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Research article

Efficacy of salicylic acid incorporated with lukewarm water immersion on postharvest quality of 'Leb Mue Nang' bananas (*Musa* AA) on shelf life storage

Pannipa Youryon[a], Ruamjit Nokkhao[a], Aphisit Suemak[a], Suriyan Supapvanich[b,*]

[a] *Department of Agricultural Technology, Prince of Chumphon Campus, King Mongkut's Institute of Technology Ladkrabang, Chumphon Province, Thailand*

[b] *Innovative Tropical Agriculture Program, Department of Agricultural Education, School of Industrial Education and Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand*

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Abstract

Importance of the work: The shelf life of 'Leb Mue Nang' bananas (*Musa* AA) is relatively short. The decline in quality with shelf life limits the market presence of bananas.
Objectives: To investigate the impact of salicylic acid (SA) incorporated with lukewarm water (LW) immersion on the quality preservation of 'Leb Mue Nang' bananas stored under shelf life conditions (27 ± 2°C, 65 ± 8% relative humidity) for 6 d.
Materials and Methods: First, the effects on banana quality were monitored of SA immersion at 0−2 mM, as well as LW immersion at 45 or 50°C for 5−15 min. Consequently, the impact was examined of SA+LW (SA at 0.25 mM combined with LW at 45°C for 15 min) on the quality preservation of bananas compared to the SA-only, LW-only and control treatments.
Results: In the preliminary study, the bananas subjected to 0.25 mM SA dipping or LW at 45°C for 15 min best preserved postharvest quality. Compared to the control, all treatments delayed senescence spots and maintained pulp firmness, as well as postponing increases in weight loss, total soluble solids content, total acidity and ripening index. Both the SA and SA+LW treatments increased the total phenols and antioxidant activity. The SA+LW treatment decreased skin damage and preserved pulp firmness compared to the SA- and LW-only treatments. All examined quality parameters were significantly and positively correlated during storage, with the exception of pulp firmness, which had a negative correlation to the other parameters.
Main finding: The SA+LW treatment effectively preserved the quality and prolonged the shelf life of the 'Leb Mue Nang' bananas compared to untreated fruits.

* Corresponding author.
E-mail address: suriyan.su@kmitl.ac.th (S. Supapvanich)

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Introduction

The 'Leb Mue Nang' banana, categorized within the *Musa* AA group (Chanaeng et al., 2017), is acknowledged as a unique variety from Thailand, recognized for its sweetness and unique texture (Reference). 'Leb Mue Nang' bananas have gained prominence in the market, accompanied by 'Nam Wa' (*Musa* AAB), 'Kluai Khai' or 'Sucrier' (*Musa* AA) and 'Hom Thong' or 'Gros Michel' (*Musa* AAA) bananas (Anupunt, 2002). 'Leb Mue Nang' bananas are small compared to standard bananas and have a sweet, firm and creamy pulp. Frequently, this cultivar is consumed fresh; however, it may also be used in desserts and traditional Thai cuisine (Pakeechai et al., 2022). Rapid ripening and bruising during handling and transportation can lead to deterioration and reduce a banana's shelf life during marketing, with the shelf life of bananas, including 'Leb Mue Nang' bananas, generally being 3–4 d following the onset of peel color change (Thompson et al., 2019). Postharvest, bananas have a climacteric ripening process marked by heightened ethylene production. Ripening commences when the internal concentration of ethylene achieves a critical threshold and throughout the ripening phase, bananas undergo numerous and swift alterations in color, texture, odor and taste (Thompson et al., 2019). Additionally, a swift conversion of starch to sugar in bananas occurs during the ripening process, affecting their taste and texture, with overripening resulting in a mushy texture (Youryon and Supapvanich, 2017a). As with 'Sucrier' bananas, black or senescence spots on the interior of 'Leb Mue Nang' banana peels generally signify over-ripeness (Youryon and Supapvanich, 2017b). The formation of black spots has been proven to be associated with the programmed cell death of stomata on banana peels (Anusornpornpong et al., 2024).

Salicylic acid (SA) is acknowledged as a plant regulator, substantially influencing postharvest physiology by improving defence mechanisms, physicochemical quality and the shelf life of fruits and vegetables (Asghari and Aghdam, 2010; Supapvanich and Promyou, 2013; Mustafa et al., 2018). Furthermore, SA postpones the ripening process by suppressing the expression and activity of ACC synthase and ACC oxidase genes (Li et al., 2022). Research has documented that the postharvest application of SA postponed the ripening process in bananas and enhanced their shelf life and chilling stress resistance during low temperature storage (Srivastava and Dwivedi, 2000; Khademi et al., 2019; Xu et al., 2019). These authors suggested that SA lowered ethylene production, the climacteric peak of respiration, starch-sugar conversion and the activities of cell wall hydrolases, as well as producing an enhanced antioxidant system and membrane integrity in bananas. Furthermore, a recent study indicated that the application of methyl salicylate postponed the formation of senescence spots in 'Sucrier' bananas by upregulating the expression of anti-apoptotic genes, which are crucial in inhibiting the activation of mitochondria-mediated programmed cell death (Chotikakham et al., 2022). Other studies have suggested that exogenous SA application enhances defence mechanisms and the antioxidant system, inducing oxidative stress and disease resistance (Mustafa et al., 2018; Surendran et al., 2018).

Postharvest heat treatments involve exposing fruits and vegetables to elevated temperatures for a short period; however, treatment duration and temperature range vary greatly from several days at 35–39°C in hot air, to a maximum of 63°C for only 1 min in hot water (Lurie and Pedreschi, 2014). In bananas, the temperatures of hot water immersion are in the range 40–55°C for 10–30 min, contingent upon the variety (Marrero Domínguez et al., 1997; Kaka et al., 2019). This method is used to improve the quality and shelf life of produce by diminishing the incidence of postharvest diseases, enhancing stress resilience and delaying ripening processes (Lurie and Pedreschi, 2014). Postharvest heat treatments impact regular protein synthesis and cellular metabolism during heat stress by causing an immediate disassociation of polyribosomes, leading to a temporary cessation of protein synthesis, which subsequently resumes with an additional set of proteins, including heat shock proteins (Spadoni et al., 2014). This alteration inhibits normal ripening processes and prevents postharvest storage disorders (Lurie and Pedreschi, 2014). Other studies have reported that hot water treatment at 45°C for 15 min or 50°C for 10 min preserved the postharvest quality of *Musa* AA bananas (Varit and Songsin, 2011) and *Musa* AAA bananas (Marrero Domínguez et al., 1997; Ummarat et al., 2011), involving delayed peel color development and black spot formation, while maintaining pulp firmness and enhancing antioxidants. Additionally, the utilization of SA combined with hot water immersion has been shown to have a synergistic impact on maintaining postharvest quality and augmenting the antioxidant system in strawberries (Niazi et al., 2021) and pomegranates (Moradi et al., 2022). Supapvanich (2015) and Supapvanich and Promyou (2017) found that the utilization of SA combined with lukewarm water (LW) immersion retarded the decrease in fresh weight and pulp softening, suppressed postharvest diseases and enhanced bioactive components and antioxidants in rambutans and papayas. Therefore, the aim

of the current research was to determine the efficacy of SA incorporated with LW immersion on the postharvest quality maintenance of 'Leb Mue Nang' bananas on shelf life storage.

Material and Methods

Raw material and experiments

'Leb Mue Nang' bananas (*Musa* AA.) were obtained from an orchard in Chumphon Province, Thailand. The bananas were harvested at the mature green stage (2 mth after flowering, approximately 80% maturity). Two hands of bananas from both the upper and lower layers of the banana bunch were discarded. Subsequently, the remaining banana hands were separated from the bunch and immersed in 200 µL/L sodium hypochlorite for 10 min. The bananas were selected for their uniform skin color and absence of defects, including damage and disease and subsequently cut into four fingers for each cluster (one replicate). In the preliminary studies (consisting of two experiments), the first experiment involved immersing the bananas in SA (Loba Chemie™; India) at concentrations of 0.25 mM, 0.5 mM, 1.0 mM or 2.0 mM for 5 min, while the second experiment involved treating the bananas with LW immersion at 45°C or 50°C for 5 min, 10 min or 15 min using a water bath. The untreated bananas were used as the control group. After treatments, the bananas were kept in shelf life condition (27 ± 2°C, 65 ± 8% relative humidity, RH) for 6 d. Three replicates of bananas were sampled at 3 d intervals during storage to investigate the postharvest quality, based on weight loss, peel color, pulp texture, ripening index (BrimA) and antioxidant activity. The outcomes of both preliminary experiments indicated that the treatments of 0.25 mM SA and LW at 45°C for 15 min better preserved the postharvest quality of the bananas than the other treatments. Consequently, the treatment of 0.25 mM SA and the LW (lukewarm 0.25 mM SA at 45ºC) was set up to examine its effect on banana quality maintenance during storage using shelf life conditions compared to the untreated group and the SA-only and LW-only treatments. Three replicates of bananas were randomly sampled at 3 d intervals to determine quality attributes of visual appearance, peel color, pulp firmness, total soluble solids content (TSS), total acidity (TA), BrimA, total phenolic compounds and antioxidant capacities. In this study, the visual appearance, peel color (10% of peel discoloration) or disease incidence (5% incidence of disease), or both, were the criteria applied to determine the end of banana shelf life.

Visual appearance and peel color determinations

The appearance of the bananas was assessed by images taken during storage. The peel color of the bananas was examined in the middle part of each fruit using a HunterLab colorimeter (MiniScan@ XE Plus; Hunter Associates Laboratory Inc.; USA). The color of the banana peel was measured using the CIE Lab color space, with particular emphasis on brightness (L^*) and yellowness (b^*) values.

Firmness measurement

The firmness of the banana pulp was assessed using a TA-XT II texture analyzer (Stable Microsystems; UK), utilizing a cylindrical probe (P/2) to penetrate the middle part of the banana at a velocity of 1 mm/s. The peak force measurement was documented and represented in newtons (N).

Total soluble solids, total acidity and ripening index determinations

The total soluble solids (TSS) of each banana pulp sample were measured as a percentage using a refractometer (ATAGO; Japan). The total acidity (TA) was quantified based on a standard procedure defined by Association of Official Analytical Chemists (2000). A mixture consisting of 5.0 mL of banana pulp juice and 2−3 drops of 1% (w/v) phenolphthalein (as an indicator) was titrated with 0.1 N NaOH. The amount of 0.1 N NaOH used to reach the titration endpoint was recorded, based on a light pink coloration lasting for a minimum of 30 s. The total acidity of the fruit juice was represented as the percentage of malic acid. BrimA has been used as an acceptable ripening indicator compared to the TSS-to-TA ratio (Magwasa and Opara, 2015). The ripening index (BrimA) of bananas was computed using Equation 1 (Magwasa and Opara, 2015):

$$\text{BrimA} = \text{TSS} - (k \times \text{TA}) \quad (1)$$

where k = 5.

Total phenolic compounds assay

A sample of banana pulp (5 g) was extracted using 30 mL of 80% ethanol. The filtrate was used to quantify the total phenolic compounds (TP), based on the methodology outlined by Slinkard and Singleton (1997). In brief, 1 mL of the extract was mixed with 1 mL of 50% (v/v) Folin-Ciocalteu reagent and

2 mL of saturated sodium carbonate solution. The mixture was allowed to incubate at room temperature (30 ± 1°C) for at least 30 min. The absorbance at 750 nm was recorded and the TP concentration was calculated using a linear equation obtained from a gallic acid standard curve. The data were presented as milligrams of gallic acid per kilogram of fresh weight.

Antioxidant capacity assays

A sample (5 gram) of the banana pulp was homogenized with 80% (v/v) ethanol and subsequently passed through Whatman No. 4 filter paper. The filtrate was used for the assessment of ferric reducing antioxidant potential (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity. The FRAP was assessed using the method outlined by Benzie and Strain (1996). The extract was reacted with FRAP reagent, which comprised acetate buffer at 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. The reaction was performed at room temperature (30 ± 1°C) for 30 min. Subsequently, the absorbance at 630 nm was measured and computed using a linear equation generated from a Trolox standard curve. Data were presented as micromoles Trolox equivalents per kilogram of fresh weight. The DPPH free radical scavenging activity was evaluated using the method established by Brand-Williams et al. (1995). The extract was mixed into 1 mM DPPH in methanol and the absorbance at 517 nm was measured (A_0). Subsequently, the mixture was maintained in a dark environment for 5 min and the absorbance at 517 nm was recorded once more (A_2). The proportion of DPPH free radical scavenging activity was determined using Equation 2:

$$\text{DPPH scavenging activity (\%)} = [(A_0 - A_2)/A_0] \times 100 \quad (2)$$

Statistical analysis

All studies were conducted using a completely randomized design. Multivariate analysis of variance and Pearson's correlation (r) were performed using the SPSS software (version 26; IBM Corporation; USA). Data were presented as mean ±SD values, based on three replicates (n = 3). Differences among treatment means were evaluated using the least significant difference *post-hoc* test at a significance level of $p < 0.05$.

Results

Quality of 'Leb Mue Nang' bananas treated with salicylic acid

Fig. 1 depicts the physicochemical quality attributes of 'Leb Mue Nang' bananas subjected to SA immersion at various concentrations at ambient temperature. The weight loss, peel color development, ripening index and antioxidant activity of the bananas significantly increased, except for the pulp firmness, which significantly declined during storage. The results revealed that the color development, including the increases in peel brightness and yellowness, was comparable in all treatments during storage (Figs. 1C and 1D). Additionally, compared to untreated bananas, the alteration in the visual appearance of the bananas, illustrated in Supplementary Fig. 1S, indicated that SA immersions delayed ripening and the formation of black spots during a shelf life of 6 d. In addition, SA slowed down the rate of weight loss. As shown in Fig. 1A, the bananas subjected to 0.25 and 0.5 mM SA immersion had a significantly lower weight loss than the control and the 2 mM SA-treated group after storage for 6 d. However, the weight loss of the bananas treated with 1 mM SA on day 6 did not differ significantly from that of groups treated with SA concentrations of 0.25 mM, 0.5 mM and 2 mM. SA immersion maintained the firmness of bananas compared to the control group; however, the firmness of all bananas treated with any of the levels of SA immersion remained comparable throughout storage (Fig. 1B). The ripening index of the bananas subjected to any of the levels of SA immersion was significantly lower than that of the control bananas after storage for 3 d (Fig. 1E). As a result, the banana ripening index was comparable in all treatments after 6 d of storage. Notably, SA immersion significantly enhanced the antioxidant activity in the banana pulp compared to the control group (Fig. 1F). Among the SA treatments, 0.25 mM SA immersion increased antioxidant activity the most. Furthermore, the application of SA at higher concentrations (0.5−2 mM) did not produce a significant difference in postharvest quality preservation compared to 0.25 mM immersion.

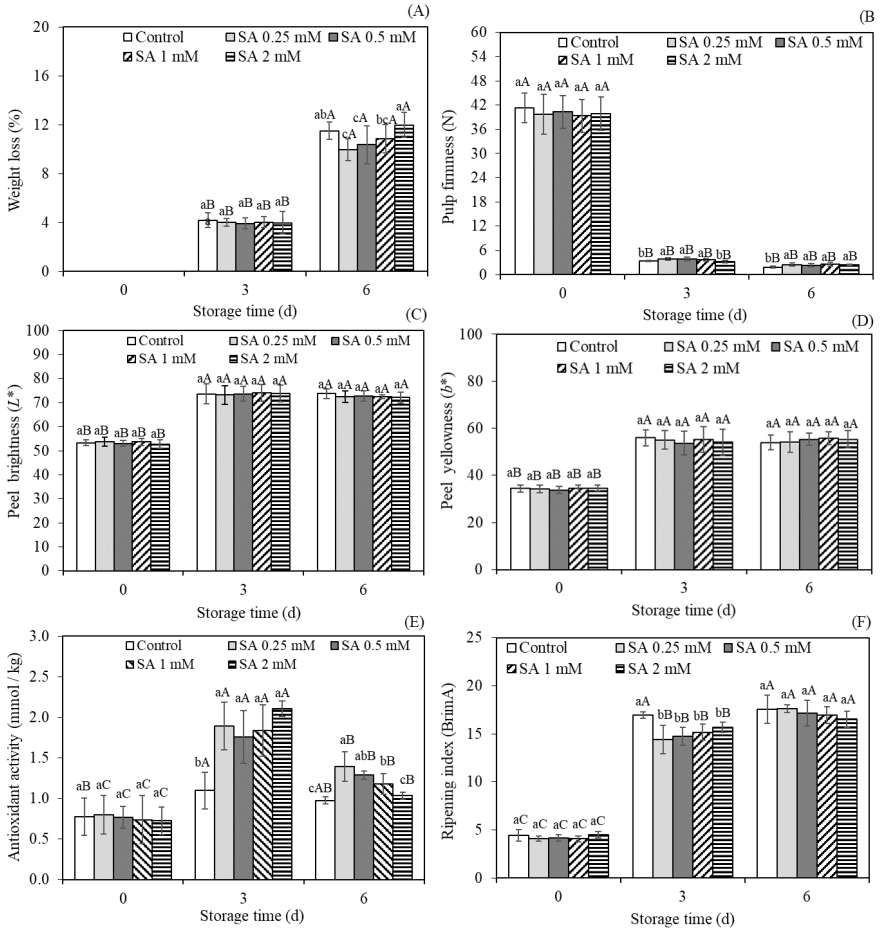

Fig. 1 'Leb Mue Nang' bananas treated with salicylic acid (SA) at various concentrations compared with untreated fruits during shelf life storage: (A) weight loss; (B) pulp firmness; (C) peel brightness; (D) peel yellowness; (E) ripening index; (F) antioxidant activity, where, error bars represent mean ± SD (n = 3), different capital letters above columns indicate significant differences ($p < 0.05$) among storage times within each treatment and different lowercase letters indicate significant differences ($p < 0.05$) among treatments on the same day

Quality of Leb Mue Nang' bananas treated with lukewarm water

Fig. 2 illustrates the impact of LW dips on the physicochemical quality of bananas. The physicochemical quality attributes of the bananas throughout storage resembled those in Fig. 1, which demonstrated that LW immersions did not influence the color development of the bananas compared to the control group. Furthermore, all LW treatments were effective in reducing the ripening index (Fig. 2E). The visual characteristics of the bananas (Supplementary Fig. 2S) suggested that LW immersion at 45°C better retarded ripening and preserved overall appearance compared to LW immersion at 50°C. Among the LW treatments at 45°C, the bananas treated with LW for 15 min had superior visual appearance compared to the other treatments after a shelf life of 6 d. The 45°C LW dip for 15 min significantly retarded increases in weight loss and BrimA values, as well as decreasing pulp firmness compared to the other treatments. Furthermore, all bananas treated with LW had significantly enhanced antioxidant activity compared to the control bananas during storage. The application of 45°C LW for 15 min appeared to augment antioxidant activity more effectively than the other treatments.

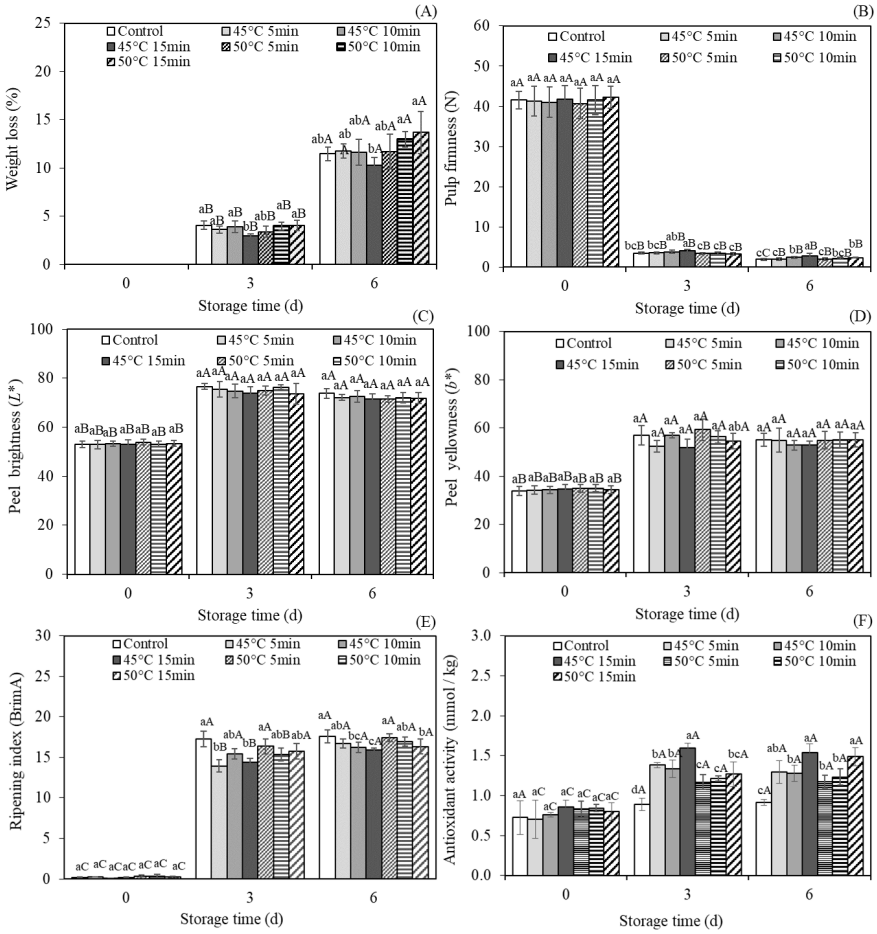

Fig. 2 'Leb Mue Nang' bananas treated with lukewarm water (45°C and 50°C) for various durations compared with untreated fruits during shelf life storage: (A) weight loss; (B) pulp firmness; (C) peel brightness; (D) peel yellowness; (E) ripening index; (F) antioxidant activity, where error bars represent mean ± SD (n = 3), different capital letters above columns indicate significant differences ($p < 0.05$) among storage times within each treatment and different lowercase letters indicate significant differences ($p < 0.05$) among treatments on the same day

Quality of 'Leb Mue Nang' bananas treated with SA+LW treatment

Visual appearance and peel color

Fig. 3 shows that the SA+LW treatment diminished the occurrence of stem end rot disease and the development of senescence spots on the bananas during the shelf life period. After storage for 3 d, the blossom end of the bananas treated with SA+LW remained greener compared to those treated with SA-only, LW-only and the control bananas, respectively. The control bananas had prominent senescence spots after 6 d of storage, followed by those subjected to LW-only. On the other hand, the bananas subjected to the SA-only and SA+LW treatments had only slight appearance of senescence spots. The peel yellowness and brightness, assessed at the midpoint of the banana, significantly increased after 3 d of storage and then remained relatively stable. No notable variations in peel yellowness and brightness among the treatments were detected during the storage period.

Weight loss and pulp firmness

The increased weight loss and decreased pulp firmness of the 'Leb Mue Nang' bananas during storage are shown in Fig. 4. All treatments slowed the rate of weight loss during storage. The weight loss of all treated groups was significantly less than that of the control bananas; however, there were no significant difference in the weight loss among all treatments after storage for 6 d. All treatments had a notable reduction in pulp firmness after 3 d of storage, decreasing from approximately 40 N on day 0 to approximately 3.5 N on day 3. All treatments maintained pulp firmness compared to the control bananas, which were significantly less firm after 3 d and 6 d of storage. Among the treatments, the bananas treated with SA+LW had pulp firmness significantly higher than for the other treatments during storage.

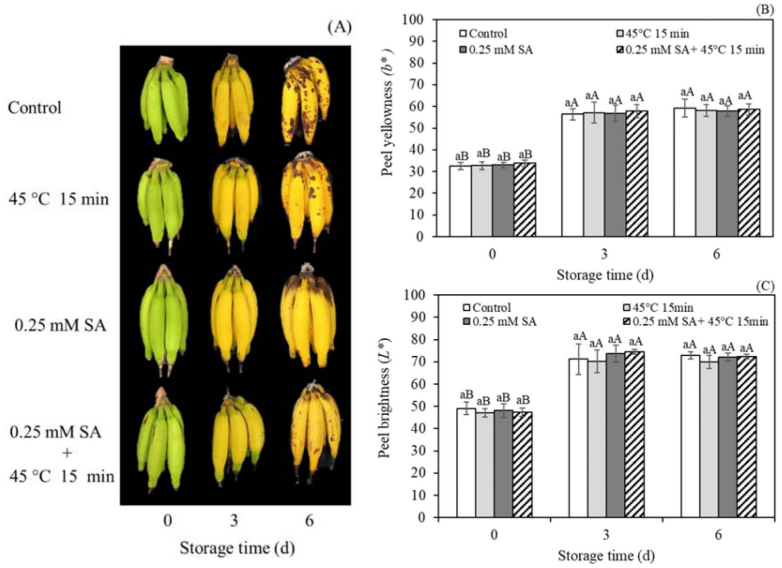

Fig. 3 'Leb Mue Nang' bananas treated with lukewarm water (LW), salicylic acid (SA) and a combination of SA and LW during 6 d of shelf life storage: (A), visual appearance; (B) peel yellowness; (C) peel brightness, where error bars represent mean ± SD (n = 3), different capital letters above columns indicate significant differences ($p < 0.05$) among storage times within each treatment and different lowercase letters indicate significant differences ($p < 0.05$) among treatments on the same day

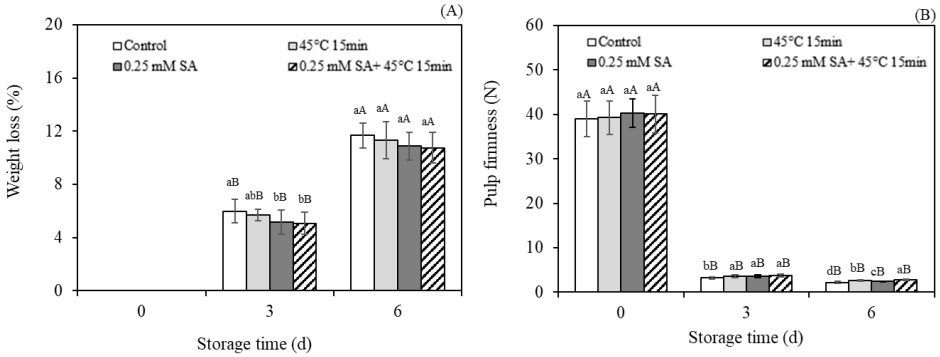

Fig. 4 'Leb Mue Nang' bananas treated with lukewarm water, salicylic acid (SA) and a combination of SA and LW during 6 d of shelf life storage: (A) weight loss; (B) pulp firmness (B), where error bars represent mean ± SD (n = 3), different capital letters above columns indicate significant differences ($p < 0.05$) among storage times within each treatment and different lowercase letters indicate significant differences ($p < 0.05$) among treatments on the same day

Total soluble solids, total acidity and ripening index

Generally, it is acknowledged that increased TSS and TA values of banana pulp are the main parameters linked to banana ripening (Reference). Fig. 5 depicts the values for TSS, TA and ripening index (expressed as BrimA), of the 'Leb Mue Nang' bananas during storage. There were significant increases in the TSS, TA and BrimA values in all banana groups during storage. After 3 d storage, the TSS content of the control bananas was significantly higher than in any of the treated bananas. There were no significant variations in the TA and BrimA values among all treatments on day 3 of storage; however, the control bananas had slightly higher values for both parameters compared to any of the treated bananas. On day 6 of storage, the SA-only and SA+LW bananas had significantly lower TSS and BrimA values than the LW-only and control treatments, although the TA contents seemed comparable, except when comparing the SA-only-treated bananas to the control bananas.

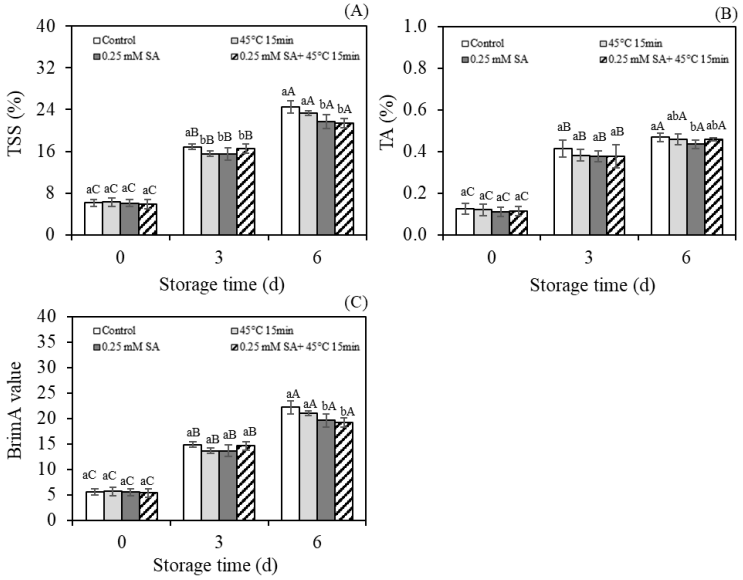

Fig. 5 'Leb Mue Nang' bananas treated with lukewarm water (LW), salicylic acid (SA) and a combination of SA and LW during 6 d of shelf life storage: (A) total soluble solids (TSS); (B) titratable acidity (TA); (C) ripening index (BrimA), where error bars represent mean ± SD (n = 3), different capital letters above columns indicate significant differences ($p < 0.05$) among storage times within each treatment and different lowercase letters indicate significant differences ($p < 0.05$) among treatments on the same day

Total phenolic compounds and antioxidant activity

Throughout the storage period, for all treatments, there was a significant increase in TP and the levels of FRAP and DPPH radical scavenging activity (Fig. 6). The SA-only and SA+LW treatments significantly enhanced TP relative to LW-only and the control treatments. During storage, the level of TP in the LW-only banana treatment was slightly higher than in the control bananas, but there was no significant difference. In addition, the change in FRAP levels during storage was similar to that of TP. The level of DPPH scavenging activity in all treated bananas was significantly higher than that of the control bananas after 3 d and 6 d of storage. The increased DPPH scavenging activity level of the SA+LW treated bananas was comparable to the SA-only and LW-only treatments during the storage period. Based on these results, the SA treatments enhanced the levels of TP, FRAP and DPPH scavenging activity in the banana pulp. The LW treatment mostly enhanced the level of DPPH scavenging activity.

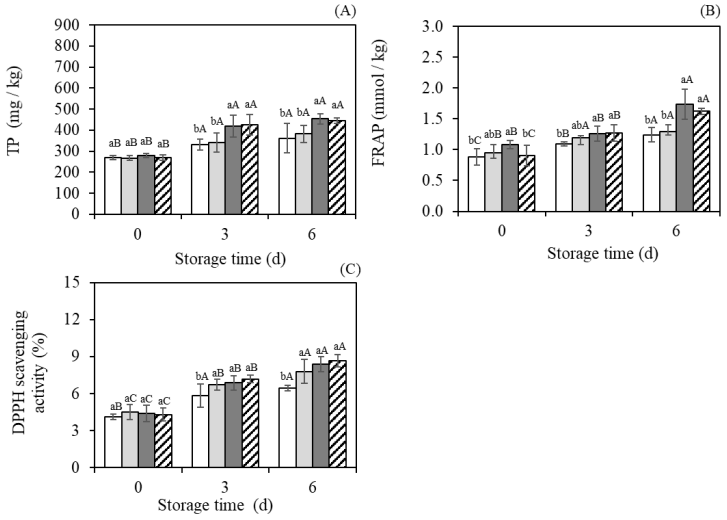

Fig. 6 'Leb Mue Nang' bananas treated with lukewarm water (LW), salicylic acid (SA) and a combination of SA and LW during 6 d of shelf-life storage: total phenolics (TP); (B) ferric reducing antioxidant power (FRAP); (C) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (C), where error bars represent mean ± SD (n = 3), different capital letters above columns indicate significant differences ($p < 0.05$) among storage times within each treatment and different lowercase letters indicate significant differences ($p < 0.05$) among treatments on the same day

Pearson's correlations

Table 1 displays the correlations among parameters related to the physicochemical quality of bananas during storage. Based on the Pearson's correlation analysis, there was a significant correlation between the increased ripening index and increases in peel color development (r = 0.910 for *L** and r = 0.950 for *b**), TSS (r = 0.985), TA (r = 0.796), weight loss (r = 0.831), TP content (r = 0.776) and antioxidants (r = 0.776 for DPPH and r = 0.637 for FRAP)), as well as a decrease in the pulp firmness of the bananas (r = -0.96). Furthermore, there was a close positive correlation between the TP content and FRAP (r = 0.833) compared to DPPH (r = 0.752).

Discussion

There are numerous problems associated with the quality and marketability of 'Leb Mue Nang' bananas, a favorite banana variety in Thailand. In particular, Overripening during transportation and sale leads to considerable postharvest losses for 'Leb Mue Nang' bananas (Youryon and Supapvanich, 2017). Typically, bananas typically undergo marketing in an ambient temperature environment due to their high susceptibility to chilling injury, with a shelf life of approximately 3-4 d after the peel color break (Lima et al., 2014). Based on the results of the current research, both the SA and LW immersions preserved the postharvest quality of the bananas during storage at an ambient temperature (27 ± 2°C, 65 ± 8% RH). The optimum levels for SA and LW immersion to preserve the postharvest quality of bananas were a 0.25 mM SA dip for 5 min (Fig. 1) and a 45°C LW dip for 15 min (Fig. 2), respectively. Postharvest SA immersion resulted in a reduction in weight loss, a delay in the ripening index, preservation of pulp firmness and enhanced antioxidant activity in the bananas. It is widely acknowledged that the application of SA to bananas can have several interesting effects, especially concerning their ripening process and longevity (Alali et al., 2018; Xu et al., 2019). SA is a plant hormone that contributes to numerous physiological processes, including stress responses and fruit ripening (Asghari and Aghdam, 2010; Mustafa et al., 2018). Bananas are classified as climacteric fruits, with increased ethylene production and starch-sugar conversion closely associated with accelerated ripening and senescence processes, as well as the pulp taste and softening during shelf life storage (Youryon and Supapvanich, 2017a). Based on the current results, the SA treatments of the 'Leb Mue Nang' bananas postponed the changes in ripening-related parameters such as the pulp firmness (Fig. 1B) and ripening index (Fig. 1E). Yuan et al. (2023) reported that SA suppressed ethylene biosynthesis in fruits, which was linked to decreased expression of the ACS and ACO genes, thereby limiting ethylene production. Furthermore, they discovered that the SA application postponed the starch-sugar conversion process in fruits, coinciding with the down-regulation of starch and sugar-related genes, including *AMY, Bam, SPS, SUSY, NINV* and *AINV*. In addition to the delayed conversion of starch to sugar, Srivastava and Dwivedi (2000) discovered that SA inhibited certain cell wall hydrolase activities, including polygalacturonase and xylanase, in bananas. In a similar vein, compared to the untreated bananas, the application of SA delayed an increase in the ripening index (BrimA value), as shown in Fig. 1E and in pulp softening (Fig. 1B) of 'Leb Mue Nang' bananas, indicating that postharvest SA treatment might postpone the starch-sugar conversion, as well as cell wall depolymerization in the pulp of 'Leb Mue Nang' bananas during shelf life.

Table 1 Pearson's correlation coefficients among biological parameters of 'Leb Mue Nang' bananas during 6 d of shelf life storage

	Weight loss	Pulp firmness	Peel *L**	Peel *b**	Pulp TSS	Pulp TA	Ripening index	Pulp TP	Pulp FRAP	Pulp DPPH
Weight loss	1	-0.792**	0.714**	0.768**	0.869**	0.807**	0.831**	0.546**	0.454**	0.757**
Pulp firmness		1	-0.978**	-0.986**	-0.967**	-0.867**	-0.96**	-0.857**	-0.713**	-0.788**
Peel (*L**)			1	0.975**	-0.923**	0.850**	0.910**	0.898**	0.686**	0.742**
Peel (*b**)				1	-0.962**	0.869**	0.950**	0.847**	0.685**	0.789**
Pulp TSS					1	0.883**	0.985**	0.783**	0.673**	0.804**
Pulp TA						1	0.796**	0.719**	0.591**	0.746**
Ripening index							1	0.776**	0.673**	0.776**
Pulp TP								1	0.833**	0.752**
Pulp FRAP									1	0.744**
Pulp DPPH										1

*L** = brightness value, *b** = yellowness value, TSS = total soluble solids; TA = total acidity, TP – total phenols, FRAP = ferric reducing antioxidant potential, DPPH = 2,2-diphenyl-1-picrylhydrazyl free radical scavenging activity.

** = correlation significant at the 0.01 level (2-tailed).

Furthermore, the current results revealed that the SA-treated 'Leb Mue Nang' bananas had greater levels of antioxidants in their pulp than the control bananas (Fig. 1F). Other reports have documented the effects of SA on the augmentation of both antioxidant enzymes and non-enzymatic antioxidants (Asghari and Aghdam, 2010; Supapvanich and Promyou, 2013; Supapvanich et al., 2017; Xu et al., 2019; Yuan et al., 2023). SA induced non-enzymatic antioxidants via the phenylpropanoid pathway by enhancing the expression of phenylalanine ammonia-lyase (Asghari and Aghdam, 2010; Supapvanich and Promyou, 2013) and antioxidant enzymes by up-regulating the expression levels of the *Cyt Cu/ZnSOD, FeSOD, APX, CAT* and *POD* genes (Wang et al., 2022).

Mild hot water or LW treatment is a technique applied to postpone the ripening of bananas, involving immersing bananas in hot water in the range 45–55°C for around 10–20 min (Varit and Songsin, 2011; Kaka et al., 2019). The mechanisms through which LW treatments preserve the postharvest quality of fruits and vegetables include enzyme inactivation, improved cell membrane integrity, activation of defense systems, delayed ripening processes and preserved edible quality (Fallik, 2004). Based on the current results, LW dips effectively hindered weight loss and ripening index increases, maintaining the pulp firmness and enhancing the antioxidant activity of 'Leb Mue Nang' bananas, particularly the LW dip at 45°C for 15 min (Fig. 2). Similarly, Ummarat et al. (2011) reported that the treatment of hot water immersion at 50°C for 10 min delayed the ripening process of 'Gros Michel' bananas (*Musa acuminata*, AAA), including slowing peel color development and softening and enhancing the levels of bioactive compounds and antioxidants during storage. Marrero Domínguez et al. (1997) documented that hot water dipping at 50-55°C impeded the normal ACC oxidase activity in the peel and pulp of 'Santa Catarina Prata' (AAB) and 'Dwarf Cavendish' (AAA) bananas. These works indicated that hot water treatment was efficient at delaying the ripening of bananas. In addition, the results indicated that hot water immersion, particularly at 45ºC for 15 min, delayed alterations in specific parameters associated with ripening, such as pulp firmness (Fig. 2B) and ripening index (Fig. 2E), of 'Leb Mue Nang' bananas throughout the shelf life period. Furthermore, Niaz et al. (2024) observed the impact of hot water immersion on enhancing the antioxidant system and chilling stress tolerance in 'Cavendish' bananas exposed to 52°C for 5 min. Although some studies have indicated that an LW dip at 50-55°C delayed ripening and prolonged the shelf life of *Musa* AAA and *Musa* AAB bananas (Ummarat et al.,2011; Kaka et al., 2019), the current findings demonstrated that the LW dip at 45°C more effectively postponed ripening and enhanced the antioxidant activity of 'Leb Mue Nang' (*Musa* AA) bananas compared to the LW dip at 50°C (Fig. 2F). Similarly, Varit and Songsin (2011) reported that immersing 'Sucrier' (*Musa* AA) bananas in hot water at 45°C for 15 min could prolong their shelf life. Based on these observations, the optimal temperature for hot water immersion varies according to the banana subspecies.

Compared to the treatments based solely on SA or LW, the combined SA+LW treatment effectively preserved visual appearance by reducing the incidence of black spots and stem end rot disease after 6 d of storage (Fig. 3A). In addition, the SA+LW treatment delayed peel color development, with the stem and blossom ends remaining greener than in the other groups after 3 d storage. It is widely known that banana peels turn yellow when they ripen and develop black spots when they overripen. For example, Pongprasert et al. (2021) and Anusornpornpong et al. (2024) noted that black spots developed from the collapsed cells surrounding the stomata of the ripened banana skin, resulting from the programmed cell death of the stomata. Plant cell death is regulated by cellular oxidation and a decrease in antioxidant capacity (Van Breusegem and Dat, 2006). The current data indicated that higher levels of total phenolic compounds and antioxidant activities (FRAP and DPPH scavenging activity) in both the SA-only and SA+LW treatments of the bananas, compared to LW-only treated and control bananas (Fig. 6), could be associated with lower levels of black spot occurrence (Fig. 3A). Chotikakham et al. (2022) reported that the postharvest application of methyl salicylate mitigated black spot formation in 'Sucrier' bananas (*Musa* AA group) by inhibiting the activation of mitochondrial-mediated programmed cell death through an enhanced expression of anti-apoptotic genes. In addition, the current findings demonstrate that the SA+LW treatment effectively maintained the firmness of the banana pulp, compared to the SA-only and LW-only treatments.

Additionally, the application of both the SA-only and SA+LW treatments delayed the ripening index (BrimA) and increased the total phenolic compounds and antioxidants compared to the LW-only treatment. Moradi et al. (2022) proposed that postharvest treatments using hot water or SA immersion could maintain the quality and augment the bioactive compounds, including antioxidants, as well as the oxidative stress resistance of pomegranates under refrigeration. They also documented that postharvest SA treatment improved the levels of antioxidants and bioactive compounds compared to a hot water treatment.

Other studies have incorporated treatments of SA and mild hot water immersion to preserve postharvest quality and enhance the levels of bioactive compounds and antioxidants in peaches (Cao et al., 2010), rambutans (Supapvanich, 2015), papayas (Supapvanich and Promyou, 2017) and strawberries (Niazi et al., 2021). Furthermore, in the current study, Pearson's correlation analysis revealed a significant link between the loss of pulp firmness and the ripening parameters such as TSS, TA and peel color development, as well as the increased fresh weight loss of the bananas during storage. The increases in bioactive compounds, including total phenolic compounds and antioxidants, were concomitant with the ripening increase. In a similar vein, Laryea et al. (2024) and Sittiprasert et al. (2025) documented the relationship between the reduction of pulp firmness and increases in fruit ripening, peel yellowness development and antioxidant activity in *Musa* AA bananas kept at ambient temperature.

Conclusion

The treatments with SA and LW immersions delayed weight loss, maintained pulp firmness, postponed ripening and enhanced the antioxidant activity of 'Leb Mue Nang' bananas during shelf life. Preliminary investigations showed that the optimal SA treatment involved a 0.25 mM SA immersion for 5 min, while the optimal LW immersion was at 45°C for 15 min. Compared to treatments with only SA or LW, the SA+LW treatment retarded the development of senescence spots and stem end rot disease and maintained pulp firmness. Both the SA-only and SA+LW treatments delayed increases in the ripening index and TSS and they enhanced the TP content and antioxidant activities (both FRAP and DPPH scavenging activity) compared to the LW-only treatment. Correlation analysis revealed a significant negative correlation between pulp firmness and weight loss, peel color attributes, TSS, TA, ripening index, TP content and antioxidant activity, while other parameters produced significant positive correlations. These findings indicated that postharvest SA application could preserve the postharvest quality of 'Leb Mue Nang' bananas during shelf life. In contrast to the treatment with SA alone, the SA+LW treatment preserved pulp firmness and visual appearance, reducing the incidence of stem end rot disease and the development of senescence spots.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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