STUDY ON UV-C IRRADIATION IN FRUITS: RIPENING QUALITY AND BIOACTIVE COMPOUNDS IN MANGO AND DEFENSE MECHANISM IN TANGERINE

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IN

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ABSTRACT

In the experiment I, effects of UV-C irradiation in Nam Dok Mai mangoes and Sai Nam Phung tangerine were investigated. Mango fruit at commercial stage was treated with UV-C (4.93 kJ/m^2) then stored at 14 ^{o}C and 90% of relative humidity for 20 days. The UV-C treated fruit was higher in a* value but lower in L* and hue values appeared as blackened lenticel and skin browning on its peel compared to that in control. However, UV-C treatment did not significantly affects respiration rate, texture, total soluble solids, and titratable acidity. Antioxidant capacity measured as total phenolic compounds, DPPH, and FRAP were decreased after UV-C treatment compared to that in control. This study suggested that 4.93 kJ/m^2 of UV-C may not suitable to be applied in conserving Nam Dok Mai Si Thong, thus further evaluation on the efficacy of UV-C in mango, both in flesh and peel are required.

In the experiment II, effects of UV-C postharvest treatments on DPPH values, total phenolic concentrations, superoxide dismutase (SOD) enzyme activity, jasmonic acid concentrations, and disease development were investigated in pathogen-

inoculated tangerine (*Citrus reticulata Blanco* cv. Sai Nam Phung). For the *in vitro* test, 7-day old *Penicillium* spp. mycelium was placed on potato dextrose agar and was exposed to 5 kJ/m^2 and 10 kJ/m^2 of UV-C treatments. Tangerine at commercial stage used in *in vivo* test was artificially inoculated with *Penicillium* spp. and then subjected to UV-C similarly in *in vitro* experiment. All experiments were done at room temperature. Infected and non-infected areas of the peel from each fruit were separated and collected for different analysis purposes. Both of UV-C treatments, 5 kJ/m^2 and 10 kJ/m^2, delayed mycelium growth *in vitro* and mycelium density in fruit exposed to high dosage of UV-C compared to that in the control. The values of DPPH, total phenolic concentrations, and jasmonate concentrations at first 24 h were significantly increased in UV-C treated fruit compared to that in the control. SOD values of UV-C treated samples were higher during the measured period. These results suggest UV-C irradiation of 5 and 10 kJ/m^2 possess direct germicidal effect and provoke some alteration in the bioactive compounds and jasmonic acid concentrations that may disrupt *Penicillium* spp. development.

In the experiment III, effects of UV-C postharvest treatments on quality attributes, DPPH values, total phenolic concentrations, superoxide dismutase (SOD) enzyme activity, and jasmonic acid content were investigated in tangerine (*Citrus reticulata Blanco* cv. Sai Nam Phung). Tangerine fruit at commercial stage was treated with 5 and 10 kJ/m^2 of UV-C then stored at 10 ^{o}C and 90% of relative humidity for 4 weeks. The peel from each fruit was collected for bioactive compound analysis. There was no significant difference in color index observed between control and UV-C treated fruit. Both of UV-C treatments, 5 kJ/m^2 and 10 kJ/m^2 did not produce such harmful effect on fruit skin surface. The values of DPPH, total phenolic concentrations, SOD enzyme activity, and jasmonate concentrations were in general significantly higher in UV-C treated fruit compared to that in the control. These results suggest UV-C irradiation of 5 and 10 kJ/m^2 possess hormetic effects, which

involve in the bioactive compounds accumulation and generate jasmonic acid production that may useful for preserving tangerine.

Keywords: Bioactive Compounds/*Citrus reticulata*/*Mangifera indica*/*Penicillium* spp./Ultraviolet Radiation

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CHAPTER 1

INTRODUCTION

1.1 Background

Nowadays, the consumption of tropical fruit is increasing due to growing recognition of its nutritional value (Gonzalez-Aguilar, Villa-Rodriguez, Ayala-Zavala, & Yahia, 2010; Moo-Huchin et al., 2014). However, tropical fruits are very susceptible to qualitative and quantitative deterioration, including sensorial, microbial and nutritional losses. It was estimated that worldwide about one third of all fruits and vegetables produced are never consumed by humans due to postharvest losses (Kader and Arpaia, 2002). Major causes of losses are attributed to fungal decay and rapid maturation that enhance senescence process (Chan and Tian, 2006). There is a need to develop technologies that can ensure the delivery of high-quality products containing high levels of desired bioactive compounds (Cisneros-Zevallos, 2006), which have been associated with various classes of compounds with antioxidant properties, a concept that has gained in popularity over the recent years (Jagadeesh et al., 2011).

Hormesis is the application of potentially harmful agents at low doses to living organisms in order to induce stress responses and within ultraviolet (UV) radiation (Shama and Alderson, 2005). These made UV-C become an attention as its effect on enhancing beneficial compounds (Maharaj and Ayoub, 2012), controlling postharvest disease (Alothman, Bhat, & Karim, 2009; Ribeiro Canada, & Alvarenga, 2012), and shelf life extension (Ribeiro et al., 2012) of fresh horticultural produce. Moreover, UV properties can obtain a desired quality changes but without neglecting microbial safety (Falguera, Pagán, Salvador, Garvin, & Ibarz, 2011; Ribeiro et al., 2012).

The UV treatment has been shown to produce both positive and detrimental effects. One of its positive effects is its role as ripening and disease control. As ripening control, UV delayed chlorophyll degradation (Costa, Vicente, Civello, Chaves, &

Martínez, 2006; Pongprasert, Sekozawa, Sugaya, & Gemma, 2011), suppressed ethylene production (Bu, Yu, Aisikaer, & Ying, 2013; Stevens et al., 1998), lowered respiration rate (Maharaj, Arul, & Nadeau 1999; Costa et al., 2006), reduced weight loss (Srilaong, Aiamla-or, Soontornwat, Shigyo, & Yamauchi, 2011; Syamaladevi et al., 2014), and maintained fruit firmness (Pombo, Dotto, Martínez, & Civello, 2009; Bu et al., 2013). As disease control, UV decreased disease incidence (Bu et al., 2013; Stevens et al., 1998), and induced phytoalexin accumulation (Charles, Mercier, Makhlouf, & Arul, 2008).

The UV treatment was found to have positive effects, however, it also caused undesirable effects upon its application. In higher dose, UV reduced organic acid (Gonzalez-Aguilar, Wang, Buta, & Kriziek, 2001). In some cases, small UV dose reduced β-carotene level (Gonzalez-Aguilar, Villegas-Ochoa, Martínez-Téllez, Gardea, & Ayala-Zavala, 2007a), delayed color development (Liu, Cai, Lu, Han, & Ying, 2012), and caused skin scald (Cia, Pascholati, Benato, Camili, & Santos, 2007).

However, ultimately, the question of whether treating fruit with low doses of UV will impair either their wholesomeness or safety can only be established once the appropriate investigations have been carried out. The present work helps to gain some physiological and pathological response understandings that observed in mango and citrus as a fruit model following UV-C irradiation treatments. The study also provides information on whether UV-C is affordable and applicable technology for prolonging the shelf life and facilitating retention of nutrition. Since Nam Dok Mai mango and Sai Num Phueng tangerine are one of the important commodities in Thailand (Roongruangsri, 2012; Watanawan, Wasusri, Srilaong, & Kanlayanarat, 2014), hence, exploring these facts has an importance in making tropical fruit, specifically Thailand's cultivar, available for downstream activities of value-added products.

1.2 Objectives

1.2.1 To investigate the effect of UV-C irradiation and its combination with abscisic acid on associated ripening parameter and antioxidant components in Nam Dok Mai mango.

1.2.2 To investigate impact during post-treatment handling of UV-C treated tangerines on the important bioactive compounds.

1.2.3 To investigate defense mechanism against *Penicillium* spp. after UV-C irradiation.

1.3 Scope of Studies

1.3.1 Experiment I: Effect of UV-C Treatment in Mango cv. Nam Dok Mai

The study investigated ripening quality and antioxidant properties in Nam Dok Mai mango subjected to 4.93 kJ/m^2 of UV-C irradiation. Quality attributes (including surface appearances, acidity, total soluble solids, firmness), respiration rate, and antioxidant properties was evaluated.

1.3.2 Experiment II: Effect of UV-C Treatment in pathogen-inoculated tangerine

The experiment involved *in vitro and in vivo* approaches for revealing molecular mechanisms involved in plant-defense induced by 5 and 10 kJ/m^2 of UV-C on inoculated-pathogen tangerine. Mycelium development (*in vitro* and *in vivo*), antioxidant properties, superoxide dismutase (SOD) enzyme activity, and stress-related plant hormone were investigated in pathogen-inoculated tangerine (*Citrus reticulata* Blanco cv. Sai Nam Phung).

1.3.3 Experiment III: Effect of UV-C treatment in tangerine cv. Sai Nam Phung

The experiment was to evaluate quality attributes (including surface appearances, acidity, total soluble solids), antioxidant properties, superoxide dismutase (SOD) enzyme activity, and stress-related plant hormone (jasmonic acid) generated from 5 and 10 kJ/m^2 of UV-C irradiation in tangerine (in the absence of any pathogen attack).

1.4 Expected Outcomes

The present work is arranged to treat harvested mango and tangerine fruits with hormic doses of UV-C light. It is hypothesized that metabolism of stressed fruits become active to cope with the stress leading to the production of various secondary metabolites. This change is expected to have an impact on ripening behavior and physicochemical quality of treated produce.

1.5 Research Location

1.5.1 Postharvest technology laboratory, Scientific and Technological Instruments Center, Mae Fah Luang University, Chiang Rai, Thailand.

1.5.2 Pomology laboratory, Bioresource Science Department, Graduate School of Horticulture, Chiba University, Matsudo, Japan.

CHAPTER 2

LITERATURE REVIEW

2.1 General Characteristics of Mango

Mango (*Mangifera indica*) belongs to the family Anacardiaceae, which comprises more than 70 genera (Ribeiro and Schieber, 2010). Mangoes are produced world wide for approximately 39 million tonnes (FAOSTAT, 2013) and it is the second largest tropical fruit production, after banana (Yahia, 2011; FAOSTAT, 2013). Thailand is the top-three exporter country of mango with almost 153.000 tones of its mangoes being traded worldwide (FAOSTAT, 2013). 'Nam Dok Mai' cultivar (Figure 2.1) is Thailand's number one mango variety for domestic and export markets (Watanawan et al., 2013; Sirisomboon and Pornchaloempong, 2011).

Figure 2.1 'Nam Dok Mai' Mangoes at Commercial Stage

Mango belongs to the category of climacteric fruit and its ripening is initiated by a burst in ethylene production and a dramatic rise in the rate of respiration (Mercadante, Rodriguez-Amaya, & Britton, 1997). Ripe mango peel shows a wide range and mixture of colors from green to greenish-yellow, red, violet, and yellow. The popularity of mango is due to its excellent flavor, attractive fragrance, beautiful color, taste, and nutritional properties (Arauz, 2000). The main antioxidants in mangoes are carotenoids, ascorbic acid, and phenolic compounds (Ornelas-Paz, Yahia, & Gardea, 2007; Yahia 2011; Ribeiro et al., 2007).

2.2 General Characteristics of Citrus

Citrus fruit, belonging to the genus Citrus of the family Rutaceae, are well known for their refreshing fragrance, thirst-quenching ability and their capability to provide the recommended adequate vitamin C (Ladaniya, 2008). In Thailand, tangerine provides 80 percent of the national citrus production in which the main production area is located in the northern part of Thailand, including Chiang Mai, Chiang Rai, Phare, and Naan provinces. In those region the citrus industry comprises about 11,200 hectare, of which 'Sai Num Phung' tangerine is the major cultivar (Office of Commercial Affairs Chiang Mai, 2008; Roongruangsri 2012).

Citrus is non-climacteric fruit that its respiration and ethylene production changes are slow and gradual, unlike many other fruits such as bananas and avocadoes (Ladaniya, 2008; El-Otmani and Ait-Oubahou, 2011). Mature citrus fruits evolve very low amounts of ethylene during ripening, but respond to exogenous ethylene by ripening-related pigment changes and accelerated respiration (Katz, Lagunes, Riov, Weiss, & Goldschmidt, 2004).

Table 2.1 Top Tangerines, Mandarins, Clementines, Satsumas Productions

Rank	Area	Production (MT)
1	China, mainland	13,600,000

Table 2.1 (continued)

Rank	Area	Production (MT)
2	Spain	1,873,900
3	Brazil	959,672
4	Turkey	889,365
5	Egypt	877,111
7	Japan	846,300
15	Thailand	375,000

Source FAOSTAT (2013)

2.3 Harvesting, Postharvest Handling, and Postharvest Disease of Citrus

Indices of maturity for citrus fruit are color (yellow or orange) on 75 % of fruit surface and also the ratio of soluble solids and acid of 6.5 or higher (Kader and Arpaia, 2002). In tropical and subtropical countries, the temperature affects the development of the fruit, thus in many cases, the fruit is edible even when the rinds still remains green.

Tangerine is moderately perishable fruit with a short postharvest life, about 2 to 6 weeks at 5-8 ^{o}C depending on the cultivar, stage at harvest, and postharvest treatment (Kader and Arpaia, 2002). Fungal diseases are the main pathological disorder and the major cause of postharvest losses in most citrus fruits. The most common postharvest fungal diseases and their corresponding causal agents are listed in Table 2.2. The incidence of each particular fungus is very variable and highly dependent on climate, growing conditions, handling, storage conditions, and transport (Palou, 2009).

Penicillium digitatum Sacc, one of the economically important pathogens, infects the fruit through rind wounds or injuries inflicted during harvest, transportation, and postharvest handling. With regards to the abundant rainfall, Southeastern Asia suffers higher incidence of postharvest decay than any other production area (Palou, 2014).

Table 2.2 Causal Agents for Disease in Citrus

Disease	Causal pathogen	Infection site
Stem-end rot	*Diplodia gregaria*	Flower, young fruit
Stem-end rot	*Phemopsis citri*	Flower, young fruit
Black rot	*Alternaria citri*	Flower, young fruit
Botrytis rot	*Botrytis cinerea*	Flower, young fruit
Brown rot	*Phytophthora citrophthora*	Fruit surface
Anthracnose	*Colletrotrichum gloeosporioides*	Fruit surface
Green mold	*Penicillium digitatum*	Fruit injury
Blue mold	*Penicillium italicum*	Fruit injury

Source Palou (2009)

Figure 2.2 *Penicillium* spp. Infection in Tangerine

2.4 Alternative Strategies for Postharvest Disease Control in Citrus

It has been shown that the non-toxic applications, such as the treatment with hot water (Inkha and Booyakiat, 2010), combination of electrolyzed oxidizing water and ozone (Whangchai, Saengnil, Singkamanee, & Uthaibutra, 2010), ozone (Boonkorn et al., 2012), blue light treatment (Liao, Alferez, & Burns, 2013), and ultraviolet (UV) treatment (Chalutz, Droby, Wilson, & Wisniewski, 1992; D'hallewin, Schirra, Pala, & Ben-Yehoshua, 2000; D'hallewin, Schirra, & Manueddu, 1999; Kinay, Yildiz, Sen, Yildiz, & Karacali, 2005; Canale, Benato, Cia, Haddad, & Pascholati, 2011) can retard fungal disease development in citrus.

Heat treatment is the most common and popular physical treatment to control postharvest disease in citrus. However, upon its application, product sensitivity to the temperatures is required for effective treatment (Couey, 1989). Another important reason is that the range of effective yet non-phytotoxic temperature is very narrow (Palou, 2009). Moreover, immersion in water for several minutes requires the use of large high volume of tanks and both the implementation and maintenance of this equipment are expensive. In addition, energy costs needed to heat up a very large volume of water are also high.

Apart from sterilizing equipment and production area, ozone is used in the fresh fruit to eliminate microorganism from the surface of the produces as well as in the preservation and extension of their shelf life. There are some limitations upon ozone application. According to Gonçalves (2009), ozone has short half-life in water and high inhalation toxicity. In addition, its complexity and capital cost are also high.

Irradiation treatments are designed to provoke very similar mechanisms in fruit physiology and to obtain similar beneficial fruit responses, not only for pathogenic decay control but also for delay of fruit senescence and overall extension of storage potential and shelf life. Irradiation, specifically UV-C, penetrates only 5-30 microns of the tissue (Maharaj and Ayoub, 2012), thus, this application is mainly limited used as a surface treatment. UV treatment constitutes an alternative to thermal treatment that is being studied and developed to obtain a better final product sensory quality, without neglecting microbial safety (Falguera et al., 2011). Regarding the mechanism and its effects during applications (especially UV-C) will be further discussed at subchapter 2.5 – 2.12.

2.5 Properties of UV-C

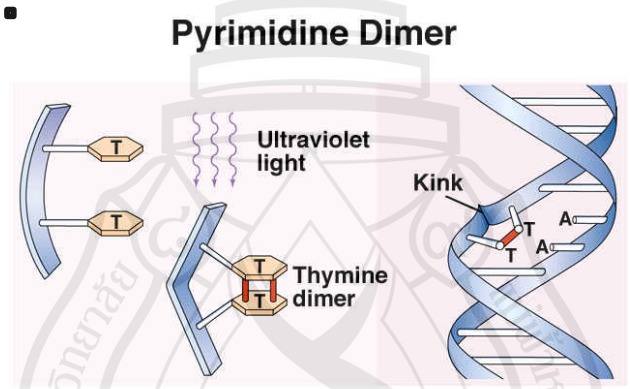
The wavelength for UV processing ranges from 50-400 nanometer (nm) as shown in Table 2.3. However, in nature, UV-C is almost absorbed in the air within few hundreds meter. Koutchma, Forney, & Moraru (2009) stated that there are two critical conditions that must be met for a photochemical reaction to proceed. Firstly, protons must have sufficient energy to promote reaction and secondly, photon energy must be absorbed to promote reactions. Nucleic acids, proteins, lipids, indole acetic acids, flavoproteins, and phytochromes are molecules that contain conjugated double bonds and absorb energy in the UV region (Maharaj, 2015). The UV C photons have enough energy to destroy chemical bonds (Koutchma et al., 2009) and are absorbed by a double bond in pyrimidine bases (such as thymine and cytosine in DNA) (Goodsell, 2001). As a result of photochemical reaction by UV-C, pyrimidine dimers might be formed (Fig. 2.3); this distorts the DNA helix and blocks cell replication (Lado and Yousef, 2002).

Table 2.3 Spectrum of Electromagnetic Radiation and Corresponding Photon Energies

Radiation Type	Wavelength (nm)	Photon Energy (kJ/Einstein)
Gamma rays	< 0.1	> 10^6
X-rays	0.1-50	= 10^6 - 2400
UV light	50-400	2400-300
UV A	330-400	300-170
UV B	270-330	360-300
UV C	200-270	600-440
Visible	400-700	300-170
Infrared	400-10^7	170-0.01
Microwaves	10^7-10^8	0.01-0.001
Radio waves	10^8-10^{13}	0.001-10^{-8}

Source Koutchma et al. (2009)

UV-C application possesses biphasic dose response, which nowadays has been referred as hormesis (Calabrese, 2003). Hormesis is observed as low dose stimulation while inhibition occurs at higher doses (Calabrese, 2003). Similarly, Stevens et al. (1996) also expressed the effect of UV-C on the degree of infection would be quadratic, meaning that the effectiveness of the UV-C treatment declined beyond a certain optimum dose. Depending on UV dose, these treatments may also deleteriously affect plant tissues (Palou, 2009), which include damage in tissue structural and also changes in cytomorphology and water permeability of inner epidermal cells (Lichtscheidl-Schultz, 1985).



Note. TT dimer (in red) is shown within a DNA double helix. Cyclobutane ring (in brown) is formed between the two-thymin bases. The dimier causes local distortions in the helix, weakening the interaction with the paired adenine bases and kinking the backbone slightly

Source Goodsell (2001)

Figure 2.3 Pyrimidine Dimer in DNA

The degree to which the destruction or inactivation of microorganisms occurs by UV radiation is directly related to the UV dose. The irradiance (I) or intensity of UV radiation is expressed as Watts per square meter (W/m^2), while the dose (D) is expressed in Joules per square meter (J/m^2) in SI units (Table 2.4).

Table 2.4 Basic Radiometric Terminologies

Term	SI Unit
Wavelength	nanometer (nm)
Radiant energy	Joule (J)
Radiant flux	Watt (W)
Irradiance	W/m^2
Radiant exposure	J/m^2

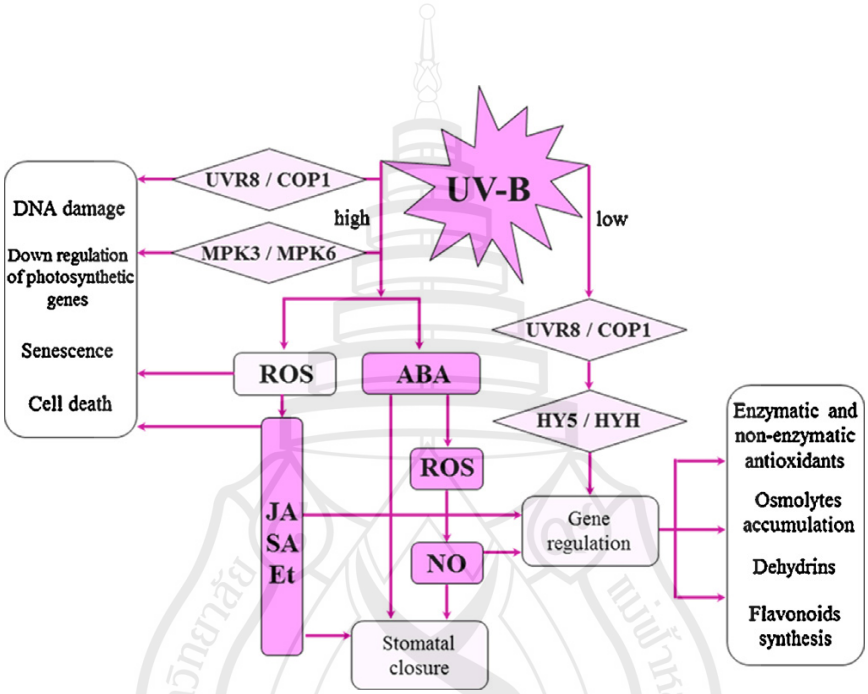
Source Civello, Vicente, & Martinez (2006)

The UV dosage is a function of the irradiance and time of exposure and it is calculated as $D = I \times t$, where: D = UV Dose (J/m^2); I = Irradiance (W/m^2); t = Treatment time (s) (Civello et al., 2006). UV-C irradiation at low doses (0.25 - 8.0 kJ/m^2) affects the DNA of microorganisms (Terry and Joyce, 2004; Harm, 1980).

According to Koo, Gao, Jones, & Howe (2009), physiologically active substances such as ethylene, abscisic acid (ABA), and jasmonates (jasmonic acid (JA) and methyl jasmonate (MeJA) play a role in signal transduction substances for stress and induce the production of secondary metabolites through the expression of tolerant genes against stress, which is shown in Fig 2.4. If the cells that are unable to repair their radiation-damaged DNA die (Fig 2.4). Thus, the exposure should be arranged for each specific commodity in order to maximize effectiveness without causing phytotoxicity (Ben-Yehoshua and Mercier, 2005).

The US Food and Drug Administration (2000) allows the use of the UV-C light for surface microorganism control in food and food products under condition stated in the

Code of Federal Regulations Title 21, Section 179.39. Moreover, treatment with ultraviolet energy offers several advantages to food processors such as it does not leave any residue, does not have legal restrictions, is easy to use and lethal to most types of microorganism, and does not require extensive safety equipment to be implemented (Artés, Gómez, Aguayo, Escalona, & Artés-Hernández, 2009).



Note. UVR8/COP1 for Regulators of UV-B Responses, HY5/HYH for Transcription Factors, MPK3/MPK6 for Mitogen-activated Protein Kinases, ABA for Abscisic Acid, Et for Ethylene, JA for Jasmonic Acid, SA for Salicylic Acid, ROS for Reactive Oxygen Species, NO for Nitric Oxide

Source Bandurska, Niedziela, & Chadzinikolau (2013)

Figure 2.4 Simplified Model Showing Signaling Pathways in Plant Response to UV-B Radiation

2.6 Effect of UV-C on Fruit Quality Attributes

Many studies have been conducted on the effects of UV-C irradiation on ripening associate processes such as delaying the loss of firmness and changes of composition and structure of cell wall, delaying in ripening, and senescence (Table 2.5). The results obtained in general are quite variable depending on the commodity, ripening stage, harvest season, and dose applied (Civello et al., 2006).

Bioactive compounds in plant, which nowadays is responsible for human health, are actually produced to provide the plant itself with unique or survival or adaptive strategies. They can act as shield between plant tissue and the environment, as a response against abiotic stress such as UV-B radiation (Jaganath and Crozier, 2008). Carotenoid protects the chlorophyll from damage when photosynthesis is light saturated, by directly accepting electronic excitation energy from triplet chlorophyll. UV damages these systems (Kovács and Keresztes, 2002). Studies on bioactive compounds in response to UV-C irradiation are shown in Table 2.6.

Table 2.5 Effect of UV Treatment on the Quality Attributes in Fruit

Effect	Investigated dose (kJ/m^2)	Optimum dose (kJ/m^2)	Commodity	Reference
Delay chlorophyll degradation	0.003	0.003	Banana	(Pongprasert et al., 2011)
	8.8 3.4	8.8	Lime	(Srilaong et al., 2011)
Suppress ethylene production	0.003	0.003	Banana	(Pongprasert et al., 2011)
Maintain fruit firmness	4.1	4.1	Strawberry	(Pombo et al., 2009)
	0.003	0.003	Banana	(Pongprasert et al., 2011)

Table 2.6 Effect of UV-C Treatment on Bioactive Compounds in Fruit

Fruit (cultivar)	Investigated dose (kJ/m^2)	Details	Reference
Tomato (*Solanum lycopersicum* cv. Zhenfen 202)	2, 4, 8, 16	4 and 8 kJm^{-2} significantly increased total phenolic content and antioxidant activity; promoted the accumulation of total flavonoids	(Liu et al., 2012)
Blueberry (Bluecrop var.)	0 - 4	Increased total anthocyanin content and FRAP (2 or 4 kJ/m^2)	(Perkins-Veazie et al., 2008)
Strawberry (*Fragaria x ananassa Duch*.)	0.43, 2.15, 4.30	Promoted the antioxidant capacity, enzyme activities; Increased total anthocyanin content during storage in all treatment	(Erkan et al., 2008)

2.7 Effect of UV-C on Oxidative Stress Defense Enzyme

In plant tissues including ripening fruit, reactive oxygen species (ROS) such as superoxide radical ($O2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-) etc. are inevitably generated via a number of metabolic pathways. However, plant cells have been provided with antioxidative defense systems, which scavenge ROS and thus provide protection against their deleterious effects. These include antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR) and glutathione reductase (GR) (Mondals et al., 2009).

UV-C treatment has been found increasing antioxidant enzyme activity as reported by Jiang, Jahangir, Jiang, Lu, & Ying (2010). It was mentioned that 4 kJ/m^2 of UV-C increased the antioxidant enzyme activities of CAT, SOD, APX, and GR in mushroom for 15 days. Whereas, in strawberry, UV-C radiation of 0.5 and 0.75 kJ/m^2 showed the best results for enhancing antioxidant enzyme activity including SOD and CAT (Mohammadi, Mohammadi, Abdossi, Mashhadi, & Boojar, 2012).

2.8 Effect of UV-C on Disease Development in Fruit

UV-C irradiation (200–270 nm) is a novel postharvest treatment, which shows an interesting result for its germicidal effect. The UV-C treatment as disease control in fruits acts in two ways, directly and indirectly.

Table 2.7 Effect of UV-C Treatments on Disease Development in Fruit

Disease (or commodity)	Investigated dose (kJ/m^2)	Details	Reference
Botrytis cinerea in strawberry	5 and 10	No surviving spores were found after irradiation	Marquenie et al., 2002
Colletotrichum gloeosporioides in 'Niagara Rosada' grapes *(Vitis labrusca)*	1.05, 2.09, 4.18, and 8.35	The dose of 4.18 and 8.35 kJ m-2 provided the best level control of *C. gloeosporioides*	Cia et al., 2009
Penicillium digitatum in orange	7.92	Reduced rate infection on oranges by threefold; Effectively inactivate spores of *P. digitatum* under *in vivo* and *in vitro* condition	Gündüz & Pazir, 2013
Star Ruby grapefruit	0.5, 1.5, 3.0	Phytoalexin (as Scoparone and Scopoletin) increased only in UV-C treated samples	D'hallewin et al., 2000

The UV-C can directly act on the microorganisms on the surface by inactivating them (damaging microbial DNA) and reducing their growth, and it also exhibits indirectly action by inducing the synthesis of compounds related with defense mechanisms and antimicrobial activity, such as phenols, flavonoids, phytoalexins, and polyamines (Marquenie et al., 2002; Rivera-Pastrana et al., 2007). However, this kind of inactivation is limited only to the surface of the fruit, as UV has extremely limited

penetration to the solids (Gardner and Shama, 2000). Various dose of UV-C treatments that have been tested for controlling natural infections and decay caused by microorganism in fruit, are shown in Table 2.7

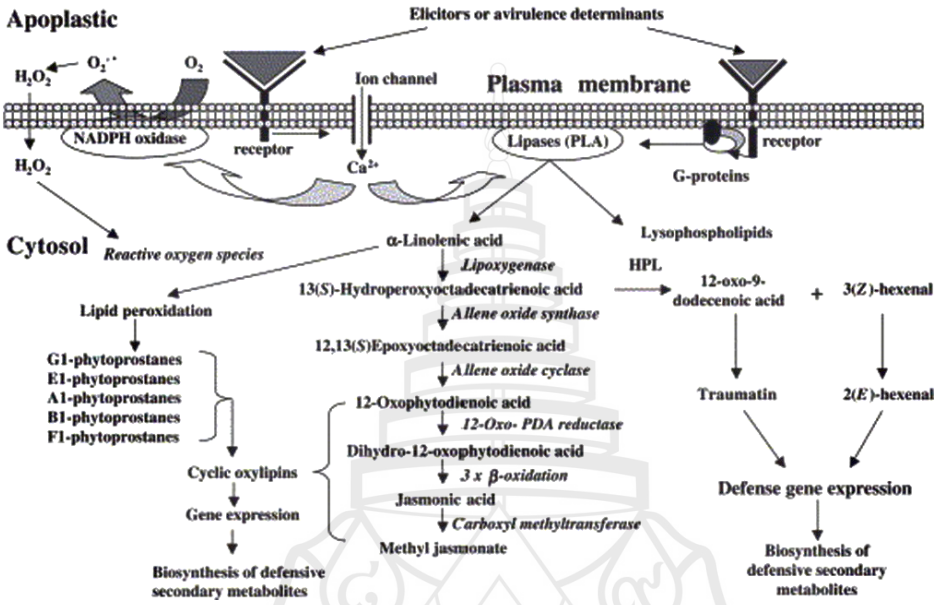
2.9 Undesired Effect After UV-C Treatment

UV radiation can directly or indirectly affect almost all cellular components. Consequently, the UV-C dosage should be carefully selected in order to obtain the beneficial effects desired, without causing detrimental changes on quality attributes. Deleterious effect of UV radiation on surface appearances has been reported as a negative effect of UV-C treatments in some cases. Skin discoloration or external browning is dose dependent (Maharaj, 2015) with the effect being more pronounced as the dose is increased and has been reported in several crops notably papaya (Cia et al., 2007), peaches (Stevens et al., 1998), and Star Ruby grapefruit (D'hallewin et al., 2000). Small dose of UV reduced ascorbic acid content in Satsuma Mandarin (Shen et al., 2013). Similar findings in mango by González-Aguilar et al. (2007a) stated that every single dose of UV would cause a decreasing on β-carotene level.

2.10 Jasmonic Acid (JA)

At low or moderate concentration, ROS has been implicated as the second messenger of the intracellular signaling cascades that mediate several plant responses in plant cells, including accumulation of JA (Sharma, Jha, Dubey, & Pessarakli, 2012). Different kinds of stresses like salt stress (Dombrowski, 2003; Pedranzani et al., 2003), wounding, drought, osmotic (Kramell et al., 1995), UV irradiation (Civello et al., 2006; Demkura, Abdala, Baldwin, & Ballare, 2010; Kondo et al., 2011), ozone (Rao, Lee, Creelman, Mullet, & Davis, 2000), and exposure to elicitors like chitins, oligosaccharides, oligogalaturonides (Doares, Syrovets, Weiler, & Ryan, 1995), are known to induce the jasmonate signaling in plants. At the onset of stress conditions like

herbivore attack, jasmonate synthesis occurs through the oxylipin biosynthetic pathway (Wasternack, 2007; Gfeller, Lietchie, & Farmer, 2010).



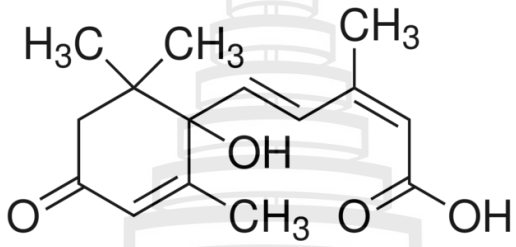
Source Reignault et al. (2013)

Figure 2.5 Schematic Illustration of Biosynthetic Pathway of JA and Other Related Oxylipins

2.11 Abscisic Acid (ABA)

The compound that is known as 'abscisic acid' (Fig. 2.6) plays a regulatory role in many physiological processes in plants. Different stress conditions, such as water, drought, cold, light, and temperature, result in increased amounts of ABA (Swamy and Smith, 1999). Abscisic acid (ABA) becomes one of the interesting hormones to explore about; with respect to its relationship between environmental response, developmental stages, and ethylene status in fruit (Setha, 2012; Zhang, Yuan, & Leng, 2009). Exogenous

applications of ABA have been found to be highly effective in the hastening fruit ripening by increasing of PAL activity, anthocyanin concentration, accelerating ethylene production, respiration rate, enhancing fruit skin color development and softening (Kondo, Ponrod, Kanlayanarat, & Hirai, 2002; Zaharah, Singh, Symons, & Reid, 2012). ABA treatment in fruit found to be involved on physiological changes and several metabolisms during fruit ripening, and also contributed to its nutritional content (Kondo and Fukuda, 2001; Setha, 2012).



Source Sigma-Aldrich (2016)

Figure 2.6 Abscisic Acid Molecular Structures

2.12 Superoxide Dismutase Enzyme

Superoxide dismutase (SOD, 1.15.1.1) plays central role in resistance against oxidative stress in plants (Alscher, Erturk, & Heath, 2002). According to Beauchamp and Fridovich (1971), the SOD enzyme belongs to the group of metalloenzymes and **t**he reaction catalyzes may be represented as follows: $O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$

It is present in most of the subcellular compartments that SOD generates activated oxygen. Three isozymes of SOD, copper/zinc SOD (Cu/Zn-SOD), manganese SOD (Mn-SOD), and iron SOD (Fe-SOD) are reported in plants (Alscher et al., 2002). SOD activity has been reported to increase in plants exposed to various environmental stresses,

including drought stress (De Calvalho, 2008), salinity stress (Gao et al., 2008), and UV-C exposure (Erkan, Wang, & Wang, 2008). Increased activity of SOD is often correlated with increased tolerance of the plant against environmental stresses.

CHAPTER 3

METHODOLOGY

3.1 Effects of UV-C Treatment in Mango

3.1.1 Mango Material

Nam Dok Mai Si Thong mangoes (*Mangifera indica* L., cv Nam Dok Mai Si Thong) were obtained on May 2014 from an orchard in Chiang Mai, Thailand at commercial stage (100-105 days after flowering). All the fruits were washed with 200 ppm of chlorine and rinsed with tap water, and then left until dry well. Fruits were then selected for uniformity in size and shape, and free from defects. They were randomly divided into four groups of 80 fruits each; Fruits were randomly selected for the treatment. Irradiation and ABA treatment were carried out on the next day after harvest under ambient condition. The treatments were labeled as T1= Control; T2= UV-C; T3= ABA; T4= combination ABA and UV-C. The mangoes were packed into boxes and stored for 20 d at 14 ^{o}C and 80–90% of relative humidity (RH).

3.1.2 UV-C Irradiation Treatment in Mango

The UV-C radiation chamber with two side of radiation source (Sylvania Ultraviolet G8W) of the rack (Fig. 3.1) was used as described by Allende et al. (2006). Mangoes were placed under the lamps at a distance of 25 cm and illuminated from both upper and lower side. Ultraviolet irradiation was evaluated using a digital UV-C meter (Lutren UVC-254SD) to determine the intensity. The average intensity was 2.2 mW/ cm^2.

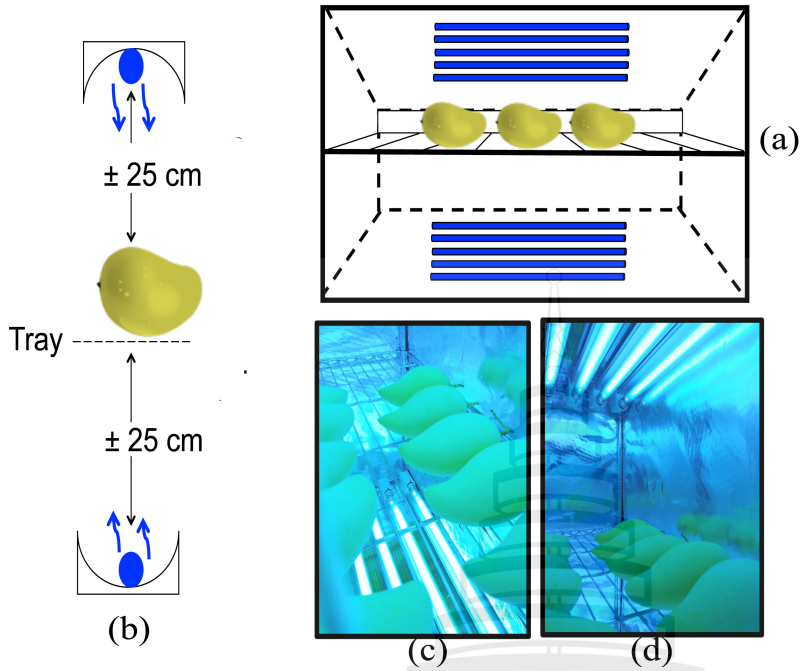


Figure 3.1 UV-C Irradiation Device; (a) Schematic of UV equipment using 10 UV-C lamps; (b) Schematic distance between lamp and fruit surface; (c) and (d) Irradiated fruits arranged on adjustable rack of UV-C equipment

Following to González-Aguilar et al. (2007b), which used 'Haden' variety that has similar peel color (yellow reddish) with 'Nam Dok Mai Si Thong' mango (yellow) and has positive correlation with higher levels of certain bioactive compounds, 4.93 kJ/ m^2 was the dosage used in this investigation. At 112 sec intervals (half of the tested exposure time), each fruit was rotated 180° vertically so that their ends had equal chance to face the lamp to ensure uniform irradiation. All samples were then stored in a refrigerator at 14 ^oC and 90% RH for 20 days in dark. For ripening quality assessment, analyses were carried out at 0, 5, 10, 15, and 20 days of storage. The samples were then frozen and stored for antioxidant analysis.

Example for UV-C dose calculation (targeted dose: 4.93 kJ/ m^2)

*) The UV meter is a machine measured the intensity power of UV lamp. In this case, UV meter (Lutren UVC-254SD) has mW/ cm^2 as displayed unit.

(where 1 mW.s/ cm^2 = 1 mJ/ cm^2 = 10 J/ m^2).

Equation for UV-C dose:

Dose = intensity x time

493 mW.s/ cm^2 = 2.2 mW/ cm^2 x time

224 sec = time

3.1.3 Abscisic Acid Treatment in Mango

The steps for ABA treatment were according to Li et al. (2014), the two groups of fruit were dipped in ABA for 5 min, after air-drying, the first group was labeled as T3. The second group was combined with UV-C irradiation and labeled as T4. According to Zaharah et al. (2013) 0.1 mM of ABA was selected as the dipping concentration. Fruit were dipped for 5 min in an aqueous solution containing ABA and 0.05% Tween 20 as a surfactant.

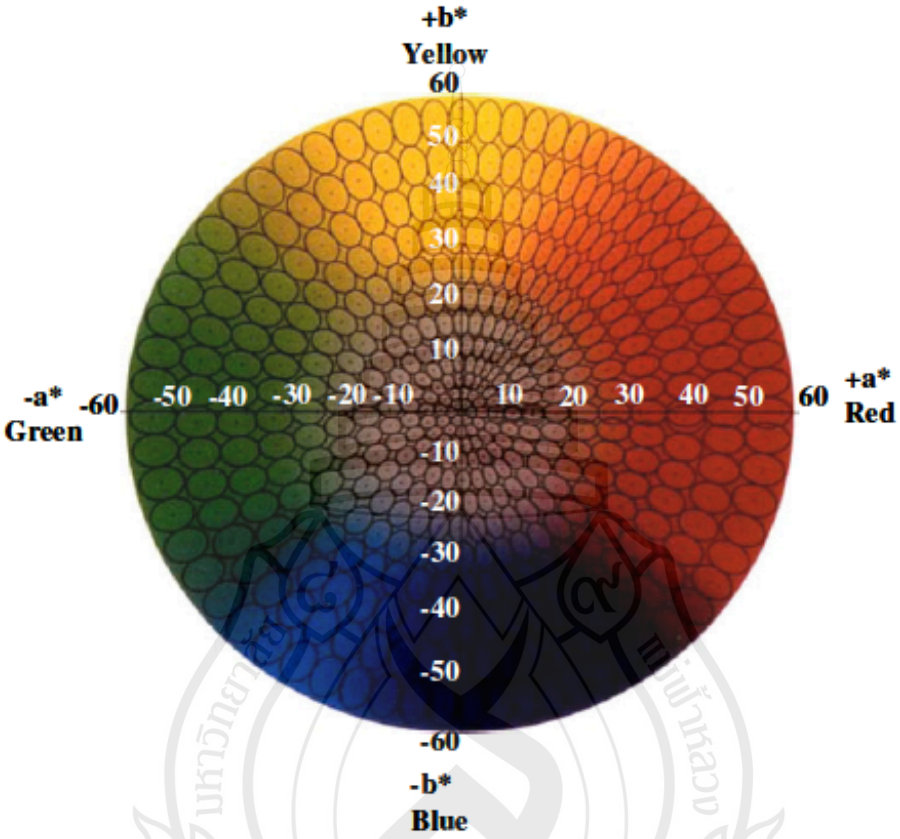
3.1.4 Ripening Quality Assessment

Changes in peel color were expressed by lightness (L*), redness (a*), and hue angle (^{o}hue) with chroma meter (Mini Scan EZ 45/0 Hunter Lab) at 4 points on each side of 9 fruits per treatment. Chromaticity L* represents the lightness of the fruit color, which ranges from 0 (black) to 100 (white). Chromaticity a* indicates the redness (+a*) or greenness (-a*), and chromaticity b* indicates the yellow (+b*) or blue (-b*) color of fruit skin (Figure 3.2). For color interpretation by using hue angle, red was at an angle of 0^o or 360^o, yellow at 90^o, green at 180^o, and blue at 270^o (Figure 3.2).

Respiration rate was determined with gas chromatography (model 7890A, Agilent, Agilent Technologies, US) using 4 fruit per treatment. Each mango fruit were sealed in plastic container at 21 ^{o}C for 3 h. Respiration rate was expressed as mL CO_2/ kg/ h.

Fruit firmness was determined in Newton (N) by using texture meter (Texture Analyzer, TA.XT plus, Stable Micro Systems Texture Technologies) equipped with 2 mm cylindrical probe. Each fruit was penetrated 5 mm at a speed of 1.5 mm/ s and the

maximum force developed during the test was recorded. Three measures were made in each side of fruit at different points along equatorial zone. Results were means of 9 fruits for each sampling period and for each treatment.



Source HunterLab (1998)

Figure 3.2 Color chart – Commission International de L'Eclairage (CIE) L*, a*, and b*

Total soluble solid (TSS) was determined by digital hand-held pocket refractometer (Model PAL1, Atago, Japan). Titratable acidity (TA) was determined by titration fruit juice with 0.1 N of NaOH (phenolphthalein as indicator) and the result was expressed as % of citric acid equivalent. Results were means of 3 replications (a total of 9 fruits) for each sampling period and for each treatment.

The dry matter content (DM) was gravimetrically determined following Kienzle et al. (2011) with a slight modification. Dry matter was resulted as the residue from drying 5.0 ± 0.5 g of mesocarp cubes in a dry aluminium dish at 105 ^{o}C for 3 h and recooling in a desiccator until constant weight.

3.1.5 Sample Preparation

Frozen pulps (5.0 ± 0.5 g) were homogenized in 95% methanol 20 mL, using an Ultra Turrax for 2 min at room temperature. Then, the preparation was centrifuged in 4 ^{o}C; 5,000 g_n for 10 min. The supernatant was taken and filtered through Whatmann filter paper No.1, then the volume was adjusted to 25 mL. The extract was diluted to get five fold.

3.1.6 Antioxidant Capacity Assessment

Analysis of DPPH (2,2-diphenyl 1-picrylhidrazyl) scavenging activity was determined using spectrophotometer (517 nm) with trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic) as standard. The concentration of standard solution ranging from 0 – 1000 µM ($y = 15.339\ x - 10.156$; $r^2 = 0.9998$). The results were expressed as µmol trolox per 100 g of dry basis.

Total reducing power of fruit extract was determined using spectrophotometer (725 nm) as described by Saha, Alam, Akter, & Jahangir (2008). The concentration of standard solution ranging from 0 – 1000 µg/ mL ($y = 0.2586\ x - 0.1669$; $r^2 = 0.9931$). The results were expressed as mg ascorbic acid equivalents (AA) per 100 g of dry basis.

Total phenolics were determined according to ISO 14502-1 (International Organization of Standardization, 2005) using spectrophotometer (G10S UV-Vis, Thermo Fisher Scientific, USA) (700 nm) and gallic acid was used as standard. The concentration of standard solution ranging from 0 – 100 µg/ mL ($y = 0.1996\ x - 0.1876$; $r^2 = 0.9996$). The results were expressed as mg gallic acid equivalents (GAE) per 100 g of dry basis.

3.1.7 Statistical Analysis

All samples were analyzed in triplicate, and expressed as mean ± SD. Analysis of variance (ANOVA) was conducted and compare significance of difference within

samples using Duncan's multiple range tests. To compare significance of difference between two samples, the t-test was conducted. Differences of $P < 0.05$ were considered to be significant (SPSS 16.0 for Windows, SPSS Inc., IL, USA).

3.2 Effects of UV-C in Pathogen-inoculated Tangerine

3.2.1 Tangerine Materials

Tangerine fruit (*Citrus reticulata* Blanco cv Sai Nam Phung) were obtained in January 2015 from commercial orchard in Chiang Mai, Thailand, at commercial stage. The fruit were transported to Mae Fah Luang University, Chiang Rai, within 3 h. All fruit were then selected for uniformity in size, washed with tap water, and dried for 24 h at room temperature prior to treatment. The fruit were divided into three groups of 375 tangerines for *in vivo* experiment; one of the groups was used as the control.

3.2.2 Germicidal Effect of UV-C: *In Vitro* Study

Penicillium spp. was isolated from naturally infected tangerine and maintained on potato dextrose agar (PDA) after confirmed the causal pathogen through Koch's postulates. Then, a 0.5 cm of mycelia disc from 7 day-old pure culture was placed in the center of PDA plate. The UV-C radiation chamber with two side of radiation source (Sylvania Ultraviolet G8W) was used in this experiment. The plates were uncovered and exposed immediately thereafter to UV-C lights. With all ten UV-C lamps powered from a working distance of 25 cm, the intensity at the surface of the plate was 2.1 mW/ cm^2. The intensity of UV-C light was measured with a digital UV-C meter (Lutren UVC-254SD, Lutren Electronic Enterprise Co, Ltd, Taiwan). Three groups of 15 plates were exposed directly to doses of UV-C at 0, 5, and 10 kJ/ m^2. After the treatments were done, the plates were closed and covered with parafilm® M (Sigma Aldrich). The plates were stored at 22 ± 2 °C in the dark room and mycelium diameter per plate was measured every 24 h.

3.2.3 Germicidal Effect of UV-C: *In Vivo* Study

The fungal pathogen that was used for *in vivo* experiment was obtained similarly with 3.2.3. A 7 days-old colony was collected using a spreader and suspended one time in sterile distilled water with 0.1% of Tween 80 followed by centrifugation at 2000 g_n for 10 min. The spore count was determined with a haemacytometer, and the concentration was adjusted with sterile distilled water to 3.67 x 10^6 spore/ mL. The fruit were then wounded (± 1 mm depth) at the equator with a sterile needle and inoculated with 10 µL of the spore suspensions. Three hours was allowed for the spores to settle into the wounds prior to UV-C treatments.

Artificially infected tangerines were placed at 25 cm of distance from UV-C light source and were submitted to two different irradiation doses, 5 kJ/ m^2 and 10 kJ/ m^2, and unexposed fruit was used as control. After irradiation, inoculated and control fruit were kept in a humid plastic chamber.

Fruit peel was removed every 24 h after exposure to UV-C, to provide varying stages of fungal penetration. Infected and uninfected tissues were collected and separated. The infected tissue was used for further observation on disease development. These samples were preserved in formaldehyde–acetic acid–alcohol (FAA) solution and examined under a scanning electron microscope (LEO 1450, VP, England), while the portion of uninfected tissues were collected, freeze-dried, grounded, and kept at -30 ^oC for bioactive compound analysis.

3.2.4 Antioxidant Capacity Assessment

Powder peels (0.300 ± 0.050 g) were soaked in 20 mL of 80% methanol and shaked 150 rpm (IKA - KS 4000 I; IKA®-Werke GmbH & Co.; Germany) at room temperature for 24 h. The preparation was then centrifuged at 4 ^oC; 10,000 g_n for 10 min. The supernatant was taken and filtered through Whatmann filter paper no.1, then evaporated by rotary evaporator (Eyela Rotary Evaporator N-1000, Eyela Oil Bath OSB-2000, Tokyo Rikakikai Co., Ltd, Japan) in vacuum condition at 40 ^oC until reached the final volume at 1 mL. The concentrated samples were then dissolved in 10 mL MeOH 100% using volumetric flask. The extract was diluted with distilled water to get 10 fold (for DPPH analysis) and 15 fold (for TPC analysis).

Analysis of DPPH scavenging activity (2,2-diphenyl 1-picrylhidrazyl) was determined using spectrophotometer (517 nm) with trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic) as standard. The concentration of standard solution ranging from 0 – 1000 μM ($y = 0.0881 x + 1.3257$; $r^2 = 0.9903$). The results were expressed as μmol trolox equivalent per g dry weight. Total phenolics were determined according to ISO 14502-1 (International Organization for Standardization, 2005) spectrophotometer (G10S UV-Vis, Thermo Fisher Scientific, USA) (765 nm) using gallic acid as standard. The concentration of standard solution ranging from 0 – 100 μg/ L ($y = 0.0101 x + 0.0117$; $r^2 = 0.9991$). The results were expressed as mg gallic acid equivalents (GAE) per g dry weight.

3.2.5 Jasmonic Acid Analysis

Extraction and analysis of JA were performed according to the procedure described in a previous paper (Kondo, Kittikorn, & Kanlayanarat, 2005) with GC–MS–SIM. Powder peels (0.300 ± 0.050 g) was homogenized with 100 μL of ibuprofen 10^{-4} M as an internal standard in 10 mL of saturated NaCl solution and 20 mL of diethyl ether containing 0.005% butylated hydroxytoluene (BHT) as an antioxidant. The aqueous layer was extracted 2 times with 20 mL of diethyl ether containing 0.005% BHT. The pooled ether extract was dried under warm air and then the residue was dissolved in 200 μL of chloroform/isopropylethylamine, 1:1 (v/v), and derivatized at 50 ^{o}C for 60 min with pentafluorobenzyl bromide. The ions were measured as m/z 390, 264, and 209. The concentration of JA in the original extract was determined from the ratio of peak areas for *m/z* 209 (jasmonic acid)/ 264 (ibuprofen).

3.2.6 Superoxide Dismutase (SOD) Enzyme Activity Assessment

The part of uninfected peel 0.300 (± 0.050) g were homogenized with 20 mL of 50 mM Na-phosphate buffer (pH 7.2) containing 3 mM ethylenediaminetetraacetic acid (EDTA) and 1% polyvinylpolypyrrolidone (PVPP). After centrifugation for 15 min at 10,000 g_n and 4 ^{o}C, the supernatant was collected and used as the crude enzyme extract for the SOD assays. Preparations and procedure are shown in Table 3.1 and 3.2, respectively.

Superoxide dismutase (SOD; EC 1.15.1.1) activity was determined

spectrophotometrically based on the inhibitory effect of SOD over the reduction of nitro blue tetrazolium (NBT) by the O_2- generated by xanthine/xanthine oxidase system according to Beauchamp and Fridovich (1971). One unit of SOD enzyme activity was defined as the quantity of enzyme that reduced the absorbance reading of samples to 50%. Protein analysis was performed according to previous report (Bradford, 1976). The SOD activity was expressed as units per mg protein. The concentration of standard protein (Bovine Serum Albumin; Wako Pure Chemical Industries, Ltd, Japan) solution ranging from 0 – 800 μg/ mL ($y = 0.0007x + 0.0389$; $r^2 = 0.9935$)

Table 3.1 Superoxide Dismutase Enzyme Activity Solution Preparations

Label	Solution	Detail	Volume usage (mL)
(A)	$Na_2CO - NaHCO_3$ buffer	pH 10.2; 50 mM	2.3
(B)	Nitroblue tetrazolium (NBT)	1 mM	0.1
(C)	Xanthine	4 mM	0.1
(D)	EDTA	3 mM	0.1
(E)	Bovine Serum Albumin	0.15% (w/v)	0.1
(F)	Phosphate buffer	pH 7.0; 50 mM	0.1
(G)	Crude enzyme (plant extract)		0.1
(H)	Xanthine oxidase	56 mu/ mL	0.1
(I)	Destilled water		0.1
(J)	$CuCl_2$	8 mM	0.2

Note. Xanthine Oxidase (Sigma Aldrich) has 13 mg protein/ mL; >0.4 unit/ mg protein; 0.8 unit/ mg protein

Table 3.2 Superoxide Dismutase Analysis Procedure

Eb (Enzyme blank)	**Eb_1** (Enzyme $blank_1$)	**Es** (Enzyme sample)	**Es_1** (Enzyme $sample_1$)
mixed (A) to (E)	mixed (A) to (E)	mixed (A) to (E)	mixed (A) to (E)
+	+	+	+
(F)	(F)	(G)	(G)
mixed (A) to (E)	mixed (A) to (E)	mixed (A) to (E)	mixed (A) to (E)
+	+	+	+
(F)	(F)	(G)	(G)
+	+	+	+
(H)	(I)	(I)	(I)
Incubation 30 ^{o}C 15 min			
+	+	+	+
(J)	(J)	(J)	(J)
Absorbance at 560 nm			

3.2.7 Statistical Analysis

All samples were analyzed and expressed as mean ± standard error (SE). Analysis of variance (ANOVA) was conducted and compared significance of difference within samples using Tukey HSD (honest significant difference). Differences of $P < 0.05$ were considered to be significant (SPSS 23.0 for Macintosh, SPSS Inc., IL, USA).

3.3 Effects of UV-C Treatment in Tangerine

3.3.1 Tangerine Material

Tangerine that was used for the experiment was obtained similarly with 3.2.1. A subsample of 15 fruits was taken for initial analyses of surface color.

3.3.2 UV-C Treatment

The UV-C radiation chamber with two side of radiation source (Sylvania Ultraviolet G8W) of the rack (Fig. 3.1) was used as described by Allende et al. (2006). Tangerines were placed under the lamps at a distance of 25 cm and illuminated from both upper and lower side. Ultraviolet irradiation was evaluated using a digital UV-C meter (Lutren UVC-254SD) to determine the intensity. The average intensity was 2.2 mW/cm^2. 5 and 10 kJ/m^2 were the dosage used in this investigation. All samples were then stored in a refrigerator at 10 ^oC and 90% RH for 4 weeks in darkness. For ripening quality assessment, analyses were carried out in every week. The samples were then frozen and stored for antioxidant analysis.

3.3.3 Quality Assessment

Fruit samples were analyzed for total soluble solids (TSS), titratable acidity (TA), and external color. Changes in peel color were expressed by lightness (L*), redness (a*), and hue angle (^{o}hue) with chroma meter (Mini Scan EZ 45/0 Hunter Lab) at 3 points on each side of 15 fruits per treatment.

For total soluble solids (TSS) and titratable acidity (TA) measurements, samples were obtained by transforming all the fruits in a replication into juice using a hand juice maker. Total soluble solid (TSS) was determined by digital hand-held pocket refractometer (Model PAL1, Atago, Japan). Titratable acidity (TA) was determined by titration fruit juice with 0.1 N of NaOH (phenolphthalein as indicator) and the result was expressed as % of citric acid equivalent. Results were means of 3 replications (a total of 15 fruits) for each sampling period and for each treatment.

3.3.4 Antioxidant Capacity Assessment

This method is already mentioned in sub 3.2.4

3.3.5 Jasmonic Acid Analysis

This method is already mentioned in sub 3.2.5

3.3.6 Superoxide Dismutase (SOD) Enzyme Activity

This method is already mentioned in sub 3.2.6

3.3.7 Statistical Analysis

This method is already mentioned in sub 3.2.7

CHAPTER 4

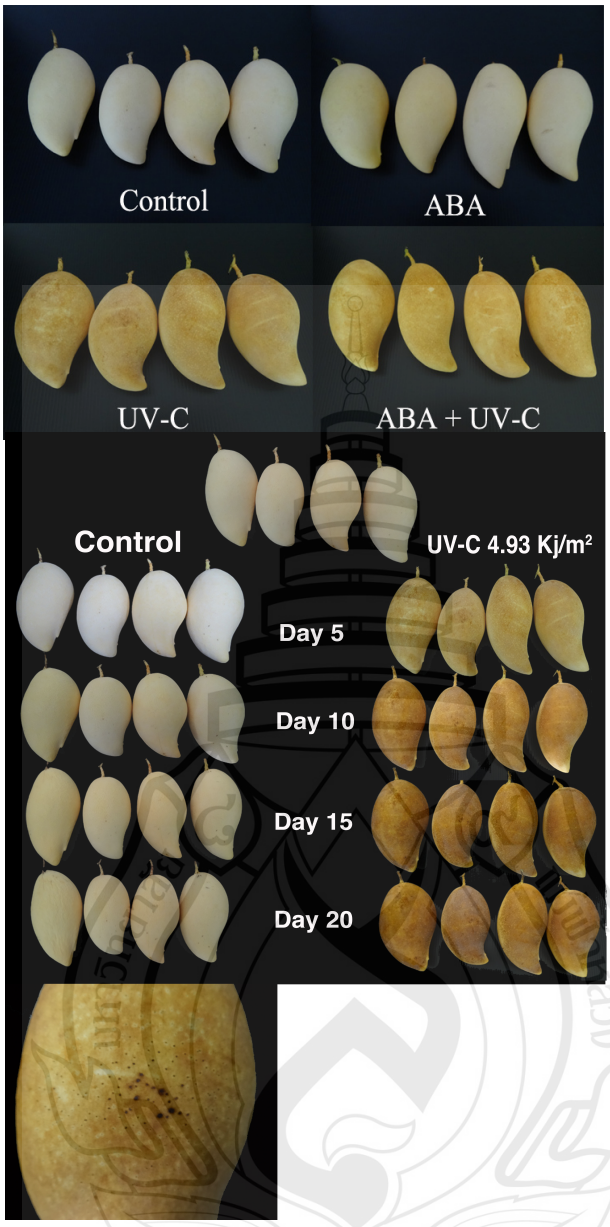
RESULTS AND DISCUSSION

4.1 Initial Quality of Mangoes

At commercial stage, mango cv Nam Dok Mai Si Thong had 0.981 ± 0.074 g/ mL of specific gravity with yellow peel color quantified as 76.88 ± 2.49 of L* value, 4.78 ± 0.97 of a* value, and 81.63 ± 1.58 of hue angle. The mangoes had initial respiration rate at 0.51 ± 0.10 mL CO_2/ kg/ h. The fruit presented 24.41 ± 1.39 N of firmness, 8.2 ± 0.9 ^{o}Brix of total soluble solid, 1.84 ± 0.6 % of titratable acidity, and 19.24 ± 2.19 % of dry matter.

4.2 Effect of UV-C on Surface Appearance in Mango

Figure 4.1 and Figure 4.2 depict the changes in peel color on the normal ripening and UV-C treated Nam Dok Mai Si Thong. During ripening process, the lightness of control samples, which showed as L* value remained relatively steady at 75. By day-20, it had decreased slightly to 72.04. On the other hand, the L* values of UV-C treated samples had suffered a significant decline ($P < 0.05$), particularly by day 10 when numbers fell to 57.38. There was also a significant decline ($P < 0.05$) in hue angle from 81.63 at 0 day to 71.79 at first 5 day of observation. As ripening progressed, the hue angle value decreased steadily throughout the period. Similar to L* value and hue angle trends, the changes of a* value was also significantly different ($P < 0.05$) between control and UV-C treated mangoes.



(A)

(B)

(C)

Figure 4.1 Visual appearance of Nam Dok Mai Si Thong mango; (A) for Appearance comparison at day 5; (B) for head-to-head visual appearance comparison between control and UV-C treated sample during storage; (C) for blackened lenticel and abnormal skin browning appeared in the peel

In UV-C samples, the a* values rose significantly ($P < 0.05$) from 4.78 to 13.70. The value was still increasing to 18.11 at the end of storage, but at a slower pace. The a* value of control samples rose slightly over the whole storage period. However, by the

end of storage time, the values were still less than 9. This showed that UV-C treatment produced a significant difference ($P < 0.05$) in L* value, a* value, and hue angle compared with control.

The external color of mango is an important factor in consumer preference (González-Aguilar et al., 2001). Overall, the UV-C treated mangoes had poor visual quality due to accumulation of skin browning and blackened lenticels throughout the peel with the color worsening as ripening progressed (Figure 4.1C). However, no harmful effects were exhibited on the mango pulp, regardless of the investigated dose used. Similar UV-C damage to peel color was found in papaya (Cia et al., 2007). Likewise, Maharaj et al. (1999) reported that higher dose of UV caused abnormal browning and manifested as sun scalding on the surface of tomato.

The investigated dose was following a report by Gonzales et al. (2007b), which did not produce any harmful effect on 'Haden' mango peel. Considering harmful damage that also occurred when UV-C applied on thick-skinned fruit such as grapefruit (Chalutz et al., 1992; D'Hallewin et al., 2000) indicated that skin thickness is not the main factor that determined UV-C-induced damage produced on fruit peel.

Following a study in strawberry by Li et al. (2014), which involved combination 1 mM of ABA and 4.1 kJ/m^2 of UV-C, had shown that ABA promotes strawberry ripening, while in contrast, UV-C has working oppositely although no surface damage was reported. However, it is mentioned that ABA might be involved in other defense systems to withstand UV-C radiation stress, which in this study, the author believe, ABA would be able to protect fruit from undesirable color change regardless of UV-C treatment application. Consumers have developed distinct correlations between color and the overall quality of specific products (Kays, 1999) As severe peel browning was found among the UV-C treatments (with or without ABA; Figure 4.1A), which affect the preference of the consumer, thus, control and UV-C were the only treatment that observed at the rest of this study.

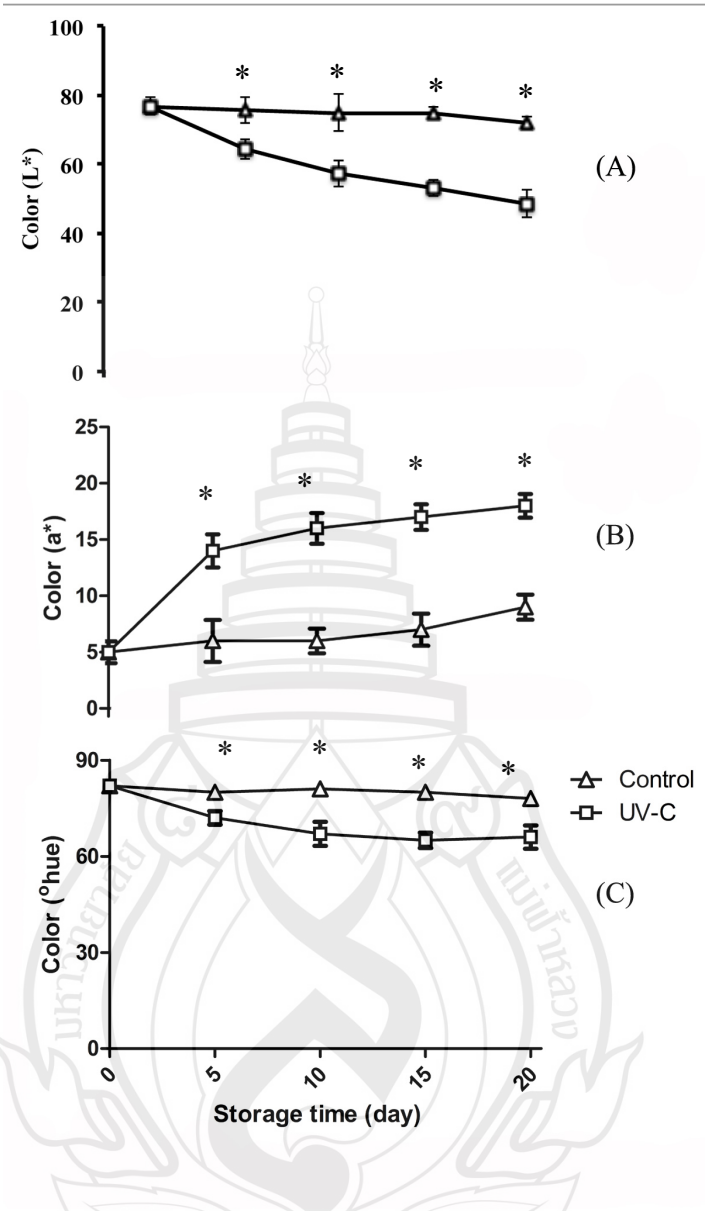


Figure 4.2 Changes in color parameters in the peel of Nam Dok Mai Si Thong mango between control and UV-B treatment during storage at 14 °C; (A) Changes in lightness parameter (The L* value indicated black=0 to white=100); (B) Changes in a* parameter (The a* value indicated positive number indicates red and a negative number indicates green); (C) Changes in hue angle parameter (°hue). Vertical bars represent the SD. (*) Asterisk represents significant difference between samples using t-test ($P < 0.05$)

4.3 Effect of UV-C on Respiration Rate in Mango

Study on the pattern of respiration rate after UV-C irradiation in Nam Dok Mai Si Thong mangoes is shown in Fig. 4.3. Started at day 10, the respiration rate of control samples sharply increased from 2.11 to 6.91 mL CO_2/ kg/ h. Following this sharp increase, the respiration rate of control samples rose more slowly to 7.35 mL CO_2/ kg/ h at day 15 and declined to 4.96 mL CO_2/ kg/ h at the end of storage time. The respiration rate in UV-C treated mango rose steadily from day 5 to day 15, reaching a peak of 6.69 mL CO_2/ kg/ h at day 15. The respiration rate then dropped to 3.85 mL CO_2/ kg/ h by the end of storage period.

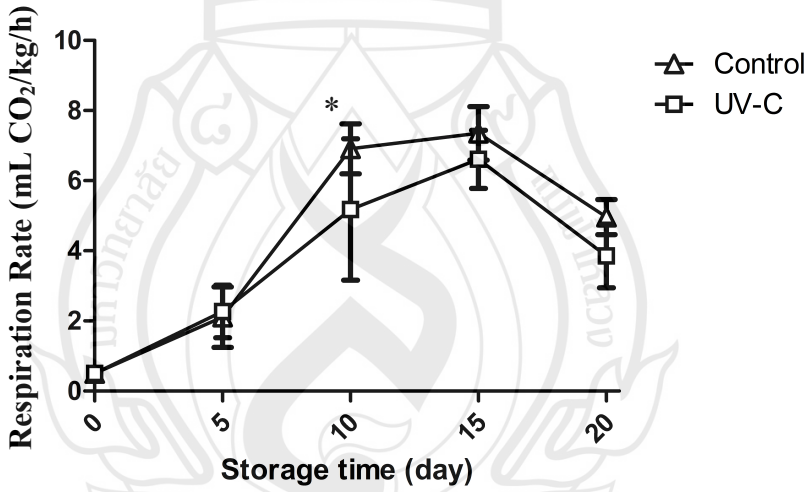


Figure 4.3 Effects of UV-C irradiation on respiration rate of Nam Dok Mai Si Thong mango during storage at 14 ^{o}C. Vertical bars represent standard deviation (SD). (*) Asterisk represents significant difference between samples using t-test ($P < 0.05$)

The differences between treated and untreated fruits on respiration rate were, however, not statistically significant ($P > 0.05$). These patterns indicated that at the investigated dose, UV-C treatment did not significantly suppress respiration rate in

mango. In contrast, Maharaj et al. (1999) reported that UV-C treatments at 3.7 kJ/m^2 and 24.4 kJ/m^2 were able to shift tomato's respiration peak to the right and lowered its peak. Furthermore, Costa et al. (2006) also mentioned that at day 4 after UV-C irradiation (10 kJ/m^2) in broccoli, the respiration rate found significantly different compared to control, while at 0 day and 2 day after irradiation, there was no significant UV-C effect to respiration rate.

4.4 Effect of UV-C on Total Soluble Solid (TSS), Titratable Acidity (TA), and Firmness in Mango

The firmness of the mangoes (Fig. 4.4A) during storage showed no significant overall difference between control and irradiated samples despite a significant difference ($P < 0.05$) in the day 10, when control fruit was firmer than UV-C treated fruit. In pear (Syamaladevi et al., 2014) and sweet cherries (Marquenie et al., 2002) also showed no significant firmness changes due to irradiation.

Khademi, Zamani, Poor-Ahmadi, & Kalantari (2013) suggested that UV-C treatment must be integrated with other effective postharvest treatments for maintaining firmness in persimmon fruit. On the other hand, several reports of UV-C treatment showed positive effect for maintaining fruit firmness in tomato (Bu et al., 2013) and strawberry (Pombo et al., 2009). However, Marquenie et al. (2002) reported that although UV-C can retard softening in strawberry, the same doses also contributed negative effect on calyx color, causing drying and staining of the leaves.

The changes of TSS and TA in Nam Dok Mai mangoes after UV-C irradiation are shown in Fig.4.4 B and C, respectively. It showed that there were no significant differences in total soluble solid and titratable acidity changes during storage between the control and irradiated fruits. This is in agreement with results reported by Syamaladevi et al. (2014) for pears and Perkins-Veazie, Collins, & Howard (2008) for blueberry. A decrease in acidity during storage coincide with the reports by Jacobi, MacRae, & Hetherington (2000) and Tovar, García, & Mata (2001) that fruit acids are used as substrates for respiration during storage.

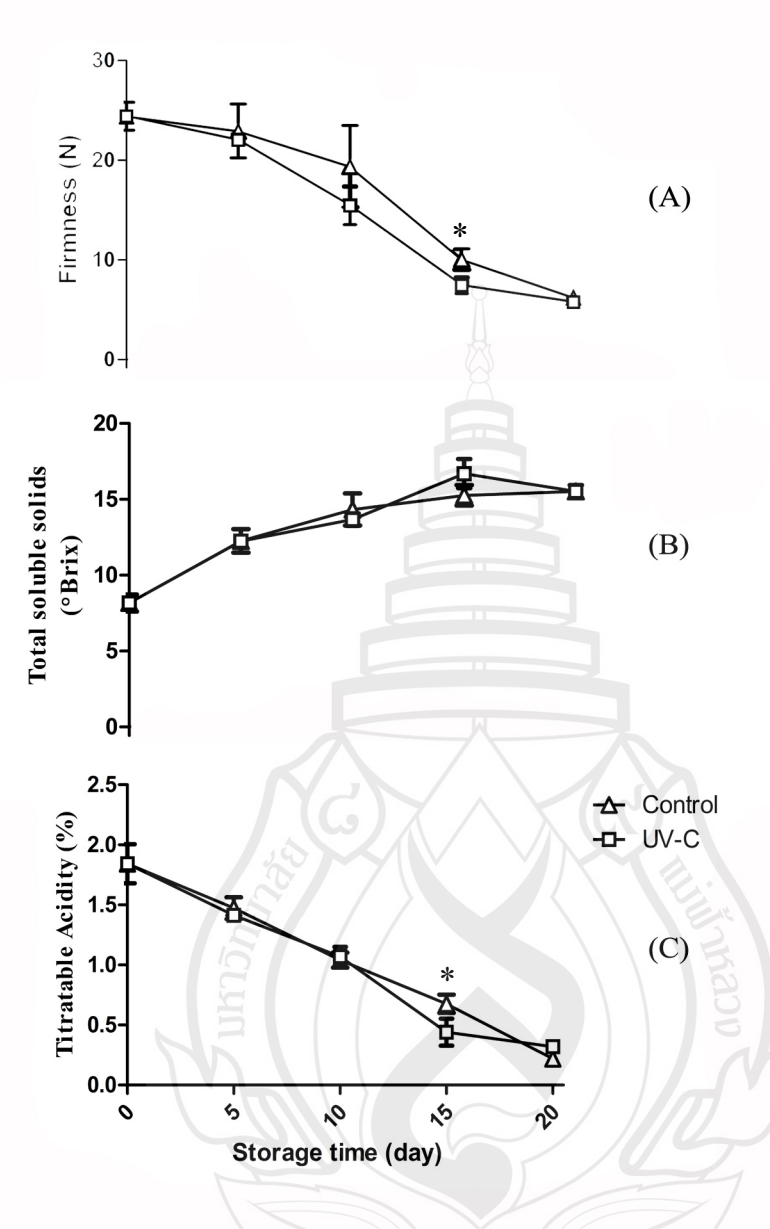


Figure 4.4 Effects of UV-C irradiation on (A) firmness; (B) total soluble solids; (C) titratable acidity of Nam Dok Mai Si Thong mango during storage at 14 ^{o}C. Vertical bars represent standard deviation (SD). (*) Asterisk represents significant difference between samples using t-test ($P < 0.05$)

It has been established that maturity stage at the time of treatment significantly impacts the response of tomato to UV-C (Charles, Corcuff, Roussel, & Arul, 2003; Liu

et al., 1993). The data reported in tomato (Charles et al., 2016) indicated that breaker fruits were more responsive to UV-C, given the overall trend toward higher in organic acid contents and lower glucose and sucrose contents in those fruits. According to Charles et al. (2016), genotype and maturity stage as well as the history of the plant organs must be taken into account when implementing UV-C to improve the postharvest shelf life of horticultural crops.

4.5 Effect of UV-C on Antioxidant Properties in Mango

Changes in antioxidant capacity of mango treated with UV-C irradiation were presented in Figure 4.5. By day 5 both treatments showed consistent decline in all 3 parameters then, the antioxidant capacity values of both samples had increase significantly ($P < 0.05$) by day 10. The authors are unable to explain regarding the occurring phenomenon at day 5 and day 10. However, overall, these 3 parameters showed consistent trend on the difference between irradiated and control fruits.

The gap emphasized UV-C, at the investigated dose, caused lower in DPPH values compared to the control. The result differs from González-Aguilar et al. (2007a), who found that exposure to UV-C for 10 minutes (dose not reported) contributed to the high DPPH value (as % radical scavenging activity) in fresh cut ‘Tommy Atkins’ mango.

Similar to DPPH, FRAP analysis in UV-C treated mango also show the same downward trend (Fig. 4.5B). Even though at day 10, UV-C seem to have higher FRAP value, but the effect was no longer. Along with ripening, UV-C treated sample had lower value significantly ($P < 0.05$) than control. In contrast, control sample also show the decreasing trend, but the value was higher than UV-C treated sample. Overall, UV-C treatment at the investigated doses was causing negative response of antioxidant capacity. This negative response was described as 5.7 mg AAE per 100 g of dry basis and 11.8 mg AAE per 100 g of dry basis for UV-C treated fruit and control fruit, respectively. It was double that what was in UV-C.

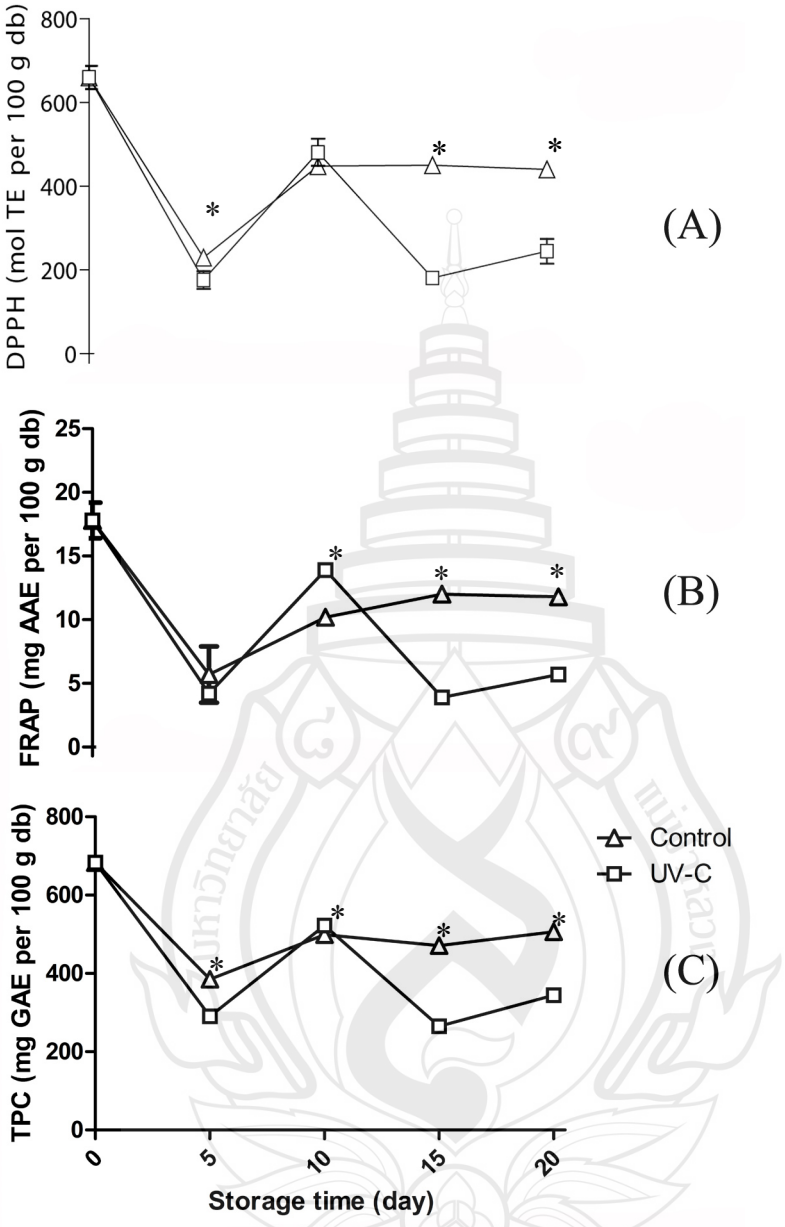


Figure 4.5 Effects of UV-C irradiation on (A) DPPH scavenging activity; (B) Ferric reducing power; (C) total phenolic concentrations of Nam Dok Mai Si Thong mango during storage at 14 ^{o}C. Vertical bars represent standard deviation (SD). (*) Asterisk represents significant difference between samples using t-test ($P < 0.05$)

However, a study on minimally processed citrus fruit found that UV-C treatment did not affect the antioxidant activity, as ascorbic acid was not greatly affected by UV-C (Shen et al., 2010). Previous study in mango shows that ascorbic acid was highly correlated with antioxidant capacity (Shivashankara, Isobe, Al-Haq, Tekanaka, & Shiina, 2004). On the other hand, Gonzales et al. (2007a) reported that application of UV-C had negative effect which causing declining in ascorbic acid content in fresh-cut mangoes during storage.

Whereas Liu et al. (2012), showed that UV-C irradiation significantly increased phenolic compounds in tomato, Fig. 4.6C shows UV-C treated fruit undergoing significant loss ($P < 0.05$) throughout the storage time. The result is also in contrast with González-Aguilar et al. (2007b), who found that UV-C treatment (4.93 kJ/m^2) in 'Haden' mango increased total phenols during storage at 25 ^{o}C for 18 days. In addition, Perkins-Veazie et al. (2008) reported that, effective UV-C dosage depends on crop types, and doses that are too high may cause deleterious effects on fruit quality.

4.6 Germicidal Effect of UV-C: *In Vitro* and *In Vivo* Studies

The control of postharvest diseases by UV treatments may contain two phenomena: a direct germicidal effect on the pathogen and indirect hormetic reactions that enhance the host disease resistance (Rodov, Tietel, Vinokur, Horev, & Eshel, 2010). In our study, the changes in mycelium diameter after UV-C irradiation were depicted in Figure 4.6. Both of UV-C treatments delayed the mycelium growth significantly ($P < 0.05$) compared to that in control samples. No fungal growth was noticeable for any UV-C treatments after two days of storage, regardless of irradiation dose. However, no UV-C treatment resulted in complete inhibition of fungal growth.

In addition, the effects of UV-C irradiation on the pathogen infection in tangerine fruits were also investigated by observing the germicidal effects of UV-C on the *Penicillium* spp. that was inoculated to tangerine. Symptoms of fungal growth first appeared on the first day with soft and watery around the inoculated spot.

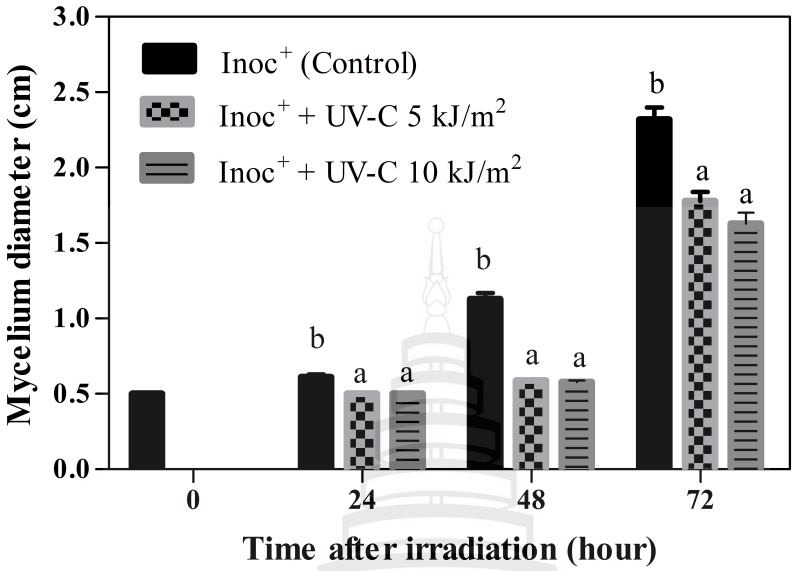


Figure 4.6 Effect of UV-C treatments on *in vitro* development of *Penicillium spp.* Vertical bars represent standard error (SE). Different letters represent significant differences between treatments separated using Tukey ($P < 0.05$)

However, both of UV-C treatments were not able to eliminate the presence of fungi on the fruit surface. Besides treatment parameters, the sensitivity of target microorganism to UV-C exposure is affected by the cell size and structure (Palou, 2009), which in general, complex microorganisms are more resistant than simple microorganisms; molds are much more resistant to UV-C damage than yeast and bacteria (Civello et al., 2006). Rodov et al., (2010) also reported that exposure of Penicillium inoculated onion bulbs to UV light (1.2 - 3.6 kJ/m^2) reduced the growth of *Penicillium* spp., but did not completely prevent the infectious process. Thus, results of the *in vitro* and *in vivo* experiments involving the evaluation of the effect of UV-C irradiation on *Penicillium* spp. have shown that both investigated dosages, 5 and 10 kJ/ m^2, can delay the mycelium growth.

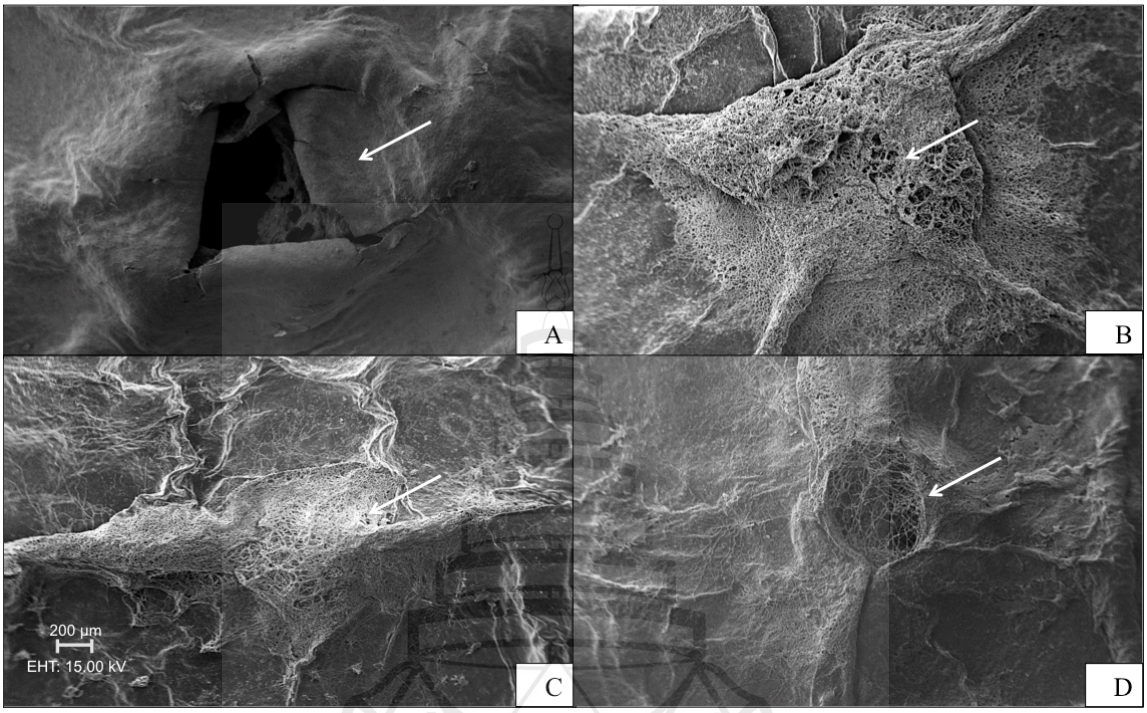


Figure 4.7 Effect of UV-C treatment on surface characterization of pathogen-inoculated fruit; (A) Control 0 h; (B), (C), and (D) Presence of hyphae of fungi at 48 h in control, UV-C 5 kJ/m^2, and 10 kJ/m^2, respectively. Arrows indicate inoculated spot

4.7 Effect of UV-C Application on Antioxidant Properties in Pathogen-inoculated Fruit

Changes in antioxidant capacity and total phenolic concentrations of pathogen-inoculated fruit with UV-C irradiation were presented in Figure 4.9 At first 24 h, DPPH value in UV-C treated fruit was significantly higher ($P < 0.05$) than that in control samples, but the antioxidant capacity values of both UV-C samples had slightly decreased at 48 h after irradiation.

Especially, the value of UV-C 10 kJ/m^2 samples decreased significantly compared to those in control and UV-C 5 kJ/m^2 samples. Similarly with DPPH, total

phenolic concentrations in UV-C treated sample at 48 h after irradiation were significantly lower ($P < 0.05$) than control. UV-C treatments may weaken the fungal through direct germicidal power, which caused the plant sensed a lesser stress resulting lower values of DPPH and total phenolic concentrations at 48 h.

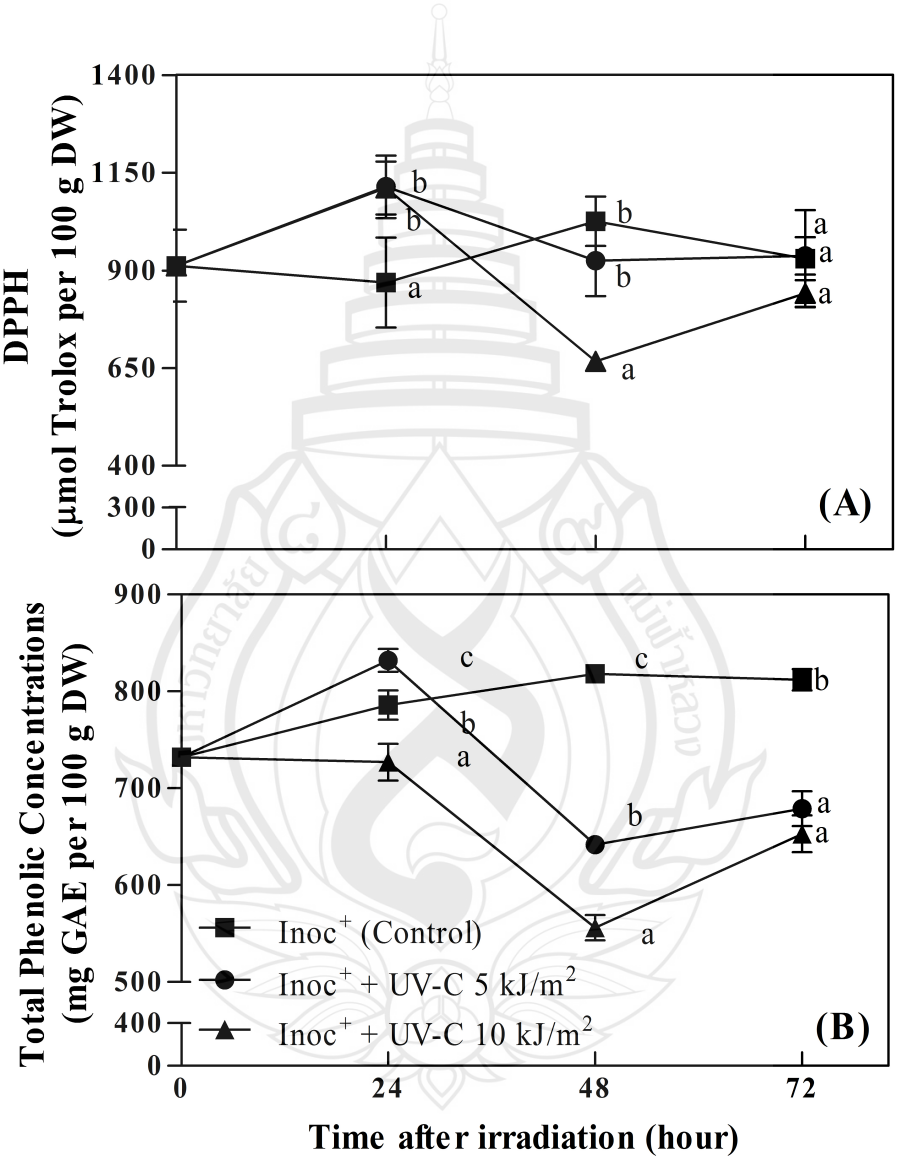


Figure 4.8 Effect of UV-C treatments on DPPH (A) and total phenolic concentrations (TPC; B) in pathogen-inoculated fruit. Vertical bars represent standard error (SE). Different letters represent significant differences between treatments separated using Tukey ($P < 0.05$)

Figure 4.9 illustrates the SOD enzyme activity decreased steadily and after 48 h it was slightly upward. In our study, SOD activity in UV-C treated fruit, especially 10 kJ/m^2 of UV-C treated fruit, had significantly higher value compared to that in control throughout observation period.

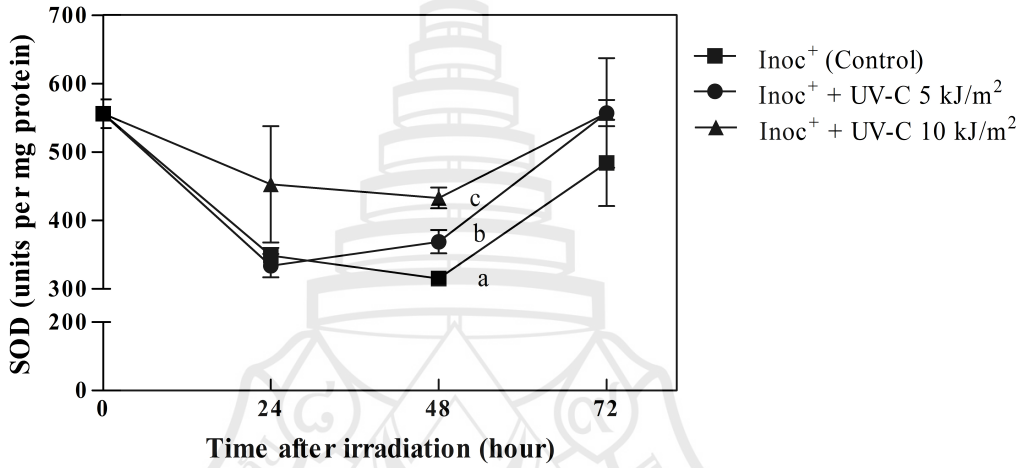


Figure 4.9 Effect of UV-C treatments on SOD enzyme activity in pathogen-inoculated fruit. Vertical bars represent standard error (SE). Different letters represent significant differences between treatments separated using Tukey ($P < 0.05$)

After pathogen infection, plant generates reactive oxygen species (ROS) that used in various aspects of the plant defense response (Wojtazsek, 1997). General defense reactions, like plant structure reinforcement (Métraux et al., 2007; Amil-Ruiz, Blanco-Portales, Muñoz-Blanco, & Caballero, 2011), involve accumulation of enzyme involved in antioxidant metabolism (Torres, Valentines, Usall, Viñas, & Larrigaudiere 2003; Oliviera et al., 2014) and production of phytoalexin (Ahuja, Kissen, & Bones, 2011). In the absence of any attack, these defense mechanisms may be induced by physical or chemical elicitation (Ruiz Garcia and Gomez-Plaza, 2013). The increased antioxidant accumulation has been found in fruit subjected to UV-C light, such as in

citrus (Patil, Vanamala, & Hallman, 2004), tomato (Liu et al., 2012), strawberry (Erkan et al., 2008), and mango (González-Aguilar et al., 2007b).

According to Labavitch (1997), sufficient irradiation of UV-C can emulate beneficial responses similar to those of fruit wounding; for example the endogenous JA was increased in apple seedlings subjected to UV-C (Kondo et al., 2011) and in wounded plants as reviewed by Howe (2004). In our study, the endogenous JA concentrations in all treatments were increased at first 24 h. Especially, 10 kJ/m^2 UV-C treated fruit increased JA concentrations significantly, which may be caused by strong stress depending to high level of UV-C. However, after 24 h, UV treated fruit had a significant decline ($P < 0.05$). In contrast, control sample showed a significantly higher value possibly indicating suffering inflicted by pathogen infection.

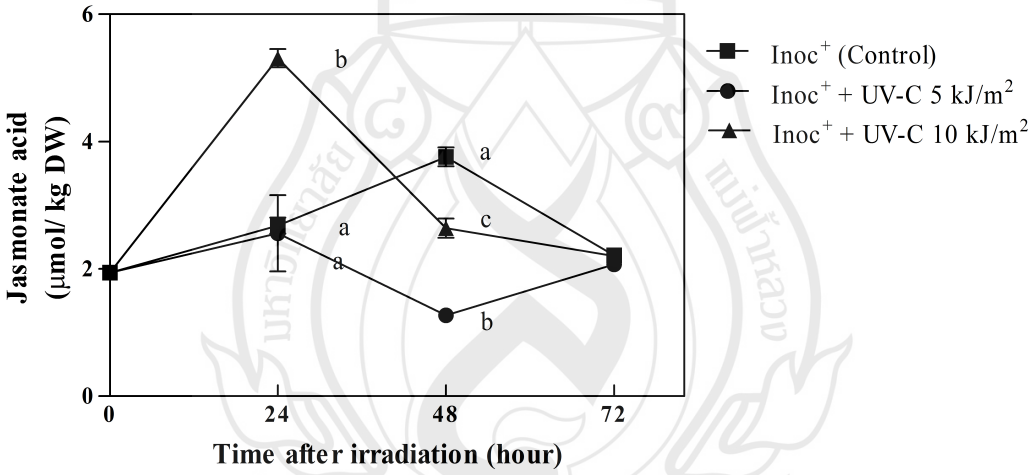


Figure 4.10 Effect of UV-C treatments on jasmonate content in pathogen-inoculated fruit. Vertical bars represent standard error (SE). Different letters represent significant differences between treatments separated using Tukey ($P < 0.05$)

As shown in Figure 4.10, the increase of jasmonate at first 24 h in our study may be related to the defense mechanism of citrus fruit in reacting to the stress induced by UV-C illumination. Joyce, Johnson, & Gosbee (1997) stated that response of stress on host-pathogen interaction may be considered as a composite of processes, that can

include stress perception, degree of injury, and capacity of defense. In our study, UV-C could partially eliminate the presence of fungal pathogen compared to that in control. Thus, after 24 h, the plant may not produce more signals to produce JA as response to pathogen attack due to partial elimination by the UV-C.

4.8 Effect of UV-C on Quality Attributes in Tangerine

Figure 4.8 shows the changes in peel color of tangerine fruit after irradiation in low temperature. The lightness (L*) of all treatments decreased during storage. The same trend for yellowness (b*) and surface color saturation (chroma) also decreased as storage time progressed, but this change being slightly rapid by exposure to UV-C. However, no significant difference was observed between control and UV-C irradiated fruit ($P < 0.05$). Another study using gamma irradiation by Ahmed, Knapp, & Dennison (1966) stated that the color of Navel oranges were generally lighter than that in control fruit after irradiated and stored for 30 days. Canale et al. (2011) observed 'Valencia' orange both in control and treated with low UV-C doses (0 - 7.28 kJ/m^2) had a good yellow appearance whereas orange treated with higher UV-C doses (15.66 and 31.2 kJ/m^2) became darker and senescent.

Both dosages of UV-C treatment (5 kJ/m^2 and 10 kJ/m^2) did not produce any harmful such as browning or blackened lenticel in tangerine fruit during storage period at low temperature. These results were in contrast with a report by Kinay et al. (2005) stated that UV-C treatment (3.38 kJ/ m^2) caused injuries that appeared as burning and browning on the fruit surface of Satsuma mandarins. In Star Ruby grapefruit, D'hallewin et al. (2000) found that UV-C treatment damage appeared as rind browning and tissue necrosis. In addition, upon its application at 1.5 kJ/m^2 damage to fruit ranged from 3.2 to 4.2% while the percentage of damaged fruit after irradiation at 3.0 kJ/m^2 was high about 13.7% (D'hallewin et al., 2000).

A case in gamma irradiation which producing exocarp browning in 'Valencia' oranges after the treatment, Belli-Donini, Baraldi, & Taggi (1974) stated the damage is caused by the irradiation that facilitate the diffusion of terpene compounds in exocarp

cells. These evidences proofed that each species producing different response upon UV-C application.

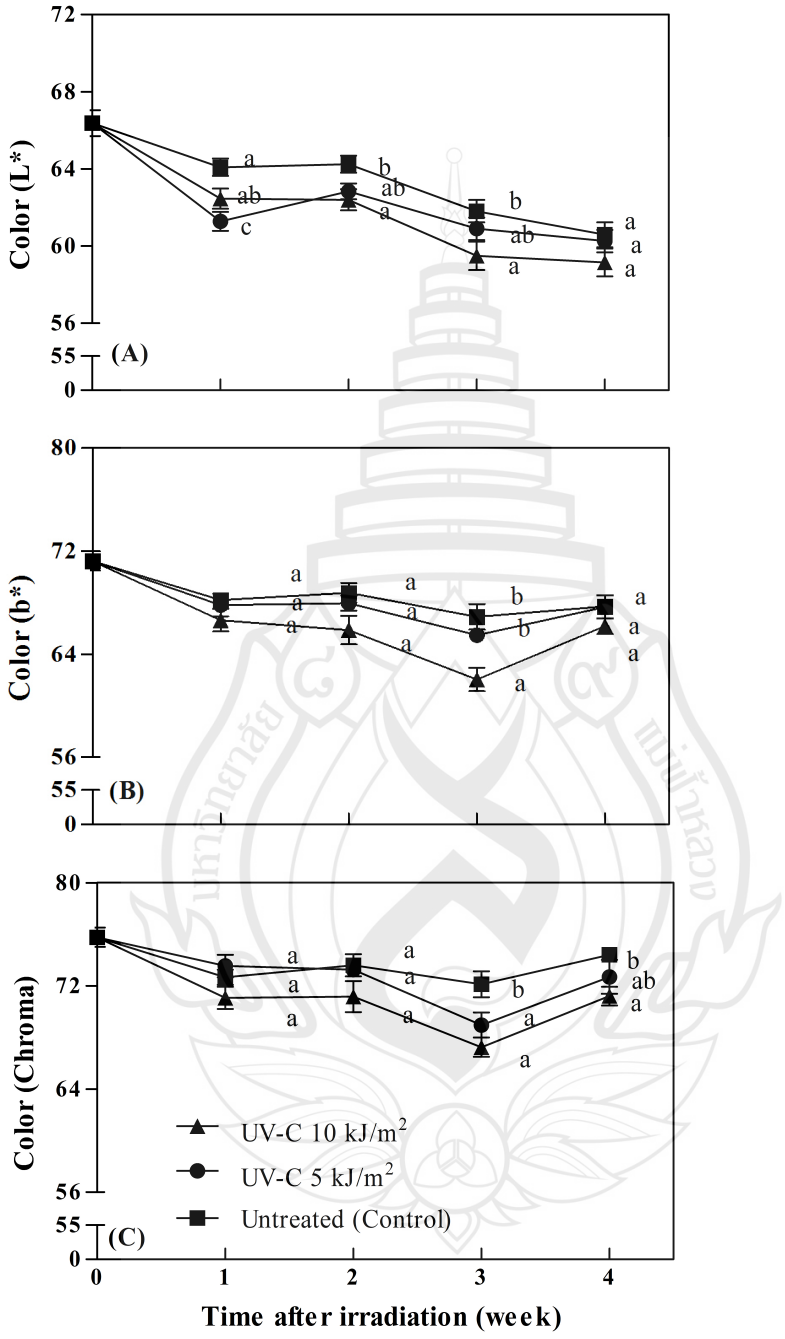


Figure 4.11 Effect of UV-C treatments on the color index during storage at low temperature; (A) L*=lightness; (B) b*=yellowness; (C) Chroma. Vertical bars represent standard error (SE). Different letters represent significant differences between treatments separated using Tukey ($P < 0.05$).

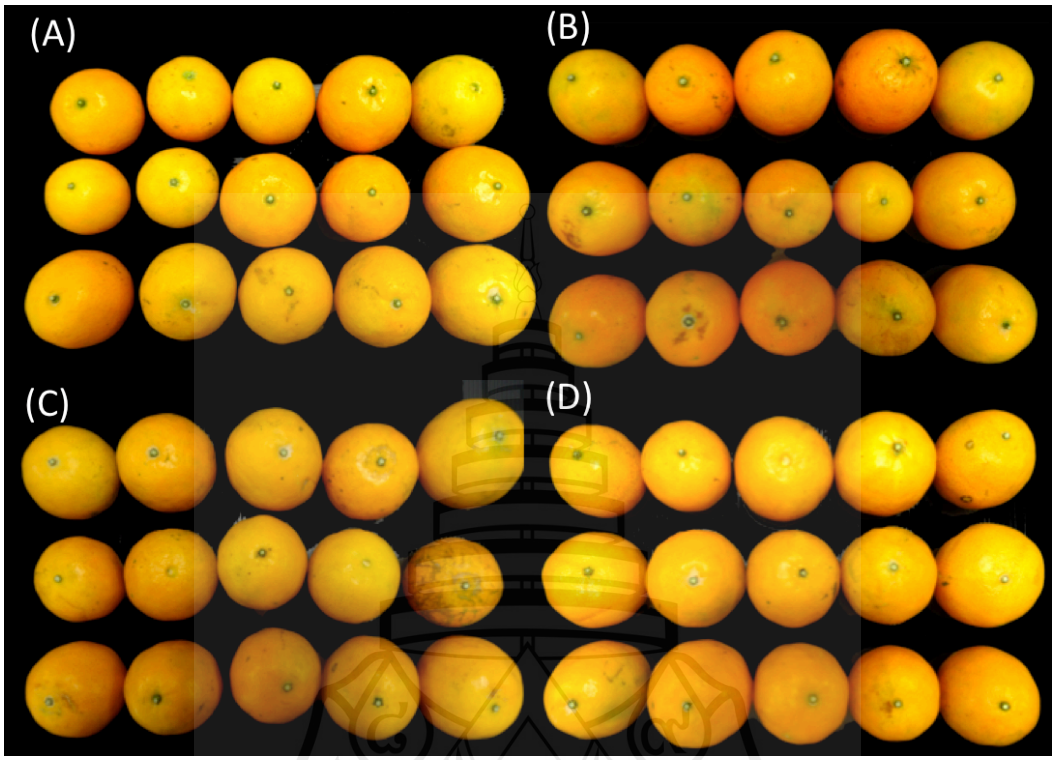


Figure 4.12 Effect of UV-C treatments on the surface color during storage at low temperature; (A) Control week 0; (B), (C), and (D) surface color at week 4 in control, UV-C 5 kJ/ m^2, and UV-C 10 kJ/ m^2, respectively.

4.9 Effect UV-C on Antioxidant Properties and Endogenous Jasmonate in Tangerine

The changes in total phenolic content of control and UV-C-treated tangerine fruit are shown in Figure 4.13. Both control and UV-C-treated tangerine fruit showed increasing trend in total phenolic content during first week of observation period. In untreated sample, the total phenolic content slightly decreased in the following week, and remained stable until the end of storage. Both of UV-C treated samples showed a significant increase, specifically 10 kJ/m^2 of UV-C treated sample during the whole

storage, this increase was significantly higher ($P < 0.05$) in UV-C-treated fruit as compared that in control fruit.

Among them, 10 kJ/m^2 UV-C-treated tangerine fruit had the highest total phenolic content, followed by 5 kJ/m^2 UV-C treatment. In our study, the trend of response caused by UV-C were similar with those in tomato (Jagadeesh et al., 2011), mango (González-Aguilar et al., 2007a); Erkan et al. (2008) in strawberry fruit. In contrast, previous studies have shown that total phenols in broccoli were significantly lower than the control after UV-C irradiation and storage, but this result could be due to a less advanced developmental stage (Costa et al., 2006).

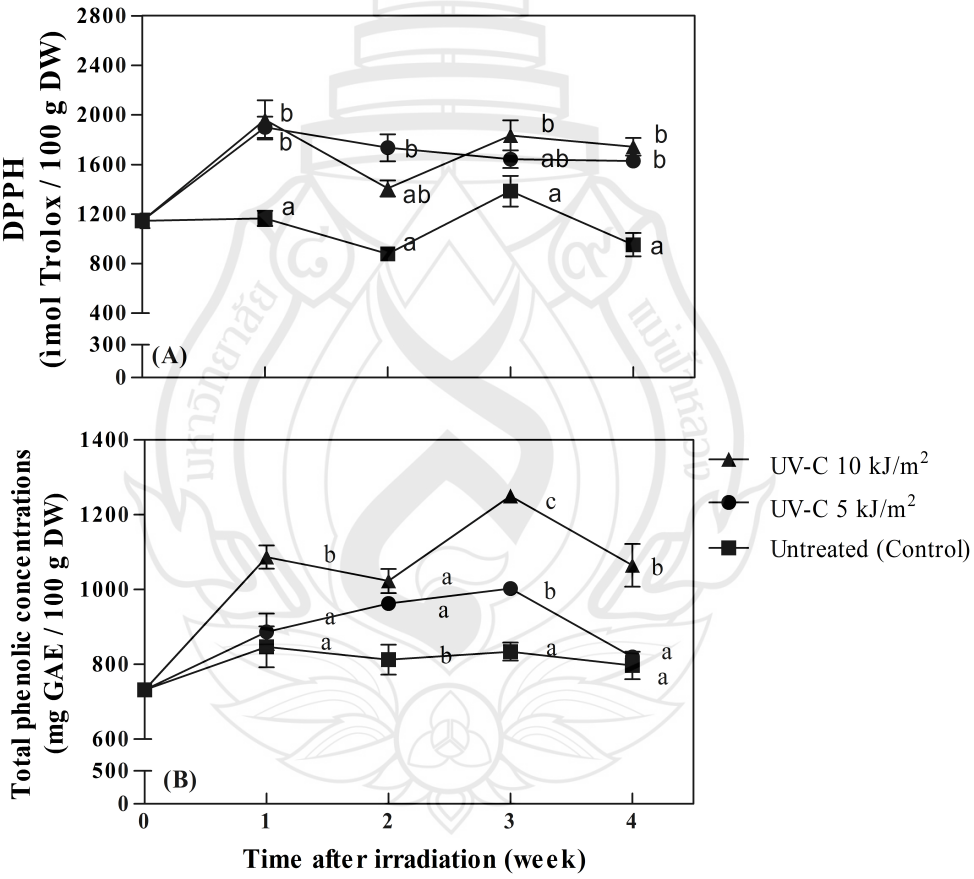


Figure 4.13 Effect of UV-C treatments on DPPH (A) and total phenolic concentrations (TPC; B) in tangerine. Vertical bars represent standard error (SE). Different letters represent significant differences between treatments separated using Tukey ($P < 0.05$).

Changes in SOD enzyme activity after UV-C treatment are shown in Figure 4.14. There was no significant effect until week 1 and after that, SOD activity in UV-C treated samples increased gradually, especially week 3 in which activity was significantly higher in UV-C treated samples compared to that in control. However, there was no significant difference between treatments at the end of measured period.

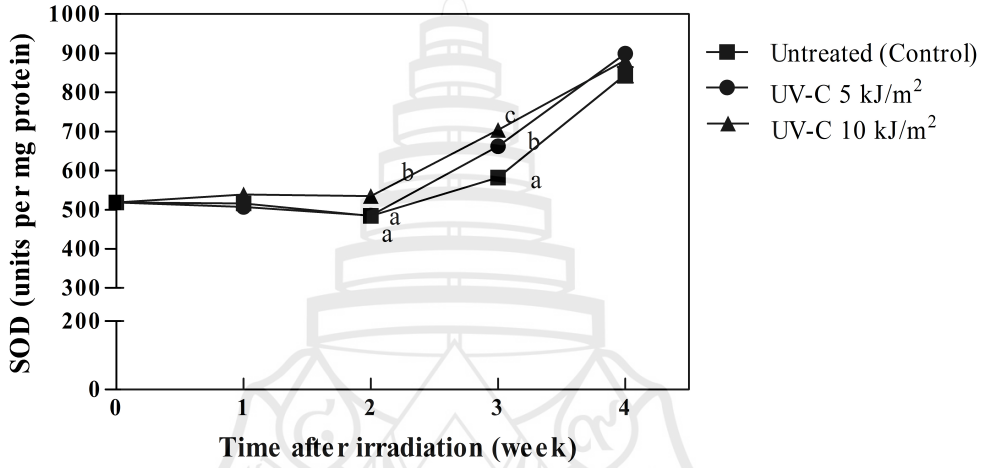


Figure 4.15 Effect of UV-C treatments on superoxide enzyme activity in tangerine peel. Vertical bars represent standard error (SE). Different letters represent significant differences between treatments separated using Tukey ($P < 0.05$).

The effect of UV-C on SOD enzyme activity may vary depending on fruit species and applied dose (Maharaj, 2015), thus, the general trend remains unsure. For example, there were no significant differences of SOD activity in *Turmera diffusa* Willd (*Damiana* medicinal plant) after subjected to 1.14 – 4.56 kJ/m^2 of UV-C treatments (Soriano-Melgar et al., 2014). Previous study in UV-C treated strawberry showed SOD level increased but then declined during storage (Erkan et al., 2008)

Jasmonate content from different UV-C illumination dosages is shown in Figure 4.15. Jasmonate content from tangerine fruit for all UV-C illuminations and control treatment increased during 1-week storage period. However, the increase was relatively

lower in control fruit when compared to illuminated fruit. Tangerine fruit illuminated with 10 kJ/ m^2 UV-C had the highest jasmonate content followed by 5 kJ/ m^2.

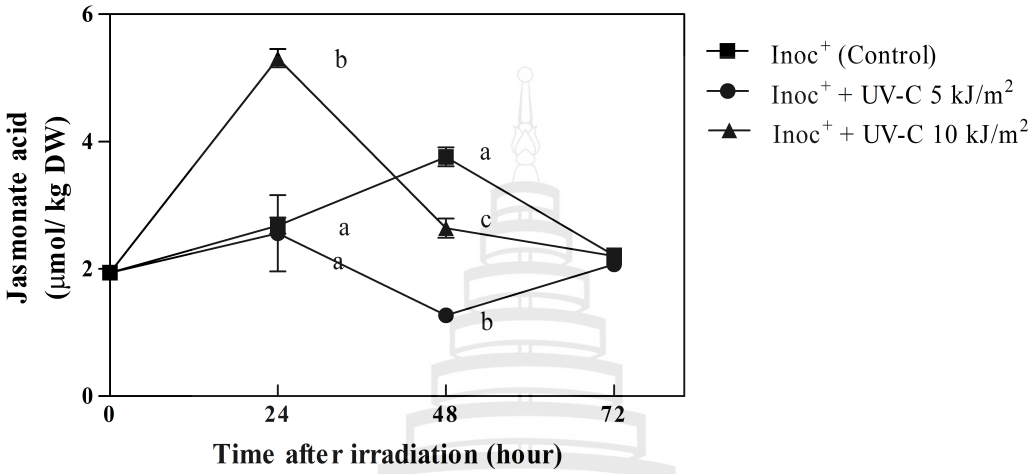


Figure 4.15 Effect of UV-C treatments on jasmonate concentrations in tangerine peel. Vertical bars represent standard error (SE). Different letters represent significant differences between treatments separated using Tukey ($P < 0.05$).

CHAPTER 5

CONCLUSION

In the experiment I, fruit color and antioxidant capacity changes were the most marked effects of the 4.93 kJ/m2 UV-C treatment. UV-C irradiation, at the investigated dose, was not able to retain antioxidant compounds in Nam Dok Mai Si Thong and caused harmful effects to the visual appearances, but not contributes to the internal color quality. The changes in respiration rate, texture, TSS, and TA values were not much affected by the irradiation.

In the experiment II, 5 and 10 kJ/m^2 of UV-C treatment had fungicidal effect on *Penicillium* spp. *in vitro* and *in vivo*, which the occurrence is suggested to be due to accumulation of bioactive compounds and jasmonate at first 24 hours after UV-C irradiation. Both of UV-C dosages producing adequate degree of protection, specifically 10 kJ/m^2 of UV-C, which provides better result against *Penicillium* spp. compared to that in 5 kJ/m^2. However, in terms of energy efficiency, 5 kJ/m^2 of UV-C would be better to be applied since it also generates as similar as responses of the fruit to 10 kJ/m^2 of UV-C irradiation. In case of disease control application, UV-C treatments have a potential to effectively reduce mold infection on the surface of tangerine fruits and may be applied as one of the steps in postharvest handling systems.

In the experiment III, in the absence of pathogen attack, both of 5 and 10 kJ/m^2 UV-C treatments were able to emulate the same response as those in fruit wounding; such as increase in bioactive compound accumulation and endogenous jasmonate concentrations. Both of investigated doses did not produce any harmful on tangerine skin surface, make it possible to be commercially applied as postharvest treatment for tangerine.

CHAPTER 6

SUGGESTION

1. The appropriate dose and mechanism of UV-C on quality attributes and antioxidant properties need to be further study in mango and tangerine.

2. The mechanism of UV-C on defense mechanism against pathogen in tangerine needs to be further study in tangerine.

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APPENDICES

APPENDIX A

STANDARD CURVE

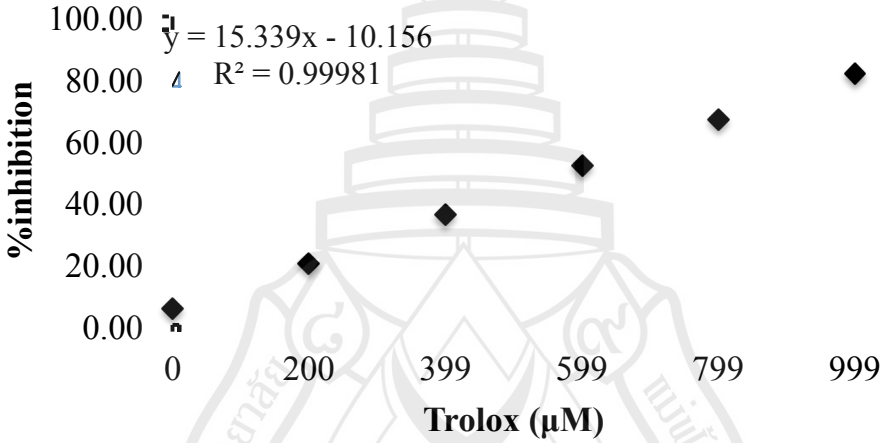


Figure A1 Standard curve of DPPH in mango experiment

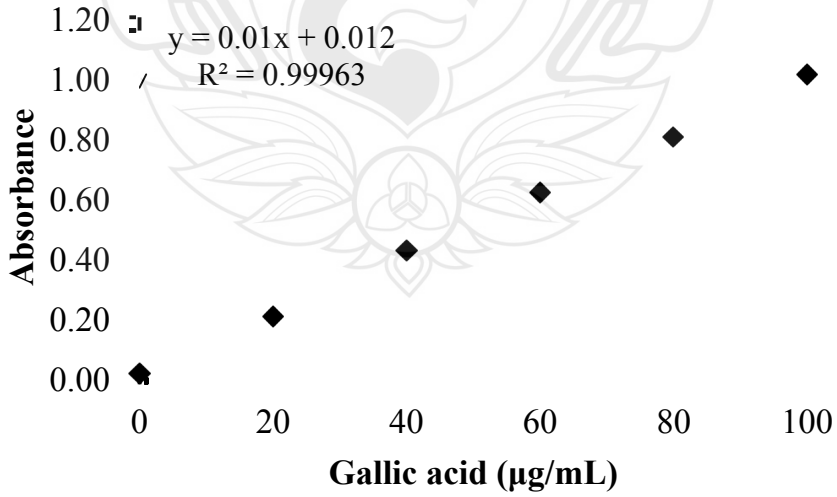


Figure A2 Standard curve of total polyphenol content in mango experiment

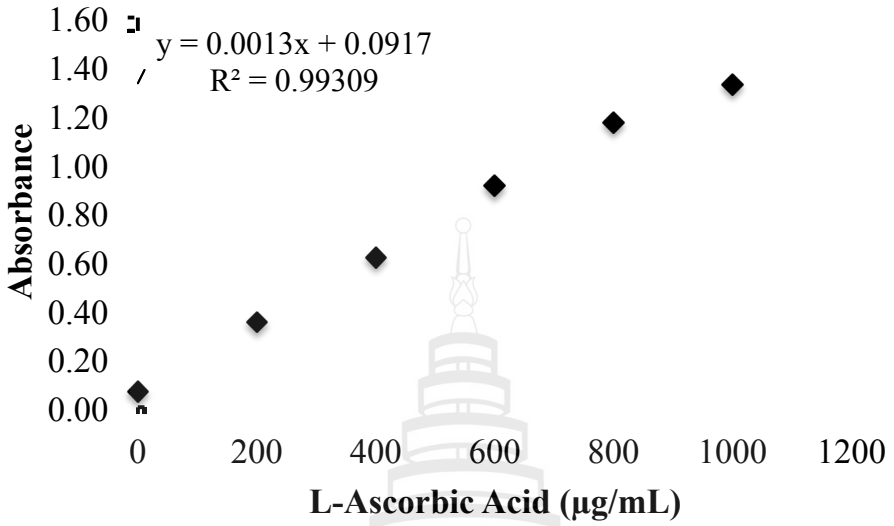


Figure A3 Standard curve of FRAP in mango experiment

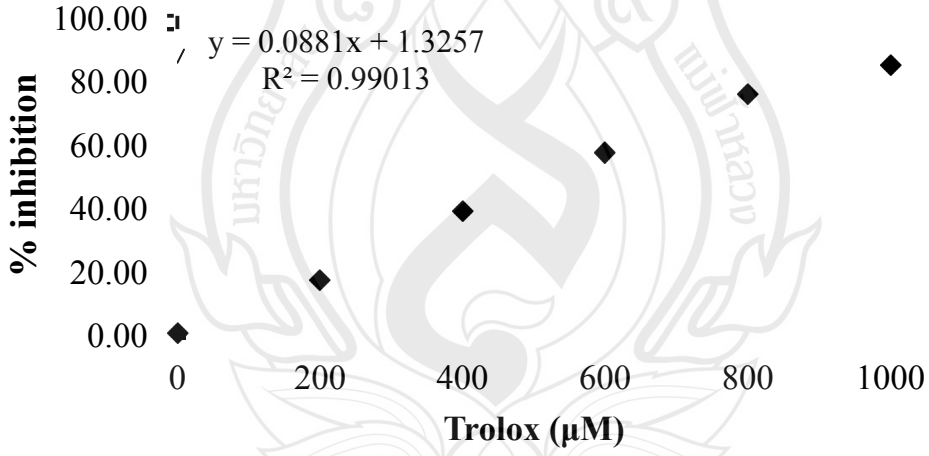


Figure A4 Standard curve of DPPH in citrus *in vivo* experiment

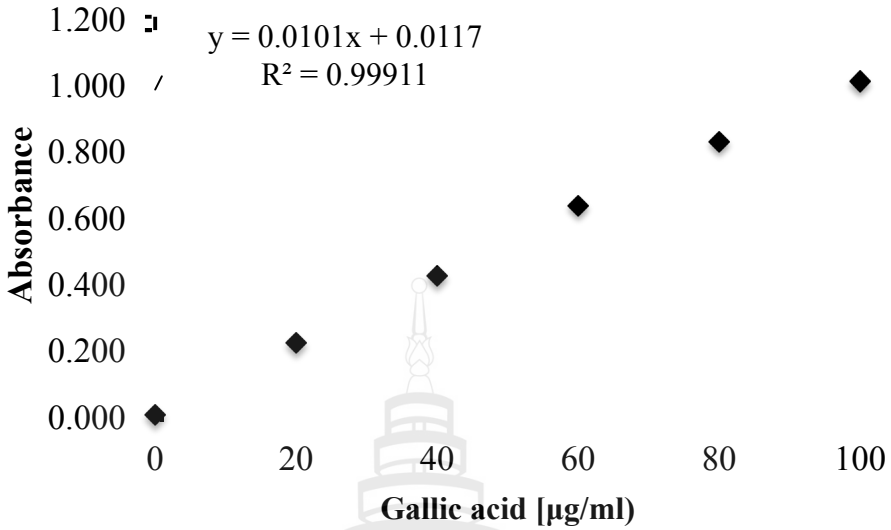


Figure A5 Standard curve of total polyphenol concentrations in citrus *in vivo* experiment

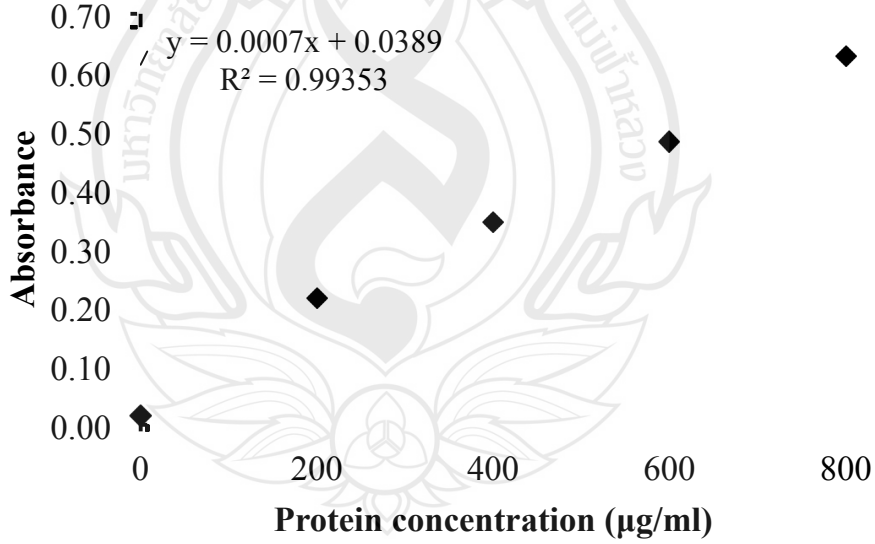


Figure A6 Standard curve of protein

Table A1 Peak Area Ratio for Determining Jasmonate Acid Standard Curve

Replication	Peak Area	
	Jasmonate acid (JA)	Ibuprofen (IBP)*
1	120,863	4,823
2	214,253	3,488
3	317,670	2,329
4	181,827	1,502
5	135,908	3,846
6	141,688	2,140
Average	185,368	3,021
Area ratio (JA/IBP) 61.35		

Note (*) as internal standard

Content ratio:

1. 100 µL of JA 10^{-4} M contained 2.10 x 10^{-6} g of JA (2.10 µg)
2. 100 µL of IBP 10^{-4} M contained 2.06 x 10^{-6} g of IPB (2.06 µg)

Ratio (JA/IBP): 1.02

The equation model: y = ax; y is peak area ratio (61.35); x content area ratio (1.02); Then, a = 60.18

Standard equation: $\underline{y = 60.18x}$

APPENDIX B

STATISTICAL ANALYSIS

Table B1 Effects of UV-C Irradiation on Respiration Rate of Nam Dok Mai Si Thong Mango During Storage at 14 ^{o}C

Storage Period (Day)	Control (mL CO_2/kg/h)	UV-C (mL CO_2/kg/h)
0	0.507 ± 0.104^{a}	0.507 ± 0.104^{a}
5	2.109 ± 0.859^{b}	2.269 ± 0.749^{b}
10	6.909 ± 0.711^{d}	5.182 ± 2.017^{cd}
15	7.350 ± 0.759^{d}	6.609 ± 0.829^{d}
20	4.961 ± 0.499^{c}	3.854 ± 0.911^{bc}

Note. The values are expressed as mean ± SD

Different letters represent significant differences within treatment separated using Duncan, $P < 0.05$. (*) Asterisk represents significant differences between treatments using t-test, $P < 0.05$

Table B2 Effects of UV-C Irradiation on Firmness Quality of Nam Dok Mai Si Thong Mango During Storage at 14 ^{o}C

Storage Period (Day)	Control (N)	UV-C (N)
0	24.413 ± 1.391^{c}	24.413 ± 1.391^{d}
5	22.933 ± 2.707^{bc}	22.009 ± 0.544^{c}

Table B2 (continued)

Storage Period (Day)	Control (N)	UV-C (N)
10	19.392 ± 4.105^{b}	15.448 ± 1.911^{b}
15	10.058 ± 1.042^{a}*	7.428 ± 0.814^{a}*
20	6.234 ± 0.610^{a}	5.759 ± 0.535^{a}

Note. The values are expressed as mean ± SD

Different letters represent significant differences within treatment separated using Duncan, $P < 0.05$. (*) Asterisk represents significant differences between treatments using t-test, $P < 0.05$

Table B3 Effects of UV-C Irradiation on Titratable Acidity of Nam Dok Mai Si Thong Mango During Storage at 14 ^{o}C

Storage Period (Day)	Control (as % citric acid)	UV-C (as % citric acid)
0	1.84 ± 0.16^{a}	1.84 ± 0.16^{d}
5	1.47 ± 0.09^{b}	1.41 ± 0.03^{c}
10	1.04 ± 0.06^{c}	1.07 ± 0.08^{b}
15	0.67 ± 0.08^{d}*	0.44 ± 0.11^{a}*
20	0.22 ± 0.06^{e}	0.38 ± 0.04^{a}

Note. The values are expressed as mean ± SD

Different letters represent significant differences within treatment separated using Duncan, $P < 0.05$. (*) Asterisk represents significant differences between treatments using t-test, $P < 0.05$

Table B4 Effects of UV-C Irradiation on Total Soluble Solid of Nam Dok Mai Si Thong Mango During Storage at 14 ^{o}C

Storage Period (Day)	Control (^{o}Brix)	UV-C (^{o}Brix)
0	8.2 ± 0.6^{a}	8.2 ± 0.6^{a}
5	12.3 ± 0.8^{b}	12.2 ± 0.4^{b}
10	14.3 ± 1.1^{c}	13.7 ± 0.4^{c}
15	15.3 ± 0.7^{c}	16.7 ± 0.9^{d}
20	15.5 ± 0.4^{c}	15.5 ± 0.8^{d}

Note. The values are expressed as mean ± SD

Different letters represent significant differences within treatment separated using Duncan, $P < 0.05$. (*) Asterisk represents significant differences between treatments using t-test, $P < 0.05$

Table B5 Effects of UV-C Irradiation on DPPH of Nam Dok Mai Si Thong Mango During Storage at 14 ^{o}C

Storage Period (Day)	Control (µmol Trolox/100 g DW)	UV-C (µmol Trolox/100 g DW)
0	676 ± 28^{e}	676 ± 28^{c}

Table B5 (continued)

Storage Period (Day)	Control (µmol Trolox/100 g DW)	UV-C (µmol Trolox/100 g DW)
5	$235 \pm 9^{a*}$	$194 \pm 9^{a*}$
10	437 ± 16^{c}	509 ± 33^{b}
15	$460 \pm 11^{d*}$	$172 \pm 14^{a*}$
20	$448 \pm 11^{b*}$	$220 \pm 30^{a*}$

Note. The values are expressed as mean ± SD

Different letters represent significant differences within treatment separated using Duncan, $P < 0.05$. (*) Asterisk represents significant differences between treatments using t-test, $P < 0.05$

Table B6 Effects of UV-C Irradiation on Total Phenolic Concentrations of Nam Dok Mai Si Thong Mango During Storage at 14 ^{o}C

Storage Period (Day)	Control (mg gallic acid/ 100g DW)	UV-C (mg gallic acid/ 100g DW)
0	683 ± 6^{e}	683 ± 6^{d}
5	$385 \pm 3^{d*}$	$285 \pm 8^{a*}$
10	$499 \pm 8^{c*}$	$521 \pm 5^{c*}$
15	$471 \pm 10^{b*}$	$265 \pm 2^{a*}$
20	$505 \pm 12^{a*}$	$346 \pm 5^{b*}$

Note. The values are expressed as mean ± SD

Different letters represent significant differences within treatment separated using Duncan, $P < 0.05$. (*) Asterisk represents significant differences between treatments using t-test, $P < 0.05$

Table B7 Effects of UV-C Irradiation on FRAP of Nam Dok Mai Si Thong Mango During Storage at 14 ^{o}C

Storage Period (Day)	Control	UV-C
0	0.178 ± 0.014^{d}	0.178 ± 0.014^{e}
5	0.057 ± 0.022^{a}	0.087 ± 0.034^{a}
10	$0.102 \pm 0.002^{b*}$	$0.139 \pm 0.002^{d*}$
15	$0.120 \pm 0.004^{c*}$	$0.039 \pm 0.001^{a*}$
20	$0.118 \pm 0.005^{c*}$	$0.058 \pm 0.002^{b*}$

Note. The values are expressed as mean ± SD

Different letters represent significant differences within treatment separated using Duncan, $P < 0.05$. (*) Asterisk represents significant differences between treatments using t-test, $P < 0.05$

Table B8 Effects of UV-C Irradiation on Color (Lightness) of Nam Dok Mai Si Thong Mango During Storage at 14 ^{o}C

Storage Period (Day)	Control (L*)	UV-C (L*)
0	76.88 ± 2.49^{b}	76.88 ± 2.49^{d}
5	$75.73 \pm 3.72^{b*}$	$64.29 \pm 2.78^{c*}$

Table B8 (continued)

Storage Period (Day)	Control (L*)	UV-C (L*)
10	$74.98 \pm 5.30^{ab*}$	$57.38 \pm 3.99^{b*}$
15	$74.96 \pm 1.69^{ab*}$	$53.20 \pm 2.34^{b*}$
20	$72.04 \pm 1.75^{a*}$	$48.57 \pm 3.87^{a*}$

Note. The values are expressed as mean ± SD

Different letters represent significant differences within treatment separated using Duncan, $P < 0.05$. (*) Asterisk represents significant differences between treatments using t-test, $P < 0.05$

Table B9 Effects of UV-C Irradiation on Redness Color (a*) of Nam Dok Mai Si Thong Mango During Storage at 14 ^{o}C

Storage Period (Day)	Control (a*)	UV-C (a*)
0	4.78 ± 0.97^{a}	4.78 ± 0.97^{a}
5	$6.23 \pm 1.86^{bc*}$	$13.70 \pm 1.47^{b*}$
10	$5.52 \pm 1.06^{ab*}$	$15.90 \pm 1.37^{c*}$
15	$6.88 \pm 1.43^{c*}$	$17.08 \pm 1.13^{cd*}$
20	$8.73 \pm 1.11^{d*}$	$18.11 \pm 1.06^{d*}$

Note. The values are expressed as mean ± SD

Different letters represent significant differences within treatment separated using Duncan, $P < 0.05$. (*) Asterisk represents significant differences between treatments using t-test, $P < 0.05$

Table B10 Effects of UV-C Irradiation on Hue Angle of Nam Dok Mai Si Thong Mango During Storage at 14 ^{o}C

Storage Period (Day)	Control (^{o}hue)	UV-C (^{o}hue)
0	81.63 ± 1.58^{c}	81.63 ± 1.58^{c}
5	$79.84 \pm 1.98^{b*}$	$71.79 \pm 2.16^{b*}$
10	$80.74 \pm 1.33^{c*}$	$66.80 \pm 3.75^{a*}$
15	$80.21 \pm 1.32^{bc*}$	64.97 ± 2.39^{a}
20	$78.25 \pm 1.06^{a*}$	65.62 ± 3.67^{a}

Note. The values are expressed as mean ± SD

Different letters represent significant differences within treatment separated using Duncan, $P < 0.05$. (*) Asterisk represents significant differences between treatments using t-test, $P < 0.05$

Table B11 Effects of UV-C Irradiation on Mycelium Diameter of *Penicillium digitatum* in Tangerine

Time After Irradiation (Hour)	Mycelium diameter (cm)		
	$Inoc^{+}$(Control)	$Inoc^{+}$+ UV-C 5 kJ/m^2	$Inoc^{+}$ + UV-C 10 kJ/m^2
0	0.50 ± 0.00^{aA}	0.50 ± 0.00^{aA}	0.50 ± 0.00^{aA}
24	0.61 ± 0.02^{bB}	0.50 ± 0.00^{aA}	0.50 ± 0.00^{aA}
48	1.13 ± 0.04^{cB}	0.59 ± 0.00^{bA}	0.58 ± 0.01^{bA}
72	2.32 ± 0.08^{dB}	1.78 ± 0.06^{cA}	1.63 ± 0.07^{cA}

Note. The values are expressed as mean ± SE

Statistical differences between treatments are indicated by capital font, while differences within treatment are indicated by small font, each separated using Tukey ($P < 0.05$)

Table B12 Effects of UV-C Irradiation on DPPH in Pathogen-inoculated Tangerine

Time After Irradiation (Hour)	DPPH (µmol trolox equivalent/ kg DW)		
	$Inoc^+$(Control)	$Inoc^+$+ UV-C 5 kJ/m^2	$Inoc^+$ + UV-C 10 kJ/m^2
0	912 ± 92^{aA}	912 ± 92^{aA}	912 ± 92^{bA}
24	869 ± 115^{aA}	$1{,}114 \pm 80^{bB}$	$1{,}111 \pm 68^{cB}$
48	$1{,}026 \pm 63^{aB}$	925 ± 91^{aB}	667 ± 8^{aA}
72	930 ± 124^{aA}	937 ± 48^{aA}	841 ± 34^{bA}

Note. The values are expressed as mean ± SE

Statistical differences between treatments are indicated by capital font, while differences within treatment are indicated by small font, each separated using Tukey ($P < 0.05$)

Table B13 Effects of UV-C Irradiation on Total Phenolic Concentrations in Pathogen-inoculated Tangerine

Time After Irradiation (Hour)	Total Phenolic Compounds (µg Gallic acid/ kg DW)		
	$Inoc^+$(Control)	$Inoc^+$+ UV-C 5 kJ/m^2	$Inoc^+$ + UV-C 10 kJ/m^2
0	732 ± 3^{aA}	732 ± 3^{aC}	737 ± 3^{aC}
24	786 ± 15^{bB}	832 ± 12^{cD}	727 ± 19^{bC}

Table B13 (continued)

Time After Irradiation (Hour)	Total Phenolic Compounds (µg Gallic acid/ kg DW)		
	$Inoc^{+}$(Control)	$Inoc^{+}$+ UV-C 5 kJ/m^2	$Inoc^{+}$ + UV-C 10 kJ/m^2
48	818 ± 4^{cC}	642 ± 7^{bA}	556 ± 13^{aA}
72	812 ± 11^{bC}	679 ± 18^{aB}	653 ± 19^{aB}

Note. The values are expressed as mean ± SE

Statistical differences between treatments are indicated by capital font, while differences within treatment are indicated by small font, each separated using Tukey ($P < 0.05$)

Table B14 Effects of UV-C Irradiation on Jasmonate Concentrations in Pathogen-inoculated tangerine

Time After Irradiation (Hour)	Jasmonate Acid (µmol/ kg DW)		
	$Inoc^{+}$(Control)	$Inoc^{+}$+ UV-C 5 kJ/m^2	$Inoc^{+}$ + UV-C 10 kJ/m^2
0	1.91 ± 0.17^{aA}	1.91 ± 0.17^{aA}	1.91 ± 0.17^{aA}
24	2.71 ± 0.16^{bA}	2.70 ± 0.75^{aA}	5.26 ± 0.17^{cB}
48	3.74 ± 0.18^{cC}	1.28 ± 0.01^{aA}	2.71 ± 0.24^{bB}
72	2.14 ± 0.08^{abA}	2.14 ± 0.23^{aA}	2.23 ± 0.08^{abA}

Note. The values are expressed as mean ± SE

Statistical differences between treatments are indicated by capital font, while differences within treatment are indicated by small font, each separated using Tukey ($P < 0.05$)

Table B15 Effects of UV-C Irradiation on Superoxide Dismutase Enzyme Activity in Pathogen-inoculated Tangerine

Time After Irradiation (Hour)	SOD enzyme activity (unit/ mg protein)		
	$Inoc^+$(Control)	$Inoc^+$+ UV-C 5 kJ/m^2	$Inoc^+$ + UV-C 10 kJ/m^2
0	556 ± 21^{aA}	556 ± 21^{aA}	556 ± 21^{aA}
24	349 ± 11^{cA}	334 ± 17^{bA}	453 ± 85^{aA}
48	315 ± 10^{dC}	369 ± 17^{bB}	433 ± 15^{bA}
72	484 ± 104^{bA}	577 ± 32^{aA}	557 ± 19^{aA}

Note. The values are expressed as mean ± SE

Statistical differences between treatments are indicated by capital font, while differences within treatment are indicated by small font, each separated using Tukey ($P < 0.05$)

Table B16 Effects of UV-C irradiation on Lightness in Tangerine

Time After Irradiation (Week)	Color (Lightness)		
	Control	UV-C 5 kJ/m^2	UV-C 10 kJ/m^2
0	66.37 ± 0.67^{cA}	66.37 ± 0.67^{cA}	66.37 ± 0.67^{cA}
1	64.09 ± 0.45^{bA}	61.28 ± 0.49^{abC}	62.46 ± 0.53^{bAB}
2	64.24 ± 0.44^{bcB}	62.83 ± 0.41^{bAB}	62.40 ± 0.55^{bA}
3	61.81 ± 0.58^{aB}	60.90 ± 0.58^{abAB}	59.50 ± 0.74^{aA}

Table B16 (continued)

Time After Irradiation (Week)	Color (Lightness)		
	Control	**UV-C 5 kJ/m^2**	**UV-C 10 kJ/m^2**
4	60.59 ± 0.65^{aA}	60.26 ± 0.58^{aA}	59.16 ± 0.72^{aA}

Note. **T**he values are expressed as mean ± SE

Statistical differences between treatments are indicated by capital font, while differences within treatment are indicated by small font, each separated using Tukey ($P < 0.05$)

Table B17 Effects of UV-C irradiation on Yellowness in Tangerine

Time After Irradiation (Week)	Yellowness (b*)		
	Control	**UV-C 5 kJ/m^2**	**UV-C 10 kJ/m^2**
0	71.23 ± 0.75^{bA}	71.23 ± 0.75^{bA}	71.23 ± 0.75^{cA}
1	68.20 ± 0.43^{aA}	67.81 ± 0.87^{aA}	66.63 ± 0.87^{bA}
2	68.76 ± 0.77^{abA}	67.95 ± 0.60^{aA}	65.87 ± 1.11^{bA}
3	66.89 ± 0.99^{aB}	65.47 ± 0.53^{aB}	62.03 ± 0.90^{aA}
4	67.70 ± 0.43^{aA}	67.66 ± 0.91^{aA}	66.18 ± 0.60^{bA}

Note. The values are expressed as mean ± SE

Statistical differences between treatments are indicated by capital font, while differences within treatment are indicated by small font, each separated using Tukey ($P < 0.05$)

Table B18 Effects of UV-C Irradiation on Chroma in Tangerine

Time After Irradiation (Week)	Chroma		
	Control	UV-C 5 kJ/m^2	UV-C 10 kJ/m^2
0	75.77 ± 0.75^{bA}	75.77 ± 0.75^{bA}	75.77 ± 0.75^{cA}
1	72.66 ± 0.59^{aA}	73.54 ± 0.87^{bA}	71.06 ± 0.86^{bA}
2	73.60 ± 0.86^{abA}	73.25 ± 0.56^{bA}	71.17 ± 1.20^{bA}
3	72.13 ± 1.01^{aB}	68.96 ± 0.97^{aA}	67.25 ± 0.74^{aA}
4	74.41 ± 0.51^{abB}	72.70 ± 1.32^{bAB}	71.21 ± 0.72^{bA}

Note. The values are expressed as mean ± SE

Statistical differences between treatments are indicated by capital font, while differences within treatment are indicated by small font, each separated using Tukey ($P < 0.05$)

Table B19 Effects of UV-C Irradiation on DPPH in Tangerine

Time After Irradiation (Week	DPPH (µmol Trolox equivalent/ 100g DW)		
	Control	UV-C 5 kJ/m^2	UV-C 10 kJ/m^2
0	1,145 ± 59^{abA}	1,145 ± 59^{aA}	1,145 ± 59^{aA}
1	1,166 ± 61^{abA}	1,900 ± 85^{bB}	1,960 ± 157^{cB}
2	878 ± 43^{aA}	1,407 ± 109^{bB}	1,407 ± 65^{abB}
3	1,385 ± 123^{bA}	1,835 ± 71^{bAB}	1,835 ± 122^{cA}
4	953 ± 94^{aA}	1,743 ± 53^{bB}	1,743 ± 71^{bcB}

Note. The values are expressed as mean ± SE.

Statistical differences between treatments are indicated by capital font, while differences within treatment are indicated by small font, each separated using Tukey ($P < 0.05$)

Table B20 Effects of UV-C Irradiation on Total Phenolic Concentrations in Tangerine

Time After Irradiation (Week)	Total phenolic concentration (mg gallic acid equivalent/ 100 g DW)		
	Control	UV-C 5 kJ/m^2	UV-C 10 kJ/m^2
0	732 ± 14^{aA}	732 ± 14^{aA}	732 ± 14^{aA}
1	847 ± 55^{bA}	887 ± 49^{bcA}	1,087 ± 31^{bB}
2	813 ± 40^{abA}	963 ± 18^{cB}	1,023 ± 32^{cB}
3	843 ± 24^{abA}	1,003 ± 10^{cB}	1,250 ± 18^{bC}
4	797 ± 37^{abA}	820 ± 17^{abA}	1,065 ± 57^{bB}

Note. The values are expressed as mean ± SE.

Statistical differences between treatments are indicated by capital font, while differences within treatment are indicated by small font, each separated using Tukey ($P < 0.05$)

Table B21 Effects of UV-C Irradiation on Superoxide Dismutase (SOD) Enzyme Activity in Tangerine

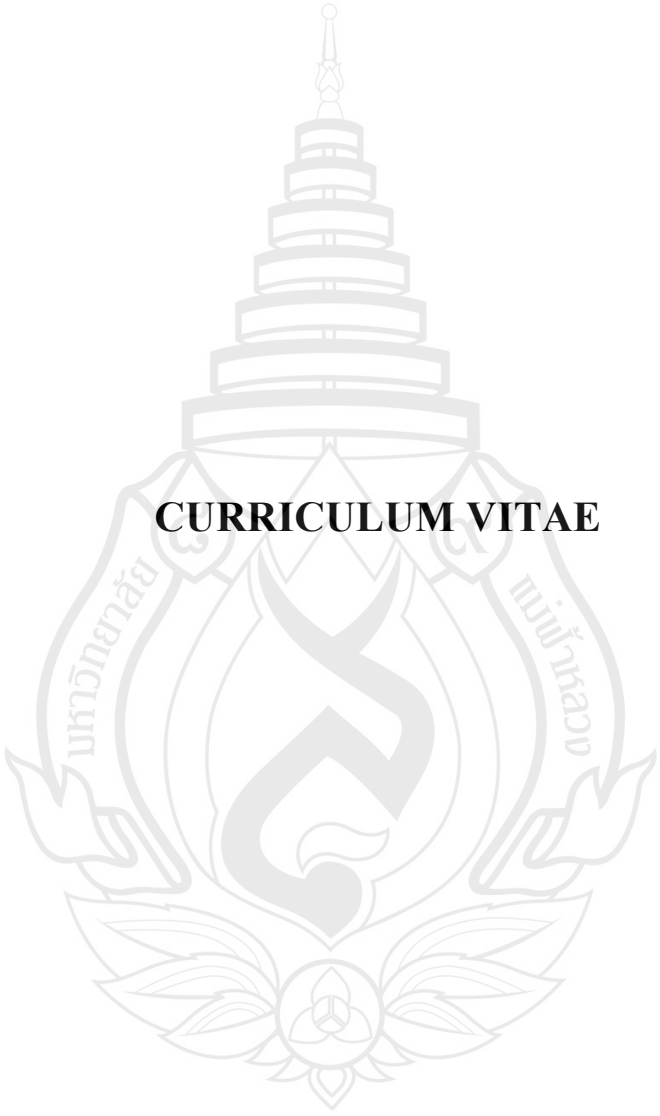
Time After Irradiation (Week)	SOD enzyme activity (Units/mg protein)		
	Control	UV-C 5 kJ/m^2	UV-C 10 kJ/m^2
0	518.9 ± 12.7^{aA}	518.9 ± 12.7^{aA}	518.9 ± 12.7^{aA}
1	516.3 ± 14.6^{aA}	506.8 ± 2.2^{aA}	538.7 ± 5.3^{aA}
2	484.2 ± 13.0^{aA}	485.3 ± 12.4^{aA}	535.1 ± 10.1^{aB}
3	582.5 ± 4.8^{bA}	662.0 ± 7.0^{bB}	703.8 ± 9.1^{bC}
4	845.0 ± 20.2^{cA}	899.2 ± 7.9^{cA}	882.2 ± 14.2^{cA}

Note. The values are expressed as mean ± SE.
Statistical differences between treatments are indicated by capital font, while differences within treatment are indicated by small font, each separated using Tukey ($P < 0.05$)

Table B22 Effects of UV-C Irradiation on Jasmonate Concentration in Tangerine

Time After Irradiation (Week)	Jasmonate acid (µmol/kg DW)		
	Control	UV-C 5 kJ/m^2	UV-C 10 kJ/m^2
0	0.83 ± 0.10^{aA}	0.83 ± 0.10^{aA}	0.83 ± 0.10^{aA}
1	1.63 ± 0.21^{bA}	3.24 ± 0.37^{cA}	3.09 ± 0.68^{bA}
2	0.93 ± 0.10^{aA}	1.97 ± 0.09^{bA}	3.40 ± 0.51^{bB}
3	0.76 ± 0.08^{aA}	3.12 ± 0.22^{cB}	3.87 ± 0.14^{bC}
4	0.79 ± 0.08^{aA}	0.93 ± 0.12^{aA}	2.07 ± 0.12^{abB}

Note. The values are expressed as mean ± SE.
Statistical differences between treatments are indicated by capital font, while differences within treatment are indicated by small font, each separated using Tukey ($P < 0.05$)



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