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Original article

Effects of simultaneous cyanocobalamin and calcium gluconate treatment on chilling injury alleviation of 'Queen' pineapple by using peduncle infiltration

P. Youryon[1], W. Keereedat[1] and S. Supapvanich[2,a]

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

[2] Department of Agricultural Education, Faculty of Industrial Education and Technology, King Mongkut's Institute of Technology Ladkrabang, Chalongkrung Road, Ladkrabang, Bangkok, Thailand

Summary

Introduction – **Chilling injury (CI) is main problem of 'Queen' pineapples during commercial storage (13 °C). The increases in calcium content and antioxidant system enhancement alleviate CI of pineapples. Cyanocobalamin (Cyn) is a novel natural agent inducing antioxidants in postharvest commodities. Therefore, the aim of this work was to investigate the efficiency of simultaneous Cyn and calcium gluconate (Ca-Glu) peduncle infiltration on CI alleviation of 'Queen' pineapple during cold storage.** *Materials and methods* – **In the first experiment, the fruits were treated with Cyn at the concentration of 0, 1, 2.5 or 5 µM. In the second experiment, simultaneous 5 µM Cys and 2 or 3% Ca-Glu treatments were determined. Biological parameters related to CI of tissue adjacent to the core were monitored after storage at 13 °C for 7 and 14 d followed by 28 ± 2 °C for 2 d.** *Results* – **5 µM Cyn peduncle infiltration relieved CI and membrane peroxidation rather than other concentrations. The simultaneous 5 µM Cys and 2 or 3% Ca-Glu treatments could alleviate CI symptoms, membrane peroxidation and enzymatic browning reaction and enhance antioxidant activities of tissue adjacent to the core of pineapples during storage. The simultaneous 5 µM Cyn and 3% Ca-Glu treatment enhanced antioxidant activities and alleviated CI of the fruit being greater than simultaneous 5 µM Cyn and 2% Ca-Glu treatment.** *Conclusion* – **The simultaneous 5 µM Cyn and 3% Ca-Glu peduncle infiltration was an effective approach alleviating CI of 'Queen' pineapples during commercial storage.**

Keywords
'Queen' pineapple, cyanocobalamin, calcium gluconate, chilling injury, internal browning

Significance of this study

What is already known on this subject?

- Chilling injury symptoms of pineapples including internal translucency and internal browning associate with membrane dysfunction and enzymatic browning reaction.

What are the new findings?

- Cyanocobalamin (vitamin B12) is a novel natural chemical alleviating CI of 'Queen' pineapples.
- Simultaneous cyanocobalamin (vitamin B12) and calcium gluconate peduncle-infiltration prevents membrane lipids peroxidation and enzymatic browning reaction and enhances antioxidant activity in tissue adjacent to the core of pineapples.

What is the expected impact on horticulture?

- Simultaneous cyanocobalamin (vitamin B12) and calcium gluconate peduncle-infiltration is a novel postharvest treatment alleviating chilling injury of 'Queen' pineapples during commercial cold storage (13 °C).

Introduction

Pineapples (*Ananas comosus* L. Merr.) have been widely accepted as a commercially important tropical fruit in the world (Lobo and Yahia, 2017). Pineapples are thought to be indigenous to South America, an area encompassing Brazil and Paraguay, and had been dispersed across tropical and subtropical regions (Collins, 1960). In Thailand, two groups of pineapples, 'Smooth Cayenne' and 'Queen', have been commercially produced for industrial processing and fresh consumption (Sangprayoon *et al.*, 2019). 'Queen' pineapples are mainly produced for fresh consumption (Quyen *et al.*, 2013) and highly susceptible to chilling injury (CI) when compared to 'Smooth Cayenne' pineapples (Om-Arun and Siriphanich, 2004). CI is commonly acknowledged as the major problem limiting marketability and eating quality of 'Queen' pineapples due to translucency and internal browning (IB) of tissue adjacent to the core which is noticeable after commercial refrigerated storage (13 °C) for 1 week (Youryon *et al.*, 2018). Typically, tissue translucency firstly occurs due to membrane dysfunction and then IB incidence appears afterwards (Paull and Rohrbach, 1985). It is commonly acknowledged that chilling stress induces the accumulation of reactive oxygen species (ROS) leading to the peroxidation of membrane lipids. This results to the loss of membrane integrity and the accumulation of malondialdehyde (MDA) concentration (Marangoni *et al.*, 1996). The loss of membrane integrity causes to translucency of tissue adjacent to the core of pineapples and then the activities of PPO and POD enzymes are induced and reacted with phenolic compounds leading to brown-

[a] Corresponding author: suriyan.su@kmitl.ac.th.

ing (Scandalios, 1993). Therefore, postharvest treatments maintaining membrane integrity and enhancing antioxidant system have been considered to induce CI tolerance of fresh commodities during cold storage.

Our previous study introduced the application of a novel natural agent named cyanocobalamin (Cyn) or vitamin B12 on bioactive compounds enhancement in baby vegetables (Supapvanich *et al*., 2020). Moreover, Samaan *et al*. (2011) reported that Cyn application could maintain quality and induce CI tolerance of mangoes during cold storage. Lo'ay (2017) suggested that Cyn treatment improved skin colour of 'Crimson seedless' grapes due to the increment of anthocyanin content. Cyn is recognised as a form of vitamin B12 which cobalamin is typically found in higher plants especially in cytosol, mitochondria and plastids (Roje, 2007). Cobalamin plays an important role to photosynthesis and to transfer methyl group during methionine biosynthesis. It also induces antioxidant system and polyamines biosynthesis in higher plants (Burguieres *et al*., 2007).

Hewajulige *et al*. (2003) suggested that CI tolerance of pineapples was accompanied with calcium concentration in flesh adjacent to the core which markedly decreased during cold storage. Youryon *et al*. (2013) introduced $CaCl_2$ peduncle infiltration to alleviate CI of 'Queen' pineapples during commercial storage. Calcium is commonly known as an element playing a key role on cell wall strengthening and membrane integrity (Picchioni *et al*., 1998). Moreover, Youryon *et al*. (2018) had reported that calcium gluconate (Ca-Glu) could alleviate CI incidence of pineapples being greater than $CaCl_2$. Therefore, we were interested in the investigation of simultaneous Cyn and Ca-Glu peduncle infiltrations on CI alleviation of 'Queen' pineapples during commercial storage at 13 °C.

Materials and methods

Raw materials

Pineapples cv. 'Sawi' fruit, a commercial 'Queen' pineapple, were derived from a commercial orchard in Chumphon province, southern Thailand. The pineapples were harvested after the induction of flowering for 5 months when the fruit peel turned to yellow approximately 25% of fruit (commercial harvesting stag). The pineapples were delivered to Crop Science Laboratory, King Mongkut's Institute of Technology Ladkrabang within 1 h after harvest. The fruit were cleaned by water spraying and then air-dried for 30 min. Pineapple crown was then half-cut and peduncle was cut and leftover approximately 3 cm length.

Preliminary experiment

The pineapples were vertically peduncle-infiltrated with 1, 2.5 and 5 µM Cyn for 3 d at 13 °C and then stored at 13 °C. Untreated pineapples stored at 13 °C were used as negative control treatment. Ten fruits of each treatment were sampled after storage for 7 and 14 d and then left at 28±2 °C (RT) for 2 d. Biological parameters related to CI such as L^* value, browning index (BI), electrolyte leakage (EL), MDA content, lipoxygenase (LOX) activity and antioxidant activity of tissue adjacent to the core, were monitored.

Simultaneous Cyn and Ca-Glu treatments

Regarding to the results of preliminary experiment (3.1), 5 µM Cyn alleviated CI incidence of 'Queen' pineapples being greater than other concentrations. Moreover, our previous work suggested that 2 or 3% Ca-Glu peduncle infiltration was recommended for CI alleviation of 'Queen' pineapples during cold storage (Youryon *et al*., 2018). Thus, the simultaneous 5 µM Cyn and Ca-Glu at the concentration of 2 or 3% peduncle infiltrations were determined. Pineapples were peduncle infiltrated with 5 µM Cyn + 2% Ca-Glu or 5 µM Cyn + 3% Ca-Glu solutions for 3 d at 13 °C and then stored at 13 °C for 14 d. The untreated pineapples stored at 13 °C were used as negative control treatment. Ten fruits of each treatment were sampled after storage at 13 °C for 7 and 14 d. Biological parameters related to CI of pineapples were investigated in tissue adjacent to the core after storage followed by left at RT for 2 d.

IB appearance and the measurements of L^* and BI values

IB appearance of pineapples was observed using the photograph of longitudinal half-cut fruit. Colour attributes such as L^*, b^* and a^* values of tissue adjacent to the core were measured using a Hunter Lab MiniScan colorimeter (Hunter Associates Laboratory Inc., USA). BI value was calculated using the equation of Palou *et al*. (1991) as shown below:

$$\text{BI value} = 100 \times \frac{(X-0.31)}{0.172}$$

$$\text{where: } X = \frac{(a^* + 1.75L^*)}{(5.645L^* + a^* - 3.012b^*)}$$

EL measurement

Tissue adjacent to the core of 'Queen' pineapple was bored using a 5-mm diameter cork borer and then cut into 2 mm-thick disc. Ten discs were washed with deionized water and then incubated in 20 mL of 0.4 M mannitol at RT for 1 h. After incubation, conductivity of the solution was measured using a conductivity meter (Consort C830, Belgium) and was then frozen at -20 °C for 24 h. After thawing, the sample was autoclaved at 120 °C for 30 min and the total conductivity of solution (C_{total}) was measured. The EL from the tissue was calculated using the equation as shown below:

$$\text{EL (\%)} = \frac{\text{Conductivity at 1 h}}{\text{total conductivity}} \times 100$$

MDA content assay

Tissue adjacent to the core was homogenised with 0.1% (w/v) of trichloroacetic acid. The homogenate was centrifuged at 12,000 × *g* for 15 min. The supernatant was used to assay MDA content using the method of Wang *et al*. (2004). The reaction began when the extract was mixed with the solution of 1.5% (w/v) trichloroacetic acid and 0.5% (w/v) thiobarbituric acid. The mixture was incubated at 90 °C for 10 min and then immediately placed in an ice-bath. The optical density (OD) at 532 and 600 nm wavelengths were recorded and then calculated using the equation shown below:

$$\text{MDA content (mmole)} = \frac{\text{OD at 532 nm} - \text{OD at 600 nm}}{155}$$

LOX activity assay

LOX activity was assayed using the method of Pérez *et al*. (1999). The tissue was homogenized with Tris-HCl buffer, pH 7.0, at 4 °C. The homogenate was then centrifuged at 12,000 × *g* for 25 min. The reaction was started when the extract was mixed with 0.01 M linoleic acid sodium salt. The increase in OD at 234 nm wavelength during incubation at RT for 5 min was recorded in every 30 sec. Unit (U) of LOX activity was defined as 1 µmole of H_2O_2 formed per min. LOX activity was expressed as U g^{-1}.

PPO and POD activities assays

PPO and POD enzymes in the tissue were extracted with 0.1 M sodium phosphate buffer, pH 7.0 containing 0.5 g of polyvinylpyrrolidone (PVPP) and then centrifuged at the speed of 12,000 × *g* at 4 °C for 25 min. Supernatant was used to determine PPO activity using the method of Galeazzi *et al*. (1981) and POD activity using the method of Zhang *et al*. (2005). Catechol and guaiacol were used as substrate of PPO and POD reactions, respectively. Unit of PPO activity was defined as the changes of 0.01 in OD at 420 nm wavelength per min and the data was expressed as U g^{-1}. Unit of POD activity was defined as the changes of 0.01 in OD at 470 nm wavelength per min and the data was expressed as U g^{-1}.

Total phenols content assay

Total phenolic compound in the tissue was extracted using 60% (v/v) ethanol. The homogenate was filtered using a Whatman No. 1 filter paper. The filtrate was used to determine total phenols concentration by using the method of Slinkard and Singleton (1997). The filtrate was reacted with 50% (v/v) Folin-Ciocalteu reagent and saturated Na_2CO_3 solution in the ratio of 1:1:2 and then incubated at RT for at least 30 min. The OD at 750 nm wavelength was measured and the concentration of total phenols was computed by using a linear equation of gallic acid standard curve. Data were expressed as mg gallic acid per kilogram fresh weight (mg kg^{-1}).

Determination of antioxidant activities

The same filtrate of total phenols determination was used to evaluate both ferric reducing antioxidant potential (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity. FRAP was determined using the method of Benzie and Strain (1996). FRAP reagent consisted of acetate buffer pH 3, 10 mM 2,4,6-tripyridyl-1,3,5-triazine (TPTZ) and 20 mM ferric chloride hexahydrate in the ratio of 10:1:1. The reaction was started when the extract was mixed with FRAP reagent and then incubated at RT for 30 min. The OD at 630 nm wavelength was measured and FRAP was reckoned using a linear equation of Trolox standard curve. Data were expressed as mmole Trolox equivalent per kilogram fresh weight (mmol kg^{-1}). Free radical scavenging activity was assayed using the method described by Brand-Williams *et al*. (1995). The extract was reacted with 1 mM DPPH in methanol. The reaction was operated in a dark place and the OD at 517 nm wavelength was measured at 0 min and 3 min of reaction. The percentage to DPPH free radical scavenging activity during incubation for 3 min was calculated.

Statistical analysis

Complete randomized design was performed in both experiments. Analysis of Variance (ANOVA) was used for statistical analysis and significant differences between the means of each treatment were compared using Least Significant Difference (LSD) test at the level of $P < 0.05$.

Results and discussion

Effects of cyanocobalamin concentrations on CI related parameters

The effects of Cyn at various concentrations on CI alleviation of 'Queen' pineapple cv. 'Sawi' fruit were shown in Figure 1. It was found than 5 µM Cyn peduncle infiltration

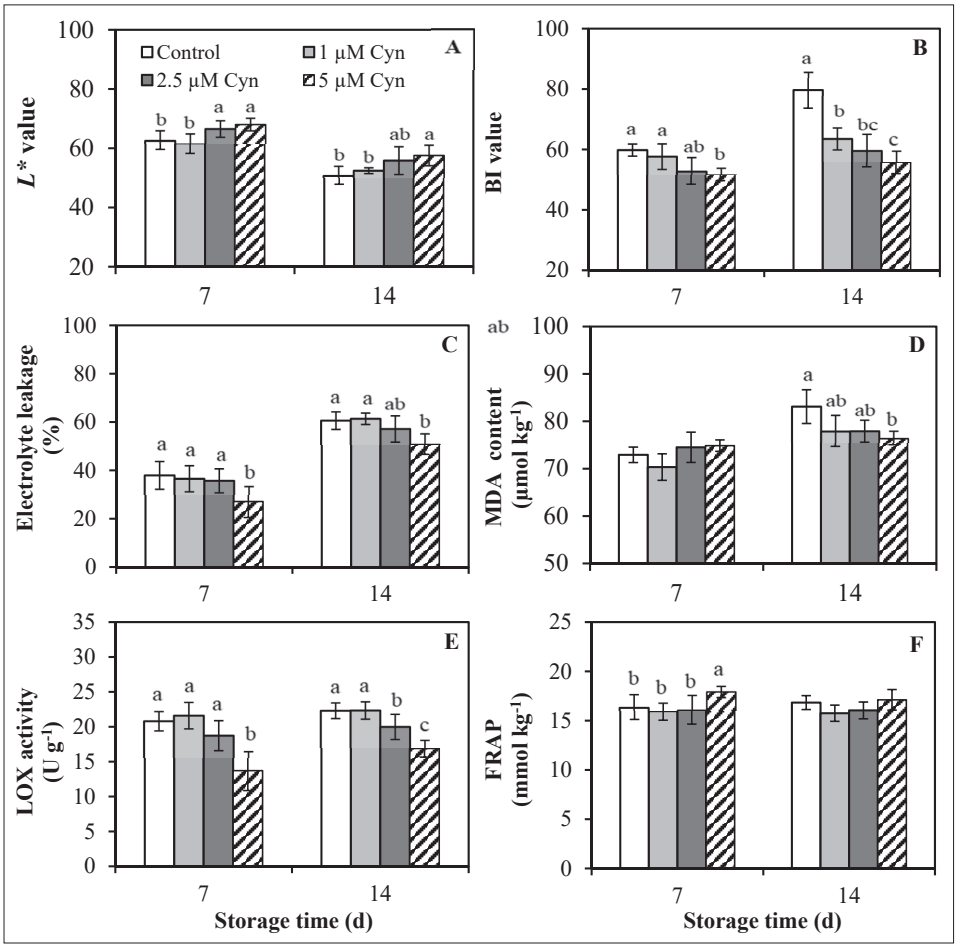

Figure 1. *L** (A), BI (B), EL (C), MDA content (D), LOX activity (E) and antioxidant activity (F) of tissue adjacent to the core of 'Queen' pineapples cv. 'Sawi' treated with cyanocobalamin at various concentrations after storage at 13 °C for 7 and 14 d followed by at RT for 2 d. The vertical bar shows the standard deviation of mean ($n = 10$). Different letters within the same figure of each day represent significant difference at $P < 0.05$.

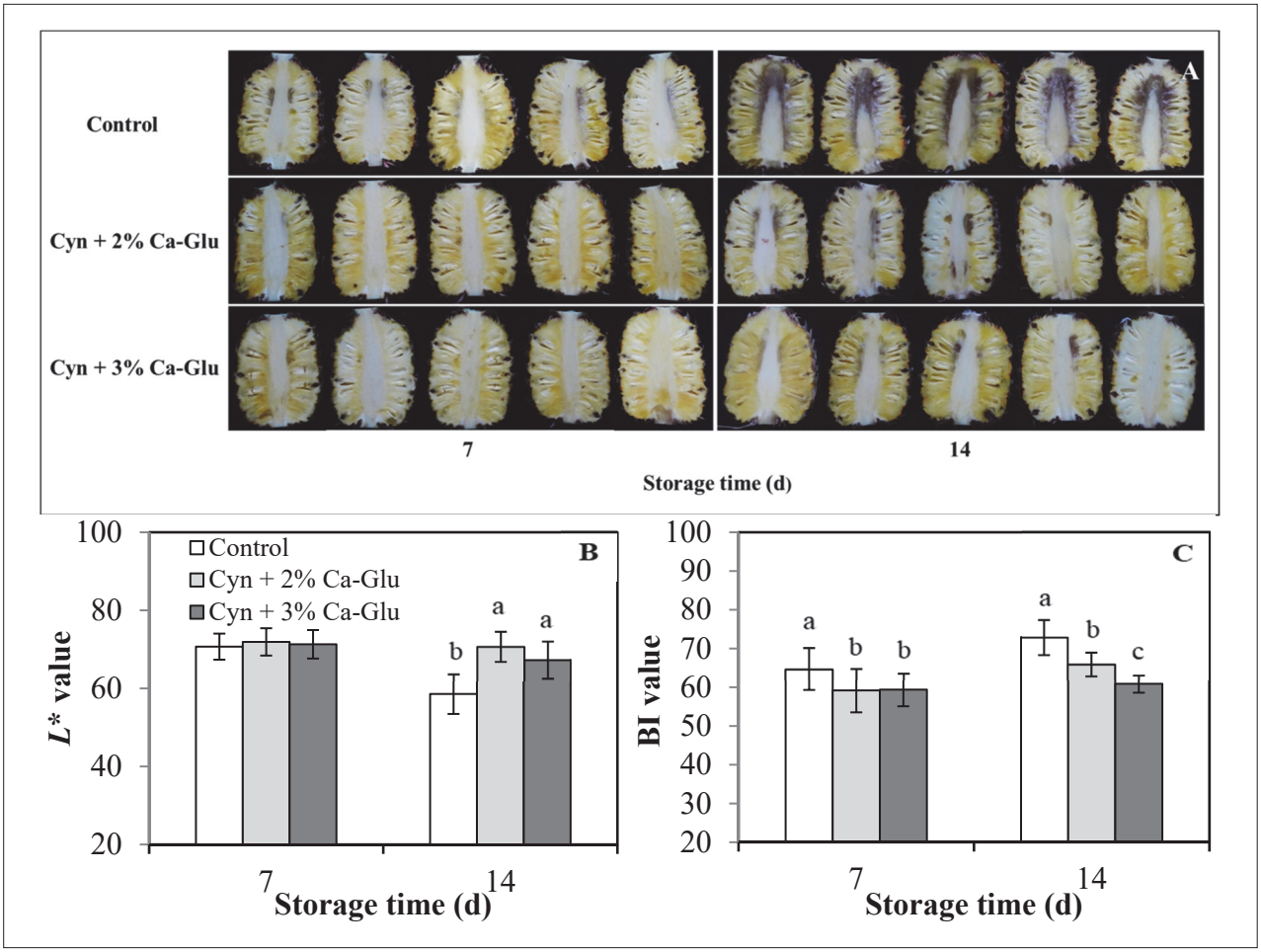

Figure 2. Visual appearance (A), *L** value (B) and BI value (C) of tissue adjacent to the core of 'Queen' pineapples cv. 'Sawi' treated with cyanocobalamin at various concentrations after storage at 13 °C for 7 and 14 d followed by at RT for 2 d. The vertical bar shows the standard deviation of mean ($n = 10$). Different letters within the same figure of each day represent significant difference at $P < 0.05$.

significantly inhibited *L** value decrease and BI value increase of tissue adjacent to the core when compared to control and 1 µM Cyn treatments ($P < 0.05$) The 5 µM Cyn treatment retarded the increases in EL MDA content when compared to other treatments. Both EL and MDA content of 5 µM Cyn-treated pineapples were significantly lower than those of control fruit after cold storage for 14 d ($P < 0.05$). The LOX activity of 5 µM Cyn-treated fruit was significantly lower than other treatments after cold storage for 7 and 14 d ($P < 0.05$). We also found that the antioxidant activity of 5 µM Cyn treated fruit was significantly higher than other treated fruit after cold storage for 7 d; however, after cold storage for 14 d, no significant difference in antioxidant activity of each treatment was observed. These suggested that Cyn peduncle infiltration could alleviate CI symptom of 'Queen' pineapples due to the retardation of membrane dysfunction and the enhancement of antioxidant activity. Burgueres *et al.* (2007) suggested that vitamin B group can stimulate phenolic compounds and proline biosynthesis leading to the inducement of antioxidant system and defence mechanism in plants. Supapvanich *et al.* (2020) reported the enhancement of antioxidant activity in baby vegetables during storage by using Cyn immersion. Samaan *et al.* (2011) reported that preharvest Cyn treatment at 1 mg L^{-1} alleviated CI of 'Zibda' and 'Hindi' mango fruits during cold storage at 5 ± 1 °C due to the enhancement of antioxidant system. The recent results indicated that 5 µM Cyn peduncle infiltration evidently alleviated CI of the pineapples compared to other concentrations. Therefore, 5 µM Cyn was selected to study the efficiency of simultaneous Cyn and Ca-Glu peduncle infiltration on CI alleviation of the pineapples during cold storage.

Visual appearance and colour attributes

The effects of simultaneous Cyn and Ca-Glu peduncle infiltrations on CI appearance, *L** and BI values of tissue adjacent to the core of 'Queen' pineapples were presented in Figure 2. After cold storage for 7 d, IB appearance was obviously observed in control fruit whilst that of Cyn + 2% Ca-Glu and Cyn + 3% Ca-Glu was found slightly (Figure 2A). After cold storage for 14 d, the IB symptom of control fruit was markedly severe. Meanwhile IB of both treated pineapples was obviously lower than control fruit. We also found that IB of Cyn + 3% Ca-Glu treated pineapples was lower than that of Cyn + 2% Ca-Glu treated fruit. The IB appearance of pineapples was concomitant with the *L** and BI values of tissue adjacent to the core as shown in Figures 2B and 2C. After cold storage for 7 d, the *L** value of all treatments was close but after cold storage for 14 d, the *L** value of control fruit was significantly lower than that of Cyn + 2% Ca-Glu and Cyn + 3% Ca-Glu treated pineapples ($P < 0.05$). No significant difference in *L** value of between Cyn + 2% Ca-Glu and Cyn + 3% Ca-Glu treated pineapples was found over the storage. The BI

value of all treatments increased during storage and that of control fruit was significantly higher than that of Cyn + 2% Ca-Glu and Cyn + 3% Ca-Glu treated pineapples ($P < 0.05$). After cold storage for 14 d, the BI of 3% Ca-Glu treated pineapples was significantly lower than that of other treatments ($P < 0.05$). The recent results indicated that simultaneous Cyn and Ca-Glu peduncle infiltration effectively alleviated CI of pineapples due to lower IB and to prevent the decreased L^* value and increased BI value of tissue adjacent to the core during cold storage. Thus, the parameters related to CI such as membrane dysfunction, enzymatic browning reaction and antioxidant activities of tissue adjacent to the core were considered.

EL, MDA content and LOX activity

It is widely recognised that membrane dysfunction is the main incidence of CI in plants. Factors related to membrane dysfunction such as EL, MDA content and LOX activity of tissue adjacent to the core of 'Queen' pineapples were shown in Figure 3. The EL of both simultaneous Cyn and Ca-Glu treated pineapples was significantly lower than that of control fruit after the storage for 7 and 14 d ($P < 0.05$); however, that of both treated pineapples was not significantly different during storage. In the similar vein, the MDA content of control fruit was significantly higher than that of both treated pineapples during storage ($P < 0.05$). The changes in both EL and MDA content were concomitant with LOX activity during storage. Both of the simultaneous Cyn and Ca-Glu treatments significantly lowered LOX activity during storage ($P < 0.05$). No significant difference in LOX activity between Cyn + 2% Ca-Glu and Cyn + 3% Ca-Glu treated pineapples were observed throughout the storage. However, after the storage for 14 d, the EL, MDA content and LOX activity of Cyn + 3% Ca-Glu treated pineapples was likely to be lower than those of Cyn + 2% Ca-Glu treated pineapples. It is commonly acknowledged that chilling stress stimulates the peroxidation of membrane fatty acids and the degradation of phospholipids and lactolipids leading to the decrease of membrane fluidity and functions (Rui *et al.*, 2010). Increased EL indicates the loss of membrane integrity and the lipid peroxidation is evaluated by MDA content and LOX activity. Increases of both MDA content and LOX activity are responsible for membrane dysfunction caused by oxidative stress during cold storage (Marangoni *et al.*, 1996). The recent results indicated that the simultaneous Cyn and Ca-Glu treatment maintained membrane integrity due to reduce membrane peroxidation of tissue adjacent to the core during cold storage. Previous works reported that Cyn induces proline biosynthesis (Burguieres *et al.*, 2007) and antioxidant capacity of Cyn (Samaan *et al.*, 2011) and Ca-Glu enhances wall strengthening and antioxidant activity (Youryon *et al.*, 2018). These might be related to membrane maintenance of pineapples by the simultaneous Cyn and Ca-Glu peduncle-infiltration.

Browning enzymes activities and total phenols content

IB of pineapples is recognised as CI symptom, with browning enzymes such as PPO and POD and phenolic compounds playing the key role of browning reaction. Both of the PPO and POD activities and total phenols content of tissue adjacent to the core were shown in Figure 4. After cold storage for 7 d, the highest PPO activity was observed in control pineapples which was significantly higher than that of Cyn + 3% Ca-Glu treated pineapples ($P < 0.05$). Meanwhile, the PPO activity of Cyn + 2% Ca-Glu treated pineapples was likely to be higher than that of Cyn + 3% Ca-Glu treated pineapples and no significant difference was found. After cold storage for 14 d, PPO activity of both simultaneous Cyn and Ca-Glu treatments was similar and significantly lower than that of control pineapples ($P < 0.05$). Interestingly, POD activity of both simultaneous Cyn and Ca-Glu treatments was significantly higher than that of control pineapples after cold storage for 7 d ($P < 0.05$). The highest POD activity was observed in Cyn + 3% Ca-Glu treated pineapples and it was significantly higher than that of Cyn + 2% Ca-Glu treated pineapples. After cold storage for 14 d, POD activity of control pineapples markedly increased and was significantly higher than that of both simultaneous Cyn and Ca-Glu treated pineapples ($P < 0.05$). Whereas, the POD activity of both simultaneous Cyn and Ca-Glu treated pineapples was not significantly different. The total phenols content of both simultaneous Cyn and Ca-Glu treated pineapples was likely to be higher than that of control during the storage. It was found that total phenols content of Cyn + 3% Ca-Glu treated pineapples was significantly higher than other samples after cold storage for 7 d ($P < 0.05$). At the

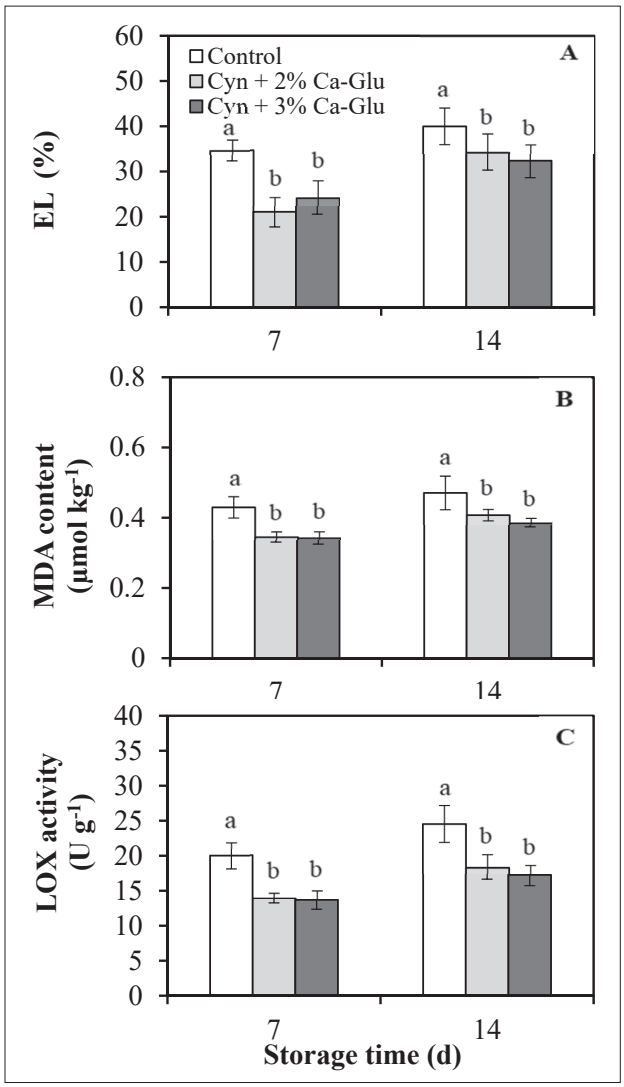

Figure 3. EL (A), MDA content (B) and LOX activity (C) of tissue adjacent to the core of 'Queen' pineapples cv. 'Sawi' treated with cyanocobalamin at various concentrations during refrigerated storage at 13 °C for 14 d. The vertical bar shows the standard deviation of mean ($n = 10$). Different letters within the same figure of each day represent significant difference at $P < 0.05$.

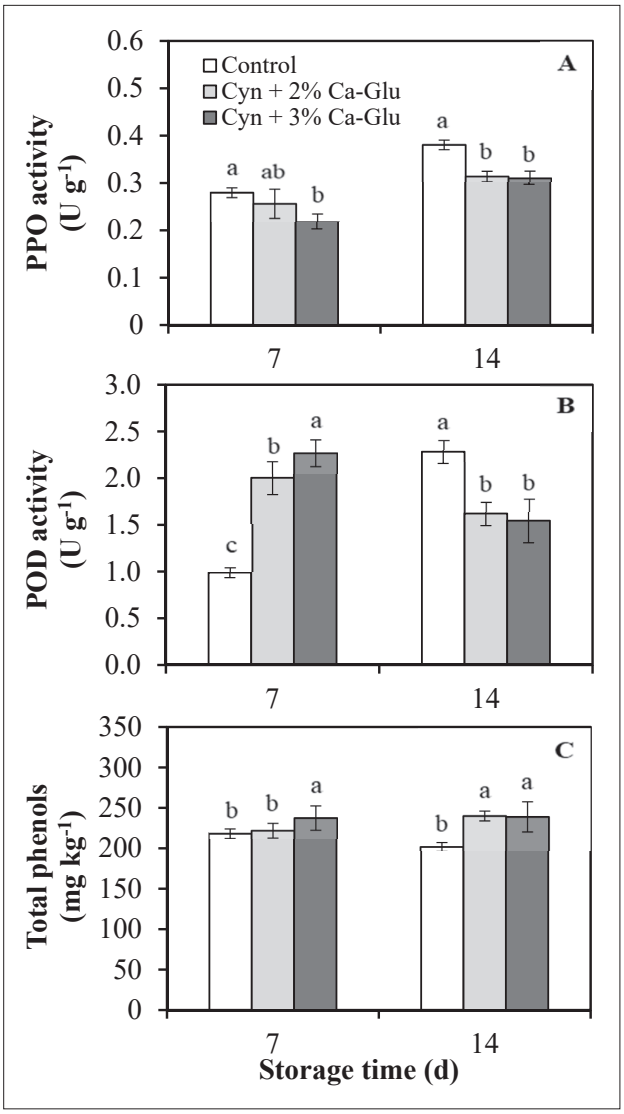

Figure 4. PPO activity (A), POD activity (B) and total phenols content (C) of tissue adjacent to the core of 'Queen' pineapples cv. 'Sawi' treated with cyanocobalamin at various concentrations during refrigerated storage at 13 °C for 14 d. The vertical bar shows the standard deviation of mean ($n = 10$). Different letters within the same figure of each day represent significant difference at $P < 0.05$.

end of storage, total phenols content of both simultaneous Cyn and Ca-Glu treated pineapples was significantly higher than that of control pineapples ($P < 0.05$). This indicated that PPO activity played a key role on IB caused by CI of 'Queen' pineapples. Meanwhile, POD activity might play roles both controlling and inducing browning reaction in tissue adjacent to the core of pineapples. We found the markedly high POD activity of both simultaneous Cyn and Ca-Glu treated pineapples when compared to control pineapples after cold storage for 7 d whilst the slight IB incidence was observed in both of the treated pineapples. Yang *et al.* (2011) reported that the enhancement of antioxidant enzymes activities including POD by nitric oxide treatment was concomitant with the lower CI symptom of cucumber during cold storage. The recent study indicated that simultaneous Cyn and Ca-Glu treatment induced POD activity after cold storage for 7 d. The enhancement of POD activity by Cyn has not been recently reported, whereas Youryon *et al.* (2018) reported that Ca-Glu could enhance POD activity in tissue adjacent to the core of 'Queen' pineapples during storage. However, after cold storage for 14 d, the obvious increases in both PPO and POD activities were observed and concomitant with increased IB of control pineapples. These suggested that PPO and POD activities of tissue adjacent to the core were markedly increased at longer period of cold storage due to the breakdown of tissues cause by CI. The recent study also found that increased total phenols content of both simultaneous Cyn and Ca-Glu treatments might not be the main factor relating to the increased IB incidence of 'Queen' pineapples during storage. It is acknowledged that PPO activity is specific to phenolic substrates and some phenolic compounds have antioxidant potential against oxidative reaction (Chimvaree *et al.*, 2019). In this case, simultaneous Cyn and Ca-Glu treatments could induce total phenols content in tissue adjacent to the core and associated with the lower IB of both treated pineapples compared to control fruits. The effect of Cyn inducing total phenols content was reported for baby vegetables (Supapvanich *et al.*, 2020) and 'Crimson seedless' grape (Lo'ay, 2017). Youryon *et al.* (2018) reported that Ca-Glu peduncle infiltration could induce total phenols content of 'Queen' pineapples after cold storage for 1 week. This suggested that simultaneous Cyn and Ca-Glu peduncle infiltration could reduce enzymatic browning reaction of tissue adjacent to the core of 'Queen' pineapples during cold storage.

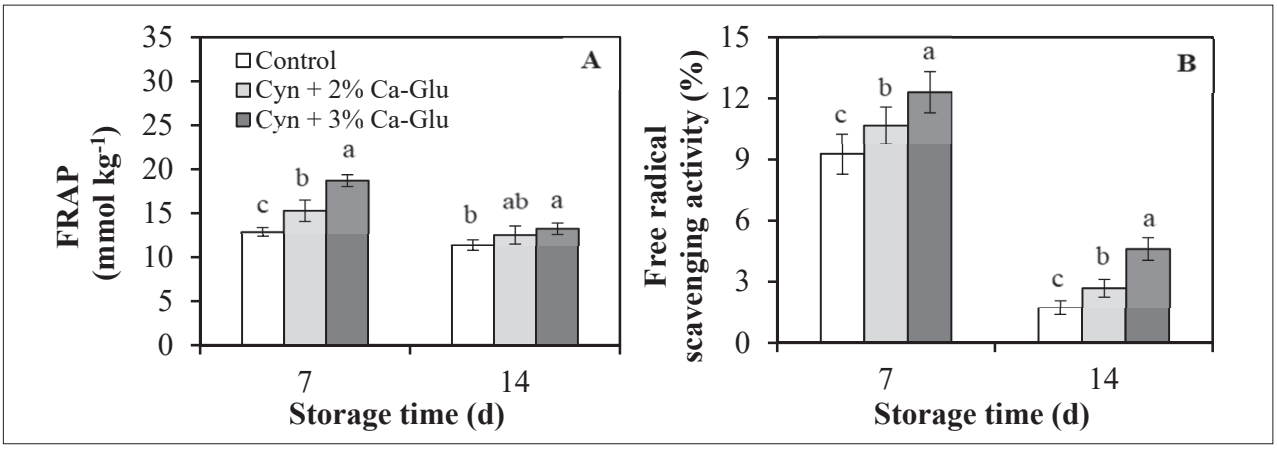

Figure 5. FRAP (A) and free radical scavenging activity (B) of tissue adjacent to the core of 'Queen' pineapples cv. 'Sawi' treated with cyanocobalamin at various concentrations during refrigerated storage at 13 °C for 14 d. The vertical bar shows the standard deviation of mean ($n = 10$). Different letters within the same figure of each day represent significant difference at $P < 0.05$.

Antioxidant activities

The effects of simultaneous Cyn and Ca-Glu peduncle infiltration on antioxidant activities in tissue adjacent to the core of 'Queen' pineapples were shown in Figure 5. Both of the simultaneous Cyn and Ca-Glu treated pineapples had FRAP and free radical scavenging activity being evidently higher than control fruit over the storage. After cold storage for 7 d, FRAP and free radical scavenging activity of Cyn + 3% Ca-Glu treated pineapples were significantly higher than those of Cyn + 3% Ca-Glu treated and control pineapples, respectively ($P < 0.05$). The evident reduction of FRAP and free radical scavenging activity were observed in all treatments after cold storage for 14 d. The FRAP and free radical scavenging activity of Cyn + 3% Ca-Glu treated pineapples were also obviously higher than those of other pineapples. Meanwhile, the lowest levels of FRAP and free radical scavenging activity were found in control pineapples. These indicated that the simultaneous Cyn and Ca-Glu peduncle infiltration could enhance as well as maintain both antioxidant activities. Previous works had reported that Cyn enhanced antioxidant activity in mangoes (Samaan *et al*., 2011) and baby vegetables (Supapvanich *et al*., 2020). Youryon *et al*. (2018) reported that Ca-Glu peduncle infiltration enhanced antioxidant activity including antioxidant enzymes activities in tissue adjacent to the core of pineapples during cold storage. In the recent results, we found that the simultaneous Cyn and Ca-Glu treatment might have synergistic effects stimulating antioxidant system against oxidative chilling stress of 'Queen' pineapples during cold storage.

Conclusion

IB caused by CI of 'Queen' pineapple could alleviate by using Cyn peduncle infiltration. From the preliminary study, 5 µM Cyn could alleviate CI due to inhibit the loss of membrane integrity, to enhance antioxidant activity and to reduce increased IB incidence of tissue adjacent to the core of pineapples. The simultaneous Cyn and Ca-Glu peduncle infiltration exhibited the effectiveness alleviating CI of 'Queen' pineapples during storage, especially Cyn + 3% Ca-Glu peduncle infiltration. Both simultaneous Cyn and Ca-Glu peduncle infiltrations reduced IB and enzymatic browning reaction, maintained membrane integrity due to the reduction of membrane lipids peroxidation, and induced antioxidant system in tissue adjacent to the core of pineapples. In conclusion, the simultaneous Cyn and Ca-Glu peduncle infiltration is an alternative approach alleviating CI of 'Queen' pineapples during commercial cold storage.

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