Improving the storage quality of Tahitian limes (*Citrus latifolia*) by pre-storage UV-C irradiation

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Abstract UV-C (180–280 nm) has been shown to extend the postharvest shelf-life of many horticulture crops. In this study, Tahitian limes (*Citrus latifolia*) were exposed to 0, 3.4, 7.2 and 10.5 kJ m⁻² UV-C then stored for 28 days in air at 10 °C and 80% RH. Weight loss, peel colour, calyx abscission, ethylene production, respiration rate, total soluble solids (TSS), titratable acidity (TA) and acceptability index were assessed. The results showed that UV-C treatment maintained lime peel green colour and retained calyx attachment after 28 days storage. UV-C treatment also affected endogenous ethylene production and respiration rate, where the highest UV-C treatment (10.5 kJ m⁻²) maintained low ethylene production and low respiration rates after 28 days storage with no differences between the different UV-C intensities. In terms of fruit acceptability, limes were exposed to 10.5 kJ m⁻² UV-C had a 60% acceptability index after 28 days storage, while untreated control fruit retained acceptability of 39%. In general, the pre-storage UV-C treatments did not affect fruit weight loss, TSS or TA contents during storage.

Keywords *Citrus latifolia* · TSS · Colour · Ethylene · Respiration · Calyx abscission

Introduction

Tahitian limes are harvested when the rind is green and the postharvest maintenance of the skin green colour is paramount (Sri-laong et al. 2011). Retention of the green colour of the skin is a key commercial trait for Tahitian limes (*Citrus latifolia*). Therefore many studies have aimed to retain peel green colour using a range of physical and chemical methods, such as heat treatments (Kaewsuksaeng et al. 2015; Opio et al. 2017), UV-B treatments (Aiamla-or et al. 2010; Kaewsuksaeng et al. 2011; Sri-laong et al. 2011), lignin extracts (Jonglertjunya et al. 2014) and 1-methylcyclopropene (Win et al. 2006).

Ultraviolet-C (UV-C) (180–280 nm) irradiation has been reported to have beneficial effect on maintaining postharvest quality of horticultural produce. For example, UV-C irradiation has been shown to delay degreening of broccoli (Buchert et al. 2011), spinach (Artés-Hernández et al. 2009) and limes during storage (Pristijono et al. 2018a). In addition, UV-C irradiation treatment has been reported to reduce pathogen growth (Guerrero-Beltrán and Barbosa-Cánovas 2004) and decay in capsicums (Mercier et al. 2001) and also to delay ripening and senescence in oranges (D'hallewin et al. 1999), grapefruit (D'Hallewin et al. 2000), tomatoes (Tiecher et al. 2013), potatoes (Pristijono et al. 2018b) and mangoes (Pristijono et al. 2019). Therefore, postharvest UV-C treatment has the potential to become a low cost, low technology for reducing fruit and vegetable loss in the supply chain.

The optimum storage temperature for Tahitian limes is 10 °C (Burns 2016). When fruits are stored at ambient temperature (20 °C), the peel degreening process is more rapid and the fruit become yellow more quickly. Therefore, to store limes at 10 °C is essential to maintain limes quality after harvest. To date, the effect of UV-C irradiation,

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followed by storage at 10 °C on the postharvest life has not been reported. The objective of this study was to evaluate the effect of UV-C irradiation followed by storage in air at 10 °C on lime fruit quality for up to 28 days.

Materials and methods

Produce

Commercial fresh green Tahitian limes (*Citrus latifolia*), free from damage and uniform in colour, shape and size were randomly selected and sorted into experimental units of 16 fruits in each treatment unit. All experiments were conducted on three separate occasions (three replicates) with different batches of fruit used for each replicate.

UV-C treatment and storage conditions

The UV-C treatments were conducted using a custom made light proof box fitted with two germicidal lamps (Sahkyo Denki Co. Ltd G20T10 20 Watt, Low Pressure Mercury). A SED008/W detector with PIR irradiance calibration at 254 nm was used to monitor UV-C intensity. UV-C intensity was determined prior to treatment by measuring the light intensity (kJ m^{-2}) using an International Light Technologies 1700 series research radiometer. UV-C dosage (kJ m^{-2}) was calculated by multiplying the emitting UV light intensity with treatment time in seconds. Light intensity was evaluated several times throughout the experiment to ensure consistent output. The surface of each lime was irradiated by placing it approximately 20 cm from the UV-C light source, with exposure occurring sequentially on each side of the fruit (by rotating through 180 °C) to ensure complete coverage. Irradiation treatment was carried out at room temperature (20 ± 1 °C) and at a relative humidity (RH) of 80%, temperature and relative humidity were recorded using calibrated TinyTag data loggers. Pre-storage treatments consisted of non-irradiated (0 kJ m^{-2} UV-C or control) and irradiated fruit with different intensities of 3.4, 7.2 and 10.5 kJ m^{-2} UV-C. After treatment, the limes from each unit were packed into unsealed low density polyethylene (LDPE) bags and stored at 10 °C and 80% RH for up to 28 days. During storage, fruit were removed at 7, 14, 21 and 28 days and stored for an additional 3 days at 20 °C to simulate commercial shelf-life before quality assessment was conducted.

Determination of Fruits quality attributes

Fruits were removed from storage at 7, 14, 21, 28 days and after simulated shelf-life for 3 days at 20 °C, fruit were assessed for weight loss, calyx detachment, skin colour,

respiration rate, ethylene production, total soluble solids (TSS), titratable acidity (TA) and overall acceptability index. Lime weight loss was measured according to Van Dijk et al. (2006), where weight loss was expressed as a percentage, relative to the initial weight of each lime as shown in Eq. 1.

$$\text{Weight loss (\%)} = \left(\frac{W_0 \times W_t}{W_0} \right) \times 100 \quad (1)$$

W_0 is the average of initial weight and W_t is the average weigh on removal day.

Calyx condition was assessed using a scoring system based on being attached (1) or detached (0) to the fruit. Skin colour was estimated by Hue angle (°Hue) using a Minolta colorimeter (Minolta CR-400, Osaka). The results were the means of four points of fruit surface and expressed as Hue angle (°Hue) as shown in Eq. 2.

$$\text{Hue} = \text{artangent} \left(\frac{b^*}{a^*} \right) \quad (2)$$

The ethylene production was determined according to Pristijono et al. (2019), limes were transferred to a sealed, 1500 mL glass jar at 20 °C for 3 h, ethylene concentration was measured by injecting a gas sample (1 mL) into a flame ionization gas chromatograph (Gow-Mac 580, Bridgewater NJ) fitted with a stainless steel column (2 m × 3.2 mm OD × 2.2 mm ID) packed with Porapak Q (80–100 mesh) (Altech, Sydney), with 110, 90 and 70 °C as the operating temperature of the detector, column, and the injector, respectively. Nitrogen, hydrogen and air were used as carrier and combustion gases at flow rates of 60, 30 and 300 mL min^{-1} , respectively. The ethylene production rate was calculated by the Eq. (3) and expressed as $\mu\text{L C}_2\text{H}_4 \text{ kg}^{-1} \text{ h}^{-1}$.

$$\begin{aligned} \text{Ethylene production } (\mu\text{L C}_2\text{H}_4 \text{ kg}^{-1} \text{ h}^{-1}) \\ = \frac{C_2H_4 (\mu\text{L L}^{-1}) \times \text{volume of container (L)}}{\text{Initial produce weight (kg)} \times \text{time (h)}} \end{aligned} \quad (3)$$

Respiration rate was measured by the method described by Pristijono et al. (2017a), where 4 limes from each replicate were allocated into 1500 mL hermetic glass jars with a septum in the lid at 20 °C, and headspace gas sample (5 mL) was collected by a syringe after 3 h, and transferred to an ICA40 series low-volume gas analysis system (International Controlled Atmosphere Ltd., Kent, UK). Respiration rate was calculated by the Eq. (4) and expressed as $\text{mL CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$.

$$\begin{aligned} \text{Respiration } (\text{mL CO}_2 \text{ kg}^{-1} \text{ h}^{-1}) \\ = \frac{\% \text{CO}_2 \times \text{Volume of container (mL)}}{\text{Initial produce weight (kg)} \times 100 \times \text{time (h)}} \end{aligned} \quad (4)$$

Total soluble solids (TSS), expressed as °Brix, was measured from the pressed juice of fruit with a digital

refractometer (ATAGO Inc., Bellevue, WA, USA). Titratable acidity (TA), expressed as percentage of citric acid, was determined by titrating 1 mL lime juice to pH 8.2 with a 0.1 N NaOH standard solution using an automatic titrator (Mettler Toledo T50, Switzerland).

The overall acceptability index for limes was calculated according to the method of Pristijono et al. (2017b), with some modifications. Overall acceptability index was assessed visually, based skin colour, skin glossiness or/and calyx intact using the scores of 1 = severe degreening or calyx detached; 2 = severe degreening, dull skin or calyx detached; 3 = slight degreening, shiny skin and calyx detached; 4 = green, shiny skin and calyx intact; and 5 = fresh as just harvested and was calculated as follows:

$$\text{Acceptability index (\%)} = \frac{\sum (\text{acceptability score (1–5)} \times \text{number of fruit at that level})}{\text{highest level} \times \text{total number of fruits}} \times 100 \quad (5)$$

Statistical analysis

The two-way ANOVA and the Least Significance Difference (LSD) calculations were performed using the SAS software package (SAS Ver. 9.4, USA). Differences among means were analysed at a significance level of $p < 0.05$ and compared by using least significant difference (LSD).

Results and discussion

The fruit used in this experiment were uniformly green where the skin hue angle was 119.0 (indicating green colour) and showed no significant difference ($p < 0.05$) between individual batches denoting high homogeneity between the three batches (replicates). The overall peel colour, ethylene production, respiration rate, TSS and TA of the lime fruit at the beginning experiment is presented in Table 1. However, there was no pattern in quality

Table 1 Quality parameters of limes at the beginning of experiment

Parameter	Value
Colour (°Hue)	119.0 ± 0.2
Ethylene production ($\mu\text{L C}_2\text{H}_4 \text{ kg}^{-1} \text{ h}^{-1}$)	0.019 ± 0.005
Respiration rate ($\text{mL CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$)	14.56 ± 0.16
TSS (°Brix)	7.9 ± 0.2
TA (% citric acid)	5.95 ± 0.27
Ratio TSS to TA	1.34 ± 0.07

Values represent the mean and standard error (S.E.) of three replicates consisting of 19 limes in each replicate

parameters during storage affected by UV-C treatments and storage time due to the quality assessment was conducted on different fruits of each removal time lead to varied in quality parameters in assessment time, thus the effect of UV-C treatments on limes was tested in each removal time to compare the effect of UV-C treatments. The variation of quality may be due to physio chemical reaction of internal substances, for example sugar variation in strawberry affected by UV-C treatment (Baka et al. 1999), where presumably the sugar variation is related to the ultraviolet stress and the stimulation of intercellular synthetic enzymes, such as invertase or phosphorylase (Stitt and Steup 1985). Imaizumi et al. 2018 also reported that there was variation of ascorbic acid levels in cucumbers following UV-C treatments, they observed that there was possible effect of UV-C irradiation on ascorbic acid metabolic pathway.

Weight loss

Weight loss is related to the respiration and transpiration of the fruits, being a combination of water loss and solutes (Cosme Silva et al. 2017). Results for the weight loss after storage are presented in Table 2 and show the average weight loss increased with storage time. However there was no effect on weight loss of fruit pre-storage irradiated with UV-C treatment at different intensities from 3.4 to 10.5 kJ m^{-2} , followed by storage at 10 °C and relative humidity of 80%. This observation was contrary to Pinheiro et al. (2015) who reported that tomatoes irradiated with UV-C radiation at 4.83 kJ m^{-2} followed by storage at 10 °C for 15 days resulted in 5% weight loss compared to 14% weight loss in the untreated control fruit. The difference may be due to the difference in the respiration rate

Table 2 Weight loss and calyx intact of limes after treatment with UV-C, followed by storage up to 28 days in air at 10 °C

UV-C treatments	Storage time (days) + 3 days at 20 °C			
	7	14	21	28
Weight loss (%)				
0.5 kJ m^{-2}	1.2 ^a	2.2 ^a	1.3 ^b	2.5 ^a
3.4 kJ m^{-2}	1.3 ^a	1.8 ^{ab}	1.5 ^{ab}	2.2 ^a
7.2 kJ m^{-2}	1.1 ^a	1.8 ^{ab}	2.0 ^a	2.7 ^a
10.5 kJ m^{-2}	0.9 ^a	1.4 ^b	1.0 ^b	1.5 ^a
Calyx intact (%)				
0.5 kJ m^{-2}	88 ^a	75 ^a	75 ^a	75 ^c
3.4 kJ m^{-2}	100 ^b	63 ^a	100 ^b	75 ^c
7.2 kJ m^{-2}	100 ^b	100 ^b	75 ^a	88 ^b
10.5 kJ m^{-2}	100 ^b	100 ^b	100 ^b	100 ^a

Values are the mean of 3 replicates. Different letters indicate mean values at the same columns that are statistically different at $p < 0.05$

between tomatoes and fruit used for this experiment (limes) during storage. The changes of fruits weight losses results from fruit physiological activities include transpiration and respiration rates. Daiuto et al. (2013) reported that the application of UV-C at high intensity was effective in delaying respiration rate of avocado fruit, which retards the increase of fruit weight loss.

Calyx abscission

Consumers often use lime fruit with calyx (button) attach as an indicator of lime freshness. The effect of UV-C treatment on calyx detachment in limes are presented in Table 2 and show that pre-storage UV-C treatment had a significant ($p < 0.05$) positive effect on the retention of the calyx onto the lime fruit. The calyxes on fruit treated with the highest UV-C treatment (10.5 kJ m^{-2}) did not abscise and all calyxes were still attached after 28 days storage, whilst the untreated control fruit retained 75% of calyxes. There was no interaction between UV-C treatment and storage time on calyx abscission.

Ethylene production

Limes are a non-climacteric fruit which means they do not produce a characteristic burst of endogenous ethylene during ripening. In this experiment, ethylene production rates generally increased at all removal and assessment times during storage, and were affected by pre-storage UV-C treatment (Table 3).

There was an interaction impact between UV-C treatments and storage time on endogenous ethylene

production, where after 28 days storage the highest UV-C intensities (10.5 kJ m^{-2}) produced the lowest ethylene production of $0.050 \mu\text{L C}_2\text{H}_4 \text{ kg}^{-1} \text{ h}^{-1}$ than other treatments.

After 21 and 28 days storage, untreated limes produced significantly higher levels of ethylene production rates than UV-C treated fruit, where the highest UV-C treatment (10.5 kJ m^{-2}) had significantly lower ethylene production rates than the lower UV-C intensity treatments. Higher ethylene production rates in the untreated control fruits were associated with senescence resulting in more rapid deterioration of physical characteristics and visual assessment of skin colour (color meter) turned low Hue values indicative of severe degreening at 10°C . The suppression of endogenous ethylene production in UV-C treated lime fruit has also been reported in peaches (Stevens et al. 1998), tomatoes (Bu et al. 2013) and lime fruit (Pristijono et al. 2018a). This result suggests that suppression of endogenous production may be a mechanism of UV-C treatment in delaying lime fruit senescence.

The period in storage also had an effect on fruits ethylene production rates, where the average ethylene production at the end experiments (28 days) was $0.076 \mu\text{L C}_2\text{H}_4 \text{ kg}^{-1} \text{ h}^{-1}$, which was fourfold increase from the beginning of the experiment.

Respiration rate

Limes are a non-climacteric fruit where the natural ripening of citrus fruit is not accompanied by any rises in respiration rates (Eaks 1970). The pre-storage UV-C treatments followed by storage at 10°C resulted in lower respiration rates than untreated control fruit after 28 days storage (Table 3). However, there were no differences between the different UV-C doses. These results are in agreement with the previous reports which showed that UV-C treatment resulted in lower respiration rates in peaches (Lu et al. 1991), strawberry (Baka et al. 1999), pepper (Vicente et al. 2005), broccoli (Costa et al. 2006), and avocado (Daiuto et al. 2013).

There was an interaction effect between UV-C treatment and storage time on respiration rates, where the 10.5 kJ m^{-2} UV-C had the lowest respiration rate of $13.44 \text{ mL CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$ after 28 days storage. However, the effects of UV-C treatment on respiration rate were not as marked as the effect of ethylene production.

Peel colour

The green peel colour is the most important factor for marketing of Tahitian limes. As expected that the peel degreening was affected by storage time, where the longer storage times resulted in lower hue values (less green—

Table 3 Ethylene production and respiration rate of limes after treatment with UV-C, followed by storage up to 28 days in air at 10°C

UV-C treatments	Storage time (days) + 3 days at 20°C			
	7	14	21	28
Ethylene ($\mu\text{L C}_2\text{H}_4 \text{ kg}^{-1} \text{ h}^{-1}$)				
0 kJ m^{-2}	0.039 ^a	0.052 ^a	0.182 ^a	0.123 ^a
3.4 kJ m^{-2}	0.028 ^a	0.050 ^a	0.135 ^b	0.079 ^b
7.2 kJ m^{-2}	0.026 ^a	0.048 ^a	0.113 ^b	0.055 ^c
10.5 kJ m^{-2}	0.030 ^a	0.050 ^a	0.062 ^c	0.050 ^c
Respiration ($\text{mL CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$)				
0 kJ m^{-2}	17.36 ^a	14.90 ^{ab}	17.71 ^a	17.91 ^a
3.4 kJ m^{-2}	15.08 ^a	15.06 ^a	16.97 ^{ab}	13.96 ^b
7.2 kJ m^{-2}	15.24 ^a	14.43 ^b	16.24 ^b	13.59 ^b
10.5 kJ m^{-2}	15.10 ^a	13.57 ^c	16.25 ^b	13.44 ^b

Values are the mean of 3 replicates. Different letters indicate mean values at the same columns that are statistically different at $p < 0.05$

more yellow) (Table 5). There was no interaction impact between UV-C treatments and storage time on peel colour.

Limes treated with different UV-C intensities, followed by storage at 10 °C showed that pre-storage UV-C treatments retained the green peel colour during storage. Fruits treated with different UV-C treatments were significantly greener than untreated fruit. This effect was observed from 7th day of storage and remained until the end of the experiment (28 days). The different UV-C intensities significantly affected the peel colour, where the highest UV-C treatment (10.5 kJ m^{-2}) retained the higher hue value (i.e. greener skin) than other UV-C intensities after 7 days storage, however the effect of different intensities was lower over storage time. UV-C treatment has been reported to delay degreening in other horticultural produce such as on broccoli, which showed delayed yellowing for 6 days following exposure to 10 kJ m^{-2} UV-C treatment (Costa et al. 2006). Similarly, after exposure of green tomato fruit to UV-C lights and the results showed that UV-C treatment delayed the ripening of tomatoes by maintaining green colour (Liu et al. 2009; Tiecher et al. 2013; Pristijono et al. 2017a). In addition, Artés-Hernández et al. (2009) also reported that UV-C treatment maintained green colour of spinach leaves.

TSS and TA

The effect of the pre-storage UV-C treatments on TSS and TA of limes juice after storage are presented in Table 4, and show that both TSS and TA were not generally affected by the pre-storage UV-C treatments. These results are consistent with those previously reported by Liu et al.

Table 4 TSS and TA of limes after treatment with UV-C, followed by storage up to 28 days in air at 10 °C

UV-C treatments	Storage time (days) + 3 days at 20 °C			
	7	14	21	28
TSS (°Brix)				
0 kJ m^{-2}	8.2 ^{ab}	8.2 ^a	8.2 ^{ab}	7.9 ^a
3.4 kJ m^{-2}	8.4 ^a	8.2 ^a	7.9 ^b	8.2 ^a
7.2 kJ m^{-2}	7.5 ^b	8.3 ^a	8.5 ^a	8.0 ^a
10.5 kJ m^{-2}	7.9 ^{ab}	8.6 ^a	8.1 ^{ab}	8.1 ^a
TA (% citric acid)				
0 kJ m^{-2}	5.73 ^a	4.85 ^b	5.72 ^a	5.20 ^a
3.4 kJ m^{-2}	4.55 ^a	5.84 ^a	4.86 ^b	5.56 ^a
7.2 kJ m^{-2}	5.61 ^a	5.45 ^{ab}	5.604 ^a	5.80 ^a
10.5 kJ m^{-2}	5.62 ^a	5.32 ^{ab}	5.63 ^a	5.85 ^a

Values are the mean of 3 replicates. Different letters indicate mean values at the same columns that are statistically different at $p < 0.05$

(2009) who observed that TSS did not change in tomatoes (cv 'Red Ruby') after treated with 22.8 W m^{-2} UV-C lights stored at 12–14 °C for 21 days. However UV-C treatments have been reported to decrease the levels of TSS and increase TA levels in strawberry (Baka et al. 1999) and tomatoes (Charles et al. 2016), whilst Erdinc and Demir (2009) reported that kiwifruit treated with UV-C lights increased in TSS levels during storage. The difference responses of UV-C treatments of sugar level may be due to defense mechanism.

Limes, untreated and UV-C treated stored at 10 °C (optimum temperature storage for limes) up to 28 days had similar TSS and TA. The results showed that pre-storage UV-C treatments did not affect TSS or TA which are important quality parameters of citrus.

Overall acceptability

The pre-storage UV-C treatments (3.4, 7.2 and 10.5 kJ m^{-2} UV-C) did not cause any phyto-toxicity symptoms or damage on the fruit. The fruit overall acceptability was visually assessed based on the skin colour, skin glossiness or/and calyx intact. The effect of pre-storage UV-C treatments on overall visual acceptability is presented in Table 5 and show that the UV-C treated fruit had higher overall acceptability index than untreated fruits after 14, 21 and 28 days storage. There was significant interaction between UV-C treatment and storage time, where the highest acceptability (60%) after 28 days storage reached by 10.5 kJ m^{-2} UV-C.

The different UV-C intensities also had a significant impact on acceptability index within the 7 days storage

Table 5 Peel colour and overall acceptability index of limes after treatment with UV-C, followed by storage up to 28 days in air at 10 °C

UV-C treatments	Storage time (days) + 3 days at 20 °C			
	7	14	21	28
Peel colour (°Hue)				
0 kJ m^{-2}	115.4 ^a	111.5 ^a	111.9 ^a	110.6 ^a
3.4 kJ m^{-2}	117.4 ^b	116.3 ^b	115.9 ^b	114.7 ^b
7.2 kJ m^{-2}	117.2 ^b	116.0 ^b	117.1 ^c	116.0 ^c
10.5 kJ m^{-2}	119.0 ^c	117.1 ^c	115.6 ^b	116.7 ^c
Acceptability index (%)				
0 kJ m^{-2}	68 ^b	44 ^a	45 ^c	39 ^b
3.4 kJ m^{-2}	75 ^a	58 ^b	58 ^b	54 ^a
7.2 kJ m^{-2}	68 ^b	70 ^c	65 ^a	54 ^a
10.5 kJ m^{-2}	63 ^c	76 ^d	56 ^b	60 ^a

Values are the mean of 3 replicates. Different letters indicate mean values at the same columns that are statistically different at $p < 0.05$

time. However the largest positive effect was observed after 14 days storage where 76% acceptability was observed following the 10.5 kJ m^{-2} UV-C treatment, compared to 44% acceptability of the untreated control fruit at the same storage time. These overall acceptability results are associated with the high level of peel degreening during storage, where after 14 days storage the hue value of UV-C treated was higher (greener peel) than untreated fruits (Table 5). The higher acceptability index is probably related to the high hue value (green peel) and lower in calyx detachment. These results show that limes treated with at least 3.4 kJ m^{-2} UV-C combined with storage at 10°C maintained fruit acceptability.

Conclusion

Application of pre-storage UV-C irradiation (from 3.4 to 10.5 kJ m^{-2} of UV-C) maintained the quality of lime fruit during storage in air at 10°C by delaying peel yellowing, reduced ethylene production, lowered respiration rate, reduced calyx detachment and increased overall acceptability. UV-C treatment had no adverse effects on external skin condition or internal composition (TSS or TA). More study is required to assess the effect of fruit exposed to UV-C irradiation and stored at 10°C , followed by different storage conditions, to determine if the mode of action of UV-C is similar with this study.

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