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## Effects of pre-process elicitor immersion on the physicochemical qualities of fresh-cut papaya fruits cv. 'Holland' during cold storage

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**Abstract** Postharvest treatment of elicitors such as salicylic acid (SA) and methyl jasmonate (MeJA) improved postharvest quality of fruit and vegetables. The effect of pre-process immersions on SA or MeJA on the physicochemical quality changes of fresh-cut papaya fruits cv. 'Holland' during cold storage is hereby reported. The fruits were immersed in 2 mM SA or  $10^{-4}$  M MeJA for 1 hour and then stored at room temperature ( $26 \pm 1$  °C) for 24 hours before fresh-cut processing. The biochemical parameters such as firmness, total soluble solids (TSS), colour attributes, antioxidant activities and bioactive compounds of fresh-cut papaya fruit during storage at 4 °C for 8 days were monitored. Both SA and MeJA treatments effectively maintained firmness but had no influence on the changes in TSS and colour attributes on storage. Antioxidant capacity of the fresh-cut papaya was enhanced by SA or MeJA immersion. While, free radical scavenging activity was induced by SA immersion. The amounts of total phenols and flavonoids of SA treated fresh-cut papaya fruit were higher than in the control. MeJA immersion induced flavonoids content but not total phenols. The pre-process immersion of SA and MeJA could maintain texture and improve nutritional quality of fresh-cut papaya fruit during storage.

**Keywords:** Elicitors, fresh-cut, papaya, physicochemical quality

### Introduction

Papaya (*Carica papaya* L.) is one of commercial fruits in Thailand. Most papaya fruit has been produced for domestic market and the demand for the fruit has increased continuously. In Thailand, the immature fruits are consumed for cooking and the ripe fruits for fresh consumption and processing. The most popular commercial cultivars of Thai papaya for commercial production are: 'Kaek Dam', 'Kaek Nuan' and 'Holland'. Recently, the demand for 'Holland' papaya fruit in market has increased in which the papaya fruit is being consumed as table papaya in the forms of intact and fresh-cut products. Previous studies had investigated the exogenous applications of

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elicitors such as heat treatment and salicylic acid (SA) immersion on the physicochemical qualities of intact 'Kaek Dam' and 'Holland' papaya fruits (Promyou and Supapvanich, 2016; Supapvanich and Promyou, 2017). The results found that 2 mM SA prolonged their shelf-life and maintained quality, especially texture, and improved nutritional value of the papaya fruits (Promyou and Supapvanich, 2016; Supapvanich and Promyou, 2017). González-Aguilar *et al.* (2003) suggested that the exogenous application of methyl jasmonate (MeJA) at the concentration of  $10^{-4}$  and  $10^{-5}$  M retarded the loss of firmness and maintained postharvest quality of 'Sunrise' papaya fruit during cold storage at 10°C. Boonyaritthongchai and Supapvanich (2017) had studied the effects of MeJA immersion on physicochemical qualities of pineapple fruit wherein MeJA maintained flesh firmness and enhanced antioxidant system including antioxidant activity, bioactive compounds and the activities of antioxidant enzymes during storage. These indicate that both SA and MeJA applications maintain postharvest qualities as well as improve nutritional quality of intact fruits. It is commonly acknowledged that both SA and MeJA induce defense mechanism in plants through the stimulation of phenylpropanoids pathway (Aghdam and Bodbodak, 2013). Moreover, SA is also recognized as an ethylene inhibitor in which certain ethylene-related reactions involved in the ripening process is delayed (Asghari, 2006).

Fresh-cut fruit tissues are more susceptible to more rapid deterioration than its intact counterpart. Karakurt and Huber (2003) reported that the softening of fresh-cut papaya fruit was greater than that of intact fruits with marked increase in water-soluble polyuronides, cellwall hydrolase activities, membrane degrading enzyme activities and ethylene production as compared to intact fruits. However, the enhanced deterioration of fresh-cut papaya fruit did not reflect chilling injury. Moreover, they also suggest that fresh-cut processing stimulates proteins expressions involved in the degradation of membrane structure and free radical generation in fresh-cut papaya fruit (Karakurt and Huber, 2007). The application of SA or MeJA to control deterioration of fresh-cut papaya fruit during storage has not been reported yet, thus this research.

The purpose of this study was to determine the effect of pre-process application of SA or MeJA on the physicochemical qualities including antioxidant activity and bioactive compounds contents of fresh-cut papaya fruit cv. 'Holland' during cold storage.

## **Materials and methods**

### ***Plant materials***

'Holland' papaya fruits were acquired from a retail fruit and vegetable market named "Talad Thai" in Pathum Thani province. The fruits were

screened for maturity (50 % yellowness on peel) and uniformity without physical damages and diseases. Afterwards, the fruits were cleaned with tap water and air-dried for 30 min. Based on previous studies, we found that 2 mM SA dip for 1 h maintained postharvest qualities of intact papaya fruit (Promyou and Supapvanich, 2016; Supapvanich *et al.*, 2017). Thus, in the first experiment, the papaya fruits were immersed in 2 mM SA solution or tap water (control) for 1 h and then left at room temperature (25 °C) for 24 h before fresh-cut processing. In the second experiment, the papaya fruits were immersed in 0.1 mM MeJA or tap water for 1 h and then left at room temperature for 24 h before processing. The concentration of MeJA was selected based on the report of Rivera-Domínguez *et al.* (2012).

### ***Fresh-cut processing***

The treated papaya fruits were dipped into 500 µL<sup>-1</sup> of sodium hypochlorite for 5 min. The fruits were then peeled and the seeds were removed using a sharp knife. The papaya flesh was cut into cubes (2.5 x 2.5 x 2.5 cm, approximately). Ten cubes of fresh-cut papaya were packed in a clear hinged PET box (8 Oz size) and then stored at 4 °C for 8 d. Three boxes in each treatment (triplicate) were sampled every 2 days during storage to investigate texture, total soluble solids (TSS) content, superficial colour, ferric reducing antioxidant capacity, DPPH free radical scavenging activity and bioactive compounds contents.

### ***Texture, TSS content and colour attributes measurements***

Firmness of the fresh-cut papaya fruits was measured using a TA Plus Texture Analyzer (Lloyds, England) with a 6 mm cylindrical probe. The firmness of the sample was expressed as maximum force of the measurement. The unit of the data was presented as Newton (N). The TSS content was measured using a hand-held refractometer (ATAGO MNL-112, Japan). Data were shown in percentage (%). The measurement of superficial colour attributes was performed using a Minolta (CR-300; Minolta Camera Co., Japan). The colour attributes such as lightness ( $L^*$ ), redness ( $a^*$ ) and yellowness ( $b^*$ ) value of sample were recorded.

### ***Antioxidant capacity assay***

The antioxidant capacity of the fresh-cut papaya was assayed using ferric reducing antioxidant potential as described by Benzie and Strain (1996). A 5g

papaya flesh was homogenized with 60 % (v/v) ethanol and then filtered through a Whatman No. 1 filter paper. The filtrate was collected. Exactly 0.1 mL of the filtrate was reacted with 2.9 mL FRAP reagent, consisting of acetate buffer pH 3, 10 mM 2,4,6-tripyridyl-1,3,5-triazine (TPTZ) and 20 mM ferric chloride hexahydrate in a ratio of 10:1:1 (v/v/v). The mixture was left for 30 min at room temperature. The absorbance at 630 nm wavelength was recorded. The antioxidant capacity of the sample was calculated using a linear equation of Trolox standard curve. The data was presented as  $\mu\text{mole Trolox equivalents per g fresh weight } (\mu\text{mol g}^{-1})$ .

#### ***Free radical scavenging activity assay***

DPPH free radical scavenging activity was assayed using the method described by Brand-Williams *et al.* (1995) with slight modifications. The 100  $\mu\text{L}$  filtrate was reacted with 2.9 mL of mM DPPH in 95% ethanol. The absorbance at 517 nm wavelength was immediately recorded ( $A_0$ ) and the mixture was then incubated in a dark place for 10 min and the absorbance at 517 nm wavelength was again measured ( $A_1$ ). The percentage of free radical scavenging activity was calculated compared with  $A_0$ .

#### ***Total phenols and flavonoids assays***

The sample was extracted as described in antioxidant capacity assay. Total phenols concentration was determined according to the method of Slinkard and Singleton (1977). One mL of filtrate was reacted with 1 mL 50 % (v/v) Folin–Ciocalteu reagent followed by 2 mL concentrated  $\text{Na}_2\text{CO}_3$  solution. The reaction was left at room temperature for 30 min and then the absorbance at 750 nm wavelength was recorded. The data was presented in term of mg gallic acid per g fresh weight ( $\text{mg g}^{-1}$ ). Flavonoids concentration of the fresh-cut papaya fruit was assayed using the method of Jia *et al.* (1999). One mL of the filtrate was mixed with 3 mL of distilled water, 225  $\mu\text{L}$  of 0.5 %  $\text{NaNO}_2$  and then left for 6 min. After that, 450  $\mu\text{L}$  of 10 %  $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$  was added and left for 5 min before the addition of 1.5 mL of 1 M NaOH. The absorbance at 510 nm wavelength was recorded. The data was expressed as  $\mu\text{g catechin equivalents per g fresh weight } (\mu\text{g g}^{-1})$ .

#### ***Statistical analysis***

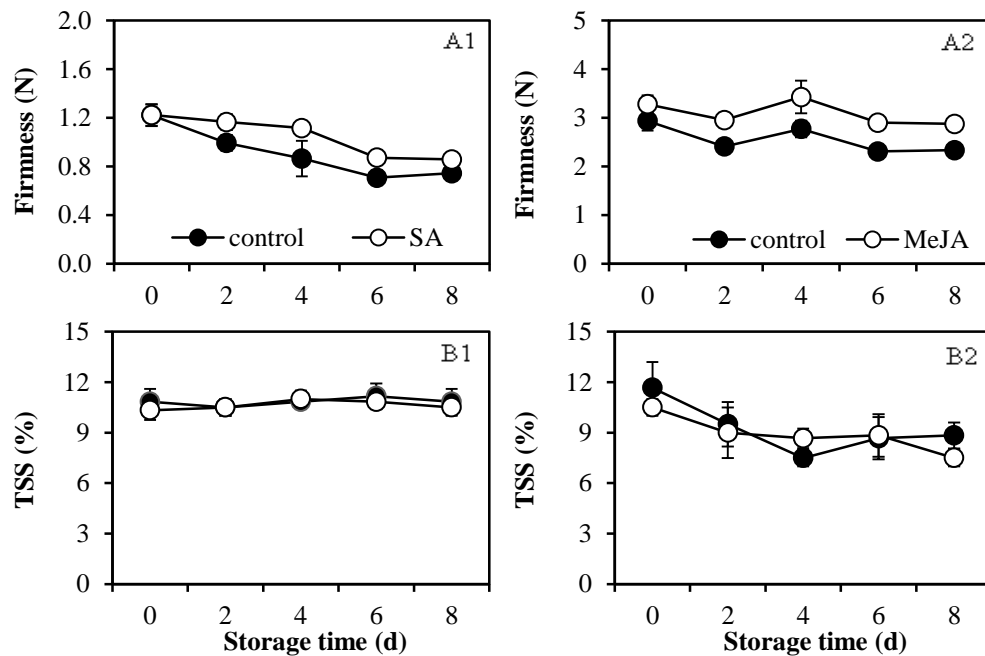
All experiments were performed according in Completely Randomized Design (CRD) and Analysis of Variance (ANOVA) was performed using the

SPSS version 18 software. Significant differences between means were compared using the DMRT at 5% level. The data for measurements were expressed as the mean ( $n=3$ )  $\pm$  standard deviation (SD).

## Results

### *Texture and TSS content*

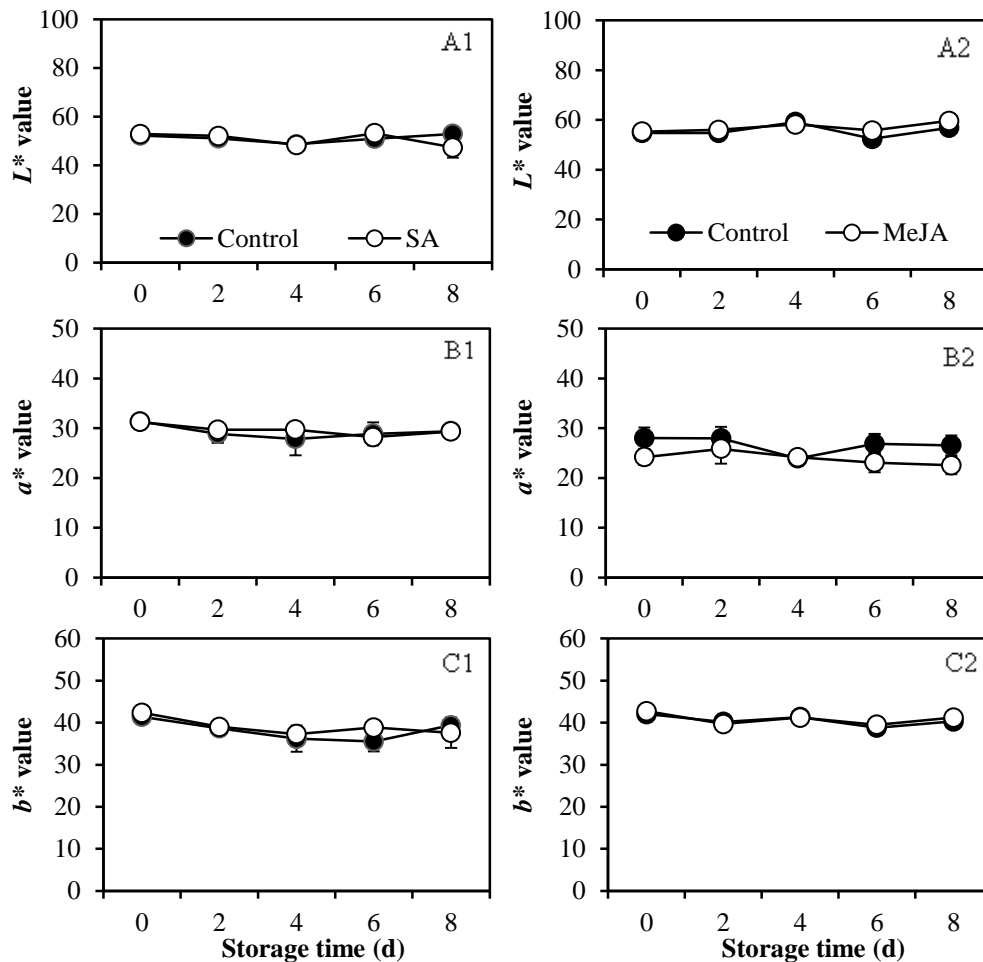
The changes in firmness and TSS content of both fresh-cut papaya fruits treated with SA and MeJA during storage is shown in Fig. 1. It was found that the firmness of fresh-cut papaya fruits declined throughout the storage. Both SA and MeJA pre-process treatments maintained the flesh firmness of fresh-cut papaya fruits compared with the control. The firmness of both treated fresh-cut papaya fruits was significantly higher than control ( $p < 0.05$ ). Both treatments did not affect TSS content during storage. These indicated that pre-process SA or MeJA treatment could delay the flesh softening and did not impact on the sweetness of fresh-cut ‘Holland’ papaya fruits during cold storage.



**Figure 1.** Firmness (A) and total soluble solids (TSS) (B) of fresh-cut ‘Holland’ papaya fruit treated with 2 mM SA (1) and  $10^{-4}$  M MeJA (2) during storage at  $4 \pm 1$  °C for 8 days. Vertical bars represent standard deviation (SD) of the means ( $n=3$ )

### Superficial colours

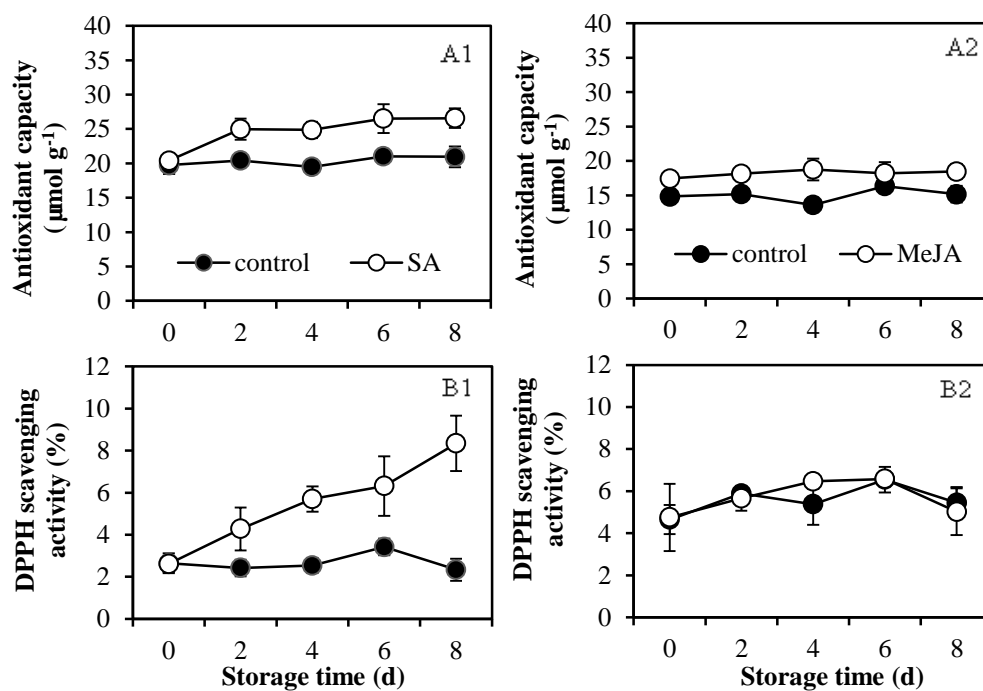
All colour attributes: lightness ( $L^*$ ), redness ( $a^*$ ) and yellowness ( $b^*$ ) values of both SA and MeJA treated fruits remained constant over the storage (Fig. 2). The colour of fresh-cut ‘Holland’ papaya fruits was red-yellow with a high  $a^*$  and  $b^*$  observed values. Both pre-process treatments with SA and MeJA did not affect all colour attributes throughout the storage.



**Figure 2.** Colour attributes,  $L^*$  (A),  $a^*$  (B) and  $b^*$  (C) values, of fresh-cut ‘Holland’ papaya fruit treated with 2 mM SA (1) and  $10^{-4}$  M MeJA (2) during storage at  $4 \pm 1$  °C for 8 days. Vertical bars represent standard deviation (SD) of the means (n=3)

### Antioxidant activities

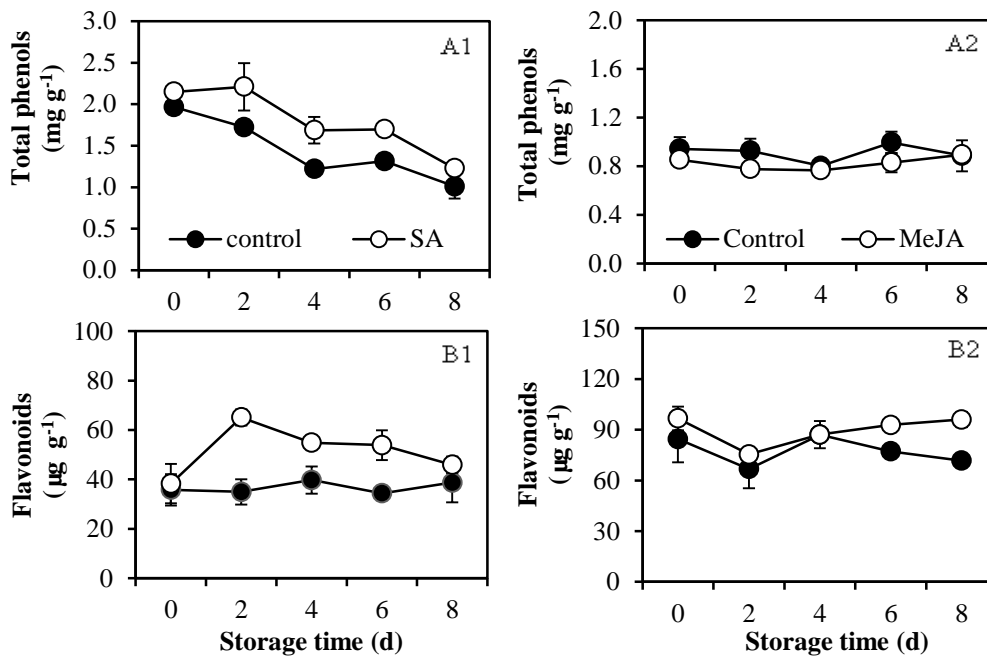
The ferric reducing antioxidant capacity and DPPH free radical scavenging activity of fresh-cut ‘Holland’ papaya fruits during storage is shown in Fig. 3. Pre-process SA treatment clearly showed the enhancement of antioxidant capacity and free radical scavenging activity of the fresh-cut papaya fruits during storage. While, pre-process MeJA treatment markedly induced antioxidant capacity after 24 h compared to control. During storage, antioxidant capacity of the both control and MeJA treated fresh-cut papaya fruits remained constant. However, the antioxidant capacity of MeJA treated papaya fruit was significantly higher than that of control ( $p < 0.05$ ). It was found out that pre-process MeJA treatment, however, did not affect the change of free radical scavenging activity when compared to control.



**Figure 3.** Antioxidant activity(A) and free radical scavenging activity (DPPH scavenging activity) (B) of fresh-cut ‘Holland’ papaya fruit treated with 2 mM SA (1) and 10<sup>-4</sup> M MeJA (2) during storage at 4 ± 1 °C for 8 days. Vertical bars represent standard deviation (SD) of the means (n=3)

### Bioactive compounds

Both the pre-process treatments of SA and MeJA could maintain or induce bioactive compounds content such as total phenols and flavonoids of ‘Holland’ fresh-cut papaya fruits during storage (Fig. 4). Pre-process SA treatment delayed the reduction of total phenols content and enhanced flavonoids content of the fresh-cut papaya fruits throughout the storage. The bioactive compounds contents of SA treated fresh-cut papaya fruits were significantly higher than those of control over the storage ( $p < 0.05$ ). Pre-process treatment of MeJA did not affect total phenols content as it remained constant during storage. However, pre-process MeJA treatment delayed the reduction of flavonoids content after 4 days of storage, flavonoids content of MeJA treated samples was significantly higher than the control.



**Figure 4.** Total phenolic compounds (A) and flavonoids (B) concentrations of fresh-cut ‘Holland’ papaya fruit treated with 2 mM SA (1) and  $10^{-4}$  M MeJA (2) during storage at  $4 \pm 1$  °C for 8 days. Vertical bars represent standard deviation (SD) of the means ( $n=3$ )

### Discussion

It is commonly accepted that the texture, taste and superficial colour are the main factors affecting the eating quality of fresh-cut products. Generally,



firmness of fresh-cut fruit decreases rapidly during storage caused by the three main factors: loss of moisture content, cell wall component degradation and membrane dysfunction (Supapvanich and Tucker, 2013). As shown in the results, both pre-process applications of SA and MeJA maintained the loss of firmness of the fresh-cut papaya fruits as compared to the control. SA and MeJA are recognized as plant regulators inducing plant defense mechanism and antioxidant systems (Ashari and Aghdam, 2010). It was known that the increment of antioxidants system in plant prevent the dysfunction of membrane brought about by the prevention of lipid peroxidation. Likewise, Supapvanich and Promyou (2013) suggested that exogenous SA application delay fruit softening due to the maintenance of membrane function and structure, reduction of cell wall hydrolase activities and inhibition of ethylene production. Promyou and Supapvanich (2016) also reported that postharvest SA immersion at the concentration of 2 mM for 1 h delayed the softening of 'Kaek Dam' papaya fruit due to the retardation of membrane dysfunction and pectin depolymerisation during storage. Moreover, Supapvanich *et al.* (2017) also found that 2 mM SA immersion preserved the firmness of 'Kimju' guava fruits due to the reduction of soluble pectin increase during cold storage for 18 d. In the case of MeJA treatment the maintainance of firmness of the fresh-cut papaya fruits, Reyes-D áz *et al.* (2016) suggested that exogenous MeJA treatment improves fruit firmness by decreasing the activities of cell wall hydrolases which hydrolyse glycosidic linkages among cell wall components. Moreover they also addressed that MeJA improves membrane integrity and function caused by the increment of antioxidant system in fruit. The recent work also found that both pre-process SA and MeJA treatments did not affect the sweetness and superficial colour of fresh-cut papaya fruits wherein the quality attributes remained constant throughout storage. These were in agreement with our previous works wherein: postharvest SA treatment has no impact on the changes in TSS and flesh colour of papaya fruits (Promyou and Supapvanich, 2016; Supapvanich *et al.*, 2017), MeJA immersion did not affect the TSS content of 'Kim Ju' guava fruits (Supapvanich *et al.*, 2019) and the TSS content and pulp yellowness of 'Queen' pineapple fruit during cold storage (Boonyaritthongchai and Supapvanich, 2017; Sangprayoon *et al.*, 2019).

Antioxidant activities such as reducing antioxidant capacity and free radical scavenging activity and bioactive compound contents such as total phenols and flavonoids are widely recognised as the important nutritional values of intact and fresh-cut fruit and vegetables (Watada and Qi, 1999). The recent work revealed that both pre-process SA and MeJA treatments could maintain as well as enhance antioxidant activities and bioactive compounds of fresh-cut 'Holland' papaya fruits during storage. The pre-process SA treatment

obviously induced or maintained the antioxidant activities and the bioactive compounds contents than MeJA treatment. The changes in antioxidant capacity and free radical scavenging activity of the fresh-cut papaya fruit were positively concomitant with the changes in bioactive compounds contents. Interestingly, pre-process MeJA treatment could induce reducing antioxidant capacity and flavonoids content in the fresh-cut papayas but it did not affect free radical scavenging activity and total phenols content. Therefore, this case needs to be investigated in further studies. However, both SA and MeJA are theoretically recognised as phytohormones which modulate antioxidant systems in plants (Supapvanich and Promyou, 2013; Reyes-D áz *et al.*, 2016). Both elicitors stimulate the increment of the key enzyme in phenylpropanoid pathway, named phenylalanin amonialyase (PAL), resulting in the increment of bioactive compounds such as phenolic compounds and flavonoids as well as antioxidant enzymes activities such as catalase (CAT), superoxide dismutase (SOD), ascorbic acid peroxidase (AsA-POD) and glutathione reductase (Supapvanich and Promyou, 2013; Reyes-D áz *et al.*, 2016; Boonyaritthongchai and Supapvanich, 2017; Sangprayoon *et al.*, 2019). These bioactive compounds are commonly acknowledged as having reducing antioxidant properties and free radical scavenging activities which are beneficial to health. Our previous works also reported that exogenous SA immersion induced antioxidant activity and the concentrations of total phenols and flavonoids in ‘Kaek Dam’ (Promyou and Supapvanich, 2016) and ‘Holland’ (Supapvanich *et al.*, 2017) papaya fruits during storage. Boonyaritthongchai and Supapvanich (2017) reported that MeJA immersion induced certain bioactive compounds in pineapple fruit during cold storage. In the similar way, Gonz ález-Aguilar *et al.* (2004) and Supapvanich *et al.* (2019) reported that postharvest MeJA treatment enhanced antioxidant systems as well as bioactive compounds in guava fruits during storage. Sangprayoon *et al.* (2019) suggested that both SA and MeJA immersion enhanced antioxidant activities and antioxidative enzymes such as CAT, AsA-POD and SOD in the pulp ‘Sawi’ pineapples during cold storage.

In conclusion, both pre-process SA and MeJA treatments could retard the flesh softening of fresh-cut ‘Holland’ papaya fruits but they did not affect the sweetness and superficial colour of the fresh-cut papaya fruit during storage at 4 °C for 8 d. The pre-process SA treatment enhanced the antioxidant capacity and free radical scavenging activity as well as flavonoids contents and delayed the reduction of total phenols content. While, pre-process MeJA treatment induced antioxidant capacity and flavonoids contents but it did not affect the changes of free radical scavenging activity and total phenols content of the fresh-cut papaya fruit during storage. These suggested that both pre-process SA

and MeJA are feasible alternatives to maintain quality especially texture and improve nutritional values of fresh-cut ‘Holland’ papaya fruits during storage.

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