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Effects of UV–C Treatment on Green Mold Rot (*Penicillium digitatum*) and Physico–Chemical Properties of Post–Harvest Strawberry Using UV–C Conveyer Machine

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We investigated the effects of Ultraviolet–C (UV–C) treatment on inactivation of green mold rot (*Penicillium digitatum*) and physico–chemical properties of strawberry fruit. Although, there was no significant difference ($P \geq 0.05$) in weight loss for all samples after immediately UV–C treatment, however, the loss dramatically increased during storage ($P < 0.05$). During storage, inoculated strawberry treated with UV–C (1 and 6 min) showed the better inactivation effect as evidenced by lower decay and number of microorganism than control sample; however, UV–C treatment for 6 min caused quality damage. This result agreed with browning calyx shriveling and drying of the strawberry simultaneously with firmness decreasing. Thus, the low UV–C treatment (1 min) was recommended to use as a postharvest mild treatment.

Key words: UV–C, UV–C conveyer machine, *Penicillium digitatum* and physico–chemical properties of strawberry

INTRODUCTION

The market sales of fresh cut fruit and vegetables have grown rapidly in recent years partially due to the health benefits associated with the consumption of these foods (Pataro *et al*., 2015). Strawberry is an economically important fruit in many countries due to its unique flavor and aroma, exceptionally rich nutrient content and potential health benefits (Wei *et al*., 2018). Strawberry usually tends highly to be perishable and susceptible to physiological change and pathogen infection at all stages of supply chain affecting economic loss (Maraei and Elsawy, 2017, Liu *et al*., 2018).

The issue of fruit losing in agri–food chain (post–harvest, processing and distribution processes) has gained high attention. Most evidences related to their loss are caused by microorganism (Trivittayasil *et al*., 2016, Ballester and Lafuente, 2017, Wei *et al*., 2018). Green mold rot caused by *Penicillium digitatum*, which is one of the main microorganisms, presented on the surface of fruit resulting in limited of shelf life of the fruit and also economic loss (Bautista–Baños *et al*., 2003). Thus, there is an urgent requirement for reducing decay and extending the postharvest life of fruit (Liu *et al*., 2018). Recently, various emerging postharvest treatments including ultrasonic treatment (Cao *et al*., 2010a, Gani *et al*., 2016), hypobaric treatment (Hashmi *et al*., 2016), Gramma–irradiation (Maraei and Elsawy, 2017), pulse light treatment (Duarte–Molina *et al*., 2016), wax coating (Li *et al*., 2018, Oregel–Zamudio *et al*., 2017), melatonin treatment (Liu *et al*., 2018), have been applied to postharvest preservation to extend shelf life of fruit. Nevertheless, some of these methods are not commonly used in daily post–harvesting process due to its expensive, a need for verifying the effectiveness and complicated process. The use of different fungicides (aniline pyrimidine, cyprodinil, phenylpyrrole and fludioxonil) is probably the most commonly used method to control postharvest decay (Wei *et al*., 2018), but it leaves residues that have potential risks to humans, environment and regulation usage (Liu *et al*., 2018). For these reasons, the identification of alternative antifungal agents to extend postharvest shelf life and maintain quality of fruit is largely gaining attention.

UV treatment of fresh produce is considered as an alternative method to chemical approaches because it has great potential for controlling postharvest diseases (Charles and Arul, 2007). The germicidal effect of UV–C presents in a wavelength ranging from 100 to 280 nm. Many benefits of UV–C, which are cheap, easy to operate and can be readily incorporated in the processing line of UV–C, resulting in it is widely implemented to decontamination in horticulture products (Trivittayasil *et al*., 2016).

There are numerous reports of UV–C treatment for extending the shelf life and maintain the quality of abundant fruits. However, no information regarding using of UV–C conveyor treatment for extending the shelf life of strawberry in the processing line exists. Also our previous study, we proposed the UV–C conveyor model for strawberry decontamination. Therefore, the present study aimed to investigate the effect of UV–C treatment in a conveyor system at various levels of treatments times on properties of strawberry for being use in the real processing line.

MATERIALS AND METHODS

Preparation of strawberry sample

Strawberry was obtained from the JA Chikuzen

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Asakura, Amagi, Fukuoka, Japan and transported to our laboratory, Kyushu University, Fukuoka, Japan. The strawberry was sorted to eliminate damaged or unripe fruit, and selected for uniform size and color, and then randomly divided into 3 groups (Group 1: Control, Group 2: In+1 min and Group 3: In+6 min; 27 strawberries/groups)) placed in a polyethylene tray (B9, Yurikago, Ohishi Sangyo Co. Ltd.), and kept at room temperature (20°C) until used.

Preparation of cell suspension

Penicillium digitatum NBRC 33116 obtained from the collection of the National Institute of Technology and Evaluation Biological Resource Center, Japan (NBRC, Chiba, Japan), were used in this study. The cell suspensions of *Penicillium digitatum* NBRC 33116 were prepared according to the method of (Trivittayasil *et al*., 2015) with a slight modification and was grown on PDA plates (Nissui Pharmaceutical Co. Ltd., Tokyo, Japan) at 25°C for 72 h. The spore was harvested by scraping off from the PDA surface with detergent (mixing of 30 mL of sterile distilled water and 1 ml of TWEEN 80). A spreader was used to gently scrap the spores into the suspension. The suspension of mycelium and spores was filtered through a funnel filled with glass wool to remove mycelium fragments. The collected suspension was mixed with 30 mL of distilled water followed by centrifuge at 3000 rpm for 10 min at 20°C (3 times). The suspension was adjusted to 10^7 CFU/mL by measurement of the optical density (OD=1) at 600 nm.

Preparation of inoculation on the strawberry and treatment

Samples were immersed in the cell suspension (10^7 CFU/ml) for 1 min. The strawberry was allowed to stand at 4°C until dry. The immersed strawberry was subjected to treat with ultraviolet (UV–C) for 1 and 6 min using a UV–C conveyor machine (Fig. 1A) at a conveyor speed of 1 m/min (In+1 min and In+6 min). The average intensity of UV–C treatments for 1 min and 6 min were 0.76 kJ/m^2 and 4.54 kJ/m^2, respectively. The distance between lamp and sample was 9.7 cm (Fig. 1B). The strawberry immersed in cell suspension without treatment with UV–C was used as the control (Control). Then, all strawberries were stored at 25°C and taken out after 0, 3 and 6 days for measurements of weight loss, color, firmness, decay incidence, pH, °Brix, calyx deterioration, and microbial load.

Determination of weight loss

Weight loss was determined by weighting the strawberry before and after UV treatment, and storage period using an electronic balance (FX – 3000i, A & D Corporation, Tokyo).

Weight loss was calculated as follows:

$$\text{Weight loss (\%)} = [(A–B)/A] \times 100$$

where: A = initial weight (before treatments)

B = weight after inoculation, followed by treatments after storage

Determination of calyx deterioration

In accordance with the evaluation method by Trivitayasil (2014) all samples were evaluated calyx deterioration by visual assessment base on its turgidity and green color using an index of 1–5, where 1=fresh and green, 2=fairly wilted and green, 3=fairly wilted and a little brown, 4=fairly wilted and fairly brown, 5=wilted and brown.

Determination of decay incidence

Decay incidence of strawberry fruit was considered from fruit appearing decay symptoms (rot, lesions or visible fungal growth) compared to total number of fruit and reported in percentage (%) (Liu *et al*., 2018). The all samples were evaluated decay severity by observing using a 1–5 scale: 0, healthy fruit; 1, 1–20% fruit surface infected; 2, 21–40% fruit surface infected; 3, 41–60% fruit surface infected; 4, 61–80% fruit surface infected; 5, ≥81% fruit surface infected and showing sporulation according to Liu *et al*. (2018).

Microbiological evaluations

Strawberry was taken out after 0, 3 and 6 days of storage to measure the surface decontamination using the standard medium agar (PT 1010, Eiken Chemical Co., Ltd., Tochigi, Japan). The standard medium agar was used for stamping on the surface of strawberry and then incubated at 25°C for 3 days. After 3 days, the number of colonies on the medium was measured and calculated. The result was reported as signs: – = clean (not detect), ± = very low contaminated (<20 Colonies on an agar plate), + = low contaminated (20–60 Colonies on an agar plate), ++ = mid contaminated (60–200 Colonies on an agar plate), +++ = high contaminated (>200 Colonies on an agar plate) (Kaneko, 1999)

Determination of color

Color was assessed by using a portable color reader (CR–20, Konica Minolta Japan Co., Ltd.) with CIELAB color mode. Color of strawberry was determined and expressed as L^* (lightness), a^* (–greenness/ + redness), b^* (–blueness/+ yellowness). The color was measure on the surface of fruit at same area (near ostiole) during the storage.

Determination of texture firmness

The strawberry was cut along the central axis into two halves. Each half of the sample was placed firmly with turned the outer surface up on the sample holder. The measurement was performed using a rheometer (RE–3305, Yamaden Co., Ltd., Tokyo, Japan) with a 7.9 mm diameter cylindrical probe. The probed penetrated and held perpendicular 5 mm of the fruit flesh at a crosshead speed of 1 mm/s and the peak force was recorded. The value of the flesh firmness was expressed in Newton (N).

Determination of pH and Brix

The strawberry was squeezed by hand to obtain the fruit juice. 1 mL of fruit juice was dropped on to the

sample holder of pH meter (twin pH B–211, HORIBA). 1 mL of fruit juice was dropped on to the sample holder of Brix meter (MASTER–T, ATAGO CORPORATION, Tokyo)

Statistical analysis

Analysis were conducted in triplicate. Data were subjected to analysis of variance (ANOVA). Comparison of means was performed by the Duncan's multiple range test. Significance of differences was defined at $P<0.05$. Statistical analysis was carried out using SPSS package.

RESULTS AND DISCUSSION

Weight loss

Postharvest weight loss is an important indicator of the freshness of fruits, and the control of the loss is a challenge for their commercialization (Oregel–Zamudio *et al*., 2017). Weight loss of strawberry after treated with different levels of UV–C exposure during storage is shown in Fig. 2A. At day 0, weight loss of strawberry generally increased significant ($P<0.05$) when treated with UV–C, regarding increased treatment time. There is the possibility that, when strawberry is exposed to UV for a long time, the free water was easily removed from fruit to the environment and surface shriveling observed, in which occurred weight loss of strawberry. Furthermore, when the storage time increased, the weight loss of all strawberry samples increased significantly ($P<0.05$). The highest weight loss was observed at 3 and 6 days of the storage in control and In+1min sample. The highest weight loss during storage might be related with transpiration, respiration of the fruit and also microbial growth which are causing physiological damage of fruit. The obtained results are in agreement with similar research conducted by Pinheiro *et al*. (2015). They reported that weight loss of tomato treated with UV–C treatments (0.32, 0.97, 2.56,4.16 and 4.83 kJm^{-2} at 254 nm) increased during storage for 15 days. Moreover, the weight loss of those samples related with visual fungal attack evidence (Fig. 3) and survival microorganism result (Fig. 2C) which showed the highest decay strawberry and microbial load. Despite the fact that the high intensity of UV–C treatment (6 min) showed moderate weight loss after storage at 3 and 6 days, it caused a negative effect of visual appearance (shriveling and loss of brightness as indicated by lowering firmness and high calyx freshness score. Thus, UV–C treatment on the surface of strawberry with optimum levels must be considered.

Calyx deterioration

The calyx was a decisive parameter of fruit quality, as browning of the calyx leaves was the most relevant damage (Allende *et al*., 2007). At the first day of the storage. No significance ($P\geq0.05$) were observed in all samples (Fig. 2B). The calyx freshness of strawberry in all samples increased significantly during storage ($P<0.05$). The highest calyx deterioration was found in In+6 min sample because of excessive of UV–C treatment. Nigro *et al*. (2000) reported that strawberry treated with irradiation at 4.00 kJ m^{-2} induced caused browning of the calyx and loss of color brightness. Allende *et al*. (2007) revealed that using higher UV–C (1 kJm^{-2}) and O_3 (5000 mg L^{-1}) treatment had the negative effect of browning and drying of the strawberry calyx.

% Decay incidence

%Decay incidence of strawberry after treated with UV–C at different treatment levels during storage is illustrated in Fig. 2C. %Decay incidence of strawberry fruit in all samples increased significantly ($P<0.05$) with risen storage time, regardless with treatment times. The quality of strawberries during storage has been limited by its highly perishable nature including susceptibility to postharvest diseases associated with bacteria, yeasts and fungal infections (Sogvar *et al*., 2016). At the end of storage, the highest decay incidence, but no significance ($P\geq0.05$) was found in control sample followed by

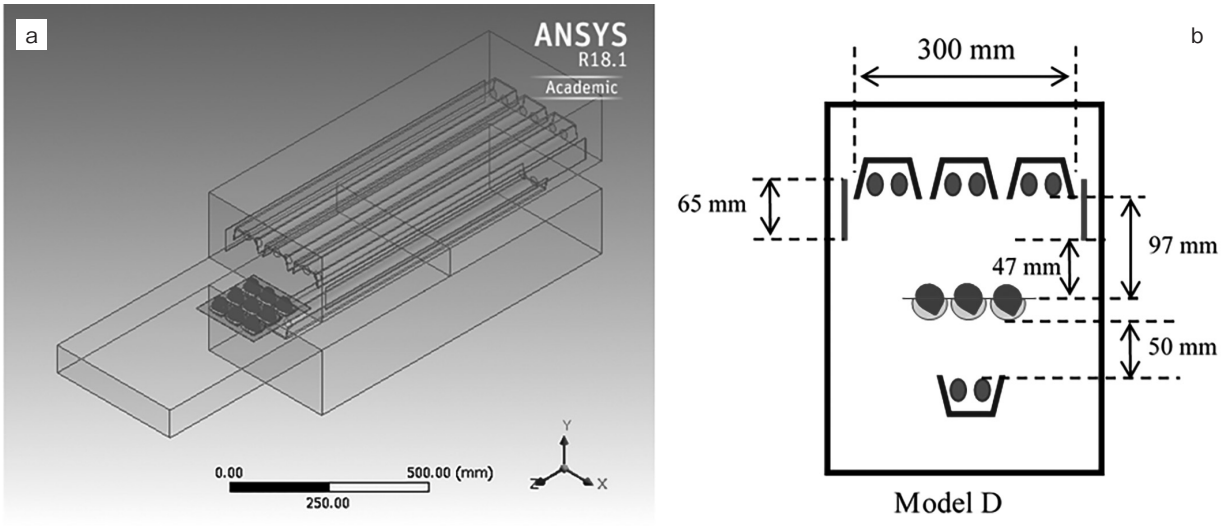

Fig. 1. The 3D model of UV–C conveyor configuration (a), UV–C conveyor configuration models (b).

In+1 min and In+6 min, respectively. It was postulated that poor UV–C treatment allowed to survive, survival microorganism and some metabolic activity affecting decay on fruit. Thus, using UV–C treatment alone can reduce the decay symptom for a while. This method still requires integration effect with other post–harvest treatments.

Microbiological evaluations

The effect of UV–C treatment to strawberry samples on the total microorganism are mentioned in Table 1. No significance ($P \geq 0.05$) was overserved in all samples after immediately treated with UV–C (day 0). In +1 min and In+6 min samples showed the significant reduction ($P<0.05$) of microbial growth compared with inoculated sample at 3 and 6 days of the storage times. There was indicated that the UV–C treatment effectively reduced the microorganisms. Ultraviolet irradiation inactivates cells by altering the physicochemical nature of the DNA, which eventually affects downstream expression of essential proteins involved in biochemical processes (Estilo and Gabriel, 2018). It is noteworthy that although UV–C treatment delayed the microorganism, combination with other method for improving efficacy in part of decontamination still required.

Color

Color of strawberry after treated with UV–C at different treatment levels during storage is illustrated in Table 2. The color factor is a crucial criterion related to consumer acceptance of strawberry fruit. To evaluate the decay of fruit, L^* and a^* values is a useful parameters to indicate the color change (brownness and darkness) of strawberry fruit during storage (Liu *et al.*, 2018). For all treatments, L^* value exhibited decreased significantly ($P<0.05$), when the storage time increased. Those results indicated darkening of the fruit samples as a consequence of the pink reddish color (Nunes *et al.*, 2006). During increased storage time, the anthocyanin

Table 1. Microbial load on the strawberry surface after treated with UV–C at different treatments time during storages (0, 3 and 6 days) at 25°C

Parameters	Storage Times	Treatment		
		Control	In+1 min	In+6 min
Microorganism	0	+	+	+
	3	+	+	+
	6	+	+	+

Noted: Control: inoculated strawberry; In+1 min: inoculated strawberry treated with 1 min of UV–C; In+6 min: inoculated strawberry treated with 6min of UV–C. The symbol – = clean (not detect), + = very low contaminated (<20 colony on agar plate), + = low contaminated (20–60 colony on agar plate), ++ = mid contaminated (60–200 colony on agar plate), +++ = high contaminated (>200 colony on agar plate)

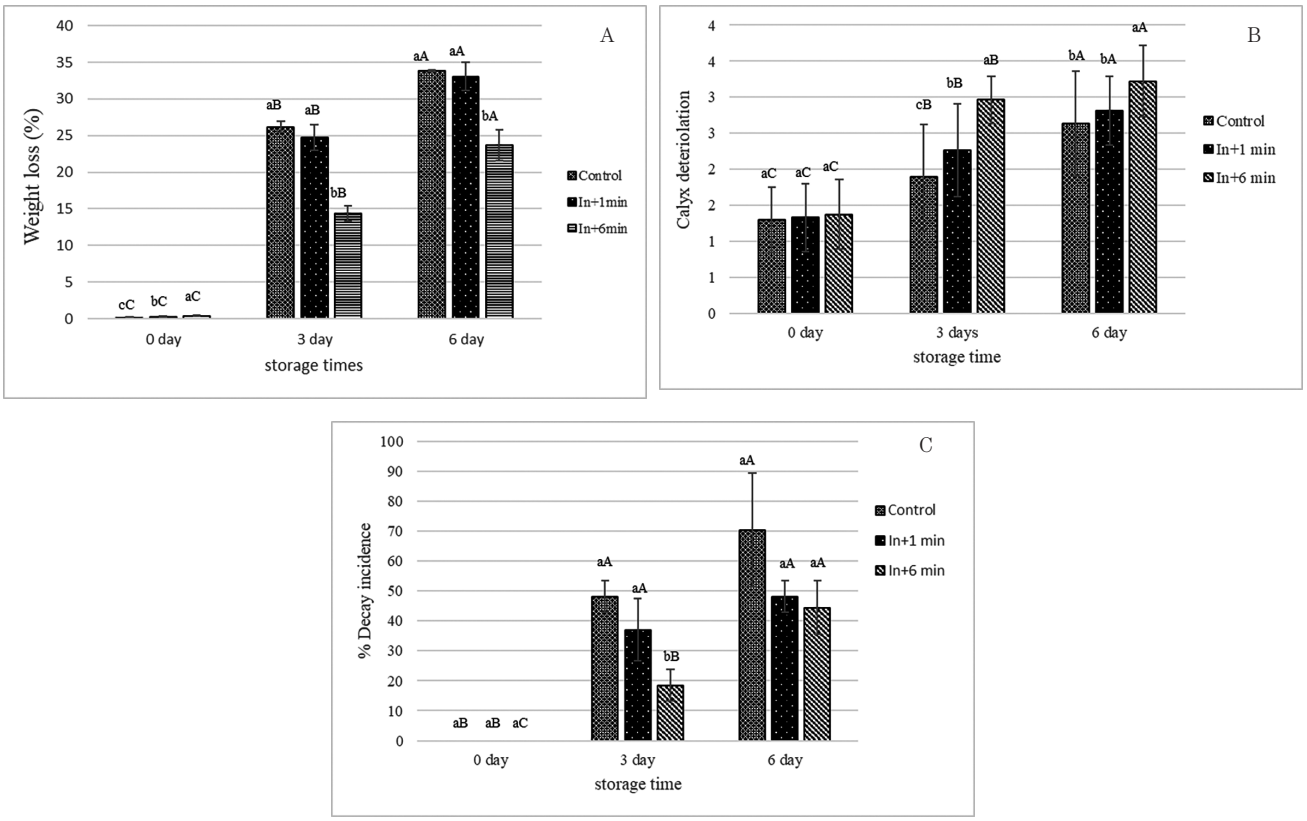

Fig. 2. Weight loss (A), Calyx deterioration (B) and % Decay incidence (C) of strawberry after treated with UV–C at different treatment times during storage. Note: Control: strawberry without treatment; Control: inoculated strawberry; In+1 min: inoculated strawberry treated with 1 min of UV–C; In+6 min: inoculated strawberry treated with 6 min of UV–C. Different lowercase letters on the bars indicate significant differences in treatments at the same storage times ($P<0.05$). Different uppercase letters on the bars indicate significant differences in same treatment at different storage times ($P<0.05$). Bars represent the standard deviation (n=27).

synthesis pathway was taken place. It led to change the color of fruit. Xu *et al*. (2017) revealed that UV–C irradiation can increase total anthocyanin and phenolic compound levels of strawberry.

Strawberry in all treatments turned to be red after storage. It was coincidental with the increase in a^* value (Table 2). The red color in strawberry fruit is derived from the orange–brown anthocyanin pigments of pelargonidin glycosides (Perkins – Veazie, 2010). Several reports have indicated that UV light exposure promotes anthocyanin synthesis in strawberries and sweet cherries (Maraei and Elsawy, 2017). The result was in accordance with Pinheiro *et al*. (2015) who reported that tomato treated with UV at various levels turned to be redder during storage. When compared at the end of storage (6 day), the lowest a^* value was observed in In+6 min sample. The result pointed out that high intensity of UV–C can be retarded senescence stage of the strawberry similarly with Pan *et al*. (2004) reported that UV light illumination has effectiveness to delay anthocyanin accumulation in strawberry fruit. Also, this phenomenon has been reported in strawberries where high doses of UV light exposure are thought to cause too much stress and possibly result in injury. Furthermore, there was no significant difference ($P \geq 0.05$) in b^* value. Thus, using UV–C high intensity (6 min) treated strawberry can retard reaching ripening and senescence stage of strawberry.

Texture firmness

Firmness of stored strawberry after treated with UV–C with different levels is illustrated in Table 3. Texture is a critical quality attribute in the consumer acceptability of fresh fruit and vegetables (Cao *et al*., 2010b). Strawberry is a soft fruit that suffers a rapid loss of firmness during storage, which contributes greatly to its short postharvest life and susceptibility to fungal contamination (Hernandez–Munoz *et al*., 2008). Statistical analysis did not show significant differences ($P \geq 0.05$) in the firmness between all samples immediately after treatments (0 day). In present study, fruit firmness of strawberries decreased significantly during storage ($P < 0.05$) due to these samples reached for the mature and senescence stage. However, at 6 days of the storage, the firmness of control and In+1 min samples had slightly decreased. It might be related to the activities of microorganism growing during the storage. The insufficient of UV–C treatment caused softening and decay on fruit surface. Hashmi *et al*. (2016) reported that strawberry with lower firmness and higher respiration rate were also higher in rots. Those results were coincident with high weight loss (Fig. 2A) and visual fungal attack evidence (Fig. 3). At 6 days of the storage, the reduction of firmness was found in In+6 min sample because the surface of strawberry shriveled. When the shriveling surface of fruit occurred, those shriveled surface was less resistant to force applied leading to the lower of firmness. Therefore, treatment of strawberry using high UV–C has adversely been affecting their firmness.

pH and ºBrix

pH plays an important role in food safety and quality, since it affects the growth and type of microbial flora spoiling the product as well as its sensory and organoleptic properties (Pataro *et al*., 2015). No significance ($P \geq 0.05$) was noticeable in all samples at day 0 (Table 3). In general, pH of strawberry (6.60–6.70) was significantly decreased ($P < 0.05$) with increasing storage time, regardless of UV–C treatment times. The pH reduction resulted might be due to the increase of fungal population, growth of which produced a large number of metabolites and acid mucus (Vieira *et al*., 2016). Sangkasanya (2013) revealed that the acid accumulation in longkong was probably due to anaerobic or fermentative metabolism during storage.

ºBrix value is used as an indicator of strawberry quality in part of sweet taste. At 0 day, ºBrix value of all

Table 2. Color of strawberry after treated with UV–C at different treatment time during storage at 25ºC

Parameters	Storage Times	Treatments		
		control	In+1 min	In+6 min
L^*	0	37.06 ± 6.54^{aA}	36.80 ± 6.82^{aA}	38.47 ± 5.47^{aA}
	3	36.24 ± 3.97^{aA}	35.77 ± 4.88^{aA}	36.55 ± 5.27^{aA}
	6	33.07 ± 3.75^{bA}	35.01 ± 4.47^{aA}	33.73 ± 4.79^{bB}
a^*	0	36.63 ± 4.59^{aC}	36.00 ± 4.91^{aB}	35.84 ± 3.31^{aB}
	3	44.67 ± 2.99^{aA}	40.74 ± 4.34^{bA}	39.15 ± 3.19^{bA}
	6	41.87 ± 3.58^{aB}	40.66 ± 4.02^{abA}	38.61 ± 4.27^{bA}
b^*	0	26.29 ± 4.29^{bB}	28.32 ± 6.13^{bA}	31.80 ± 4.37^{aA}
	3	30.89 ± 4.92^{aA}	25.18 ± 6.43^{bB}	27.50 ± 5.08^{abB}
	6	25.67 ± 4.87^{aB}	25.55 ± 6.04^{aB}	24.00 ± 4.64^{aC}

† Mean ± SD (n=27).
Control: inoculated strawberry; In+1 min: inoculated strawberry treated with 1 min of UV–C; In+6 min: inoculated strawberry treated with 6 min of UV–C. Different lowercase superscripts in the same row indicate the significant differences treatments ($P < 0.05$). Different uppercase superscripts in the same treatment under the same state of parameters indicate the significant differences ($P < 0.05$).

samples did not differ significantly ($P<0.05$) as shown in Table 3. When the storage time increased, °Brix value slightly increased without significance ($P\geq0.05$), regardless of treatments. Ripening stage of strawberry might be caused of total soluble solid increased (Hernandez–Munoz *et al.*, 2008). The solubilization of the cell wall polyuronides and hemicelluloses in mature strawberry might also contribute to the increase in soluble solid content (Hernandez–Munoz *et al.*, 2008). Thus, UV–C treatment did not affect pH and °Brix of strawberry.

Table 3. Firmness, pH and °Brix of strawberry after treated with UV–C at different treatment time during storage at 25°C

Parameters	Storage Times	Treatments		
		control	In+1 min	In+6 min
Firmness (N)	0	19.26±0.12^{aA}	19.30±0.08^{aA}	19.25±0.12^{aA}
	3	19.22±0.14^{aA}	19.19±0.42^{aAB}	19.21±0.15^{aA}
	6	18.60±1.41^{aB}	18.73±1.40^{aB}	18.86±0.77^{aB}
pH	0	0.88±0.13^{aA}	0.88±0.13^{aA}	0.92±0.11^{aA}
	3	0.79±0.10^{aA}	0.79±0.10^{aB}	0.83±0.10^{aB}
	6	0.77±0.0.8^{aB}	0.77±0.08^{aB}	0.79±0.12^{aB}
°Brix	0	8.80±0.70^{aB}	9.24±0.83^{aA}	9.01±0.84^{aA}
	3	9.26±0.75^{aAB}	9.25±0.82^{aA}	9.08±0.49^{aA}
	6	9.41±1.09^{aA}	8.81±0.56^{aA}	9.22±0.66^{aA}

† Mean ± SD (n=27).
Control: inoculated strawberry; In+1 min: inoculated strawberry treated with 1 min of UV–C; In+6 min: inoculated strawberry treated with 6 min of UV–C. Different lowercase superscripts in the same row indicate the significant differences treatments ($P<0.05$). Different uppercase superscripts in the same treatment under the same state of parameters indicate the significant differences ($P<0.05$).

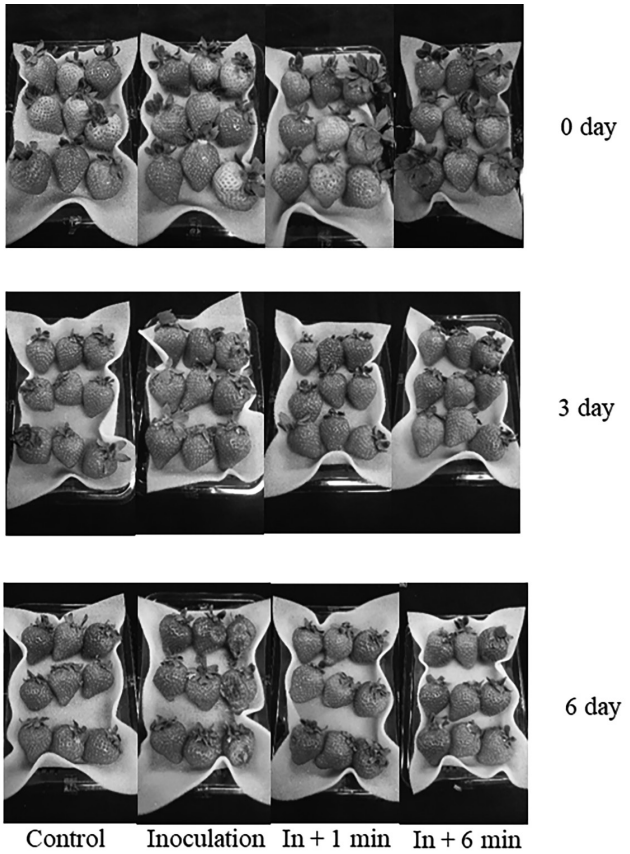

Fig. 3. Visual fungal attack evidence of strawberry after treated with UV–C at different treatment times during storage. Note: Control: inoculated strawberry; In+1 min: inoculated strawberry treated with 1 min of UV–C; In+6 min: inoculated strawberry treated with 6 min of UV–C.

CONCLUSION

The UV–C treatment had potential to improve quality of strawberry during storage. The efficiency of UV–C treatment in improving the fruit quality was governed by treatment time, which determined based on the decay incidence and microbial population on the strawberry surface. Strawberry treated with UV–C for 6 min (In+6 min) showed the highest efficacy to reduce microbial growth; however, the treatment caused negative effect on calyx freshness and decreased shear force. Thus, combination of different postharvest treatments still required to improve the quality of the fruit and reduce food loss during food process chain.

AUTHOR CONTRIBUTION

P. KINGWASCHARAPONG and Y. IIDA designed the study, performed data analysis and wrote paper. F. TANAKA and F. TANAKA designed the study, supervised the work. All authors assisted in editing the manuscript and approved the final version.

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