

Ripening characteristics of vapour heat treated 'Frangi' papaya (*Carica papaya* L. cv. Frangi) as affected by maturity stages and ethylene treatment

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ABSTRACT: 'Frangi' papaya is a F1 hybrid cultivar produced by Malaysian Agrifood Corporation Bhd in 2006. Since then, 'Frangi' papaya is a new hybrid and its physiology may differ from another cultivars. Several importer countries, such as Japan and China, have made the vapour heat treatment (VHT) an export requirement for disinfestation of fruit flies in Malaysia. The exporting country is also required to carry out ripening treatments with ethylene before export. Therefore, the objective of this work was to determine the effects of maturity stages (4, 5 and 6) and ethylene treatments on ripening characteristics of vapour heat treated papaya during storage at 25 °C. Papaya fruits were treated with VHT systems. After VHT, the fruits were exposed with 100 $\mu\text{L}\cdot\text{L}^{-1}$ ethylene gas at 20 °C for 24 h. Non-ethylene treated fruits (control) were kept separately at

20 °C for 24 h. After 24 h, the fruits were removed from the ripening rooms, and stored at 25 °C. The ripening characteristics of fruits that reached maturity stages 4, 5, and 6 were recorded. Results showed that the fruits ripened normally at 25 °C with or without ethylene following VHT with respect to peel and pulp color, edible firmness and soluble solids concentration (SSC). Fruits at maturity stage 5 were considered at the edible stage by taking into account the firmness, SSC, titratable acidity, and ascorbic acid contents. It is recommended that no ethylene treatment is needed to ripen vapour heat treated fruits, since the ethylene treatment did not affect the ripening process of the fruits.

Key words: *Carica papaya* L. cv. Frangi, postharvest quarantine treatment, disinfestations, fruit quality.

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INTRODUCTION

The new F_1 hybrid papaya called 'Frangi', or commercially known as 'Paioia', has all the quality characteristics of a desirable fruit. Its golden-yellow peel, sweet tasting pulp and palm-sized fruit are appealing to consumers, especially those who are aware of the health and nutritional benefits of papaya. In addition, the thickness of the skin and firmness of the pulp enabled it to be stored longer than other varieties, such as 'Subang', 'Sekaki', 'Eksotika' and 'Solo' (Chan and Baharuddin 2010). In fact, the first two varieties mentioned are too big to be consumed by an individual. The fruits have to be cut into smaller portions and stored in the refrigerator, thus, they are no longer fresh when eaten at a later date. On the other hand, the 'Frangi' fruit is small and its often cut into two halves to consume the whole fruit. These favourable characteristics of 'Frangi' provide opportunities for market expansion locally and abroad (Carmen et al. 2013).

Currently, the production of papaya has become popular in Malaysia. This is due to the high demand both in the domestic market, and for export, mainly to China and Japan. However, fruit fly infestation has become a serious problem, especially when the papaya fruit peel has turned to 25% yellow. The fruit flies cause fruit surface blemishes, edible flesh destruction and spoilage (Lobo and Pastor 2012). China and Japan quarantine laws require vapour heat treatment (VHT), established by the World Trade Organization (WTO), to disinfect fruit flies of papaya (TQAU 2008).

Postharvest heat treatments of fruit are used for insect disinfestation, disease control, to modify fruit responses to cold stress and maintain fruit quality during storage (Lurie and Pedreschi 2014). Meanwhile, vapour heat is a quarantine treatment technique of heating fruits with water vapour at temperatures of 40 °C – 50 °C to kill insect eggs and larvae (Lurie 1998). VHT can modify fruit responses to other stresses, such as chilling injury, while maintaining fruit quality during storage (Paull and Chen 2000). The advantage of using VHT is the sterilizing of commodities without the use of chemicals such as ethylene dibromide and methylbromide (Armstrong and Mangan 2007). There is no negative effect for human and the fruit is preserved naturally. The VHT has the advantage of being chemical and residue free, thus it will give added value to the papaya to meet consumer's preferences for good quality and safe fruits, in accordance with the standard of the 'Malaysia's Best' brand (Department of Standards Malaysia 2012).

Ethylene initiates and control ripening in climacteric fruits (Bapat et al. 2010). The fruits that are harvested at the mature green stage need to be ripened with ethylene gas in order to make the fruit edible with uniform ripening when they reach the target market. The ripening process can be accelerated by applying exogenous ethylene treatment ($100 \mu\text{L}\cdot\text{L}^{-1}$) and papayas are reported to ripen satisfactorily between 20 °C and 25 °C (Broughton et al. 1977).

In domestic market, the 'Frangi' papaya is usually harvested at the mature green stage (maturity stage 1) and then allowed to ripen naturally at 25 °C, without using ethylene to induce fruit ripening. The ethylene concentration in climacteric fruits increases quickly to saturation levels after ripening process was started. Then, the exogenous ethylene has no further effect on ripening of climacteric fruits (Minas et al. 2015). Ethylene is one of the plant hormones that plays an important role in ripening process of climacteric fruits and its perception is needed for the expression of specific ripening genes even at advance stages of fruit ripening (Paul and Pandey 2014). Meanwhile, ripening process of fruits was delayed after VHT because the endogenous ethylene production is inhibited, thus extending storage life of fruits (Paull and Chen 1990). According to Lurie and Pedreschi (2014), during application of heat stress there is a rapid change in polyribosomes and protein synthesis, which inhibit and delay normal ripening processes, especially if the commodity is then placed in low temperature, the inhibition persists for some time. When rewarmed after storage ripening resumes. However, information on the response of 'Frangi' to ethylene following pretreatment with vapour heat is still lacking. Thus, there is a need to find the effects of the ethylene treatment of vapour heat treated 'Frangi' papaya VHT on its quality for the domestic market.

The objective of this study was to determine the effects of ethylene treatment on vapour heat treated 'Frangi' papaya ripened at 25 °C. Postharvest physical and chemical quality characteristics, including the peel and pulp color, firmness, soluble solids concentration (SSC), titratable acidity (TA), and ascorbic acid (AA), were evaluated when fruits reached maturity stages 4, 5 and 6. Physiological quality characteristics, including ethylene and carbon dioxide (CO_2) production, were also determined at the respective maturity stages so as to explain the ripening behaviour of the vapour heat treated papaya fruits. It is important to determine that the most suitable maturity stages need to be ripened with ethylene gas, in order to make the fruit edible with uniform ripening when they reach the target market.

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MATERIALS AND METHODS

Sample preparation

Papaya fruits were obtained from a commercial farm in Lanchang, Pahang, Malaysia. The fruits were treated with 0.1% Octave® (imidazole) fungicide for 5 min. Then, fruits were individually wrapped with a pure white paper and packaged into corrugated paper boxes. Then they were transported to the Department of Agriculture, Serdang, for vapour heat treatment (VHT).

Vapour heat treatment (VHT)

The VHT system (EHK-500MC, Sanshu Sang You Co. Ltd., Japan) consisted of three main functions: heating, cooling and drying. Each chamber had its own heater and humidity controller. Twenty fruits were arranged onto a tray, and six trays were loaded into the chamber. For measuring the fruit core temperature, a fruit sensor probe was inserted in the selected fruits in each chamber. Then, the VHT system was turned on. The temperature of the chamber and fruit core was raised to 48 °C and 46.5 °C, respectively. The respective temperatures were maintained for 20 min, and followed by air and water cooling for 35 min and 10 min, respectively. Next, the fruits were dried for 5 min and removed from the VHT system for repacking into corrugated paper boxes, each one containing nine fruits.

Ethylene treatment

After VHT, the fruits were putted in a ripening room and treated with 100 $\mu\text{L}\cdot\text{L}^{-1}$ ethylene gas at 20 °C for 24 h at 95% relative humidity. Non-ethylene treated fruits (control) were also kept at 20 °C for 24 h at 95% relative humidity in another ripening room. After 24 h, the papaya fruits were removed from the respective ripening rooms and allowed to ripen at ambient temperature (25 °C \pm 2 °C). Measurements of quality characteristics of two fruits (per box), from three boxes (one box / replication), were taken when the fruits reached maturity stages 4, 5 and 6, respectively. The quality characteristics measured were peel and pulp color (L^* , C^* , h°), firmness (N), SSC (%), TA (% malic acid), and AA (mg/100 g), carbon dioxide (CO_2 $\text{mL}\cdot\text{kg}^{-1}\text{FW}\cdot\text{h}^{-1}$) and ethylene (C_2H_4 $\mu\text{L}\cdot\text{kg}^{-1}\text{FW}\cdot\text{h}^{-1}$) production.

Physical quality characteristics

Determination of peel and pulp color (L^* , C^* and h°)

Peel color was measured on opposite sides of the mid-equatorial region of each fruit surface. Then, a 2-3 mm thick peel was removed from the same area where peel color was measured with a sharp knife to expose the pulp. The exposed pulp color was measured. The fruit peel and pulp color were determined with a chromameter (CR 300, Minolta Corp., Japan). The meter was equipped with a measuring head of 8-mm-diameter and calibrated with a standard white tile. Color was reported as lightness (L^*), chroma (C^*), and hue (h°). Values of L^* indicate lightness (0 = black to 100 = white), C^* indicates vividness, and h° indicates color in a 360° wheel, where 0, 90, 180 and 270 degrees represent red, yellow, green and blue, respectively (McGuire 1992). The mean of two readings from the peel and pulp measurements were calculated.

Determination of firmness

Fruit firmness was measured on the opposite sides of the mid-equatorial region of each papaya fruit. Firmness was measured using an Instron (Model 5543 Load Frame, Instron Corp, USA), equipped with a 6 mm diameter cylindrical probe, and a Merlin Software (version M12-13664-EN) to process the readings. The Instron was set at a speed of 20 mm / min. The mid-equatorial region of the whole fruit was placed under the Instron probe and two readings were taken on opposite sides of each fruit. The mean of two readings per fruit was calculated to obtain fruit firmness (N).

Chemical quality characteristics

Determination of SSC

Each fruit was cut longitudinally into two halves. The peel, placenta, and seeds were removed. One half of the fruit was chopped and mixed. A spoonful of the mixed fruit was extracted using a manual juice extractor. Then, 2 drops of the extracted juice were placed on the prism of a digital refractometer (Model N1, Atago Co. Ltd., Japan) and the measurement was recorded. Three measurements were made on each sample. A mean of the readings was calculated and converted to a standard temperature (20 °C) by adding a correction factor of 0.28% to obtain percentage of SSC at 27 °C.



Determination of TA

TA was determined according to the titration method by Ranganna (1986). Twenty grams of mixed sample (prepared for the SSC determination) was homogenized in 80 mL distilled water using a blender (model MX V2N, National, Malaysia) for 1 min. Then, the homogenate was filtered using a filter paper (Whatman No.2). Five mL of the filtrate was put into a conical flask, and 2 – 3 drops of 1% of phenolphthalein indicator were added. The filtrate was titrated with 0.1 N NaOH, until the color turned pink at the end point of pH 8.2. The titre volume was recorded and the result was expressed in percentage of malic acid (67 g equivalent weight). The malic acid percentage was calculated by using Eq. 1:

$$\% \text{ malic acid} = \frac{\text{mL NaOH} \times 0.1 \text{N NaOH} \times \text{product vol. (100mL)} \times \text{malic acid eq. weight (67)} \times 100}{\text{sample vol. for titration (5 mL)} \times \text{sample weight (20g)} \times 100} \quad (1)$$

Determination of AA

AA was determined according to the titration method by Ranganna (1986). Twenty grams of chopped sample (prepared for the SSC determination) was homogenized with 80 mL metaphosphoric acid (HPO₃) for 1 min using a blender (model MX V2N, National, Malaysia). Then, the homogenate was filtered using a filter paper (Whatman No.2). Five mL of the filtrate was titrated with 2-6 dichlorophenol-indophenol dye until the filtrate changed to pink which persisted for 15 s. The titre volume of dye solution used was recorded, and the AA content (mg/100 g) was calculated as Eq. 2:

$$\text{Ascorbic acid (mg/100g)} = \frac{\text{mL used dye} \times \text{dye factor} \times \text{product vol. (100mL)} \times 100}{\text{papaya sample weight (20g)} \times \text{sample vol. for titration (5mL)}} \quad (2)$$

Standardization of dye

Five mL of standard AA solution was added to 5.0 mL of 3% HPO₃ and titrated with the dye solution until pink which persisted for 15 s. The dye factor was determined as (Eq. 3):

$$\text{Dye factor} = \frac{0.5}{\text{titration volume (mL)}} \quad (3)$$

Physiological changes during ripening

Determination of ethylene and carbon dioxide production

Two representative fruits were taken out for evaluation at maturity stages 4, 5 and 6. Each papaya fruit was incubated in an hermetically airtight container (1900 mL volume; 19.5 cm length × 12.5 cm width × 12 cm height) (Lock and Lock, Korea) fitted with a rubber septum. After 2 h of incubation, 1.0 mL of gas sample was withdrawn from the container headspace with a 1.0 mL syringe through the rubber septum. Ethylene production was determined according to the method of Fuggate et al. (2010), with modification. Ethylene production was measured by injecting the gas sample into a gas chromatograph (Clarus-500, Perkin Elmer, USA) equipped with a combination of flame ionization detector (FID) and thermal conductivity detector (TCD) and fitted with a stainless steel porapak Q column (3 m × 1 / 8 in; 50 / 80 mesh) for determination of ethylene and carbon dioxide production, respectively. Nitrogen was used as carrier gas with a flow rate of 30 mL·min⁻¹, while flow rates of hydrogen and air were 45 mL·min⁻¹ and 400 mL·min⁻¹, respectively. Temperatures for the oven, injector, FID and TCD were 100 °C, 200 °C, 200 °C and 200 °C, respectively.

The ethylene production was calculated based on areas of standard gas with Eq. 4:

$$\mu\text{L C}_2\text{H}_4 \cdot \text{kg}^{-1} \text{FW} \cdot \text{h}^{-1} = \text{ppm C}_2\text{H}_4 \times \frac{\text{container vol. (ML)} - \text{fruit vol. (mL)}}{\text{fruit weight (kg)} \times \text{time (h)} \times 100} \quad (4)$$

The CO₂ production was calculated based on areas of standard gas with Eq. 5:

$$\text{mL CO}_2 \cdot \text{kg}^{-1} \text{FW} \cdot \text{h}^{-1} = \% \text{CO}_2 \times \frac{\text{container vol. (ML)} - \text{fruit vol. (mL)}}{\text{fruit weight (kg)} \times \text{time (h)} \times 100} \quad (5)$$

Experimental design and data analysis

The experiment was conducted using a randomized complete block design in a factorial arrangement of treatments (2 ethylene levels × 3 maturity stages), with three replications and two fruits per replication. The data were subjected to an

analysis of variance (ANOVA), and means were separated by Duncan's multiple range test (DMRT) at $p \leq 0.05$ (SAS version 9.4).

RESULTS AND DISCUSSION

Peel and pulp color (L^* , C^* and h°)

In this experiment, vapour heat treated papaya fruits were ripened with $100 \mu\text{L}\cdot\text{L}^{-1}$ ethylene and stored at 25°C . Papaya fruits without ethylene were also stored at 25°C as control group. The peel and pulp color, represented by L^* , C^*

and h° values, were measured when fruits reached maturity stages 4, 5 and 6 (Table 1).

There was no significant interaction effect between ethylene treatment and fruit maturity stages on peel and pulp L^* , C^* and h° . The peel L^* of fruits at maturity stage 4 was significantly lower than fruit peel L^* at maturity stages 5 and 6 (Table 2). This indicates that the surface color of papaya at maturity stage 4 was darker than maturity stages 5 and 6, as the peel was still green-yellow (Figure 1 and Table 1). The peel C^* was significantly higher in fruits treated without ethylene than in fruits with ethylene (Table 2). However, the peel C^* showed significant increases with the advancement

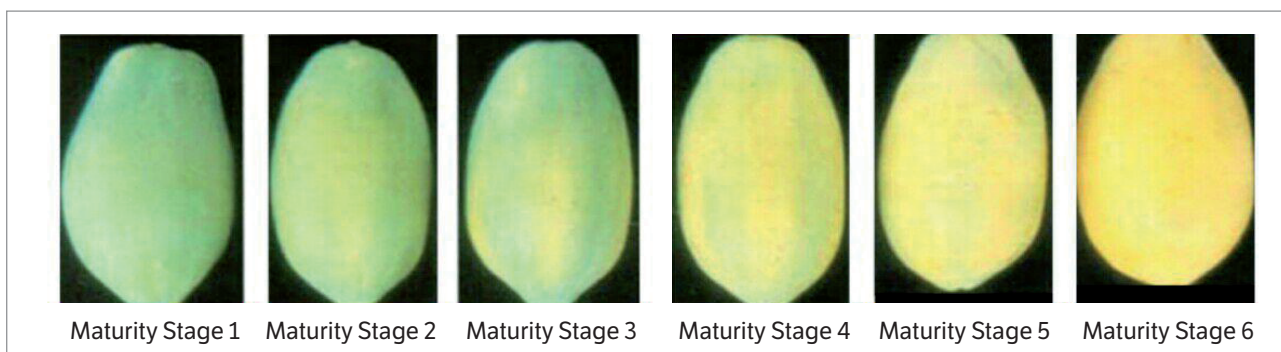


Figure 1. Visual aspect of representative 'Frangi' papaya fruit at each maturity stage.

Table 1. Color index, maturity stage and descriptions according to fruit skin color under room temperature ($25^\circ\text{C} \pm 2^\circ\text{C}$).

Color index	Maturity stage	Description
0	1	Mature green. 100% green skin colour.
1	2	Green with trace of yellow. Less than 15% yellow color of the fruit surface.
2	3	More green than yellow. $\frac{1}{4}$ mature with up to 25% of the surface yellow.
3	4	More yellow than green. $\frac{1}{4}$ mature with up to 50% of the surface yellow.
4	5	Yellow with trace of green. $\frac{3}{4}$ mature with 50-75% of the surface yellow.
5	6	Fully yellow. Mature with 76-100% of the surface yellow.

Table 2. Effect of ethylene treatments on peel and pulp color (L^* , C^* and h°) at three maturity stages of vapour heat treated 'Frangi' papaya (*Carica papaya* L.) stored at 25°C .

Ethylene (E)	Peel			Pulp		
	L^*	C^*	h°	L^*	C^*	h°
With	65.52 a	45.22 b	85.35 a	61.21 a	42.00 a	63.82 a
Without	65.40 a	49.27 a	85.34 a	62.69 a	44.74 a	63.74 a
Maturity stage (MS)						
4	62.77 b	37.84 c	95.65 a	65.61 a	40.74 b	66.79 a
5	66.53 a	49.33 b	83.44 b	61.01 b	44.14 a	63.83 b
6	67.08 a	54.56 a	76.96 c	59.23 c	45.23 a	60.72 c
CV	1.82	4.43	1.95	2.04	2.66	1.61
SE	0.69	1.21	0.96	0.73	0.67	0.59

Means within column and factor, followed by the same letter, are not significantly different by DMRT at $p \leq 0.05$. L^* = lightness; C^* = chromaticity; and h° = hue of color.

in maturity stages from 4 to 6, irrespective of fruits treated with or without ethylene. These results indicated that the development of fruit peel chromaticity was not affected by the pre-VHT followed by storage at 25 °C. The higher peel C^* at maturity stages 5 and 6 was due to the more intense yellowish orange peel color of the papaya compared to fruits at maturity stage 4. The more intense yellowish orange peel color showed that papaya at maturity stage 6 was at fully ripe stage, while papaya at maturity stage 5 was at three-quarter ripe stage and maturity stage 4 was at half ripe stage. The peel h° was not significantly different, irrespective of ripening the fruits with or without ethylene at 25 °C (Table 2). The peel h° values decreased significantly from maturity stages 4 to 6, indicating that peel color changed from yellow to orange. Peel color of fruits at maturity stage 5 was yellow with trace of green, while peel color of fruits at maturity stage 6 were more vivid yellowish orange, when values of L^* , C^* and h° were considered together.

The pulp L^* was significantly higher at maturity stage 4 than at maturity stages 5 and 6. This indicates that the orange pulp color of the papaya at maturity stage 4 was darker than at maturity stages 5 and 6. Papaya at maturity stage 4 possessed a darker color since fruits were only at half-ripe stage compared to maturity stages 5 (three-quarters ripe) and 6 (fully ripe). The pulp C^* was significantly lower at maturity stage 4 than at maturity stages 5 and 6, whereas the pulp C^* values of fruits at maturity stages 5 and 6 were not significantly different. The pulp C^* values showed that the color intensity of pulp changes from less intense to more intense color as fruits ripened. The h° values of pulp increased significantly as maturity stage increased from 4 to 6. There was no effect of exogenous ethylene treatment since the pulp h° of fruits ripened with or without ethylene was not significantly different.

The results of L^* , C^* and h° of vapour heat treated papaya showed a normal color development throughout ripening process. According to Paull and Chen (1990), exposure to temperatures higher than 40 °C for a short period could result in a rapid loss of 1-aminocyclopropane-1-carboxylate oxidase (ACC oxidase) in papaya, but after fruits are removed from the heat and transferred to ambient condition for three days, the ACC oxidase activity recovered. The inhibition of ripening due to lack of ethylene is reversible if the heat treatment is not too extended and does not cause damage (Lu et al. 2010). This could explain why ethylene treatment had no effect on ripening characteristic of papaya.

In this study, changes in L^* , C^* and h° values were due to increases in maturity stages of papaya as the fruit changes color during ripening. The increase in lightness and intensity of color was indicated by an increase in L^* and C^* values. The peel color developed from green-yellow (maturity stage 4) to yellow-orange (maturity stage 6), as shown by a decrease in h° throughout the ripening process.

The increase in L^* and C^* and decrease in h° was similar to the results with control papaya fruits cultivar 'Pluk Mai Lie', in which, the values of L^* ranges from 60 to 64, C^* ranges from 46 to 54 and h° ranges from 65 to 89, indicating color changes from yellow to orange-red during ripening (Fuggate et al. 2010). Also, the results were in agreement with 'Golden' papaya, that showed increases in L^* and C^* and a decrease in h° with the increase of fruit maturation, which represent the changes from green to yellow (Pereira et al. 2009). This showed that skin color development was not affected by the VHT. At maturity stage 6, the peel showed fully yellowish orange color.

Both VHT (47 °C for 15 min) and hot water treatment (53 °C for 5 min) were reported to accelerate fruit ripening and enhance skin color changes in a number of mango varieties (Jacobi and Giles 1997). High humidity and hot-air treatment at 46.5 °C for 10 min enhanced skin color development of mangoes, as shown by the significantly higher L^* and C^* and lower h° in treated mangoes than in non-treated mangoes (Jacobi et al. 1995).

Paull and Chen (1990) found that the exposure of fruits to 42 °C for 30 min, and then at 49 °C for up to 70 min, allowed papaya to develop normal yellow peel and pulp color. The increase in intensity of yellow-orange of the pulp color was indicated by the increase in C^* and the reduction in L^* and h° throughout the ripening process. At maturity stage 6 or fully ripe fruits, which was the edible stage for papaya, the values of L^* , C^* and h° showed that the pulp color of the vapour heat treated papaya was orange-red. This results was in agreement with control papaya fruits cultivar 'Pluk Mai Lie', in which the values of L^* , C^* and h° showed that the pulp color was orange-red for fully ripe fruits (Fuggate et al. 2010).

Studies on mango fruits cultivar 'Nam Dokmai', 'Dashehari' and 'Kaew' showed a decrease in L^* and C^* during ripening (Jha et al. 2006; Mahayothee et al. 2004; Saranwong et al. 2004). In a climacteric fruit such as mango, respiration rate increased, which consequently increased the carotenoid synthesis, hence, increasing fruit colouration in the pulp (Saltveit 1999).

The changes in peel and pulp colors (L^* , C^* and h°) of papaya were due to the chlorophyll degradation, formation of carotenoids (yellow, orange and red colors) and development of other phenolic compounds during ripening process (Wang 1990; Workneh et al. 2012). When the greenness of papaya peel was lost, the fruit peel turned yellow during the ripening, thus leading to changes in L^* , C^* and h° . Loss of chlorophyll makes yellow and red tones more evident, whereas carotenoids and other pigments are responsible for these colors (Sancho et al. 2010). Carotenoids (when not masked by chlorophylls) can produce h° ranging from pale yellow to red, and lycopene was the major pigment that contributes to the papaya pulp reddish color (Wall 2006).

The peel color progression was from dark green in immature fruits to orange red in fully-ripe fruits and, usually in climacteric fruits, it is due to the degradation of chlorophyll structure. The main factor for this degradation is pH changes (because of organic acid leakage from vacuoles), oxidative systems and chlorophyllase enzymes (Yang et al. 2009).

Bron et al. (2004) reported the changes in chlorophyll fluorescence, skin color (h°) and fruit firmness of 'Golden' papaya during the ripening process. The decline in chlorophyll fluorescence and h° was due to the chlorophyll degradation and degree of chloroplast membrane injury during ripening and senescence of papaya fruits. Moreover, the high linear correlation between chlorophyll fluorescence and h° value was also observed during fruit ripening in that study.

Fan et al. (2011) stated that the chlorophyll fluorescence of 'Jonagold' and 'Cortland' apples at different harvest maturity was decreased after fruits were treated at 46 °C for 12 h in the heating chamber. In the same study, the chlorophyll fluorescence also decreased when the fruit maturation

increased at ambient temperature after three months of storage at 0 °C.

Firmness

There was no significant interaction effect between ethylene treatment and fruit maturity stages on firmness (Table 3). Ethylene treated papaya were able to ripen faster and more uniformly in which the fruit firmness reached the edible condition although the skin color was only 60% yellow (Wang 1990). However, the results indicated that fruit firmness was not affected by the ethylene treatment. According to Lurie (1998), heat treatments not only inhibit ethylene production within hours but also fruits will not respond to exogenous ethylene. Irrespective of the ethylene treatments, firmness were significantly lower at maturity stage 6 than at maturity stages 5 and 4 (Table 2). The decrease in firmness was due to increase in maturity stage of papaya as the fruit tended to ripen. It has been reported that the edible firmness for papaya was 20 N (Bron and Jacomino 2006). In this study, maturity stage 6 was considered as the edible or palatable stage to eat and the firmness value obtained showed that the fruits were able to soften at 25 °C. According to Almora et al. (2004), hydrolytic enzymes are initiated during the ripening process, resulting in breakdown of cell walls that lead to fruit softness. This result is in agreement with that for hot water treated papaya, in which the fruits were able to soften to 16 N during 9 days of postharvest shelf life (Chávez-Sánchez et al. 2013). Fruit ripening leads to the softening and it is catalyzed by the enzymes, pectin methyl esterase (PME), β -galactosidase, polygalacturonase (PG), cellulose and endo- β -mannanase (Wang et al. 2013; Archbold et al. 2003). There

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Table 3. Effects of ethylene treatment on firmness, soluble solids concentration, titratable acidity, ascorbic acid content and pH at three maturity stages of vapour heat treated 'Frangi' papaya (*Carica papaya* L.) stored at 25 °C.

Ethylene (E)	Firmness (N)	Soluble solids concentration (%)	Titratable acidity (% malic acid)	Ascorbic acid (mg·100g ⁻¹)
With	44.95 a	10.34 a	0.04 b	64.34 a
Without	44.73 a	10.03 a	0.05 a	63.36 a
Maturity stage (MS)				
4	71.05 a	9.71 b	0.04 b	53.44 c
5	41.82 b	10.32 a	0.04 b	64.90 b
6	21.65 c	10.53 a	0.05 a	73.22 a
CV	10.29	1.87	9.38	4.99
SE	2.66	0.11	0.0025	1.84

Means within column and factor, followed by the same letter, are not significantly different by DMRT at $p \leq 0.05$.

is a positive relationship between polygalacturonase and xylanase activity and fruit softening (Prasanna et al. 2007). Cell wall components keep the cells attached to each other (pectins), and when cell-to-cell links become weak, the tissue rigidity will be reduced, along with the firmness.

Soluble solids concentration (SSC)

There was no significant interaction effect between ethylene treatment and fruit maturity stages on SSC (Table 3). The SSC was significantly lower at maturity stage 4 than at maturity stages 5 and 6. There was no significant difference in SSC between fruits at maturity stages 5 and 6 within each ethylene treatment.

Reyes and Paull (1995) reported that on day 11 after harvest, both ethylene and non-ethylene treated of non-heated guava fruits did not show any change in SSC. They further suggested that SSC is a function of fruit age rather than its stage of ripeness. The increases in SSC from maturity stages 4 to 5 and 6 could be attributed to hydrolysis of starch into simple sugars due to the ripening process. The range in SSC obtained in this study is similar to the results obtained by other researchers. Arina et al. (2010) reported that the SSC of hot-water (47 °C for 1 min) treated 'Eksotika' papaya was between 10.3% and 11.3%. At edible stage, the SSC of papaya cultivar 'Solo' was 9% – 10% (Gomez et al. 2002), 'Maradol' was 9.6% (Sancho et al. 2010) and 'Pluk Mai Lie' was 11.7% (Fuggate et al. 2010). The range in SSC varied from 9% to 12%, depending upon the papaya cultivar. The results obtained in this study were within the predicted and range reported, thus, indicating that the SSC was not affected by heat treatment and the commodities can tolerate heat treatment without significant quality loss. Therefore, the effects of heat treatment can be variable depending upon cultivars, temperature used and treatment duration.

Titrateable acidity (TA)

TA in papaya was expressed as the percentage of malic acid content since it was the predominant acid in papaya fruits. There was no significant interaction effect between ethylene treatment and fruit maturity stages on TA. As the results showed, there were significant effects of ethylene as well as maturity stage on TA of papaya (Table 3). The TA was significantly lower in fruits treated with ethylene than those without ethylene treatment. Meanwhile, TA at maturity

stage 6 was significantly higher than at maturity stages 4 and 5. At edible stage, TA of papaya was found to be between 0.09% and 0.24% (Bron and Jacomino 2006; Fuggate et al. 2010; Sancho et al. 2010).

In this study, the TA was 0.04% – 0.05%, which was unusually low compared to 'Maradol' papaya treated with hot water at 55 °C, which showed 0.09% – 0.24% of malic acid (Chávez-Sánchez et al. 2013). However, the value of TA obtained was similar to another result reported on 'Maradol' papaya (Sancho et al. 2010). Also, TA of 'Pluk Mai Lie' and 'Golden' papaya did not change significantly during ripening at 23 °C (Bron and Jacomino 2006; Fuggate et al. 2010). According to Addai et al. (2013), TA increases with increasing fruit maturity.

Vicente et al. (2002) reported that hot-air treated strawberry at 45 °C had a lower TA than the control treatment. The composition of the organic acids varies with different fruit cultivars and the acidity of fruit arises from the organic acids that are stored in the vacuole. The decrease in acidity during later ripening could be due to a rapid utilization of acids for respiration. The increase in fruit temperature during heat treatment resulted in an increase of respiration rate during which organic acid are utilized as substrates for respiration, thus, resulting in low TA in fruits (Lurie and Klein 1990). Also, the process of converting starch into sugar caused the decrease in TA in fruits (Vicente et al. 2002).

Ascorbic acid (AA)

There was no significant interaction effect between ethylene treatment and fruit maturity stages on AA. Meanwhile, there were significant ($p \leq 0.05$) increases in AA content when fruits matured from stages 4 to 6 irrespective of ethylene treatment (Table 3). AA content was significantly higher at maturity stage 6 than at maturity stages 5 and 4. Also, the AA content was significantly higher at maturity stage 5 than at maturity stage 4. There was a significant increase in ascorbic acid content at each maturity stage.

This result was as expected, since ascorbic acid content would normally increase with increasing maturity stage. However, there was no significant interaction between ethylene treatment and maturity stage of the fruits. According to Bron and Jacomino (2006), AA content of papayas increased 20% – 30% during ripening, independent of the maturity stages at harvest. It has been reported that in many horticultural crops, DHA (L-dehydroascorbic acid), that represents less

than 10% of total vitamin C, tends to increase during storage (Lee and Kader 2000).

Mannose and L-galactose are key substrates for AA synthesis in plants and ascorbate oxidase is a copper containing enzymes that oxidizes AA to DHA in the presence of molecular oxygen (Conklin 2001). Under stress condition, ascorbate oxidase levels were increased, thus, contributing to increasing AA content. At the edible stage, AA content for papaya cultivar 'Golden' was 1.0 g·kg⁻¹ fresh weight (Bron and Jacomino 2006), and for other papaya variety it was 46.1 mg·100 g⁻¹ (Shakila and Anburani 2010). Whereas, the 'Hawaiian' papaya (1/8 ripe) contained 45-55 mg·L⁻¹ AA per 100 g fresh weight (Wall 2006). Lee and Kader (2000) reported that the 'Solo' papaya harvested at green stage had 72.0 mg·100 g⁻¹ fresh weight of AA content, which is similar to the results of vapour heat treated papaya obtained in this study. This indicates that AA content was not affected by heat treatment, which is in agreement with Dea et al. (2010), who reported that there was no significant difference in AA content of hot water treated (46 °C for 90 min) and control Kent mango.

The level of AA content was found in response to the heat treatment. The effects of heat treatment could be due to the treatment duration as fruits exposed to a high temperature for a shorter period were able to increase or maintain the AA content compared to fruits exposed to a high temperature for a longer period.

Ethylene production rates

There was no significant interaction effect between ethylene treatment and fruit maturity stages on ethylene production rates of the fruits (Table 4). According to Sancho et al. (2010), there was no significant difference in ethylene production between 'Maradol' papaya when fruits have 50% – 75% and 75% – 100% yellow skin color. Also, the 'Golden' papaya obtained peak ethylene production when pulp firmness reached the edible stage of < 20 N, together with changes of other quality parameters (Bron and Jacomino 2006).

Ethylene production peaks in 'Maradol' papaya on the eighth day of postharvest, followed by a gradual decrease until day 13 after harvest (Basulto et al. 2009). Ethylene production rates of control 'Maradol', 'Pluk Mai Lie' and 'Frangi' papayas were 0.9 – 3.6, 2.5 – 22.0 and 8.0 – 81.3 µL C₂H₄·kg⁻¹FW·h⁻¹, respectively (Basulto et al. 2009; Fuggate et al. 2010; Ong et al. 2013).

Table 4. Effects of ethylene treatment on ethylene and carbon dioxide production rates at three maturity stages of vapour heat treated 'Frangi' papaya (*Carica papaya* L.) stored at 25 °C.

Ethylene (E)	Ethylene (µL C ₂ H ₄ ·kg ⁻¹ FW·h ⁻¹)	Carbon dioxide (mL CO ₂ ·kg ⁻¹ FW·h ⁻¹)
With	0.16 a	15.01 a
Without	0.15 a	14.72 a
Maturity stage (MS)		
4	0.14 a	14.52 a
5	0.16 a	14.88 a
6	0.17 a	14.75 a
CV	7.43	4.72
SE	0.56	0.44

Means within column and factor, followed by the same letter, are not significantly different by DMRT at p ≤ 0.05.

In the present study, the ethylene production was the highest at maturity stage 6 for both fruits treated with or without ethylene. The production rates ranged between 0.12 – 0.19 µL C₂H₄·kg⁻¹FW·h⁻¹, and this range is exceptionally low when compared to production rates of control fruits reported in previous studies. These results could be attributed to the pretreatment of fruits with vapour heat before ethylene was used to induce fruit ripening. However, these rates did not completely affect all fruit ripening parameters, especially peel and pulp C* and h° color values as fruits showed normal color changes.

Carbon dioxide production rates (CO₂)

There was no significant interaction effect between ethylene treatment and fruit maturity stages on CO₂ production rates (Table 4). The ethylene treatment following pretreatment with vapour heat did not cause changes in CO₂ production. Similarly, the CO₂ production for fruit at maturity stages 4, 5 and 6 was not significantly different from each other. This might be due to the response of the fruits towards heat treatment. In contrast, it has been reported that heat treatment of 35 °C to 40 °C can either increase or decrease the CO₂ production as well as advancing or delaying it (Klein and Lurie 1990; Lurie 1998). The CO₂ production rates were not affected by the treatments. However, CO₂ production rates were similar to those reported in 'Maradol' and 'Solo' papaya. Sancho et al. (2010) reported that the CO₂ production of 'Maradol' papaya with 50% – 75% and 75% – 100% yellow skin ranged between 15 – 35 mL·kg⁻¹FW·h⁻¹

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at 20 °C, similar to the results reported in 'Solo' papaya (Paull et al. 1997).

During heat treatment at 43 °C – 48 °C, ripening fruit showed an elevated CO₂ production in papayas (Paull and Chen 1990) and mangoes (Mitcham and McDonald 1993), then declined to the same level or below that of non-heated fruit. The onset of ethylene evolution coincided with an increase in respiration and a rapid decline in firmness. Ethylene and CO₂ production increased as ripening progressed and reached maximum values three days after harvest. The peak in ethylene and CO₂ production coincide with the highest activities of both ACS and ACO enzymes as well as the maximum flesh ACC content. As ACC content increased, malonyl ACC (MACC) content declined, suggesting a regulation of ethylene production by malonylation of ACC (Koslanund et al. 2005). Therefore, the climacteric development of ethylene production may be regulated by an increase of ACS and ACO activities as well as a decrease in ACC malonyl transferase activity.

Heat treatment by hot water immersion at 46 °C for 30 min and 60 min slowed the ripening process by disrupting ethylene and CO₂ production. A heat treatment, depending on the length of exposure, can decrease or increase the climacteric respiration peak as well as advance or delay it after treatment (Lurie 1998).

CONCLUSION

Fruits ripened normally at 25 °C with or without ethylene following VHT. The ripening characteristics of fruits at

maturity stages 4, 5 and 6 were normal with respect to peel and pulp color, edible firmness and SSC. The fruits at maturity stage 5 were at the edible stage, considering the firmness, SSC, TA and AA content. The level of TA and AA content was found to increase with increasing maturity in response to the VHT. The low production rates of ethylene and CO₂ were adequate to stimulate normal ripening processes as indicated by ripening progression from maturity stages 4 to 6. The exogenous ethylene treatment did not significantly affect the ripening process of the fruits since there was no significant interaction between ethylene treatment and maturity stages of the fruits. It can be recommended that no ethylene treatment is needed to ripen fruits that had been treated with VHT, since the vapour heat treated fruits will not respond to exogenous ethylene within hours after heat treatments.

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