แอกทิวิตีของเอนไซม์แอนติออกซิเดนต์ การเปลี่ยนแปลงของโปรตีนและการสะสมทรานสคริปต์ ของเซลล์วอลล์ไฮโดรเลสที่สัมพันธ์กับมะม่วงพันธุ์น้ำดอกไม้และพันธุ์อกร่องที่ผ่านการจุ่มน้ำร้อน

นางสาวศรัญญา ยิ้มย่อง

ศูนย์วิทยทรัพยากร
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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

ANTIOXIDANT ENZYME ACTIVITIES, PROTEIN CHANGES AND TRANSCRIPT ACCUMULATION OF CELL WALL HYDROLASES ASSOCIATED WITH HOT WATER IMMERSION TREATMENT OF 'NAM DOK MAI' AND 'OK RONG' MANGOES

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A Dissertation Submitted in Partial Fulfillment of the Requirements
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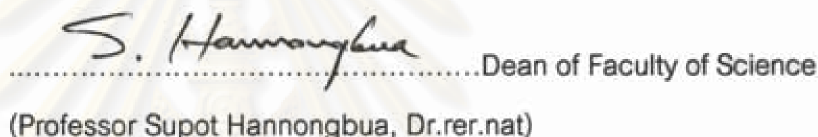
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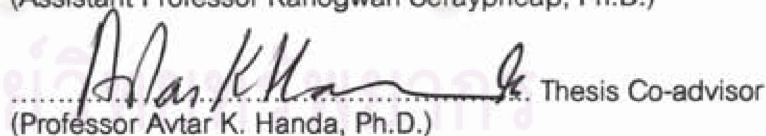
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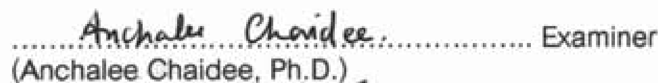
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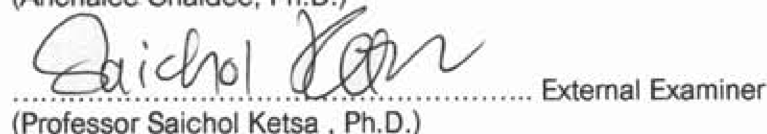
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ศรัญญา ยิ้มย่อง: แอกทิวิตีของเอนไซม์แอนติออกซิแดนต์ การเปลี่ยนแปลงของโปรตีนและการสะสมทรานสคริปต์ของเซลล์วอลล์ไฮโดรเลสที่สัมพันธ์กับมะม่วงพันธุ์น้ำดอกไม้และพันธุ์อกร่องที่ผ่านการจุ่มน้ำร้อน (ANTIOXIDANT ENZYME ACTIVITIES, PROTEIN CHANGES AND TRANSCRIPT ACCUMULATION OF CELL WALL HYDROLASES ASSOCIATED WITH HOT WATER IMMERSION TREATMENT OF 'NAM DOK MAI' AND 'OK RONG' MANGOES) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ดร. กนกวรรณ เสรีภาพ, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: Prof. Avtar K. Handa, Ph.D., 110 หน้า.

มะม่วงพันธุ์น้ำดอกไม้และอกร่องเก็บเกี่ยวระยะที่ผลเจริญเต็มที่ และแบ่งเป็นสองกลุ่มโดยกลุ่มแรกจุ่มในน้ำร้อนที่อุณหภูมิ 50 องศาเซลเซียส เป็นเวลา 10 นาที อีกกลุ่มจุ่มในน้ำที่อุณหภูมิ 30 องศาเซลเซียส เป็นเวลา 10 นาที นำไปเก็บรักษาที่อุณหภูมิ 8 และ 12 องศาเซลเซียส บันทึกการเปลี่ยนแปลงของผลทุกห้าวันเป็นเวลาสิบห้าวัน จึงย้ายออกมาเพื่อให้สุกที่อุณหภูมิห้อง หลังจากนั้นเก็บผลทุกวันจนถึงวันที่ยี่สิบของการเก็บรักษา โดยทำการวิเคราะห์หาค่าการสูญเสียน้ำหนักสด ค่าความแน่นเนื้อ สีเปลือก ปริมาณของแข็งที่ละลายน้ำได้ และปริมาณกรด พบว่ามะม่วงมีค่าการสูญเสียน้ำหนักสดและปริมาณของแข็งที่ละลายน้ำได้เพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติ ในขณะที่ค่าความแน่นเนื้อและปริมาณกรดลดลงอย่างมีนัยสำคัญทางสถิติ มะม่วงเก็บรักษาที่อุณหภูมิ 8 องศาเซลเซียสคงความเขียวมากกว่ามะม่วงที่เก็บรักษาที่อุณหภูมิ 12 องศาเซลเซียสจนถึงวันที่ยี่สิบของการเก็บรักษา การจุ่มน้ำร้อนมีผลต่อการสูญเสียน้ำหนักสด ปริมาณกรดและปริมาณของแข็งที่ละลายน้ำได้ในมะม่วงทั้งสองสายพันธุ์ มะม่วงที่ผ่านการจุ่มน้ำร้อนก่อนการเก็บรักษามีการผลิตเอทิลีนลดลงในระหว่างการเก็บรักษา การวิเคราะห์โปรตีน lipoxygenase (LOX) และเอนไซม์ในกลุ่มแอนติออกซิแดนต์ในเปลือกและเนื้อมะม่วงพบว่ามะม่วงที่ผ่านการจุ่มน้ำร้อนมีแอกทิวิตีของเอนไซม์ CAT, APX และ GR สูงกว่ามะม่วงชุดควบคุมในวันที่สิบห้า เปลือกมะม่วงมีการตอบสนองต่อการจุ่มน้ำร้อนมากกว่าเนื้อมะม่วง รูปแบบของโปรตีนที่ผ่านการตรวจสอบโดยวิธี SDS-PAGE ความเข้มข้น 10% พบแถบโปรตีนที่เห็นได้ชัดจำนวน 29 แถบ ซึ่งมีน้ำหนักโมเลกุลระหว่าง 12-347 กิโลดาลตัน การจุ่มน้ำร้อนสามารถเพิ่มการสะสมของโปรตีน LOX ในผลมะม่วง การตรวจสอบการสะสมทรานสคริปต์ของพบว่ามีการสะสมทรานสคริปต์ของ SOD ในมะม่วงที่ผ่านการจุ่มน้ำร้อนในระหว่างการเก็บรักษาที่อุณหภูมิต่ำ การจุ่มน้ำร้อนชะลอการสะสมทรานสคริปต์ของ pectate lyase (PL) และ β-galactosidase ในระหว่างการสุกที่อุณหภูมิห้อง จากผลการทดลองทั้งหมดเป็นหลักฐานสนับสนุนว่าการจุ่มน้ำร้อนก่อนการเก็บรักษามีผลต่อการสุกของผลมะม่วงน้ำดอกไม้และอกร่อง

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4873893023 : MAJOR BIOLOGICAL SCIENCES
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SARUNYA YIMYONG: ANTIOXIDANT ENZYME ACTIVITIES, PROTEIN CHANGES AND TRANSCRIPT ACCUMULATION OF CELL WALL HYDROLASES ASSOCIATED WITH HOT WATER IMMERSION TREATMENT OF 'NAM DOK MAI' AND 'OK RONG' MANGOES. THESIS ADVISOR: ASST. PROF. KANOGWAN SERAYPHEAP, Ph.D., THESIS CO-ADVISOR: PROF. AVTAR K. HANDA, Ph.D., 110 pp.

'Nam Dok Mai' and 'Ok Rong' mango were harvested at the green-mature stage. The fruits were divided into two groups and treated with hot water at 50±1°C for 10 min and 30±1°C for 10 min as control, and then stored at 8°C and 12°C. Mango fruits were randomly sampled for physiological change analysis every 5 days for 15 days and then transferred to ripen at ambient temperature and sampled everyday for 5 days. Weight loss, pulp firmness, peel color, total soluble solids (TSS) and titratable acidity (TA) were analyzed. The results showed that weight loss and TSS of mangoes increased significantly, while firmness and TA decreased significantly in all treatments during storage. The peel of fruit stored at 8°C remained green longer than that of fruit stored at 12°C until the end of 20 days. Heat treatment affected weight loss, TA and TSS in both cultivars of mangoes. Ethylene production of HWT fruit both stored at 8°C and 12°C were reduced during the storage period. Mango peel and pulp were collected for lipoxygenase (LOX) protein and antioxidant enzyme assays. The activities of catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) in HWT fruit were much higher than those in control fruit on day 15. Mango peel exhibited greater physiologically response to hot water treatment than pulp of mango. Protein pattern of mango peel and pulp were detected by 10% SDS-PAGE showing 29 visible bands with the range of molecular weight from 12 to 347 kDa. Hot water treatment enhanced the accumulation of LOX protein in mango fruit. Transcript accumulations in mango pulp were also investigated. The increase in transcript levels of superoxide dismutase (SOD) was observed in HWT fruit during low temperature storage. Hot water treatment delayed transcript accumulation of pectate lyase (PL) and β-galactosidase during ripening at ambient temperature. Taken together, these results provided supporting evidence that hot water treatment affected 'Nam Dok Mai' and 'Ok Rong' mango fruit ripening.

Field of study: Biological Sciences......Student's signature....Sarunya Yimyong.......
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CHAPTER I

INTRODUCTION

1.1 Rationale

Mango (*Mangifera indica* L.) is a popular tropical fruit crop which has a great value in the world market, and Thailand is one of the major producers and exporters of mango. Among mango cultivars presently cultivated in Thailand, 'Nam Dok Mai' and 'Ok Rong' mangoes are the leading commercial varieties which are mainly offered as fresh fruits both raw as well as ripe fruit. Mango is a climacteric fruit which shows rapid ripening after harvest due to its high perishable nature. Storage and ripening of mango are concerned with many factors, such as cultivar, stage of maturity, size grading, method of harvesting, handling, packing, and mode of transport affect the storability of mango fruits (Tharanathan, Yashoda and Prabha, 2006). Methods to extend shelf life of fresh fruit have included low temperatures storage (Dang, Singh and Swinny, 2008; Shivashankara *et al*., 2006) , hot water treatment (Dang *et al*., 2008; Djioua *et al*., 2009; Jacobi, MacRae and Hetherington, 2001a; Kim, Brecht and Talcott, 2007; Kim, Lounds-Singleton and Talcott, 2009), inhibition of ethylene action (Singh and Dwivedi, 2008; Wang *et al*., 2009), application of plant growth regulators (Ding *et al*., 2007; Wang *et al*., 2008) and modified and controlled atmospheres storage (Kim *et al*., 2007; Pesis *et al*., 2000). Hot water treatment is one of methods in postharvest treatments that is effectively used for insect disinfestation, disease control, delayed ripening and modification of fruit responses to other stresses (Lurie, 1998; Paull and Chen, 2000). Manipulation of storage at low temperature is also used to delay fruit ripening and maintain fruit quality during long distant shipment, storage and marketing.

The process of textural softening is of the commercial importance as it has direct consequence on fruit shelf life quality and the consumer acceptability. Fruit ripening is genetically controlled developmental process that regulates changes in gene expression including changes in transcripts and proteins (Prasanna, Prabha and Tharanathan, 2007). Mango undergoes extensive textural softening during ripening that are associated with production of cell wall hydrolases such as pectate lyase (PL), beta-

galactosidase, beta-1,3-glucanase. Most of these enzymes are present in low levels throughout fruit development, while they increase in activity and show a peak activity at climacteric stage during ripening. Published reports have investigated in cell wall hydrolases on the molecular basis of tissue softening of ripening mango including expansin gene (Sane, Chourasia and Nath, 2005), pectate lyase gene (Chourasia, Sane and Nath, 2006) and endo-β-1,4-glucanase (Chourasia, Sane, Singh and Nath, 2008). The observation with different cell wall degrading enzymes reveal that heat disruption of cell wall breakdown may be associated with mRNA synthesis and stability, or protein synthesis and degradation. In addition to fruit ripening process, mechanical stress, temperature stress, injury and pathogen attack induce the excess production of reactive oxygen species (ROSs) that cause membrane damage and trigger plant defense responses. Lipoxygenase (LOX) catalyses lipid peroxidation that is responsible for membrane degradation due to oxidative deterioration. Plants contain antioxidant enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT) and glutathione reductase (GR) for protection against ROSs (Blokhina, Virolainen and Fagerstedt, 2003). The protective function of these enzymes against different stress conditions has been reported in climacteric fruits such as tomato (Yahia *et al.*, 2007), peach (Zheng *et al.*, 2007), apple (Ahn, Paliyath and Murr, 2007) and also in mango (Kondo, Kittikorn and Kanlayanarat, 2005; Singh and Dwivedi, 2008; Wang *et al.*, 2008, 2009; Zhao *et al.*, 2009). However, the effects of hot water treatment in combination with low temperature storage and the resultant changes in quality, antioxidant enzymes, protein and cell wall hydrolases have not been investigated in mango.

1.2 Hypothesis

This study is focused on the evaluation and the comparison in ‘Nam Dok Mai’ and ‘Ok Rong’ cultivars of mangoes in response to hot water treatments during low temperature storage and ripening at ambient temperature. The hypothesis is that if fruit were treated at appropriate temperature and duration of hot water immersion prior to storage at low temperature, the fruit quality can be enhanced resulting in the prolonging shelf life of fruit. Besides, it is also hypothesized that hot water treatment will promote

antioxidant enzyme activities and delay fruit ripening resulting in low accumulation in transcript level of cell wall hydrolases.

1.3 Objectives

1. To determine the effects of hot water immersion treatment on physiological changes of mango fruits during low temperature storage
2. To evaluate the relationship between hot water treatment and the antioxidant enzyme activities in mango fruits during low temperature storage
3. To investigate the effects of hot water treatment on the changes in protein patterns during low temperature storage of mango fruits by SDS-PAGE and Western blots
4. To examine the effects of hot water treatment on the RNA metabolism including changes in transcripts of Mn-SOD and cell wall hydrolases during low temperature storage of mango fruits by Northern blots

1.4 Anticipated benefit

This study provided understanding at basic level of the physiological and biochemical responses of mango fruit to temperature stress that can enable the development of effective strategies for heat treatment and marketing of this fruit throughout the world.

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CHAPTER II

LITERATURE REVIEWS

2.1 Mango

Mango is one of the tropical fruits known for its flavor and attractive appearance. Morphologically, mango fruit is classified as drupe type which contains a single large seed surrounding with fleshy mesocarp, and the pericarp is divided into three layers: a thin outer skin, the epicarp; an edible fleshy middle layer, the mesocarp; and an inner hard shell, the endocarp (Jacobi, MacRae and Hetherington, 2001b). Nowadays, mango is normally marketed in most countries. The prominent mango producing countries are India, China, Thailand, Pakistan, Mexico, Indonesia, Philippines and Brazil that have data of top mango production based on FAO statistics for the year 2005 as shown in Table 2.1. In addition, There are data of top exporters: mangoes, mangosteens and guavas based on FAO statistics for the year 2007 as shown in Table 2.2.

Table 2.1 Top production of mango in the world (Statistics Division, 2005)

Rank	Commodity	Production(Int$1000)	Production(MT)
1	India	2,629,692	10,800,000
2	China	840,040	3,673,000
3	Thailand	438,282	1,800,000
4	Pakistan	407,578	1,673,900
5	Maxico	365,968	1,503,010
6	Indonesia	359,928	1,478,204
7	Phillippines	231,316	950,000
8	Brazil	206,966	850,000
9	Nigeria	177,748	730,000
10	Egypt	92,526	380,000
11	Viet Nam	77,917	320,000
12	Peru	65,255	268,000
13	Haiti	63,307	260,000
14	Bangladesh	59,168	243,000
15	Cuba	56,003	230,000
16	Madagascar	51,133	210,000
17	Democratic Republic of the Congo	49,494	203,270
18	United Republic of Tanzania	48,698	200,000
19	Sudan	47,481	195,000
20	Guatemala	45,533	187,000

Production in Int$1000 have been calculated based on 1999-2001 international prices.

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Table 2.2 Top exporters: mangoes, mangosteens and guavas in the world (Statistics Division, 2007)

Rank	Commodity	Quantity(tonnes)	Value(1000$)	Unit value($/tonne)
1	India	240,858	163,622	679
2	Maxico	236,004	119,187	505
3	Netherlands	80,598	114,408	1419
4	Brazil	116,271	90,102	775
5	Peru	82,512	63,674	772
6	France	17,165	48,223	2,809
7	Belgium	19,327	45,376	2,348
8	Phillippines	27,068	35,469	1,310
9	Thailand	61,026	34,231	561
10	Israel	16,591	21,742	1,310
11	Pakistan	62,057	20,038	323
12	Ecuador	41,379	19,812	479
13	Spain	6,558	11,747	1,791
14	China	4,943	9,664	1,955
15	Germany	6,040	9,010	1,492
16	Burkina Faso	3,261	8,753	2,684
17	Côte d'Ivoire	16,877	7,999	474
18	Haiti	8,408	6,652	791
19	Guatemala	20,490	6,626	323
20	Yemen	11,404	6,237	547

2.1.1 Cultivars and characteristics

Mango is a member of the family Anacardiaceae which has originated in the Indo-Burma region. Many cultivars of mango are classified into two groups due to their reproduction from seeds to be monoembryonic or polyembryonic. Monoembryonic cultivars are hybrid in origin and must be reproduced by asexual propagation. They are mostly of India origin such as 'Dashehari', 'Alphonso', 'Bombay', 'Neelum' and 'Langra', and some cultivars in Florida such as 'Irwin', 'Tommy Alkins', 'Zill', 'Ruby' and 'Kent'. Polyembryonic cultivars are many embryos which develop from diploid parent nucellar tissue after fertilization of the egg cell. They are mostly of Philippine or Indochinese origin such as, 'Golek', 'Gadung' and 'Arumanis' cultivars in Indonesia; 'Carabao', 'Pico' and 'Pahutan' cultivars in Philippines; 'Khiew Sawoey', 'Nung Klang Wun', 'Nam Dok Mai' and 'Tong Dum' cultivars in Thailand; and 'Kensington' cultivar in Australia (Yahia, 2005; Tharanathan *et al.*, 2006).

In Thailand, there are some cultivars of mango which are preferred to consume in ripe fruit, such as 'Nam Dok Mai', 'Ok Rong', 'Nung Klang Wun', 'Tong Dum' and

‘Maha Chanok’. Cultivar of ‘Nam Dok Mai’ and ‘Ok Rong’ mangoes are the most important varieties on both domestic and international markets. The botanical characteristic of both varieties are described as follows (Department of Agriculture, 2009):

Nam Dok Mai: Oval with a sharp-pointed tip. Ripe fruit has golden-yellow to deep yellow of pulp with a sweet, slightly sour and scented taste.

Ok Rong: Oval with rounded tips. Ripe fruit has high fiber, light yellowish orange of pulp with an absolute sweet and scented taste.

Figure 2.1 ‘Nam Dok Mai’ (A) and ‘Ok Rong’ (B) cultivars of mangoes (http://www.kajohnfresh.com)

2.1.2 Nutritive value

Mango fruit is a rich source of minerals and vitamins essential for human body, especially vitamin C and vitamin A contents as shown in Table 2.3. Ripe mango has the highest level of beta-carotene (pro-vitamin A), while green mango is higher in vitamin C. Mango is a rich source of various polyphenolic compounds including mangiferin, catechins, quercetin, kaempferol, rhamnetin, anthocyanins, gallic and ellagic acids, propyl and methyl gallate, benzoic acid, and protocatechuic acid which are varied in each part (pulp, peel, seed, bark, leaf and flower) (Masibo and He, 2008). Mango peel is a good source of tropical fruit fiber (Larrauri *et al.*, 1996). Ripe peel contained higher amount of anthocyanins and carotenoids than raw peel, whereas raw mango peel had a high of polyphenol content. The mango peel extract showed good antioxidant activity, and may be used in nutraceutical and functional foods (Ajila *et al.*, 2007). In addition, it has also been used for its medicinal value. In India, a drink made from unripe

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mango fruit is used as a remedy for exhaustion and heat stroke, and fruit sap has been used to treat the pain of bee and scorpion stings (Bally *et al*., 2006).

Table 2.3 Nutritional value of raw mango per 100 grams of edible portion (Agricultural Research Service, 2009)

Nutrients	Quantity
Proximates:	
Water	81.71 g
Energy	272 kJ
Protein	0.51 g
Total lipid (fat)	0.27 g
Ash	0.50 g
Carbohydrate	17 g
Fiber, total dietary	1.8 g
Sugars, total	14.80 g
Minerals:	
Calcium, Ca	10 mg
Iron, Fe	0.13 mg
Magnesium, Mg	9 mg
Phosphorus, P	11 mg
Potassium, K	156 mg
Sodium, Na	2 mg
Zinc, Zn	0.04 mg
Copper, Cu	0.110 mg
Manganese, Mn	0.027 mg
Vitamins:	
Vitamin C, total ascorbic acid	27.2 mg
Vitamin B1 (thiamine)	0.058 mg
Vitamin B2 (riboflavin)	0.057 mg
Niacin	0.584 mg
Pantothenic acid	0.160 mg
Vitamin B6	0.134 mg
Carotene, beta	445 mcg
Vitamin A	765 IU
Vitamin E (alpha-tocopherol)	1.12 mg
Lipid:	
Fatty acids, total saturated	0.066 g

2.1.3 Diseases and insect pests

Mango is susceptible to many postharvest diseases such as anthracnose caused by *Colletotrichum gloeosporioides*, alternaria rot (*Alternaria alternata*), black-mold rot (*Aspergillus niger* and *Aspergillus* spp.), stem-end rot (*Lasioidplodia theobromae* Pat.) powdery mildew (*Oidium mangiferae* Berthet and *Oidiopsis* spp.) and die back (*Botryodiplodia theobromae* Pat.). Anthracnose is the most serious disease of mango in areas where rain is widespread during flowering and fruit set because it can

cause destruction of the inflorescences, and infection or drop of young fruit (Pitkethley and Conde, 2007). These can obviously lead to postharvest losses. The insects which infest mango are the mango hopper (*Idioscopus clypeatu*), the mango mealy bug (*Drosicha mangiferae*), the mango fruit fly (*Daccus dorsalis* and *Strumata ferrugineus*), the mango seed borer (*Nozorda albizonalis*), the mango bud mite (*Aceria mangiferae*) and the mango seed weevil (Sternochetus mangiferae Faust). Fruit flies of the family Tephritidae are the main insect of concern with a quarantine risk in many importing markets, fresh mango fruit must be treated to ensure that it is free of fruit flies (Jacobi *et al.*, 2001b).

Figure 2.2 The oriental fruit fly and anthracnose in mango (http://www.padil.gov.au)

2.1.4 Harvest maturity indices

Harvesting at a physiological mature stage considers both postharvest life of fruit and its quality. A distinction between "mature fruit" and "ripe fruit" can be distinguished that a mature fruit has completed its growth phase and shows typically hard, sour and astringent. During ripening the tissues in fruit show typically change colors, soften, decrease in acidic chemicals and have starch converted to sugars. Mango which is harvested before getting to the optimum maturity will not either ripe or reach the optimum quality. Physiological maturity of climacteric fruits indicates their continual processes in ripening and the optimum eating quality off the tree. Mango fruit gets the physiological maturity in about 90 days and the increase in size and weight almost stop for 4-5 weeks before harvesting (Tharanathan *et al.*, 2006). The commercial mango fruit trade for consumption as ripe fruit is harvested at a mature-green stage, and then ripened after harvesting. Harvesting should be done at the appropriate time depending

on cultivars based on several factors, such as the age of fruit after flower opening (full bloom), after 50% of fruit set, the creamy-white appearance on fruit peel and the use of floating in the water (DOA, 2009). However, fruits on the same tree or the position where fruit hang on the tree have various maturity resulting in harvesting with uniform maturity. It also depends on cultivar, region, environmental conditions, type and purpose of market.

2.2 Fruit development and ripening physiology

Fruit development and ripening are genetically as well as biochemically and physiologically programmed processes. Fruit involved in these complex processes including maturation: series of changes that occur after the cessation of growth of the fruit and after certain physiological changes; ripening: changes that occur in fruit from the end of the maturation period to the beginning of senescence; and senescence: irreversible changes that follow fruit ripening, ultimately leading to death. Mango is a climacteric fruit which its ripening characteristics change dramatically including chemical compositions, respiration rate, ethylene production, texture, color, flavor and aroma.

In 2010, Pandit *et al*. studied on expression profiling of various genes during the fruit development and ripening of mango. They isolated eighteen genes related to the physiology and biochemistry of the fruit (terpenoid metabolism related genes (IPPI, GPPS, MTPS, GGPPS, FPPS, SqTPS, IsoCH and GT), and genes related to the dynamic environment of fruit (MDHAR, 14-3-3, MT, MeTr, sHSP, CysPI, Chitinase, LOX, ERF, UbqPL)) from all 12 tissues including leaf, flower and fruits (5, 15, 30, 60 and 90 days after pollination (DAP) ,and 2, 5, 10, 15 and 20 days after harvesting (DAH)) of 'Alphonso' mango, and then profiled their expression by using relative quantitation PCR. They found that most of the tissues, genes related to primary metabolism, abiotic stress, ethylene response and protein turnover showed high expression as compared to that of the genes related to flavor production; metallothionin and/or ethylene-response transcription factor showed highest level of transcript abundance in all the tissues; expressions of mono- and sesquiterpene synthases and 14-3-3 lowered during ripening while, that of lipoxygenase, ethylene-response factor and ubiquitin-protein ligase

increased during ripening; flower showed better positive correlation with developing and ripening fruits than leaf based on these expression profiles; most of the genes showed their least expression on the second day of harvest (2DAH) suggesting that harvesting signals significantly affect the fruit metabolism; the stage at 90DAP considered as a perfect physiological maturity for harvesting; and the stage at 15DAH could be marked as a perfect ripe stage by the expression peaks of FPPS, LOX, MeTr, Chitinase and UbqPL genes.

2.2.1 Compositional changes during fruit ripening

Fruit ripening involves in set of physiological, biochemical and organoleptic changes leading to development of a soft and edible ripe fruit. Compositional changes associated with mango fruit ripening compose of peel color changes from green to yellow in some cultivars; pulp color changes from greenish yellow to yellow to orange in all cultivars; decrease in chlorophyll and increase in carotenoid contents; increase in flavor and aroma; decrease in pulp firmness and increased juiciness; starch in converted to sugars; increase in soluble solids content; decreased titratable acidity; increase in characteristic aroma volatiles; carbon dioxide production rate increases from 40-50 to 160-200 mg/kg.hr at 20°C and ethylene production rate increases from 0.2-0.4 to 2-4 μl/kg.hr at 20°C; increase activity of cell wall degrading enzymes. The color is changed during fruit ripening by degradation of chlorophyll and synthesis of anthocyanin, carotenoids such as β-carotene, xanthophylls and lycopene. Flavor and aroma are increased by production of a complex mixture of volatile compounds, and degradation of bitter principles, flavonoids, tannins and related compounds (Yahia, 2005; Tharanathan *et al*., 2006; Prasanna *et al*., 2007).

2.2.2 Role of ethylene in fruit ripening

Ethylene is the gaseous plant hormone, specifically a fruit ripening hormone which can trigger various events of cell metabolism including initiation of ripening and senescence. It is autocatalytically synthesized at low level prior to the initiation of ripening, which in turn triggers the entire process of changes during ripening. Mango, banana, apple, tomato, papaya and guava are grouped as climacteric fruit, harvested at

fully maturity, can be ripened off the parent plant. The respiration rate and ethylene formation dramatically raise to a climacteric peak during ripening. Non-climacteric fruit, such as strawberry, orange, grape, lemon and litchi are not able to continue their ripening process after they are detached from the parent plant. These fruits produce a very small quantity of endogenous ethylene, and do not respond to external ethylene treatment. Ethylene has many effects on the growth, development and storage life of many fruits, vegetables and ornamental crops which is effective at part-per-million (ppm, $\mu l\ 1^{-1}$) to part-per-billion (ppb, $nl\ 1^{-1}$) concentrations. Both the synthesis and action of ethylene associated with complicated metabolic processes need oxygen and respond to elevated concentrations of carbon dioxide. Ethylene induces numerous responses during ripening through a signaling cascade, and thousand of genes cooperate which not only arrays in ripening but also responsible for its spoilage. Slowing down post ripening process in fleshy fruit has been the first point of ripening-related research (Saltveit, 1999; Nath *et al*., 2006; Tharanathan *et al*., 2006; Prasanna *et al*., 2007; Bapat *et al*., 2010). Sane *et al.* (2005) cloned and characterized an expansin gene, *MiExpA1*, from ripening ‘Dashehari’ mango which was correlated with softening in mango. The expression of this gene was under dual control, being triggered by ethylene treatment within 90 minutes followed by a ripening associated peak on the third day after ethylene treatment.

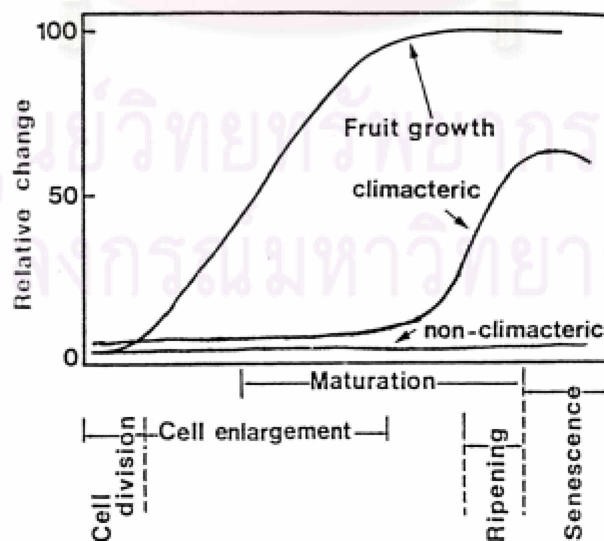

Figure 2.3 Fruit development and ethylene production in fruit (Ketsa, 1984)

2.2.3 Textural softening during fruit ripening

The metabolic events responsible for the textural changes in fruit involved in loss of turgor pressure, physiological changes in the composition of membranes, degradation of starch, and modifications in cell wall structure and dynamics. The major textural change that occur during fruit ripening is softening of fruit by enzyme-mediated modification in the structure and composition of cell wall, partial or complete solubilization of cell wall polysaccharides (Prasanna *et al.*, 2007; Goulao and Oliveira, 2008). Therefore, ripening of mango is characterized by a gradual change as a result of progressive depolymerization of pectic and hemicellulosic polysaccharides with significant loss of galactose, arabinose and mannose residues at the ripe stage. Yoshoda, Prabha and Tharanathan (2005) elucidated the depolymerization changes in ripening 'Alphonso' mango by using GC-MS analysis, IR and ^{13}C NMR measurements. They revealed that the major CWS fractions of both unripe and ripe mangoes to be of variable molecular weights and having a 1,4-linked galactan/galacturonan backbone, which is infrequently involved in side chain branches consisting of single residues of galactose and arabinose or oligomeric 1,5-linked arabinofuranose residues linked through 1,3-linkages; whereas the major hemicellulosic fractions of unripe mango to be of xyloglucan-type having 1,4-linked glucan backbone with branching by non-reducing terminal arabinose and xylose residues. In addition, they examined the extent of correlation with textural softening in 'Alphonso' mango fruit during ripening, and found that the starch content came down from 18% to 0.1%; pectin from 1.9% to 0.5%; cellulose from 2% to 0.9% and hemicelluloses from 0.8% to 0.2% from unripe to ripe stage, respectively. Simultaneously, the total soluble solids increased from 7%to 20%, total soluble sugars from 1% to 15% and pH increased from 2.8 to 5.1. The activity of carbohydrate-degrading enzymes increased resulting in solubilization of the various polysaccharide fractions which were correlated with fruit softening phenomenon. Efficient distribution of ^{14}C-starch into glucose, fructose and sucrose showed considerable sugar interconversions indicating active gluconeogenesis during mango fruit ripening (Yoshoda, Prabha and Tharanathan, 2006). On the other hand, the excessive softening of fruit increase its susceptibility to the pathogen attack resulting in limitation of quality and shelf life.

2.2.4 Cell wall hydrolases in relation to fruit softening

Fruit ripening is characterized by progressive depolymerisation of pectic and hemicellulosic polysaccharides of the cell wall. Several cell wall hydrolases have been studied and their role implicated in the softening of various fruits. Chourasia *et al.* (2006) studies pectate lyases (PL), which degrade pectins in mango fruit tissue. They cloned a PL homologue, *MiPel1* from ripening mango (*Mangifera indica* var. Dashehari), which shows sequence similarity to higher plant PL genes. Mango fruit pulp from different ripening stages, 1-MCP treated fruits, control (ethylene untreated) fruits, different developmental stages and vegetative tissues were used for analysis, and the result showed that the expression was specific to fruits and triggered only during ripening. Moreover, the increase in transcript accumulation of *MiPel1* during ripening was associated with an increase in the Ca^{+2} dependent PL activity and pectin solubilization. So, it is proposed that expression of PL may be closely associated with pectin degradation during ripening and play an important role in mango softening.

2.3 Postharvest handling

The postharvest life of mango fruit generally does not exceed 2-3 weeks due to its physiological deterioration or pathogen infection. Therefore, several methods of postharvest handling have been applied to extend the shelf life of mango fruit and reduce losses, through inhibition of respiration and ethylene production. These include refrigeration or cold storage, wax or film coating, controlled and modified atmosphere storage, irradiation, heat treatment, and the use of various chemicals (Nair and Singh, 2003). A combination of them can also use for prolonging the shelf life of fruit.

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2.3.1 Low temperature storage

The basic concept of storage of horticultural crops is to extend their shelf life by storing them in appropriate conditions to maintain their quality. Low temperature storage is a common technique for prolonging shelf life of many kinds of fruit as it can reduce metabolic activity, delay ripening and senescence, reduce water loss, prevent or reduce disease and insect activity. Due to mango's highly perishable nature and susceptibility to postharvest disease and injury, it is necessary to efficiently control the temperature

throughout the storage period. Cold storage is used to delay the period of fruit ripening by slowing down its physiological activities. Many commodities which stored at low temperature are subjected to damage caused by chilling. Mango is a tropical fruit, therefore sensitive to chilling when stored below a critical minimum. It has been reported that 'Okrong' mango can safely be stored at 12°C for up to 25 days (Phakawatmongkol, Ketsa and Van Doorn, 2004). However, recommended temperatures are in the range of 10-15°C for 2-3 weeks of storage life. Mango fruit usually remain green at temperatures of 28°C or higher but do not ripen when maintained at temperatures of 33°C or higher for a long period (Tasneem, 2004; Yahia, 2005).

2.3.2 Heat treatments

Heat treatment is often used for disinfestation and disinfection of fruits to satisfy the quarantine requirements of some importing markets. Many fruits and vegetables tolerate exposure to water temperatures of 50-60°C for up to 10 minutes (Lurie, 1998).

2.3.2.1 Vapor heat treatment (VHT)

Vapor heat, referred to as high humidity air heating, is a method of heating fruit with air saturated with water vapor at temperature of 40-50°C to kill insect eggs and larvae as a quarantine treatment. The heat from the fruit surface is transferred toward the fruit center. Protocols of VHT disinfestations, accepted for mango, acceptable to the high value markets such as Japan are at 46.5°C fruit core temperature held for 10 minutes for 'Nung Klang Wun' mango and 47°C fruit core temperature held for 10 minutes for 'Nam Dok Mai', 'Pimsen Dang' and 'Rad' mangoes, Thailand. Recently, the vapor heat contains forced air that circulates through the pallets and heats the commodity more quickly than vapor heat without forced air (Lurie, 1998; Jacobi *et al.*, 2001b).

2.3.2.2 Hot air treatment (HAT)

Placing fruit or vegetables in a heated chamber with a ventilating fan, or by applying forced hot air where the speed of air circulation is precisely controlled are the application of Hot air. For HAT with or without forced, heat more slowly than hot water

immersion or forced vapor heat, even forced hot air can produce heat faster than a regular heating chamber. The hot air chamber has been used to study physiological changes in fruits and vegetables in response to heat (Lurie, 1998). Jacobi *et al.* (2001a) studied on effect of hot air condition of 'Kensington' mango fruit on the response to hot water immersion treatment (HWT) by incubating fruit at temperatures from 22°C to 42°C for 4-16 hours, prior to HWT of 45°C for 30 minutes or 47°C for 15 minutes, before ripening at 22°C. They reported that conditioning at 40°C prior to HWT accelerated the ripening of fruit, increased weight loss, reduced fruit firmness, increased °brix and lowered titratable acidity compared to untreated fruit and fruit receiving other heat treatments. It demonstrated that 'Kensington' fruit can be artificially conditioned prior to HWT protocols required for disinfestation without injuries occurring to render the fruit unmarketable.

2.3.2.3 Hot water immersion treatment (HWT)

Hot water immersion quarantine treatments have been used to disinfest mangoes of fruit flies. The treatments of 48-55°C for 3-15 minutes were used for decay control which depended on the variety. HWT is cheaper than other heat treatment and is also effective on commercial scale in the USA. They can apply for only a few minutes for the heat transfer to take place from the water to the fruit peel, and from the fruit peel through the pulp to the center. The peel heat transfer from the water is faster than the heat transfer from the fruit peel to center transfer. The temperature of hot water should be controlled and water should be circulating to support temperature uniformity. After HWT, fruits should immediately be cooled in ambient temperature (Lurie, 1998; Jacobi *et al*., 2001b; Yahia, 2005).

2.4 Physiological fruit responses to heat treatments

2.4.1 Fruit heat tolerance

Fruit heat tolerance differs in a set of factors including species, genotypic variability within species, stage of fruit maturity, fruit size, exposure to different environmental and/or preharvest factors, the type of heat treatment applied, and

whether postharvest conditioning treatments have been given before a heat treatment (Jacobi *et al*., 2001b).

2.4.2 Fruit damage caused by heat treatments

When harvested fruit is transferred from ambient growth temperature to an elevated temperature, it induces stress. Although mango has more tolerant than many other fruits but it is susceptible to heat injury such as peel browning, peel scalding, pulp darkening and lenticel damage. Preconditioning fruit can also reduce heat damage (Jacobi *et al*., 2001b; Yahia, 2005).

2.4.3 Reducing chilling injury (CI) symptoms by using heat treatments

Mangoes, as one of the main tropical fruits which are very perishable during storage at ambient temperature so they are usually stored under refrigeration at a temperature below 13°C resulting in visual symptoms of chilling injury (CI). Zhao *et al.* (2009) evaluated the effect of mature stage on chilling tolerance of 'Zihua' mangoes and the mechanism involved. The fruit were categorized into three stages of ripeness: green (100% green fruit), preyellow (10-20% yellow fruit) and yellow (45-55% yellow fruit) after harvest and stored at 2°C for 12 days and then incubated at 25°C for 2 days for chilling injury (CI) development. They found that CI index in pre-yellow and yellow fruit was significantly lower than that of the green fruit, as a rapid increase in ion leakage was observed in the green fruit. Activities of superoxide dismutase, catalase, ascorbate peroxidase and polyphenoloxidase of pre-yellow and yellow fruit were higher than those of the green from day 6 to day 12 during cold storage. A lower content of malondialdehyde but higher levels of glutathione and ascorbic acid were maintained in pre-yellow and yellow fruit than that in green fruit. These results suggested that stronger resistance of pre-yellow and yellow mangoes to CI compared to green fruit was due to their higher antioxidant capacity involved in the tolerance to chilling temperature. Alleviating CI in mangoes during storage may be achieved by storing the fruit when peel color is beginning to change to yellow from green.

2.4.4 Fruit ripening affected by heat treatments

The effect of heat treatments on fruit ripening can be variable, but can be categorized as either inhibiting, promoting or disrupting fruit ripening. Heat treatment affects various aspects of fruit ripening, such as respiration rate; ethylene production, softening and cell wall metabolism; chlorophyll and carotenoid metabolism; flavor and volatile production; protein metabolism; and nucleic acid metabolism (Lurie, 1998; Paull and Chen, 2000; Jacobi *et al*., 2001b). Ketsa *et al.* (1999) examined ethylene synthesis in heat treated 'Nam Dok Mai' mango fruit. The fruits were divided into two lots, one was placed directly at 25°C, and the other was held for 3 days at 38°C and then transferred to 20°C. They found that fruits placed directly at 25°C after harvest had the peak of ethylene production after 4 days, the highest of ACC content occurred on day 4 and the highest of ACS activity was also on day 4; while the heated fruit had the peak of ethylene production after 9 days, ACC content continued to increase for 8 days and the activity of ACS was undetectable following heat treatment, but recovered partially during the ripening period. Finally, they concluded that the inhibition of ethylene production found during heat treatment is due to inhibition of both ACS and ACO. Moreover, ACO recovers to its full activity following heat, whereas ACS activity recovers only partially, but enough to allow the heated fruit to achieve an ethylene peak.

2.5 Oxidative stress in plant

Oxidative stress is a general term used to explain the steady state level of oxidative damage in a cell, tissue, or organ, which is caused by the reactive oxygen species (ROSs). Plants are damaged with the excess production of ROSs during severe environmental conditions resulting in losses of crop quality and productivity. The various adverse environmental conditions include drought stress and desiccation, salt stress, chilling, heat shock, heavy metals, ultraviolet , mechanical stress, nutrient deprivation, pathogen attack and high light stress. In addition, plant injury usually involves an imbalance between the production and elimination of ROSs that are inevitably generated in plant cells as a consequence of normal metabolism (Inzé and Montagu, 1995; Mittler, 2002).

2.5.1 Generation of ROSs

Oxidative stress occurs when the concentrations of reactive oxygen species, such as the superoxide radical, hydrogen peroxide and the hydroxyl radical, increase in cells (Vierling and Kimpel, 1992). There are various sources of ROSs in plant. Some reaction are involved in normal metabolism, such as photosynthesis and respiration as unavoidable as by products of aerobic metabolism. Other sources of ROSs are pathways enhanced during abiotic stresses, such as glycolate oxidase in peroxisomes during photorespiration (Mittler, 2002). The effect of ROSs formation depends on the intensity of the stress and on the physiochemical conditions in cell, such as antioxidant status, redox state and pH. It has been proved that ROSs produced under stress is a detrimental factor, which causes lipid peroxidation, enzyme inactivation and oxidative damage to DNA (Blokhina, 2000).

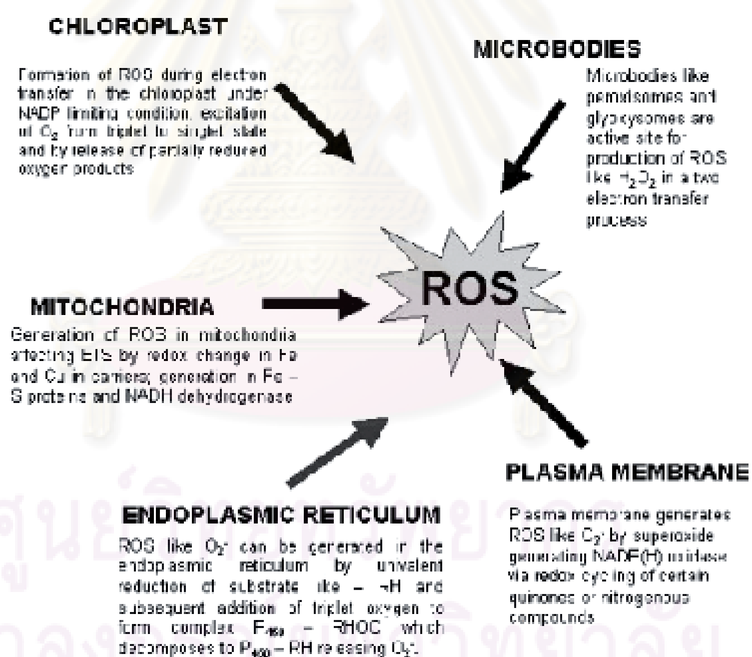


Figure 2.4 Different sources of ROS in plants and their reactivities with cellular components (Panda and Choudhury, 2005)

2.5.2 Lipid peroxidation

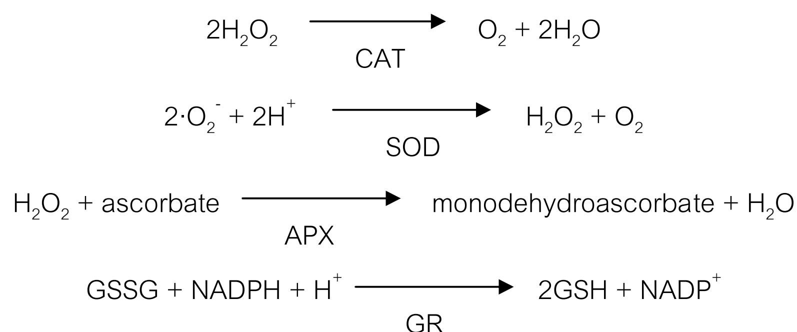
Lipid peroxidation is one of the major investigation resulting from ROSs action on the membrane structure and function. Polyunsaturated fatty acids (PUFA), the main components of membrane lipids, are susceptible to peroxidation which catalysed by

lipoxygenases. The metabolism of PUFA via LOX-catalyzed step following to the reactions are aggregately named LOX pathway (Blokhina, 2000; Liavochanka and Feussner, 2006). Lipoxygenases (LOXs; EC 1.13.11.12) form a family of non-heme-iron-containing fatty acid dioxygenases which are widely distributed in plants and animals (Feussner, Kühn and Wasternack, 2001). Lipoxygenase mediates the conversion of polyunsaturated fatty acids (linolenic and linoleic acids, in plant) to their conjugated hydroperoxydiene derivatives using molecular oxygen. They present in a widespread biological organs and tissues which are specifically abundant in grain legume seeds (beans and peas) and potato tubers. In plants, LOX has been correlated with some processes in many developmental stages, and with the mobilization of storage lipids during germination, and also used as a storage protein during vegetative growth (Lynch and Thompson, 1984; Porta and Rocha-Sosa, 2002; Baysal and Demirdöven, 2007).

2.5.3 Antioxidant system

The antioxidant defense system including enzymes like superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT) and glutathione reductase (GR), and a network of low molecular mass antioxidants, such as ascorbate, glutathione, phenolic compounds and tocopherols, help in scavenging of ROSs. SOD catalyses the dismutation of superoxide to H_2O_2, while H_2O_2 is predominantly destroyed by APX and CAT. SOD has three types which classified by their metal cofactors, found in living organisms, and are structurally similar including Fe-SOD (prokaryotic organisms and chloroplast stroma), Mn-SOD (prokaryotic organisms and the mitochondrion of eukaryotes); and the unrelated CU/Zn-SOD (cytosolic and chloroplast enzyme, gram-negative bacteria (Blokhina *et al.* 2003). Previously, Kim *et al.* (2009) investigated polyphenolic and antioxidant changes to mature, green mangoes following varying times of HWT and their changes during short-term storage. 'Tommy Atkins' mango fruit were immersed in 46.1°C water from 70 to 110 minutes; half evaluated within 2 h of treatment, while the remainder was evaluated after 4 days of storage at 25°C for changes in polyphenolics, antioxidant capacity and fruit quality. The result showed that two major polyphenolics in mango, gallic acid and gallotannins, as well as total soluble phenolics, decreased as a result of prolonged HWT, while the antioxidant capacity

remained unchanged in all heat-treated mangoes immediately after HWT. However, during 4 days storage, only minor changes were observed in gallic acid and gallotannin concentrations whereas total soluble phenolics and antioxidant capacity in all hot water-treated fruits decreased. The optimum hot water immersion times did not affect the external quality and polyphenolics of mangoes but all heat treatments reduced total soluble phenolics and antioxidant capacity, regardless of the duration of treatment times, during 4 days storage. Yahia *et al.* (2007) investigated postharvest hot air treatment effects on the antioxidant system in stored mature-green tomatoes. They found that 'Rhapsody' tomatoes exposed to air at 34 or 38°C and stored at 20°C had higher cysteine, "reduced glutathione", CAT, and glutathione *S*-transferase, but lower isoascorbic acid and APX compared to control fruit. Fruit exposed to 38°C developed slight heat injury, and had slightly lower β-carotene, lycopene, cysteine, APX, CAT, and "reduced glutathione" compared to fruit exposed to 34°C. Fruit stored at 4°C had less color development, lower β-carotene, lycopene, ascorbic acid, isoascorbic acid, dehydro-ascorbic acid, cysteine and "reduced glutathione", and higher α-tocopherol, dehydroascorbate reductase, peroxidase, CAT and GR than those stored at 20°C. Of the two heat treatments, 34°C for 24 hours caused little injury, and had less negative effects on antioxidants during storage at 4 or 20°C than did prior exposure to 38°C. Sala (1998) examined changes in activated oxygen scavenging enzymes, SOD, CAT, APX and GR during low temperature storage of mandarin fruits and found that no symptoms of CI occurred in cold-stored fruits of 'Clemenules' and 'Clementine' cultivars. In 'Nova' and 'Fortune' cultivars, CI increased sharply after 4 weeks at 2.5°C and the peel surface was severely damaged. SOD activity increased during cold storage in both chilling-sensitive and -tolerant cultivars. However, CAT, APX and GR activities were higher in tolerant cultivars ('Clemenules' and 'Clementine') at low temperature.



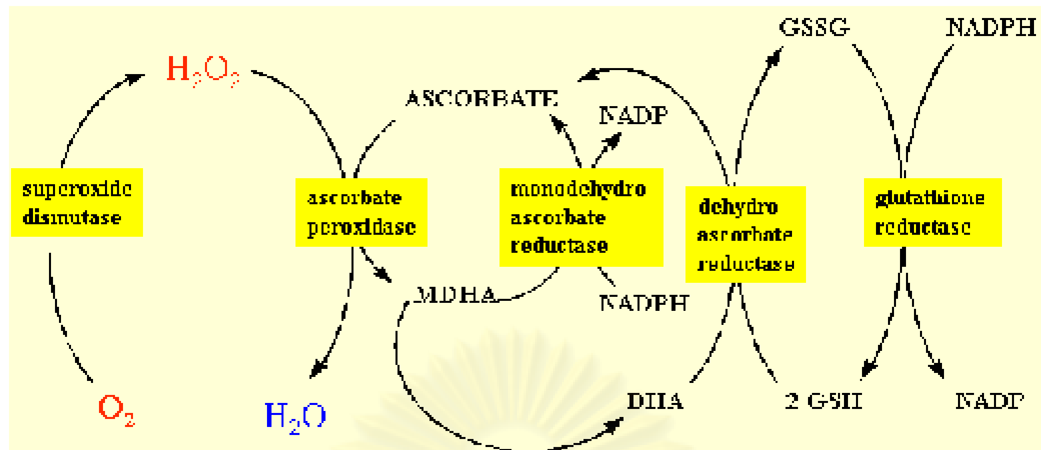


Figure 2.5 The Halliwell and Asada pathway of ROSs generation and antioxidant enzymes (Arora, Sairam and Srivastava, 2002)

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CHAPTER III

MATERIALS AND METHODS

3.1 Plant materials

'Nam Dok Mai' and 'Ok Rong' mature-green mangoes were harvested 90 days after fruit set and collected from a commercial orchard in Nakonratchasima province, Thailand. Fruits were transported to the laboratory within 2-3 hours and selected for uniformity of size, color and absence of blemishes or infections.

3.2 Hot Water Treatment (HWT)

Fruits were randomly divided into 2 groups; treated group (160 fruits) was dipped in hot water (50°C) for 10 minutes and untreated group (160 fruits) was dipped in tap water (30°C) for 10 minutes as a control. Hot water treatment was carried out in a large water bath and water temperature varied by no more than 0.5°C from the set temperature. Following treatment, fruits were placed at room temperature and allowed to dry before storage at 8°C and 12°C for 15 days in a temperature-controlled chambers. Fruits were randomly sampled every 5 days and transferred to room temperature and sampled everyday until day 20. Fruits were immediately analyzed or cut in small pieces, frozen in liquid nitrogen and stored at -80°C until use. Both peel and pulp tissue samples were lyophilized and used for antioxidant enzyme assays, protein and RNA analysis.

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3.3 Fruit quality analysis

The parameters used to monitor the quality of mango fruit throughout the storage period were percentage of weight loss, pulp firmness, peel color, total soluble solids and titratable acidity.

3.3.1 Weight loss

Thirty-two fruits were weighted using a top pan balance and recorded following treatment (day 0) and at the different sampling dates. The same fruits were used to evaluate weight loss at the beginning of the experiment and throughout the storage time.

It was calculated by the following formula: $(W_1 - W_2) / W_1 \times 100$, where W_1 is the initial weight (day 0) and W_2 is the final weight of each storage time. Cumulative weight losses were expressed as means±S.E. of percentage loss of original weight.

3.3.2 Pulp firmness

Firmness was measured on four peeled fruit for each treatment using a penetrometer (Effegi, Alfonsine, Italy). For each fruit measurements were taken at three different points on the equatorial region of fruit pulp and recorded as Newtons (N).

3.3.3 Peel ground color

Color attributes of fruit peel were quantified using a Konica Minolta colorimeter CR-10 model (Minolta, Japan) with results obtained in terms of color space L* (lightness), a* (red-green axis), b* (yellow-blue axis), C* (chroma/saturation) and h (hue/shade) (see Appendix A). The same fruits were evaluated at the beginning of the experiment and throughout the storage time. The measurements were taken at three different points on the equatorial region of fruit peel and recorded as L, a, b values, chroma and hue angle.

3.3.4 Total soluble solids (TSS)

Soluble solids content was determined using a hand refractometer N-1E model (Atago, Japan) with a scale of 0-32% °Brix as modified by Ratule *et al.* (2006). Twenty grams of fruit pulp in 40 ml distilled water was grounded using a Waring blender for 2 min and filtered through a muslin cloth. New piece of cloth was used for filtering each sample. A drop of the filtrate was placed on the prism glass of the refractometer. The °Brix of TSS was directly recorded, and the recorded value was multiplied by a dilution factor of three.

3.2.5 Titratable acidity (TA)

Titratable acidity was measured according to the procedure of Ratule *et al.* (2006). Ten grams of fruit pulp was blended with 50 ml distilled water and boiled for 1 hour. After boiling, the sample was cooled and transferred to 100 ml volumetric flask

and made up to volume. Filtered sample aliquot was used for analysis. Ten ml of clear sample aliquot was titrated against 0.01 M NaOH using 2-3 drops of phenolphthalein as indicator. The titration value was noted and the results were expressed as percent malic acid using the following equation:

$$\text{Titratable acidity (\%)} = \frac{\text{Titre} \times \text{molarity of alkali} \times \text{volume made up} \times 67\text{ g} \times 100}{\text{Volume taken for estimation} \times \text{weight of sample} \times 1000}$$

3.4 Ethylene production

Individual fruit per each treatment were kept in airtight glass jars (2.4 litres volume) fitted with a rubber septum for collecting the gases. After 1 h incubation of the fruit, the head space gas (1 ml) was withdrawn from each jar using a syringe and injected into a Gas Chromatograph-8A (Shimadzu, Japan) equipped with a flame ionization detector (FID) fitted with a Porapack Q 80/100 column held at 80°C with nitrogen as the carrier gas. Ethylene was estimated and expressed as $\mu L\ kg^{-1}\ h^{-1}$.

3.5 Analysis of the antioxidant enzyme activities (CAT, APX and GR) of hot water treated mango fruits

Fruit peel and pulp (0.1 g dried weight) were separately ground in a mortar and pestle under liquid nitrogen, and then homogenized in 1 ml of an extraction buffer containing 45 mM potassium phosphate buffer (pH 7.0), polyvinylpyrrolidone (PVP) 0.5% (w/v), 1,4-dithiothreitol (DTT) 6.5 mM and 1 mM phenylmethyl sulfonyl fluoride (PMSF) (see Appendix C). The homogenate was centrifuged at 4°C for 25 minutes at 10 000×*g* and the supernatant was collected for enzyme activities analysis.

3.5.1 Catalase

Catalase (CAT, EC 1.11.1.6) activity was measured by a decrease in the absorbance at 240 nm according to Zhao *et al.* (2006) with slight modifications. The reaction mixture contained (final concentrations) 1.8 ml potassium phosphate buffer (40 mM, pH 7.0), 0.2 ml H_2O_2 (40 mM) and 0.05 ml enzyme extract in a total volume of 2.05 ml. Enzyme solution containing hydrogen peroxide-free phosphate buffer was used as control. CAT activity was expressed in units per mg protein, which decomposed one

μmol of H_2O_2 per minute (extinction coefficient of hydrogen peroxide at 240 nm = 43.6 M^{-1} cm^{-1}).

3.5.2 Ascorbate peroxidase

Ascorbase peroxidase (APX, EC 1.11.1.11) activity was spectrophotometrically assayed by a decline in the absorbance at 290 nm following to Zheng *et al.* (2007) with slight modifications. The assay mixture contained (final concentrations) 1.56 ml potassium phosphate buffer (40 mM, pH 7.0), 0.2 ml L-ascorbic acid (0.5 mM), 0.2 ml H_2O_2 (0.1 mM) and 0.05 ml of enzyme extract in a total volume of 2.01 ml. APX activity was expressed as units per mg protein, that oxidized one μmol of AsA per minute (extinction coefficient of ascorbic acid at 290 nm = 2.8 mM^{-1} cm^{-1}).

3.5.3 Glutathione reductase

Glutathione reductase (GR, EC 1.6.4.2) activity was determined by the decline in the absorbance at 340 nm for 3 minutes according to the method of Shen *et al.* (2008) with slight modifications. The reaction mixture consisted of (final concentrations) 1 ml potassium phosphate buffer (50 mM, pH 7.6) containing EDTA (1 mM), 0.75 ml deionized water, 0.1 ml NADPH (0.15 mM), 0.1 ml oxidized glutathione (0.5 mM GSSG) and 0.15 ml of enzyme extract in a total volume of 2.1 ml. GR activity was expressed as units per mg protein, that catalysed the oxidation of one μmol of NADPH per minute (extinction coefficient of NADPH at 340 nm = 6.2 mM^{-1} cm^{-1}).

3.5.4 Determination of protein

Protein content of enzyme extracts was measured according to the method of Bradford (1976), using bovine serum albumin (BSA) as the standard protein (see Appendix E).

3.6 Statistical design

All statistical analyses were performed with SPSS software. Experiments were carried out in a completely randomized design (CRD). Data were analyzed by a one-way analysis of variance (ANOVA), and the mean differences were compared by the

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least significant difference (LSD) test or Duncan's new multiple range test. Significant differences are given at $P < 0.05$. Experimental data were the means±standard errors (S.E.) with three replicates of the determinations for each sample.

3.7 Examination of changes in protein patterns and LOX in hot water treated mango fruits by SDS-PAGE and Western blots

3.7.1 Protein extraction of both fruit peel and pulp

Total soluble proteins of lyophilized tissue which had a storage time at 0, 5, 10, 15, 16, 18 and 20 days were extracted according to Biggs, Harriman and Handa (1986) with slight modifications. Six milligrams of sample was ground to a fine powder with liquid nitrogen, and then suspended in 200 µl of Laemmli's buffer (1X) (see Appendix C). The homogenate was placed in a boiling water bath for 3 minutes, and centrifuged at 14 000×*g* for 10 minutes. The supernatant was collected for separation by SDS-PAGE.

3.7.2 Protein separation by SDS-PAGE

Total proteins of both peel and pulp tissue were separated by SDS-Polyacrylamide Gel Electrophoresis as described by Sambrook, Fritsch and Maniatis (1989) with some modifications. Ten micrograms of total protein from each sample was run on a 10% acrylamide gel (see Appendix C). Prestained molecular weight markers (Bio-Rad) were used. Gels were fixed and stained with 50% (v/v) methanol, 10% (v/v) acetic acid containing 0.5% (w/v) Coomassie blue R-250, destained twice in 40% (v/v) methanol, 10% (v/v) acetic acid. Gels were scanned using a scanner, and intensities of the strained bands were quantified using Imagine software (Srivastava *et al.,* 2007).

3.7.3 Western Blot analysis

Separated polypeptides were equilibrated with transfer buffer (see Appendix C) and then blotted on the nitrocellulose membrane using a Semi-Dry Electrophoretic Transfer Cell (Bio-Rad) according to the recommendations of the manufacturer. The membrane was blocked with 3% non-fat dried milk in tris buffered saline (TBS) for 1 hour at room temperature. TBS contained 20 mM Tris and 500 mM sodium chloride

(NaCl), adjusted to pH 7.5. The membrane was incubated at 4°C overnight with tomato's LOX–specific antibodies (1:200 dilution) in antibody buffer (prepared with 1% (w/v) in TTBS). The membrane was washed twice for 5 minutes each with tris buffered saline containing 0.5% Tween-20 (TTBS). Goat Anti-Rabbit IgG(H+L) conjugated with alkaline phosphatase was used as the second antibody in 1:3000 dilution. After an incubation of 1 hour at room temperature, the blot was washed two times for 5 minutes each with TTBS and once for 5 minutes with TBS. The blot was developed with bromochloroindolylphosphate/nitro blue tetrazolium (BCIP/NBT) solution (see Appendix C). The intensity of the immunoreactive signal was quantified by using Imagine software (Biodiscovery).

3.8 Investigation of the transcript accumulation of Mn-SOD and cell wall hydrolases in hot water treated mango fruits by Northern blots

3.8.1 RNA extraction and probe preparation for Northern hybridization

RNA extraction was carried out following López-Gómez and Gómez-Lim (1992) method with slight modifications. Two hundred milligrams of lyophilized pulp tissue was homogenized using a hand mixer with 2 ml of lyses buffer (2% SDS, 50 mM EDTA and 150 mM Tris base with pH 7.5 adjusted with 1 M boric acid at room temperature) prepared from DEPC (diethyl pyrocarbonate) treated water, with the addition of 1% β-mercaptoethanol. The homogenate was mixed with 0.25 ml of absolute ethanol and 0.11 ml of 5 M potassium acetate and continuously vortexed for 1 minute following by chloroform: isoamyl alcohol (49:1) extraction and centrifugation (10 000×*g* for 10 minutes). The recovered aqueous phase was extracted once with equal volumes of phenol: chloroform (1:1) and once with chloroform: isoamyl (49:1) according to centrifugation (10 000×*g* for 10 minutes) in each time. The aqueous phase was carefully pipetted out and RNA was precipitated overnight at -20°C after addition of lithium chloride (3 M final concentration). The RNA was pelleted by centrifugation (10 000×*g* for 15 minutes at 4°C), washed once with 70% (v/v) ethanol by centrifugation (10 000×*g* for 10 minutes) and dried. The RNA pellet was resuspended in sterile DEPC treated water and stored at -80°C. Finally, the RNA was quantified by measuring absorbance at

260/280 nm with a Nanodrop spectrophotometer ND-1000 model (NanoDrop Technologies, USA) and the quality was examined by electrophoresis on a 1% agarose gel.

The primers for mango genes encoding manganese superoxide dismutase (Mn-SOD) and cell wall hydrolases were constructed by using a database of the National Center for Biotechnology Information (NCBI). The oligonucleotide primers specific for Mn-SOD, pectate lyase (PL), β-galactosidase, β-1,3-glucanase transcripts were used for cDNA synthesis and shown as follows:

MNSD-F 5´-ACCGAATCCGTCCGCCTTGG-3´

MNSD-R 5´-CTTTGGTGGCTCCCCGCCTC-3´

PL-F 5´-CGAAGCGGCAACAATGGCGG-3´

PL-R 5´-CCATCACGGCCACCAATGGCA-3´

bGal-F 5´-GGACTGAGGCTTGGACCGGC-3´

bGal-R 5´-AAAGCAGCACAGCCTCCCGA-3´

b1,3G-F 5´-GCCGGGGGTGCACCAGAGAA-3´

b1,3G-R 5´-CGTCAGCGGAGTAGGCCAGGA-3´

Each primer was composed of about 20 nucleotides with melting temperatures around 60°C. A total of 2 µg RNA were used for the template by using in the ratio of 0.5 µg primer per µg RNA template. Heated to 70°C for 5 minutes. Chilled the tube on ice for 5 minutes and centrifuged briefly to collect the solution at the bottom of the tube. Added the components to the annealed primer/template as follows:

5X Reaction Buffer	5	µl
dNTP mixtures	2.5	µl
Ribonuclease Inhibitor, 40 units	2.5	ul
AMV Reverse Transcriptase, 30 units	3	ul

Nuclease-free water to final volume 25 µl

Then, incubated for 60 minutes at 42°C and chilled on ice. Added 1 µl of RNase H and incubated 30 minutes at 37°C. Afterthat, cDNA diluted 1:20 was used in PCR.

Added the components to the PCR reaction tube as follows:

10X PCR Buffer	2.5	µl
10 mM dNTP mixtures	1	µl
20 mM $MgCl_2$	2	µl
Primer mix (125 ng/ µl each)	1.25	µl
Template cDNA as required	2	ul
Taq polymerase	0.5	µl

Autoclaved distilled water to final volume 25 µl

PCR products were checked on a 1% agarose gel, extracted and cleaned by using QIAGEN Gel Extraction kit. Final product was used as a probe for Northern hybridization. The gene sequences were analysed by using the Basic Local Alignment Search Tool (BLAST) which searched for their homology with the sequence of genes already available in databases of NCBI (see Appendix B).

3.8.2 Northern Blot analysis

Ten micrograms of total RNA from each sample was electrophoresed on a 1% agarose-formaldehyde gel, and blotted onto a Hybond-N membrane (Amersham) overnight in 10X SSC as described by Sambrook *et al.* (1989). 10X SSC contained 1.5 M NaCl and 150 mM sodium citrate, adjusted to pH 7.0. The RNA was covalently cross-linked to the membrane by using an UV stratalinker. The probe was labeled using a random primer labeling kit (DECA PrimeII, Ambion) and radiolabeled probe purified on a Sephadex G-50 column used. The membranes were hybridized with a ^{32}P-labeled probe of Mn-SOD, PL, β-galactosidase, β-1,3-glucanase and ribosomal RNA at 37°C in 50% (v/v) formaldehyde, 5X SSPE, 0.1% (w/v) SDS, 5X Denhardt's solution (see Appendix C). Hybridized membranes were washed two times in 5X SSPE and 0.1% (w/v) SDS at 37°C for 15 minutes followed by a 10 minutes wash in 2X SSPE at 62°C. The blots were exploded on the phosphor-imaging cassette (Molecular Dynamics, Sunnyvale, CA, USA) for overnight and scanned in the Typhoon Phosphorimager (Amersham Biosciences, Pistacaway, NJ) and the signal intensities were quantified using Imagine software (Biodiscovery).

CHAPTER 4

RESULTS

4.1 Effect of hot water treatment on fruit quality analysis of mango fruits

Weight loss, pulp firmness, total soluble solids and titratable acidity in both ‘Nam Dok Mai’ and ‘Ok Rong’ mango fruits were presented in Table 4.1 and 4.2, respectively. Changes in color attribute of fruit peel in both cultivars of mango throughout the entire storage time were given in Table 4.3 and 4.4, respectively.

Weight loss in all treatments of both mango cultivars gradually increased during the whole storage period. Hot water treated (HWT) ‘Nam Dok Mai’ fruit both stored at 8°C and 12°C showed significantly higher percentage of weight loss than control fruit on day 5, 16 and 17. ‘Ok Rong’ fruit stored at 12°C were significantly higher percentage of weight loss than fruit stored at 8°C on day 5, 10, 15, 16 and 17. There were no significant difference between HWT and control fruit of ‘Ok Rong’. The highest weight loss was observed on control fruit upon removal from 12°C storage.

Pulp firmness in both mango cultivars started to decrease after transferring to ripen at ambient temperature on day 15. ‘Nam Dok Mai’ fruit stored at 12°C showed significantly lower firmness than fruit stored at 8°C on day 10, 16 and 20, whereas ‘Ok Rong’ fruit stored at 12°C showed significantly lower firmness than fruit stored at 8°C on day 15, 16 and 20. ‘Ok Rong’ fruit showed a decline in pulp firmness more rapidly than those of ‘Nam Dok Mai’ fruit during ripening.

Total soluble solids contents continued to increase constantly in relation to the entire storage period. ‘Nam Dok Mai’ fruit stored at 12°C had significantly higher percentage of TSS than fruit stored at 8°C on day 15, 16 and 18. The highest percentage of TSS was found in HWT ‘Nam Dok Mai’ fruit stored at 12°C (17%) on day 18. ‘Ok Rong’ fruit stored at 12°C had significantly higher percentage of TSS than fruit stored at 8°C on day 10, 15, 17 and 20. They showed the highest percentage of TSS at the end of storage (16.50%), while HWT ‘Ok Rong’ fruit exhibited the lowest percentage of TSS (14.20%).

Titratable acidity (TA) decreased constantly with the storage period but at different extent. HWT ‘Nam Dok Mai’ fruit both stored at 8°C and 12°C showed

significantly lower percentage of TA than control fruit on day 10 and 20. 'Ok Rong' fruit stored at 12°C showed significantly lower percentage of TA than fruit stored at 8°C on day 5, 10, 16 and 18. TA of 'Ok Rong' fruit decreased faster than 'Nam Dok Mai' fruit throughout the whole storage.

Skin reflectance or lightness in both mango cultivars had slightly changed throughout the prolonging shelf life period. The highest of L-values was obtained from the HWT 'Ok Rong' fruit stored at 12°C on day 16 (61.81); and fruit stored at 8°C were significantly different from the fruit stored at 12°C during ripening at ambient temperature. Decreasing of green color on skin of mango was observed in the increasing of a-values and it turned yellow which reflected by the increasing of b-values. Fruit stored at 8°C showed lower amount of a and b values than those of fruit stored at 12°C, as well as the fruits stored at 8°C remained green longer than fruit stored at 12°C until day 20. Color intensity or chroma increased with the storage period. The highest of c-values was presented in the control of 'Ok Rong' fruit stored at 12°C on day 18 (46.96); and fruit stored at 8°C showed a significant difference from fruit stored at 12°C on day 16 and 17. Hue angle decreased throughout the storage time. HWT 'Nam Dok Mai' fruit stored at 12°C of showed a significant difference from other treatments on day 17. Heat treatment delayed color changes of 'Ok Rong' fruit stored at 8°C. The pictures of color changes in 'Nam Dok Mai' fruit were shown in Figure D.1 to Figure D.16; and also of 'Ok Rong' fruit in Figure D.17 to Figure D.32, respectively (see Appendix D).

4.2 Effect of hot water treatment on ethylene production

The changes in ethylene production of HWT and control fruit were shown in Figure 4.1. For both cultivars, ethylene production of HWT fruit stored both at 8°C and 12°C had slightly changed during the storage period. A delay in ethylene production was also detected in HWT fruit both stored at 8°C and 12°C during ripening at ambient temperature. The control of 'Nam Dok Mai' fruit demonstrated a climacteric peak on day 19 (stored at 8°C) and day 16 (stored at 12°C), while the control of 'Ok Rong' fruit showed a peak on day 17 (stored at 8°C) and day 19 (stored at 12°C) correlated with the normal ripening. HWT fruit of both cultivars did not show climacteric peak and reached a lower values than those of the control fruit at the end of the storage.

Table 4.1 Weight loss, pulp firmness, total soluble solids and titratable acidity of control and hot water treated (HWT) 'Nam Dok Mai' fruit during low temperature storage (8°C and 12°C) for 15 days and ripening at ambient temperature for 5 days

Time (days)	Treatments	Weight loss (%)	Pulp firmness (N)	TSS (%)	TA (%)
0	control at 8°C	0	0.84 ± 0.01 a	7.13 ± 0.62 a	2.04 ± 0.30 a
	HWT at 8°C	0	0.84 ± 0.01 a	7.30 ± 0.62 a	1.91 ± 0.22 a
	control at 12°C	0	0.84 ± 0.01 a	6.30 ± 0.52 a	1.84 ± 0.25 a
	HWT at 12°C	0	0.84 ± 0.01 a	7.28 ± 0.51 a	2.15 ± 0.35 a
5	control at 8°C	3.71 ± 0.26 b	0.84 ± 0.01 a	7.28 ± 0.79 b	1.83 ± 0.06 a
	HWT at 8°C	4.04 ± 0.26 a	0.84 ± 0.01 a	8.78 ± 0.45 a	1.89 ± 0.06 a
	control at 12°C	3.47 ± 0.20 b	0.83 ± 0.01 a	8.80 ± 0.17 a	1.72 ± 0.03 a
	HWT at 12°C	4.02 ± 0.31 a	0.83 ± 0.00 a	8.50 ± 0.87 a	1.74 ± 0.31 a
10	control at 8°C	5.42 ± 0.33 a	0.83 ± 0.00 ab	8.70 ± 0.00 b	1.89 ± 0.33 a
	HWT at 8°C	5.64 ± 0.37 a	0.83 ± 0.01 a	9.98 ± 0.67 a	1.49 ± 0.06 b
	control at 12°C	5.56 ± 0.32 a	0.82 ± 0.01 c	9.98 ± 0.62 a	1.90 ± 0.20 b
	HWT at 12°C	5.78 ± 0.45 a	0.82 ± 0.01 bc	10.70 ± 0.62 a	1.73 ± 0.22 ab
15	control at 8°C	7.22 ± 0.40 a	0.82 ± 0.01 a	10.05 ± 1.02 bc	1.73 ± 0.29 a
	HWT at 8°C	7.36 ± 0.48 a	0.82 ± 0.01 a	9.60 ± 0.60 c	1.71 ± 0.28 a
	control at 12°C	7.34 ± 0.39 a	0.81 ± 0.01 a	11.33 ± 0.83 ab	1.85 ± 0.27 a
	HWT at 12°C	7.71 ± 0.55 a	0.81 ± 0.01 a	11.60 ± 0.69 a	1.70 ± 0.17 a
16	control at 8°C	9.13 ± 0.50 b	0.80 ± 0.00 a	12.60 ± 0.79 b	1.64 ± 0.13 a
	HWT at 8°C	9.39 ± 0.59 ab	0.81 ± 0.01 a	12.40 ± 0.35 b	1.89 ± 0.15 a
	control at 12°C	9.44 ± 0.52 ab	0.76 ± 0.02 b	13.28 ± 0.90 ab	1.84 ± 0.24 a
	HWT at 12°C	9.80 ± 0.66 a	0.77 ± 0.02 b	14.30 ± 0.96 a	1.80 ± 0.27 a
17	control at 8°C	10.98 ± 0.57 b	0.69 ± 0.02 a	14.60 ± 0.69 a	1.36 ± 0.20 a
	HWT at 8°C	11.29 ± 0.69 ab	0.67 ± 0.07 a	13.95 ± 0.71 a	1.39 ± 0.09 a
	control at 12°C	11.48 ± 0.60 ab	0.67 ± 0.05 a	14.25 ± 0.79 a	1.29 ± 0.10 a
	HWT at 12°C	11.76 ± 0.75 a	0.65 ± 0.02 a	15.00 ± 0.00 a	1.20 ± 0.05 a
18	control at 8°C	11.87 ± 0.59 a	0.56 ± 0.03 a	15.30 ± 0.81 b	0.89 ± 0.31 a
	HWT at 8°C	12.15 ± 0.74 a	0.55 ± 0.02 a	15.83 ± 1.08 ab	0.70 ± 0.19 a
	control at 12°C	12.37 ± 0.61 a	0.54 ± 0.05 a	15.60 ± 0.60 ab	0.70 ± 0.13 a
	HWT at 12°C	12.58 ± 0.79 a	0.58 ± 0.05 a	17.00 ± 0.62 a	0.29 ± 0.09 b
19	control at 8°C	13.20 ± 0.63 a	0.43 ± 0.02 a	15.60 ± 0.65 a	0.29 ± 0.09 a
	HWT at 8°C	13.44 ± 0.79 a	0.42 ± 0.01 a	16.10 ± 0.46 a	0.32 ± 0.12 a
	control at 12°C	13.71 ± 0.65 a	0.44 ± 0.01 a	15.38 ± 0.29 a	0.20 ± 0.10 a
	HWT at 12°C	13.79 ± 0.87 a	0.45 ± 0.04 a	15.10 ± 1.35 a	0.25 ± 0.03 a
20	control at 8°C	14.27 ± 0.68 a	0.43 ± 0.01 ab	16.60 ± 1.05 a	0.13 ± 0.03 a
	HWT at 8°C	14.47 ± 0.78 a	0.45 ± 0.02 a	16.58 ± 0.29 a	0.14 ± 0.01 a
	control at 12°C	14.80 ± 0.73 a	0.41 ± 0.00 b	16.80 ± 0.79 a	0.09 ± 0.01 b
	HWT at 12°C	14.84 ± 0.92 a	0.40 ± 0.03 b	16.30 ± 0.46 a	0.09 ± 0.01 b

The values represented means and their respective standard errors. The different letters within treatments and columns indicated significant differences by using DMRT at $P<0.05$.

Table 4.2 Weight loss, pulp firmness, total soluble solids and titratable acidity of control and hot water treated (HWT) 'Ok Rong' fruit during low temperature storage (8°C and 12°C) for 15 days and ripening at ambient temperature for 5 days

Time (days)	Treatments	Weight loss (%)	Pulp firmness (N)	TSS (%)	TA (%)
0	control at 8°C	0	0.82 ± 0.01 a	6.00 ± 0.49 a	2.39 ± 0.14 a
	HWT at 8°C	0	0.83 ± 0.01 a	6.30 ± 0.35 a	2.33 ± 0.14 a
	control at 12°C	0	0.82 ± 0.00 a	5.85 ± 0.57 a	2.40 ± 0.09 a
	HWT at 12°C	0	0.83 ± 0.01 a	6.00 ± 0.49 a	2.33 ± 0.14 a
5	control at 8°C	2.75 ± 0.63 b	0.82 ± 0.02 ab	7.20 ± 0.60 a	2.03 ± 0.02 a
	HWT at 8°C	2.78 ± 0.60 b	0.83 ± 0.00 a	6.80 ± 0.35 a	2.03 ± 0.04 a
	control at 12°C	3.77 ± 0.58 a	0.81 ± 0.01 b	7.60 ± 0.35 a	0.28 ± 0.01 b
	HWT at 12°C	3.52 ± 0.52 a	0.82 ± 0.01 ab	7.40 ± 0.35 a	0.31 ± 0.03 b
10	control at 8°C	4.26 ± 1.00 b	0.82 ± 0.02 a	8.80 ± 0.35 b	0.26 ± 0.03 a
	HWT at 8°C	4.22 ± 0.92 b	0.82 ± 0.00 a	8.70 ± 0.35 b	0.28 ± 0.03 a
	control at 12°C	5.97 ± 0.94 a	0.81 ± 0.02 a	9.80 ± 0.69 a	0.22 ± 0.02 b
	HWT at 12°C	5.47 ± 0.81 a	0.81 ± 0.01 a	9.30 ± 0.60 ab	0.22 ± 0.01 b
15	control at 8°C	5.68 ± 1.34 b	0.81 ± 0.01 a	9.40 ± 0.35 b	0.21 ± 0.02 a
	HWT at 8°C	5.44 ± 1.20 b	0.82 ± 0.01 a	9.75 ± 1.14 b	0.21 ± 0.02 a
	control at 12°C	7.93 ± 1.25 a	0.66 ± 0.12 b	11.33 ± 0.79 a	0.18 ± 0.02 a
	HWT at 12°C	7.28 ± 1.08 a	0.75 ± 0.07 ab	9.60 ± 0.49 b	0.19 ± 0.02 a
16	control at 8°C	8.34 ± 1.51 b	0.65 ± 0.03 a	11.40 ± 0.60 a	0.15 ± 0.01 ab
	HWT at 8°C	7.90 ± 1.51 b	0.63 ± 0.03 a	11.20 ± 0.35 a	0.17 ± 0.01 a
	control at 12°C	10.07 ± 1.48 a	0.53 ± 0.05 b	11.85 ± 0.90 a	0.13 ± 0.02 b
	HWT at 12°C	9.27 ± 1.32 a	0.54 ± 0.06 b	11.70 ± 0.77 a	0.15 ± 0.02 ab
17	control at 8°C	10.20 ± 1.69 ab	0.50 ± 0.01 a	11.20 ± 1.25 b	0.10 ± 0.01 a
	HWT at 8°C	9.82 ± 1.75 b	0.50 ± 0.02 a	12.40 ± 0.35 ab	0.08 ± 0.01 a
	control at 12°C	11.77 ± 1.62 a	0.48 ± 0.04 a	13.73 ± 0.51 a	0.06 ± 0.02 a
	HWT at 12°C	10.89 ± 1.46 ab	0.49 ± 0.03 a	12.40 ± 0.92 ab	0.08 ± 0.04 a
18	control at 8°C	12.87 ± 2.12 a	0.45 ± 0.01 a	13.13 ± 0.62 a	0.07 ± 0.01 a
	HWT at 8°C	12.64 ± 2.06 a	0.45 ± 0.01 a	13.65 ± 1.92 a	0.05 ± 0.01 b
	control at 12°C	14.05 ± 1.85 a	0.45 ± 0.02 a	14.40 ± 1.10 a	0.06 ± 0.01 ab
	HWT at 12°C	13.21 ± 1.62 a	0.44 ± 0.01 a	14.18 ± 0.67 a	0.05 ± 0.01 ab
19	control at 8°C	14.61 ± 2.33 a	0.46 ± 0.01 a	13.43 ± 1.23 a	0.04 ± 0.01 a
	HWT at 8°C	14.27 ± 2.32 a	0.46 ± 0.03 a	13.95 ± 1.65 a	0.03 ± 0.01 a
	control at 12°C	15.61 ± 1.96 a	0.45 ± 0.03 a	15.00 ± 0.49 a	0.03 ± 0.01 a
	HWT at 12°C	14.93 ± 1.74 a	0.42 ± 0.03 a	14.63 ± 0.29 a	0.04 ± 0.01 a
20	control at 8°C	15.59 ± 2.46 a	0.45 ± 0.02 ab	15.15 ± 1.02 ab	0.03 ± 0.00 a
	HWT at 8°C	15.25 ± 2.43 a	0.46 ± 0.01 a	14.20 ± 1.25 b	0.03 ± 0.01 a
	control at 12°C	16.99 ± 2.10 a	0.43 ± 0.01 b	16.50 ± 1.73 a	0.03 ± 0.01 a
	HWT at 12°C	15.66 ± 1.47 a	0.44 ± 0.01 ab	15.53 ± 0.38 ab	0.03 ± 0.01 a

The values represented means and their respective standard errors. The different letters within treatments and columns indicated significant differences by using DMRT at $P<0.05$.

Table 4.3 Color parameters (L*, a*, b*), chroma (C*) and hue angle (h) of control and hot water treated (HWT) 'Nam Dok Mai' fruit during low temperature storage (8°C and 12°C) for 15 days and ripening at ambient temperature for 5 days

Time (days)	Treatments	L*	a*	b*	C*	h
0	control at 8°C	71.48 ± 1.28 a	15.25 ± 1.13 a	37.74 ± 0.97 a	40.46 ± 1.41 ab	112.15 ± 1.30 a
	HWT at 8°C	68.31 ± 1.67 b	15.23 ± 1.09 a	36.58 ± 0.63 b	39.65 ± 0.67 b	112.39 ± 1.50 a
	control at 12°C	69.17 ± 2.20 b	14.86 ± 0.70 a	38.15 ± 0.62 a	41.06 ± 0.72 a	111.66 ± 1.35 a
	HWT at 12°C	70.17 ± 1.51 ab	14.66 ± 1.30 a	37.80 ± 1.17 a	40.88 ± 0.96 a	111.04 ± 1.33 a
5	control at 8°C	70.67 ± 1.12 a	14.90 ± 1.15 a	38.19 ± 0.88 a	41.00 ± 1.01 a	111.20 ± 1.52 a
	HWT at 8°C	68.60 ± 1.26 b	14.54 ± 1.23 a	37.58 ± 1.48 a	40.33 ± 1.37 a	111.20 ± 1.94 a
	control at 12°C	68.22 ± 2.16 b	14.46 ± 1.08 a	38.15 ± 0.66 a	40.94 ± 0.70 a	111.67 ± 0.86 a
	HWT at 12°C	68.33 ± 2.18 b	14.32 ± 1.54 a	37.17 ± 1.60 a	39.82 ± 1.68 a	110.06 ± 1.57 a
10	control at 8°C	68.52 ± 1.38 a	14.81 ± 1.13 a	39.86 ± 2.33 a	42.55 ± 1.86 a	110.02 ± 2.33 a
	HWT at 8°C	66.51 ± 1.87 a	14.34 ± 1.95 a	40.77 ± 2.78 a	43.29 ± 2.92 a	109.02 ± 2.33 a
	control at 12°C	67.60 ± 1.94 a	15.37 ± 1.12 a	40.12 ± 2.05 a	42.84 ± 1.89 a	109.37 ± 1.15 a
	HWT at 12°C	68.06 ± 2.05 a	14.08 ± 1.73 a	39.00 ± 2.05 a	41.55 ± 2.05 a	109.52 ± 1.87 a
15	control at 8°C	70.40 ± 1.30 a	13.81 ± 1.38 a	39.18 ± 0.84 a	41.60 ± 0.99 a	109.55 ± 1.77 a
	HWT at 8°C	68.99 ± 1.56 a	13.48 ± 1.76 a	38.89 ± 1.09 a	41.21 ± 0.98 a	109.13 ± 2.53 a
	control at 12°C	69.25 ± 2.44 a	14.39 ± 1.11 a	39.50 ± 1.77 a	41.86 ± 1.01 a	109.71 ± 1.34 a
	HWT at 12°C	69.98 ± 2.33 a	12.47 ± 2.36 a	39.77 ± 1.34 a	41.76 ± 1.23 a	107.24 ± 3.30 a
16	control at 8°C	69.16 ± 1.62 ab	13.75 ± 1.58 b	41.74 ± 3.93 a	42.84 ± 1.78 b	108.11 ± 2.54 a
	HWT at 8°C	67.92 ± 1.50 b	11.72 ± 2.97 ab	42.94 ± 2.02 a	45.87 ± 3.01 a	105.83 ± 4.41 a
	control at 12°C	68.82 ± 2.79 ab	13.42 ± 1.07 b	41.89 ± 2.62 a	43.84 ± 1.94 ab	106.32 ± 5.57 a
	HWT at 12°C	70.29 ± 1.44 a	10.49 ± 2.62 a	43.40 ± 1.75 a	44.62 ± 1.78 ab	104.5 ± 3.76 a
17	control at 8°C	69.85 ± 1.23 a	12.25 ± 1.91 b	43.85 ± 1.39 b	45.98 ± 0.81 a	105.29 ± 2.45 a
	HWT at 8°C	68.41 ± 1.75 a	11.83 ± 2.35 b	45.29 ± 2.03 ab	47.01 ± 1.74 a	104.70 ± 3.20 a
	control at 12°C	68.75 ± 2.45 a	11.08 ± 1.85 b	45.74 ± 2.10 ab	47.01 ± 1.57 a	103.81 ± 2.48 a
	HWT at 12°C	69.10 ± 1.37 a	8.31 ± 3.17 a	46.50 ± 1.94 a	47.39 ± 1.32 a	100.10 ± 4.11 b
18	control at 8°C	69.29 ± 1.12 a	10.40 ± 2.58 a	44.68 ± 1.26 a	46.36 ± 0.63 a	103.12 ± 3.23 a
	HWT at 8°C	67.54 ± 1.31 a	9.07 ± 2.96 a	45.48 ± 2.26 a	46.21 ± 2.00 a	101.35 ± 3.95 a
	control at 12°C	67.53 ± 2.43 a	8.61 ± 2.51 a	46.33 ± 1.78 a	47.33 ± 1.51 a	100.62 ± 3.29 a
	HWT at 12°C	68.33 ± 1.56 a	7.63 ± 3.33 a	46.47 ± 1.55 a	47.21 ± 1.91 a	99.29 ± 4.26 a
19	control at 8°C	68.29 ± 1.15 a	8.73 ± 2.68 b	45.77 ± 1.28 a	46.71 ± 1.09 a	100.83 ± 3.39 a
	HWT at 8°C	67.11 ± 1.16 a	7.60 ± 3.27 ab	45.94 ± 2.05 a	46.47 ± 1.74 a	99.48 ± 4.21 ab
	control at 12°C	67.38 ± 2.03 a	6.90 ± 1.95 ab	47.16 ± 2.33 a	47.93 ± 2.18 a	98.50 ± 2.59 ab
	HWT at 12°C	67.27 ± 1.84 a	5.25 ± 3.38 a	46.21 ± 1.71 a	46.71 ± 1.50 a	96.45 ± 4.25 b
20	control at 8°C	67.28 ± 1.07 a	6.99 ± 2.43 a	45.86 ± 1.26 a	46.97 ± 0.43 a	99.49 ± 3.69 a
	HWT at 8°C	66.01 ± 1.11 a	7.29 ± 3.57 a	45.60 ± 1.88 a	46.02 ± 1.61 a	98.40 ± 4.30 a
	control at 12°C	66.36 ± 1.98 a	5.85 ± 1.67 a	46.88 ± 2.09 a	47.33 ± 1.91 a	97.24 ± 2.25 a
	HWT at 12°C	66.53 ± 1.63 a	4.20 ± 3.65 a	46.54 ± 2.02 a	46.95 ± 1.87 a	95.21 ± 4.61 a

The values represented means and their respective standard errors. The different letters within treatments and columns indicated significant differences by using DMRT at $P<0.05$.

Table 4.4 Color parameters (L*, a*, b*), chroma (C*) and hue angle (h) of control and hot water treated (HWT) 'Ok Rong' fruit during low temperature storage (8°C and 12°C) for 15 days and ripening at ambient temperature for 5 days

Time (days)	Treatments	L*	a*	b*	C*	h
0	control at 8°C	51.66 ± 1.67 a	16.89 ± 1.20 a	24.93 ± 1.62 a	29.73 ± 2.32 a	126.25 ± 3.02 a
	HWT at 8°C	51.52 ± 1.99 a	17.35 ± 0.85 a	23.65 ± 2.80 a	29.07 ± 2.30 a	126.99 ± 3.39 a
	control at 12°C	50.68 ± 1.33 a	17.29 ± 1.20 a	24.01 ± 2.92 a	27.82 ± 2.30 a	128.00 ± 3.05 a
	HWT at 12°C	51.65 ± 1.70 a	17.15 ± 1.04 a	24.60 ± 1.56 a	28.90 ± 1.66 a	127.92 ± 3.03 a
5	control at 8°C	49.60 ± 1.81 a	18.41 ± 0.81 a	29.66 ± 1.91 a	34.13 ± 2.81 a	122.66 ± 1.50 a
	HWT at 8°C	50.71 ± 2.20 a	18.53 ± 1.13 a	26.87 ± 3.88 a	32.60 ± 3.58 a	123.98 ± 3.73 a
	control at 12°C	49.74 ± 1.62 a	18.08 ± 1.33 a	27.18 ± 2.59 a	31.60 ± 2.23 a	123.80 ± 2.05 a
	HWT at 12°C	50.93 ± 2.41 a	18.47 ± 0.83 a	27.82 ± 2.17 a	32.79 ± 1.41 a	124.41 ± 2.34 a
10	control at 8°C	50.03 ± 1.50 a	18.33 ± 1.20 ab	31.77 ± 1.87 a	35.97 ± 2.65 a	121.03 ± 1.01 a
	HWT at 8°C	51.15 ± 2.95 a	19.00 ± 0.60 b	32.47 ± 2.86 a	37.77 ± 2.74 a	120.88 ± 1.68 a
	control at 12°C	51.32 ± 2.14 a	17.51 ± 1.51 a	29.48 ± 3.70 a	34.84 ± 2.59 a	120.12 ± 3.84 a
	HWT at 12°C	52.20 ± 2.58 a	18.27 ± 0.88 ab	30.48 ± 2.41 a	35.20 ± 2.13 a	121.58 ± 2.15 a
15	control at 8°C	50.83 ± 2.29 a	19.23 ± 0.92 b	36.50 ± 2.33 a	40.58 ± 3.27 a	118.30 ± 1.01 a
	HWT at 8°C	49.81 ± 2.32 a	18.87 ± 1.63 ab	34.25 ± 3.74 a	39.21 ± 3.52 a	119.20 ± 3.18 a
	control at 12°C	53.31 ± 3.69 a	17.56 ± 1.11 ab	34.85 ± 4.82 a	40.27 ± 3.73 a	117.45 ± 3.85 a
	HWT at 12°C	52.96 ± 2.59 a	17.81 ± 1.69 a	36.72 ± 2.68 a	40.64 ± 2.17 a	116.16 ± 3.18 a
16	control at 8°C	54.00 ± 2.69 bc	16.96 ± 2.13 b	31.68 ± 4.05 b	36.45 ± 3.53 b	119.69 ± 3.89 a
	HWT at 8°C	53.02 ± 2.01 c	17.52 ± 1.28 b	31.22 ± 2.67 b	35.93 ± 2.54 b	119.57 ± 2.02 a
	control at 12°C	57.93 ± 4.81 b	13.36 ± 3.77 a	39.60 ± 7.13 a	42.48 ± 5.82 a	110.68 ± 7.34 b
	HWT at 12°C	61.81 ± 3.65 a	13.25 ± 3.52 a	42.42 ± 5.56 a	44.39 ± 4.15 a	107.06 ± 6.75 b
17	control at 8°C	53.60 ± 4.04 b	16.35 ± 2.57 b	33.90 ± 4.63 b	38.75 ± 3.76 b	117.43 ± 5.03 a
	HWT at 8°C	53.58 ± 1.95 b	17.10 ± 1.22 b	32.68 ± 2.83 b	36.42 ± 2.15 b	118.18 ± 2.01 a
	control at 12°C	58.20 ± 4.60 a	12.16 ± 4.46 a	41.86 ± 7.22 a	44.35 ± 5.82 a	108.58 ± 7.83 b
	HWT at 12°C	61.04 ± 3.63 a	12.33 ± 4.01 a	43.47 ± 5.07 a	45.10 ± 3.83 a	103.77 ± 7.17 b
18	control at 8°C	54.63 ± 3.59 b	15.43 ± 2.74 b	38.33 ± 4.57 b	42.42 ± 3.68 bc	113.79 ± 4.91 a
	HWT at 8°C	53.85 ± 2.35 b	16.51 ± 1.26 b	36.88 ± 2.81 b	40.05 ± 2.51 c	114.22 ± 2.62 a
	control at 12°C	59.52 ± 4.51 a	10.36 ± 5.00 a	45.13 ± 5.91 a	46.96 ± 4.77 a	104.88 ± 7.25 b
	HWT at 12°C	61.16 ± 3.52 a	10.37 ± 4.22 a	44.59 ± 3.33 a	45.76 ± 2.47 ab	102.23 ± 5.68 b
19	control at 8°C	55.46 ± 3.03 b	14.14 ± 3.14 b	40.55 ± 3.54 bc	43.56 ± 2.76 ab	110.97 ± 4.63 a
	HWT at 8°C	54.52 ± 2.50 b	15.50 ± 1.50 b	38.37 ± 2.61 c	41.38 ± 2.44 b	111.95 ± 2.34 a
	control at 12°C	60.81 ± 3.33 a	9.43 ± 4.98 a	45.10 ± 5.75 a	46.69 ± 4.76 a	103.80 ± 6.94 b
	HWT at 12°C	59.89 ± 2.60 a	9.48 ± 4.09 a	44.18 ± 3.36 ab	45.19 ± 2.71 a	101.01 ± 5.49 b
20	control at 8°C	55.28 ± 2.47 b	13.48 ± 3.12 b	42.05 ± 3.46 bc	43.88 ± 3.22 ab	110.13 ± 2.56 a
	HWT at 8°C	54.89 ± 2.63 b	14.34 ± 1.64 b	40.17 ± 1.93 c	42.72 ± 1.71 a	109.62 ± 2.54 a
	control at 12°C	59.76 ± 4.02 a	8.26 ± 4.82 a	45.15 ± 5.46 ab	46.46 ± 4.61 a	102.26 ± 6.52 b
	HWT at 12°C	61.30 ± 2.08 a	8.17 ± 3.79 a	46.41 ± 2.83 a	47.10 ± 2.32 a	98.73 ± 4.67 b

The values represented means and their respective standard errors. The different letters within treatments and columns indicated significant differences by using DMRT at $P<0.05$.

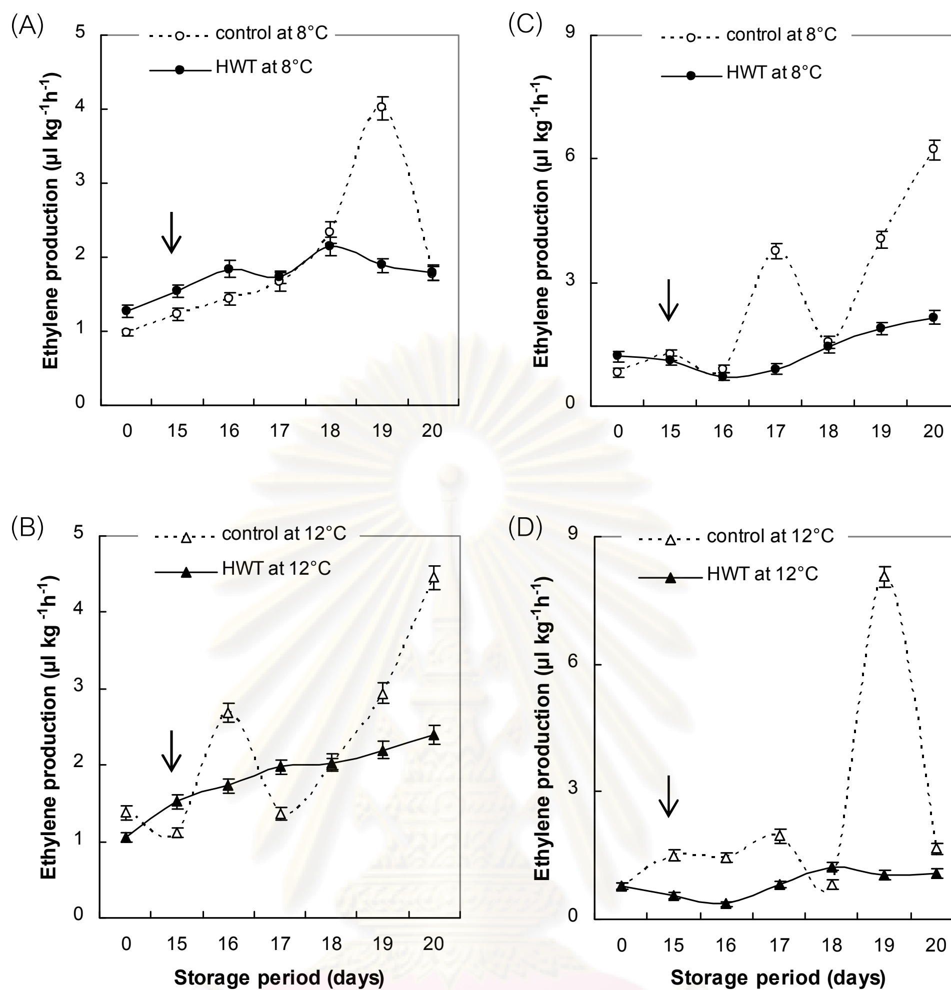

Figure 4.1 Ethylene production of control and HWT 'Nam Dok Mai' (A, B) and 'Ok Rong' fruit (C, D) during low temperature storage (8°C and 12°C) for 15 days and ripening at ambient temperature for 5 days. Arrows indicated a day which fruits were transferred to store at ambient temperature.

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4.3 Effect of hot water treatment on CAT, APX and GR activities in mango fruits

Activities of CAT, APX and GR in peel and pulp of 'Nam Dok Mai' cultivar were shown in Figure 4.2 to 4.4, respectively; and those of 'Ok Rong' mango peel and pulp in Figure 4.5 and 4.7, respectively. In 'Nam Dok Mai' mango stored at 8°C, the peel of HWT fruit had significantly increased in CAT activity on day 5, day 15 and day 18 compared to the peel of control fruit, while the pulp of HWT fruit showed significantly increased in CAT activity only on day 20 of storage (Fig. 4.2A). In addition, CAT activity in the pulp of HWT fruit stored at 12°C exhibited significantly higher activity than those of the pulp of control fruit on day 15 of storage (Fig. 4.2B). The CAT activity in 'Ok Rong' mango stored at 8°C had significantly increased in the peel of HWT fruit on day 5, day15 and day 20 of storage period (Fig. 4.5A), while the peel of HWT fruit stored at 12°C had significantly higher activity than those of the peel of control fruit on day 5 of storage (Fig. 4.5B).

APX activity in 'Nam Dok Mai' mango stored at 8°C had slightly increased in the peel of HWT fruit on day 15 compared to the peel of control fruit, while the pulp of HWT fruit were significantly different than those of the pulp of control fruit on day 20 at the end of storage (Fig. 4.3A). There were similar pattern of changes in peel and pulp of mango stored at 12°C (Fig. 4.3B). In 'Ok Rong' mango stored at 8°C, the peel of HWT fruit showed significantly higher activity than those of the peel of control fruit on day 5 and day 15 of storage (Fig. 4.6A), but the peel of control fruit stored at 12°C had significantly higher activity than those of the peel of HWT fruit on day 5 during low temperature storage (Fig. 4.6B).

GR activity in 'Nam Dok Mai' mango stored at 8°C exhibited significantly higher activity in the peel of HWT fruit than those of the peel of control fruit on day 5 and day 20 of storage, while the pulp of HWT fruit had significantly higher activity than those of the pulp of control fruit on day 10 and day 20 of storage (Fig. 4.4A). In addition, the peel of HWT fruit stored at 12°C showed significantly higher activity than those of the peel of control fruit on day 15, and the pulp of HWT fruit stored at 12°C had significantly higher activity than those of the pulp of control fruit on day 16 during ripening at ambient temperature (Fig. 4.4B). The GR activity in 'Ok Rong' mango stored at 8°C had significantly higher activity in the peel of HWT fruit than those of the peel of control fruit on day 5 and day 10 during low temperature storage (Fig. 4.7A), while the peel of HWT

fruit stored at 12°C had significantly higher activity than those of the peel of control fruit only on day 10 of storage (Fig. 4.7B).

4.4 Effect of hot water treatment on the protein patterns and LOX protein

The SDS-PAGE of total proteins extracted from peel and pulp tissues in 'Nam Dok Mai' mango were shown in Figure 4.8 and 4.9, respectively; and those of 'Ok Rong' mango peel and pulp in Figure 4.10 and 4.11, respectively. Total protein were extracted from peel and pulp of both varieties with and without heat treatment and storing at 8°C and 12°C followed by ripening at ambient temperature. These protein extracts were separated on 10% SDS-PAGE and protein bands visualized with followed by staining with Commassie brilliant blue R-250. Protein bands ranging from molecular weight of 12 to 347 kD were quantified using an Imaging program (Image Quant version 5.1) For 'Nam Dok Mai' mango peel, 20 bands including 347, 212, 155, 99, 93, 83, 74, 69, 63, 59, 53, 47, 43, 37, 33, 27, 25, 22, 15 and 12 kD were quantified; and also found 22 bands from 'Nam Dok Mai' pulp including 253, 186, 168, 143, 127, 112, 99, 91, 83, 80, 74, 69, 59, 53, 47, 43, 37, 27, 22, 19, 17 and 12 kD. For 'Ok Rong' mango peel, 22 bands including 347, 228, 212, 186, 155, 99, 96, 93, 83, 74, 69, 63, 53, 47, 43, 37, 33, 27, 25, 22, 19, 15 and 12 kD were investigated; and also found 29 bands from 'Ok Rong' pulp, including 253, 228, 198, 186, 168, 143, 127, 112, 99, 91, 83, 80, 74, 69, 59, 53, 47, 43, 37, 31, 27, 25, 22, 19, 17, 15, 14, 13 and 12 kD were quantified. Changes in pattern of these proteins (% control) are shown on blue to red bars on heat maps. Protein patterns also showed in the percent level from indicating different changes in their patterns as shown in Figure 4.16a, b and 4.17a, b. The intense red bars represented the highest protein values and the intense blue bars represented the lowest protein values. 'Nam Dok Mai' peel had a high value of 347 kD protein on day 10, and also in 155 kD protein on day 15. It showed that HWT at 50°C had an effect on a 15 kD protein level due to its high values on day 15 and 18 during ripening at ambient temperature. In 'Ok Rong' peel showed that HWT at 50°C had an effect on a 228 kD protein level due to its high values during ripening; and it also had an effect on a 19 kD protein level resulting in its high values during low temperature storage. In 'Nam Dok Mai' pulp had a high value of 253 kD protein on day 16 and 18 during ripening at ambient temperature. It also showed a high value of 19 kD on the end of storage both in

control (8 and 12°C) and HWT fruit stored at 8°C. HWT 'Ok Rong' pulp stored at 8°C had a high value of 253, 228, 198, 186 and 168 kD on day 16 indicated that hot water treatment had effects on these proteins. A 17 kD protein showed a high value during ripening in both control (8 and 12°C) and HWT fruit stored at 12°C. The HWT 'Ok Rong' fruit pulp had a high value of 15 kD on day 20, and 12 kD on day 5.

The accumulation of lipoxygenase (LOX) protein in peel and pulp of 'Nam Dok Mai' mango as shown on immunoblots were presented in Figure 4.12 and 4.13; and those of 'Ok Rong' mango in Figure 4.14 and 4.15, respectively. Although the polyclonal LOX antibodies used in these studies were raised against a tomato fruit LOX protein, they cross reacted to mango proteins with molecular weight ranging from 91 to 99 kD. In general, molecular weights of plants lipoxygenases range from 94 to 104 kD (Szymanowska *et al.,* 2009), thus suggesting that tomato lox antibodies cross reacted to mango LOX proteins. Semi-quantitation of these cross-reactive bands indicated that hot water treatment enhanced the accumulation of LOX protein in mango fruit.

4.5 Effect of hot water treatment on transcript accumulation of Mn-SOD and cell wall hydrolases

The changes of four selected genes in both mango cultivars were shown in Figure 4.18. Besides, the transcript abundance of Mn-SOD, pectate lyase (PL), β-galactosidase and β-1,3-glucanase exhibited in transcript levels from blue to red on the heat map indicating different changes in the expression patterns of selected genes as shown in Figure 4.19. The intense red bars represented the highest expression values and the intense blue bars represented the lowest expression. The steady state levels of Mn-SOD transcript had a range from 85 to 225 percent of zero time in 'Nam Dok Mai' cultivar, and the pulp of control fruit accumulated higher levels of transcript than the pulp of HWT fruit during ripening at ambient temperature. In 'Ok Rong' cultivar, the steady state levels of Mn-SOD transcripts ranged from 71 to 227 percent of zero time with high of transcript levels during low temperature storage in the pulp of HWT fruit especially, on day 10 of storage.

The steady state levels of PL transcript had a range from 85 to 393 percent of zero time in 'Nam Dok Mai' cultivar with the pulp of HWT fruit stored at 12°C accumulating higher transcript levels than other treatments from day 10 to the end of

storage. Mostly, fruits showed high of transcript levels during ripening at ambient temperature. The steady state PL transcript levels in ‘Ok Rong’ fruit pulp ranged from -342 to 541 percent of zero time with high transcript accumulation during low temperature storage in the HWT fruit. However, during ripening at ambient temperature, the pulp of HWT fruit exhibited reduced transcript levels than the pulp of control fruit.

The accumulation of β-galactosidase transcript had a range from 38 to 226 percent of zero time in ‘Nam Dok Mai’ cultivar with the pulp of control fruit accumulating higher levels than the pulp of HWT fruit during ripening at ambient temperature. In ‘Ok Rong’ cultivar, they ranged from -342 to 526 percent of zero time in transcript accumulation of β-galactosidase with high of transcript levels in the pulp of HWT fruit during low temperature storage. Later, the pulp of HWT fruit accumulated less transcripts than the pulp of control fruit during ripening at ambient temperature.

The accumulation of β-1,3-glucanase transcript had a range from -1492 to 461 percent of zero time in ‘Nam Dok Mai’ cultivar, with the pulp of HWT fruit accumulating higher in transcript levels than the pulp of HWT fruit in the storage period except on day 15. ‘Ok Rong’ fruit pulp exhibited a range from 45 to 293 percent of zero time in transcript accumulation of β-1,3-glucanase with the highest of transcript levels in the pulp of HWT fruit on day 10 during low temperature storage.

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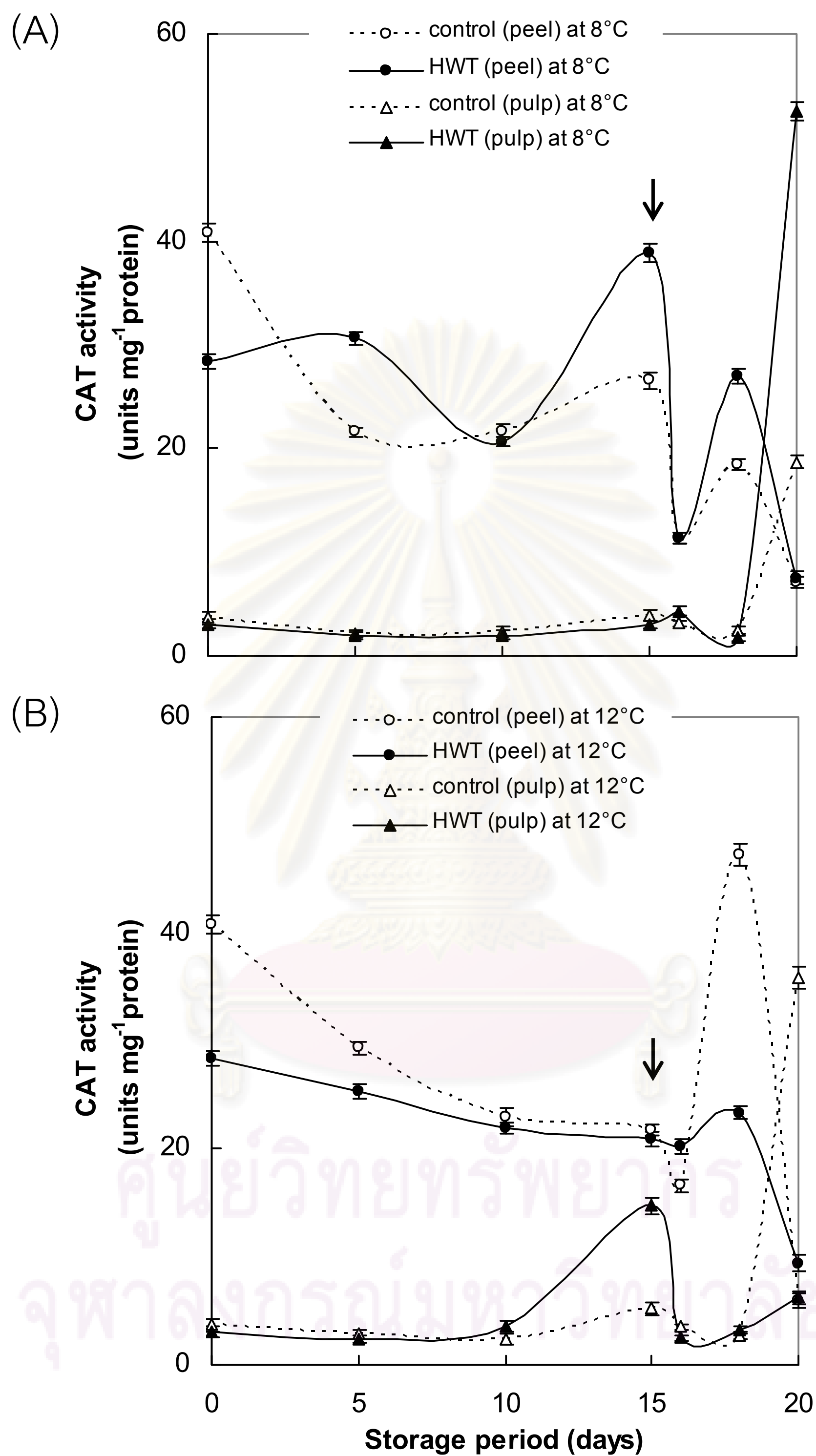

Figure 4.2 The activity of CAT in 'Nam Dok Mai' fruit peel and pulp during low temperature storage at 8°C (A), 12°C (B) and ripening at ambient temperature. Arrows indicated a day which fruits were transferred to store at ambient temperature. Vertical bars represented the standard errors of the means.

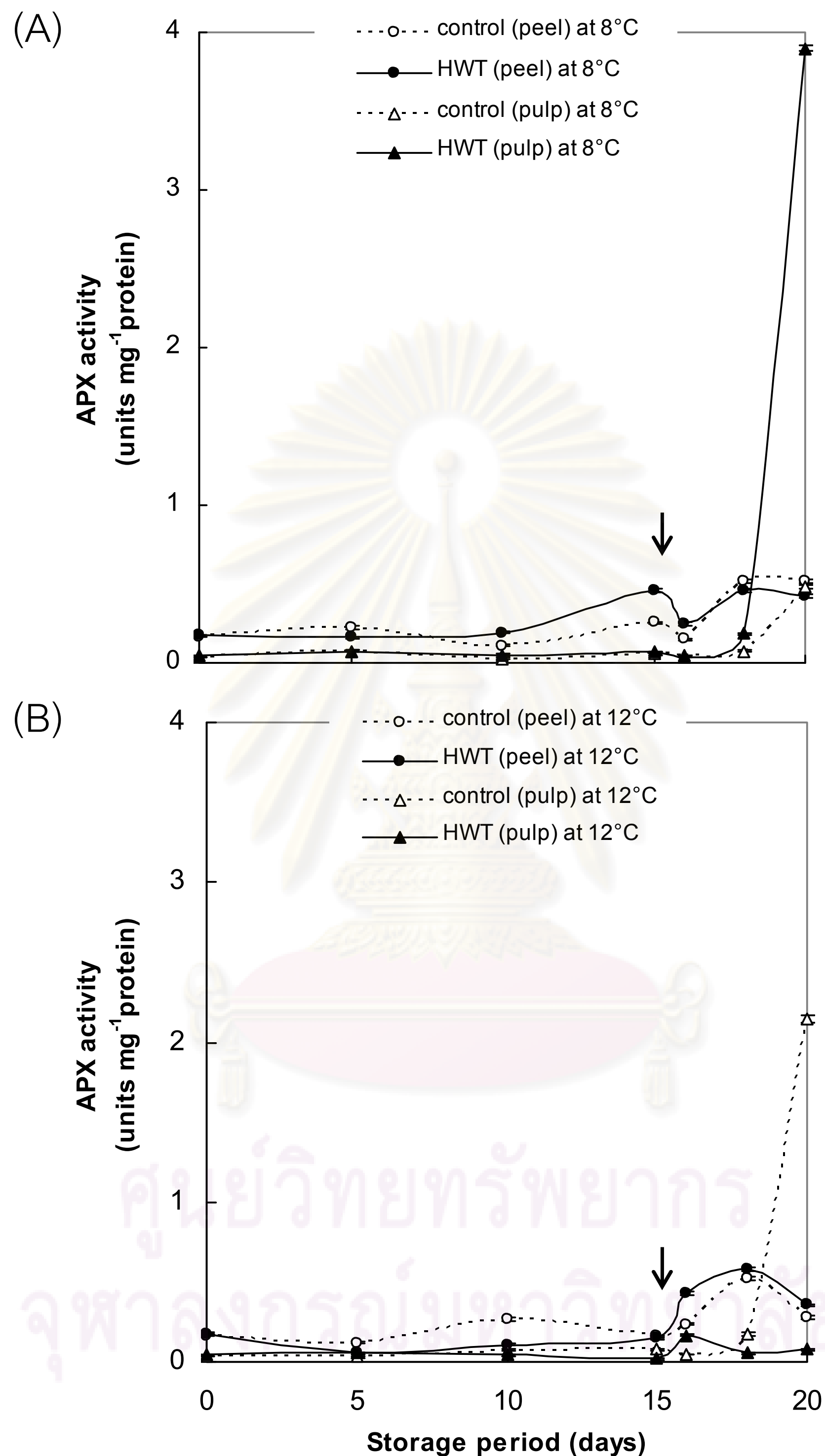

Figure 4.3 The activity of APX in 'Nam Dok Mai' fruit peel and pulp during low temperature storage at 8°C (A), 12°C (B) and ripening at ambient temperature. Arrows indicated a day which fruits were transferred to store at ambient temperature. Vertical bars represented the standard errors of the means.

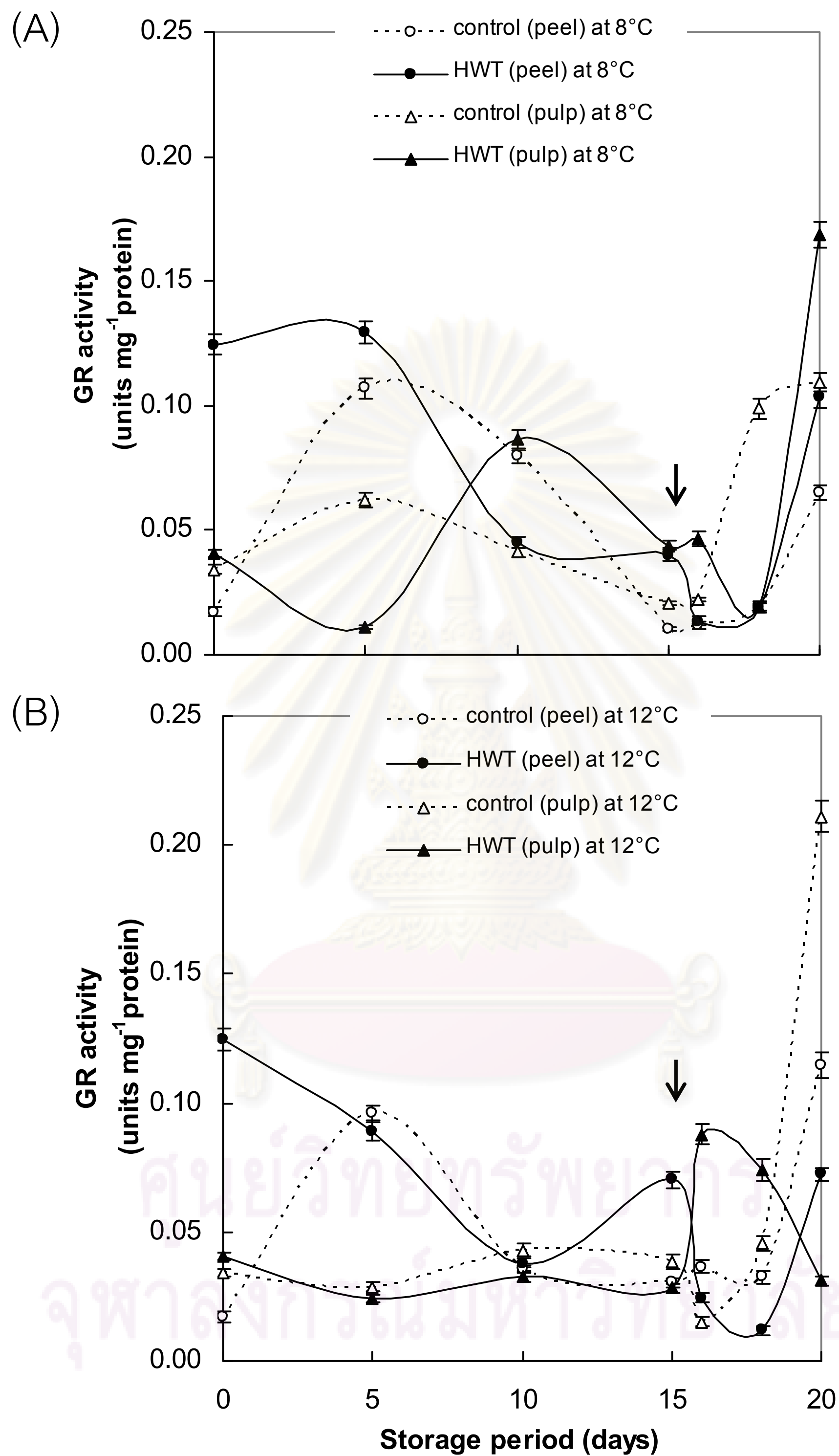

Figure 4.4 The activity of GR in 'Nam Dok Mai' fruit peel and pulp during low temperature storage at 8°C (A), 12°C (B) and ripening at ambient temperature. Arrows indicated a day which fruits were transferred to store at ambient temperature. Vertical bars represented the standard errors of the means.

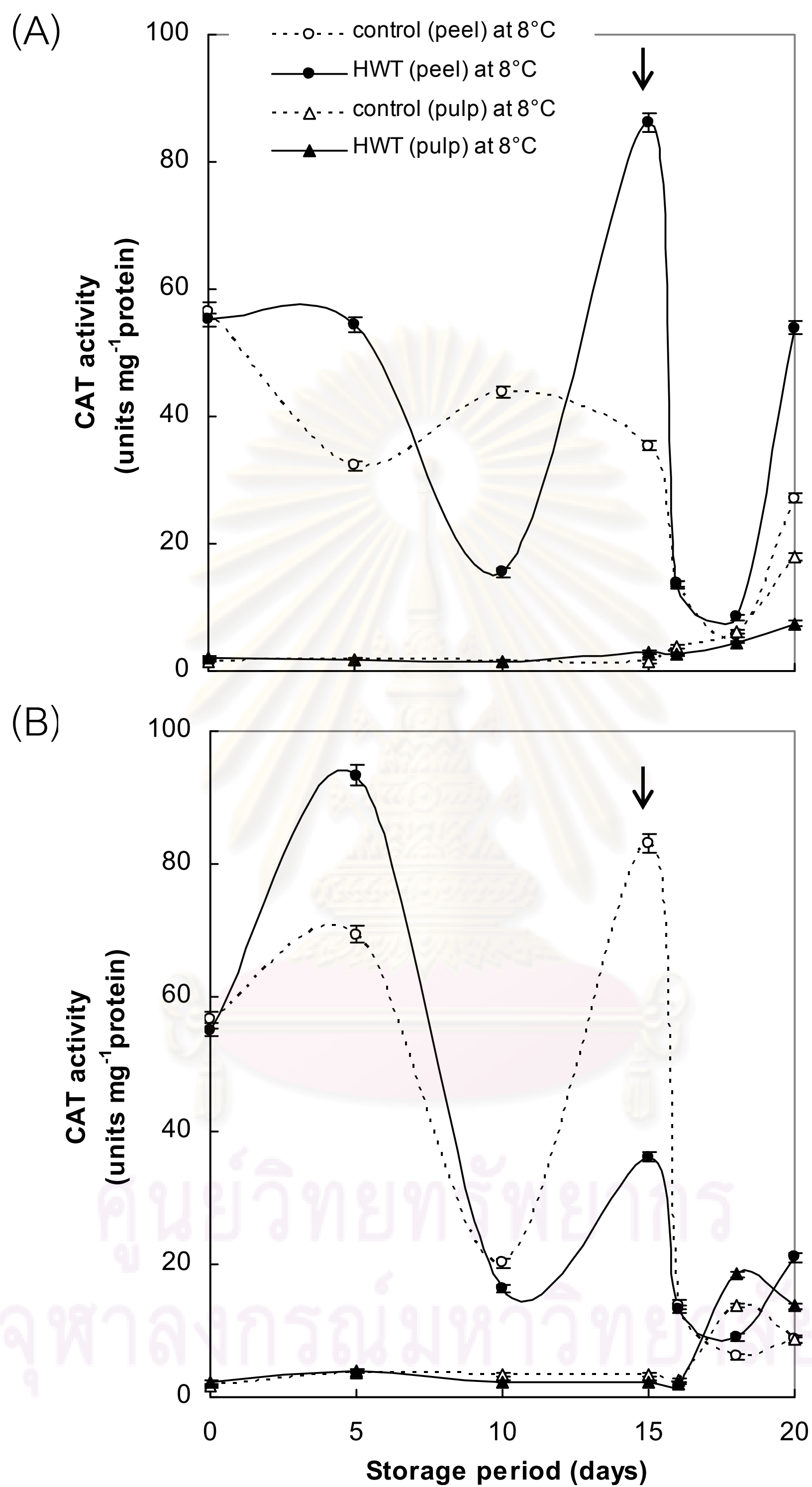

Figure 4.5 The activity of CAT in 'Ok Rong' fruit peel and pulp during low temperature storage at 8°C (A), 12°C (B) and ripening at ambient temperature. Arrows indicated a day which fruits were transferred to store at ambient temperature. Vertical bars represented the standard errors of the means.

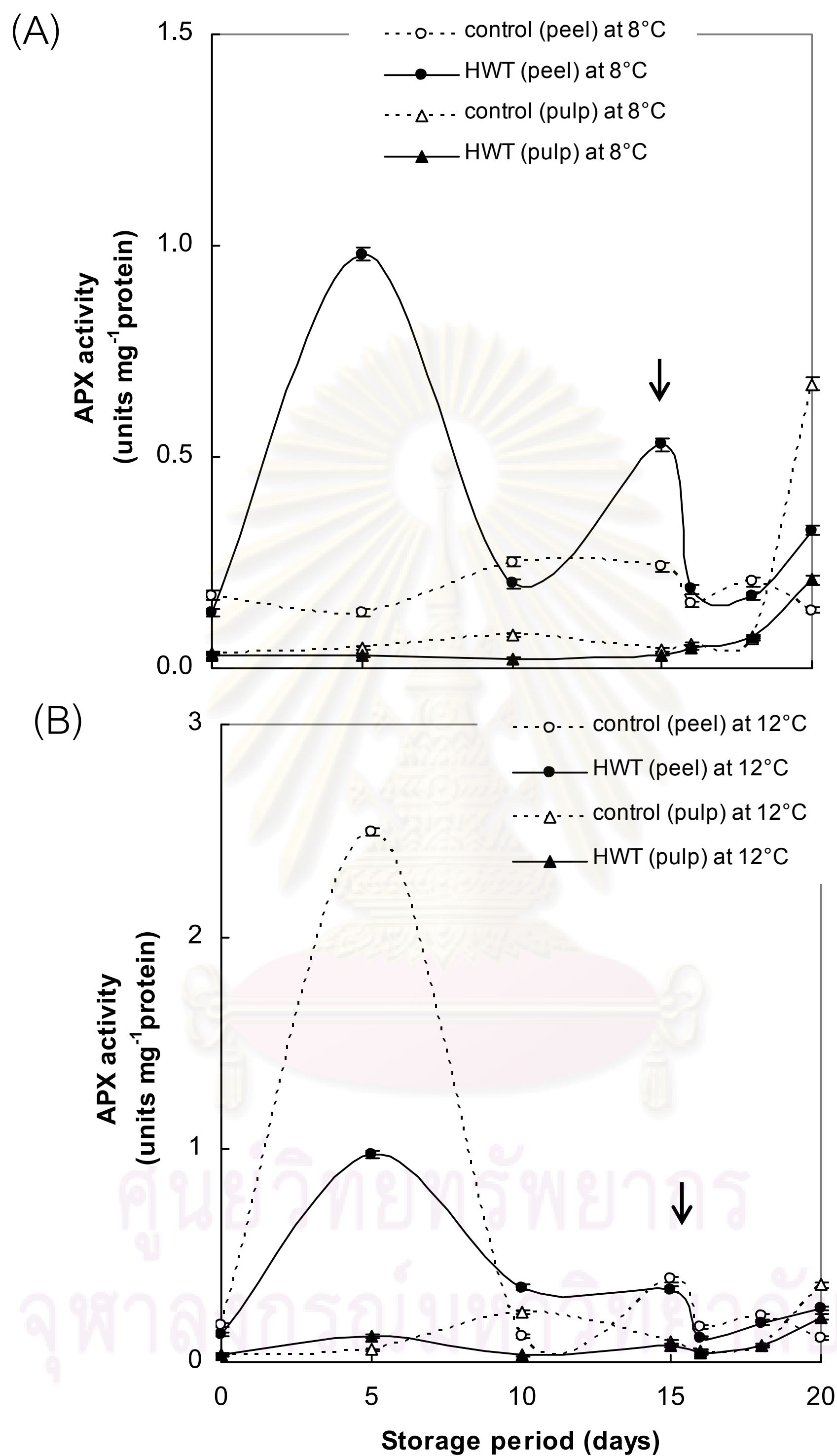

Figure 4.6 The activity of APX in 'Ok Rong' fruit peel and pulp during low temperature storage at 8°C (A), 12°C (B) and ripening at ambient temperature. Arrows indicated a day which fruits were transferred to store at ambient temperature. Vertical bars represented the standard errors of the means.

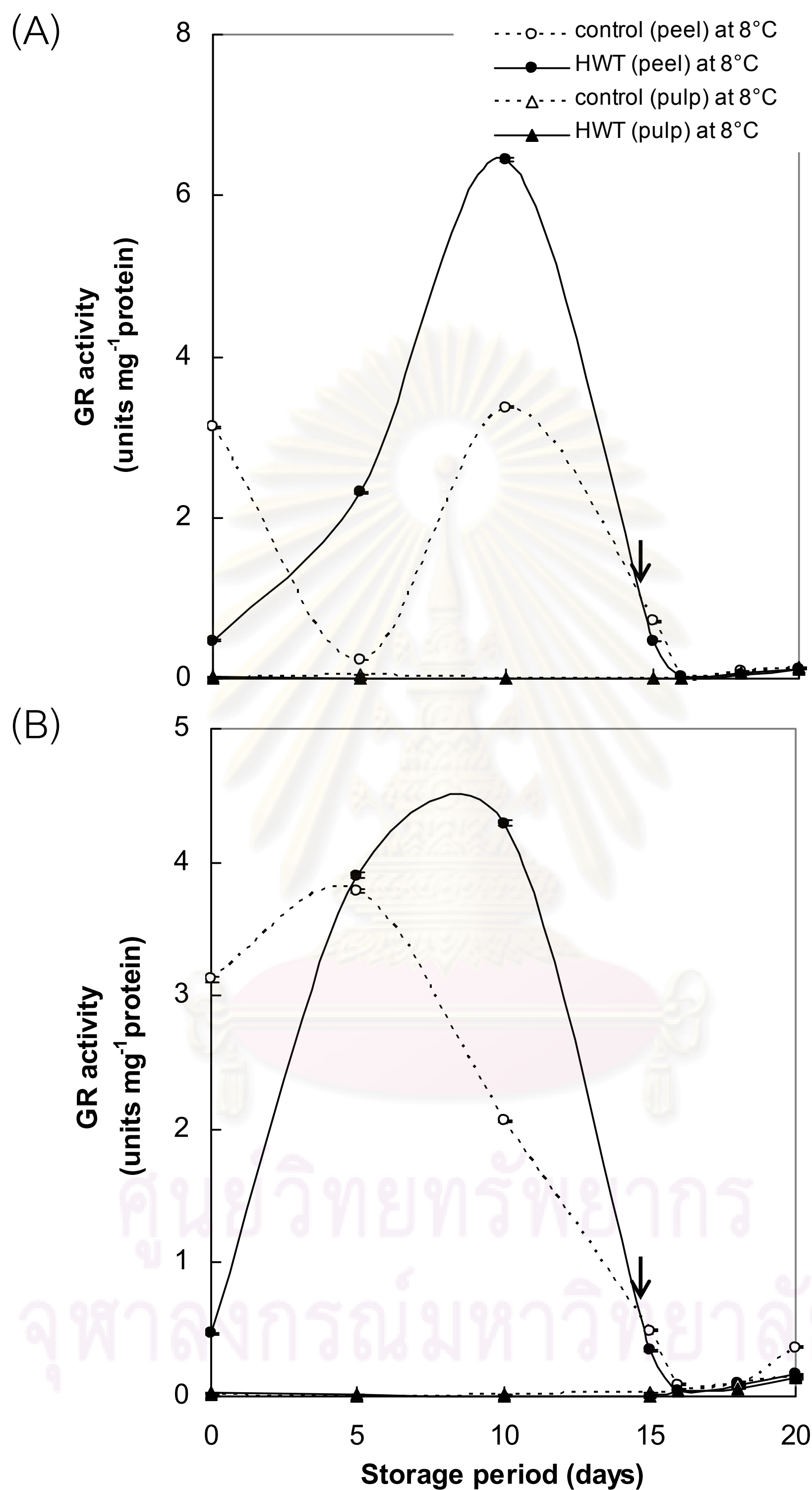

Figure 4.7 The activity of GR in 'Ok Rong' fruit peel and pulp during low temperature storage at 8°C (A), 12°C (B) and ripening at ambient temperature. Arrows indicated a day which fruits were transferred to store at ambient temperature. Vertical bars represented the standard errors of the means.

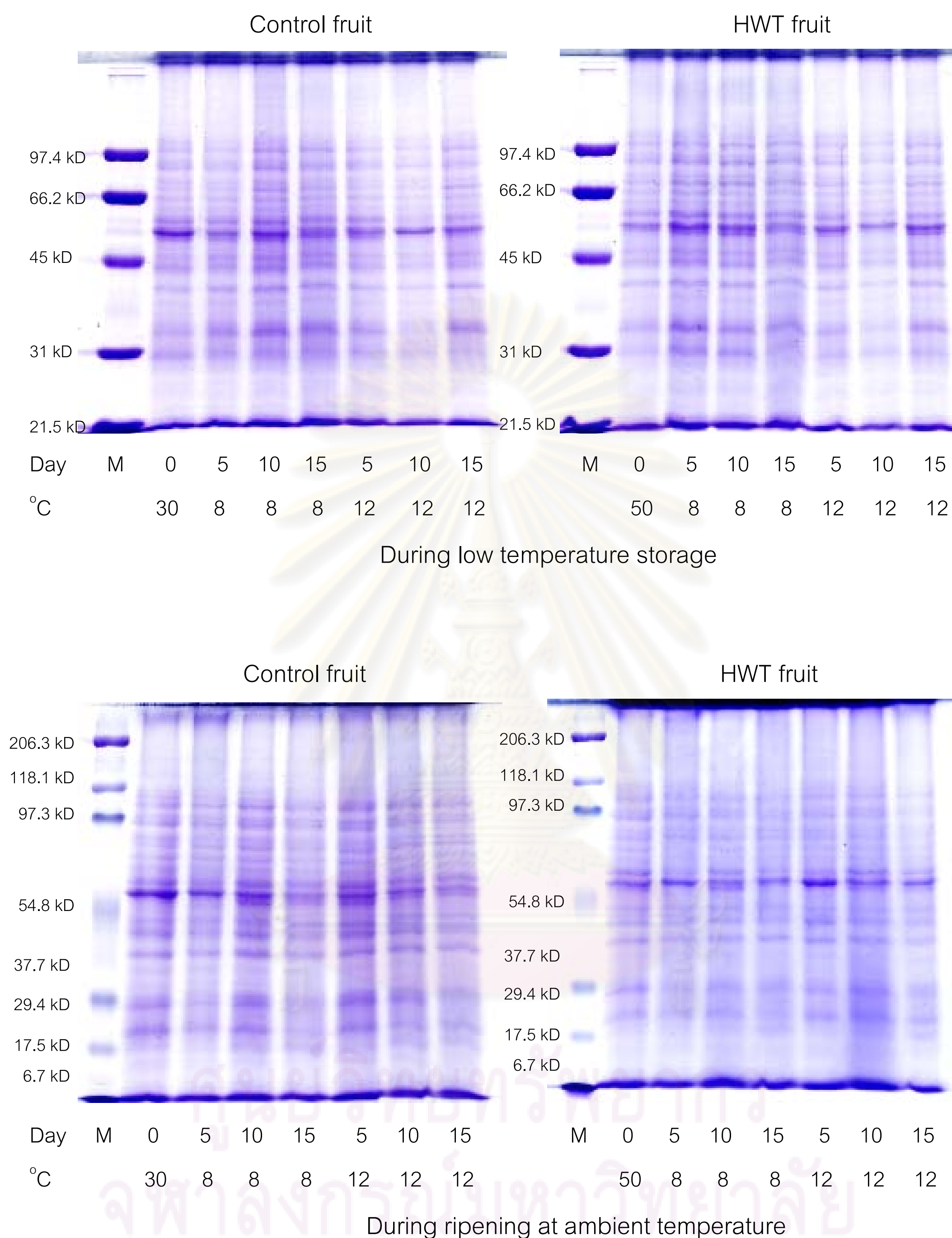

Figure 4.8 Protein profiles of peel 'Nam Dok Mai' mango during low temperature storage and ripening at ambient temperature. Equal amount of total proteins were loaded in lanes 2-7; seperated on 10% SDS-polyacrylamide gel; stained with Coomassie blue R-250 and quantified the intensity band of the profile by using Imagine software. Lane 1 was marker (molecular mass expressed in kD as marked on the left side).

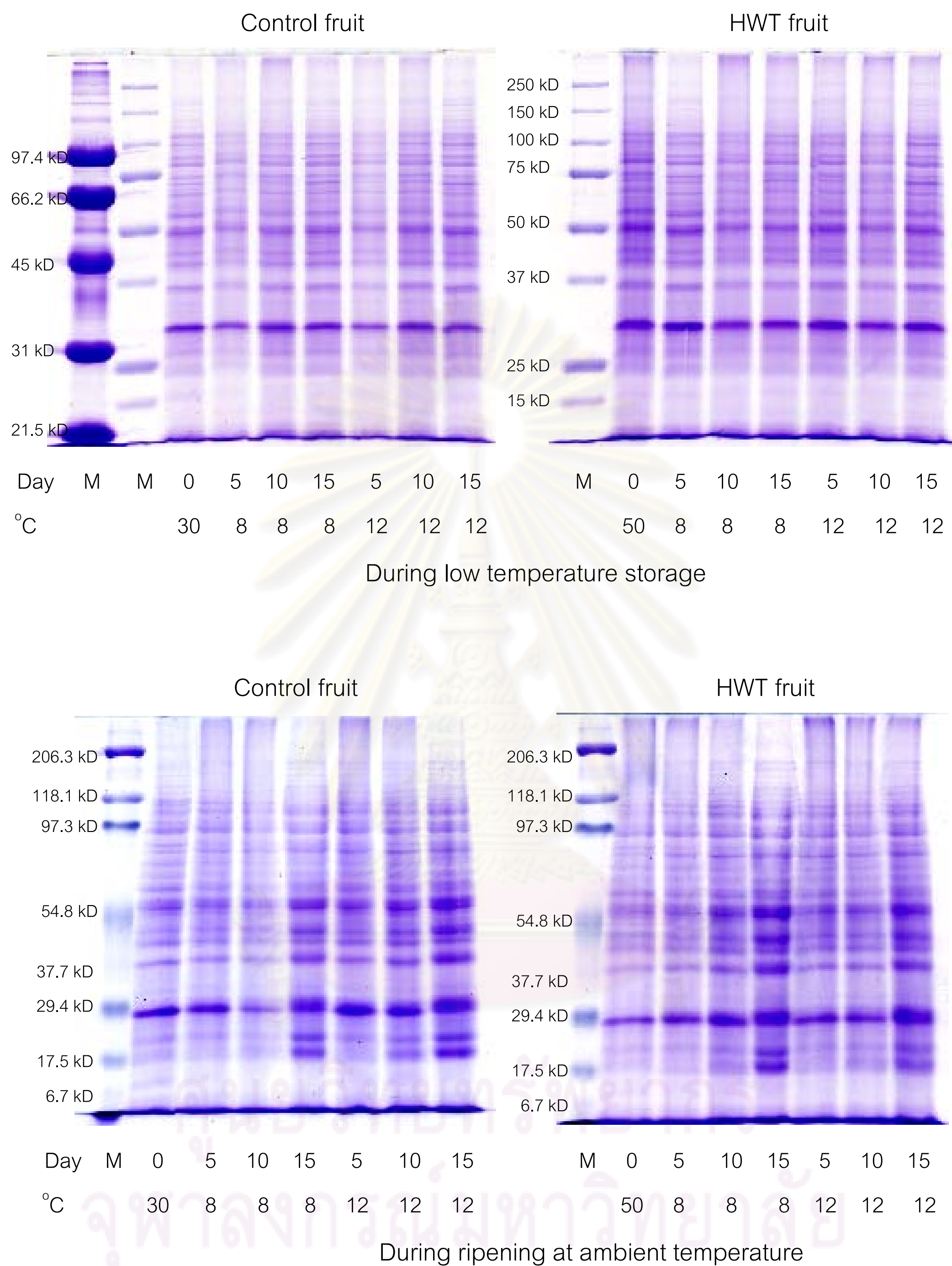

Figure 4.9 Protein profiles of pulp 'Nam Dok Mai' mango during low temperature storage and ripening at ambient temperature. Equal amount of total proteins were loaded in lanes 2-7; seperated on 10% SDS-polyacrylamide gel; stained with Coomassie blue R-250 and quantified the intensity band of the profile by using Imagine software. Lane 1 was marker (molecular mass expressed in kD as marked on the left side).

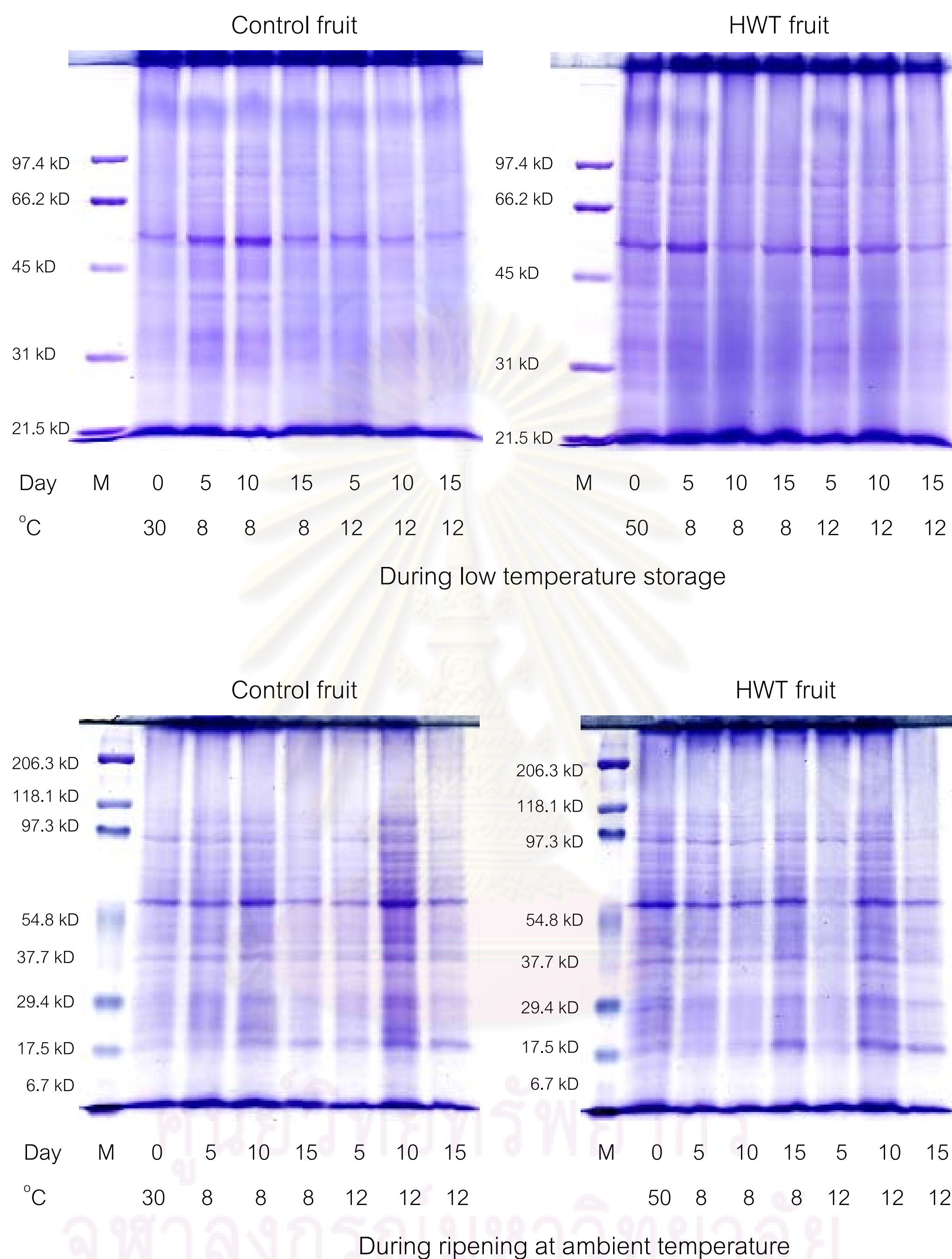

Figure 4.10 Protein profiles of peel 'Ok Rong' mango during low temperature storage and ripening at ambient temperature. Equal amount of total proteins were loaded in lanes 2-7; seperated on 10% SDS-polyacrylamide gel; stained with Coomassie blue R-250 and quantified the intensity band of the profile by using Imagine software. Lane 1 was marker (molecular mass expressed in kD as marked on the left side).

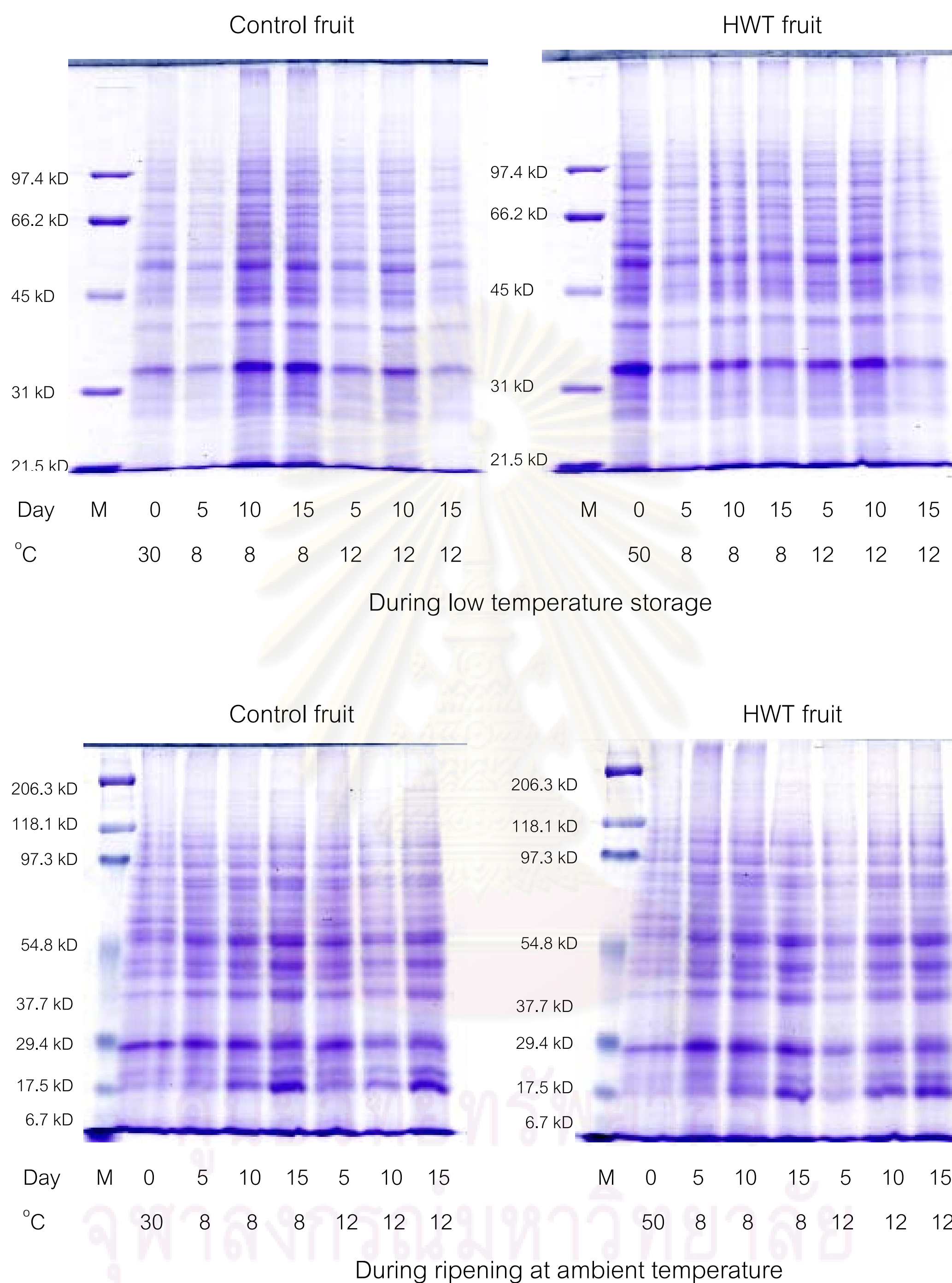

Figure 4.11 Protein profiles of pulp 'Ok Rong' mango during low temperature storage and ripening at ambient temperature. Equal amount of total proteins were loaded in lanes 2-7; seperated on 10% SDS-polyacrylamide gel; stained with Coomassie blue R-250 and quantified the intensity band of the profile by using Imagine software. Lane 1 was marker (molecular mass expressed in kD as marked on the left side).

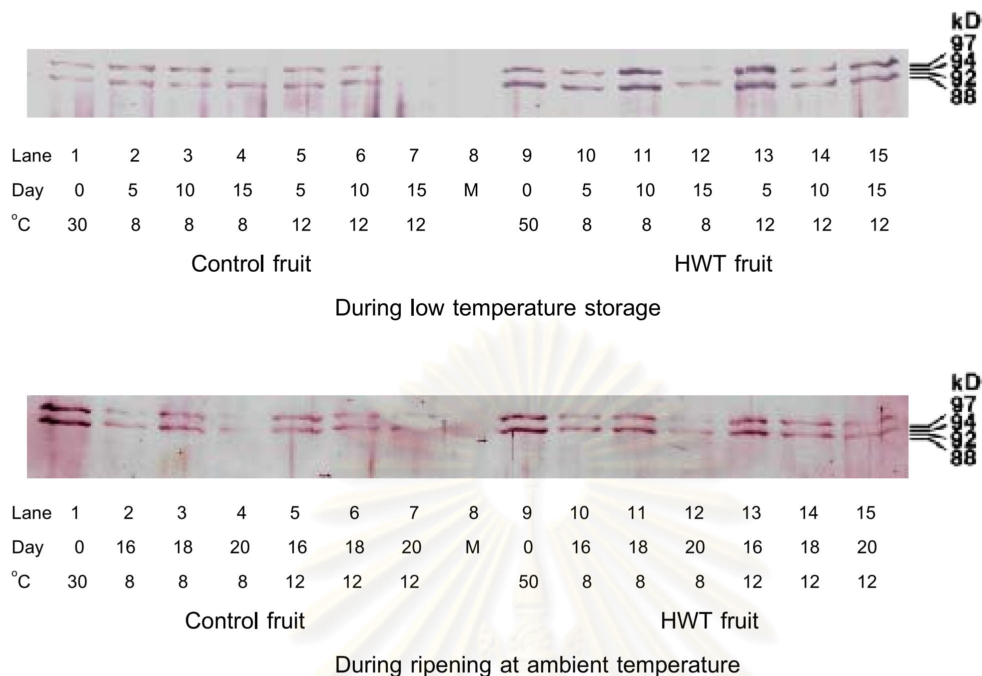

Figure 4.12 Changes in levels of LOX protein in peel 'Nam Dok Mai' mango during low temperature storage and ripening at ambient temperature. Equal total proteins were loaded in lane 1-14; seperated on 10% SDS-polyacrylamide gel; electroblotted to nitrocellulose and treated with 1:200 dilution of tomato 94 kD-specific IgG.

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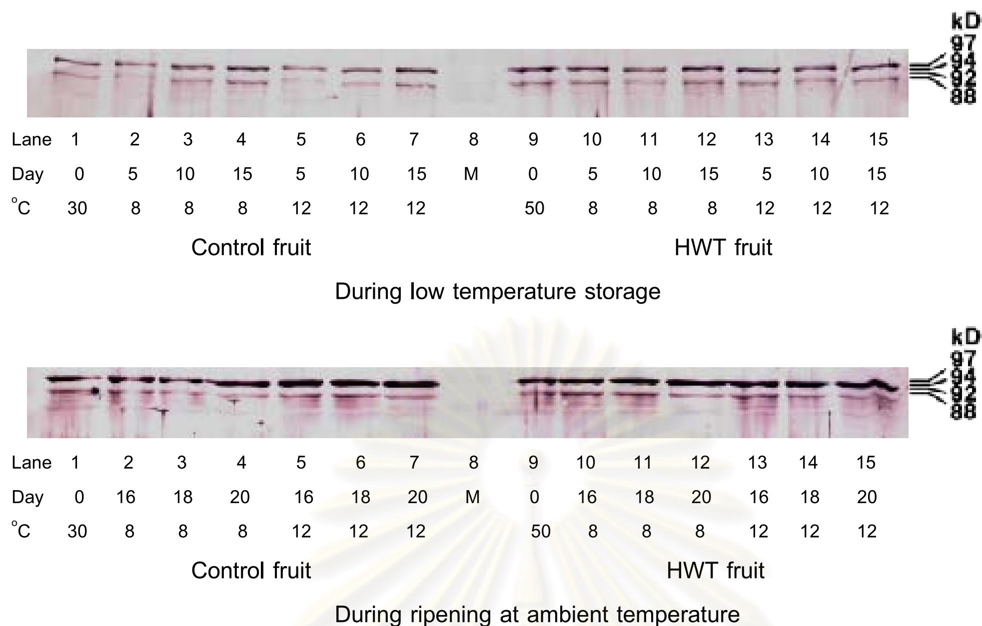

Figure 4.13 Changes in levels of LOX protein in pulp 'Nam Dok Mai' mango during low temperature storage and ripening at ambient temperature. Equal total proteins were loaded in lane 1-14; seperated on 10% SDS-polyacrylamide gel; electroblotted to nitrocellulose and treated with 1:200 dilution of tomato 94 kD-specific IgG.

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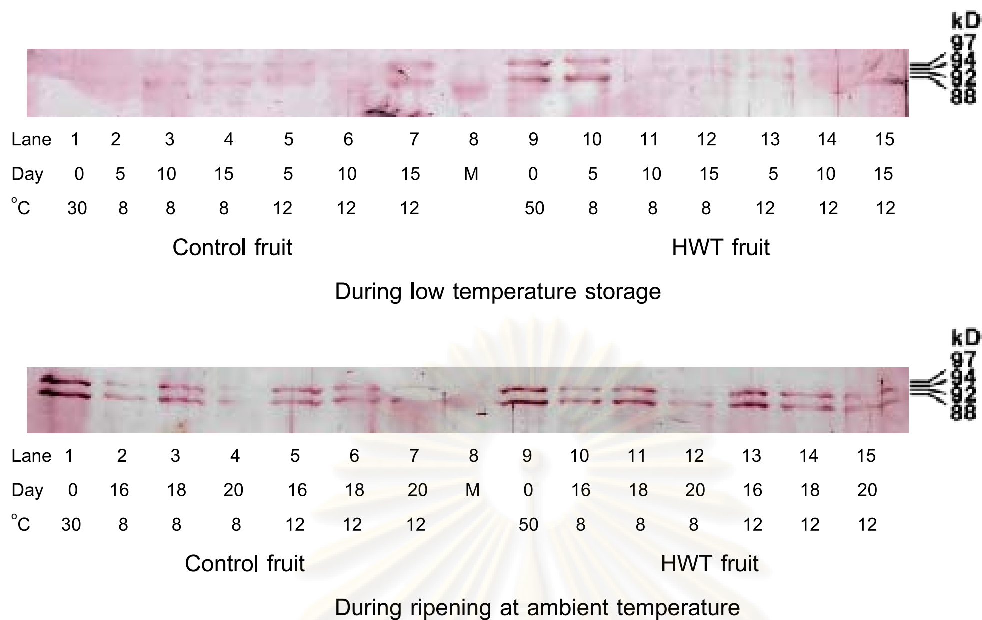

Figure 4.14 Changes in levels of LOX protein in peel 'Ok Rong' mango during low temperature storage and ripening at ambient temperature. Equal total proteins were loaded in lane 1-14; seperated on 10% SDS-polyacrylamide gel; electroblotted to nitrocellulose and treated with 1:200 dilution of tomato 94 kD-specific IgG.

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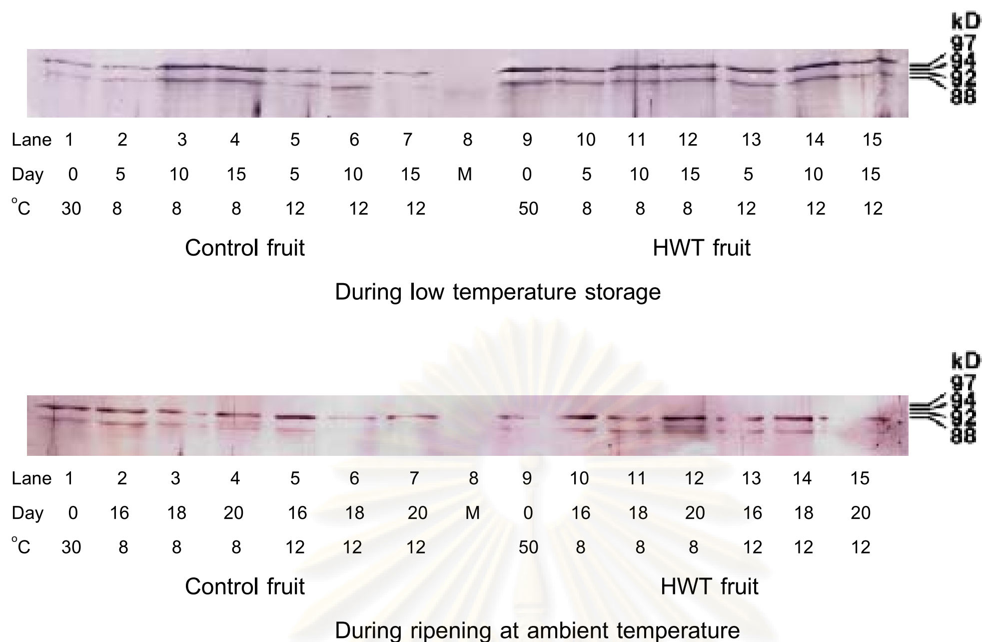

Figure 4.15 Changes in levels of LOX protein in pulp 'Ok Rong' mango during low temperature storage and ripening at ambient temperature. Equal total proteins were loaded in lane 1-14; seperated on 10% SDS-polyacrylamide gel; electroblotted to nitrocellulose and treated with 1:200 dilution of tomato 94 kD-specific IgG.

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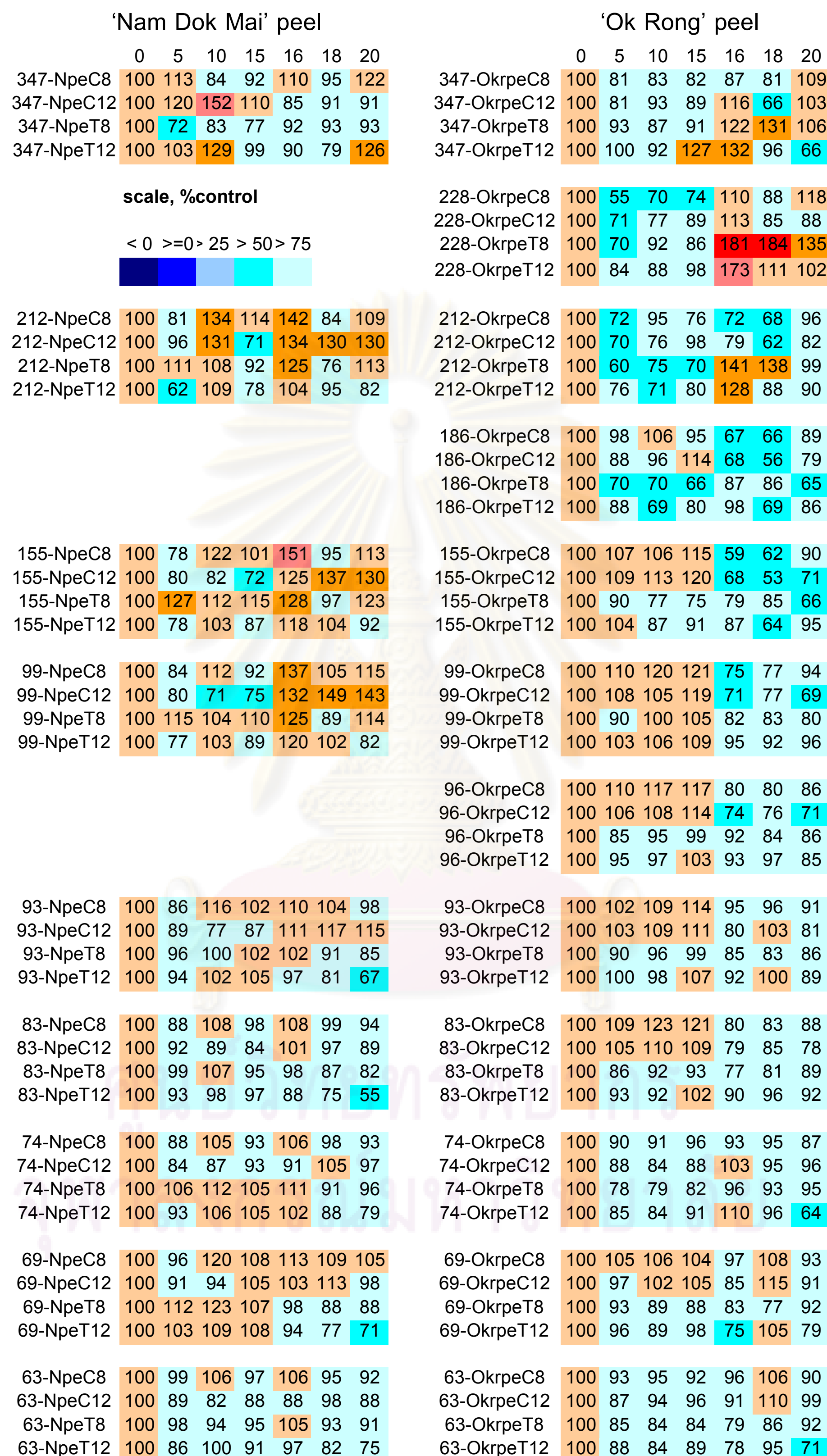


'Nam Dok Mai' peel

	0	5	10	15	16	18	20
347-NpeC8	100	113	84	92	110	95	122
347-NpeC12	100	120	152	110	85	91	91
347-NpeT8	100	72	83	77	92	93	93
347-NpeT12	100	103	129	99	90	79	126
212-NpeC8	100	81	134	114	142	84	109
212-NpeC12	100	96	131	71	134	130	130
212-NpeT8	100	111	108	92	125	76	113
212-NpeT12	100	62	109	78	104	95	82
155-NpeC8	100	78	122	101	151	95	113
155-NpeC12	100	80	82	72	125	137	130
155-NpeT8	100	127	112	115	128	97	123
155-NpeT12	100	78	103	87	118	104	92
99-NpeC8	100	84	112	92	137	105	115
99-NpeC12	100	80	71	75	132	149	143
99-NpeT8	100	115	104	110	125	89	114
99-NpeT12	100	77	103	89	120	102	82
93-NpeC8	100	86	116	102	110	104	98
93-NpeC12	100	89	77	87	111	117	115
93-NpeT8	100	96	100	102	102	91	85
93-NpeT12	100	94	102	105	97	81	67
83-NpeC8	100	88	108	98	108	99	94
83-NpeC12	100	92	89	84	101	97	89
83-NpeT8	100	99	107	95	98	87	82
83-NpeT12	100	93	98	97	88	75	55
74-NpeC8	100	88	105	93	106	98	93
74-NpeC12	100	84	87	93	91	105	97
74-NpeT8	100	106	112	105	111	91	96
74-NpeT12	100	93	106	105	102	88	79
69-NpeC8	100	96	120	108	113	109	105
69-NpeC12	100	91	94	105	103	113	98
69-NpeT8	100	112	123	107	98	88	88
69-NpeT12	100	103	109	108	94	77	71
63-NpeC8	100	99	106	97	106	95	92
63-NpeC12	100	89	82	88	88	98	88
63-NpeT8	100	98	94	95	105	93	91
63-NpeT12	100	86	100	91	97	82	75

'Ok Rong' peel

	0	5	10	15	16	18	20
347-OkrpeC8	100	81	83	82	87	81	109
347-OkrpeC12	100	81	93	89	116	66	103
347-OkrpeT8	100	93	87	91	122	131	106
347-OkrpeT12	100	100	92	127	132	96	66
228-OkrpeC8	100	55	70	74	110	88	118
228-OkrpeC12	100	71	77	89	113	85	88
228-OkrpeT8	100	70	92	86	181	184	135
228-OkrpeT12	100	84	88	98	173	111	102
212-OkrpeC8	100	72	95	76	72	68	96
212-OkrpeC12	100	70	76	98	79	62	82
212-OkrpeT8	100	60	75	70	141	138	99
212-OkrpeT12	100	76	71	80	128	88	90
186-OkrpeC8	100	98	106	95	67	66	89
186-OkrpeC12	100	88	96	114	68	56	79
186-OkrpeT8	100	70	70	66	87	86	65
186-OkrpeT12	100	88	69	80	98	69	86
155-OkrpeC8	100	107	106	115	59	62	90
155-OkrpeC12	100	109	113	120	68	53	71
155-OkrpeT8	100	90	77	75	79	85	66
155-OkrpeT12	100	104	87	91	87	64	95
99-OkrpeC8	100	110	120	121	75	77	94
99-OkrpeC12	100	108	105	119	71	77	69
99-OkrpeT8	100	90	100	105	82	83	80
99-OkrpeT12	100	103	106	109	95	92	96
96-OkrpeC8	100	110	117	117	80	80	86
96-OkrpeC12	100	106	108	114	74	76	71
96-OkrpeT8	100	85	95	99	92	84	86
96-OkrpeT12	100	95	97	103	93	97	85
93-OkrpeC8	100	102	109	114	95	96	91
93-OkrpeC12	100	103	109	111	80	103	81
93-OkrpeT8	100	90	96	99	85	83	86
93-OkrpeT12	100	100	98	107	92	100	89
83-OkrpeC8	100	109	123	121	80	83	88
83-OkrpeC12	100	105	110	109	79	85	78
83-OkrpeT8	100	86	92	93	77	81	89
83-OkrpeT12	100	93	92	102	90	96	92
74-OkrpeC8	100	90	91	96	93	95	87
74-OkrpeC12	100	88	84	88	103	95	96
74-OkrpeT8	100	78	79	82	96	93	95
74-OkrpeT12	100	85	84	91	110	96	64
69-OkrpeC8	100	105	106	104	97	108	93
69-OkrpeC12	100	97	102	105	85	115	91
69-OkrpeT8	100	93	89	88	83	77	92
69-OkrpeT12	100	96	89	98	75	105	79
63-OkrpeC8	100	93	95	92	96	106	90
63-OkrpeC12	100	87	94	96	91	110	99
63-OkrpeT8	100	85	84	84	79	86	92
63-OkrpeT12	100	88	84	89	78	95	71

Figure 4.16a Heat maps of changes in peel protein patterns during storage at 8°C and 12°C after with (T) and without (C) HWT as shown in percentage of zero time. The intense red bars to the intense blue bars represented the highest protein values to the lowest protein values. N: 'Nam Dok Mai', Okr: 'Ok Rong' and pe: peel.

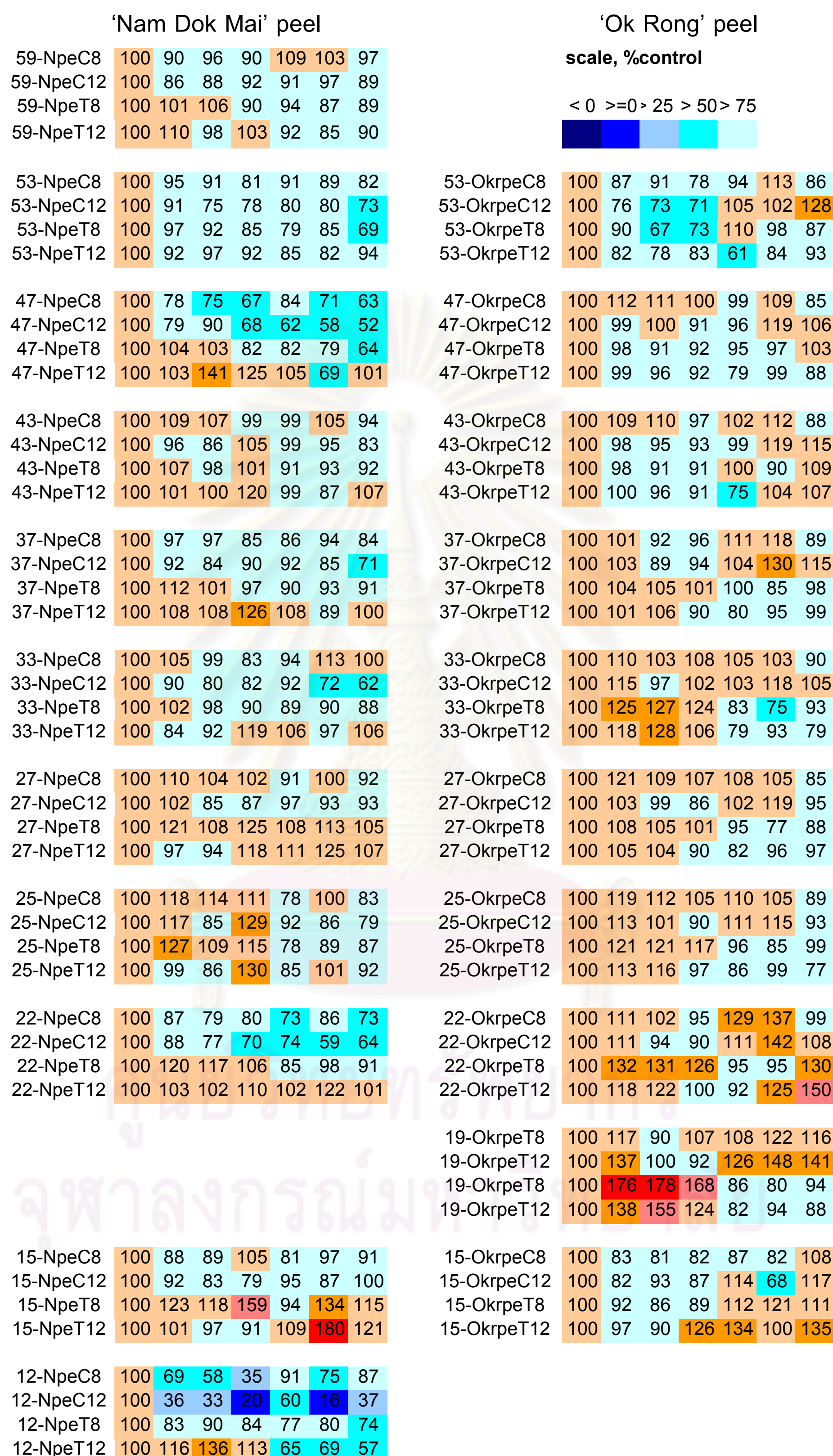
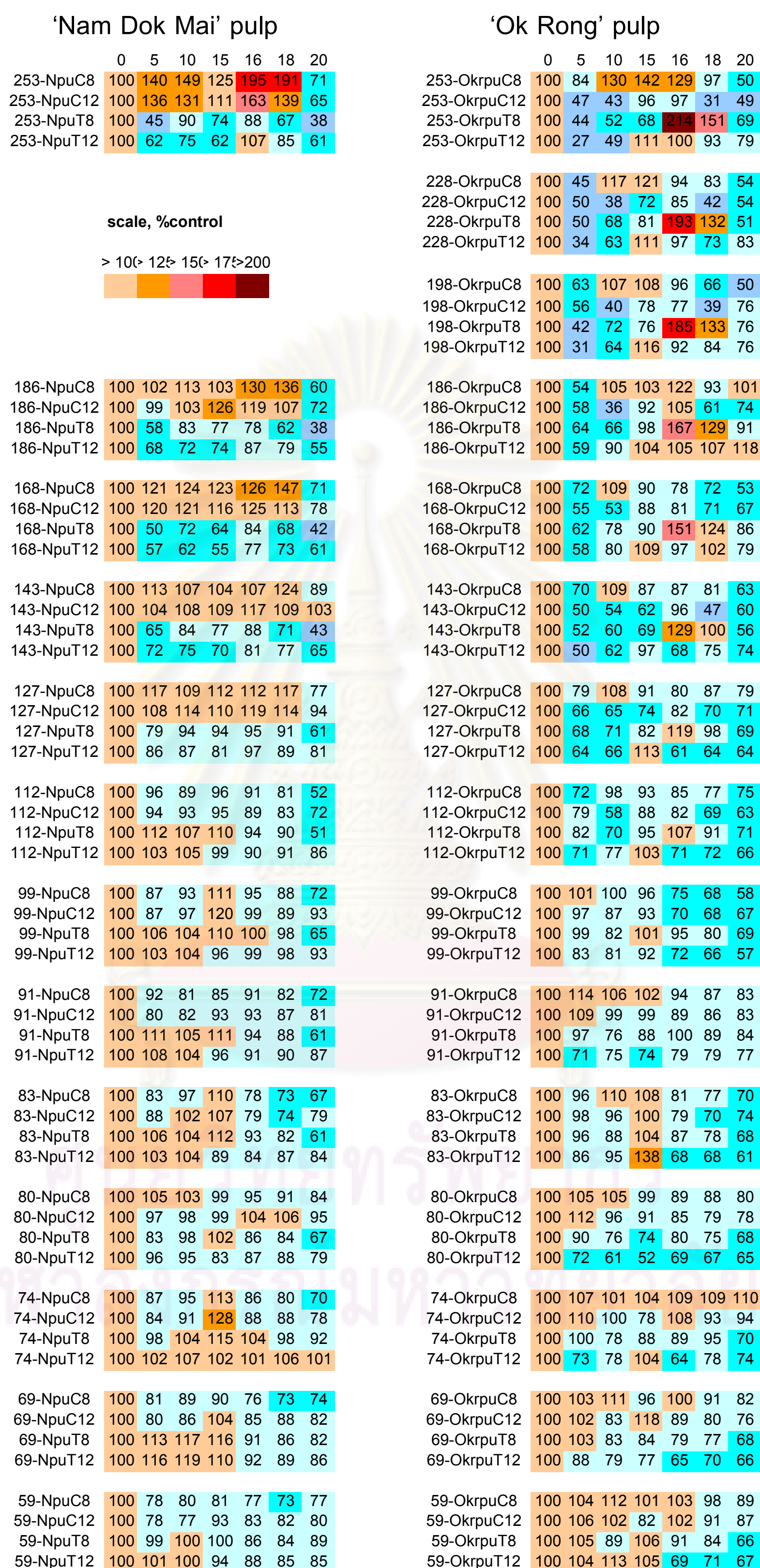


Figure 4.16b Heat maps of changes in peel protein patterns during storage at 8°C and 12°C after with (T) and without (C) HWT as shown in percentage of zero time. The intense red bars to the intense blue bars represented the highest protein values to the lowest protein values. N: 'Nam Dok Mai', Okr: 'Ok Rong' and pe: peel.



‘Nam Dok Mai’ pulp

	0	5	10	15	16	18	20
253-NpuC8	100	140	149	125	195	191	71
253-NpuC12	100	136	131	111	163	139	65
253-NpuT8	100	45	90	74	88	67	38
253-NpuT12	100	62	75	62	107	85	61
186-NpuC8	100	102	113	103	130	136	60
186-NpuC12	100	99	103	126	119	107	72
186-NpuT8	100	58	83	77	78	62	38
186-NpuT12	100	68	72	74	87	79	55
168-NpuC8	100	121	124	123	126	147	71
168-NpuC12	100	120	121	116	125	113	78
168-NpuT8	100	50	72	64	84	68	42
168-NpuT12	100	57	62	55	77	73	61
143-NpuC8	100	113	107	104	107	124	89
143-NpuC12	100	104	108	109	117	109	103
143-NpuT8	100	65	84	77	88	71	43
143-NpuT12	100	72	75	70	81	77	65
127-NpuC8	100	117	109	112	112	117	77
127-NpuC12	100	108	114	110	119	114	94
127-NpuT8	100	79	94	94	95	91	61
127-NpuT12	100	86	87	81	97	89	81
112-NpuC8	100	96	89	96	91	81	52
112-NpuC12	100	94	93	95	89	83	72
112-NpuT8	100	112	107	110	94	90	51
112-NpuT12	100	103	105	99	90	91	86
99-NpuC8	100	87	93	111	95	88	72
99-NpuC12	100	87	97	120	99	89	93
99-NpuT8	100	106	104	110	100	98	65
99-NpuT12	100	103	104	96	99	98	93
91-NpuC8	100	92	81	85	91	82	72
91-NpuC12	100	80	82	93	93	87	81
91-NpuT8	100	111	105	111	94	88	61
91-NpuT12	100	108	104	96	91	90	87
83-NpuC8	100	83	97	110	78	73	67
83-NpuC12	100	88	102	107	79	74	79
83-NpuT8	100	106	104	112	93	82	61
83-NpuT12	100	103	104	89	84	87	84
80-NpuC8	100	105	103	99	95	91	84
80-NpuC12	100	97	98	99	104	106	95
80-NpuT8	100	83	98	102	86	84	67
80-NpuT12	100	96	95	83	87	88	79
74-NpuC8	100	87	95	113	86	80	70
74-NpuC12	100	84	91	128	88	88	78
74-NpuT8	100	98	104	115	104	98	92
74-NpuT12	100	102	107	102	101	106	101
69-NpuC8	100	81	89	90	76	73	74
69-NpuC12	100	80	86	104	85	88	82
69-NpuT8	100	113	117	116	91	86	82
69-NpuT12	100	116	119	110	92	89	86
59-NpuC8	100	78	80	81	77	73	77
59-NpuC12	100	78	77	93	83	82	80
59-NpuT8	100	99	100	100	86	84	89
59-NpuT12	100	101	100	94	88	85	85

‘Ok Rong’ pulp

	0	5	10	15	16	18	20
253-OkrpuC8	100	84	130	142	129	97	50
253-OkrpuC12	100	47	43	96	97	31	49
253-OkrpuT8	100	44	52	68	214	151	69
253-OkrpuT12	100	27	49	111	100	93	79
228-OkrpuC8	100	45	117	121	94	83	54
228-OkrpuC12	100	50	38	72	85	42	54
228-OkrpuT8	100	50	68	81	193	132	51
228-OkrpuT12	100	34	63	111	97	73	83
198-OkrpuC8	100	63	107	108	96	66	50
198-OkrpuC12	100	56	40	78	77	39	76
198-OkrpuT8	100	42	72	76	185	133	76
198-OkrpuT12	100	31	64	116	92	84	76
186-OkrpuC8	100	54	105	103	122	93	101
186-OkrpuC12	100	58	36	92	105	61	74
186-OkrpuT8	100	64	66	98	167	129	91
186-OkrpuT12	100	59	90	104	105	107	118
168-OkrpuC8	100	72	109	90	78	72	53
168-OkrpuC12	100	55	53	88	81	71	67
168-OkrpuT8	100	62	78	90	151	124	86
168-OkrpuT12	100	58	80	109	97	102	79
143-OkrpuC8	100	70	109	87	87	81	63
143-OkrpuC12	100	50	54	62	96	47	60
143-OkrpuT8	100	52	60	69	129	100	56
143-OkrpuT12	100	50	62	97	68	75	74
127-OkrpuC8	100	79	108	91	80	87	79
127-OkrpuC12	100	66	65	74	82	70	71
127-OkrpuT8	100	68	71	82	119	98	69
127-OkrpuT12	100	64	66	113	61	64	64
112-OkrpuC8	100	72	98	93	85	77	75
112-OkrpuC12	100	79	58	88	82	69	63
112-OkrpuT8	100	82	70	95	107	91	71
112-OkrpuT12	100	71	77	103	71	72	66
99-OkrpuC8	100	101	100	96	75	68	58
99-OkrpuC12	100	97	87	93	70	68	67
99-OkrpuT8	100	99	82	101	95	80	69
99-OkrpuT12	100	83	81	92	72	66	57
91-OkrpuC8	100	114	106	102	94	87	83
91-OkrpuC12	100	109	99	99	89	86	83
91-OkrpuT8	100	97	76	88	100	89	84
91-OkrpuT12	100	71	75	74	79	79	77
83-OkrpuC8	100	96	110	108	81	77	70
83-OkrpuC12	100	98	96	100	79	70	74
83-OkrpuT8	100	96	88	104	87	78	68
83-OkrpuT12	100	86	95	138	68	68	61
80-OkrpuC8	100	105	105	99	89	88	80
80-OkrpuC12	100	112	96	91	85	79	78
80-OkrpuT8	100	90	76	74	80	75	68
80-OkrpuT12	100	72	61	52	69	67	65
74-OkrpuC8	100	107	101	104	109	109	110
74-OkrpuC12	100	110	100	78	108	93	94
74-OkrpuT8	100	100	78	88	89	95	70
74-OkrpuT12	100	73	78	104	64	78	74
69-OkrpuC8	100	103	111	96	100	91	82
69-OkrpuC12	100	102	83	118	89	80	76
69-OkrpuT8	100	103	83	84	79	77	68
69-OkrpuT12	100	88	79	77	65	70	66
59-OkrpuC8	100	104	112	101	103	98	89
59-OkrpuC12	100	106	102	82	102	91	87
59-OkrpuT8	100	105	89	106	91	84	66
59-OkrpuT12	100	104	113	105	69	71	67

Figure 4.17a Heat maps of changes in pulp protein patterns during storage at 8°C and 12°C after with (T) and without (C) HWT as shown in percentage of zero time. The intense red bars to the intense blue bars represented the highest protein values to the lowest protein values. N: ‘Nam Dok Mai’, Okr: ‘Ok Rong’ and pu: pulp.

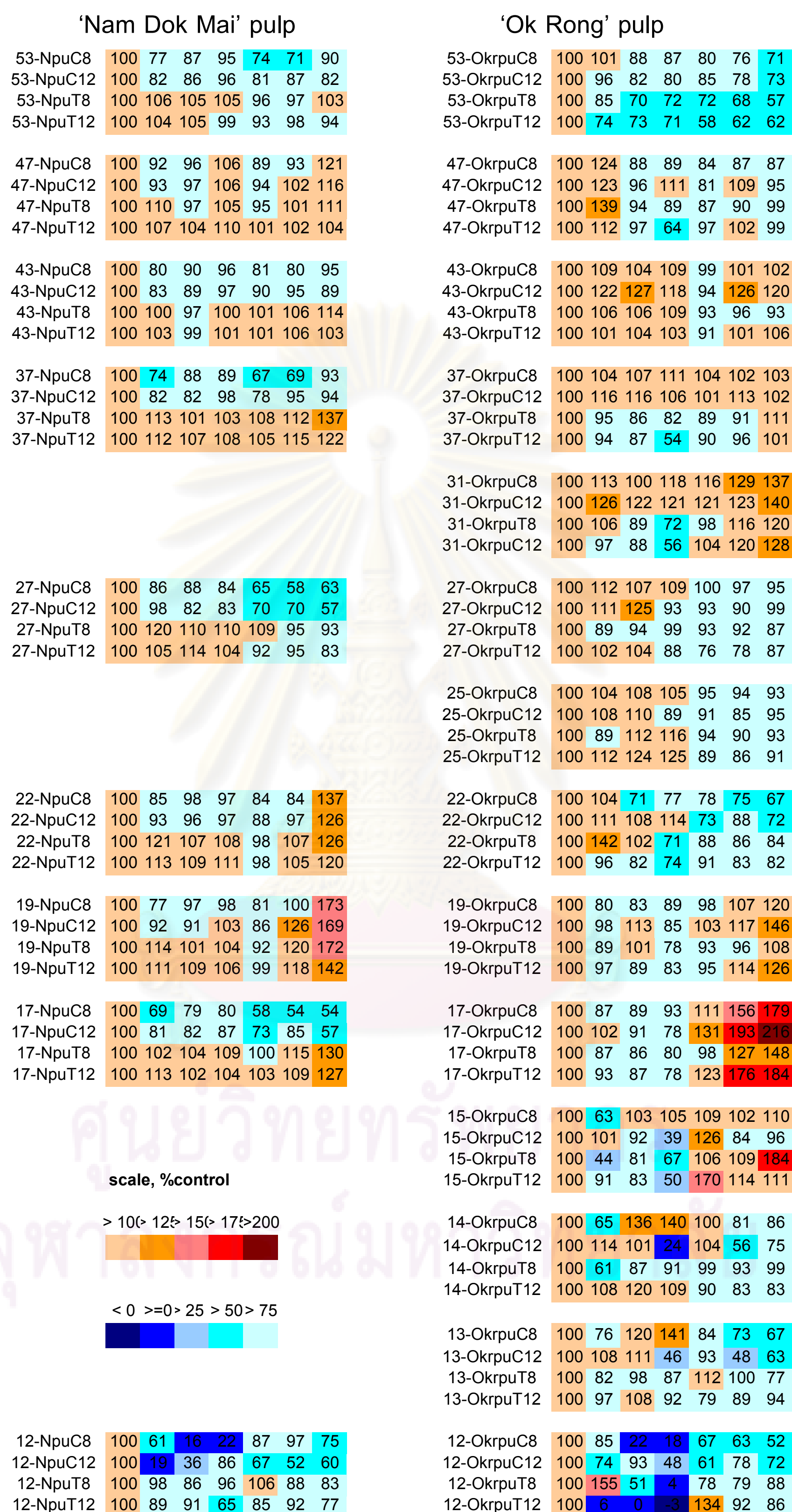


Figure 4.17b Heat maps of changes in pulp protein patterns during storage at 8°C and 12°C after with (T) and without (C) HWT as shown in percentage of zero time. The intense red bars to the intense blue bars represented the highest protein values to the lowest protein values. N: 'Nam Dok Mai', Okr: 'Ok Rong' and pu: pulp.

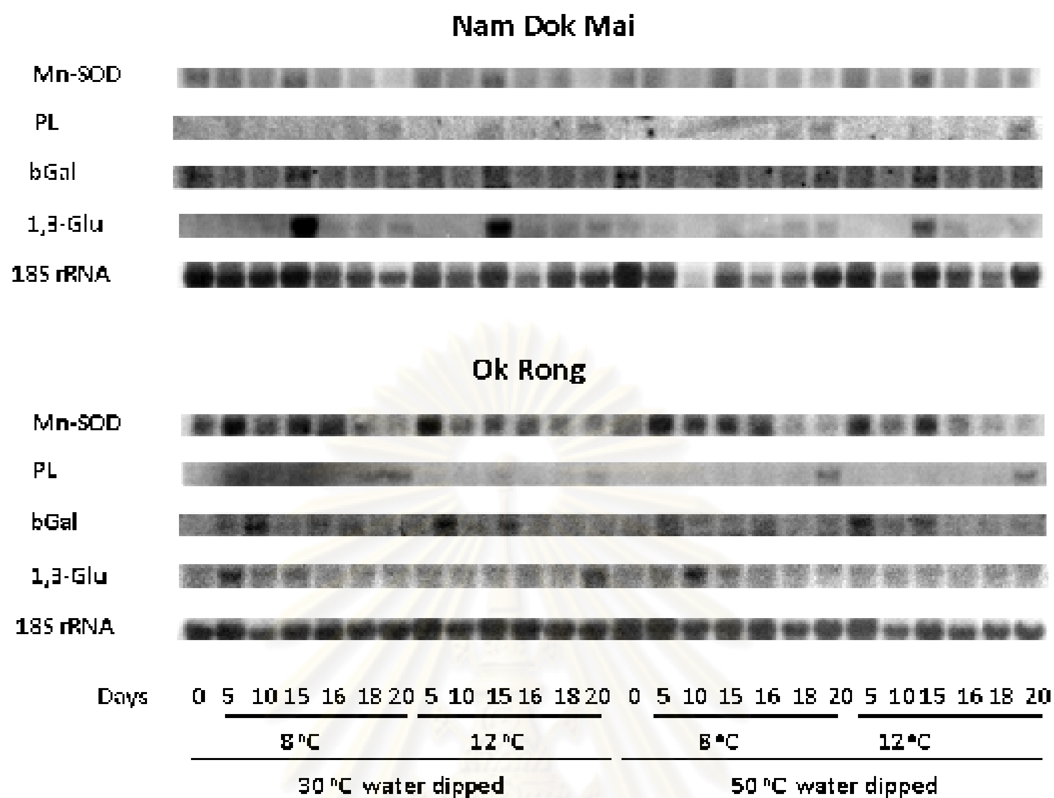


Figure 4.18 Autoradiogram of RNA gel blot analysis of four selected genes in 'Nam Dok Mai' and 'Ok Rong' mango during storage after hot water treatment and storage at 8°C and 12°C for increasing lengths of time.

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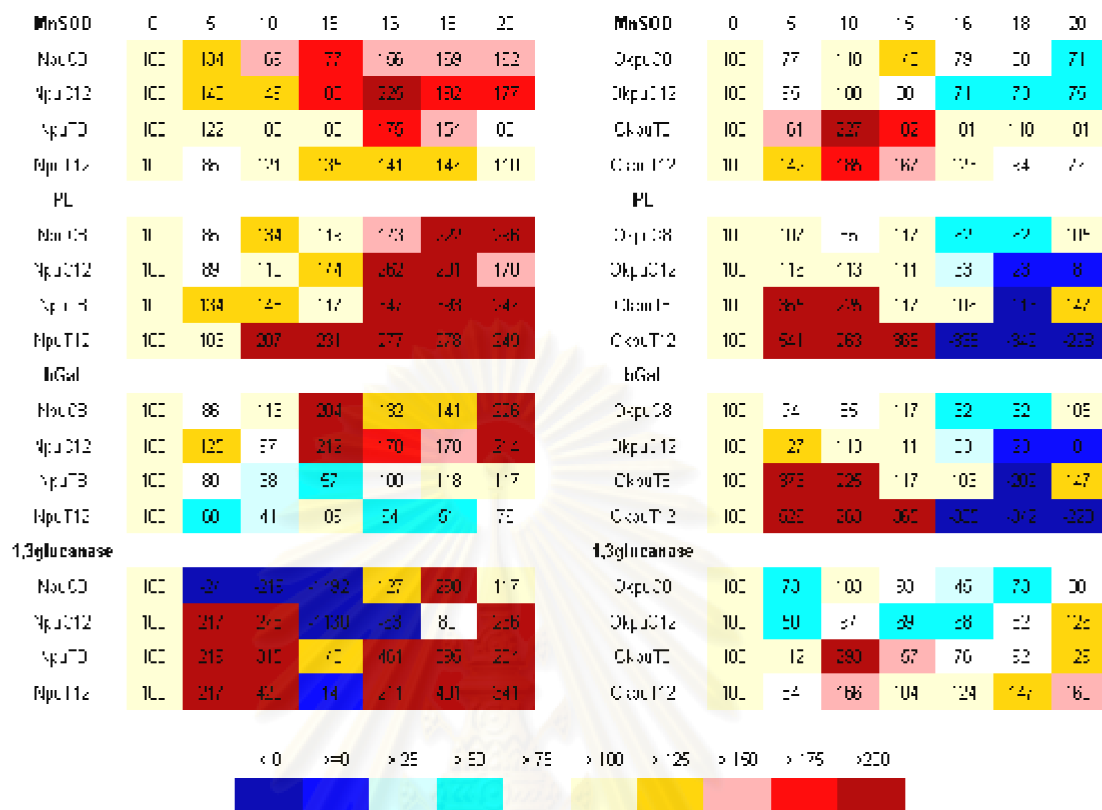

Figure 4.19 Heat maps of changes in the expression patterns of four selected transcripts in pulp of ‘Nam Dok Mai’ and ‘Ok Rong’ mango during storage at 8°C and 12°C after with (T) and without (C) HWT as shown in percentage of zero time. The intense red bars to the intense blue bars represented the highest expression values to the lowest expression. N: ‘Nam Dok Mai’, Okr: ‘Ok Rong’ and pu: pulp.

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Chapter 5

DISCUSSION AND CONCLUSIONS

5.1 Effect of hot water treatment on fruit quality analysis of mango fruits

Mango is a commercially important tropical fruit which is also classified as a climacteric fruit based on its rate of respiration and ethylene production followed by a series of biochemical changes such as decreased acidity, accumulation of sugars, chlorophyll degradation and increased activity of cell wall hydrolases. Mango fruit has a limited shelf life, the quality of the fruit rapidly decreases once fully ripe fruit. However, hot water immersion treatment has been developed to modify fruit response to other stresses and maintain fruit quality during storage. Our results for the effect of hot water treatment on fruit quality analysis of mango fruit have shown that HWT ‘Nam Dok Mai’ fruit exhibited more reduction in percentage of weight loss than control fruit, however there were no significant difference between HWT and control fruit of ‘Ok Rong’. Previous researches which are supported these results in our study were hot air conditioning treatments prior to a hot water treatment (47°C fruit core temperature held for 15 minutes) of ‘Kensington’ mango fruit increased percentage of weight loss during storage at 22°C compared to control fruit (Jacobi, MacRae and Hetherington, 2000); ‘Keitt’ and ‘Tommy Atkins’ mangoes intermittently warmed at 34°C for 1 or 2 days prior to HWT of 46 to 46.5°C for up to 12 minutes lost more weight than fruits that were not warmed prior to HWT (Nyanjage, Wainwright and Bishop, 1998). Baloch, Morimoto and Hatou (2006) reported that HWT tomato at 39°C for 45 minutes, 41°C for 30 and 45 minutes, and 45°C for 20, 25 and 30 minutes showed higher water losses than control fruit after 10 days storage at 15°C, however, hot air treated fruit at 40°C for 6 hours minimized the rate of water loss. It was suggested that the internal heat resistance in tomato during water heating acts as a dominant factor in controlling the heat transfer than in hot air (Wang, Tang and Cavalieri, 2001). The higher water loss at 45°C was mainly due to the effect of high temperature exposure which increased the water loss of the fruit and also due to protein denaturation, disruption of protein synthesis and loss of membrane integrity. Accelerated weight loss may be associated with transpiration

occuring on the fruit surface as a result of the fruit transpiring too much water which in turn cause a loss in turgidity that may appear slightly shriveled (Hong *et al.*, 2007). In addition, treatments at higher temperature levels indicated the epicuticular waxes melt completely and may be removed that results in higher water loss in fruits (Dimitris *et al.*, 2005). On the other hand, the short heat treatment may have stimulated an increase in the synthesis of wax to protect the surface, resulting in less water loss (Fallik *et al.*, 2001). 'Nam Dok Mai' and 'Ok Rong' fruits that stored at 8°C showed lower percentage of pulp firmness that fruits stored at 12°C, however, no significant different between HWT and control fruit. Sams (1999) concluded that firmness of apples (Haller, 1941; Blanpied *et al.*, 1978), strawberries (Rose, Haller and Harding, 1934), and pears (Hartman, 1924) is higher at lower temperatures. Ketsa, Chidtragool and Lurie (2000) revealed that no change occurred in firmness of heated (38°C for 3 days), cold-stored mango fruit, whereas nonheated, cold-stored fruit softened after first held for 3 weeks at 4°C and then stored during the first 2 days at 25°C. However, there was no difference in firmness among any of the treatments after 6 days at 25°C of storage. TSS (°brix) and TA are indicators of ripening within the mango mesocarp as well as the conversion of starch reserves to sugars and organic acids used in respiration. In 'Nam Dok Mai' cultivar, HWT fruit stored at 12°C had the highest °brix and the lowest TA on day 18 during ripening at ambient temperature, while the highest °brix and the lowest TA were found in the control fruit of 'Ok Rong' stored at 12°C. These results indicate that HWT 'Nam Dok Mai' fruit stored at 12°C or the control fruit of 'Ok Rong' stored at 12°C were ripen faster than other treatments, and fruit stored at 12°C was induced to ripen faster than fruit stored at 8°C. Increased TSS and decreased TA levels within heat-conditioned fruit have been reported in the mango cv. 'Kingsington' that was treated with conditioning for 8 hours at 38°C, 40°C and 42°C followed immediately by a HWT (47°C fruit core temperature held for 15 minutes) before storage at 22°C (Jacobi *et al.*, 2000).

The color change is a reliable parameter to measure as indicating fruit ripening, the results obtained in terms of color space L* a* b* that relate to human perception. The changes in skin color of 'Nam Dok Mai' fruit revealed that HWT fruit stored at 12°C had significantly higher reflectance or lightness (L), a-value and b-value than other treatments on day 16 and day 17 during ripening at ambient temperature. Furthermore,

the change in skin color of 'Ok Rong' fruit showed that HWT fruit stored at 12°C had significantly higher L-value than other treatments on day 16 of storage and after transferring to ripen at ambient temperature (day 15 to day 20) showed significantly different L-value, a-value and b-value between the fruit stored at 8°C and fruit stored at 12°C. The fruit stored at 12°C were significantly higher a and b values than the fruit stored at 8°C resulting in appearance of a yellow pigment of the mango skin during fruit ripening. Similarly, high-humidity hot air treated (HT) 'Kingsington' mango fruit and mature fruit generally colored at a faster rate than the untreated and immature fruit (Jacobi *et al*., 1995). The yellow pigment in mango peel and pulp is attributed to carotenoids which is associated with the climacteric increase in respiration by the action of ethylene (Saltveit *et al.*, 1999).

5.2 Effect of hot water treatment on ethylene production

Prestorage hot water treatment at 50°C had a dramatic effect on ethylene production in both cultivars of mango fruit. The HWT fruit both stored at 8 and 12°C exhibited a decrease in ethylene production throughout the whole storage. The similar observation occur in hot water rinsing and brushing (HWRB) treated apple fruit which was rinsed with hot water at for 55±1°C for 15 seconds, showing significantly the lowest ethylene production compared with all other treatments during 10 days incubation at 20°C (Fallik *et al*., 2001); and 'Satsuma' mandarin fruit which were dipped at 50°C for 2 minutes and stored in cold storage for 8 weeks had significantly suppressed ethylene evolution and a lower respiration rate compared to other treatments (Ghasemnezhad *et al*., 2008). Budde *et al.* (2006) found that air heat treatment (39±1°C for 0, 24 and 36 hours) of 'Dixiland' peaches in both maturity grades (M1-for export and M2-for domestic market) slightly increased or maintained some ethylene production as compared to control fruit supporting in the exposure of fruit to high temperatures induces the inhibition of ethylene production, and this inhibition is maintained during the period of exposure. Several researches have revealed that heat treatment can alter ripening and senescence of fruit by reducing the rates of ethylene production (Lurie, 1998). ACC synthase activity in heated fruit (held for 3 days at 38°C and then transferred to 20°C) remained much lower than that found in unheated fruit (Ketsa *et al*., 1999). Ketsa *et al*.

(1999) concluded that the inhibition of ethylene production found during heat treatment is due to inhibition of both 1-aminocyclopropane-1-carboxylic acid synthase (ACS) and 1-aminocyclopropane-carboxylic acid oxidase (ACO). Moreover, ACO recovers to its full activity following heat, whereas ACS activity recovers only partially, but enough to allow the heated fruit to achieve an ethylene peak. It is possible that heat treatment limited the action of ACC synthase and ACC oxidase enzymes responsible for ethylene production in 'Nam Dok Mai' and 'Ok Rong' mangoes in this experiment as it occurs in previous studies.

5.3 Effect of hot water treatment on CAT, APX and GR activities in mango fruits

Fruit ripening may be considered as an oxidative process involving alterations in fruit metabolism and the activities of enzymatic systems relating to the regulation of reactive oxygen species (ROSs) (Wang *et al.*, 2009). Naturally, plant cells have several enzymes scavenging ROSs including superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT) and glutathione reductase (GR) that are observed in the experiment. Exposing plants to moderate heat treatments also causes weak oxidative stress that modulates antioxidant levels and induces tolerance to a subsequent severe stress (Li, 2003) including stress induced by low temperature storage. Generally, activity of antioxidant enzymes increased when the oxidant level increased with environmental stress (Ren *et al.*, 1999). Previous studies have reported that the response of the antioxidant enzyme system in mango fruit to low-temperature stress increased with the maturity stage advanced due to activities of SOD and CAT in the yellow fruit were markedly higher than those in the green and pre-yellow fruit after 9 days of the storage (Zhao *et al.*, 2009). The effect of hot water treatment on CAT, APX and GR activities during low temperature storage in both 'Nam Dok Mai' and 'Ok Rong' mango fruits were more obvious in mango peel than mango pulp which may be due to the direct exposure of the peel to hot water immersion. The observed effects of hot water treatment on increase in antioxidant enzymes activities in mango fruit were in agreement with Yahia *et al.* (2007), who reported that hot water immersion was a major factor leading to an antioxidant protective system in tomato. Exposing 'Rhapsody' tomatoes to mild heat treatment at 34 or 38°C and 95% relative humidity (RH) for 24

hours, which is recommended to ameliorate chilling injury (CI) and delay ripening, increased the activity of CAT and GST (Yahia *et al.*, 2007); and heat treated (45°C for 3 hours) strawberry had higher SOD and APX activities than the respective control after 7 days of storage at 0°C, and remained after 1-2 days of storage at 20°C indicating that the heat treatment produced changes in the oxidative metabolism of the fruit (Vicente *et al.*, 2006).

Oxidative damage is also considered to be an early response of sensitive tissues to low temperature stress (Hariyadi and Parkin, 1991). It has been reported that low temperature storage raises the level of reactive oxygen species, which are highly reactive oxidant molecules that can damage plant cellular components (Elstner, 1991). Acclimation of plants to other stresses may be partly related to an enhanced antioxidant system that prevents the accumulation of ROSs. In this study we observed higher levels of CAT, APX and GR following heat treatment. Prestorage activities of antioxidant enzymes AsPX, CAT, DHAR, GR, MDHAR, POX and SOD in honeydew melon fruit or spinach leaves which were collected in varies types including fresh, frozen at -80°C, frozen with liquid nitrogen, freeze-dried and acetone powder precipitate suggested that fresh versus frozen to -80°C or flash-frozen in liquid N_2 result in a significant change in the activities of seven of the most important antioxidant enzymes (Lester *et al.*, 2004) and prestorage application of oxalic acid (OA) in 'Zill' mango fruit peel and pulp could limit postharvest deterioration of refrigerated mango fruit and result in a decreased lipoxygenase (LOX) activity and increased SOD activity in the peel, and increased APX activity in both peel and pulp, which concluded with a decrease in ROSs (Zheng *et al.*, 2007). Sala and Lafuente (1999) revealed that hybrid 'Fortune' mandarins developed CI upon cold storage, unless the fruits were conditioned at 37°C for 3 days before they were held at low temperature (2°C for up to 8 weeks). This heat treatment induced 2.5-, 1.2-, and 1.4-fold increases in the activities of CAT, APX, and SOD, respectively, and reduced the activity GR. The differences in the activities afforded by the heat treatment were, in general, maintained during cold storage. They summarized that CAT may be a major antioxidant enzyme operating in the heat-induced chilling tolerance of cold-stored 'Fortune' mandarin fruits.

5.4 Effect of hot water treatment on the protein patterns and LOX protein

Protein profiles of mango peel and pulp showed various protein bands including several molecular weights ranging from 12 to 347 kDa. HWT 'Ok Rong' pulp stored at 8°C had an increase in varied sizes of protein during fruit ripening, indicating that hot water treatment had effects on protein patterns. Heat treatment affects protein metabolism by suspending the synthesis of housekeeping proteins to produce heat-shock proteins (Vicente *et al.*, 2002). Two-dimensional gel electrophoresis of the boiled soluble protein fraction showed two new polypeptides that were involved in heat-induced chilling tolerance, although it is not certain that these polypeptides were so called heat shock proteins (Zhu *et al.*, 2003). Despite the short time of exposure to high temperature, the protection mechanism of heat treatment may also include the induction and accumulation of a 105 kDa protein in grapefruit that cross-reacted with an antibody raised against a bovine heat shock protein and 18 and 21 kDa proteins that cross-reacted with pea Hsp18 and Hsp21 antibodies (Porat *et al.*, 2000; Pavoncello *et al.*, 2001). The heat pretreatment of grape berries (*Vitis vinifera* cv. Jingxiu) also induced expression of Hsp70 transcripts.

It has been reported that low temperature storage induces peroxidation of unsaturated fatty acids and degradation of peroxidized fatty acids which catalysed by many enzymes including LOX (Berger *et al.*, 2001). It had been reported the molecular weight of LOX protein including 93 kDa of pea seeds (Szymanowska *et al.*, 2009) and 94 kDa of tomato fruits (Kausch and Handa, 1997) thus suggesting that tomato LOX antibodies cross reacted to mango LOX proteins. Semi-quantitation of these cross-reactive bands indicated that hot water treatment enhanced the accumulation of LOX protein in mango fruit. The activity of LOX has been used as an indicator of membrane injury. Similarly in 'Gros Michel' banana, the hot water treatment resulted in a lower LOX activity throughout the experiment (Promyou, Ketsa and Van Doorn, 2008). An increase in LOX enzyme activity correlated with the level of lipid peroxidation. Ali, Hahn and Paek (2005) determined whether exposure to temperature-stress (25-40°C) with different exposure periods (4, 24 and 48 hours) would lead to oxidative stress in leaf and roots segments of *Phalaenopsis*. They found that temperature-stress caused oxidative damage to leaf and root segments (high MDA and high LOX activity), and that oxidative

damage may play a primary role in the decrease of photochemical efficiency in leaf subjected to high (40°C) temperature-stress period with observed increases in MDA levels and LOX activity in leaf segments. The increases in CAT in leaf, DHAR in leaf and root, GR in leaf, G-POD in root segments at 40°C compared to 25°C could also provide increased protection.

Our results obtained that HWT 'Nam Dok Mai' fruit peel showed a pale visible band on day 15 of storage which related to an increase in antioxidant activities, supported by Demiral and Türkan (2005) in studied on comparative lipid peroxidation and antioxidant defense systems in roots of two rice cultivars (Pokkali and IR-28). They found that Pokkali had lower level of lipid peroxidation than of IR-28 suggested that it may have better protection against oxidative damage under salt stress. The improved protection in Pokkali may reflect a more efficient antioxidative system as evidenced by a higher activity of SOD (under 120 mol m^{-3} NaCl), CAT and APX enzymes, however, significant increased in MDA level in roots of IR-28 appeared to be correlated with a decrease in activity of CAT and GR and a non-induced activity of SOD and APX enzymes under salt stress. In addition, the level of MDA, produced during peroxidation of membrane lipids, is often used as an indicator of oxidative damage (Demiral and Türkan, 2005). Singh and Dwivedi (2008) studied the effect of 1-methylcyclopropene (1-MCP) and ethrel on antioxidant levels in mango fruit during ripening. They found that 1-MCP treatment led to decreased levels of H_2O_2 and lipid peroxidation, concomitant with increased activities and isozymes of CAT and SOD, as compared to respective controls. On the other hand, Ethrel treatment led to an increase in H_2O_2 and lipid peroxidation, concomitant with a decrease in the activities and isozymes of catalase and SOD. Activity of APX was found to drastically increase in the presence of Ethrel while 1-MCP treatment led to only a marginal increase in APX.

5.5 Effect of hot water treatment on transcript accumulation of Mn-SOD and cell wall hydrolases

'Nam Dok Mai' fruit pulp showed high accumulation of Mn-SOD transcript levels during ripening at ambient temperature, while the pulp of HWT 'Ok Rong' fruit showed high transcript levels during low temperature storage. It has been reported that Mn-SOD

may play an important role in the drought tolerance of rice. Mn-SOD gene, *pMnSOD*, from pea was used to express in rice. Results suggested that SOD is a critical component of ROSs scavenging system in plant chloroplasts and the expression of Mn-SOD can improve drought tolerance in rice due to the induction of *pMnSOD* transgene by drought stress mediated by polyethylene glycol (PEG) 6000, whereas under normal conditions its expression was almost undetectable (Wang *et al*., 2005).

Mango fruit showed rapid decrease in pulp firmness after transferring to ripen at ambient temperature. The pulp firmness of 'Ok Rong' fruit decreased faster than those of 'Nam Dok Mai' fruit, and fruit stored at 12°C decreased more than fruit stored at 8°C. It is probable that the increase in mango fruit softness associated with conditioning treatments as well as the duration and temperature of hot water immersion leads to an increase in the activity of cell wall enzymes (Jacobi *et al*., 2000). Effect of hot water treatment on transcript accumulation of cell wall hydrolases revealed that the mango pulp of 'Ok Rong' cultivar had high accumulation of PL and β-galactosidase in transcript levels on day 5 and on day 10 during low temperature storage. In addition, pulp of 'Nam Dok Mai' fruit showed high accumulation of PL, β-galactosidase and β-1,3-glucanase in transcript levels during ripening at ambient temperature. It has been reported that expression of PL gene, *MiPel 1*, from 'Dashehari' mango correlated with the progress of ripening as evident from the increased expression during ripening (Chourasia *et al*., 2006). Also, in strawberry, PL gene, *Fapl C* showed significantly higher firmness values than control but no differences in other quality attributes such as color and soluble solids, and the expression of this gene was low in green fruit, increased at the white stage, and showed the maximum level at the red stage (Youssef *et al*., 2009). In addition, we observed less accumulation of β-galactosidase transcript both stored at 8 and 12°C in the pulp of HWT fruit than the pulp of control fruit of 'Nam Dok Mai' both stored at 8 and 12°C. This result indicated that HWT affected the pulp of 'Nam Dok Mai' fruit at both low temperature storage and ripening at ambient temperature. Moreover, in similar studies, it was shown that the transcript accumulation of β-gal gene, *TBG 4*, from tomato fruit was not affected by cold storage (Rugkong *et al*., 2010). Tateishi *et al.* (2007) isolated three new β-gal cDNA clones, *PaGAL2*, *PaGAL3* and *PaGAL4* from the fruit of ripening avocado in addition to the *AV-GAL1* clone previously obtained. Based

on the results obtained, it seemed that *AV-GAL1*, which may be encoded AV-GAL III, was important for postharvest fruit softening while *PaGAL2*, *PaGAL3* and *PaGAL4* may be involved in galactose metabolism of cells or cell walls during development and ripening. According to the results, both cultivars of fruit pulp showed high accumulation of β-1,3-glucanase in transcript levels during low temperature storage especially on day 10 of storage which correlated with the transcript accumulation of β-1,3-glucanase gene, *Vcgns 1*, from grape exhibiting drastically increased levels during low temperature storage and remained stable throughout the storage (Romero *et al*., 2006). Sanchez-Ballesta *et al.* (2006) identified a full-length cDNA encoding an acidic class III β-1,3-glucanase (*CrGlcQ*) from the flavedo of the chilling-sensitive 'Fortune' mandarin. The results showed changes in *CrglcQ* gene expression were linked to postharvest chilling-induced cell damage in citrus fruit because the storage at 2°C had a clear effect increasing the *CrglcQ* mRNA accumulation in fruit of the chilling-sensitive citrus cultivar 'Fortune' but not in fruit of a chilling-tolerant cultivar ('Hernandina') stored at 2°C, or in 'Fortune' mandarins kept at a nonchilling temperature. The *CrGlcQ* transcript was also up-regulated by mechanical wounding and by exogenous ethylene at low temperature, which reduced CI. In 2010, Wei *et al.* investigated the activity and gene expression of β-galactosidase (β-Gal), α-l-arabinofuranosidase (α-l-Af), polygalacturonase (PG), and pectin methylesterase (PME). They demonstrated that an increase in β-Gal activity and related gene expression during ripening and softening was concomitant with a decrease in firmness. β-Gal activity was rapidly enhanced at the early ripening stage in 'Golden Delicious' fruit, and was greater than that measured in 'Fuji' fruit. Treatments with 1-MCP and 0°C dramatically postponed fruit softening and inhibited β-Gal activity and β-Gal mRNA accumulation, whereas the opposite response was found with ethephon. In addition, this change in pattern of activity did not differ from that for the control, indicating that β-Gal had an important role in apple fruit softening, particularly the initial process. They concluded that β-Gal and α-l-Af may be more closely related to the storability of apples than PG and PME, especially when fruit ripening and softening begin. The results confirmed that cell wall enzymes play an important role in fruit softening.

In conclusion, results from our studies indicated that HWT at 50±1°C for 10 minutes did not cause the heat damage in both cultivars of mango. HWT enhanced the postharvest shelf life of mango due to retard decreasing in percent of weight loss, increasing in TSS, the accumulation of β-galactosidase in transcript levels and promoting the transcript accumulation of Mn-SOD. Storage at 8°C can delay the ripening process in the mango compared to storage at 12°C. The comparison of two cultivars ('Nam Dok Mai' and 'Ok Rong') showed that 'Ok Rong' mango was effective with HWT condition, resulting to maintain fruit quality throughout the storage. Mango peel were affected by HWT more than mango pulp due to enhancement of the activities of CAT, APX and GR both low temperature storage and ripening at ambient temperature. HWT also had effect on protein changes of mango peel and pulp during fruit ripening.

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APPENDICES

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Appendix A
The CIE color space

There are several color coordinate systems such as RCB (red, green and blue), which is used in color video monitors; Hunter L a b; CIE (Commission Internationale de l'Eclairage) L* a* b*, which in English is the International Commission on Illumination; CIE XYZ, CIE L* u* v*; CIE Yxy ;and CIE LCH that are used in color measurement. According to CIE concepts, the human eye has three color receptors-red, green and blue-and all colors are combination of those. The CIE L* a* b* color space devised in 1976 to provide more uniform color differences in relation to human perception of differences (Abbott, 1999). Color is measured by colorimeters in which the sensors are filtered to respond similarly to the human eye.

Minolta chromameter can be used for determination of color attributes expressed in CIE L* a* b* co-ordinates where L* represents lightness, a* denotes red/green value and b* indicates yellow/blue value as shown in Figure A.1. From left to right a* axis run which +a direction shows shift toward red while along the b* axis which +b movement shows shift toward yellow. The center L* axis shows the degree of lightness which L=0 for black to 100 for white on a vertical axis (McGuire, 1992).

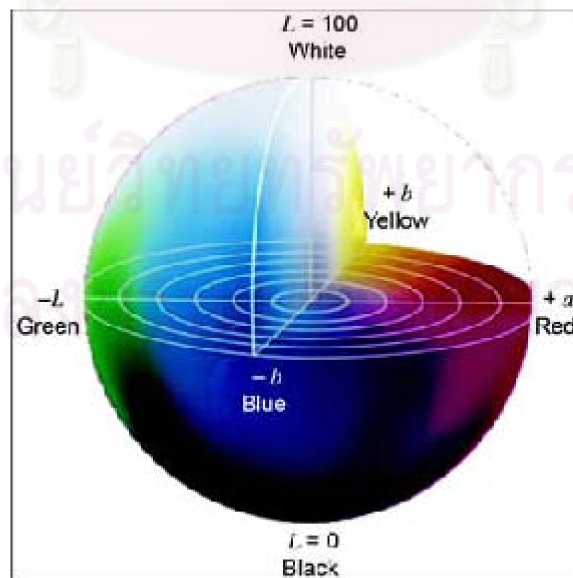

Figure A.1 Representation of a color solid for L* a* b* color space (Minolta, 1994)

The distance of a color from the center where the two dimensions cross each other indicates the chroma (C*). A hue angle (h) is a line that gotten by drawing a simple line from the center of the a* and b* plane through the location of the paint.

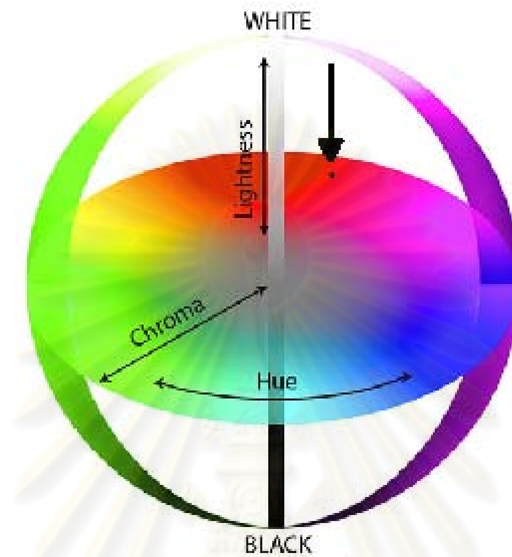

Figure A.2 The geometrical arrangement of color attributes (Tasneem, 2004)

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Appendix B

The mango gene sequences

Manganese Superoxide Dismutase (Mn-SOD)

Forward

MNSD-F

5´-CGTTGCCCGATCTTCCCTATGATTATGGCGCTTTGGAGCCAGCGATTAGC

GGTGAGATTATGCAGTTGCATCACCAGAAGCACCACCAGACTTACATTAC

TAATTACAACAAGGCACTTGAGCAGCTCGACCAAGCCATGAACAAAGGCG

ACGCTTCTACCATTGTCAAGTTGCAAAGTGCCATCAAGTTCAACGGCGGA

GGTCATGTCAACCACTCAATTTTCTGGAAGAATCTGACTCCTATTAATGA

AGGAGGCGGGAGCGCACCAAA-3´

Reverse

MNSD-R

5´-TTGACATGACCTCCGCCGTTGAACTTGATGGCACTTTGCAACTTGACAAT

GGTAGAAGCGTCACCTTTGTTCATGGCTTGGTCGAGCTGCTCAAGTGCCT

TGTTGTAATTAGTAATGTAAGTCTGGTGGTGCTTCTGGTGATGCAACTGC

ATAATCTCACCGCTAATCGCTGGCTCCAAAGCGCCAAAATCATAGGGAAG

ATCGGGCAACGTGAAGGTCTGAAGTCCACGGACGTGCGACATCAATCCAA

GGCAGACGGAT-3´

Pectate Lyase (PL)

Forward

PL-F

5´-CATAGCGGACAGTTCGGATGATTCATGGAGCGAGCACGCTGTTGATAATC

CAGAGGAGGTGGCTGCTATGGTCGACATGTCAATTCGTAATAGTACTGAA

AGAAGAAGGTTAGGATATTTCTCATGTGAAACTGGAAACCCAATCGATGA

TTGTTGGCGTTGTGACCCTAAATGGCATTTGCACCGAAAACATCTTGCTG

ATTGTGCCATTGGTTTTGGGCGCAATGCCATTGGTGGCCGTGAT-3´

Reverse

PL-R

5´-CGCAACGCCGACAATCATCGATTGAGTTTCCAGTTTCACATGATAAATAT

CCTAACCTTCTTCTTTCAGTACTATTACCAATTGATATGCCCACCATAGC

AGCCACCTCCTCTGGATTATCACCAGCGTGCTGGCTCCATGAATCATCCG

AACTGTGCGCTATGGATGTATTTTTGGAGTTCAACAACCTATAGTTTATC

TCGTGCTTCACTGTCGATGCTTCGACGCCGAGAAATAGACATAGAATCAG

TAATGCACATACAGAACTTATCCATCTAGGAGAAACCGCCATTGTTGCCG

CTTC-3´

β-galactosidase

Forward

bGal-F

5´-TAGCATTTGCAGTTGCACGATTCATTCAAGCTGGCGGCTCTTTTGTTAAT

TACTACATGTATCATGGAGGAACAAACTTTGGAAGAACTGCTGGAGGCCC

TTTCATTACCACCAGCTATGATTATGATGCTCCCATCGATGAATATGGCT

TAATAAGACAACCAAAGTATGATCATTTAAAGGAACTCCATCAGGCTGTT

AAATTATGTGAAACAGCTTTGCTTTATGCAGATCCTTATGTCATGTCCTT

GGGAAATTATGAACAGGCGCACGTGTTCTCTTCAACATCGGGAGGCTGTG

CTGCTT-3´

Reverse

bGal-R

5´-ATTTCGCAAGGACATGACATAAGGATCTGCATAAAGCAAAGCTGTTTCAC

ATAACTTAACAGCCTGATGGAGTTCCTTTAAATGATCATACTTTGGTTGT

CTTATTAAGCCATATTCATCGATGGGAGCATCATAATCATAGCTGGTGGT

AATGAAAGGGCCTCCAGCAGTTCTTCCAAAGTTTGTTCCTCCATGATACA

TGTAGTAATTAACAAAAGAGCCTCCAGCTTGAATGAATCGTGCAACTGCA

AATGCTAAATCTTCAACTGGTCGTTGATAAATTGGCCCACCGAATTCTGT

GAACCAGCCGGTCCAAGCCTCAGTC-3´

β-1,3-glucanase

Forward

b1,3G-F

5´-TACAAGGACAACAACATTCGGGCAGATGCGAATTTACGATCCGGATCAAG

CAACCCTCCAGGCCCTGAGAGGATCCAACATTGAACTCATCCTCGACGTC

CCAAAAGACAAACTCCAGGAACTGACGGATTCTGCAAAAGCCGGAGATTG

GGTCCAGACAAACGTCCTG-3´

Reverse

b1,3G-R

5´-ATCCTGGAGTTTGTCTTTTGGGACGTCGAGGATGAGTTCAATGTTGGATC

CTCTCAGGGCCTGGAGGGTTGCTTGATCCGGATCGTAAATTCGCATCTGC

CCAATGTTGTTGTCCTTGTAGAGAGTCACAACTTCAGCTTGTGGTGGTAA

ATTGTTGCCATTTCTTCCATAGCATACACCTATGGCTCCTGATGTTTGTA

TGTGGGACATCAACAACCCAAAGAGCAACAACATTGCAGCCCTGGAAAGT

TTTTCACTCGCAGAATAAGAGGAAACAGCCATGGATCAGAAAATAAAAAC

CAAAATTAAATTTTCTCTGGTGCACCCCC-3´

Appendix C

Reagent recipes

A. Enzyme extraction

1. Extraction buffer

autoclaved 50 mM KH_2PO_4, pH 7.0	8.90	ml
100 mM PMSF	100	µl
1 mg/ml DTT	1	ml
PVP	0.05	g

Adjusted total volume to 10 ml with autoclaved distilled water.

2. 50 mM potassium phosphate buffer (KH_2PO_4), pH 7.0

KH_2PO_4	6.80	g
Distilled water	800	ml

Adjusted pH to 7.0 with NaOH and then adjust total to 1,000 ml with distilled water.

3. 100 mM phenylmethyl sulfonyl fluoride (PMSF)

PMSF	0.035	g

Dissolved in 2 ml isopropanol and stored at -20°C.

B. Protein extraction and separation by SDS-PAGE

1. 1X Laemmli's buffer

1.0 M Tris-HCl, pH 6.8	6.25	ml
10% SDS	20	ml
Glycerol	15	ml
0.5 M EDTA, pH 8.0	250	µl

Adjusted total volume to 100 ml with distilled water.

2. Preparation of 10% acrylamide gel

Sealing the end of gel

Deionized water	2	ml

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30% acrylamide mix	1.65	ml
1.5 M Tris-HCl, pH 8.8	1.25	ml
10% SDS	50	µl

Took 1 ml of mixed solution and then added as follows:

10% APS (freshly prepared)	50	µl
TEMED	5	µl

Immediately sealed the end because it was so fast to set up.

Seperating gel

Deionized water	3.95	ml
30% acrylamide mix	3.35	ml
1.5 M Tris-HCl, pH 8.8	2.50	ml
10% SDS	100	µl
10% APS (freshly prepared)	100	µl
TEMED	4	µl

Stacking gel

Deionized water	3.40	ml
30% acrylamide mix	850	µl
0.5 M Tris-HCl, pH 6.8	625	µl
10% SDS	50	µl
10% APS (freshly prepared)	50	µl
TEMED	5	µl

3. 1.0 M Tris-HCl, pH 6.8

Tris base	12.11	g
Distilled water	80	ml

Adjusted pH to 6.8 with HCl and adjusted total volume to 100 ml with distilled water.

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4. 10% sodium dodecyl sulfate (SDS)

SDS (sodium salt)	5	g

Dissolved in distilled water and adjusted total volume to 50 ml.

5. 0.5 M ethylenedinitrilo tetraacetic acid (EDTA), pH 8.0

EDTA (disodium salt, dihydrate)	18.61	g

Dissolved and adjusted pH to 8.0 with NaOH in the total volume of 100 ml with distilled water.

6. 1.5 M Tris-HCl, pH 8.8

Tris base	18.15	g
Distilled water	80	ml

Adjusted pH to 8.8 with HCl and adjusted total volume to 100 ml with distilled water. Stored at 4°C.

7. 0.5 M Tris-HCl, pH 6.8

Tris base	6.05	g
Distilled water	80	ml

Adjusted pH to 8.8 with HCl and adjusted total volume to 100 ml with distilled water. Stored at 4°C.

8. 10% ammonium persulfate (APS)

APS	0.1	g

Dissolved in distilled water and adjusted total volume to 1 ml.

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9. Electrode buffer for running a gel

Tris base	3	g
Glycine	14	g
SDS	1	g

Dissolved and adjusted total volume to 1,000 ml with deionized water.

C. Western blot analysis

1. Transfer buffer

Tris base	5.82	g
Glycine	2.93	g
Methanol	200	ml
10% SDS	3.75	ml

Dissolved and adjusted total volume to 1,000 ml with distilled water. Stored at 4°C.

2. Alkaline phosphate (AP) buffer

Tris base	6.06	g
NaCl	2.92	g
$MgCl_2.6H_2O$	1.02	g

Dissolved and adjusted total volume to 500 ml with distilled water.

3. AP buffer with BCIP and NBT

AP buffer	15	ml
BCIP	49.5	µl
NBT	99	µl

Mixed them together and stored at -20°C in the dark.

C. Northern blot analysis

1. 5X Denhardt's solution

Ficoll	0.10	g
BSA	0.10	g
PVP	0.10	g

Dissolved and adjusted total volume to 10 ml with DEPC treated water.

2. 20X SSPE

NaCl	175.3	g
NaH_2PO_4.H2O	27.6	g
EDTA	7.4	g
DEPC treated water	800	ml

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Adjusted pH to 7.4 with NaOH and adjusted total volume to 1,000 ml with DEPC treated water.

3. Hybridization solution

20X SSPE	25	ml
Formamide	50	ml
10% SDS	1.25	ml
5X Denhardt's solution	10	ml

Mixed them together and adjusted total volume to 100 ml with DEPC treated water.

4. 20X SSC

NaCl	175.3	g
$Na_3C_6H_5O_7.2H_2O$	88.2	g
DEPC treated water	800	ml

Adjusted pH to 7.0 with NaOH and adjusted total volume to 1,000 ml with DEPC treated water.

5. Wash A

20X SSPE	25	ml
10% SDS	1.25	ml

Mixed them together and adjusted total volume to 100 ml with DEPC treated water.

6. Wash B

20X SSPE	10	ml

Adjusted total volume to 100 ml with DEPC treated water.

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Appendix D

Color changes of 'Nam Dok Mai' and 'Ok Rong' fruits

Figure D.1 Control 'Nam Dok Mai' fruit stored at 8°C on day 0

Figure D.2 HWT 'Nam Dok Mai' fruit stored at 8°C on day 0

Figure D.3 Control 'Nam Dok Mai' fruit stored at 12°C on day 0

Figure D.4 HWT 'Nam Dok Mai' fruit stored at 12°C on day 0

Figure D.5 Control ‘Nam Dok Mai’ fruit stored at 8°C on day 15

Figure D.6 HWT ‘Nam Dok Mai’ fruit stored at 8°C on day 15

Figure D.7 Control ‘Nam Dok Mai’ fruit stored at 12°C on day 15

Figure D.8 HWT ‘Nam Dok Mai’ fruit stored at 12°C on day 15

Figure D.9 Control 'Nam Dok Mai' fruit stored at 8°C on day 18

Figure D.10 HWT 'Nam Dok Mai' fruit stored at 8°C on day 18

Figure D.11 Control 'Nam Dok Mai' fruit stored at 12°C on day 18

Figure D.12 HWT 'Nam Dok Mai' fruit stored at 12°C on day 18

Figure D.13 Control 'Nam Dok Mai' fruit stored at 8°C on day 20

Figure D.14 HWT 'Nam Dok Mai' fruit stored at 8°C on day 20

Figure D.15 Control 'Nam Dok Mai' fruit stored at 12°C on day 20

Figure D.16 HWT 'Nam Dok Mai' fruit stored at 12°C on day 20

Figure D.17 Control 'Ok Rong' fruit stored at 8°C on day 0

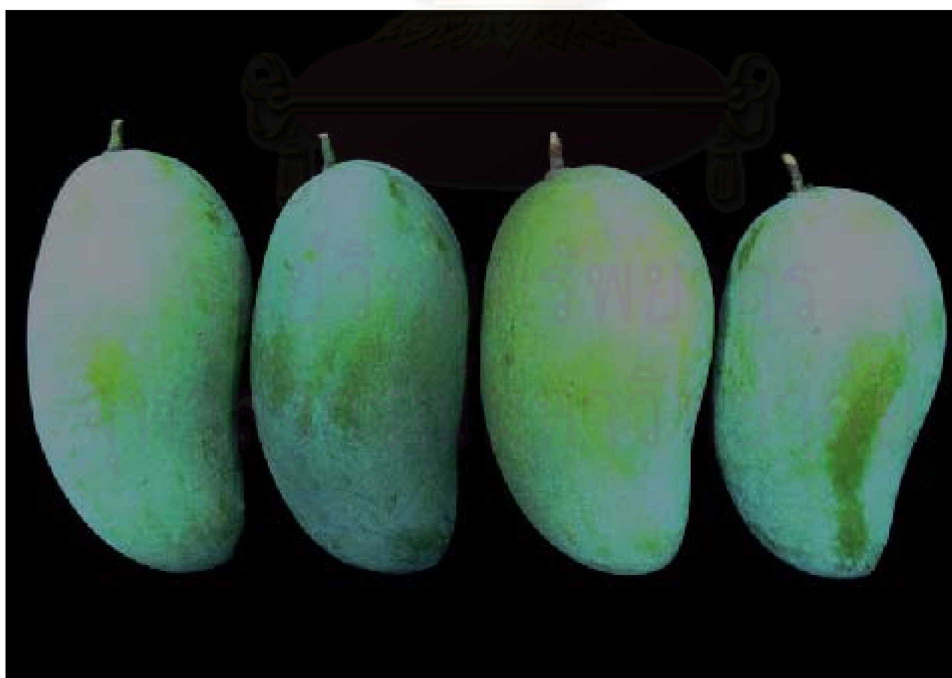


Figure D.18 HWT 'Ok Rong' fruit stored at 8°C on day 0

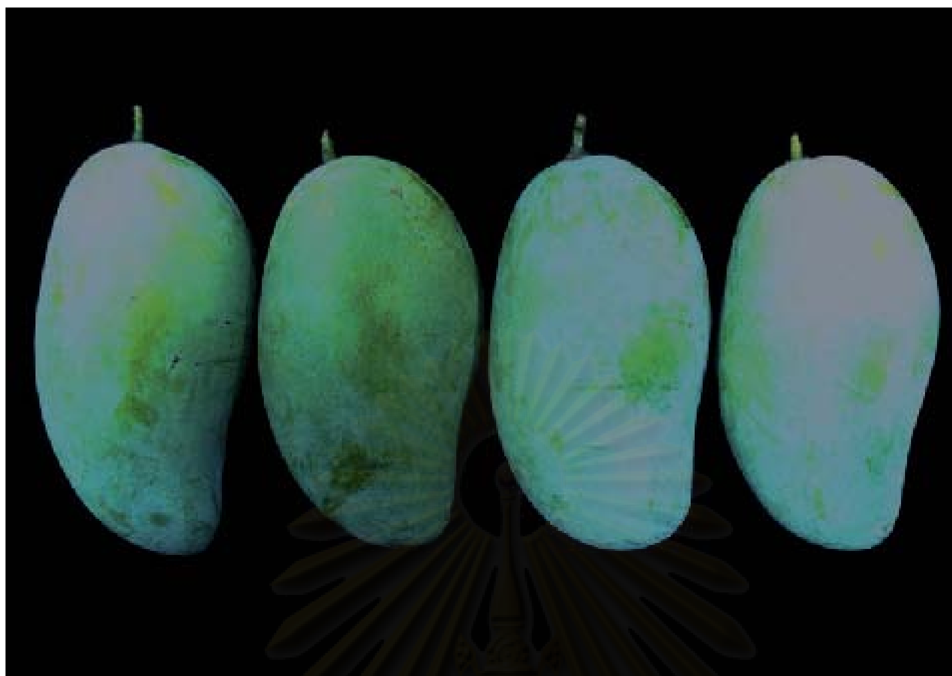


Figure D.19 Control 'Ok Rong' fruit stored at 12°C on day 0

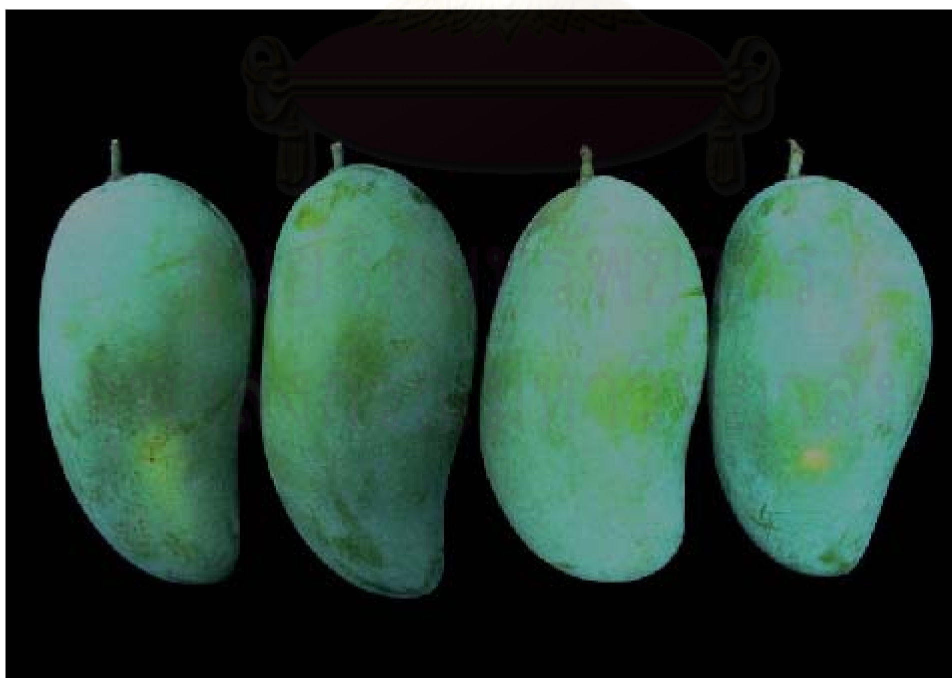


Figure D.20 HWT 'Ok Rong' fruit stored at 12°C on day 0

Figure D.21 Control 'Ok Rong' fruit stored at 8°C on day 15

Figure D.22 HWT 'Ok Rong' fruit stored at 8°C on day 15

Figure D.23 Control 'Ok Rong' fruit stored at 12°C on day 15

Figure D.24 HWT 'Ok Rong' fruit stored at 12°C on day 15

Figure D.25 Control 'Ok Rong' fruit stored at 8°C on day 18

Figure D.26 HWT 'Ok Rong' fruit stored at 8°C on day 18

Figure D.27 Control 'Ok Rong' fruit stored at 12°C on day 18

Figure D.28 HWT 'Ok Rong' fruit stored at 12°C on day 18

Figure D.29 Control 'Ok Rong' fruit stored at 8°C on day 20

Figure D.30 HWT 'Nam Dok Mai' fruit stored at 8°C on day 20

Figure D.31 Control 'Ok Rong' fruit stored at 12°C on day 20

Figure D.32 HWT 'Ok Rong' fruit stored at 12°C on day 20

Appendix E

Biorad Protein Assay: Bradford

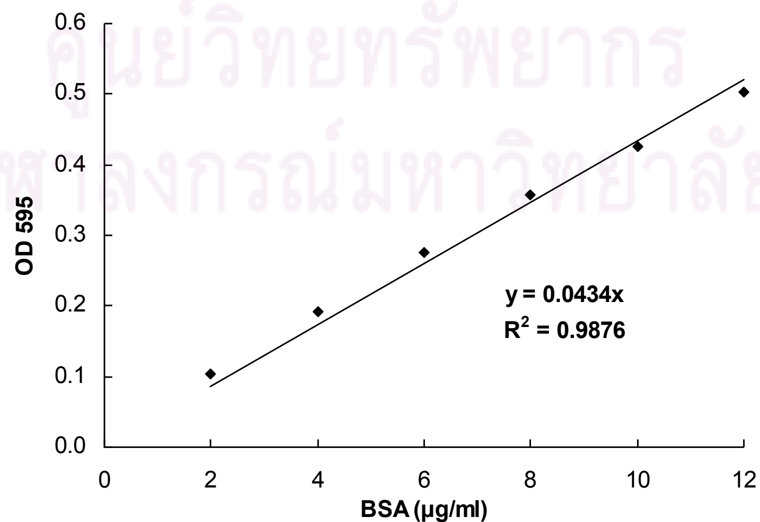
Preparation of standard: 1 mg/ml BSA Stock

Dilution in 10-fold to get (0.1 mg/ml) 100 µg/ml BSA

Final Concentration of BSA (µg/ml)	100 µg/ml BSA (µl)	DDI H_20 (µl)
Blank	0	800
2	20	780
4	40	760
6	60	740
8	80	720
10	100	700
12	120	680

1. Prepared BSA concentrations: 2, 4, 6, 8, 10 and 12 µg/ml
2. Added 200 µl of concentrated Bio-Rad dye reagent and incubated at room temperature for 15 minutes
3. Measured the absorbane at 595 nm

Standard curves for protein determination by Bradford's assay



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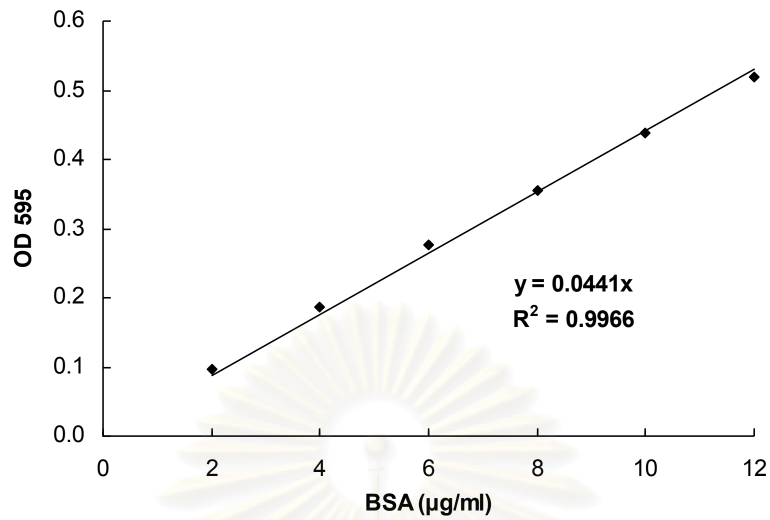

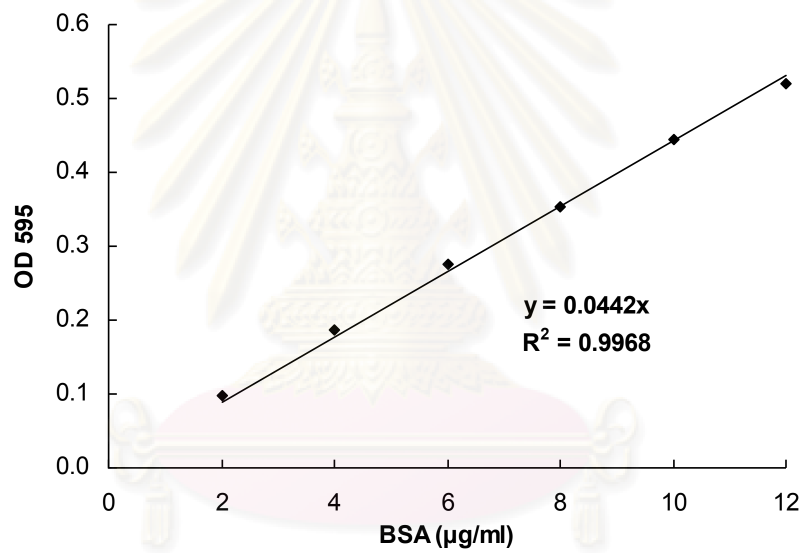

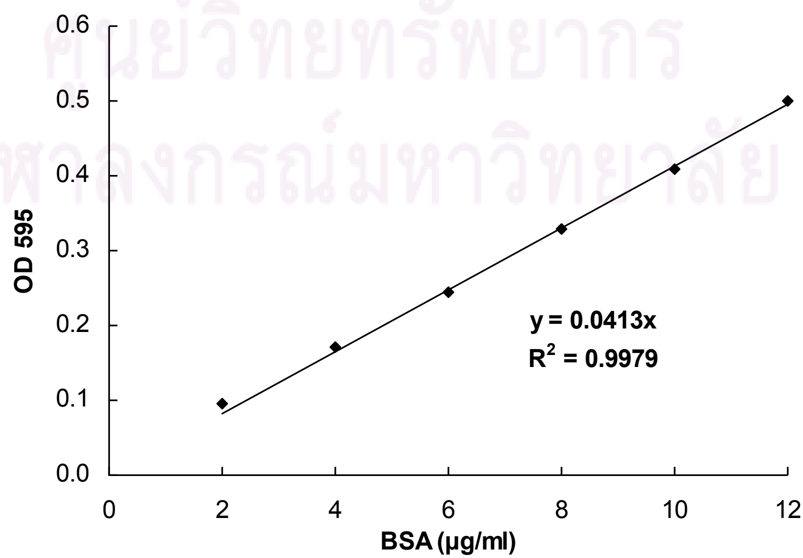

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