to delayed enzyme and gene expression in chlorophyll catabolism such as PAO, CHL1 and RCCR in fruit peel (Harpaz-Saad et al., 2007; Jacob-Wilk et al., 1999). On the other hand, application of AVG and 1-MCP-MBs retarded a reduction of weight loss compared to the control. Chemical composition in pulp including; total carotenoid, beta-carotenoid, lycopene, vitamin C, DPPH free radical scavenging activity and total phenolic content were maintained and were significantly different when compared with the control treatment. This suggest that the ethylene inhibitory action of 1-MCP and AVG is involved in suppression of the generation of free radicals of plant tissues through the inhibition of ethylene. In conclusion, AVG and 1-MCP-MBs treatments could maintain both external and internal postharvest quality of 'Tubtim siam' pummelo fruit.

REFERENCES

Alós, E., Cercós, M., Rodrigo, M.J., Zacarías, L., Talón, M., 2006. Regulation of color break in citrus fruits. Changes in pigment profiling and gene expression induced by gibberellins and nitrate, two ripening retardants. J. Agric. Food Chem. 54, 4888–4895.

Alquézar, B., Rodrigo, M.J., Zacarías, L., 2008. Regulation of carotenoid biosynthesis during fruit maturation in the red-fleshed orange mutant Cara Cara. Phytochemistry. 69, 1997–2007.

Alquézar, B., Zacarías, L., Rodrigo, M.J., 2009. Molecular and functional characterization of a novel chromoplast specific lycopene beta-cyclase from Citrus and its relation to lycopene accumulation. J. Exp. Bot. 60, 1783–1797.

Alquézar, B., Rodrigo, M.J., Lado, J., Zacarías, L., 2013. A comparative physiological and transcriptional study of carotenoid biosynthesis in white and red grapefruit (*Citrus paradisi* Macf.). Tree Genet Genomics. 9, 1257–1269.

Cunningham, F.X., 2002. Regulation of carotenoid synthesis and accumulation in plants. Pure Appl. Chem.74, 409–417.

Dayal, V., Dubey, A.K., Singh, S.K., Sharma, R.M., Dahuja, A., Kaur, C., 2016. Growth yield and physiology of mango (*Mangifera indica* L.) cultivars as affected by polyembryonic rootstocks. Sci Hortic. 199, 186–197.

Duran-Zuazo, V.H., Raya, A.M., Aguilar-Ruiz, J. and Tarifa, D.F., 2004. Impact of salinity on macro and micronutrient uptake in mango (*Mangifera indica* L.) with different rootstocks. Span. J. Agric. Res. 2, 121–133.

Fanciullino, A.L., Cerćos, M., Dhique, M.C., Froelicher, Y., Talón, M., Ollitrault, P., Morillon, R., 2008. Changes in carotenoid content and biosynthetic gene expression in juice sacs of four orange varieties (*Citrus sinensis*) differing in flesh fruit color. J. Agric. Food Chem. 56, 3628–3638.

Fanciullino1, A. L., Bidel, L. P., Urban L., 2014. Carotenoid responses to environmental stimuli: integrating redox and carbon controls into a fruit model. Plant Cell Environ. 37, 273–289.

Fish, W.W., Perkins, V.P., Collins, J.K., 2002. A quantitative assay for lycopene that utilizes reduced volumes of organic solvents. J. Food Comp. Anal. 15, 309–317.

Food and Agriculture Organization of United nations (FAO), 2004. Products year report. The State of Food Insecurity in the World, Rome, Italy.

Gupta, S.K., Sharma, S., Santisree, P., Kilambi, H.V., Appenroth, K., Sreelakshmi, Y., Sharma, R., 2014. Complex and shifting interactions of phytochromes regulate fruit development in tomato. Plant Cell Environ. 37, 1688–1702.

Haldankar, P.M., Parulekar, Y.R., Kireeti, A., Kad, M.S., Shinde, S.M. and Lawande K.E., 2015. Studies on influences of bagging of fruits at marble stage on quality of mango cv. Alphonso. J. Plant Stud. 4, 12–20.

Kaewtubtim, M. and Issarakraisila, M., 2011. Effects of nitrogen and zinc on fruit quality of pummelo cv. Tubtim Sayam. In Commission on Higher Education Congress IV University Staff Development Consortium, Chanburi. 44.

Kato, M., Ikoma, Y., Matsumoto, H., Sugiura, M., Hyodo, H., Yano, M., 2004. Accumulation of carotenoids and expression of carotenoid biosynthetic genes during maturation in citrus fruit. Plant Physiol.134, 824–837.

Khalid, S., Malik, A.U., Saleem, B.A., Khan, A.S., Khalid, M.S., Amin, M. 2012. Tree age and canopy position affects rind quality, fruit quality and rind nutrient content of ‘Kinnow’ mandarin (*Citrus nobilis* Lour × *Citrus deliciosa* Tenora). Sci Hortic. 135, 137–144.

Kumar, M.N. and Ram, A., 2018. Tree age affects physicochemical, functional quality and storability of Amrapali mango (*Mangifera indica* L.) fruits. J. Sci. Food Agric. 98, 3255–3262.

Kumar, M.N. and Ram, A., 2018. Tree age affects postharvest attributes and mineral content in amrapali mango (*Mangifera indica*) fruits. Hortic. Plant J. 4, 45–88.

Lado, J., Cronje, P., Alque´zar, B., Page, A., Manzi, M., Go´mezCadenas, A., Stead, A.D.,

Zacarı´as, L., and Rodrigo, M.J., 2015. Fruit shading enhances peel color, carotenes accumulation and chromoplast differentiation in red grapefruit. Physiol. Plant. 154, 469–484.

Lee, H.S., 2001. Characterization of carotenoids in juice of red navel orange (*Cara Cara*). J. Agric. Food Chem. 49, 2563–2568.

Maccarone, E., Maccarone, A., Perrin, G., Rapisarda, P., 1983. Anthocyanin of the Moro orange juice. Ann. Chim. 73, 533–539.

Mendes, A.F.S., Chen. C., Gmitter. F.G. J.r., Moore, G.A., Costa, M.G.C., 2011. Expression and phylogenetic analysis of two new lycopene β-cyclases (*LCYBs*) from *Citrus paradisi* Macf. Physiol. Plant. 141,1–10.

Nanakhon and Chalumpak .2016. Effect of tree age and fruit age on fruit development and fruit quality of pummelo Var. Tabtimsiam. International Journal of Agricultural Technology. 12, 541–549.

Rao, A.V. and Rao, R.G., 2007. Carotenoids and human health. Pharm. Res. 55, 207-216.

Rodriguez-Concepcion, M., 2010. Supply of precursors for carotenoid biosynthesis in plants. Arch. Biochem. Biophys. 504, 118–122.

Rodrigo, M.J., Marcos, J.F., Zacarías, L., 2004. Biochemical and molecular analysis of carotenoid biosynthesis in flavedo of orange (*Citrus sinensis* L) during fruit development and maturation. J. Agric. Food Chem. 52, 6724–673.

Toledo-Ortiz, G., Huq, E., Rodríguez-Concepción, M., 2010. Direct regulation of phytoene synthase gene expression and carotenoid biosynthesis by phytochrome-interacting factors. Proceedings of the National Academy of Sciences, USA. 107, 11626–11631.

Wu, J.X., Su, S.Y., Fu, L.L., Zhang, Y.J., Chai, L.J., Yi, H. L., 2014. Selection of reliable reference genes for gene expression studies using quantitative real-time PCR in navel orange fruit development and pummelo floral organs. Sci. Hortic. 176, 180–188.

Xu, C.J., Fraser, P.D., Wang, W.J., Bramley, P.M., 2006. Differences in the carotenoid content of ordinary citrus and lycopene-accumulating mutants. J. Agric. Food Chem. 54, 5474–5481.

Alquézar, B., L. Zacarías and M.J. Rodrigo. 2009. Molecular and functional characterization of a novel chromoplast specific lycopene beta-cyclase from Citrus and its relation to lycopene accumulation. J. Exp. Bot. 60, 1783–1797.

Alquézar, B., M.J. Rodrigo, J. Lado and L. Zacarías. 2013. A comparative physiological and transcriptional study of carotenoid biosynthesis in white and red grapefruit (*Citrus paradisi* Macf.). Tree Genet. Genomes. 9, 1257–1269.

Azari, R., Y. Tadmor, A. Meir, M. Reuveni, D. Evenor, S. Nahon, H., Shlomo, L. Chen and I. Levin. 2010. Light signalling genes and their manipulation towards modulation of phytonutrient content in tomato fruits. Biotechnol. Adv. 28, 108–118.

Cronje, P.J.R., G.H. Barry and M. Huysamer. 2011. Postharvest rind breakdown of "Nules Clementine" mandarin is influenced by ethylene application, storage temperature and storage duration. Postharvest Biol. Technol. 60, 192–201.

Cronje, P.J.R., G.H. Barry and M. Huysamer. 2013. Canopy position affects pigment expression and accumulation of flavedo carbohydrates of "*Nules Clementine*" mandarin fruit, thereby affecting rind condition. J. Am. Soc. Hortic. Sci.138, 217–224.

Cunningham, F.X. 2002. Regulation of carotenoid synthesis and accumulation in plants. Pure Appl. Chem. 74, 409–417.

Fanciullino, A.L., M. Cerćos, M.C. Dhique, Y. Froelicher, M. Talón, P. Ollitrault and R. Morillon. 2008. Changes in carotenoid content and biosynthetic gene expression in juice sacs of four orange varieties (*Citrus sinensis*) differing in flesh fruit color. J. Agric. Food Chem. 56, 3628–3638.

Fish, W.W., V.P. Perkins and J.K. Collins. 2002. A quantitative assay for lycopene that utilizes reduced volumes of organic solvents. J. Food Comp. Anal. 15, 309–317.

Fuentes, P., L. Pizarro, J.C. Moreno, M. Handford, C.M. Rodriguez and C. Stange. 2012. Light-dependent changes in plastid differentiation influence carotenoid gene expression and accumulation in carrot roots. Plant Mol. Biol. 79, 47–59.

Hamauzu, Y., Y. Miyamoto and K. Chachin. 1995. Effect of High Temperatures on the Change of Carotenoid Contents in Tomato Fruit after Harvest. J. Jpn. Soc. Hortic. Sci. 63, 675–684.

Holland, N. A., H. C. Menezes and M. A. T. Lafuente. 2005. Carbohydrate metabolism as related to high-temperature conditioning and peel disorders occurring during storage of citrus fruit. J. Agric. Food Chem. 53, 8790–8796.

Huang, C.H., M.L. Chai, Z.M. Pan, B.Yu, Z.M. Jiang, J.L. Hu and Y.W. Teng. 2007. Effects of bagging on fruit skin features and quality of Cuiguan pear cultivar. J. Fruit Sci. 24, 747–751.

Huang, C.H., B.Yu, Y.W. Teng, J. Su, Q. Shu and Z.Q. Cheng. 2009. Effects of fruit bagging on coloring and related physiology, and qualities of red Chinese sand pears during fruit maturation. Sci. Hort. 121, 149–158.

Inskeep, W. P., and P. R. Bloom. 1985. Extinction coefficients of chlorophyll a and b in N, N dimethylformamide and 80% acetone. Plant Physiol. 77, 483–485.

Iorizzo, M., S. Ellison and D. Senalik. 2016. A high-quality carrot genome assembly provides new insights into carotenoid accumulation and asterid genome evolution. Nature Genetics. 48, 657–666.

Kaewtubtim, M. and M. Issarakraisila. 2011. Effects of nitrogen and zinc on fruit quality of pummelo cv. Tubtim Sayam. In Commission on Higher Education Congress IV University Staff Development Consortium. 44. Chanburi.

Kapur, A., A. Hasković, A. Čopra-Janićijević, L. Klepo, A. Topčagić, I. Tahirović and E. Sofić. 2012. Spectrophotometric analysis of total ascorbic acid content in various fruits and vegetables Bulletin of the Chemists and Technologists of Bosnia and Herzegovina. 38, 39–42.

Kato, M., Y. Ikoma, H. Matsumoto, M. Sugiura, H. Hyodo and M. Yano. 2004. Accumulation of carotenoids and expression of carotenoid biosynthetic genes during maturation in citrus fruit. Plant Physiol. 134, 824–837.

Kato, M., H. Matsumoto, Y. Ikoma, H. Okuda. and M. Yano. 2006. The role of carotenoid cleavage dioxygenases in the regulation of carotenoid profiles during maturation in citrus fruit. J. Exp. Bot. 57, 2153–2164.

Kondo, S., and A. Jitratham. 2004. Relationships between Jasmonates and Chilling Injury in Mangosteens Are Affected by Spermine HortScience. 3, 1346–1348.

Kondo, S., H. Tomiyama, A. Rodyoung, K. Okawa, H. Ohara, S. Sugaya, N. Terahara and N. Hirai. 2014. Abscisic acid metabolism and anthocyanin synthesis in grape skin are affected by light emitting diode (LED) irradiation at night. J. Plant Physiol. 171, 823–829.

Kondo, S., H. Yoshikawa and R. Katayama. 2004. Antioxidant activity in astringent and non-astringent persimmons. J. Hortic. Sci. Biotech. 79, 390–39.

Lado, J., P. Cronje, B. Alque´zar, A. Page, M. Manzi, A. Go´mez-Cadenas, A.D. Stead, L. Zacarı´as and M.J. Rodrigo. 2015. Fruit shading enhances peel color, carotenes accumulation and chromoplast differentiation in red grapefruit. Physiol. Plant. 154, 469–484.

Lee, H.S. 2001. Characterization of carotenoids in juice of red navel orange (*Cara Cara*). J. Agric. Food Chem. 49, 2563–2568.

Lindshield, B.L., K. Canene-Adams, J.W. Erdman. 2007. Lycopenoids: are lycopene metabolites bioactive. Arch. Biochem. Biophys. 458, 136–140.

Liu, Y., Q. Liu, N.G. Tao and X.X. Deng. 2006. Efficient isolation of RNA from fruit peel and pulp of ripening navel orange (*Citrus sinensis* Osbeck). J. Huazhong Univ. Sci. Technol. 25, 300–304.

Liu, Q., J. Xu, Y.Z. Liu, X.L. Zhao, X.X. Deng, L.L. Guo and J.Q. Gu. 2007. A novel bud mutation that confers abnormal patterns of lycopene accumulation in sweet orange fruit (*Citrus sinensis* Osbeck). J. Exp. Bot. 58, 4161–4171.

Liu, L., Z. Shao, M. Zhang and Q. Wang. 2015. Regulation of carotenoid metabolism in tomato. Mol. Plant. 8, 28–39.

Mendes, A.F.S., C. Chen, F.G. Gmitter, G.A. Moore, M.G.C. Costa. 2011. Expression and phylogenetic analysis of two new lycopene β-cyclases from Citrus paradise. Physiol. Plant. 141, 1–10.

Noro, S., N. Kudo and T. Kitsuwa. 1989. Differences in sugar and organic acid contents between bagged and un-bagged fruits of the yellow apple cultivars, and the effect on development of anthocyanin. J. Jpn. Soc. Hortic. Sci. 58, 17–24.

Pizarro, L. and C. Stange. 2009. Light-dependent regulation of carotenoid biosynthesis in plants. Cienc Invest Agrar. 36, 143–162.

Porras, I., A. García, C. Egea, A. Conesa and M.F. García. 1996. Internal and external colour changes in different grapefruit varieties. Proc. Intl. Soc. 2, 1113–1116.

Rodríguez C.M. and C. Stange. 2013. Biosynthesis of carotenoids in carrot: an underground story comes to light. Arch. Biochem. Biophys.1, 1–7.

Roldan-Gutierrez, J.M. and M.D.L. Castro. 2007. Lycopene: the need for better methods for characterization and determination. Trends Anal. Chem. 26, 163–170.

Schofield, A. and G. Paliyath. 2005. Modulation of carotenoid biosynthesis during tomato fruit ripening through phytochrome regulation of phytoene synthase activity. Plant Physiol. Biochem. 43, 1052–1060.

Urban, L., R. X. Le, H. Sinoquet, S. Jaffuel and M. Jannoyer. 2003. A biochemical model of photosynthesis for mango leaves: evidence for the effect of fruit on photosynthetic capacity of nearby leaves. Tree Physiol. 23, 289–300.

Villalón, A. R., E. Gas and M.R. Concepción. 2009. Phytoene synthase activity controls the biosynthesis of carotenoids and the supply of their metabolic precursors in dark grown Arabidopsis seedlings. Plant J. 60, 424–435.

Walker, A.J. and L.C. Ho. 1977. Carbon translocation in the tomato: effects of fruit temperature on carbon metabolism and the rate of translocation. Ann. Bot. 41, 825–832.

Wu, J.X., S.Y. Su, L.L. Fu, Y.J. Zhang, L.J. Chai, H. L. Yi. 2014. Selection of reliable reference genes for gene expression studies using quantitative real-time PCR in navel orange fruit development and pummelo floral organs. Sci. Hortic. 176, 180–188.

Xu, C.J., P.D. Fraser, W.J. Wang and P.M. Bramley. 2006. Differences in the carotenoid content of ordinary citrus and lycopene-accumulating mutants. J. Agric. Food Chem. 54, 5474–5481.

Xu, Q., Y. Liu, A. Zhu, X. Wu, J. Ye, K. Yu, W. Guo and X. Deng. 2010. Discovery and comparative profiling of micro RNAs in a sweet orange red-flesh mutant and its wild type. BMC Genomics. 11, 1471–2164.

Yamada H., K. Hamamoto, A. Sugiura, T. Tomana. 1988. Effects of controlled fruit temperature on maturation of apple fruits. J. Jpn. Soc. Hortic. Sci. 57, 173–177.

Youryon, P.S. and S. Supapvanich. 2019. Effect of canopy positions on physicochemical quality of Mandarin Fruit cv. 'Shogun' during Storages. Int. J. Automot. Technol. 15, 183–194.

Zhishen, J., T. Mengcheng and W. Jianming. 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 64, 555-559.

Amarante, C., Banks, NH. and Ganesh, S. 2001. Relationship between character of skin cover of coated pears and permeance to water vapour and gases. Posthar Biol Tech. 21, 291–301.

Ara, N. and Nur, H. 2009. In vitro antioxidant activity of methanolic leaves and flowers extracts of Lippia alba. Res J Medicine Med Sci. 4, 107-110.

Arnao, MB., Cano, A. AND Acosta, M. 2001. The hydrophilic and lipophilic contribution to total antioxidant activity. Food Chem. 73, 239-244.

Asrey, R., Sasikala, C. and Singh, D. 2012. Combinational impact of Debaryomyces hansenii bioagent and 1-MCP on shelf life and quality attributes of Kinnow mandarin. Hort. Flor Res Spec. 1, 103-109.

Autio, WR. and Bramgage, WJ. 1982. Effects of AVG on maturation, ripening, and storage of apples. J. Amer Soc Hort Sci 107, 1074–1077.

Azoulay Shemer, T., Harpaz-Saad, S., Belausov, E., Lovat, N., Krokhin, O., Spicer, V., Standing, K.G., Goldschmidt, E.E., and Eyal, Y. 2008. Citrus chlorophyllase dynamics at ethylene-induced fruit color-break: A study of chlorophyllase expression, post-translational processing kinetics and in-situ intracellular localization. Plant Physiol. 148, 108–118.

Black, A.; White, B. Page title. URL (http://etc.) (most recent access date). Blankenship, S.M. and Dole, J.M. 2003. 1-Methylycyclopropene: a review. Postharvest. Biol. Technol. 28, 1–25.

Blankenship, S.M. and Dole, J.M. 2003. 1-Methylycyclopropene: a review. Postharvest. Biol. Technol. 28, 1–25.

Bregoli, AM., Scaramagli, S., Costa, G., Sabatini, E., Ziosi, V., Biondi, S. and Torrigiani, P. 2002. Peach (*Prunus persica*) fruit ripening: aminoethoxyvinylglycine (AVG) and exogeneous polyamines affect ethylene emission and flesh flrmness. Physiol Plant 114, 472–481.

Byers, RE. 1997. Effects of aminoethoxyvinylglycine (AVG) on preharvest fruit drop and maturity of 'Delicious' apples. J. Tree Fruit Prod. 2, 53–75.

Cancel, JD., Larsen, PB. 2002 Loss-of-function mutations in the ethylene receptor ETR1 cause enhanced sensitivity and exaggerated response to ethylene in Arabidopsis. Plant Physiol., 129,1557–1567.

Chervin, C., El-Kereamy, A., Roustan, JP., Latche, A., Lamon, J. and Bouzayen, M. 2004. Ethylene seems required for the berry development and ripening in grape, a non-climacteric fruit. Plant Sci. 167, 1301–1305.

Curl, A. I. and Bailey, G. F. 1957. The carotenoids of Ruby Red grapefruit. Food Res. 22, 63-68.

Di Mascio, P., Kaiser, S. and Sies, H. 1989. Lycopene as the most efficient biological carotenoid singlet oxygen quencher. Arch. Biochem. & Bioph. 274, 532-538.

Cin, VD., Rizzini, FM., Botton, A. and Tonutti, P. 2006. The ethylene biosynthetic and signal transduction pathways are differently affected by 1-MCP in apple and peach fruit. Posthar Biol Tech 42, 125–133.

Elfving, D.C., Drake., S.R., Reed, A.N. and Visser, D.B. 2007. Preharvest applications of sprayable 1-methylcyclopropene in the orchard for management of apple harvest and postharvest condition. HortScience 42, 1192–1199.

Fan, X., Blankenship, S.M. and Mattheis, J.P. 1999. 1-methylcyclopropene inhibits apple ripening. Journal of the American Society for Horticultural Sciences 124, 690-695.

Fish, W.W., Perkins-Veazie, P., Collins, J.K., 2002. A quantitative assay for lycopene that utilizes reduced volumes of organic solvents. J. Food Comp. Anal. 15, 309-317.

Food and Agriculture Organization of United nations (FAO). 2004. Products year report. The State of Food Insecurity in the World, Rome, Italy.

García-Luis, A., Fornés, F. and Guardiola, J.L. 1986. Effects of gibberellin A3 and citoquinins on natural and post-harvest, ethylene-induced pigmentation of Satsuma mandarin peel. Physiol. Plant. 68, 271-274.

Greene, DW. and Schupp, JR. 2004. Effect of aminoethoxyvinylglycine (AVG) on preharvest drop fruit quality and maturation of 'McIntosh' apples. Hort. Sci. 39, 1036–1041.

Garner, D., Crisosto, CH. and Otieza, E. 2001. Controlled atmosphere storage and aminoethoxyvinylglycine postharvest dip delay post cold storage softening of 'Snow King' peach. HortTech. 11, 598–602.

Goldschmidt, EE. 1997. Ripening of citrus and other non-climacteric fruits: a role for ethylene. Acta Hort. 463, 335–340.

Goldschmidt, EE., Huberman, M. and Goren, R. 1993. Probing the role of endogenous ethylene in degreening of citrus-fruit with ethylene antagonists. J Plant Growth Regul. 1993. 12, 325–329.

Golding, J.B., Shearer, D., Wyllie, S.G. and McGlasson, W.B. 1998. Application of 1-MCP and propylene to identify ethylene-dependent ripening processes in mature banana fruit. Postharvest Biol. Technol. 14, 87–98.

Harpaz-Saad, S., Azoulay, T., Arazi, T., Ben-Yaakov, E., Mett, A., Shiboleth, YM., Hörtensteiner, S., Gidoni, D., Gal-On, A. and Goldschmidt, EE. 2007. Chlorophyllase is a rate-limiting enzyme in chlorophyll catabolism and is posttranslationally regulated. Plant Cell. 19, 1007–1022

Henry, L. K., Catignani, G. L.; Schwartz, S. J. 1998. Oxidative degradation kinetics of lycopene, lutein, 9-cis and all-trans â-Carotene. J. Am. Oil Chem. Soc. 75, 823-829.

Hirayama, O., Nakamura, K., Hamada, S. and Kobayasi, Y. 1994. Singlet oxygen quenching ability of naturally occuring carotenoids. Lipids. 29, 149-150.

Hoeberichts, FA. and Woltering, EJ. 2002. Multiple mediators of plant programme cell death: interplay of conserved cell death mechanisms and plant-specific regulators. Bioassays. 25, 47–57.

Iannetta, PPM., Laarhovenb, LJ., Medina-Escobar, N., James, EK., McManuse, MT., Davies, HV. and Harren, FJM. 2006. Ethylene and carbon dioxide production by developing strawberries show a correlative pattern that is indicative of ripening climacteric fruit. Physiologia Plantarum 127, 247–259

Jacob-Wilk, D., Holland, D., Goldschmidt, EE., Riov, J. and Eyal, Y. 1999. Chlorophyll breakdown by chlorophyllase: isolation and functional expression of the Chlase1 gene from ethylene-treated Citrus fruit and its during development. The Plant Journal, 20, 653-661.

Kaewsuksaeng, S. and Sangwanangkul, P. 2015. Bioactive compound contents in citrus family locally cultivated in southern Thailand. AER. J., Khon Kaen, Thailand.

Kaewtubtim, M. and Issarakraisil. 2011. Effects of nitrogen and zinc on fruit quality of pummelo cv. Tubtim Sayam. In Commission on Higher Education Congress IV University Staff Development Consortium (Page 44), Chanburi

Kapur A, Hasković A, Čopra-Janićijević A, Klepo L, Topčagić A, Tahirović I, Sofić E. 2012. Spectrophotometric analysis of total ascorbic acid content in various fruits and vegetables. Bull Chem Technol Bosnia Herzegovina. 38, 39–42.

Katz, E., Lagunes, PM., Riov, J., Weiss, D. and Goldschmidt, EE. 2004. Molecular and physiological evidence suggests the existence of a system II-like pathway of ethylene production in non-climacteric citrus fruit. Planta. 219, 243–252.

Krings, U., and Berger, R. G. 2001. Antioxidant activity of some roasted foods. Food Chemistry. 72, 223-229.

Khan, M. U. D. and MacKinney, G. 1953. Carotenoids in grapefruit, *Citrus paradisi*. J. Plant Physiol. 28, 550-552.

Khan, AS. and Singh, Z. 2007. 1-MCP regulates ethylene biosynthesis and fruit softening during ripening of 'Tegan Blue' plum. Posthar Biol Tech. 43, 298-306.

Kumbhar, B.K., Agarwal, R.S. and Das, K. 1981. Thermal properties of fresh and frozen fish. International Journal of Refrigeration. 4: 143-146.

Laamim, M., Ait Oubahou, A. and Benichou, M. 2005. Effect of 1-methylcyclopropene on the quality of Clementine mandarin fruit at ambient temperature. J Food Agr Environ. 3, 34-36.

Lee, H. S. and Castle, W. S. 2001. Seasonal changes of carotenoid pigments and color in Hamlin, Earlygold, and Budd Blood orange juices. J. Agric. Food Chem. 49, 877-882.

Machado, M.F., Oliveira, F.A.R. and Gekas, V. 1997. Modelling water uptake and soluble solids losses by puffed breakfast cereal immersed in water or milk. In Proceedings of the Seventh International Congress on Engineering and Food, Brighton, UK.

Martinez, XC., Alonso, JM., Sanchez, B. and Granel ,A. 2002. Ethylene perception and response in citrus fruit. In: Kanellis AK and Grierson D. Ed. Kluwer Academic Publisher. 137-142, The Netherlands.

Moran, R., 1982. Formulae for determination of chlorophyll pigments extracted with N,N-dimethylformamide. Plant Physiol. 69, 1376–1381.

Minguez-Mosquera, M.I. and Garrido-Ferntmdez, J. 1989. J. Agric. Food Chem. 37,1

Minguez-Mosquera, M. I., Hornero-Mendez, D. 1993. Separation and Quantification of the Carotenoid Pigments in Red Peppers (*Capsicum annuum* L.) Paprika, and Oleoresin by Reversed Phase HPLC. J. Agric. Food Chem. 41, 1616-1620.

Minguez-Mosquera, M.I. and Hornero-Mendez, D. 1994. Changes in carotenoid esterification during the fruit ripening of Capsicum annuum Cv. Bola. J. Agric. Food Chem. 42, 640–644.

Neter, J., Kutner, M.H., Nachtsheim, C.J. and Wasserman, W. 1966. Applied linear statistical models Irwin, Chicago, 1289-1293.

Olson, J. A. 1989. Provitamin-A function of carotenoids: the conversion of β-carotene into vitamin-A. J. Nutr. 119, 105–108.

Porat, R., Weiss, B., Cohen, L., Daus, A, Goren, R. and Droby, S. 1999. Effects of ethylene and 1-methylcyclopropene on the postharvevst qualities of 'Shamouti' oranges. Postharvest Biol. & Technol. 15, 155-163.

Palozza, P. and Krinsky, N. I. 1991. Free Radical Biol. Med. 11, 407

Pongprasert, N. and Srilaong, V. 2014. A novel technique using 1-MCP microbubbles for delaying postharvest ripening of banana fruit. Postharvest Biology and technology. 95, 42-45.

Pongprasert, N., Boonyaritthongchi, P. and Srilaong, V. 2012. 1-MCP Micro and Nanobubbles using as a Novel Posthavest Technique for Delay Posthavest Ripening of Banana Fruits. 10th National Posthavest Conference, Khon Khen, Thailand

Promkaew, P., Pongprasert, N. and Srilaong, V. 2015. Effect of 1-Methylcyclopropene Microbubbles on Delaying of Senescence in 'Khai' Banana by Spraying. Agricultural Sci. J. 46, 841-844.

Purvis, A. and Barmore, Ch. 1981. Involvement of ethylene in chlorophyll degradation in peel of Citrus fruits. Plant Physiol. 68, 854-856.

Rouseff, R. L., Sadler, G. D., Putnam, T. J. and Davis, J. E. 1992. Determination of β-carotene and other hydrocarbon carotenoids in red grapefruit cultivars. J. Agric. Food Chem. 40, 47-51.

Sigal-Escalada, V. 2006. Interactions of AVG, MCP and heat treatment on apple fruit ripening and quality after harvest and cold storage. Doctoral Dissertations (pp.448). University of Kentucky, USA.

Singleton, V.L., Orthofer, R., Lamuela-Raventos, R.M., 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods in Enzymology. 299, 152-178.

Sisler, EC. and Serek, M. 1997. Inhibitors of ethylene responses implants at the receptor level: recent developments. Physiol Plant. 100, 577-582.

Stahl, W. and Sies, H. 1996. Perspectives in biochemistry and biophysics. Lycopene: A biologically important carotenoid for humans. Arch. Biochem. & Biophys. 336, 1-9

Stewart, I. and Wheaton, TA. 1972. Carotenoids in citrus their accumulation induced by ethylene. J Agric Food Chem. 20:448–449.

Tadmala, N. 2014. Chlorophyll degradation mechanism and its control I lime fruit. Master degree thesis (pp.53). King Moungkut's University of Technology Thonburi university, Bangkok, Thailand.

Takahashi, M., 2005. Potential of microbubbles in aqueous solutions: electrical properties of the gas-water interface. J. Phys. Chem. 109, 21852-21868.

Tavallali, V., dan, M., Moghadam, M. 2015. Postharvest application of AVG and 1MCP enhance quality of 'Kinnow' mandarin during cold storage. International Journal of Farming and Allied Sciece. 4, 526-535.

Tavares, S. et al. 2003. Qualidade ós-Colheita em tangor 'Murcott' tratado com biorregulador 1-metilciclopropeno e armazenado sob refrigeração.Revista Laranja. 24, 491-506.

Thomson, F. M. 1984. Storage of particulate solids. In M. E. Fayed and L. Otten (Eds.). Handbook of powder science and technology Van Nostrand Reinhold, New York. 365-463.

Trainotti, L., Pavanello, A. and Casadoro, G. 2005. Different ethylene receptors show an increased expression during the ripening of strawberries: does such an increment imply a role for ethylene in the ripening of these non-climacteric fruits. J Exp Bot. 56, 2037–2046.

Tian, M. S. et al. 2000. Responses of strawberry fruit to 1methylcyclopropene (1-MCP) and ethylene. Plant Growth Regulation. 32, 83-90.

Trebitsh, T., Goldschmidt, E.E. and Riov, J. 1993. Ethylene induces de novo synthesis of chlorophyllase, a chlorophyll degrading enzyme, in citrus fruit peel. Proc. Natl. Acad. Sci, USA. 90, 9441-9445

Vera, J., Pongprasert, N., Jitareerat P., Boonyaritthongchai, P. and Srilaong, V. 2017. 1-MCP Microbubbles Influence in Fruit Color and Quality of Dragon Fruit. Agricultural Sci. J. 48, 237--240

Office of Agricultural Economics. (2015). Information of Pomelo production. (cited 12 April 2015) Available from: URL: http://www.oae.go.th/maim.php

Office of Agricultural Economics. (2013). Basic Information of Agricultural Economics in 2013. (cited 5 November 2016) Available from: URL: http://www.oae.go.th/download/download_journal/yearbook56.pdf

Athipanyakul, T., and S. Chancharat. 2014. Production system and marketing of pomelo in the upper Northeast region, Thailand project. Full report: Thailand Research Fund.

Cunningham, F. X. and E. Gantt. 1998. Genes and enzymes of carotenoid biosynthesis in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 49, 557–583.

Cunningham, F. X., B. Pogson, Z. Sun, K. A. McDonald, D. DellaPenna and E. Gantt. 1996. Functional analysis of the β and ε lycopene cyclase enzymes of Arabidopsis reveals a mechanism for control of cyclic carotenoid formation. Plant Cell 8, 1613–1626.

Ronen, G., M. Cohen, D. Zamir and J. Hirschberg. 1999. Regulation of carotenoid biosynthesis during tomato fruit development: expression of the gene for lycopene epsilon-cyclase is downregulated during ripening and is elevated in the mutant Delta. Plant J. 17, 341–351.

Alquézar, B., L. Zacarías and M. J. Rodrigo. 2009. Molecular and functional characterization of a novel chromoplast-specific lycopene β-cyclase from Citrus and its relation to lycopene accumulation. J. Exp. Bot. 60, 1783–1797.

Kato, M., Y. Ikoma, H. Matsumoto, M. Sugiura, H. Hyodo and M. Yano. 2004. Accumulation of carotenoids and expression of carotenoid biosynthetic genes during maturation in citrus fruit. Plant Physiol. 134, 824–837.

Kita, M., M. Kato, Y. Ban, C. Honda, H. Yaegaki, Y. Ikoma and T. Moriguchi. 2007. Carotenoid accumulation in Japanese Apricot (*Prunus mume* Siebold & Zucc.): molecular analysis of carotenogenic gene expression and ethylene regulation. J. Agric. Food Chem. 55, 3414–3420.

Park, H., S. Kreunen, A. J. Cuttriss, D. DellaPenna and B. J. Pogson. 2002. Identification of the carotenoid isomerase provides insight into carotenoid biosynthesis, prolamellar body formation, and photomorphogenesis. Plant Cell 14, 321– 332.

Pogson, B., K. A. McDonald, M. Truong, G. Britton and D. DellaPenna. 1996. Arabidopsis carotenoid mutants demonstrate that lutein is not essential for photosynthesis in higher plants. Plant Cell 8, 1627–1639.

Fraser, P. D., M. R. Truesdale, C. R. Bird, W. Schuch and P. M. Bramley. 1994. Carotenoid biosynthesis during tomato fruit development. Plant Physiol. 105: 405–413.
Giuliano, G., G. E. Bartley and P. A. Scolnik. 1993. Regulation of carotenoid biosynthesis during tomato development. Plant Cell 5, 379–387.

Isaacson, T., G. Ronen, D. Zamir and J. Hirschberg. 2002. Cloning of tangerine from tomato reveals a carotenoid isomerase essential for the production of β-carotene and xanthophylls in plants. Plant Cell 14, 333–342.

Ronen, G., M. Cohen, D. Zamir and J. Hirschberg. 1999. Regulation of carotenoid biosynthesis during tomato fruit development: expression of the gene for lycopene epsilon-cyclase is downregulated during ripening and is elevated in the mutant Delta. Plant J. 17, 341–351.

Bramley, P. M. 2002. Regulation of carotenoid formation during tomato fruit ripening and development. J. Exp. Bot. 53, 2107– 2113.

Pecker, I., R. Gabbay, F. X. Cunningham and J. Hirschberg. 1996. Cloning and characterization of the cDNA for lycopene βcyclase from tomato reveals decrease in its expression during fruit ripening. Plant Mol. Biol. 30, 807–819.

Stewart, I. and Wheaton, T.A. 1973a Carotenoids in citrus. Proceedings of the 1st International Citrus Congress 3, 325-330

Gross, J., Gabay, M. and Lifshitz, A. 1972. A comparative study of the carotenoid pigments in juice of Shamouti, Valencia and Whasington oranges, three varieties of *Citrus sinensis*. Phytochemistry 11, 303-308.

Stewart, I. 1977a. High performance liquid chromatographic determination of provitamin A in orange juice. Journal of the Association of Official Analytical Chemists 60, 132-136.

Stewart, I. 1977b. Provitamin A and carotenoid content of citrus juices. Journal of Agricultural and Food Chemistry 25, 1132-1137.

Stewart, I. 1980. Color as related to quality in citrus. In: Nagy S, Attaway A (Eds) Citrus Nutrition and Quality, American Chemical Society, Washington, DC, 129-150.

Dhuique Mayer C, Caris Veyrat C, Ollitrault P, Curk F, Amiot MJ (2005) Varietal and interspecific influence on micronutrient contents in citrus from the Mediterranean area. Journal of Agricultural and Food Chemistry 53, 2140-2145

Matsumoto, H., Ikoma, Y., Kato, M., Kuniga, T., Nakajima, N. and Yoshida, T. 2007. Quantification of carotenoids in Citrus fruit by LC-MS and comparison of patterns of seasonal changes for carotenoids among Citrus varieties. Journal of Agricultural and Food Chemistry 55, 2356-2368.

Meléndez Martínez, A.J., Britton, G., Vicario, I.M. and Heredia, F.J. 2005. Identification of zeinoxanthin in orange juices. Journal of Agricultural and Food Chemistry 53, 6362-6367.

Fanciullino, A.L., Dhuique-Mayer. C., Luro,, F., Morillon, R. and Ollitrault, P. 2007. Carotenoid biosynthetic pathway in the Citrus genus: number of copies and phylogenetic diversity of seven genes. Journal of Agricultural and Food Chemistry 55, 7405-7417.

Oberholster, R., Cowan, A.K., Molnar, P. and Toth, G. 2001. Biochemical basis of color as an aesthetic quality in Citrus sinensis. Journal of Agricultural and Food Chemistry 49, 303-307.

Gross, J. and Timberg, R. 1983. Pigment and ultrastructural changes in the developing pummelo Citrus grandis 'Goliath'. Botanical Gazette 144, 401-406.

Murata, T. 2001. Citrus, In S. K. Mitra, en. Postharvest physiology and storage of tropical and subtropical fruits. CAB international, p. 21-46.

Kader, A.A., 2008. Flavor quality of fruit and vegetables. J. Sci. Food Agric. 88, 1863–1868.

Porat, R., Weiss, B., Cohen, L., Daus, A., Goren, R., Droby, S., 1999. Effects of ethylene and 1-methylcyclopropene on the postharvest qualities of Shamouti oranges. Postharvest Biol. Technol. 15, 155–163.

Saltveit, M.E., 2016, "Ethylene Effects", In Agriculture Handbook Number 66: The Commercial Storage of Fruits Vegetables and Florist and Nursery Stocks, U.S. Department of Agriculture, Agricultural Research Service, Beltsville, Maryland, pp. 76-82.

Saltveit, M.E., 1999, "Effect of Ethylene on Quality of Fresh Fruits and Vegetables", Postharvest Biology and Technology, Vol. 15, No. 3, 279-292.

Kammapana, L., 2014, Study on Chlorophyll Degradation Mechanism of Dragon Fruit Bract and its Control by Uv Treatment, Ph.D. thesis, Postharvest Technology, Bioresources and Technology, King Mongkut's University of Technology, 218.

Blasiak, J. and Musgrave, M.E., 2002, "Varietal Differences in Locular Gas Composition in Developing Fruit of Sweet and Hot Peppers, Capsicum Spp., and Evidence for Divergent Diffusion Pathways", The Journal of Horticultural Science and Biotechnology, 77, 432-437.

Díaz-Pérez, J.C., Muy-Rangel, M.D., and Mascorro, A.G., 2007, "Fruit Size and Stage of Ripeness Affect Postharvest Water Loss in Bell Pepper Fruit (*Capsicum annuum* L.)", Journal of the Science of Food and Agriculture, 87, 68-73.

Lownds, N., Banaras, M., and Bosland, P., 1993, "Relationships between Postharvest Water Loss and Physical Properties of Pepper Fruit (*Capsicum annuum* L.)", HortScience, Vol. 28, 1182-1184.

Yahia, E.M., 1991, "Production of Some Odor-Active Volatiles by 'Mcintosh' Apples Following Low-Ethylene Controlled-Atmosphere Storage.", HortScience, 26, 1183-1185.

Baritelle, A.L., Hyde, G.M., Fellman, J.K., and Varith, J., 2001, "Using 1-MCP to Inhibit the Influence of Ripening on Impact Properties of Pear and Apple Tissue", Postharvest Biology and Technology, 23, 153-160.

Tian, M., Downs, C., Lill, R., and King, G., 1994, "A Role for Ethylene in the Yellowing of Broccoli after Harvest", Journal of the American Society for Horticultural Science, 119, 276-281.

Tian, M., Downs, C., Lill, R., and King, G., 1994, "A Role for Ethylene in the Yellowing of Broccoli after Harvest", Journal of the American Society for Horticultural Science, 119, 276-281.

Watkins, C.B., 2016, "1-Methylcyclopropene (1-MCP)", Agriculture Handbook Number 66: The Commercial Storage of Fruits Vegetables and Florist and Nursery Stocks, U.S. Department of Agriculture, Agricultural Research Service, Beltsville, Maryland, 83-88.

Li, L., Lichter, A., Chalupowicz, D., Gamrasni, D., Goldberg, T., Nerya, O., Ben-Arie, R., and Porat, R., 2016, "Effects of the Ethylene-Action Inhibitor 1-methylcyclopropene

on Postharvest Quality of Non-Climacteric Fruit Crops", Postharvest Biology and Technology, 111, 322-329.

Merchante, C., Vallarino, J.G., Osorio, S., Aragüez, I., Villarreal, N., Ariza, M.T., Martínez, G.A., Medina-Escobar, N., Civello, M.P., Fernie, A.R., Botella, M.A., and Valpuesta, V., 2013, "Ethylene Is Involved in Strawberry Fruit Ripening in an Organ-Specific Manner", Journal of experimental botany, 64, 4421-4439.

Villarreal, N.M., 2010, "Effect of Ethylene and 1-MCP Treatments on Strawberry Fruit Ripening", Journal of the Science of Food and Agriculture, 90, 683-689.

Deaquiz, Y.A., Álvarez-Herrera, J., and Fischer, G., 2014, "Ethylene and 1-MCP Affect the Postharvest Behavior of Yellow Pitahaya Fruits (Selenicereus megalanthus Haw.)", Agronomía Colombiana, 32, 44-51.

Blankenship, S.M., 2001, "Ethylene Effects and the Benefits of 1-MCP", Perishables Handling Quarterly, 108, 2-4.

Fernando, H.R.P., 2013, Effect of 1-Mcp Microbubble Immersion on Antioxidant Properties, Chemical Composition and Quality of Banana, MSc thesis, Postharvest Technology, Bioresources and Technology, King Mongkut's University of Technology, 118.

Biswas, P., East, A.R., Hewett, E.W., and Heyes, J.A., 2014, "Ripening Delay Caused by 1-MCP May Increase Tomato Chilling Sensitivity", New Zealand Journal of Crop and Horticultural Science. 42, 145-150.

Manganaris, G.A., Crisosto, C.H., Bremer, V., and Holcroft, D., 2008, "Novel 1-Methylcyclopropene Immersion Formulation Extends Shelf Life of Advanced Maturity 'Joanna Red'plums (Prunus salicina Lindell)", Postharvest Biology and Technology, Vol. 47, 429-433.

Takahashi, M., Chiba, K., and Li, P., 2007, "Free-Radical Generation from Collapsing Microbubbles in the Absence of a Dynamic Stimulus", The Journal of Physical Chemistry B, 111, 343-1347.

Matsumoto, M. and Tanaka, K., 2008, "Nano Bubble—Size Dependence of Surface Tension and inside Pressure", Fluid Dynamics Research, 40, 546-553.

ACKNOWLEDGEMENTS

I would like to express my sincerest appreciation to my advisor, Professor Satoru Kondo for valuable advice and suggestions, reviewing the manuscript and his valuable encouragement throughout the course of my Ph.D. program.

I would like to express my sincerest appreciation to my committee, Prof. Hitoshi Ohara, Prof. Miyoshi Kazumitsu, Prof. Yoshihiro Nishida and Associate Professor Varit Srilaong for their encouragement and assistance which were essential for completion of thesis. I also express my warm gratitude and cordial thanks, Associate Professor Dr. Varit Srilaong, at King Mongkut's University of Technology Thonburi, Thailand, for giving me the opportunity to further study in Japan and valuable advice and suggestions, reviewing the manuscript.

I would like to thank to MEXT scholarship for supporting a scholarship throughout my study in Japan. I also would like to express my sincerest appreciation to all members in the Pomology Laboratory of Graduate School of Horticulture, Chiba University, Japan, for their kindly suggestion and assistances for completion of thesis.

Moreover, I would like to thank all staffs of the Graduate School of Horticulture, Chiba University for their kindness and support. I feel very grateful for the Petchra Pra Jom Klao Ph.D. Scholarship of my colleagues King Mongkut's University of Technology Thonburi, Thailand for supporting a scholarship during study in Japan. I also would like to express my sincerest appreciation to all members in the School of Bioresources and Technology King Mongkut's University of Technology Thonburi

Last but not the least; I would like to thank my family for their love and encouragement.

LIST OF PUBLICATION AND PRESENTATION

Research articles:

1. Promkaew, P., Pongprasert, N., Wongs-Aree, C., Kaewsuksaeng, S., Opio, P., Kondo, S. and Srilaong, V., Carotenoids accumulation and carotenoids biosynthesis gene expression during fruit development in pulp of 'Tubtim siam' pummelo fruit Scientia Horticulturae. 260, 2020. (Accepted: September/2019)
2. Promkaew, P., Pongprasert, N., Wongs-Aree, C., Kaewsuksaeng. S., Srilaong, V. and Kondo, S. Lycopene synthesis and related gene expression in pummelo pulp increased in shade-grown fruit. American Society for Horticultural Science (Accepted: October/2019)
3. Promkaew, P., Pongprasert, N., Wongs-Aree, C., Kaewsuksaeng., Kondo, S. and Srilaong, V., Application of AVG or 1-MCP-MBs on Postharvest Quality of 'Tubtim siam' pummelo fruit (*Citrus maxima* (Burm.) Merr.). Food and Applied Bioscience Journal, 7, 55-71, 2019. (Accepted: March /2019)

Conferences:

I. The 3rd International Conference on Agriculture and Agro-Industry 2018 (ICAAI2018) under the theme of "Food and Agriculture: Innovation and Sustainability". Application of AVG or 1-MCP-MBs on postharvest quality of pummelo cv. 'Tubtim Siam' (*Citrus maxima* Burm.)

II. International Joint Conference on JSAM and SASJ, and CIGR VI Technical Symposium joining FWFNWG and FSWG Workshops. Postharvest ethylene influences biochemical quality of pummelo fruit under low temperatu