
Efficiency of salicylic acid immersion using fine-bubble technique on quality of *Musa* AAA fruit during ripening

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Abstract The physiological quality and bioactive compound in changes of banana (*Musa* AAA) fruit was determined by using 2 mM salicylic acid solution with 2 different methods. Dipping (SA) and immersion using fine-bubble technique (SAF) with 2 different period of time (15 and 30 minutes) were used. Peel color, fruit firmness, total acidity (TA), antioxidant, total phenol and total flavonoid were investigated. The result showed that banana treated with SAF for 15 minutes could maintain lightness (L*) and yellowness (b*) of peel color. In term of fruit firmness, there were not significantly different among the treatments. After 4 days of color break, banana treated with SAF for 15 minutes also resulted in high TA, antioxidant and total phenol. While SAF for 30 minutes resulted in high total flavonoid contents. It concluded that SAF technique can maintain and strengthen physiological quality and bioactive compound changes of banana (*Musa* AAA) fruit during ripening.

Keywords: Fine-bubble technique, *Musa* AAA, Postharvest quality, Salicylic acid

Introduction

Thailand's total land area is about 51.3 million ha which agricultural use area shares 20.97 million ha. Fruit crop area is 1.25 million ha with total production of 9.02 million tons, whereas banana area is about 76,815 ha. Banana is one of economic fruit crops which producing 117,427 tons of fruit annually. One of the most popular cultivars of banana in Thailand is "Hom Tong" (AAA group, Gros Michel). In 2012, Thailand produced banana "Hom Tong" 231,031 ton for domestic and 2,169 for export. The average farmgate price was 186 USD per metric tons for large grade. (OAE, 2012) Banana is a common fruit crop in Thailand. Thai people always use banana in many ways such as, fresh fruit, raw banana fruit is used as vegetable in Thai food, banana flowers, banana leaves, banana pastes and banana chips are also use in Thai cooking. The main varieties of banana in

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Thailand are 'Hom' banana, especially the “Hom Thong” variety (AAA group), “Kai” banana (AA group) and “Namwa” banana (AB group). The Hom Thong banana and the Kai banana are grown for export on the other hand the “Namwa” banana are grown for domestic consumption.

As we know, banana is one of the antioxidative food (Kanazawa and Sakakibara, 2000). The chemical composition of the banana has shown that it is rich in minerals and dietary fiber. It is one of the good source of vitamins C and E (von Loesecke, 1950). In term of the antioxidants gallicocatechin, catechin, and epicatechin were previously identified in banana (Someya *et al.*, 2002). Antioxidant activities in banana were studied by using DPPH, ABTS and FRAP assays (Sulaiman *et al.*, 2011 and Darsini *et al.*, 2012).

Salicylic acid is a plant hormone which played role as an inhibitor of ethylene biosynthesis and delayed plant senescence (Ozeker, 2005). It has been shown to suppress ACC oxidase activity (Fan *et al.*, 1996) then ACC was inhibited to convert to ethylene (Leslie and Romani, 1988). Salicylic acid is also related in the potential of plant pathogens resistance (Yalpani *et al.*, 1994; Kang *et al.*, 2003). Salicylic acid has been reported to correlate with postharvest diseases and can extend the shelf life. Salicylic acid and post-harvest guava fruit were reported using a 32 $\mu\text{mol/L}$ to decrease fruit rot disease which associate with the activities of enzyme catalase (CAT) and ascorbate peroxidase (AsPOD). Aghdam *et al.* (2011) also reported the same effect as those reported. Zeng *et al.* (2006) studied the effect of salicylic acid and mango fruits on the mango fruit was found to be more resistant to the disease. Yu *et al.* (2007) found that salicylic acid was effective to control infection of *Penicillium expansum* and *B. cinerea* infection which caused fruit rot in Asian pear by stimulating activity of enzyme β -1,3-glucanase, Phenylalanine ammonia lyase (PAL), Polyphenol oxidases (PPO) and Peroxidase (POD). These enzymes are involved in the production of phenolics in plants, which inhibit the growth of pathogens that cause disease. Salicylic acid played role in physiological or biochemical processes which included ion uptake, membrane permeability, enzyme activity, heat production and growth development (Arberg, 1981). Salicylic acid was used to control the firmness of harvested in peaches and strawberry during storage (Li and Han, 1999; Wang *et al.*, 2006; Shafiee *et al.*, 2010; Valero *et al.*, 2011). Junmatong *et al.* (2015) studied the effect of salicylic acid by dipping mature mango in 1 mM SA and distilled water for 10 min. To store the mango at $5 \pm 1^\circ\text{C}$ with $90 \pm 2\%$ RH for 42 days. The result showed that salicylic acid treatment exhibited significantly higher antioxidant activities and increased levels of ascorbic acid, total glutathione, total phenolic compounds, and total antioxidant capacity compared to control.

Fine-bubbles refer to all bubbles with a diameter below 100 μm (ISO20480). Recent attention has been given to industrial application of fine

bubbles for example, in agriculture, aquaculture, medical treatment, industries or household appliances. Fine bubble technology is expected to be successfully applied in agriculture from the following viewpoints 1) To improve agricultural productivity by enhancing growth rate of vegetables, 2) To improve biological or physiological conditions for soil in production sites in terms of suppression of a failure caused by continuous cropping, suppression of eutrophication in field soil caused by nitrogen chemicals, to increase a number of aerobic bacteria and microorganism, to encourage aerobic microorganism in decomposing organic matters 3) Agricultural water treatment by fine bubbles, oxygen dissolution with high efficiency, higher performance of water permeability through soil particles 4) Fine bubble water is a beneficial tool, as already used, for culture solution in hydroponics for the purpose of purification and sterilization (ozone bubbles) (Akimi, 2017). A study of fine-bubble and nano-bubble of 1-MCP treatment to delay the ripening of banana by dipping and spraying. It was found that dipping method can delay the ripening, decrease respiratory rate, ethylene production and maintain the firmness of banana during storage (Pongprasert and Srilaong, 2014). A study on the use of dynamic systems and fine-bubble technology on decontamination of *E. coli* O157: H7 on chili pepper demonstrated that using fine-bubble technology resulted in the highest possible to decontamination of *E. coli* O157: H7 in 40 minutes after treatment (Todsapong and Pokpong, 2016).

In this study, we investigated postharvest quality including physical quality, chemical quality and bioactive compounds in banana (*Musa* AAA) fruit during storage which had different application technique (dipping, fine-bubble) to involve postharvest quality during storage.

Materials and Methods

Raw Material

“Hom Thong” banana (*Musa* AAA) fruits at the full mature stage were ordered from Chumphon province. The fruit bunches were de-handled and selected for uniformity of shape, size, skin color, physical damage and disease., cleaned the fruits with tap-water and dried at room temperature.

Treatments and Application of salicylic acid

Fine-bubble technique was used to compare with the conventional method on the application of salicylic acid. “Hom Thong” banana (*Musa* AAA) fruits were subjected to the following treatments:-Treatment 1 was control, Treatment 2 was 2 mM salicylic acid by dipping for 15 minutes (SA15), Treatment 3 was 2 mM salicylic acid by dipping for 30 minutes (SA30), Treatment 4 was 2 mM salicylic acid by using fine-bubble

technique for 15 minutes (SAF15) and Treatment 5 was 2 mM salicylic acid by using fine-bubble technique for 30 minutes (SAF30). The tested fruits were dried after immersion.

Color measurement

Peel color of banana fruits were measured using HunterLab MiniScan photometer (MiniScan@ XE Plus; Hunter Associates Laboratory Inc.; Reston, VA, USA). The lightness (L^*), greenness ($-a^*$), redness ($+a^*$) and yellowness (b^*) values were recorded (Bolin and Huxsoll, 1991).

Firmness measurement

Five fingers from each hand of banana were used for firmness measurement. Peeled banana was used a Texture Analyzer (TA Plus; Lloyd Limited; Fareham, UK) with a 6 mm cylindrical probe at the middle part of fruit. The result was showed in newton unit at the maximum force.

Total acidity measurements

Five fingers from each hand of banana were selected. 10 g of banana pulp was homogenized with 20 ml. of distilled water and filtered through a cloth. Then used 5 ml aliquot and titrated with 0.1 N NaOH using 1% (w/v) phenolphthalein as the indicator. The result was expressed as % malic acid (Association of official analytical chemists, 1990).

Total antioxidant capacity measurements

Two methods were used to assay the antioxidant activity of the banana pulp; DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (ferric reducing antioxidant potential). The antioxidant activity of the extracts was determined using the method desc ribed by Brand-Williams *et al.* (1995); this methodology is based on the scavenging capacity of the DPPH free radicals. The absorbance was determined by a spectrophotometer at 517 nm. The percentage of antioxidant activity was calculated as the percentage of the DPPH radicals, using Equation: $AA\% = [(A_0 - A_{10})/A_0] \times 100$, where A_0 is the absorbance of the sample at 0 min and A_{10} is the absorbance of the sample at 10 min. To determine the FRAP of the banana pulp, the method described by Benzie and Strain (1996) was used. The absorbance of the colored complex formed with acetate buffer pH 3, 20 mM ferric chloride hexahtrate and TPTZ (2,4,6-Tri(2-pyridyl)-s-triazine) in ratio 10:1:1 (v/v/v) was measured at 630 nm using a spectrophotometer after incubated at room temperature for 30 min. The results were expressed in micromoles of Trolox equivalents per gram fresh weight ($\mu\text{mol TE/gFW}$).

Determination of total phenolic content

Total phenolic content of banana pulp extract was measured according to the method reported by Alothman *et al.* (2009) using Folin-Ciocalteu reagent with some modification. 1 ml of supernatant was mixed with 1ml of 50% (v/v) Folin-Ciocalteu's phenol reagent and 2ml of saturated Na₂CO₃ solution. The mixture was kept at room temperature for 30 min. Absorbance was measured at 750 nm using a spectrophotometer. The total phenolic contents were expressed as micrograms of gallic acid per gram fresh weight (µg GA/g FW).

Determination of total flavonoids content

Total flavonoids contents of the extracts were determined according to the procedure of Zhishen *et al.* (1999). 0.25 ml of the supernatant was added into a mixture of 1.25 ml of distilled water and 0.5% NaNO₂ 75 µl. After 6 min at room temperature, 150 µl of (10% w/v) AlCl₃ was added. After another 5 min, 0.5 ml of 1M solution of NaOH was added. The mixture was shaken vigorously and the absorbance of mixture was recorded at 510 nm using spectrophotometer. The results were expressed as µg of catechin per gram fresh weight (µg catechin/g FW).

Statistical analysis

All data analyses were carried out in ten replications \pm SD. The results were submitted to analysis of variance (ANOVA) with a significance level of 95% ($p < 0.05$) using SPSS software program (SPSS Inc.; Chicago, IL, USA).

Results

Fruit color

Lightness (L^*) and yellowness (b^*) of the banana (*Musa AAA*) peel are shown in Figure 1. After color break, the L^* value remained constant (Figure 1A). After color break for 4 days, the L^* value decreased significantly in all treatments ($P \leq 0.05$). The control had the lowest L^* value compared to the others treatments. This showed that 2mM salicylic treatment delayed the loss of L^* value in banana peel during storage especially when using fine-bubble technique for 15 minute (SAF15). The yellowness (b^*) of banana peel were increased continuously until color break ($P \leq 0.05$). In the treatment using fine-bubble technique for 15 minute (SAF15), the increased of b^* values seemed constant until 4 days after color break (Figure 1B). From the visual appearance of banana (Figure 2), it was

found that on the first day of color break, the banana peel was changed from green to yellow color. The yellow color increased in the following day on day 2. After the color break, the color changed to yellow in all treatments. After 4 days of color break, the results showed that banana peel in treatment using fine-bubble technique for 15 minute (SAF15) had better appearance than other treatments. The brownish appearance on the peel occurred less than other treatments. The control treatment showed the highest percentage of browning on the banana peel surface compared to other treatments. In figure 2 showed that salicylic treatment using fine-bubble technique for 15 minute (SAF15) maintained lightness (L^*) and yellowness (b^*) of peel color with less senescent spots until the fourth day after color break.

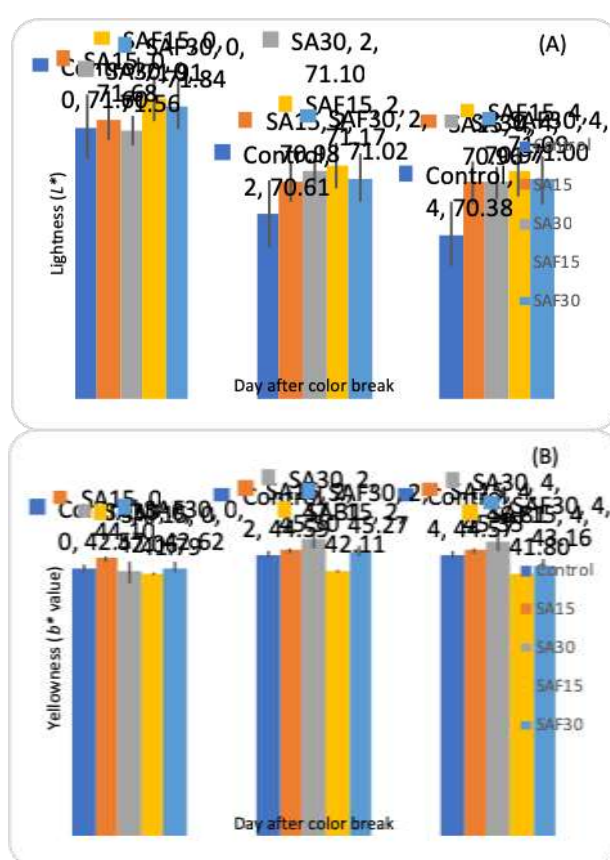


Figure 1. Peel color of banana (*Musa AAA*) after color break with different treatment; control, dipping with 2mM salicylic acid for 15 minute (SA15), Dipping with 2mM salicylic acid for 30 minute (SA30), immersion using fine-bubble technique for 15 minute (SAF15) and immersion using fine-bubble technique for 15 minute (SAF15). Each bar represents the mean \pm standard deviation of the results from 5 replicates.

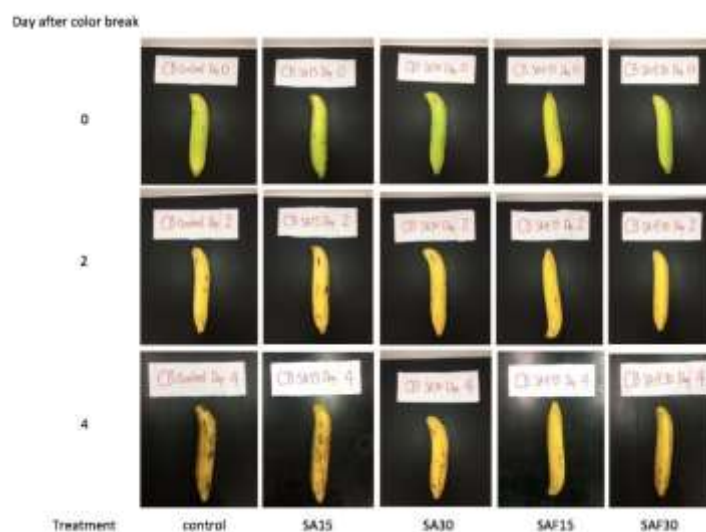


Figure 2. Appearance of banana (*Musa AAA*) fruit after color break with different treatment; control, dipping with 2mM salicylic acid for 15 minute (SA15), Dipping with 2mM salicylic acid for 30 minute (SA30), immersion using fine-bubble technique for 15 minute (SAF15) and immersion using fine-bubble technique for 30 minute (SAF30).

Firmness

Figure 3 showed the changes in pulp firmness after color break. The fruit pulp firmness decreased continuously throughout storage but there were not significantly different among the treatments. But there was higher amount of firmness in immersion using fine-bubble technique for 30 minute (SAF30) treatment compare with control.

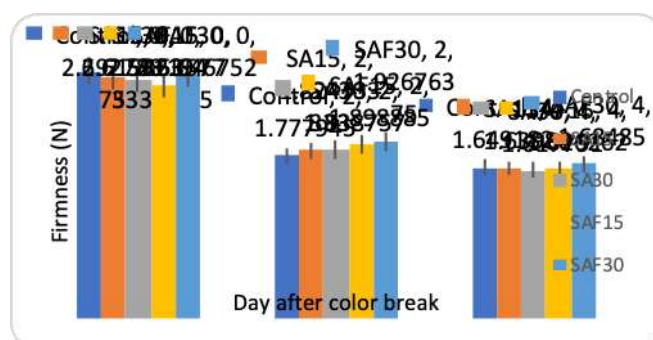


Figure 3. Firmness of banana (*Musa AAA*) pulp after color break with different treatment; control, Dipping with 2mM salicylic acid for 15 minute (SA15), Dipping with 2mM salicylic acid for 30 minute (SA30), immersion using fine-bubble technique for 15 minute (SAF15) and immersion using fine-bubble technique for 30 minute (SAF30). Each bar represents the mean \pm standard deviation of the results from 5 replicates.

Total acidity

The result was found that the total acidity content highest after 2 day of color break and then slowly decrease after 4 after 4 days of color break. After 2 and 4 days of color break, the treatment immersion using fine-bubble technique for 15 minute (SAF15) showed highest amount (0.25 and 0.24 % malic acid, respectively) of total acidity compare to others treatment (Figure 4).

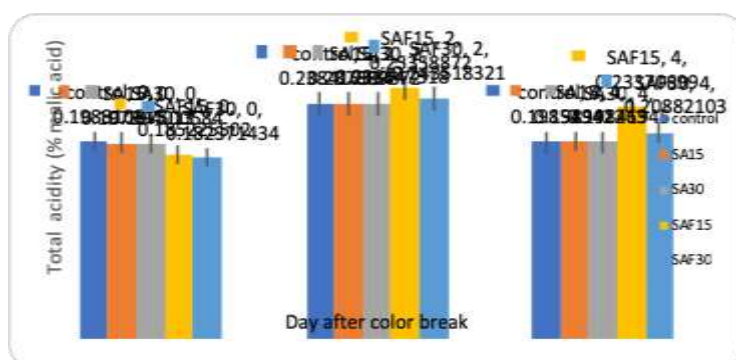


Figure 4. Total acidity (TA) of banana (*Musa AAA*) with different treatment; control, Dipping with 2mM salicylic acid for 15 minute (SA15), Dipping with 2mM salicylic acid for 30 minute (SA30), immersion using fine-bubble technique for 15 minute (SAF15) and immersion using fine-bubble technique for 30 minute (SAF30). Each bar represents the mean \pm standard deviation of the results from 5 replicates.

Bioactive compound

The changes in antioxidant capacity, total phenols and total flavonoids contents of banana (*Musa AAA*) fruit are shown in Figure 5. On the initial day after color break, the antioxidant capacity measured by FRAP assay was remained very low (Figure 5A). After 4 days of color break, the antioxidant capacity was higher in all treatment. The antioxidant capacity of the treatment using fine-bubble technique for 15 minute (SAF15) was significant higher (1.07 μ mole TE g/FW) than others treatments. The result of DPPH radical scavenging activity of banana (*Musa AAA*) fruit are shown in Figure 5B. The amount of the DPPH radical scavenging activity was significant higher in the treatment using fine-bubble technique for 15 minute (SAF15) compares to others treatment. The results are shown that the DPPH radical scavenging activity in the treatment using fine-bubble technique for 15 minute (SAF15) after 0, 2 and 4 days after color break were 19.01, 67.60 and 70.27 %, respectively. Total phenols of the treatment using fine-bubble technique for 15 minute (SAF15) was significantly highest than others treatment. After 0, 2, 4 days after color break total

phenol content were 2.00, 2.35 and 244 mg GA/g FW, respectively (Figure 5C). Total flavonoids of all the treatments decreased significantly after 2 days of color break then small increased after 4 days of color break (Figure 5D). After 4 days of color break, total flavonoids of the treatment using fine-bubble technique for 15 minute (SAF15) was 21.92 μ g catechin g/FW which showed the highest amount of total flavonoid content.

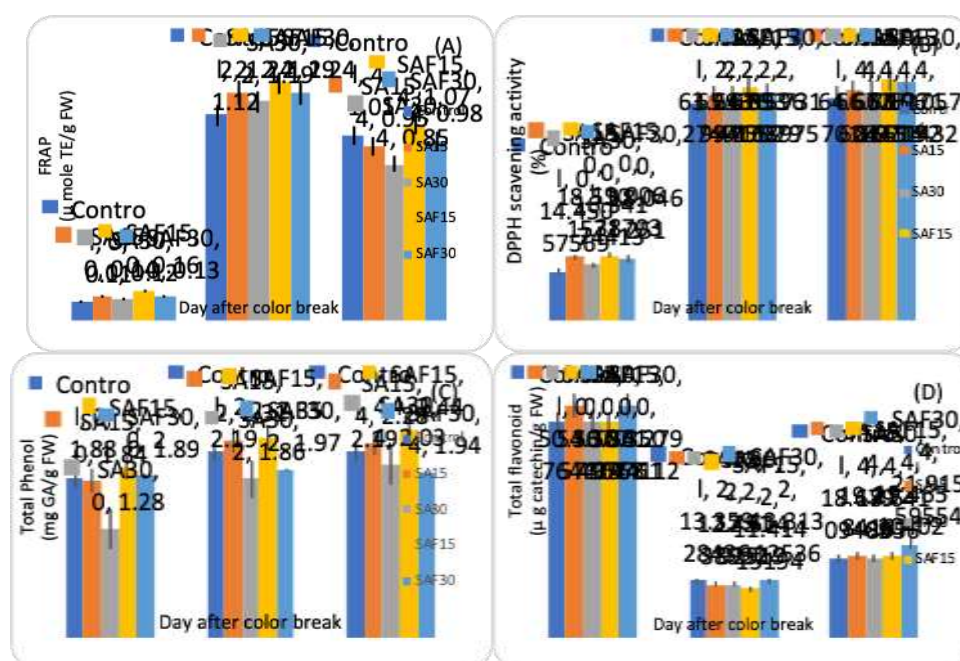


Figure 5. Antioxidant capacity by FRAP assay (A), DPPH radical scavenging activity (B), total phenolic compounds (C) and total flavonoids (D) content of banana (*Musa AAA*) control, Dipping with 2mM salicylic acid for 15 minute (SA15), Dipping with 2mM salicylic acid for 30 minute (SA30), immersion using fine-bubble technique for 15 minute (SAF15) and immersion using fine-bubble technique for 30 minute (SAF30). Each bar represents the mean \pm standard deviation of the results from 5 replicates.

Discussion

According to the result, it showed that the positive effect of salicylic acid on visual appearance and color measurement. The treatment using fine-bubble technique for 15 minute (SAF15) maintained lightness (L^*) and yellowness (b^*) of peel color with less senescent spots better than others treatments. The result from Seymour *et al.* (1987) showed the reduction of total carotenoid content in banana peel due to carotenoid biosynthesis during the early stage of ripening that caused the peel color change from green to yellow. It was found that dipping method can delay the ripening and decrease respiratory rate during storage. Wei *et al.* (2011) reported the

use of salicylic acid can delay the chlorophyll degradation in asparagus at concentration 0.1 mmol/L. In this study, the result showed that the firmness of banana fruit not significant different in each treatment. However, the treatment using fine-bubble technique was showed higher amount of firmness compare to control. Sayyari *et al.* (2011) studied the effect of salicylic acid solution on pomegranate after harvest. The salicylic acid treatment of pomegranate showed a firmness of 16-17 newton while control showed a firmness of only 9 newton. As in the bananas, the study of Srivastava and Dwivedi (2000), salicylic acid has been shown to maintain the firmness of the banana fruit. As a result of salicylic acid, the rate of ethylene production decreased. It also helps in inhibiting the activity of enzymes involved in the decay of cell walls and cell membranes. The enzymes involved, such as polygalacturonase (PG), lipoxygenase (LOX), cellulose and pectinmethylesterase (PME). The result from the treatment immersion using fine-bubble technique for 15 minute (SAF15) showed a high amount of total acidity, antioxidant capacity and total phenols. Moreover, the treatment immersion using fine-bubble technique for 30 minute (SAF30) showed a high amount of total phenol. In some previous study showed that salicylic acid treatment enhanced the activity of antioxidant system and increased the activity of antioxidant in plants which helped to prevent fruit chilling injury during storage (Hayat *et al.*, 2005). Salicylic acid solution was found to promote antioxidant activity in plants. The results showed that salicylic acid solution was able to stimulate antioxidant content in mango (Suwanna *et al.*, 2007). Same as Hooper and Cassidy (2006) that studied the effect of salicylic acid on external exposure in asparagus, it was found that salicylic acid increased the total phenolic content of asparagus (Wei *et al.*, 2011). As in the pomegranate, salicylic acid, affected to increase the total phenolic content during storage (Sayyari *et al.*, 2011). Wang *et al.* (2006) suggested that salicylic acid can prevent chilling injury of peach fruit caused the induction of antioxidant activity. According to Knorzer *et al.* (1999) reported that salicylic acid solution promoted antioxidant activity in plants. Supapvanich *et al.* (2015) reported the use of pre-harvest salicylic acid in vegetables promoted antioxidant activity and prevent the antioxidant activity of lemon basil during storage. Sulaiman *et al.* (2011) which studied regarding antioxidant activities from 8 cultivars of banana grown in Malaysia demonstrated that antioxidant activity of n-hexane, chloroform and methanol peel extracts of Nangka banana were 1.00, 2.01, 0.65 mg TE/g fresh weight and 2.26, 3.99, 3.36 mg TE/g dried weight, respectively by DPPH method and 2.5, 7.40, 3.54 mg TE/g fresh weight and 3.69, 7.50, 10.32 mg TE/g dried weight, respectively by FRAP method. Xavier *et al.* (2016) also showed the result that banana (*Musa acuminata* L. AAA group cv. Grand Naine) which treated with methyl jasmonate and salicylic acid could faster induce the production of phenolic compound. The result also showed that 2.5 mM salicylic acid

treatment had double accumulation of phenolic compounds from 31.20 to 68.75 mg/g of dry leaf within 24h. Phenolic compounds have been reported to stimulate some biological effects, included antibacterial and antioxidant activity (Mokbel and Hashinaga, 2005; Fawole *et al.*, 2012) such as banana, guava, papaya, tea and coffee (Castillo *et al.*, 2002; Thaipong *et al.*, 2006; Chan *et al.*, 2007). To compare the application of salicylic acid, dipping method and fine-bubble technique were used. The fine-bubble technique showed the greatest result compare to dipping method. Both physiological quality and bioactive compound also showed the greatest result in the treatment using fine-bubble technique. Fine bubble technique has been used in many application ways such as ozone fine-bubble treatment. It showed the effective result in remove the residual of fenitrothion and this can apply for continuous bubbling ozone fine-bubble technique that more effective than the previous one (Akimi, 2017). The previous study was conducted by Pongprasert and Srilaong (2014) used 1-MCP micro and nano-sized bubbles to delay banana ripening by dipping and spraying. Our study has been used fine-bubble technique with salicylic acid application. Salicylic acid is adsorbed at the interface with hydrophobic group towards the gas side. On still surface, organic substrate distributes uniformly over the surface at equilibrium condition. However, on moving surface, it distributes non-uniformly, which leads to non uniform surface condition. Accumulation of surfactant starts from the rear side of the bubble and smaller surface tension force with surfactant accumulated (Akimi, 2017). That's the reason why salicylic acid can easily to absorb at fine-bubble surface.

In conclusion, the research indicated that the salicylic treatment with fine-bubble technique for 15 minutes is the most effective treatment in physical quality, certain chemical quality and bioactive compounds in banana (*Musa* AAA).

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