

Effects of Preharvest Boron, Calcium Sulfate Treatment and Postharvest Calcium Chloride Peduncle Infiltration on Chilling Injury Alleviation of Queen Pineapple cv. Sawi Fruit

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Abstract

The aim of this study was to determine the incorporative effects of preharvest boron (B) or calcium sulfate (CaSO_4) application and postharvest calcium chloride (CaCl_2) peduncle infiltration on chilling injury (CI) alleviation of Queen pineapple during commercial cold storage (13°C). Pineapple fruits were sprayed with 0.25% B four times a month after one month of anthesis, or CaSO_4 (100 kg per 400 m^2) was applied during fruit development. The fruits were harvested after 135 days of flower induction. Both preharvest B and treated fruits were then peduncle-infiltrated with 2% CaCl_2 for 3 days and stored at cold temperature (CT) for 14 days. Control fruits were not peduncle-infiltrated with CaCl_2 . Visual appearance of half cut fruit, CI score, the amount of fruit having CI, colour attributes, browning index (BI) value and electrolyte leakage (EL) of tissue adjacent to the core were determined after storage at CT for 7 or 14 days, followed by leaving at room temperature (RT), $28 \pm 1^\circ\text{C}$, for 2 days. The results show that the incorporative application of preharvest CaSO_4 with CaCl_2 peduncle infiltration ($\text{CaSO}_4 + \text{CaCl}_2$) alleviated CI, delayed decrease in lightness (L^*) and chroma values, and also increased BI and total colour difference (ΔE^*) values during storage compared with control and the incorporative application of preharvest B with CaCl_2 treatment (B + CaCl_2). The treatment with $\text{CaSO}_4 + \text{CaCl}_2$ lowered CI severity and the amount of fruit having CI when compared to B + CaCl_2 and control treatments, respectively. Both treatments had no effect on the hue value over the storage period. Therefore, $\text{CaSO}_4 + \text{CaCl}_2$ treatment is an alternative method for alleviating CI of Queen pineapples.

Keywords: Queen pineapple; CaSO_4 ; boron; CaCl_2 ; chilling injury
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1. Introduction

Pineapple (*Ananas comosus* L. Merr) is recognized as one of commercial fruits in Thailand. It has been commercially grown around the country to serve the pineapple product industry and for fresh consumption. Two pineapple groups, Smooth Cayenne and Queen, have been commercially cultivated. Smooth Cayenne pineapples are mostly grown to supply the pineapple processing industry and Queen pineapples are mainly produced for fresh consumption [1]. Regarding physiological disorders of pineapples during storage, internal browning or heart blackening is a main disorder that limits the quality and consumption acceptability of both Smooth Cayenne and Queen pineapples. The disorder symptoms generally appear during refrigerated storage, and are referred to as chilling injury [2, 3]. These symptoms sometimes occur in the fruit stored at room temperature. Compared to Smooth Cayenne pineapples, Queen pineapples are more susceptible to internal browning disorder, especially during cold storage [4, 5]. Om-arun and Siriphanich [6] reported that no internal browning incidence was found in Smooth cayenne pineapple fruit during cold storage for 3 weeks whilst internal browning incidence in Queen pineapple fruit appeared within 7 days after storage at 13°C [7, 8].

Recently, most previous work has been concerned with postharvest applications including anoxia treatment [9], plant regulator application [10] and calcium peduncle infiltration [8] alleviating internal browning of Queen pineapples. Our previous work showed that calcium peduncle infiltration was an effective postharvest treatment that could alleviate the internal browning of Queen pineapple cv. Sawi during refrigerated storage [7, 8, 11, 12]. However, there have been no studies of incorporative preharvest treatment and postharvest calcium peduncle infiltration ameliorating internal browning disorder of pineapples. Nitrogen, potassium and calcium are often required as plant nutrients in the production of pineapple [13]. Moreover, boron has also been suggested as a key element for pineapple fruit formation and development of quality [13, 14]. Lack of boron can cause malformed fruit, broken core, separation and cracking of fruitlets and reduced sugar content [13]. Boron also plays a crucial role in retaining plasma membrane integrity and protecting membrane against oxidative stress [14]. Gypsum has been fertilized as a calcium source to prevent calcium deficiency during pineapple fruit development. Silva *et al.* [15] reported that the application of calcium sources such as lime, gypsum and basaltic dust increased calcium content in soil and reduced internal translucency of pineapple fruit. They also suggested that gypsum fertilization did not affect soil pH compared to lime. Kumari and Deb [16] suggested that the foliar application of 0.5 or 1.0% of boron (B) could improve the quality of 'Mauritius' pineapple. Moreover, Poovarodom and Boonplang [17] reported that the preharvest application of gypsum and 0.25% B spray could alleviate physiological disorders affecting quality of mangosteen fruit. Thus, we were interested in investigating the combinative effects of preharvest application of B or gypsum and postharvest calcium peduncle infiltration to inhibit internal browning symptom of Queen pineapples during refrigerated storage.

2. Materials and Methods

2.1 Plant materials and experiments

Queen pineapple 'Sawi' fruits were cultivated at King Mongkut's Institute of Technology Ladkrabang, Prince of Chumphon campus demonstration farm. Ammonium sulfate granular 21-0-0 fertilizer was applied to the pineapple plants every two months. The 16-month-old plants were flowered by spraying with 25 µl l⁻¹ of ethephon in aqueous solution containing 2% urea and 0.04% calcium carbonate. This was in contrast to the work of Poovarodom and Boonplang [17] in which

preharvest treatments of CaSO_4 and B were applied. After one month of anthesis, the pineapple fruits were sprayed with 0.25% of boron (B) every month until the fruits were harvested (4 times), or CaSO_4 (100 kg per 400 m^2) was applied to the pineapple plant one month after anthesis. The untreated pineapples were used as control. The fruits were harvested after 135 days of flower induction when fruit peels were yellow to about 25%. Regarding our previous work, 2% CaCl_2 peduncle infiltration was recommended as an effective approach to alleviate internal translucency and browning of Queen pineapple cv. 'Sawi' [7, 11], whereas the application of CaCl_2 at concentrations higher than 2% caused browning incidence in the fruit stem [18]. Fifty fruits per treatment were taken. The fruits were cleaned using an air-blaster. Both preharvest B and CaSO_4 treated pineapples were peduncle-infiltrated with 2% CaCl_2 at 13°C for 3 days and then continuously stored at 13°C for 14 days. In the control treatment, the fruits were stored at 13°C without CaCl_2 peduncle infiltration. The fruits were sampled every 7 days. Before investigation on various parameters, the fruits were left at room temperature (RT) ($28 \pm 1^\circ\text{C}$) for 2 days. Various biological parameters such as visual appearance of half-cut fruit, colour attributes, chilling injury (CI) severity score, browning index (BI) value, the amount of fruit having CI and electrolyte leakage (EL) value of tissue adjacent to the core were monitored. Fifty fruits (50 replicates) were used to evaluate CI incidence, including CI severity score, and the amount of fruit having CI. Five replicates per treatment (10 fruits per replicate) were used to determine colour attributes, BI, and EL values.

2.2 Visual appearance and colour attribute measurements

Vertically half-cut pineapple fruits were used to determine internal browning and translucency appearance caused by CI. Visual browning appearance of the tissue adjacent to the core was presented by photographs. L^* , hue and chroma values of the tissue were measured using a HunterLab MiniScan@ XE Plus (Hunter Associates Laboratory Inc., USA). The total colour difference (ΔE^*) value of tissue adjacent to the core compared with the fruit at initial day (0) was calculated using the equation shown below.

$$\Delta E^* = \sqrt{(L_0^{*2} - L_x^{*2}) + (\text{hue}_0^2 - \text{hue}_x^2) + (\text{chroma}_0^2 - \text{chroma}_x^2)}$$

2.3 CI score measurement

CI severity score was evaluated using a 5-point ranking test. Ten trained panellists were used to evaluate the CI severity score. The simplified CI scores ranged from 0 to 5: 0 = no symptom; 1 = small translucent spots turning brown (less than 5%); 2 = 5-10% CI symptom; 3 = 11-20% CI symptom; 4 = 21-30% CI symptom; and 5 = more than 30% CI symptom, were used.

2.4 BI measurement

BI of the fruit tissues was assessed using the method described by Supapvanich *et al.* [19] with slight modification. A 10 g sample of tissue adjacent to the core was homogenised with 30 ml of 60% (v/v) ethanol and then stirred for 1 h. The homogenate was filtered through Whatman No. 1 filter paper and the wavelength absorbance of filtrate was measured at 420 nm. Data were expressed as $\text{OD}_{420} \text{ g}^{-1}$ fresh weight ($\text{OD}_{420} \text{ g}^{-1}$).

2.5 EL measurement

EL of tissue adjacent to the core was determined using the method of Youryon *et al.* [8]. Fifteen disks (7.5 mm diameter per disk) of tissue adjacent to the core of pineapple fruit were rinsed with de-ionized water and dried using a Whatman No.1 filter paper. The disks were immersed in 30 ml of de-ionized water for 30 min and the conductivity of solution (EC_{sample}) was measured using a conductivity meter. Afterwards, the sample was frozen at -20°C for 24 h. After thawing, the sample was boiled for 30 min and after the temperature of sample had dropped to room temperature, the conductivity of solution was again measured and recorded as total conductivity (EC_{total}). The EL value of each sample was calculated by comparing with total conductivity of the sample.

2.6 Statistical analysis

The experiments were performed using a completed randomized design (CRD) and statistical data were analysed using the Analysis of Variances (ANOVA). Significant differences between data were compared using the Duncan's new multiple range test (DMRT) at $P < 0.05$.

3. Results and Discussions

3.1 Visual appearance

It is commonly recognized that translucency and browning of tissue adjacent to the core are the symptoms of CI in pineapples during cold storage. Figure 1 shows the visual appearance of half-cut Queen pineapple 'Sawi' fruits treated with B + CaCl_2 or $\text{CaSO}_4 + \text{CaCl}_2$ during storage. It was found that after storage at CT for 7 days followed by leaving at RT for 2 days, the control fruits had more CI incidence than fruits treated with B + CaCl_2 or $\text{CaSO}_4 + \text{CaCl}_2$. The lowest CI incidence was found in the fruit treated with $\text{CaSO}_4 + \text{CaCl}_2$. After cold storage for 14 days followed by leaving at RT for 2 days, $\text{CaSO}_4 + \text{CaCl}_2$ treated fruits had IB incidence much lower than other treatments. The highest CI incidence was found in the control fruits. The result shows that $\text{CaSO}_4 + \text{CaCl}_2$ could alleviate CI symptom of Queen pineapple during commercial cold storage at 13°C . Youryon *et al.* [7] suggested that the more uptake of Ca in the core of pineapple fruits, the more alleviation of internal translucency and browning were caused by chilling stress during cold storage. Youryon *et al.* [8] explained that Ca could possibly strengthen cell wall structure and maintain membrane function. Youryon and Wongs-Aree [11] reported that the postharvest application of 2 or 3% of CaCl_2 alleviated CI more than 1% CaCl_2 . The recent study confirms that calcium application could better alleviate CI of Queen pineapple as the fruit treated with $\text{CaSO}_4 + \text{CaCl}_2$ had lower internal translucency and browning than the fruit treated with B + CaCl_2 and control.

3.2 CI incidence

Table 1 shows the CI severity score and the percentage of fruit having CI symptoms. The CI severity score of the pineapple fruits increased as the storage period increased. The highest CI severity score was found in control fruit followed by B + CaCl_2 and $\text{CaSO}_4 + \text{CaCl}_2$ treated fruits, respectively. At the end of 14 days storage, the CI severity score of both control and B + CaCl_2 treated fruit was 4.8 and 4.6, respectively, while that of $\text{CaSO}_4 + \text{CaCl}_2$ treated fruit was only 2.80. The amount of fruit without CI symptom found in $\text{CaSO}_4 + \text{CaCl}_2$ treated fruits was about 70% and 16.7% after storage for 7 and 14 days, respectively. Moreover, CI symptom was detected in all control fruits after storage

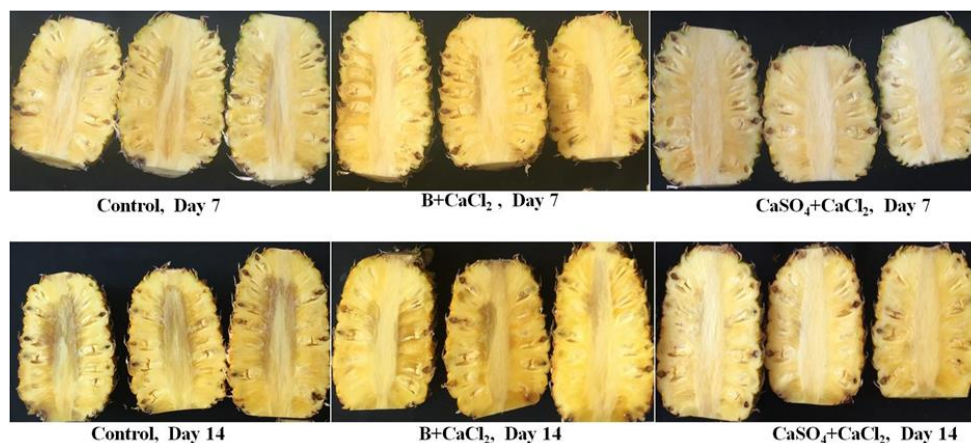


Figure 1. Visual appearance of half-cut Queen pineapple fruits preharvest-treated with B, CaSO_4 and postharvest CaCl_2 peduncle infiltration after cold storage at 13°C for 7 and 14 days

Table 1. CI severity score and percentage of fruit with CI of Queen pineapple fruits preharvest treated with boron or CaSO_4 followed by postharvest CaCl_2 peduncle infiltration after storage at CT for 7 and 14 days and by storage at RT for 2 days

Treatments	Storage time (d)	CI severity score	Percentage of fruit with CI (%)		
			no CI	< 3 score	≥ 3 score
Control	7 (CT)+2(RT)	3.00 ^b	0	50	50
	14(CT)+2(RT)	4.80 ^a	0	30	70
B+ CaCl_2	7 (CT)+2(RT)	1.80 ^c	30	50	20
	14(CT)+2(RT)	4.60 ^a	0	30	70
CaSO_4 + CaCl_2	7 (CT)+2(RT)	0.20 ^d	70	10	20
	14(CT)+2(RT)	2.80 ^b	16.7	33.3	50

Data are shown as means (n = 50).

Different superscript letters shown in the same column indicated a significant difference at $P < 0.05$.

at CT for 7 and 14 days. The fruits treated with 30 % of B + CaCl_2 had CI symptom after storage for 7 days and all of the B+ CaCl_2 treated fruit had CI after storage for 14 days. After cold storage for 7 days, the CI score of the control fruit was significantly higher than that of other treated fruits ($P < 0.05$). The lowest CI score was observed in CaSO_4 + CaCl_2 treated fruits and was significantly lower than that of other treated fruits ($P < 0.05$). After cold storage for 14 days, CI scores of the control and CaSO_4 + CaCl_2 treated fruits were similar while that of CaSO_4 + CaCl_2 treated fruits was significantly lower ($P < 0.05$). The CI severity score was concomitant with the CI incidence and the changes in colour attributes as shown in Figures 1 and 2. These results suggest that the treatment of B + CaCl_2 treatment could alleviate CI of the pineapple fruit when compared to the control, but the efficiency was less than that of CaSO_4 + CaCl_2 treatment. It is commonly recognized that B functions to maintain plant cell wall strength through its binding to the pectin polysaccharides

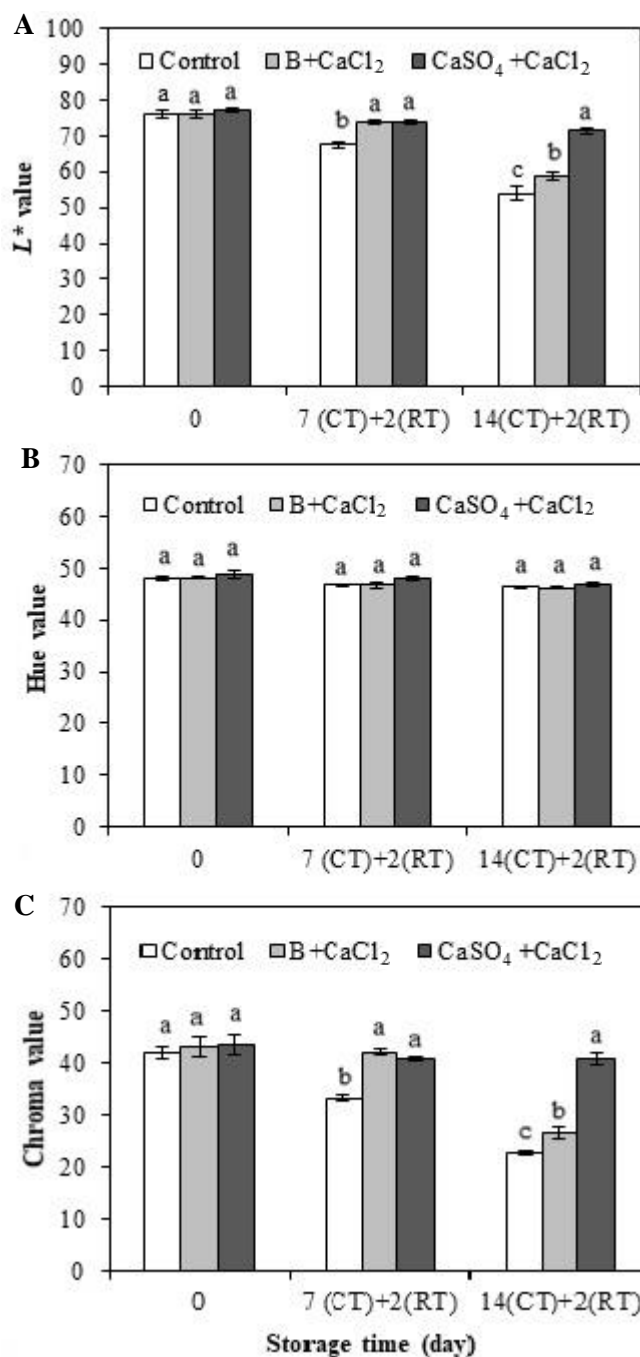


Figure 2. L^* (A), hue (B) and chroma (C) values of tissue adjacent to the core of Queen pineapple fruits preharvest treated with boron or CaSO_4 followed by postharvest CaCl_2 peduncle-infiltration after CT for 7 and 14 days and by leaving at RT for 2 days. Vertical bars represent standard deviation (SD) of means ($n = 5$). The different letters within the same day of storage are significantly different at $P < 0.05$.

that B functions to maintain plant cell wall strength through its binding to the pectin polysaccharides in collaboration with Ca^{2+} [20]. The treatment of $\text{CaSO}_4 + \text{CaCl}_2$ could alleviate severity of CI incidence as well as induce the chilling stress tolerance of the fruit rather than B + CaCl_2 alone. Hewajulige *et al.* [21] also reported that the concentration of Ca^{2+} in tissue of pineapple fruit was positively related to CI symptom, where Ca^{2+} concentration in the fruit core was lower than that in flesh and shell. These results also confirm that the concentration of Ca^{2+} in tissues influenced the CI tolerance of Queen pineapple fruit [11].

3.3 Colour attributes of tissue adjacent to the core

The changes in colour attributes of tissue adjacent to the core of Queen pineapple fruits treated with B + CaCl_2 and $\text{CaSO}_4 + \text{CaCl}_2$ are shown in Figure 2. After cold storage for 7 days followed by leaving at RT for 2 days, L^* and chroma values of control fruits were significantly lower than those of B + CaCl_2 and $\text{CaSO}_4 + \text{CaCl}_2$ treated fruits ($P < 0.05$). However, both L^* and chroma values of B + CaCl_2 and $\text{CaSO}_4 + \text{CaCl}_2$ treated fruits were similar. After cold storage for 14 days followed by leaving at RT for 2 days, both L^* and chroma values of the control fruits had evidently decreased and were significantly lower than those of B + CaCl_2 and $\text{CaSO}_4 + \text{CaCl}_2$ treated fruits ($P < 0.05$). When compared to $\text{CaSO}_4 + \text{CaCl}_2$ treated fruits, L^* and chroma values of B + CaCl_2 treated fruits were significantly lower ($P < 0.05$). While, hue values of all treatments remained constant throughout the storage periods. The hue value of tissue adjacent to the core was approximately 47.33, which presented as yellow colour. These results suggest that $\text{CaSO}_4 + \text{CaCl}_2$ treatment can prevent the losses of L^* and chroma value of tissue adjacent to the core better than B + CaCl_2 treatment. Many previous work suggested that the increase in internal translucency and browning of tissue adjacent to the core of pineapples was concomitant with the reduction of L^* value [3, 8, 10]. Moreover, we found that the reduction of chroma value was also related with the increase in CI symptom of Queen pineapple during cold storage. It is typically acknowledged that chroma value represents the intensity of colour. Therefore, the occurrence of internal translucency (Figure 1) was concomitant with the decrease of chroma value of tissue adjacent to the core of pineapples.

3.4 Browning index and total colour difference value of tissue adjacent to the core

BI and ΔE^* values of the pineapples are shown in Figure 3. Both parameters of the control fruits evidently increased and were significantly higher than those of B + CaCl_2 and $\text{CaSO}_4 + \text{CaCl}_2$ treated fruits over the storage ($P < 0.05$). After storage at CT for 7 days followed by RT for 2 days, both BI and ΔE^* values of B + CaCl_2 or $\text{CaSO}_4 + \text{CaCl}_2$ treated fruits were similar. After storage at CT for 14 days followed by RT for 2 days, both BI and ΔE^* values of B + CaCl_2 increased significantly higher than those of $\text{CaSO}_4 + \text{CaCl}_2$ treated fruits. These results indicate that $\text{CaSO}_4 + \text{CaCl}_2$ treatment could maintain colour and prevent browning incidence of tissue adjacent to the core of the pineapples during cold storage. It is commonly recognized that browning incidence occurred in tissue adjacent to the core of pineapple is caused by the reaction of PPO and phenolic compounds, namely enzymatic browning reaction. Hewajulige *et al.* [21] suggested that the severity of black heart or internal browning incidence in pineapples was associated with low calcium content in tissue adjacent to the core compared to pulp and peel. Hopfinger *et al.* [22] suggested that preharvest Ca treatment increased Ca concentration in fruit and decreased browning enzyme activity. Moreover, previous work reported that postharvest calcium treatment could alleviate internal browning of tissue adjacent to the core of Queen pineapples due to the decrease in PPO activity [7, 8, 10, 12]. The recent results indicated that preharvest CaSO_4 treatment incorporated with postharvest CaCl_2 application effectively alleviated internal browning incidence when compared to preharvest B treatment incorporated with postharvest CaCl_2 peduncle infiltration.

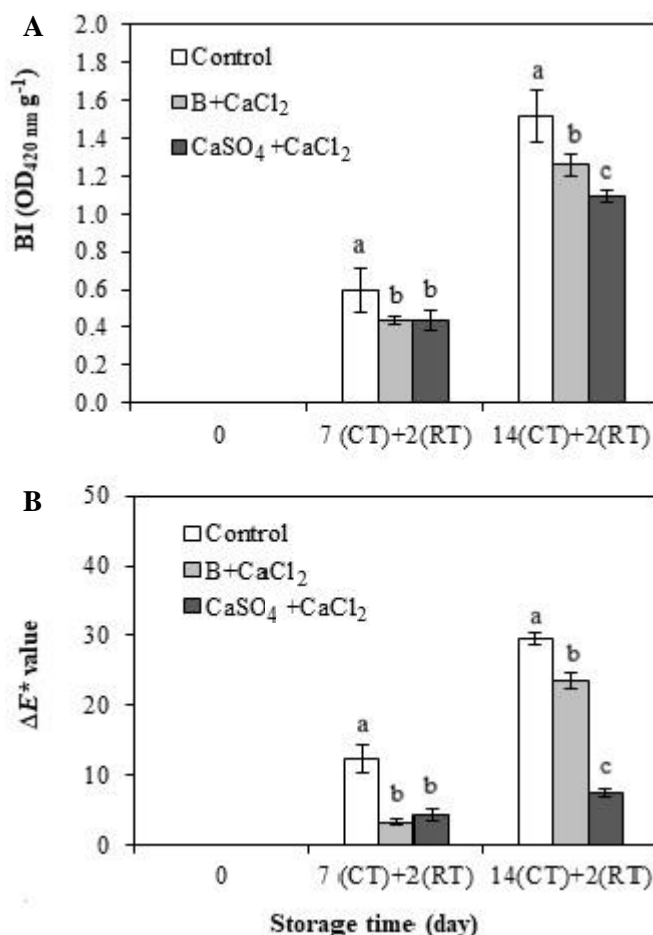


Figure 3. BI (A) and ΔE^* (B) values of tissue adjacent to the core of Queen pineapple fruits preharvest treated with boron or CaSO₄ followed by postharvest CaCl₂ peduncle-infiltration after CT for 7 and 14 days and by leaving at RT for 2 days. Vertical bars represent standard deviation (SD) of means (n = 5). The different letters within the same day of storage are significantly different at $P < 0.05$.

3.5 EL of tissue adjacent to the core

Figure 4 shows EL value of tissue adjacent to the core during storage. EL value of all treatments increased during the storage period. The highest EL value was found in the control fruits and it was significantly higher than that of B + CaCl₂ and CaSO₄ + CaCl₂ treated fruits ($P < 0.05$). CaSO₄ + CaCl₂ treatment controlled the increase in EL of tissue adjacent to the core better than B + CaCl₂ treatment. These results indicate that the treatment of CaSO₄ + CaCl₂ could alleviate the membrane dysfunction and thus inhibit the increase in EL value during the storage period. It is commonly recognized that CI is an oxidative stress caused by chilling temperature. The factors indicating CI incidence is EL value which associated with increased membrane lipid oxidation caused by chilling

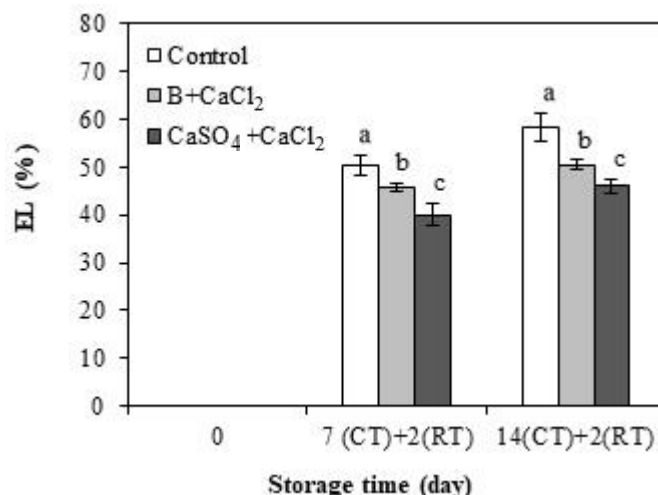


Figure 4. EL value of tissue adjacent to the core of Queen pineapple fruits preharvest treated with boron or CaSO₄ followed by postharvest CaCl₂ peduncle infiltration after CT for 7 and 14 days followed by RT for 2 days. Vertical bars represent standard deviation (SD) of means (n = 5). The different letters within the same day of storage are significantly different at $P < 0.05$.

temperature [23]. Picchioni *et al.* [24] stated that Ca in plant tissue plays a role in strengthening cell wall structure as well as regulating protein kinase signalling and inducing membrane integrity. Thus, the treatment of CaSO₄ + CaCl₂ may increase the Ca concentration in tissue adjacent to the core in a superior way to the treatment of B + CaCl₂, leading to prevent the increase in EL value. Thus, Ca application helps to maintain membrane integrity of pineapples. Regarding the cell wall and membrane strengthening by Ca treatment, the breakdown of tissue structure is low, leading to lower translucency incidence and to prevent the release of PPO [25]. The recent study showed that the lower EL value of CaSO₄ + CaCl₂ treated fruits was concomitant with lower CI incidence (Figure 1 and Table 1), higher L^* and chroma values (Figure 2) and lower BI and ΔE^* values (Figure 3) compared to B + CaCl₂ and control treatments, respectively.

4. Conclusions

The applications of preharvest B, CaSO₄ and postharvest CaCl₂ peduncle infiltration could alleviate CI of Queen pineapple cv. 'Sawi' fruits during commercial cold storage. CaSO₄ + CaCl₂ treatment could inhibit the increase in internal browning incidence. The treatment also maintained the membrane integrity of tissue adjacent to the core due to the lowered EL value when compared to B + CaCl₂ treatment. Our results suggest that CaSO₄ + CaCl₂ treatment is an alternative approach for alleviating CI of postharvest Queen pineapple during cold storage.

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