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Preharvest Salicylic Acid Incorporated with Calcium Chloride Spray Improves the Postharvest Quality of Thai Dwarf Mulberries (*Morus alba*)

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ABSTRACT

The current research evaluated the impacts of preharvest salicylic acid (SA), calcium chloride ($CaCl_2$), and SA incorporated with $CaCl_2$ (SA+$CaCl_2$) sprays on the quality of mulberries. In the preliminary experiment, the mulberries were sprayed with SA (0, 1, and 2 mM) or $CaCl_2$ (0, 0.5, and 1%) before harvest for 5 d. The results indicated that the preharvest application of 1 mM SA or 0.5% $CaCl_2$ resulted in the deceleration of the BrimA (a ripening index) increase and hindered fruit softening during storage at 5 °C for 6 d. In the major experiment, the effects of preharvest sprays of 1 mM SA, 0.5% CaCl2, and SA+$CaCl_2$ for 5 d prior to harvest were investigated. All preharvest treatments delayed fruit darkening and acidity reduction compared to the untreated fruits and did not affect redness. The SA+$CaCl_2$ spray delayed fruit darkening and enhanced firmness compared to the individual sprays. The total soluble solids content and BrimA value of treated mulberries were lower than those of untreated fruits. All treatments improved the antioxidant activity and bioactive compounds compared to the untreated fruits. The ferric-reducing antioxidant potential, DPPH radical scavenging activity, total anthocyanin, and total phenol content were all higher with the SA+$CaCl_2$ preharvest spray than with the SA or $CaCl_2$ spray alone. Thus, the preharvest SA+$CaCl_2$ spray is a promising approach for improving Thai dwarf mulberry postharvest quality.

Introduction

Mulberry, classified within the Moraceae family and the *Morus* genus, is widely distributed across various climatic zones, including tropical and temperate regions. In recent years, both the production and consumption of mulberries have increased significantly, driven by growing recognition of their pleasant taste, high nutritional value, and beneficial biological properties. In Thailand, the cultivation of *Morus alba* is primarily geared toward sericulture and fruit consumption (Saensouk et al., 2022). Traditionally, mulberries have served as a valuable dietary component due to their rich nutritional profile and have also played a role in traditional medicine (Suttisansanee et al., 2020). Recently, rising consumer demand has paralleled an upward trend in fruit consumption.

As a climacteric fruit, mulberries develop their best organoleptic qualities when harvested at full maturity (Herman et al., 2022; Saensouk et al., 2022). However, they are highly perishable, making the extension of their postharvest life essential to maintain availability and quality. Fully ripe

mulberries are notably juicy, which renders them particularly vulnerable to mechanical damage and fungal decay during postharvest handling. Park et al. (2013) reported that the pH and color of mulberries remained stable when stored at -1.5, 0, and 1.5 °C, though these cold temperatures did affect fruit softening and deterioration. Similarly, Memete et al. (2022) identified fruit softness and degradation, primarily due to moisture loss and cell wall modifications during storage, as the main factors limiting mulberry quality and shelf life.

Salicylic acid (SA) and calcium (Ca) have been widely used in postharvest treatments of fresh produce to regulate fruit softening and inhibit fungal growth. SA is a well-known plant growth regulator that activates defense mechanisms against both biotic and abiotic stresses. Plants naturally synthesize SA in response to stress, but exogenous application of SA has also been shown to enhance resistance to physiological disorders and diseases in fruits and vegetables. Asghari and Aghdam (2010) reported that SA treatment upregulates pathogenic resistance genes and strengthens antioxidant systems in horticultural crops. In addition to enhancing defense responses, SA delays ripening and reduces cell membrane oxidation. According to Supapvanich et al. (2017a), SA immersion effectively maintained the firmness of guavas by slowing cell wall degradation and preventing membrane dysfunction. It also enhanced antioxidant activity and reduced disease incidence during storage. Similar effects of SA in delaying ripening and softening have been observed in grape berries (Lo'ay, 2017), tomatoes (Kumar et al., 2021), and strawberries (El-Mogy et al., 2019). Supapvanich et al. (2017b) further demonstrated that preharvest SA spraying helped preserve the postharvest quality of rose apples by reducing physiological disorders and increasing antioxidant and bioactive compound levels.

Calcium application, particularly after harvest, is also widely recognized for its ability to maintain fruit texture and delay softening. Ca treatment inhibits cell wall degradation by increasing insoluble pectin and reducing soluble pectin. Additionally, Ca^{2+} contributes to maintaining membrane structure and fluidity (Gao et al., 2019; Supapvanich et al., 2022). Gao et al. (2019) also noted that Ca reduces ethylene production, which contributes to ripening delay. Irfan et al. (2013) found that preharvest Ca sprays delayed ripening, maintained firmness, and suppressed microbial growth in figs during storage. Similarly, Shafiee et al. (2010) reported that both pre- and postharvest Ca applications preserved firmness, slowed ripening, and reduced fruit rot in strawberries. Moreover, several studies have highlighted the synergistic effects of combined SA and Ca treatments in enhancing disease resistance and preserving postharvest quality. This combined approach has shown particularly strong effects in strawberries (Shafiee et al., 2010; Shahzad et al., 2020), eggplants (Ghahremani et al., 2021), peaches (Khalid et al., 2023), and broccoli (El-Beltagi et al., 2022; Rastegar et al., 2022). Building on previous research, the use of a preharvest spray combining salicylic acid (SA) with calcium chloride ($CaCl_2$) may offer an effective strategy for enhancing the postharvest quality of mulberries during storage. This study aimed to evaluate the effects of preharvest application of SA in combination with $CaCl_2$ on the postharvest quality of Thai dwarf mulberries (*Morus alba* cv. 'Chiang Mai') during short-term refrigerated storage.

Material and methods

Raw materials and experiments

Thai dwarf mulberries (*Morus alba* cv. 'Chiang Mai') were cultivated at the Queen Sirikit Sericulture Center in Chumphon Province, Thailand (10°52'49.2"N, 99°10'39.1"E). Preliminary trials involving preharvest applications of salicylic acid (SA; Kemaus, Australia) and calcium chloride ($CaCl_2$; Kemaus, Australia) were conducted to determine the optimal concentrations for maintaining postharvest fruit quality. Each trial included 30 mulberry trees, divided into three treatments of 10 trees each.

The preharvest SA and $CaCl_2$ applications were initiated when the fruits reached 40 d after anthesis (DAA), corresponding to the M2 stage of ripening (Fig. S1). SA was applied at concentrations of 0 (distilled water), 1, and 2 mM, while $CaCl_2$ was applied at 0 (distilled water), 0.5%, and 1.0% (w/v). Five days after treatment, at the M4 ripening stage (Fig. S1), fruits were harvested. Postharvest, 300 g of mulberries were packed in foam trays (7.5 × 5 inches) and wrapped in polyvinyl chloride (PVC) cling film. Each tray served as one replicate, and four replicates were prepared per treatment. All samples were stored at 5 °C for six days. During storage, changes in visual appearance, BrimA value, and firmness were monitored.

Based on the outcomes of the preliminary trials, the most effective SA and $CaCl_2$ concentrations were selected for a follow-up experiment to evaluate their combined effect on postharvest quality preservation. In this experiment, mulberries at 40 DAA (M2 stage) were treated with one of the following: distilled water (control), 1 mM SA, 0.5% $CaCl_2$, or a combination of 1 mM SA and 0.5% $CaCl_2$ (SA+$CaCl_2$). After five days of treatment (M4 stage), fruits were harvested, packed, and stored under the same conditions as in the preliminary trials. Samples were collected at three-day intervals throughout the storage period. For each treatment, four packages were randomly selected for analysis. Physicochemical and biological parameters were assessed, including visual appearance, superficial

color attributes, firmness, total soluble solids (TSS), total acidity (TA), BrimA value, antioxidant activity, total phenolic content, and ascorbic acid content.

Visual appearance and color measurements

The change in the visual appearance of mulberries was recorded by taking photographs during storage. Twenty fruits from each package were randomly selected to determine the fruit colour. The measurement of fruit color was conducted using a Chroma Meter CR-400 (Minolta, Japan). The brightness (L^*) and redness (a^*) values were recorded.

Total soluble solids, titratable acidity, and BrimA measurements

Ten fruits per package were randomly sampled to determine the total soluble solids (TSS) content and total acidity (TA). The TSS of the fruit juice was measured using a handheld refractometer (Atago, Japan), and the percentage of TSS (%) was recorded. The TA was determined using the titration method. The fruit juice was titrated with 0.1 N NaOH, and the endpoint of titration was pH 8.2. TA was calculated and expressed as the percentage of citric acid (%). A recent study by Magwasa and Opara (2015) suggests that BrimA is a superior ripening index to the TSS/TA ratio. The BrimA value, an indicator of fruit ripening, was calculated using the equation proposed by Magwasa and Opara (2015):

$$BrimA = TSS - (5 - TA).$$

Texture measurement

Ten fruits per package were sampled to determine firmness using the CT3 Brookfield texture analyzer (AMETEK Brookfield, Middleboro, MA, USA). In this study, a Magness-Taylor probe with a diameter of 5 mm was employed, and the probe's speed and compression depth were adjusted to 1 mm s^{-1} and 3 mm, respectively. The measurement of fruit firmness was quantified using the unit of force known as Newton (N).

Antioxidant activity assay

Five grams of the fruits were extracted by homogenising with 60% (v/v) ethanol. The antioxidant activity of the extract was assayed using the ferric reducing antioxidant potential (FRAP) method of Benzie and Strain (1996) and the DPPH radical scavenging activity assay of Brand-Williams et al. (1995), slightly modified by Supapvanich et al. (2012). The extract was reacted with FRAP reagent, and the absorbance (Abs) at 630 nm was measured after incubation for 30 min. The antioxidant activity was computed using a linear equation from the Trolox standard curve and represented in moles of Trolox equivalent per kilogram of fresh weight (mol kg^{-1}).

In the DPPH radical scavenging activity assay, a solution of 10^{-3} M DPPH in methanol (1 mL) was mixed with 2 mL of the extract. The measurement of absorbance at 517 nm was promptly conducted (Abs 0 min), followed by a 10 min incubation of this mixture in absolute darkness. The absorbance at 517 nm was again measured (Abs 10 min). DPPH free radical scavenging capacity was calculated using the following formula:

$$DPPH\ free\ radical\ scavenging\ capacity\ (\%) = \left[\frac{Abs\ 0\ min - Abs\ 10\ min}{Abs\ 0\ min}\right] \times 100$$

Total phenolic compounds assay

The same extract used for the antioxidant activity assay was employed to determine the total phenolic content, following the method described by Slinkard and Singleton (1997). The extract was mixed with 50% (v/v) Folin–Ciocalteu reagent and saturated Na_2CO_3 in a ratio of 1:1:2. After incubation in the dark for 30 min, the absorbance was measured at 750 nm. Total phenolic content was quantified using a gallic acid standard curve and expressed as grams of gallic acid per kilogram of fresh weight (g kg^{-1}).

Total ascorbic acid assay

Five grams of mulberries were extracted using 5% cold metaphosphoric acid. The total ascorbic acid content in the extract was determined following the method of Roe et al. (1948). The extract was sequentially reacted with 2% di-indophenol, 2% thiourea, and 1% dinitrophenylhydrazine. The mixture was then incubated at 37 °C for 3 h, after which 80% H_2SO_4 was added. Absorbance was measured at 540 nm, and the ascorbic acid concentration was calculated using a linear regression equation derived from a standard curve. Results were expressed as milligrams of ascorbic acid equivalent per kilogram of fresh weight (mg kg^{-1}).

Anthocyanin content assay

Five grams of mulberries were extracted with 50 mL of ethanolic HCl, prepared by mixing 95% ethanol and 1.5 M HCl in an 85:15 ratio. After filtration, the extract was used for determining the total anthocyanin content following the method of Lateef et al. (2021), with slight modifications. The absorbance of the sample was measured at 530 nm, and anthocyanin concentration was calculated using the following equation. Results were expressed as grams per kilogram of sample (g kg^{-1}).

$$Anthocyanin\ content\ (mg\ kg^{-1}) = \frac{Abs\ 530\ nm \times extraction\ solution\ volume \times total\ volume}{98.2 \times sample\ weight\ (kg)}$$

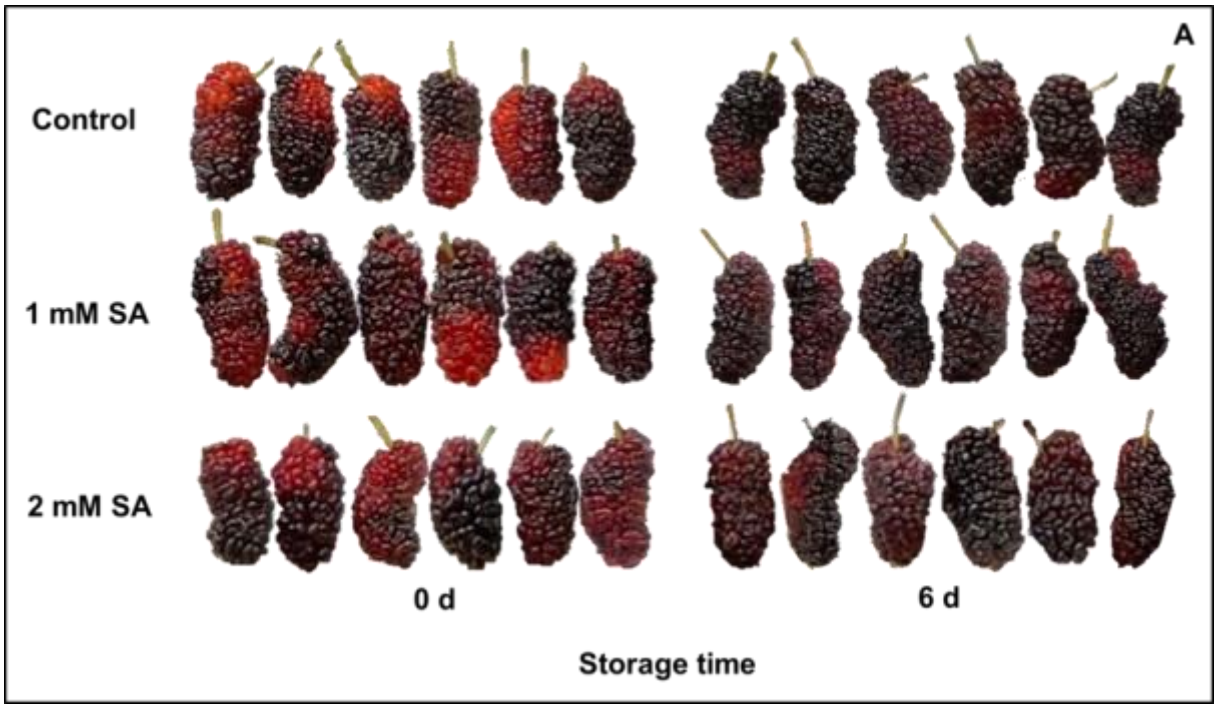
Statistical analysis

The study employed a completely randomized design (CRD) for its experimental setup. Data from four replications were analyzed using multivariate analysis of variance (MANOVA) and repeated measures. Analysis of significant differences between treatments was conducted using Duncan's New Multiple Range Test (DMRT) with a confidence level of 95%.

Results

Preliminary studies

Figure 1 shows the appearance, BrimA value, and firmness of Thai dwarf mulberries preharvest-sprayed with SA at various concentrations during refrigeration at 5 °C for 6 d. Preharvest spraying of SA at 1 and 2 mM had no effect on the visual appearance and colour development of the mulberries, both at the harvest date and during the refrigerated period. No fruit rot was observed throughout storage for 6 d. The BrimA value, an indicator of fruit ripeness and taste, was considerably lower in both 1 mM and 2 mM SA-treated mulberries compared to untreated fruits at the harvest date and during storage ($P < 0.05$). Nevertheless, the BrimA value of both SA-treated mulberries remained consistent throughout the storage period. Preharvest SA spraying enhanced the texture of the mulberries, as seen at harvest, where both SA-treated mulberries had significantly higher firmness than the control fruits ($P < 0.05$). During the refrigeration period, the softening of the control fruit increased continuously, while the firmness of both SA-treated mulberries was significantly higher ($P < 0.05$) and appeared to remain consistent. Although the firmness of 2 mM SA-treated mulberries appeared to be greater than that of 1 mM SA-treated fruits, no statistically significant difference was found. These results suggest that a preharvest spray of 1 mM SA was sufficient to improve the postharvest quality of Thai dwarf mulberries. The results showed that preharvest SA spray delayed fruit ripening, as shown by the lower BrimA and higher firmness of the treated fruits at the harvest date and during storage for 6 d.



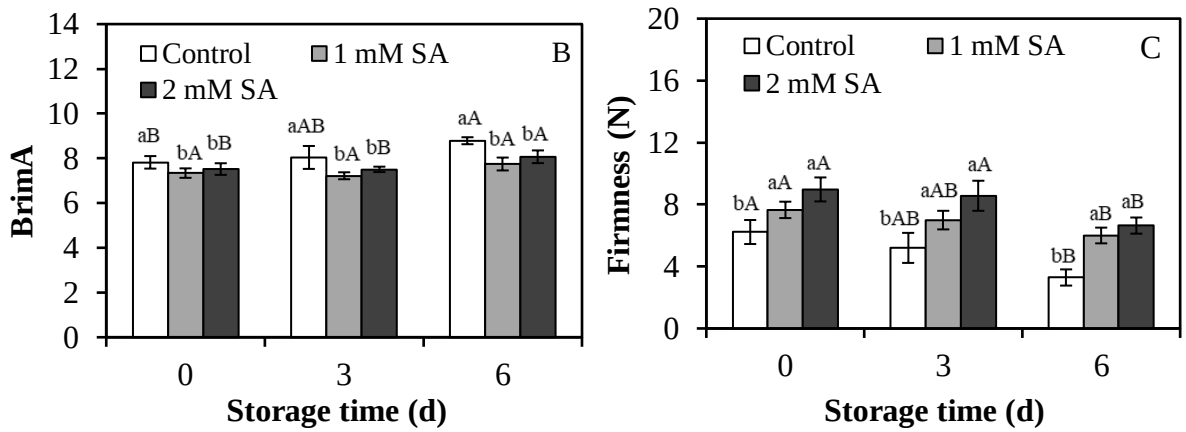
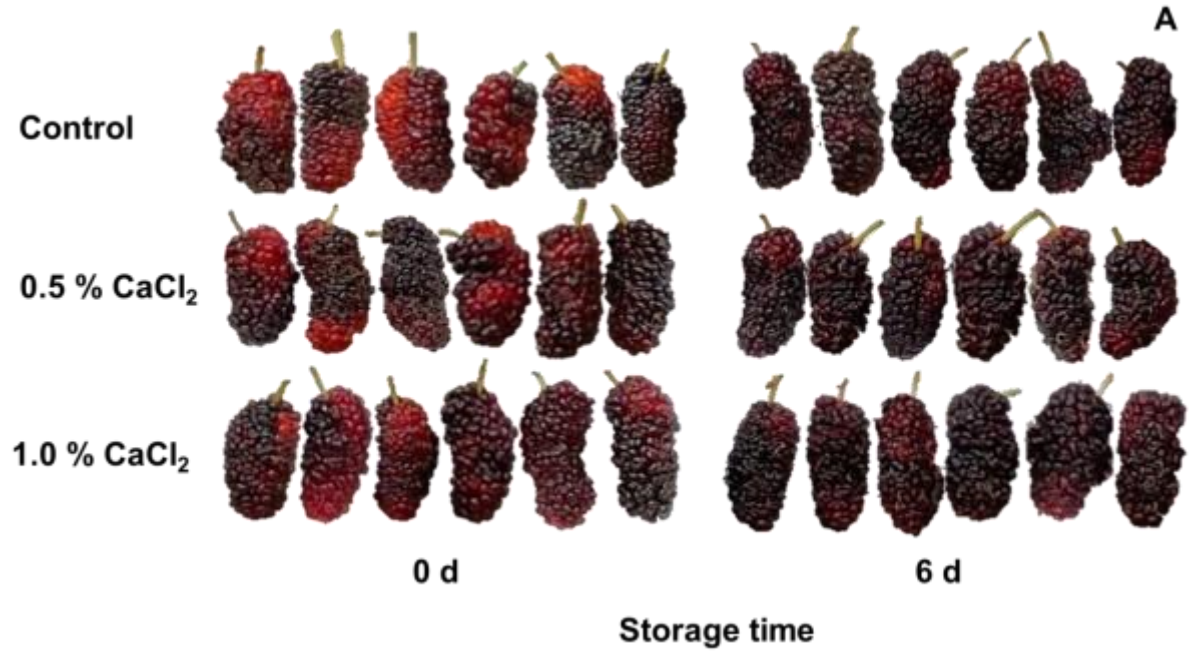


Fig. 1. Visual appearance (A), BrimA (B), and firmness (C) of the mulberries preharvest-sprayed with salicylic acid (SA) (1 mM and 2 mM) compared with untreated fruits (control) during refrigeration at 5 °C for 6 d. The values are the mean of 4 replicates. Significant change in individual treatment during storage is shown by distinct capital letters ($P < 0.05$), while substantial variation between treatments on the same day is indicated by lowercase letters ($P < 0.05$).

Figure 2 depicts the impact of preharvest $CaCl_2$ spraying at 0.5 and 1.0% (w/v) concentrations on the visual appearance, BrimA value, and texture of Thai dwarf mulberries during cold storage. Figure 2A shows that both $CaCl_2$ treatments had no impact on the visual appearance and colour development of the mulberries over the storage period. At harvest (d 0), the overall appearance of all treated mulberries matched that of the untreated fruits. At harvest, there was no significant difference in the BrimA value of the samples. During the storage period, the BrimA value of control fruits increased significantly higher than the fruits treated with 0.5 and 1.0% $CaCl_2$, which were not statistically different. The firmness of $CaCl_2$-sprayed mulberries was obviously higher than that of the control fruits. The firmness of 0.5% $CaCl_2$-treated fruits appeared significantly higher than that of 1.0% $CaCl_2$-treated fruits at harvest ($P < 0.05$). Over the refrigeration period, the firmness of untreated mulberries was significantly lower than that of both 0.5 and 1.0% $CaCl_2$-treated mulberries, which were not statistically different ($P < 0.05$).

Regarding the results shown in Figures 1 and 2, the impacts of preharvest 1 mM SA incorporated with 0.5% $CaCl_2$ spray on the physicochemical quality of mulberries during short-term storage were monitored.



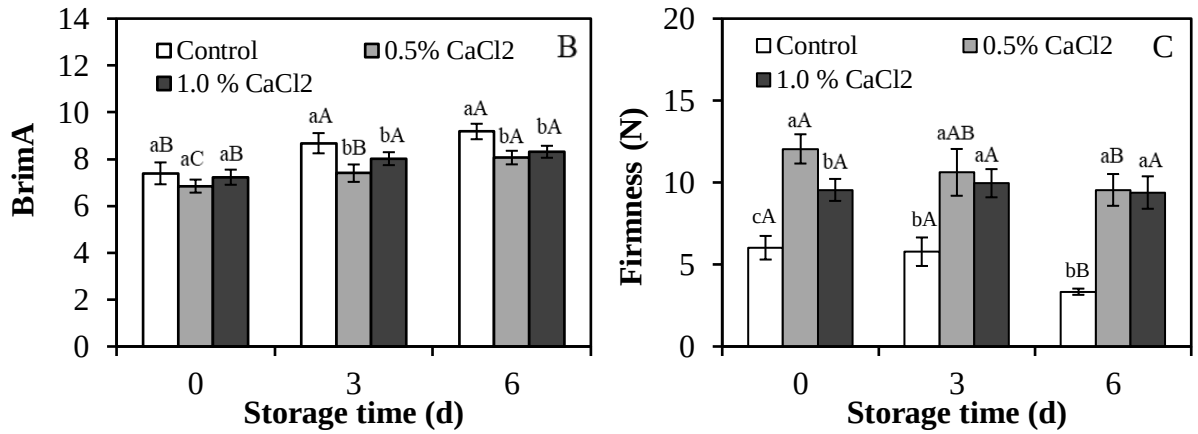
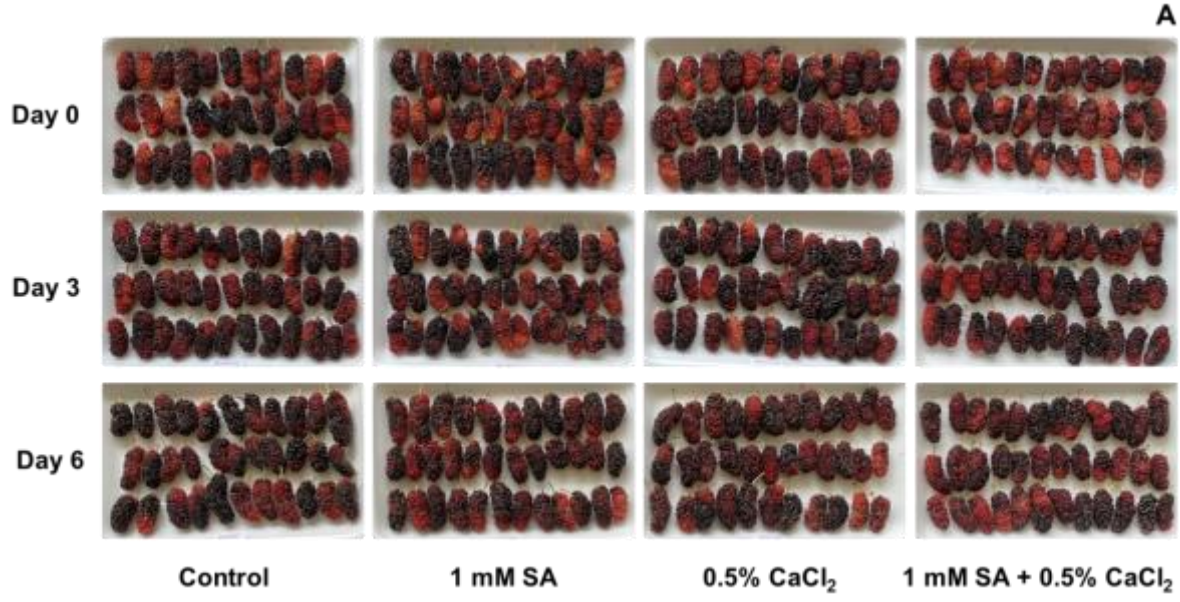


Fig. 2. Visual appearance (A), BrimA (B), and firmness (C) of the mulberries preharvest-sprayed with $CaCl_2$ (0.5 and 1.0%) compared with untreated fruits (control) during refrigeration at 5 °C for 6 d. The values are the mean of 4 replicates. Significant change in individual treatment during storage is shown by distinct capital letters ($P < 0.05$), while substantial variation between treatments on the same day is indicated by lowercase letters ($P < 0.05$).

Verification results of individual and combination treatments

Visual Appearance and Colour Attributes

Figure 3 depicts the appearance and colour attributes (L^* and a^*) values of mulberries preharvest-treated with 1 mM SA, 0.5% $CaCl_2$, and SA+$CaCl_2$ during storage. The observed alterations in the visual appearance of each treatment were consistent throughout storage, with the skin colour of the fruit exhibiting a greater intensity of darkness and redness (Fig. 3A). The lower L^* value shown in Figure 3B indicated a darker fruit skin. Throughout storage, the L^* value of the control samples (untreated fruits) was lower than that of all treated fruits. On the harvesting date (d 0), the L^* value of SA+$CaCl_2$-treated fruits was significantly higher than that of control fruits ($P < 0.05$), whereas the L^* value of all other treated fruits was not significantly different. After 3 d of storage, the highest L^* value was observed in the fruits treated with SA+$CaCl_2$, and the lowest value was found in the control fruits. On the last day of storage, the L^* value of all treated fruits was similar and significantly higher than that of the control fruits. No significant difference in the a^* value (redness) between treated and untreated fruits was found over the storage period. These results indicate that the redness value of mulberries did not change during cold storage, but the skin darkness increased noticeably. Preharvest SA+$CaCl_2$ spray delayed the increase in fruit skin darkness more than preharvest SA or $CaCl_2$ spray alone.



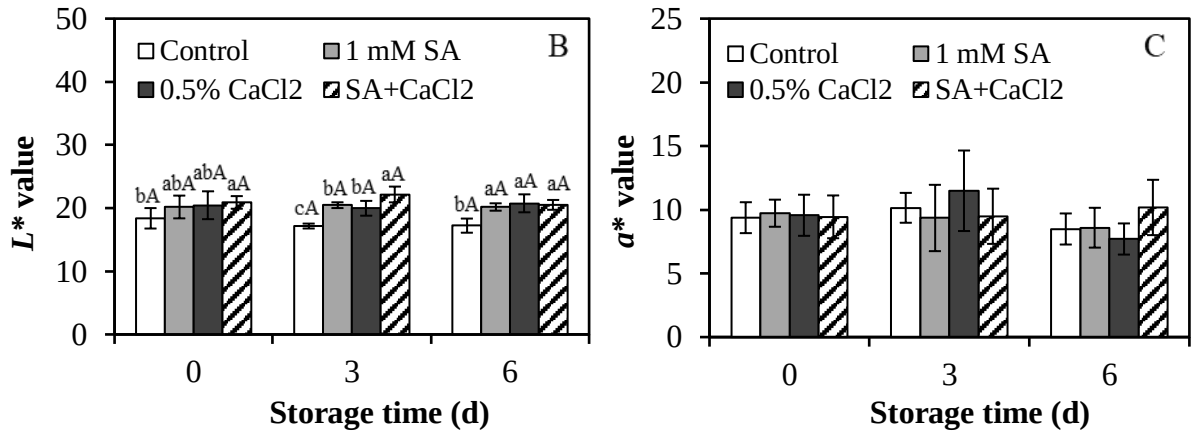


Fig. 3. Visual appearance (A) and superficial colour attributes, *L** (B) and *a** (C) values, of the mulberries preharvest-sprayed with 1 mM salicylic acid (SA), 0.5% $CaCl_2$, and SA+$CaCl_2$ compared with untreated fruits (control) during refrigeration at 5 °C for 6 d. The values are the mean of 4 replicates. Significant change in individual treatment during storage is shown by distinct capital letters ($P < 0.05$), while substantial variation between treatments on the same day is indicated by lowercase letters ($P < 0.05$).

Texture

Figure 4 shows that the firmness of mulberries during storage was preserved by the preharvest SA, $CaCl_2$, or SA+$CaCl_2$ spray. After harvest (d 0), there was no significant difference between treatments. During storage, the firmness of control fruits dramatically decreased and was significantly lower than that of all treated fruits ($P < 0.05$). This finding reveals that mulberries exhibited a rapid softening process during storage. Preharvest SA, $CaCl_2$, or SA+$CaCl_2$ spray effectively preserved the firmness of the fruits during storage. After 6 d of storage, the SA+$CaCl_2$-treated fruits exhibited the highest firmness, which was significantly higher than the firmness of the other samples ($P < 0.05$), whereas the firmness of SA-treated and $CaCl_2$-treated fruits was comparable.

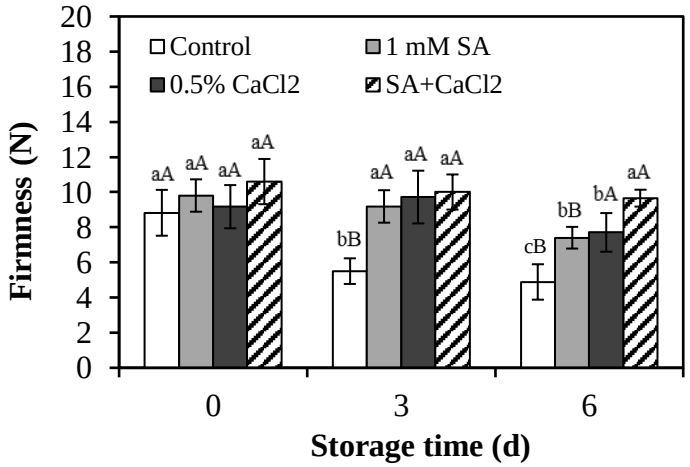


Fig. 4. Firmness of the mulberries preharvest-sprayed with 1 mM salicylic acid (SA), 0.5% $CaCl_2$, and SA+$CaCl_2$ compared with untreated fruits (control) during refrigeration at 5 °C for 6 d. The values are the mean of 4 replicates. Significant change in individual treatment during storage is shown by distinct capital letters ($P < 0.05$), while substantial variation between treatments on the same day is indicated by lowercase letters ($P < 0.05$).

TSS, TA, and BrimA Values

Figure 5 shows the changes in TSS, TA, and BrimA values of the mulberries during storage. On the harvesting date (d 0), higher TSS and BrimA values and a lower TA value were observed in untreated fruits. During the storage period, TSS and BrimA in the control fruits were significantly higher than in all treated fruits ($P < 0.05$). The TA of untreated fruits was significantly lower compared to all treated fruits ($P < 0.05$). Among SA, $CaCl_2$, and SA+$CaCl_2$ treatments, these parameters were similar throughout storage.

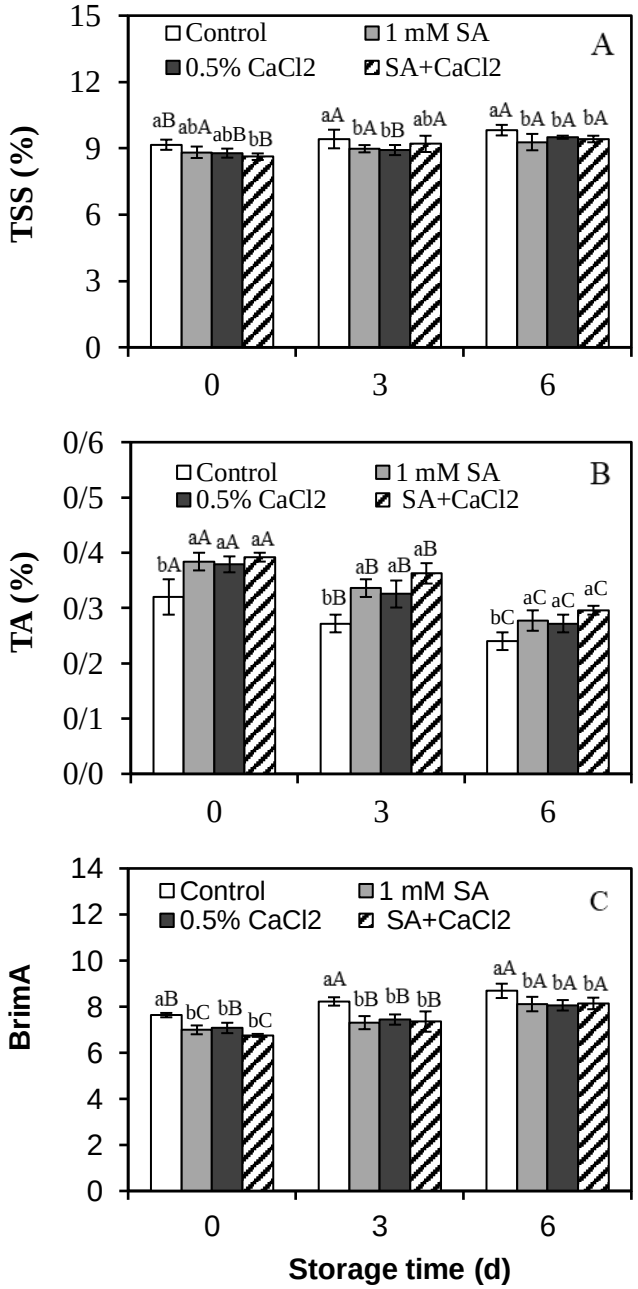


Fig. 5. Total soluble solids (TSS) (A), titratable acidity (TA) (B), and BrimA (C) of the mulberries preharvest-sprayed with 1 mM salicylic acid (SA), 0.5% $CaCl_2$, and SA+$CaCl_2$ compared with untreated fruits (control) during refrigeration at 5 °C for 6 d. The values are the mean of 4 replicates. Significant change in individual treatment during storage is shown by distinct capital letters ($P < 0.05$), while substantial variation between treatments on the same day is indicated by lowercase letters ($P < 0.05$).

Antioxidant activity, total phenolic compounds, total anthocyanin, and ascorbic acid contents

Figure 6 depicts the outcomes of preharvest applications of SA, $CaCl_2$, and SA+$CaCl_2$ sprays on the alteration in FRAP, DPPH radical scavenging activity, total phenolic compounds, total anthocyanin, and ascorbic acid in mulberries during the storage period. On the day of harvest (d 0), it was observed that all treated fruits had significantly higher levels of FRAP, DPPH radical scavenging activity, total phenolic compounds, and total ascorbic acid contents compared to the control fruits ($P < 0.05$). The highest total anthocyanin content was observed in SA+$CaCl_2$-treated fruits, and it was significantly higher than in untreated fruits ($P < 0.05$). In comparison to SA and $CaCl_2$-treated fruits, SA+$CaCl_2$-treated fruits exhibited significantly elevated levels of both antioxidant activities and total phenolic compounds ($P < 0.05$). During storage, the

FRAP and DPPH radical scavenging activity of SA+$CaCl_2$-treated fruits were significantly higher than those of the other treatments ($P < 0.05$). On the last day of storage, the lowest FRAP and DPPH radical scavenging activity were observed in the control fruits, and they were significantly lower than in the other treated fruits ($P < 0.05$). The concentration of total phenolic content in the control fruits was significantly lower compared to the other treated fruits over the storage duration ($P < 0.05$). The highest total phenolic content was exhibited in the SA+$CaCl_2$-treated fruits. Notably, on the last day of storage, this amount was significantly higher compared to the other fruits ($P < 0.05$). The total anthocyanin content of the control fruits was significantly lower than that of SA+$CaCl_2$-treated fruits during storage ($P < 0.05$).

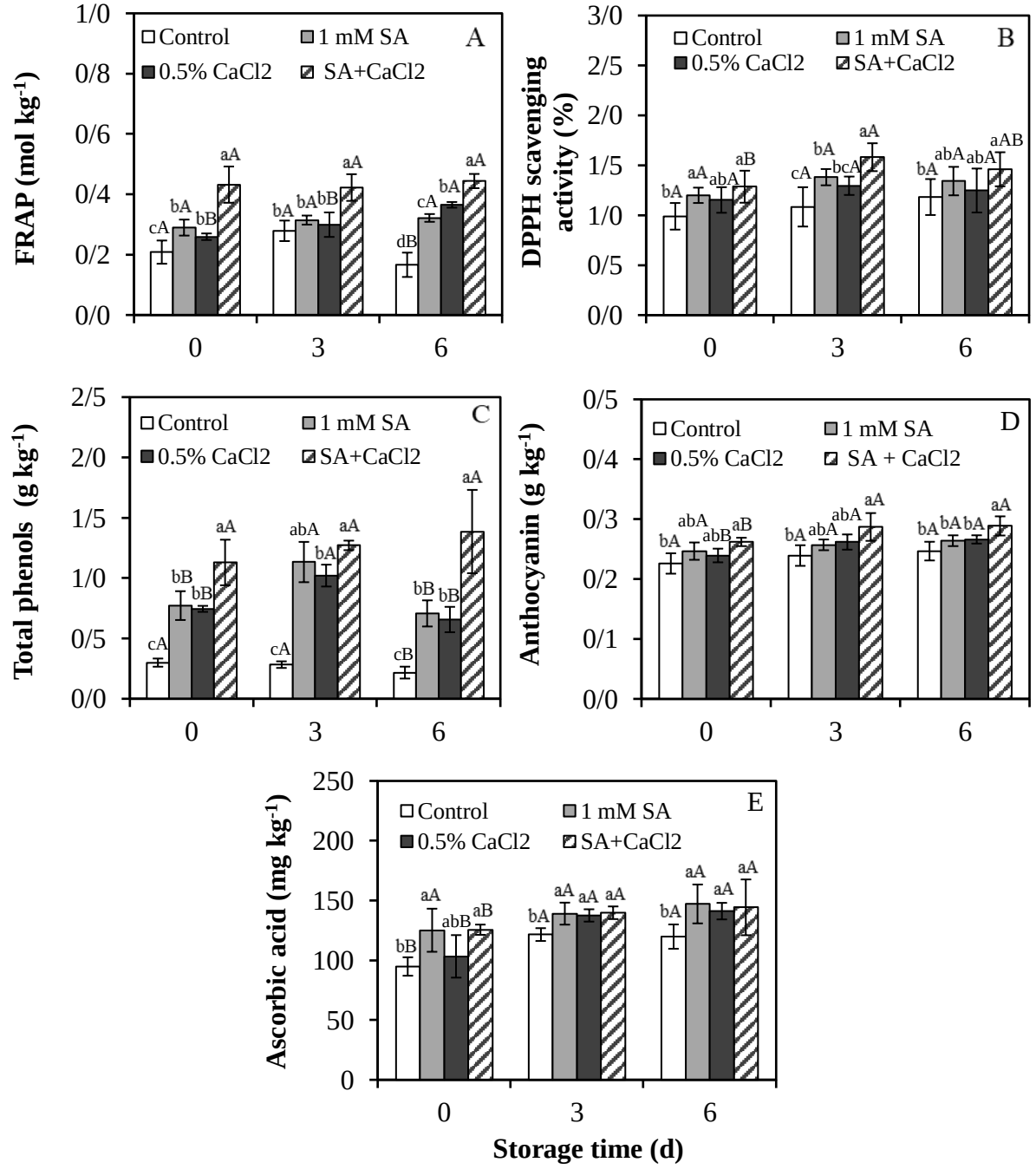


Fig. 6. Antioxidant capacity (FRAP) (A), DPPH scavenging activity (B), total phenols content (C), total anthocyanin content (D), and ascorbic acid content (E) of the mulberries preharvest-sprayed with 1 mM salicylic acid (SA), 0.5% $CaCl_2$, and SA+$CaCl_2$ compared with untreated fruits (control) during refrigeration at 5 °C for 6 d. The values are the mean of 4 replicates. Significant change in individual treatment during storage is shown by distinct capital letters ($P < 0.05$), while substantial variation between treatments on the same day is indicated by lowercase letters ($P < 0.05$).

Discussion

Preliminary studies

The preharvest sprays with SA maintained the visual appearance by delaying the increase in fruit darkness. The preharvest SA sprays delayed the fruit ripening process due to the prevention of total acid loss, as well as the increase in TSS and BrimA during storage. The preharvest spraying of SA at 1 and 2 mM significantly delayed fruit ripening, as indicated by the lower BrimA value and higher firmness. Similarly, Lo'ay (2017) documented that the use of preharvest SA spray resulted in a delay of the ripening and softening processes, along with a reduction in the increase of the soluble solids content/titratable acidity (SSC/TA) ratio in 'Superior Seedless' grape berries. El-Mogy et al. (2019) also reported that SA had no impact on strawberry SSC but preserved fruit firmness. In a study conducted by Supapvanich et al. (2017b), it was shown that the application of preharvest SA spray at doses of 0.5 or 1.0 mM effectively preserved the firmness of rose apples. However, no significant effects were observed on SSC and TA throughout the storage period. Cell wall degradation and membrane lipid peroxidation are recognised as key factors stimulating fruit softening. It has been recognized that SA is a plant elicitor controlling the ripening process by preventing the hydrolysis of pectin polymers and cell wall hydrolase (polygalacturonase and pectin methylesterase) activities (Supapvanich et al., 2017a; Kumar et al., 2021) and delaying cell membrane peroxidation by inducing the antioxidant system (Sangprayoon et al., 2019).

Regarding the results shown in Figure 2, the application of preharvest $CaCl_2$ sprays delayed the ripening process of mulberries as indicated by the lower BrimA value and preserved fruit firmness during storage. The results align with studies that have demonstrated the role of calcium in delaying fruit ripening. Gao et al. (2019) suggested that increased total intercellular Ca^{2+} in fruit delays ripening through the interaction of calcium, calmodulins, and calmodulin-like proteins. Moreover, calcium strengthens the cell wall structure by generating calcium pectate and preserves cell membrane structure and function by reducing lipid peroxidation (Gao et al., 2019; Youryon et al., 2018). Shafiee et al. (2010) and Chéour et al. (1990) reported that the foliar spray of $CaCl_2$ delayed the ripening process, increased free sugar content, and preserved the texture of strawberries during storage. The lower BrimA indicated the the ripening process of the mulberries during storage was delayed by preharvest $CaCl_2$ spray (Fig. 1B). These findings indicate that preharvest $CaCl_2$ spraying plays a crucial role in delaying the softening and ripening of Thai dwarf mulberries during cold storage.

Verification results of individual and combination treatments

The preharvest treatment with 1 mM SA and 0.5% $CaCl_2$, both individually and in combination, had a significant impact on the visual appearance and color of mulberries during short-term storage (Fig. 3). The L^* value, which indicates the lightness or darkness of the fruit skin, was significantly higher in the treated fruits compared to the untreated control fruits, suggesting that the treated fruits retained a lighter skin color for a longer period. This finding indicates that both SA and $CaCl_2$ treatments were effective in delaying the darkening of the fruit skin during cold storage. However, the lack of a significant difference in the a^* value (which represents the redness of the fruit) across treatments suggests that the redness of the mulberries was not significantly affected by these treatments during storage. The observed increase in skin darkness (lower L^* values) in the control fruits could be associated with an increase in anthocyanin content, a known pigment responsible for the red and purple colors in fruit skins. This increase in anthocyanins during ripening is typically linked to the activation of the Ethylene Response Factor (ERF5) gene, as found by Mo et al. (2022) in mulberries. It is widely acknowledged that both SA and calcium inhibit the ethylene response, a key regulator of fruit ripening and anthocyanin biosynthesis. Previous studies have reported that the application of SA and $CaCl_2$ can delay fruit ripening and slow the increase in anthocyanin content. For example, Lateef et al. (2021) observed that a foliar spray of SA combined with calcium reduced the increase in anthocyanin in strawberries during storage. Similarly, Lo'ay (2017) and Gao et al. (2019) documented the inhibitory effects of both SA and calcium on ethylene-induced ripening processes. Thus, in this study, the delay in the darkening of mulberries treated with SA+$CaCl_2$ can be attributed to the combined effects of these treatments on ethylene regulation and anthocyanin biosynthesis, offering a means to extend the shelf life and preserve the quality of mulberries during storage.

The results in Figure 4 indicate that the preharvest application of SA, $CaCl_2$, and their combination (SA+$CaCl_2$) effectively preserved the firmness of mulberries during storage. The rapid softening observed in the control fruits highlights the importance of these treatments in delaying the deterioration of fruit texture. The superior firmness observed in SA+$CaCl_2$-treated fruits after 6 d of storage, compared to the other treatments, suggests that the combination of SA and calcium had a more pronounced effect in preventing cell wall degradation and membrane breakdown. This result aligns with previous studies showing that both SA and calcium treatments can inhibit the hydrolysis of

cell wall components, such as pectin, and reduce membrane lipid peroxidation, thus slowing the softening process (Kumar et al., 2021; Gao et al., 2019; Youryon et al., 2018; Supapvanich et al., 2017a). Zhang et al. (2003) demonstrated that SA promotes cellular swelling, contributing to enhanced fruit firmness. Furthermore, Shafiee et al. (2010) reported that the application of SA (2 mM) in combination with $CaCl_2$ (1%) improved the firmness of strawberries during storage, consistent with our findings. Similarly, Ghahremani et al. (2021) found that foliar treatments with SA combined with calcium lactate improved the firmness of eggplants more effectively than when either SA or calcium lactate was applied alone. The outcomes suggest that preharvest spraying with SA and $CaCl_2$, particularly in combination, offers a practical approach to maintaining the firmness of mulberries during short-term storage. This combination likely works synergistically to strengthen the cell wall structure and reduce fruit softening.

The results shown in Figure 5 indicate that preharvest treatments with SA, $CaCl_2$, or SA+$CaCl_2$ were effective in delaying the ripening process in mulberries during storage. These findings align with those of Khalid et al. (2023), who reported that SA, $CaCl_2$, and SA+$CaCl_2$ treatments maintained TA levels and delayed the increase in TSS in peaches during storage. Similarly, Ghahremani et al. (2021) found that foliar sprays containing SA and calcium lactate led to lower TSS and higher TA levels in eggplants compared to untreated fruits. One potential explanation for this observed phenomenon involves the impact of SA and $CaCl_2$, which decelerate the process of respiration and delay the conversion of acids into sugars, leading to the observed effect. Furthermore, the lower BrimA value in all treated fruits indicated that preharvest sprays with SA, $CaCl_2$, and SA+$CaCl_2$ delayed the ripening process of mulberries during refrigeration. The lower TA level in control fruits might be associated with the higher darkness of the fruits compared to all treated fruits during storage. The association between the change in color of anthocyanin and pH, or acidity level, is widely acknowledged. Anthocyanin has a purple or blue coloration under conditions of high pH or low acidity, while it manifests a red color under conditions of low pH or high acidity.

Regarding the results shown in Figure 6, preharvest treatments with SA, $CaCl_2$, and their combination (SA+$CaCl_2$) effectively enhanced the bioactive compounds in mulberries during storage. The combination of SA and $CaCl_2$ appears to have a synergistic effect, resulting in the highest levels of antioxidant activity (FRAP and DPPH), total phenolic compounds, and anthocyanins compared to when SA or $CaCl_2$ were applied individually. These findings are consistent with studies by Li and An (2016), which highlighted that calcium plays a significant role in promoting ascorbic acid biosynthesis, while SA enhances the expression of genes involved in ascorbic acid biosynthesis in plants (Li et al., 2019). Li and An (2016) revealed that Ca^{2+} plays a positive role in promoting ascorbic acid biosynthesis by up-regulating L-galactono-1,4-lactone dehydrogenase (GalLDH) and L-galactose-1-phosphate phosphatase (GPP) in the L-galactose biosynthetic pathway, as well as dehydroascorbate reductase (DHAR) and monodehydroascorbate reductase (MDHAR) in the recycling pathway. The findings of this study reveal that preharvest SA+$CaCl_2$ spray resulted in an enhanced amount of biologically active compounds in the mulberries, surpassing the effects observed when SA or $CaCl_2$ were applied individually. The increase in FRAP and DPPH radical scavenging activity in the treated mulberries was accompanied by an increase in total phenolic compounds and anthocyanin content. It is generally recognized that both SA and Ca^{2+} induce the phenylpropanoid pathway, leading to an increase in phenolic compounds in plants (Asghari and Aghdam, 2010; Rui et al., 2021). Shahzad et al. (2010) reported that SA and $CaCl_2$ applications increased antioxidant activity and the amounts of total phenolic compounds, anthocyanins, and vitamin C in strawberries. Moreover, we found that the combination of SA and calcium may have the potential to elicit a synergistic effect on the biosynthesis of phenolic compounds. As shown in Figures 6A-D, preharvest SA+$CaCl_2$ spray resulted in a greater enhancement in both antioxidant activities (FRAP and DPPH), total phenolic compounds, and anthocyanin content compared to their individual applications. Similarly, El-Beltagi et al. (2022) reported that SA+$CaCl_2$ treatment induced antioxidant activity and bioactive compounds in broccoli florets during storage, which were greater than those from SA or $CaCl_2$ treatments alone. Rastegar et al. (2022) discovered that a Ca (2%)+SA (0.01%) treatment effectively preserved the postharvest quality of broccoli, leading to increased antioxidant activity and total phenolic compounds. Mostafa and Sultan (2018) suggested that SA+$CaCl_2$ treatment resulted in higher levels of total phenolic compounds and ascorbic acid in loquat fruit compared to $CaCl_2$ alone or $CaCl_2$+ascorbic acid treatments.

Conclusion

Individual preharvest SA (1 mM) and $CaCl_2$ (0.5%) sprays resulted in preserving the postharvest quality of Thai dwarf mulberries cv. ‘Chiang Mai’ during storage. The change in BrimA and the increased softening were delayed by these treatments. The preharvest sprays with SA, $CaCl_2$, and SA+$CaCl_2$ maintained the visual appearance by delaying the increase in fruit darkness. These treatments delayed

the fruit ripening process due to the prevention of total acid loss, as well as the increase in TSS and BrimA during storage. These treatments led to an increase in antioxidant activity, as well as the presence of bioactive compounds, including total phenols and ascorbic acid, compared to untreated fruits. Moreover, the preharvest SA+$CaCl_2$ treatment resulted in preserving fruit firmness and improving FRAP, DPPH radical scavenging activity, and total phenolic compounds, in comparison to the individual application of SA and $CaCl_2$. The treatment also significantly enhanced the total anthocyanin content of mulberries when compared to the untreated fruits. In summary, the application of a preharvest spray with SA (1 mM)+$CaCl_2$ (0.5%) presented a viable option for maintaining the postharvest quality and improving the nutritional value of Thai dwarf mulberries cv. 'Chiang Mai' during short-term storage.

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Conflict of Interest
The authors indicate no conflict of interest in this work.

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Supplemental Materials

Fig. S1. The stage of mulberry ripening (Saensouk et al., 2022).