Research Article

UV-C Enhances Phenolics Metabolism and the Production of the Related Bioactive Compounds in Green Chi-fah Chili (*Capsicum annuum* L. cv. Chi-fah Kiaw) Fruit

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Abstract
One of the fruit vegetables that is a favorite for consumers of spicy food worldwide is chili (*Capsicum annuum*). Thus, the external and internal quality attributes of harvested chili fruit, such as peel color and fruit antioxidants, are of interest for different cooking needs. UV-C is the shortest wavelength of ultraviolet radiation that can harm the living organisms. However, short-term exposure to this physical stress might have many advantages for crop species. This research studied the post-harvest effect of UV-C exposure for different times (0, 10, and 20 min) on the green fruit of Chi-fah chili. The results showed that the percentage of fruit with red peel color increased rapidly during storage after UV-C irradiation, especially in the 10-minute treatment 75% of fruit had red color after 6 days. UV-C treatment also promoted phenolic biosynthesis in green Chi-fah chili fruit as 10 and 20 min of UV-C exposure elevated phenolic contents in both the pulp and placenta with the maximum of 30 and 45 mg gallic acid/gFW, respectively. Exposure to 20 min of UV-C irradiation seemed to inhibit flavonoid production, whereas 10 min UV-C irradiation increased flavonoids in both the pulp and placenta (0.72 and 0.87 mg rutin/gFW, respectively). Of particular interest to the consumers, UV-C treatment could increase the capsaicin amount in green Chi-fah chili fruit. Only the fruit irradiated with UV-C for 10 min had the highest level of phenylalanine ammonia-lyase (PAL) activity in the pulp one day after storage, while the placenta had the highest level of PAL activity from day 1 to day 3. The 10 and 20 min UV-C irradiation led to the highest peroxidase (POD) activity in the pulp and the placenta, respectively. In conclusion, UV-C could be used to enhance the production of phenolics and related bioactive compounds, such as flavonoids and capsaicin in green Chi-fah chili fruit during postharvest storage.

Keywords: Capsaicin, Flavonoids, Phenolics, Phenylpropanoid pathway, Postharvest storage, UV-C

1 Introduction

Chili (*Capsicum annuum*), a nightshade family (Solanaceae) plant, is a fruit vegetable that exhibits either climacteric or non-climacteric behavior depending on the cultivar. It is one of the world's most economically important crops and broadly used horticultural products. As stated in the report of FAO (Food and Agriculture Organization of the United Nations), more than 39 million tons of chili fruit were consumed in 2020. In many cuisines worldwide, this kind of fruit is extensively used in food recipes, giving a good taste and high nutritional value [1]–[5].

Generally, gardeners harvest chili fruit when fully matured as the fruit color has changed from green to dark green or black. Since chili flowers bloom periodically, chili fruit at different maturation stages may be found on the same plant. This is a problem for

harvesting as gardeners often need to harvest large quantities of fruit at regular intervals, and at each harvest, there may be many fruits ranging from fully mature (dark green) to fully ripe (red). Therefore, when chili fruit are allowed to turn red naturally after harvesting, there will be chili fruit with different quality attributes, including uneven peel color development, various levels of spiciness, and different fruit shapes due to uneven wilting. The harvesting-associated problem is related to the overall poor quality of the chili fruit. Since, consumers prefer chilies of different colors, such as green or red, it is necessary to use labor (cost) to sort the harvested chili fruit by colors.

After harvest and during storage, the internal quality attributes including pulp color, nutritional value, antioxidants, and level of pungency of chili fruit may also change. For example, fully mature chili fruit (dark green) has a medium spiciness and gets spicier as it turns red. However, the spiciness will decrease slightly when the chili fruit is overripe (dark red, the fruit surface begins to wilt, and the fruit flesh is soft) [6]–[8]. Therefore, finding a method to control degreening with stimulants, such as ethylene or UV-C radiation will help to ensure a similar quality of the fruit harvested each time. For example, spraying chili plants with ethephon before harvesting has been shown to accelerate color change and reduce the rate of wilting of chili fruit after harvest and UV-C has been shown to delay the peel degreening in mango fruit [9], [10].

During ripening, many chili fruit cultivars have a high respiration rate and a very low ethylene production rate. Thus, the high respiration activity of chili fruit may result in the production of many free radicals. These free radicals are often involved in the redox process that releases superoxide anions, which can harm the structure of cell membranes. It was found that plants could have increased antioxidant capacity during fruit ripening by synthesizing antioxidants such as phenolic compounds and carotenoids to help preventing membrane damage [11]–[14]. Therefore, the production of antioxidants has been found to be stimulated using UV-C radiation in many fresh produce [15]–[17]. Besides, there are numerous advantages of using UV-C in various crop species. Tomato fruit treated with UV-C could slow down post-harvest deterioration, accompanied by higher total phenolics content, while fresh-cut carrots treated with UV-C had an increase in total phenolics content and total antioxidant capacity [18], [19]. Additionally, UV-C could possibly reduce disease activity in fresh produce. For example, grapes treated with UV-C could produce stilbenes, which are the phenolic substances associated with protection against infection by microorganisms [20], [21].

In Capsicum species, UV-C has been used to bring about many benefits. UV-C-treated pepper plants (*C. annuum*) increased the activities of antioxidant enzymes, such as catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) and anthocyanin, proline, quercetin, and rutin contents in their leaves [22], [23]. Moreover, bell pepper fruit (*C. annuum*) at the mature green stage during postharvest storage could delay the physiological changes after exposure to UV-C. Furthermore, UV-C could also reduce chilling injury in the red-colored bell pepper fruit during storage [24], [25]. So far, there have been very few studies on the effects of UV-C on postharvest changes in chili fruit quality attributes, especially on green Chi-fah chili fruit (*C. annuum* L. cv. Chi-fah Kiaw). Therefore, the present research aimed to investigate the effect of UV-C on red peel color development in chili fruit and the relationship of phenolics and capsaicin with phenylalanine ammonia-lyase (PAL) and peroxidase (POD) activities after exposure to UV-C. It was hypothesized that these UVC-induced changes could be associated with the effects of UV-C on delaying postharvest losses and improving the quality of fresh chili fruit.

2 Materials and Methods

2.1 *Plant materials and storage conditions*

Healthy green fruit of Chi-fah chili (*Capsicum annuum* L. cv. Chi-fah Kiaw) that were free of insect damage and disease (Figure 1) were purchased from Si Moom Muang (a wholesale market) in Lum Luk Ka District, Pathum Thani Province, Thailand. These fruits were cleaned with tap water and left to air dry. Subsequently, they were divided into 3 groups, 1 kg each, and spread evenly on a basket (size 22 cm width × 33 cm length × 7 cm height). The first group was the control group, which was not exposed to UV-C radiation, while the second and third groups were irradiated with UV-C for 10 and 20 min, respectively. To expose to UV-C

Figure 1: Green Chi-fah chili fruits (whole fruit and half-cut open fruit).

radiation for 10 or 20 min, a basket with green fruit was placed under the UV-C lamp (Philips TUV 15W/G15 T8, Poland) of 48 μW/cm² intensity inside a laminar flow cabinet (Yamato Scientific Co., Ltd., Japan). The distance between a basket and a UV-C lamp was 60 cm. After this, the fruit from the two UV-C treatments and the control were stored at 25 ± 2 °C and 80% RH for 0, 1, 2, 3, 4, 5, and 6 days before further investigation.

2.2 *Peel color development*

During storage, the green peel of Chi-fah chili turned red. The number of fruit that turned red at different times of storage was counted, and the percentage of red fruit was calculated using the formula below.

Red fruit (%) = (number of fruit changed to red color/ number of all fruit) × 100

2.3 *Phenolics determination*

Phenolic content was analyzed using the modified method of Chang *et al*., [26]. Chi-fah chili fruit was separated into pulp and placenta, which were then crushed and finely ground before mixing 10 g of each tissue with 10 mL of 80% (v/v) ethanol, and centrifuged at 12,000 pm for 20 min. One mL of the supernatant was then diluted with 9 mL of distilled water. After this, 1 mL of the diluted supernatant was mixed with 5 ml of Folin-Ciocalteu reagent and 4 mL of 7.5% (w/v) Na_2CO_3 before incubation in a water bath at 30 °C for 1 h, and then in an ice bath for 1 h. The absorbance of the reaction mixture was measured using a spectrophotometer (model SP-830 Plus, Metertech Inc., Taipei, Taiwan, R.O.C.) at 760 nm, and the phenolics content was calculated with reference to a gallic acid standard curve.

2.4 *Flavonoids determination*

Flavonoid content was analyzed using the modified method of Chang *et al*., [26]. Two g of finely ground Chi-fah chili pulp or placenta were mixed with 6 mL of methanol for 6 min and the slurry was then filtered through Whatman No.1 filter paper. One ml of the filtered solution was mixed with 0.2 mL of 10% (w/v) aluminum chloride, 0.2 mL of 1 M potassium acetate, and 5.6 mL of distilled water. After incubation at room temperature for 30 min, the absorbance of the mixture was measured spectrophotometrically at 415 nm and the flavonoid content was calculated with reference to a rutin standard curve.

2.5 *Capsaicin determination*

The method for capsaicin analysis was modified from Noichinda *et al*., [27].

2.5.1 Capsaicin extraction

One g of finely ground Chi-fah chili pulp or placenta was mixed with 25 mL of acetone and then refluxed at 62 °C for 1 h, before filtering through Whatman No. 1 filter paper. The volume of the filtrate was adjusted to 25 mL by adding acetone. The transparent portion (capsaicin extract) was used for capsaicin purification.

2.5.2 Capsaicin purification

A silica gel column was packed and washed with acetone. Ten mL of capsaicin extract was eluted through the column using the eluent of acetone: methanol (75:25 v/v). The eluate (containing purified capsaicin) was dried and then dissolved with 25 ml of ethanol.

2.5.3 Capsaicin content

Ten ml of purified capsaicin was diluted with 10 ml of 0.1 M HCl and 10 mL of distilled water. The absorbance of the mixture was measured using a UV/Vis

spectrophotometer (model T80+, PG Instrument Ltd., United Kingdom) at 297 nm and the capsaicin content was calculated using the following formula:

Capsaicin (mg/gFW) = [Abs297 × df × 10^3] / [(g × 10^6) × slope]

Where:
Abs 297 = absorbance at 297 nm
df = dilution factor
g = sample weight in gram
slope = slope value from the capsaicin standard curve

2.6 *Phenylalanine ammonia-lyase determination*

Phenylalanine ammonia-lyase (PAL) activity was assayed following the method of Camm & Towers [28].

2.6.1 Acetone powder preparation

Finely ground Chi-fah chili pulp or placenta (5 g) was mixed with 20 mL of cold 95% (v/v) acetone thoroughly in a blender for 5 min. The mixture was filtered through Whatman No. 2 filter paper using a vacuum pump with a suction power of approximately 10–15 psi. The filter paper was washed until no color remained. The filtrate was then dried at 40 °C and then ground with a grinder to become a fine acetone powder, which was placed in a pocket of aluminum foil for storage in a desiccator until further analysis.

2.6.2 PAL extraction

The acetone powder (0.1 g) from 2.6.1 was gently mixed with 20 ml of cold sodium borate buffer, pH 8.8, on a magnetic stirrer for 30 min at 4 °C, and then centrifuged at 14,000 g and 0 °C for 25 min. After centrifugation, the supernatant was used as a crude enzyme extract for PAL activity determination.

2.6.3 PAL activity assay

A reaction mixture was composed of 1.5 mL sodium borate buffer, pH 8.8, 1 mL phenylalanine (10 mg/mL dissolved in H_2O), and 1 mL of crude enzyme extract was incubated in a water bath at 30 °C for 1 h. The reaction was stopped by adding 0.5 mL of 5 M HCl. The absorbance of the enzyme reaction mixture was measured with a UV/Vis spectrophotometer at a wavelength of 290 nm, and 1 unit of PAL activity was the amount of cinnamic acid produced in 1 μM in 1 h.

2.7 *Peroxidase determination*

Peroxidase (POD) activity was assayed according to the method of Morita *et al*., [29].

2.7.1 POD extraction

Finely ground Chi-fah chili pulp or placenta (3 g) was mixed with 20 mg polyvinylpyrrolidone and 15 mL 0.2 M potassium phosphate buffer, pH 7.0 and was then centrifuged at 5,000 g for 20 min, after centrifugation, the clear supernatant was used for POD activity assay.

2.7.2 POD activity assay

The POD assay mixture consisted of the supernatant (0.1 mL) from 2.7.1, 1.5 mL of 0.43 mM H_2O_2 (in 0.2 M phosphate buffer, pH 7.0), and 1.4 mL of 2.5 mM 4-aminoantipyrine (in 17 mM phenol) as a substrate. For the enzyme blank, the supernatant was substituted with 0.1 mL of phosphate buffer (0.2 M, pH 7.0). Both the blank and POD assay mixtures were left at room temperature for 90 s, and the absorbances of the mixtures were measured spectrophotometrically at a wavelength of 510 nm.

2.8 *Statistical analysis*

The data from the experiments were analyzed statistically using SPSS version 26 (IBM, Chicago, IL, USA) and tested for variance (ANOVA) at a confidence level of 95%. If differences were found among the data groups, the average values of the data were compared using DMRT (Duncan's New Multiple Range Test) at a confidence level of 95%.

3 Results and Discussion

3.1 *Peel color development*

Two principal characteristics for the determination of capsicum fruit quality are color and spiciness. Moreover, the peel color development is related to the

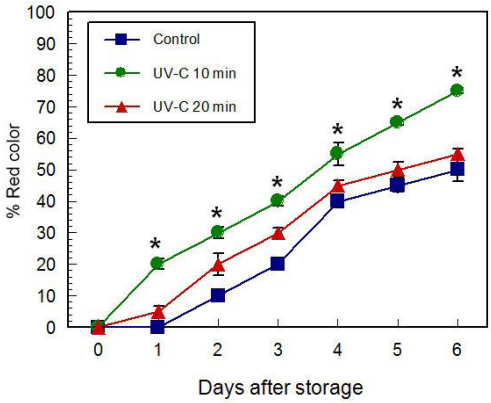

Figure 2: Fruit color development of green Chi-fah chili fruit after UV-C treatment for 0, 10, and 20 min at 25 ± 2 °C and 80% RH during 6 days after storage. Asterisks above the curve on experimental days indicate statistically significant differences (p-value < 0.05).

maturity of Capsicum fruit. In this research, the peel color of Chi-fah chili fruit treated with UV-C changed from green to reddish-brown on day 1, while that of the control began to change on day 2. Approximately 50% of green chili fruit changed to red after 4, 5, and 6 days following exposure to UV-C for 10, 20, and 0 min, respectively (Figure 2). This experiment showed that exposure to UV-C for 10 min was most effective to stimulate red peel coloration in Chi-fah chili fruit while 20 min of UV-C irradiation had only a small but significant effect over the control. Generally, the change to red color in *C. annuum* fruit occurs by a combination of increasing levels of two red pigments: carotene (red-orange pigment) and anthocyanin (red-purple pigment). In the previous reports, UV-C was also found to increase these two red pigments in *C. annuum* [8], [23], [30], [31].

3.2 *Phenolics content*

Phenolic compounds are classified as antioxidants that plants synthesize from precursors in the phenylpropanoid pathway to protect against free radical damage to membranes, especially the denaturation of lipids that are components of cell membranes. Under stress conditions, it is often found that plants produce various phenolics [32]. In this experiment, the phenolics content in both the pulp and placenta of Chi-fah chili fruit increased throughout storage and the exposure times

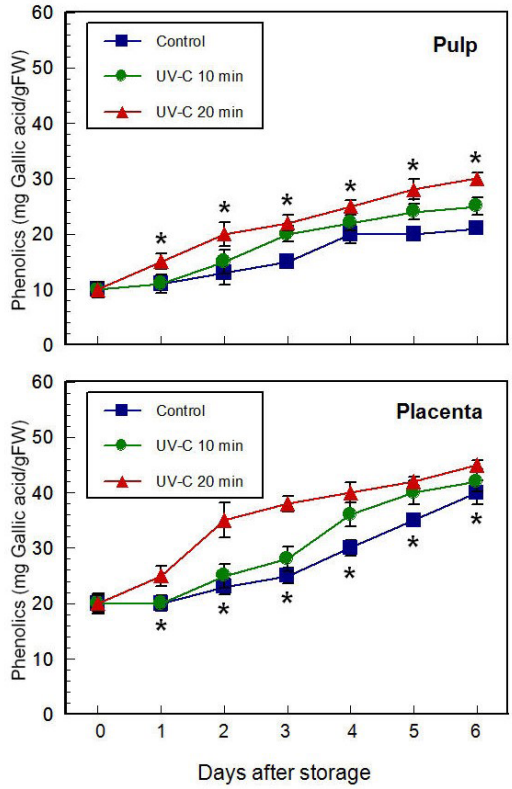

Figure 3: Phenolics content of green Chi-fah chili fruit after UV-C treatment for 0, 10, and 20 min at 25 ± 2 °C and 80% RH during 6 days after storage. Asterisks above or below the curve on experimental days indicate statistically significant differences (p-value < 0.05).

of green Chi-fah chili fruit to UV-C were 10 or 20 min before the start of storage led to higher phenolics contents than those in the control group. After 6 days of storage, there were approximately 25–30 mg gallic acid/gFW and 41–44 mg gallic acid/gFW in the pulp and placenta, respectively, of fruit exposed to 20 min of UV-C (Figure 3). It has been reported that UV-C can induce PAL activity, which can catalyze the synthesis of the precursors in the phenylpropanoid pathway with intermediate substances that lead to the synthesis of phenolics [16], [33].

3.3 *Flavonoids content*

The largest group of naturally occurring phenolic compounds that are used as attractants, UV screens,

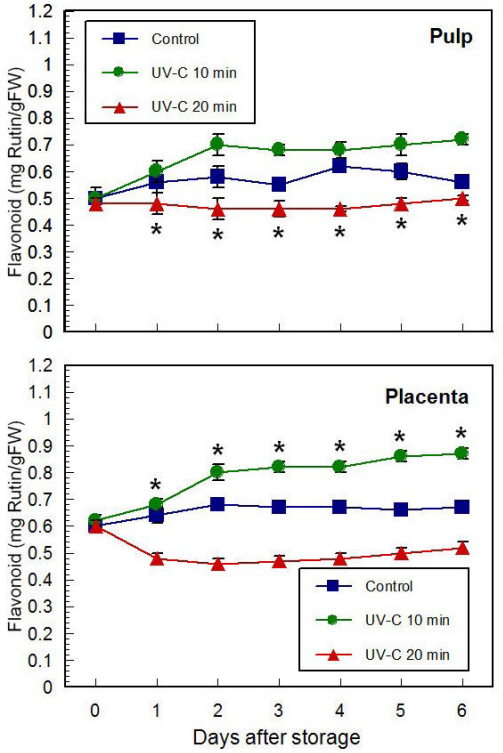

Figure 4: Flavonoids content of green Chi-fah chili fruit after UV-C treatment for 0, 10, and 20 min at 25 ± 2 °C and 80% RH during 6 days after storage. Asterisks above or below the curve on experimental days indicate statistically significant differences (p-value < 0.05).

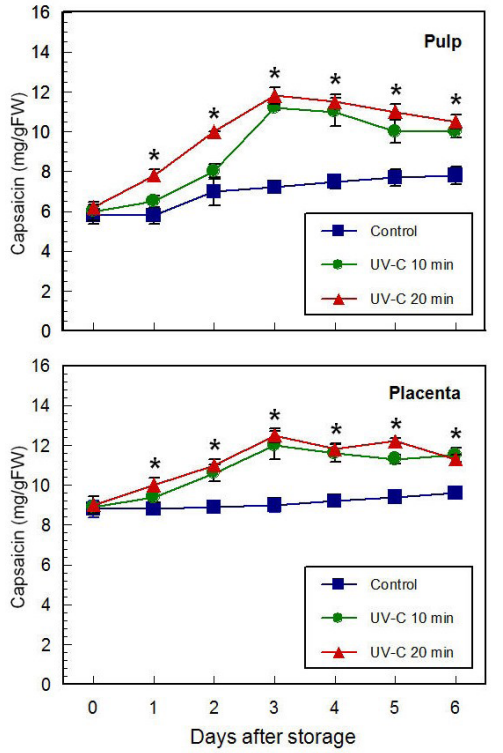

Figure 5: Capsaicin content of green Chi-fah chili fruit after UV-C treatment for 0, 10, and 20 min at 25 ± 2 °C and 80% RH for 6 days after storage. Asterisks above the curve on experimental days indicate statistically significant differences (p-value < 0.05).

and signal compounds in plants are flavonoids [34], [35]. In the present study, it was found that the duration of UV-C exposure affected flavonoid production in Chi-fah chili fruit. UV-C irradiation for 20 min suppressed flavonoid biosynthesis in both the pulp and placenta, while 10 min of UV-C irradiation could induce this biosynthesis better than the control (Figure 4). The increased amount of flavonoids appeared to be related to the red color of Chi-fah chili fruit peel since exposure to 10 min of UV-C resulted in the highest percentage of fruit with red peel color (Figure 2). Mahdavian *et al*., [23] described that pepper plants treated with UV-C had higher anthocyanin contents. Thus, it is possible that the increased anthocyanin via UV-C exposure may reflect the increased level of red color in chili fruit. Moreover, it was likely that 10 min of UV-C irradiation was the optimum duration for flavonoid biosynthesis whereas 20 min appeared to be in excess which could be harmful.

3.4 *Capsaicin content*

Capsaicin is chili's primary bioactive plant compound, which is responsible for the spicy flavor and many pharmacological advantages [36]. In the present study, 3 days after UV-C exposure to chili fruit, the capsaicin content in the pulp increased from 6–11.8 mg/gFW, while in the placenta increased from 8.9–12.5 mg/gFW (Figure 5). This is the first report that UV-C could also enhance capsaicin production in *Capsicum annuum* (Chi-fah chili fruit), and suggests that exposure of Chi-fah chili fruit to UV-C radiation for 10 or 20 min before storage could increase the pungency in the pulp and placenta of Chi-fah chili fruit. This result would

be beneficial for the consumer who likes spicy food. In another species of Capsicum (*C. chinense*), UV-C irradiation of habanero pepper fruit was also found to result in a postharvest storage increase in its capsaicin content [37].

Capsaicin is classified as a group of phenolics [8] and is synthesized from an intermediate containing phenylalanine as a precursor in the phenylpropanoid pathway. Many published data showed that UV-C can induce higher phenylalanine ammonia-lyase (PAL) activity [16], [38], [39] resulting in the formation of intermediates in the phenylpropanoid pathway, providing a precursor used in the synthesis of capsaicin in *Capsicum* species [40].

3.5 *Phenylalanine ammonia-lyase activity*

In response to a variety of stresses, including low and high-temperature stress, water and wounding stress, and UV stress, PAL activity would increase [16], [41]–[43]. As mentioned earlier, increased PAL activity is involved in the formation of intermediates in the phenylpropanoid pathway, providing precursors for the synthesis of various phenolics, such as flavonoids, capsaicin, and anthocyanin. It was found in the present study that PAL activity in the pulp increased from 0.0015–0.0018 to 0.003–0.0036 unit/min/mg-protein after 1 day from exposure to UV-C and then decreased rapidly. At the same time, PAL activity in the placenta increased from 0.005 to 0.007 unit/min/mg-protein after 1 day from UV-C treatment and remained high until day 3. Then, the PAL activity decreased rapidly (Figure 6).

Accordingly, UV-C acts as a free radical, which could damage cell membranes. However, plants have a mechanism to respond to free radicals by producing antioxidants, such as phenolic compounds formed from intermediates in the phenylpropanoid pathway, with phenylalanine as a starting substance [32]. This result is consistent with a significant increase in PAL enzyme activity and the amount of phenolics (Figures 3 and 6). Hence, UV-C can induce higher PAL activity in Chi-fah chili fruit, which is consistent with the previous studies [16], [38], [39]. In addition, the higher PAL activity in the placenta of chili fruit was maintained over a more extended period than that in the pulp, resulting in a higher phenolics content in the placenta than in the pulp (Figures 3 and 6).

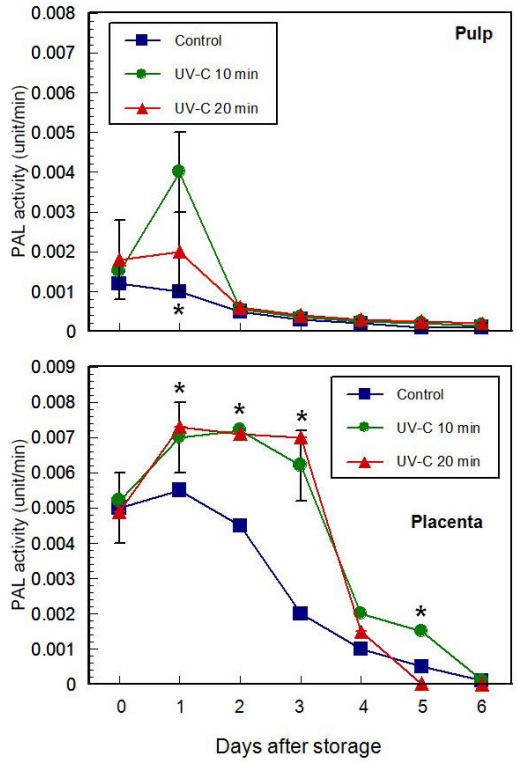

Figure 6: PAL activity of green Chi-fah chili fruit after UV-C treatment for 0, 10, and 20 min at 25 ± 2 °C and 80% RH during 6 days after storage. Asterisks above or below the curve on experimental days indicate statistically significant differences (p-value < 0.05)

3.6 *Peroxidase activity*

One of the significant antioxidant enzymes that participates in numerous crucial physiological processes of growth and development throughout the plant life cycle is peroxidase (POD). The reinforcement of the cell wall, auxin metabolism, synthesis of reactive oxygen species (ROS) and reactive nitrogen species (RNS), and phytoalexin production are essential roles of this enzyme [44], [45]. In the current research, UV-C irradiation for 10 min induced significantly higher POD activity in the pulp than 20 min of UV-C and the control throughout the first 5 days of postharvest storage. In contrast, UV-C irradiation for 20 min resulted in a significantly higher POD activity than 10 min of UV-C irradiation and the control after 4 and 5 days of postharvest storage (Figure 7).

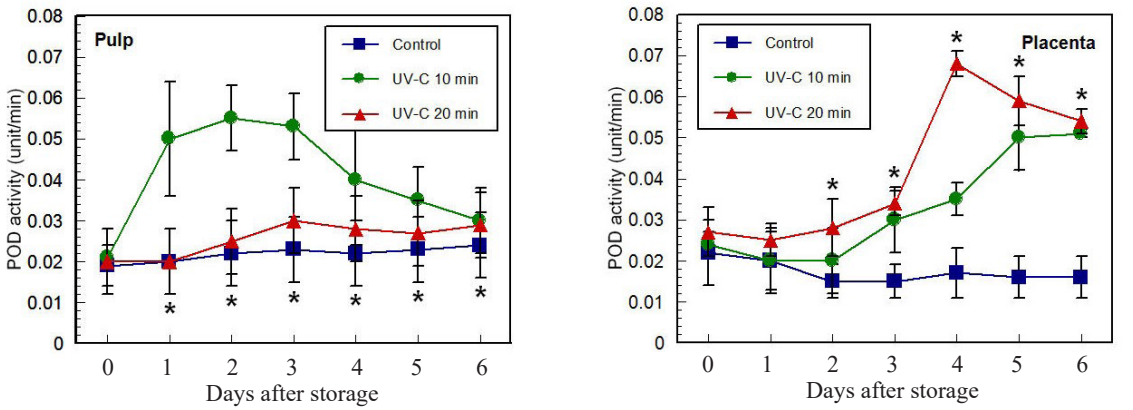

Figure 7: POD activity of green Chi-fah chili fruit after UV-C treatment for 0, 10, and 20 min at 25 ± 2 °C and 80% RH during 6 days after storage. Asterisks above or below the curve on experimental days indicate statistically significant differences (p-value < 0.05).

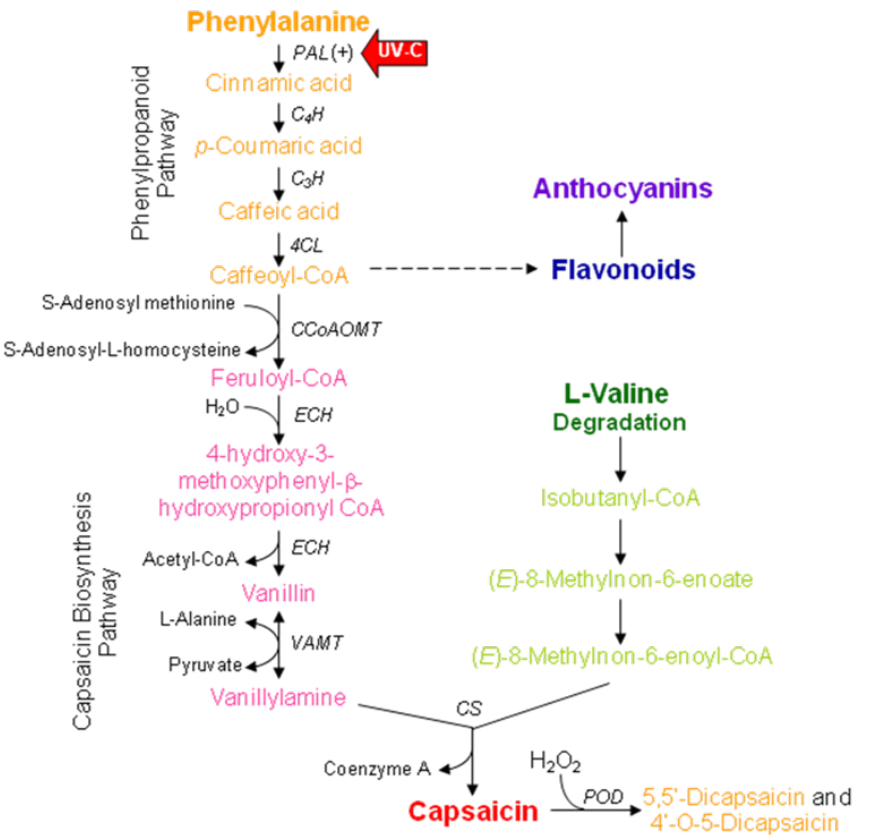

Figure 8: Capsaicin, flavonoids, and anthocyanins biosynthesis routes of UV-C treated green Chi-fah chili fruits [PAL, phenylalanine ammonia-lyase; C_4H, cinnamate 4-hydroxylase; C_3H, p-coumarate 3-hydroxylase; 4CL, 4-coumaric acid coenzyme A ligase; CCoAOMT, caffeoyl-CoA 3-O-methyltransferase; ECH, enoyl-CoA hydratase/aldolase; VAMT, vanillin transaminase; CS, capsaicin synthase; POD, peroxidase].

Generally, capsaicin could be oxidized by POD [46]. However, based on the present results, it appeared that POD might only be slightly involved in the capsaicin degradation process in Chi-fah chili fruit as the capsaicin level in the fruit was still increasing while POD activity was high. It is possible that the changes in the POD activity in Chi-fah chili fruit were similar to the phenolics content as described in many prior studies, particularly under UV stress [47]–[49]. This suggests that POD activity in Chi-fah chili fruit was closely related to production of phenolics rather than capsaicin production.

4 Conclusions

UV-C irradiation causes physiological stress in harvested Chi-fah chili fruit that could lead to improvement in the external and internal quality of the fruit during postharvest storage. Exposure to this radiation increased the red color pigment in the peel, stimulated the synthesis of fruit antioxidants, phenolics, and flavonoids, and could also elevate the spiciness via increased capsaicin production. This increase in antioxidants and capsaicin benefits consumers in terms of taste and nutritional value, which leads to better health. The activity of PAL and POD related to the biosynthesis of these compounds in Chi-fah chili fruit was also promoted by UV-C irradiation, as outlined in Figure 8. UV-C stimulated PAL activity, leading to phenolic production through the phenylpropanoid pathway, which would also provide the precursors for flavonoid, anthocyanin, and capsaicin production, suggesting that phenolics production was related to flavonoid and capsaicin biosynthesis in green Chi-fah chili fruit and all were enhanced by UV-C. The knowledge gained from this research allows us to know the appropriate time to use UV-C irradiation for maximum benefit. In addition, using UV-C irradiation would help to sterilize the surface of chili, thereby reducing the spoilage of the produce and helping to reduce the spread of pathogens. Therefore, this is incredibly important for the chili export industry.

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Author Contributions

S.N.: conceptualization, investigation, methodology, research design, data analysis, writing an original draft, reviewing; K.B.: conceptualization, investigation, methodology, research design, data analysis, writing an original draft, reviewing; D.L.: conceptualization, writing an original draft, reviewing and editing. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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