

Effects of Salicylic Acid Incorporated with Lukewarm Water Dips on the Quality and Bioactive Compounds of Rambutan Fruit (*Nephelium lappaceum* L.)

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ABSTRACT

This study investigated the quality attributes of rambutan cv. Rong-rien treated with salicylic acid (SA) solution and/or lukewarm water (LW). The fruit were dipped in distilled water (control), lukewarm water at 35°C, 1.0 mM salicylic acid solution, and 1.0 mM lukewarm salicylic acid solution (SA+LW) at 35°C for 20 min. The fruit were packed in polyethylene containers and then held at 13±2°C for 9 days. Visual appearance, weight loss, pulp firmness, external browning score, superficial colors, total soluble solids (TSS), total acidity (TA) and bioactive compounds – namely antioxidant capacity, total phenols, total flavonoids and total ascorbic acid contents – were determined. The weight loss of the fruit was small during storage and slightly delayed by salicylic acid or/and lukewarm water dips. SA and SA+LW dips delayed external browning and effectively inhibited the softening of the fruit. The reduction of lightness (L) was delayed by salicylic acid and/or lukewarm water dips. All treatments had no effect on superficial colors and taste of the fruit during storage. SA and SA+LW dips clearly enhanced bioactive compounds involving antioxidant capacity, total phenols, total flavonoids and total ascorbic acid content during storage. In conclusion, SA and SA+LW treatments delayed external browning, maintained pulp firmness and enhanced the bioactive compounds of rambutan during storage.*

Keywords: Rambutan, Salicylic acid, Lukewarm water, Bioactive compounds

INTRODUCTION

Rambutan (*Nephelium lappaceum*, L.) is an important exotic fruit indigenous to Southeast Asia, including Thailand, Malaysia and Indonesia (Lam and Kosiyachinda, 1987). Thailand is a major exporter of rambutan. 'Rong-rien' rambutan is the most popular commercial variety in Thailand, with demand increasing annually. As a non-climacteric fruit, rambutan must be harvested at the peak of maturity, because further ripening does not continue after harvest (O'Hare, 1995; Wall et

al., 2011). During storage, rambutan rapidly lose their attractiveness because of shrivelling and browning of spinterns and skin. Moreover, released juice and pulp softening are also the key factors affecting consumer acceptance.

Salicylic acid (SA) is one of the safe, natural compounds used in post-harvest processing of fruit. It is a plant hormone that induces plant defence mechanisms under abiotic and biotic stresses (Asghari and Aghdam, 2010). However, post-harvest usage of salicylic acid is limited to concentrations that are non-toxic to plants, with an optimum range of about 0.5-2 mM (Babalar et al., 2007). In postharvest application, salicylic acid delays the ripening process of fruit, maintains postharvest quality (Srivastava and Dwivedi, 2000; Zhang et al., 2003; Lu et al., 2011; Khademi and Ershadi, 2013; Razavi et al., 2014), reduces postharvest decay and enhances bioactive compounds, imparting health benefits (Supapvanich and Promyou, 2013). Lukewarm water immersion is one of the most promising approaches for controlling postharvest diseases and insect pests, maintaining postharvest quality and alleviating chilling injury to fruit and vegetables (Fallik et al., 1999; Paull and Chen, 2000; Funamoto et al., 2002; Vigneault, 2007; Promyou et al., 2012; Supapvanich et al., 2012). Recently, most post-harvest rambutan research has focused on the development of skin and spinterns browning. Research on the effects of salicylic acid and/or lukewarm water (LW) immersion of rambutan is limited. In our previous research, lukewarm water at 35°C for 20 min maintained fruit quality when compared to lukewarm water dip at 35°C for 10 or 30 min and 40°C for 10, 20 or 30 min during storage. Others have previously shown that salicylic acid treatment maintained postharvest quality and enhanced nutritional values of fruit and vegetables. Thus, we investigated the effects of incorporating lukewarm water and salicylic acid treatment on postharvest quality and certain bioactive compounds of rambutan cv. Rong-rien during cold storage.

MATERIALS AND METHODS

Plant materials

Rambutan fruit (*Nephelium lappaceum* L., cv. Rong-rien) was obtained from Phu Phan Royal Development Study Center, Sakon Nakhon Province, Thailand. The fruit were harvested at commercial maturity stage, as determined by pericarp color (redness), and immediately delivered to the laboratory. The fruit were then screened again, with those free from physical damage, insect attack and disease selected. The fruit were cleaned with tap water and air dried at room temperature.

Treatments

The fruit were divided into four groups of 16 fruits and subjected to the following four treatments: (1) control, (2) dipped in lukewarm water at 35°C for 20 min, (3) dipped in 1.0 mM salicylic acid solution for 20 min and (4) dipped in 1.0 mM lukewarm salicylic acid solution for 20 min. The treated fruit were then air dried at room temperature. Four fruit were placed in a PET plastic container (size 9x15x6 cm). Each container with four fruits was sealed and punctured (6 holes, 0.5 mm in diameter) using a needle. The fruit were stored at 13±2°C for

9 days. The relative humidity in the container (about 92 ± 2 %RH during storage) was measured using a humidity content meter MS 705SD (Lutron, Taiwan).

Weight loss and firmness

Fresh weight loss was determined before and after storage. Four fruits were weighed before packing in the container (initial weight) and during storage at 3, 6 and 9 days. The percentage of weight loss during storage was calculated and compared to the initial weight. Pulp firmness was determined using a TA-XT II texture analyser (Stable Microsystem, England), equipped with P4 probe. The maximum force-exerted was expressed as Newtons (N) and used for the firmness data.

Superficial color measurement

Superficial color of the rambutan was measured using a HunterLab MiniScan[®]XE Plus (Hunter Associates Laboratory Inc., USA). Color measurements (L^* , a^* and b^* values) were taken. Superficial color of the fruit was expressed as L^* (lightness), a^* (redness), hue angle (h°) and chroma.

Browning score

Scoring test was conducted to evaluate browning score of the rambutan described by Follett and Sanxter (2000). The appraisal was performed by 10 semi-trained panelists in a sensory room. All panelists were trained for agreement on rambutan peel browning before sensory evaluation. The browning score (1 = (best score) no spinterns darkened; 2 = spinterns darkened; 3 = all spinterns darkened and minor pericarp darkened; 4 = all spinterns darkened and 50% or less of the pericarp surface area distinctly darkened; 5 = (worst score) all spinterns darkened and > 50% of pericarp distinctly darkened) was estimated.

Total soluble solids content (TSS) and total acidity (TA)

Total soluble solids content was measured by using a hand-held refractometer (ATAGO MNL-112, Japan) and expressed as °Brix. Total acidity of the fruit juice was determined using titrimetric method. Ten mL of rambutan juice was titrated with 0.1 N NaOH using 1% (w/v) phenolphthalein as the indicator and the titer value was noted. Total acidity was calculated and defined as the percentage of citric acid (% citric acid). TSS/TA ratio was also calculated and recorded.

Total antioxidant capacity and total phenolics content

Five grams of rambutan pulp were homogenised with 25 mL cold distilled water and then centrifuged for 15 min at $4000 \times g$. The supernatant was collected to determine total antioxidant capacity and total phenolics content. Total antioxidant capacity was measured using ferric reducing antioxidant potential (FRAP) according to the method described by Benzie and Strain (1996). The FRAP reagent was a mixture of 25 mL of acetate buffer pH 3, 2.5 mL of 20mM ferric chloride hexahydrate and 2.5 mL of 10 mM 2,4,6-tripyridyl-1,3,5-triazine (TPTZ). The reaction was started when 0.3 mL of supernatant was added into 3 mL of FRAP

reagent. The mixture was then held at room temperature for 30 min before the absorbance at 630 nm was measured. The data was expressed as $\mu\text{mole Trolox equivalents/gram fresh weight}$ ($\mu\text{mole TE g}^{-1}\text{fw}$). Total phenolics content was assayed using the method described by Slinkard and Singleton (1977). The reaction began when 1 mL of extract was mixed into 1 mL of 50% (v/v) Folin-Ciocalteu reagent solution and 2 mL of saturated Na_2CO_3 solution. The reaction was held at room temperature for 30 min before the absorbance at 750 nm was recorded. The data was presented as $\mu\text{g gallic acid/ gram fresh weight}$ ($\mu\text{g GA g}^{-1}\text{fw}$).

Total flavonoids content

Five grams of rambutan pulp were homogenised with 25 mL of 80% methanol and 0.5% sodium bisulphate and then centrifuged at 4000 x g for 15 min. Total flavonoids content was determined using the method carried out by Jia et al. (1999). The reaction started when 0.25 mL of supernatant was mixed with 1.25 mL of distilled water, 75 μL of 0.5% NaNO_2 . The mixture was held at ambient temperature for 6 min and then 150 μL of 10% $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ was added and allowed to stand for 5 min. Then, 0.5 mL of 1M NaOH was added. The absorbance of the mixture was measured at 510 nm. The data were expressed as $\mu\text{g catechin equivalents/ gram fresh weight}$ ($\mu\text{g catechin g}^{-1}\text{fw}$).

Total ascorbic acid content

Five grams of rambutan pulp were extracted with 20 mL of cold 5% metaphosphoric acid by using a homogeniser and then centrifuged at 12000 x g for 15 min at 4°C. Total ascorbic acid content was assayed using the method carried out by Hashimoto and Yamafuji (2001). Then, 0.8 mL of supernatant was mixed with 0.4 mL of 2% di-indophenol. Then, 0.8 mL of 2% thiourea and 0.2 mL of 1% dinitrophenol hydrazine were added. The mixture was held at 37°C for 3 h and then 1 mL of 85% sulphuric acid was added. The mixture was again incubated at room temperature for 30 min before the absorbance at 540 nm was recorded. The data was expressed as $\mu\text{g ascorbic acid / gram fresh weight}$ ($\mu\text{g AsA g}^{-1}\text{fw}$).

Statistical analysis

All experiments were arranged in a completely randomized design. The data are shown as the means of four replications and standard deviation bar. The data were analysed statistically using analysis of variance (ANOVA) and the difference among the means were determined for significant different at $p < 0.05$ using Duncan Multiple Rang Test (DMRT) performed in SPSS software program.

RESULTS

Weight loss and external browning score

This study detected only a small, but continuously decreasing, weight loss in the fruit during storage (Figure 1). At day 9, the weight loss of the fruit had only reached 1.3%. Salicylic acid and/or lukewarm water dips slightly delayed

the loss of fresh weight, when compared to the control, although the differences were not significant.

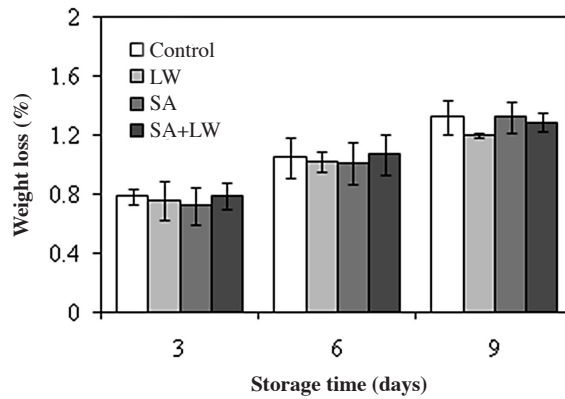


Figure 1. The percentage of weight loss of ‘Rong-rien’ rambutan dipped in lukewarm water (LW), salicylic acid solution (SA) and lukewarm salicylic acid solution (SA+LW) during storage. Each bar represents the mean \pm standard deviation of the results from four replicates.

External browning increased continually during storage, but remained relatively low based on the final browning score of approximately 2.0, with browning present at the base of the spinterns (Figure 2). At the end of storage, the highest external browning score was found in the control (2.1), followed by the lukewarm water dip (2.0) and SA and SA+LW dips (both 1.9). However, the differences were not significant.

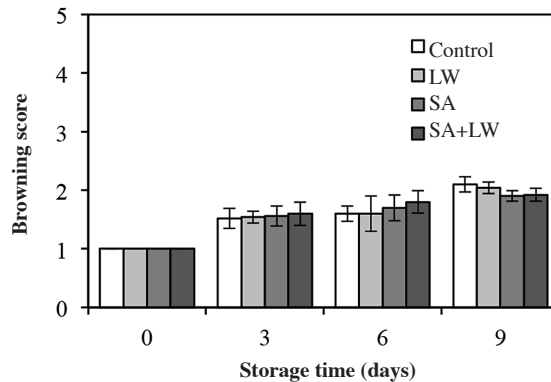


Figure 2. External browning score of ‘Rong-rien’ rambutan dipped in lukewarm water (LW), salicylic acid solution (SA) and lukewarm salicylic acid solution (SA+LW) during storage. Each bar represents the mean \pm standard deviation of the results from four replicates.

Superficial colors

Lightness (L^*), redness (a^*), hue angle (h°) and chroma of rambutan peel are shown in Figure 3. During storage for 6 days, the L^* value (approximately 33) remained constant (Figure 4A). On day 9, the L^* value decreased significantly in all treatments ($P \leq 0.05$). The control had the lowest L^* value; this difference was statistically significant ($P \leq 0.05$) compared to the LW, SA and SA+LW dips. This showed that LW, SA and SA+LW dips delayed the loss of L^* value in rambutan peel during storage. A positive a^* value represents red color of the fruit peel (Figure 3B). The redness of the fruit body increased continuously and significantly over storage ($P \leq 0.05$). After day 6, the increased a^* values seemed constant until the end of storage (day 9) and no significant difference occurred in all treatments. The h° and chroma values remained constant over storage and no significant differences between treatments were found (Figure 3C and 3D). The h° value of the fruit was approximately 46, which presented as orange-red color. Salicylic acid and/or lukewarm water dips had no effect on the color of the fruit, except its L^* value.

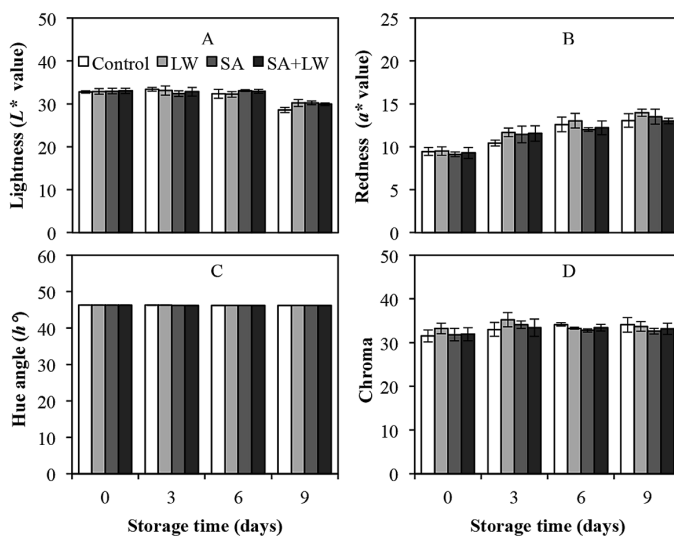


Figure 3. Superficial color of 'Rong-rien' rambutan at various intervals after treatment with lukewarm water (LW), salicylic acid solution (SA) and lukewarm salicylic acid solution (SA+LW). Each bar represents the mean \pm standard deviation of the results from four replicates.

Firmness, total soluble solids and total acidity

The firmness of the rambutan pulp decreased continuously throughout storage (Figure 4). A rapid decrease in firmness was detected in the control and lukewarm water dip samples during storage, and a much smaller decrease with the SA and SA+LW dips. After day 6, the firmness of the control and lukewarm water dip samples was significantly lower than that of the SA and SA+LW dips ($P \leq 0.05$). The salicylic acid dips maintained the firmness of rambutan.

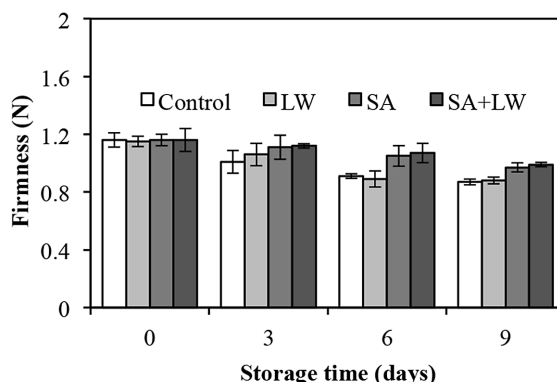


Figure 4. Firmness of ‘Rong-rien’ rambutan dipped in lukewarm water (LW), salicylic acid solution (SA) and lukewarm salicylic acid solution (SA+LW) during storage. Each bar represents the mean \pm standard deviation of the results from four replicates.

Figure 5 shows essentially no changes in TSS, TA and TSS/TA ratio of rambutan during storage. TSS of the fruit was relatively constant (17-18.5 °Brix) during storage, with no significant difference detected in each treatment throughout storage (Figure 5A). A slight decrease in TA was detected in all treatments during storage; however, the differences were not significant (Figure 5B). The calculated TSS/TA ratio of the fruit (2.45) also remained constant in all treatments during storage (Figure 5C).

Biologically active compounds

The changes in antioxidant capacity, total phenols, total flavonoids and total ascorbic acid contents of the rambutan fruit are shown in Figure 6. On the initial day of storage, the antioxidant capacity measured by FRAP assay was approximately $0.76 \mu\text{mole TE g}^{-1}\text{fw}$ (Figure 6A). At day 9, the antioxidant capacity of the control and lukewarm water dip samples remained constant, but that of SA and SA+LW dip samples ($0.81 \mu\text{mole TE g}^{-1}\text{fw}$) increased significantly ($P \leq 0.05$). Total phenols of both control and lukewarm water dip samples decreased, while those of the SA and SA+LW dip samples increased (Fig 6B). Total phenols of both salicylic acid dip samples were significantly higher than that of control and lukewarm water dip samples. Total flavonoids of both control and lukewarm water dip samples decreased significantly during storage ($P \leq 0.05$) while total flavonoids of both salicylic acid dip samples increased (Figure 6C). At day 9, total flavonoids of control and lukewarm water dip samples were 0.62 and $0.30 \mu\text{g catechin g}^{-1}\text{fw}$ and those of SA and SA+LW dip samples were 1.83 and $1.61 \mu\text{g catechin g}^{-1}\text{fw}$, respectively. A reduction of total ascorbic acid content was found in the control and lukewarm water dip samples, and an increase was detected in the SA and SA+LW dips (Figure 6D). Total ascorbic acid content of the SA+LW dip samples was significantly higher than that of the control and lukewarm water dip samples ($P \leq 0.05$). These results indicate clearly that salicylic acid dips prevented the loss and also enhanced bioactive compounds in rambutan during storage.

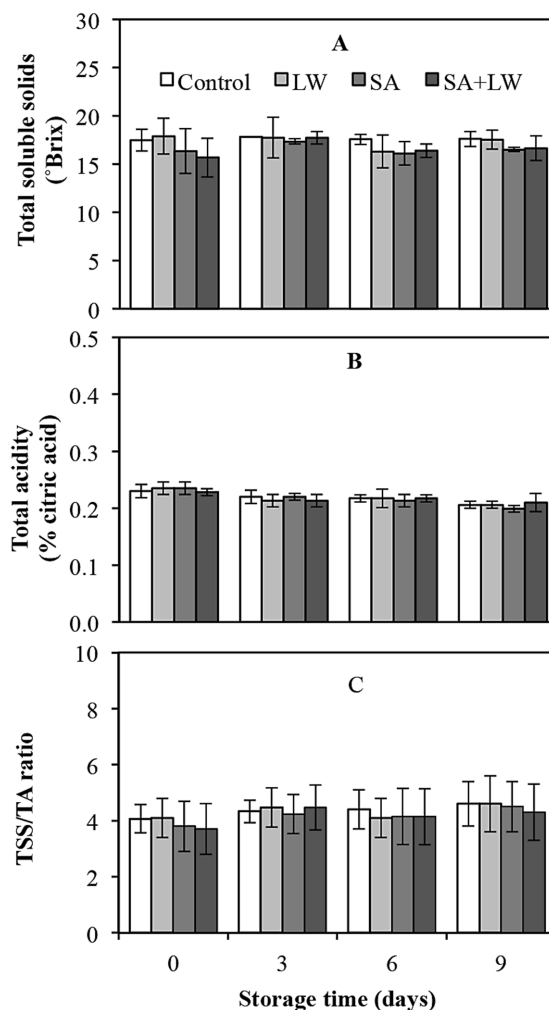


Figure 5. Total soluble solids (TSS) (A) and total acidity (TA) (B) of ‘Rong-rien’ rambutan dipped in lukewarm water (LW), salicylic acid solution (SA) and lukewarm salicylic acid solution (SA+LW) during storage. Each bar represents the mean \pm standard deviation of the results from four replicates.

DISCUSSION

Weight loss, due to loss of moisture content, is a major factor affecting visual appearance and shelf life of rambutan. The increased weight loss is positively related to the shrivelling of spinterns and browning of the skin (O’Hare, 1995). Our research showed that salicylic acid and/or lukewarm water dips slightly delayed the loss of fresh weight when compared to the control. During storage for 9 days at $13\pm 2^{\circ}\text{C}$ and $92\pm 2\%\text{RH}$, spintern shrivelling was not found and external browning was present at the base of the spinterns only. The low external browning score

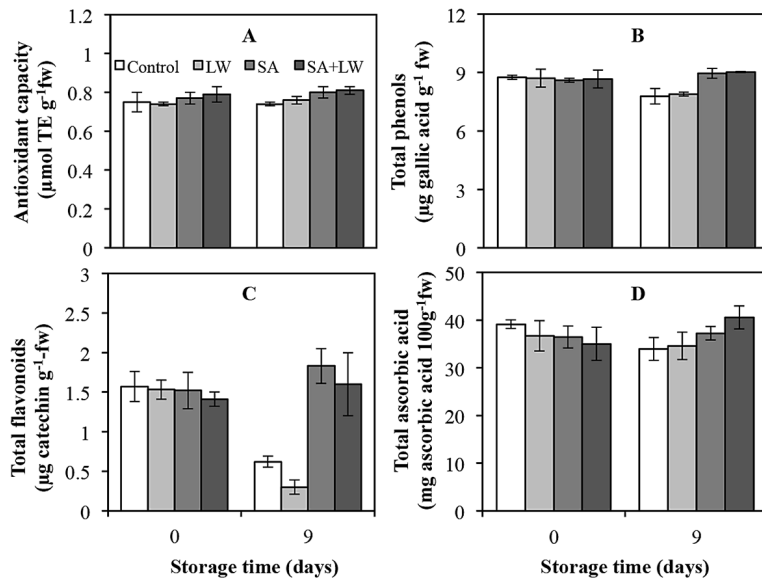


Figure 6. Antioxidant capacity (A), total phenolic compounds (B) total flavonoids (C) and total ascorbic acid (D) content of ‘Rong-rien’ rambutan dipped in lukewarm water (LW), salicylic acid solution (SA) and lukewarm salicylic acid solution (SA+LW) during storage. Each bar represents the mean ± standard deviation of the results from four replicates.

we detected was obviously related to the low weight loss, as shown in Figure 1. O’Hare (1995) showed that water loss from rambutan occurs largely through its spinterns, which have a stomata density approximately five times greater than the skin. In this study, we found the browning occurred only at the base of spinterns. Similarly, Yingsanga et al. (2008) reported that the browning of rambutan spinterns increased more than that of the skin during storage in both high and low relative humidity and the severity of browning was associated with increased weight loss. These findings indicate that the external browning of rambutan was related to the loss of moisture from the spinterns and skin. Similarly, Widjanarko et al. (2000), Latifah et al. (2009), Julianti et al. (2012), and Shao et al. (2013) reported that the browning development of rambutan packed in plastic containers was positively related to the loss of fruit weight. Srilaong et al. (2002) also reported that the development of rambutan senescence was delayed by keeping the fruit in an LLDPE bag, which maintained a high relative humidity. Browning development is also associated with increased ion leakage from the spinterns and peel of the fruit.

The browning score of the fruit treated with salicylic acid was slightly lower than that of the control and lukewarm water dips (Figure 2). This might be the effect of salicylic acid inducing plant defence mechanisms and enhancing antioxidants, which reduces free radicals and maintains cell membrane integrity (Supapvanich and Promyoo, 2013). However, this protection is not enough to

inhibit the browning of rambutan pericarp. This suggests that the prevention of moisture loss from the fruit during storage is more essential for maintaining visual appearance and reducing browning of rambutan; however, disease development during storage must be considered.

Fruit rotted rapidly in all treatments after day 11 of storage (data not shown). The occurrence of fruit rot at day 12 was associated with high relative humidity in the package and the fluctuating temperature during storage.

Superficial color is a key factor indicating the visual quality of rambutan. Landrigan et al. (1996) and Julianti et al. (2012) reported that the reduction of redness and lightness in rambutan during storage was concomitant with the increased weight loss. In our study, weight loss of the fruit was low, thus the redness of the fruit peel in each treatment remained constant. The reduction of lightness in our study was related to the increase in browning score of the fruit. Landrigan et al. (1996) suggested that external browning development and the reduced L^* value of rambutan stored in high relative humidity was involved with increased enzymatic browning reaction. Similarly, Yingsanga et al. (2008) reported that the browning of rambutan spinterns during storage at high relative humidity was related to the increase in PPO and POD activity. Latifah et al. (2009) reported an increase in redness of rambutan during storage, with no change in redness of the fruit body packed in telescopic fibreboard boxes wrapped with plastic films and a relative humidity inside the containers within a range of 90-98% RH. A similar result was also reported by Srilaong et al. (2002). Our study showed that the postharvest application of salicylic acid and/or lukewarm water did not affect visual color changes, except pericarp lightness. Similarly, Follett and Sanxter (2000) reported that a^* value of rambutan treated with hot forced-air and irradiation treatments remained constant throughout storage at 80% RH. These results suggest that the prevention of moisture loss from the fruit during storage is more essential for maintaining visual appearance and superficial color of rambutan peel than salicylic acid and/or lukewarm water dips.

Softening is one of main problems undermining the eating quality of rambutan. It is widely recognised that key factors relating to fruit softening are the depolymerisation and degradation of cell wall components and the deterioration of the cell membrane (Brummell, 2006). Postharvest application of salicylic acid is an effective approach inhibiting tissue softening in many kinds of fruit by reducing cell wall hydrolases activities and maintaining cell membrane integrity (Supapvanich and Promyou, 2013). Salicylic acid has been shown to prevent fruit softening on kiwifruit (Zhang et al., 2003), banana (Srivastava and Dwivedi, 2000), sugar apple (Mo et al., 2008) and peach (Khademi and Ershadi, 2013). Mo et al. (2008) suggested that salicylic acid treatment reduces lipoxygenase activity and superoxide free radical production in fruit, resulting in maintained cell membrane structure and integrity. Wei et al. (2011) suggested that exogenous application of salicylic acid enhances defence mechanisms and production of antioxidants in fruits during storage, leading to a decrease in lipid peroxidation of the cell membrane. Srivastava and Dwivedi (2000) reported that the use of salicylic acid inhibited certain cell wall hydrolases activities, such as polygalacturonase and xylanase in

banana fruit. Zhang et al. (2003) reported that salicylic acid effectively prevented kiwifruit softening during storage.

As the results show in Figure 5, salicylic acid and/or lukewarm water dips had no effect on the changes in taste of the rambutan. In contrast, salicylic acid treatment has an effect on the taste of climacteric fruit, as it is an ethylene inhibitor (Supapvanich and Promyou, 2013). In banana and kiwifruit, salicylic acid application maintains a low TSS by reducing invertase activity and sucrose-phosphate synthase and delaying the breakdown of starch (Srivastava and Dwivedi, 2000; Asghari and Aghdam, 2010; Aghdam et al., 2011). Kazemi et al. (2011) reported that salicylic acid treatments delayed TA decline during ripening of kiwifruit. Lu et al. (2011) reported similar results for winter pineapple fruit. However, salicylic acid dips had no effect on TSS and TA of rambutan in our study. It is widely accepted that rambutan is a non-climacteric fruit, which is generally harvested at peak maturity. Thus, TSS, TA and TSS/TA ratio of the fruit do not evidently change during storage. Follett and Sanxter (2000) and Widjanarko et al. (2000) also reported no changes in TSS, TA and pH of rambutan during storage.

Bioactive compounds in fruit and vegetables have been widely accepted as providing health benefits, beyond basic nutrients. Salicylic acid treatment has been reported to enhance bioactive compounds in many kinds of fruit and vegetables (Supapvanich and Promyou, 2013). Sarikhani et al. (2010) reported that the use of salicylic acid induced total phenols in grape berries by stimulating phenylalanine ammonia-lyase activity. Chen et al. (2006) reported a similar result. Huang et al. (2008) showed that salicylic acid activated plant defence mechanisms and biosynthesis of certain bioactive compounds, including antioxidant compounds, total phenols and flavonoid content in peach fruit during storage. Sayyari et al. (2009) suggested that the use of 2 mM salicylic acid was highly effective in reducing ascorbic acid loss in pomegranate fruit. In asparagus, salicylic acid treatment could induce ascorbic acid, antioxidants, and total phenols during storage (Wei et al., 2011). Similar to our study, Razavi et al. (2014A) reported that salicylic acid treatment enhanced postharvest quality of peach fruit by maintaining firmness, inducing bioactive compounds such as antioxidant potential, total phenols, and total flavonoids contents. Although the enhancement of bioactive compounds by lukewarm water dip had been reported for fresh-cut sweet leaf bush (Supapvanich et al., 2012) and jujube fruit (Promyou et al., 2012), the enhancement of these compounds in rambutan by using lukewarm water was not obvious. The literature and our study evidently support that the use of salicylic acid enhances biologically active compounds in fruit, including rambutan, that impart health benefits to consumers.

CONCLUSION

In summary, although the use of salicylic acid and/or lukewarm water delayed external browning of rambutan, the treatments were not enough to inhibit browning during storage, as the key factor that affected rambutan appearance and browning was relative humidity. Salicylic acid and/or lukewarm water dips

had no effect on the changes in rambutan colors during storage, but retarded the reduction of skin lightness. For eating quality, TSS, TA and TSS/TA ratio of the fruit remained constant over storage in all treatments and SA and SA+LW dips obviously inhibited rambutan softening and enhanced nutritional values involving antioxidant capacity, total phenols, total flavonoids and total ascorbic acid content. According to the results, salicylic acid dips clearly maintained eating quality and enhanced nutritional values of 'Rong-rien' rambutan fruit. However, a combination between postharvest salicylic acid use and other approaches to eliminate browning development and spintern shrivelling are required.

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