

## Intermittent warming affects postharvest quality and chilling injury of ‘Holland’ papaya fruit

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### Abstract

Papaya (*Carica papaya* L.) is available all-year round in Thailand, but the export volume is low. This may be attributed to unsuitable temperature control during postharvest management resulting to unacceptable quality. This study aimed to investigate the impact of intermittent warming (IW) on the quality of ‘Holland’ papaya during cold storage. Fruit with 25% peel yellowing were exposed to four conditions: untreated fruit stored at 5°C (T1; control), 15°C (T2) and 2 IW conditions. These were T3 where fruit was stored at 5°C for 4 days then moved to 15°C for 1 day and the process repeated. For by T3 and T4 conditions the fruit were then stored at 5°C, 90% RH until the end of storage. The results showed that, after ten days, fruit stored at 15°C, 80% RH had higher trend in L\* and Chroma. It was also found that their hue angle was significantly ( $p < 0.05$ ) lower than when the fruit had undergone other treatments. The fruit stored at 15°C showed the highest level of respiration and the highest ethylene production rate while the control fruit showed moderate levels. Under both IW conditions the respiration and ethylene production rates were suppressed up to day 9 and the rates increased after that. The control fruit failed to ripen but the IW fruit continued to yellow after 10 days. The control fruit could only be kept for 5 days as they expressed chilling injury (CI) scale from 2 to 4 as moderate (50%), moderate to severe (16.7%) and severe (16.7%) after 10 days of storage. The fruit stored under IW1 condition suffered moderate to severe CI (scale 2-4, 66.7% in total) after 20 days of storage. However the IW2 fruit exhibited only moderate and moderate to severe CI (scale 2 & 3, 33.3% in total). This research concluded that storage using IW2 condition was best at retaining the quality of stored papaya fruits for 20 days.

**Keywords:** disorder, ethylene, respiration, temperature conditioning

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### 1. Introduction

Papaya is classified as a climacteric fruit [1, 2], which its ripening can be progressed when the fruit are harvested at the optimum maturity stage, [2]. There are several papaya cultivars in Thailand such as ‘Khak Nuan’, ‘Khak Dum’ and ‘Pak Mai Lai’ or ‘Holland’ [3, 4, 5, 6]. ‘Khak Nuan’ and ‘Khak Dum’ cultivars are commonly consumed as a ripe fruit as well as a raw papaya spicy salad known as ‘Som Tam’ while the ‘Holland’ cultivar is popular in Thailand for ripe fruit consumption [5, 6]. In 2014, the production of ‘Holland’ papaya increased due to its increased yield when compared with the local cultivars. ‘Holland’ papayas are popular for the domestic and export markets due to their small size, higher yield and more

favourable price when compared to the local varieties [7]. In 2010, Thailand ranked number eight in papaya production in the world with a yield of 211,594 metric tons [8]. During the years 2012-2015, Thailand’s papaya production was reported to fluctuate between 206,475 and 166,679 metric tons [9]. However, in 2015-2016, the export quantity of fresh papaya increased from 920 to 1,150 metric tons with a value of 33,277 to 34,528 thousand THB (around 1 million USD) [10]. From these figures it can be seen that the majority of papaya production in Thailand is used for domestic consumption.

Unfortunately this tropical fruit has some limitations for exportation due to chilling sensitivity during storage and transportation. Another problem is its rapid ripening under ambient temperature conditions (~30°C) during transport and sale that leads to deterioration in less than 3 days [5]. Cold storage has

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the potential to be used to minimize the metabolic activities and in turn maintain the product quality for an extended period after harvest [11]. Generally, low temperatures are known to be the most important factor for maintaining the quality of most fresh products. Low temperatures help by delaying ripening, senescence, undesirable metabolic changes, and rot. Unfortunately, low temperature storage can be harmful for some crops, chiefly those of tropical or subtropical origin [12]. Exposing these chilling-sensitive products to cold storage temperatures for too long can lead to a series of physiological dysfunction collectively known as chilling injury (CI), resulting in a significant loss of quality [13]. This problem can be avoided if the low temperature stress is removed before irreversible damage occurs [11]. It is therefore proposed that by disturbing low temperature storage with one or shorter warming periods may be a potential strategy to avoid problems with CI. Intermittent warming (IW) can be defined as a periodic transfer of the product from a chilled temperature to a non-chilled temperature for one or more periods of time, in order to increase the resistance of the product to low temperatures, and to store the product at temperatures below their critical temperature [14].

Although IW is primarily used to alleviate CI, it also improves maintenance of fruit quality during postharvest storage. For example; 'Paraguay' peaches, (*Prunus persica* L. Batsch) were subjected to IW three cycles of 1 day at 20°C and every 6 days at 2°C. This slowed the ripening process i.e. softening, flesh color and preserved the juiciness of the fruit [15]. 'Durinta' Tomatoes (*Lycopersicon esculentum* Mill.) at breaker stage were stored at 9°C and warmed to 20°C for 1 day every week, up to 28 days. It was found that fruit from this process provided better fruit rind and flavour compared with the control fruit stored at 9°C [16].

Many studies reported the quality changes in perishable produces during cold storage however no information was found about quality changes for 'Holland' papaya grown in Thailand stored at low temperature with application of IW regime. This study, therefore, aimed to investigate the impact of different IW conditions on physicochemical quality and physiology of papaya fruit without using any harmful chemicals, as well as its impact on extending the storage life.

## 2. Materials and Methods

### 2.1. Raw materials

Papaya fruit cv. Holland was obtained from a commercial orchard located in Chiang Rai province, Thailand. It was then transported within 2 hours to the postharvest laboratory, school of Agro-Industry, Mae Fah Luang University in Chiang Rai province. Fruits with 25% peel yellowing were selected according to export grade (size code 4, 801-1100 g) [17, 18, 19]. Fruit were sanitized using chlorinated water (200

ppm) for 1 min, then left to dry for approximately 1 hour at room temperature ( $27\pm 1^\circ\text{C}$ ) before storage.

### 2.2. Methods

The fruits were selected and divided into four treatments with three replicates of each and two fruits per replicate. All the stored fruit were packed in foam boxes with size (60 cm × 45 cm × 30 cm) according to the treatments. The four treatments were; (1) control fruit continuously stored at 5°C, 90% RH, (2) fruit continuously stored at 15°C, 80% RH. (3) intermittent warming condition 1 (IW1) fruit were stored at 5°C for 4 days then subjected to 15°C for 1 day for only 1 cycle after that stored at 5°C until the end of storage, and (4) intermittent warming condition 2 (IW2) fruit were stored at 5°C, 4 days and moved to 15°C, 1 day for 2 cycles then stored at 5°C until the end of storage (Table 1). Samples were taken from each treatment for quality assessment every 5 days during the 20 days of storage.

### 2.3. Non-destructive analyses

External color was measured on opposite sides at the centre of 6 papaya fruits using a color reader (model CR-10, Konica Minolta Inc., Osaka, Japan) set for CIELAB color space and D65 light source. Color measurements were expressed in terms of Lightness ( $L^*$ ), Chroma ( $C^*$ ) and hue angle ( $h^\circ$ ). The instrument was completely in contact with the fruit to avoid any light leakage from the light emitted by the color reader

Fruit respiration rate ( $\text{CO}_2$  production) and ethylene ( $\text{C}_2\text{H}_4$ ) emission were measured at room temperature (27°C) using the static method. These were measured every 4-5 days during the storage period. The treated and untreated papaya fruits were chosen randomly and allowed to stabilize at room temperature. Measurement was started after the fruits had been removed from the cold storage for four hours. Each fruit was incubated for two hours in air tight container (5,000 ml) fitted with rubber septum and kept at room temperature.

Ethylene and  $\text{CO}_2$  production rates were measured at the same time by withdrawing 5 ml of accumulated gas with a gastight syringe through the rubber. Gas samples were injected into a gas chromatography machine (model 7890A, Agilent Technologies Inc., Wilmington, DE, USA) equipped with flame ionization detector (FID) as a capillary column (length 30 m, I.D 0.59 mm, film 15  $\mu\text{m}$ ) for  $\text{C}_2\text{H}_4$  and thermal conductivity detector (TCD) fitted with a stainless steel porapak Q column (3 m × 1/8"; 50/8 mesh) for  $\text{CO}_2$ . The helium gas flow rate was 8 ml  $\text{min}^{-1}$  for ethylene and 10 ml  $\text{min}^{-1}$  for  $\text{CO}_2$  while hydrogen gas flow rate and air were 45 and 400 ml  $\text{min}^{-1}$  respectively. The detector temperatures for FID and TCD were maintained at 200°C and the oven temperature was set at 50°C. The ethylene production rate was expressed in

**Table 1.** The details of proposed treatments for 'Holland' papaya until 20 days

Treatments	Cold storage & Days in storage	1 <sup>st</sup> Warming cycle & Day in storage [Moved to normal cold storage]	2 <sup>nd</sup> Warming cycle & Day in storage	Storage at 5°C until the end of storage
5°C	5°C (20 days)	-	-	yes
15°C	15°C (20 days or until spoilage)	-	-	no
IW1	5°C (4 days)	15°C (1 day)	-	yes (15 days)
IW2	5°C (4 days)	15°C (1 day) [moved to 5°C for 4 days]	15°C (1 day)	yes (10 days)

units of  $\mu\text{L C}_2\text{H}_4 \text{ kg}^{-1} \text{ hr}^{-1}$  and the  $\text{CO}_2$  production rate was expressed in units of  $\text{mL CO}_2 \text{ kg}^{-1} \text{ hr}^{-1}$ . The ethylene and  $\text{CO}_2$  production rate were calculated according to Saltviet [20].

#### 2.4. Destructive analyses

The total soluble solids (TSS) content was measured from fruit juice of 5g of papaya flesh, ground for 5 min in a mortar and then filtered through sheet cloth. A drop of fruit juice was used for measurement the TSS content by using a digital refractometer (Atago, Japan) and reported as % (°Brix).

The titratable acidity (TA) was analyzed using the titration method described by Ranganna [21]. The TA was calculated and expressed as percentage of citric acid.

Total chlorophyll content was measured according to the method of Witham *et al.* [22] with slight modification. 1g of plant sample (peel) was extracted in a mortar with 80% acetone, the homogenate was filtered through Whatman filter paper No.1, the extraction was repeated until no color was obtained then the final volume of extract was adjusted to 50 ml with 80% acetone. The absorbance at 652 nm was recorded from a spectrophotometer (model G10S UV-Vis, Thermo Fisher Scientific, Madison, WI USA). The chlorophyll concentration was expressed as  $\mu\text{g}$  chlorophyll per g fresh weight ( $\mu\text{g g}^{-1} \text{FW}$ ).

The carotenoids analysis was carried out using a modified version of the method described by Witham *et al.* [22] and Gayosso-García Sancho *et al.* [23]. One gram of papaya (peel) sample was added to 2.5 mL of distilled water and 25 mL of 95% hexane. It was then homogenized for 1 min and the mixture solution was centrifuged at  $2000\times g$ , 4°C for 5 min. The upper yellow layer was carefully taken for absorbance measurement at 454 nm using the same model of spectrophotometer. The total carotenoid concentration was calculated as  $\beta$ -carotene equivalents and expressed as  $\mu\text{g g}^{-1} \text{FW}$ .

#### 2.5. Physiological disorder (chilling injury, CI)

Chilling injury (CI) percentage (skin pitting and scald) was assessed on 6 fruits per treatment at each

stage. A 0 to 4 visual rating scale for symptom severity was used where the assessment was performed according to the following hedonic scale\* that was modified from Proulx *et al.* [24]. 0 = no abnormality (0%), 1 = trace symptoms, small pits (~1-10%), 2 = moderate symptoms, small to medium pits, blotchy appearance (~11-25%), 3 = moderate to severe symptoms (~26-50%), 4 = severe symptoms (> 50%). The CI percentage (%) was calculated according to following equation:

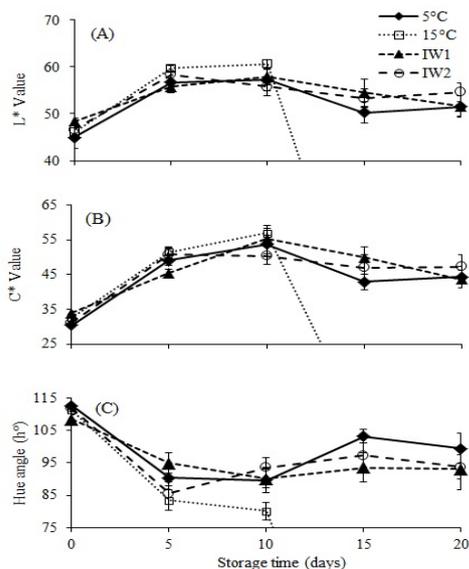
$$\text{CI}(\%) = \frac{\text{Number of affected fruit based on classified scale*}}{\text{Total number of fruit}} \times 100 \quad (1)$$

#### 2.6. Statistical analysis

The experiment was conducted using a completely randomized design (CRD) with three replicates. Data from the analytical determinations were subjected to analysis of variance (ANOVA). Mean comparisons were performed using the Duncan test at  $p < 0.05$ .

### 3. Results and Discussion

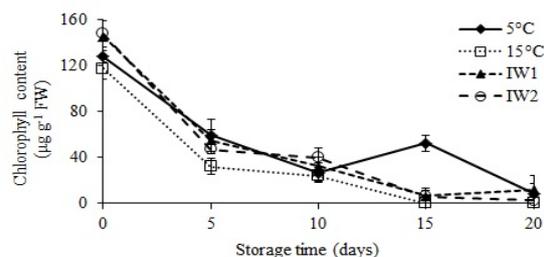
Color changes of papaya fruit were observed during storage for 20 days. The average of all of initial  $L^*$  values of fruits in this work was 46.5 (Figure 1A). The control fruit stored at 5°C showed the lowest  $L^*$  value (50.3) at day 15 (Figure 1A). This result was possibly due to the fact that the chilling temperature (5°C) caused irregular ripening when papaya fruit suffered from CI [16]. The results also showed that, after ten days, fruit stored at 15°C, 80% RH had higher trend in  $L^*$  and  $C^*$ . The results for  $C^*$  and  $h^\circ$  analysis are shown in Figures 1B and C. As illustrated in Figure 1B, the average  $C^*$  values of skin color changed significantly within 10 days from 32.0 at initial day to 54.2. The color of papaya in all cases, except the control fruit, changed from green to a brighter yellow. The fruit stored at 15°C had higher  $C^*$  value (57) compared with other treatments at day 10 of storage. For the hue angle, it was found to decrease from day 0 (112.6) until day 20 (99.4) for the fruit stored at 5°C. The  $h^\circ$  of fruit stored under IW (1 and 2) decreased from 108 and 112 to 93 and 94, respectively. For the fruit stored at 15°C, the  $h^\circ$  rapidly decreased



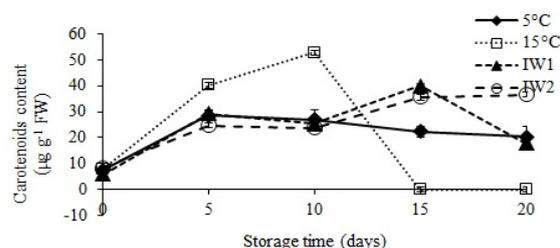
**Figure 1:** Changes in lightness (A), Chroma (B) value and hue angle (C) of the peel of 'Holland' papaya fruit stored at 5°C, 15°C and IW conditions (5°C for 4 days and 15°C for 1d) with different warming conditions for 1 (IW1) or 2 cycles (IW2) then stored at 5°C until the end of storage. Vertical bars indicate  $\pm$ SE (n= 6).

from 111 to 80 after 10 days of storage (Figure 1C). It was found that color did not change much for papaya fruit that were stored at 5°C during cold storage. This is similar to Rivera-Pastrana *et al.* [24] who observed lower carotenoids content of 'Maradol' papaya after cold storage at 1°C compared with the fruit kept at 25°C. From this work,  $h^\circ$  of 'Holland' papaya stored at 15°C decreased drastically along with the fastest peel yellowing during storage, but  $h^\circ$  changed most slowly in papaya fruit stored at lower temperature (5°C). For the IW conditions (warming at 15°C, 1 and 2 cycles for 1 day), the  $h^\circ$  of fruit changed steadily but less quickly than the control fruit until 20 days of storage. This change is normal for climacteric fruit, which ripen faster at 15°C than non-climacteric fruits. Such results were expected, considering that the rate of metabolic reactions is directly related to the temperature at which they occur. Thus, fruit stored at 15°C had the lowest  $h^\circ$  (80) which was more yellow than others. From this work,  $h^\circ$  values of fruit stored under IW1 and IW2 conditions showed slightly lower values (93 and 94, respectively) than the fruit stored at 5°C (99) whereas the changes in  $h^\circ$  of these fruit were significantly delayed compared with the fruit stored at 15°C after 10 days of storage (Figure 1C). Hence low temperatures could affect ripeness by retarding color change whereas high temperatures could increase ripeness by increasing external color in papaya [25].

Postharvest de-greening in horticultural crops, as a result of chlorophyll degradation, has been shown to be an indicator of a loss in a very important quality attributes [26]. In this work a sharp decline of ini-

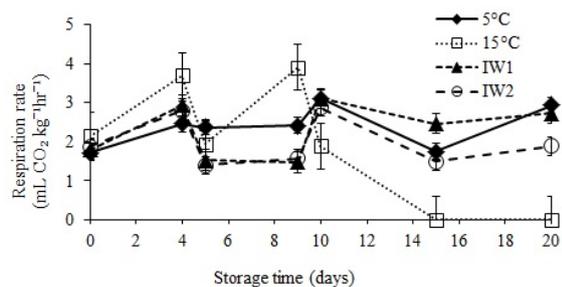


**Figure 2:** Chlorophyll content of 'Holland' papaya fruit stored at 5°C, 15°C and IW conditions (5°C for 4 days and 15°C for 1d) with different warming conditions for 1 (IW1) or 2 cycles (IW2) then stored at 5°C until the end of storage. Vertical bars indicate  $\pm$ SE (n= 6).



**Figure 3:** Carotenoid content of 'Holland' papaya fruit stored at 5°C, 15°C and IW conditions (5°C for 4 days and 15°C for 1d) with different warming conditions for 1 (IW1) or 2 cycles (IW2) then stored at 5°C until the end of storage. Vertical bars indicate  $\pm$ SE (n= 6).

tial chlorophyll content among treatments (average of 134  $\mu\text{g g}^{-1}$  FW) was observed after 5 days of storage. The losses of chlorophyll content from the initial day of the control fruit, fruit stored at 15°C and the fruit stored under two IW conditions were 56, 76 and 62%, respectively. The fruit stored at 15°C had significantly lower chlorophyll content of 32 and 24  $\mu\text{g g}^{-1}$  FW whereas the fruit stored at 5°C had chlorophyll content of 59 and 26  $\mu\text{g g}^{-1}$  FW after 5 and 10 days of storage, respectively. Fruits that were stored at 15°C lost their green color faster than other treatments within 10 days of storage (Figure 2). This result was in agreement with Khurnpoon *et al.* [27] who observed rapid decrease in chlorophyll a and b in 'Holland' papayas during storage at room temperature ( $25 \pm 1^\circ\text{C}$ ). In contrast, the chlorophyll content of papaya fruit stored at a constant low temperature (5°C) remained in the range of 26-59  $\mu\text{g g}^{-1}$  FW during the first 15 days (Figure 2) but with more CI symptoms incidence. These findings agree with Lam [28] who postulated that papaya fruit affected by CI did not change their color indices even when transferred the fruit from cold storage to 25°C. Warming the fruit in the IW1 and IW2 conditions showed similar chlorophyll contents when compared with the control fruit stored at 5°C after 10 days of storage. This achievement of slowing chlorophyll degradation on IW fruit was thought to be due to the

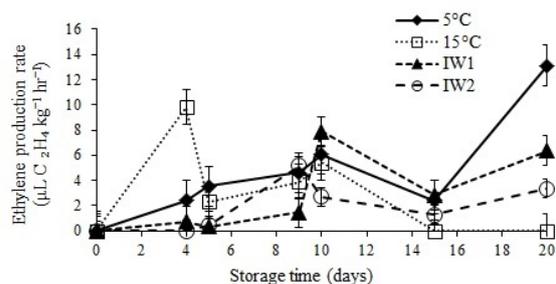


**Figure 4:** Respiration rate (measured at 27°C) of 'Holland' papaya fruit stored at 5°C, 15°C and IW conditions (5°C for 4 days and 15°C for 1d) with different warming conditions for 1 (IW1) or 2 cycles (IW2) then stored at 5°C until the end of storage. Vertical bars indicate  $\pm$ SE (n= 6).

potential effect of low temperature that has the ability to delay enzyme activities associated with chlorophyll degradation. Furthermore low temperatures could delay ethylene production in fruit that could accelerate fruit ripening. However, IW warming allows the fruit to ripen and release some toxic substances that may have accumulated during cold storage [12].

Carotenoid pigments in fruit are indicative of the ripening process and potential nutritional value. In this work, the carotenoid content of 'Holland' papaya was in the range between 8.3 and 52.8  $\mu\text{g g}^{-1}$  FW. These results were similar to the ranges of carotenoid content reported by Supapvanich and Promyou [5], Schweigert *et al.* [29], Mélo *et al.* [30] in several papaya cultivars. The results showed that carotenoid content increased proportionally to the storage temperature (Figure 3) where the highest value was found in the fruit stored at 15°C (52.8  $\mu\text{g g}^{-1}$  FW) at day 10 of storage, while the lower value was found in IW1 and the control fruit kept at 5°C about 17.9 and 20.4  $\mu\text{g g}^{-1}$  FW, respectively after 20 days of storage. IW conditions (1 and 2) significantly delayed increment of carotenoid content and in turn they controlled the ripening process (Figure 3). During ripening, chlorophyll started to reduce, coinciding with carotenoid accumulation that appeared in the form of yellow-orange color. This change is normal for several tropical fruits where color reflects the higher levels of carotenoids which play an important part in the fruit acceptability [31].

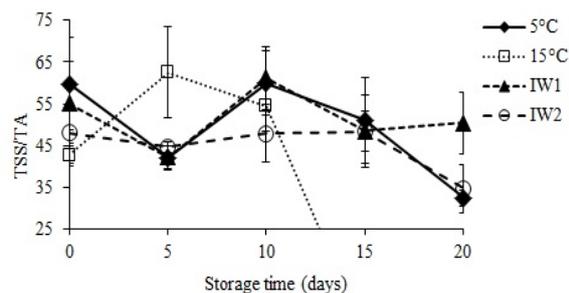
Respiration rates of 'Holland' papaya stored continuously at 5 or 15°C and the fruit stored under IW1 and IW2 conditions are shown in Figure 4. The average of all of initial respiration rates of fruits was 1.9 mL CO<sub>2</sub> kg<sup>-1</sup> hr<sup>-1</sup>. In this work, the typical climacteric peak was not obvious in the fruit stored at 15°C. However, the fruit stored at 15°C tended to have a significantly higher respiration rate (3.7-3.9 mL CO<sub>2</sub> kg<sup>-1</sup> hr<sup>-1</sup>) than the other treatments. This was especially noticeable after 4 and 9 days of storage (Figure 4). The fruit stored at 5°C had respiration rate in the range of 2.4-3.1 mL CO<sub>2</sub> kg<sup>-1</sup> hr<sup>-1</sup> after 10 days of storage. It then decreased at day 15 and finally increased



**Figure 5:** Ethylene production rate (measured at 27°C) of 'Holland' papaya fruit stored at 5°C, 15°C and IW conditions (5°C for 4 days and 15°C for 1d) with different warming conditions for 1 (IW1) or 2 cycles (IW2) then stored at 5°C until the end of storage. Vertical bars indicate  $\pm$ SE (n= 6).

to be 2.9 mL CO<sub>2</sub> kg<sup>-1</sup> hr<sup>-1</sup> at day 20 of storage. In contrast to the above treatments (5 and 15°C), the respiration rates of fruit stored under IW1 and IW2 conditions decreased during the first warming from about 2.9 mL CO<sub>2</sub> kg<sup>-1</sup> hr<sup>-1</sup> at day 4 to about 1.5 mL CO<sub>2</sub> kg<sup>-1</sup> hr<sup>-1</sup> at day 5 until day 9. After this they tended to increase. This was particularly the case in IW1 fruits where they increased to about 3.1 mL CO<sub>2</sub> kg<sup>-1</sup> hr<sup>-1</sup> after day 10 of storage and slightly decreased after that (Figure 4). Respiration rates of IW2 fruit were at similar levels as those of IW1 fruit until 9 days of storage but their levels were significantly lower at day 15 (1.5 mL CO<sub>2</sub> kg<sup>-1</sup> hr<sup>-1</sup>) and still remained lower until 20 days of storage (1.9 mL CO<sub>2</sub> kg<sup>-1</sup> hr<sup>-1</sup>). This may possibly due to the impact of the second warming on the respiratory patterns of the fruit. Overall, the respiration rates of papaya cv. Holland tested in this experiment were in the range of 1.4 to 7.0 mL CO<sub>2</sub> kg<sup>-1</sup> hr<sup>-1</sup> (or  $\sim$ 2.5 to 7.0 mg CO<sub>2</sub> kg<sup>-1</sup> hr<sup>-1</sup>). This is similar to the range of respiration rate, measured at 27 $\pm$ 2°C, of papaya cv. Holland (1.8 to 3.4 mg CO<sub>2</sub> kg<sup>-1</sup> hr<sup>-1</sup>) reported by Khurnpoon and Siriphanich [4]. It was found that our fruits' respiration rates were lower than that of Mexico papaya cv. Maradol measured at 23°C which was in the range of  $\sim$ 10 to 37 mL CO<sub>2</sub> kg<sup>-1</sup> hr<sup>-1</sup> [32] and the color break (25% yellowing) Brazil papaya cv. Golden, measured at 25°C, which was in the range of  $\sim$ 44 to 124 mg CO<sub>2</sub> kg<sup>-1</sup> hr<sup>-1</sup> [33].

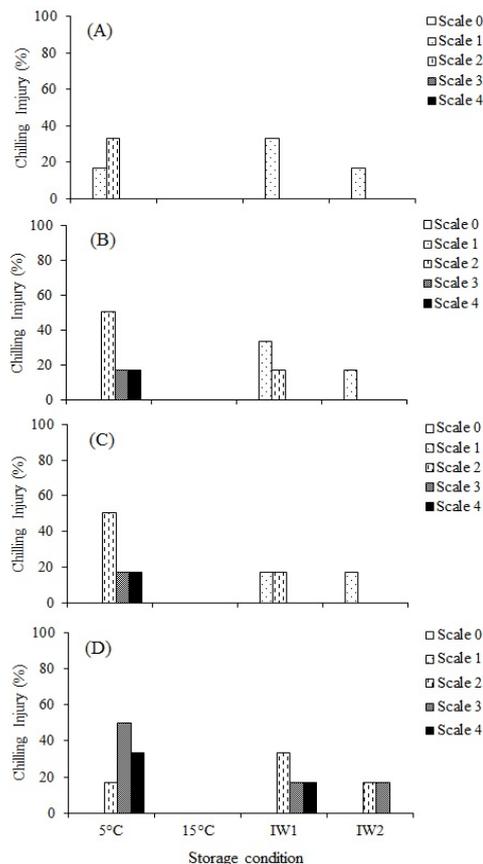
Ethylene production rates of the 'Holland' papaya fruit tested in this work were in the range of 0 to 13.1  $\mu\text{L C}_2\text{H}_4 \text{ kg}^{-1} \text{ hr}^{-1}$ . These were similar to those levels (2.5 to 13  $\mu\text{L C}_2\text{H}_4 \text{ kg}^{-1} \text{ hr}^{-1}$ ) measured at 25 $\pm$ 2°C in Thai papaya fruit cv. 'Kaek Dum' (or Khak Dum) at color break stage (<10% yellowing) and 'Red Maradol' (0.5 to 14  $\mu\text{L C}_2\text{H}_4 \text{ kg}^{-1} \text{ hr}^{-1}$ ) [3]. The authors also observed the ethylene peaks of these two fruits at day 4 of storage. They found that the ethylene level sharply decreased after that until 8 days. The 'Holland' papaya fruits that were stored at 15°C tested in this work also showed the ethylene peak at day 4 and decreases at day 5 with a slight increase at day 9 of storage. Furthermore, similar trends of ethylene



**Figure 6:** TSS/TA ratio of 'Holland' papaya fruit stored at 5°C, 15°C and IW conditions (5°C for 4 days and 15°C for 1d) with different warming conditions for 1 (IW1) or 2 cycles (IW2) then stored at 5°C until the end of storage. Vertical bars indicate  $\pm$ SE (n= 6).

production rates were also observed in other papaya cultivars such as the Brazil papaya cv. Golden. Which when measured at 25°C showed the ethylene peak at day 4 with the range of 0 to 14  $\mu\text{L C}_2\text{H}_4 \text{ kg}^{-1} \text{ hr}^{-1}$  [33]. The Mexico papaya cv. Maradol, measured at 23°C, showed slightly lower levels of between 1 and 3.5  $\mu\text{L C}_2\text{H}_4 \text{ kg}^{-1} \text{ hr}^{-1}$  [32].

As was predicted, lower levels of ethylene production rates were observed in IW1 and IW2 fruits. The lowest level was observed in IW2 fruit with the range of 0.5 to 5.2  $\mu\text{L C}_2\text{H}_4 \text{ kg}^{-1} \text{ hr}^{-1}$  with the delayed ethylene peak occurred at day 9 of storage (Figure 5). Similar to the above mentioned respiratory patterns, this proved that the IW regime could delay metabolic changes in 'Holland' papaya fruit. Romani [34] reported that the climacteric respiration may be reflected as a maximum response of mitochondria to replace the degradation effects caused by cellular senescence. Therefore, it is concluded that there are beneficial effects from IW treatment when carried out as described in this work. In contrast, the gradual increase in ethylene production rates at each sampling day was observed in the papaya fruit stored at 5°C with the values of 2.4 and 13.1  $\mu\text{L C}_2\text{H}_4 \text{ kg}^{-1} \text{ hr}^{-1}$  at day 4 and day 20 of storage, respectively. The highest ethylene production rate at the end of storage for this treatment (5°C) may be due to the physiological responses of papaya fruit to more severe CI symptoms with rot development. Based on the observation mentioned above, the fluctuation of ethylene emission of fruit during storage may possibly be the result of the cooling and warming cycles of IW. The efficacy of IW regime also depends on the number of IW cycles as well as the conditions used. These findings agree with the research of Artés *et al.* [16] who applied IW on the breaker stage of 'Durinta' tomato stored at constant temperatures of 9 and 20°C compared with fruit applied with IW treatment. In this case the IW treatment consisted of the fruit being stored at 9°C for 6 days and at 20°C for 1 day. The authors found that tomato fruit applied with the IW condition had similar metabolic rates to the control fruit stored at 9°C, however during the warm-



**Figure 7:** Incidence of CI (%) after 5 (A), 10 (B), 15 (C) and 20 (D) days of storage for 'Holland' papaya fruit stored at 5°C, 15°C and IW conditions (5°C for 4 days and 15°C for 1d) with different warming conditions for 1 (IW1) or 2 cycles (IW2) then stored at 5°C until the end of storage. (Severity scale\* refer to 2.Materials and methods; 2.5 Physiological disorder). (n= 6).

ing periods both the respiration and ethylene production rates increased [16].

Eating quality (TSS, TA) was evaluated by TSS/TA ratio as shown in Figure 6. The fruit stored at 15°C gave significantly higher TSS/TA ratio than other treatments after storage for 5 days. The TSS/TA ratio of the fruit stored at 15°C was about 62.5 at day 5 and slightly reduced until the end of storage, 20 days. The lowest ratio of 32.4 was observed at day 20 in control fruit stored at 5°C. However, the changes in TSS/TA ratio of the control fruit were quite similar to the changes in TSS/TA ratio of the fruit stored under IW1 condition during 15 days of storage. The TSS/TA ratios of fruit stored under IW 2 condition were quite stable during 15 days of storage compared with other treatments then the decline in TSS/TA ratio was observed after that. As a higher in average TSS value (12°Brix) and a lower in average TA (0.2%) were observed on the fruit stored at 15°C compared with the TSS values (~10-11°Brix) and the TA values (~0.3%) of other treatments at day 5 (data not shown). This could influence the TSS/TA ratio of the fruit stored at 15°C at 5 days of storage. The overall TA values (0.2-

0.4%) of papaya fruit in this work were quite similar in all treatments over storage periods. Overall there was no significant difference in the changes of TSS/TA ratio for all treatments except the fruit stored at 15°C at day 5.

The development of CI occurred unceasingly within 5 days in control fruit stored at 5°C, it became more visible within hours when the fruit were transferred from cold storage to ambient temperature (27±1°C) as this resulted in small pitting then developed to dark spots on the skin (Figure 7A, B, C and D). However, CI symptoms were also detected in fruit stored under IW conditions (1 and 2) ranging from scale 1 to 2 (1-25% abnormality) during 15 days of storage. The CI symptoms of those fruits stored under IW conditions were significantly lower compared with the control fruit stored at 5°C which showed CI scales of 2, 3 and 4 within 15 days of storage and collectively reached more than 80% of the symptoms (Figure 7C). This effective reduction of CI observed in both IW conditions was clearly explained by Wang [12, 13]. Wang [12, 13] reported that the;

*“potential effect of IW is to recover the metabolite concentrations during the warming phase that were disturbed during the cool storage, this restoration of metabolites could occur by allowing tissue to activate appropriate metabolic enzymes and produce compounds at an appropriate level during chilling. and, thus, allowing tissue to restore substances that were exhausted; or allowing the tissue to catabolize excess intermediates accumulated during the chilling”.*

Similar effects were also found when Thailand papaya fruits cv. Khakdum (or Khak Dum) were stored at 15°C or 20°C for 3 days, then held at 5°C for 2 days. The fruits developed less CI symptoms (< 5%) when compared with the fruits stored continuously at 5°C which showed a greater extent of pitting (22%) after 30 days and remained unripe when transferred to room temperature [35]. In this work, papaya fruit stored at 15°C continued to ripen normally within 6 days of storage and maintained acceptability for 10 days with no signs of CI. After 15 days, mycelia developed due to fruit senescence. It can therefore be concluded that temperature management is very important in preserving fruit quality and protecting fruit against CI.

#### 4. Conclusions

The application of IW regimes with different warming cycles during cold storage was found to suppress the development of CI during cold storage. These IW treatments also regulated color development of ‘Holland’ papaya fruit more effective than the constant storage at a chilled temperature (5°C) and a storage condition at 15°C. IW improved the storage quality of papaya cv. Holland and delayed the ripening. In a comparison between the IW1 (1 cycle) and IW2 (2

cycles), the results suggested that IW2 had a stronger potential for maintaining the quality of ‘Holland’ papaya grown in Thailand.

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