#### Full Length Research Paper

# Effects of wax treatment on quality and postharvest physiology of pineapple fruit in cold storage

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Pineapple (*Ananas comosus* (L.) Merr.) is an important fruit crop grown in tropical and subtropical areas. Cold storage is one of methods for prolonging postharvest storage life for pineapple fruit. A major problem of this method is that low temperature causes chilling injury symptoms in fruits and deterioration of their quality and nutritional values. This study aimed to find a new way to effectively alleviate chilling injury and maintain fruit quality during cold storage. Thus, the effects of two types of waxing treatment (Sta-Fresh 2952 wax and Sta-Fresh 7055 wax) of pineapple fruits of cultivar Paris' (a major cultivar for pineapple production) on alleviating chilling injury and their physiological responses during cold storage were examined. Sta-Fresh 2952 wax (60 g/l) was more effective in: (1) alleviating chilling injury, which delayed the changes in firmness, flesh color, weight loss and soluble protein content; (2) decreasing titratable acidity, total soluble solids, cell membrane permeability and malondialdehyde content when compared with those in control. This waxing also improved total sugars and the contents of ascorbic acid in pineapple fruits. These results suggested that this treatment might be a useful technique to alleviate chilling injury and maintain fruit quality during cold storage.

**Key words:** Pineapple, wax treatment, chilling injury, quality.

#### INTRODUCTION

Pineapple (*Ananas comosus* (L.) Merr.) is an important fruit crop grown in many tropical and subtropical countries. Fresh pineapple fruit is perishable (Chen and Paull, 2001; Avallone et al., 2003; Soares et al., 2005; Wilsonwijeratnam et al., 2005; Ko et al., 2006) and cold storage is the main method to slow the product deterioration in terms of consumer perception and nutritional value (Zhang et al., 2009; Cantín et al., 2010). However, low temperature results in chilling injury symptoms in pineapple fruits during or after cold storage (Selvarajah et al., 2001; Zhou et al., 2003). Chilling injury hygrophanous

flesh in pineapple fruits and is the major is manifested as internal browning (black heart) and postharvest limitation for the pineapple industry, as fruits rapidly develop blackheart following low temperature, which severely restricts refrigerated seafreight export (Selvarajah et al., 2001). Thus, postharvest treatments that can alleviate chilling injury and extend shelf-life of pineapple fruits are urgently needed.

The development of effective methods to alleviate chilling injury in pineapple fruits has been widely reported and these include heat treatment and controlled atmosphere (Wilsonwijeratnam et al., 2005; Nimitkeatkai et al., 2006). However, these methods do not eliminate significantly chilling injury. At present, wax (edible coatings) have been used as an effective technology to increase the quality of postharvest fruits and vegetables (Qiuping and Wenshui, 2007; Fan et al., 2009; Tzoumaki et al., 2009; Tietel et al., 2010). Coatings could effectively retard the loss of the water, titratable acidity and ascorbic acid of sweet cherries (Dang et al., 2010). Waxing could improve firmness, titratable acidity, ascorbic acidity and

**Abbreviations: CI,** Chilling injury index; **AsA**, ascorbic acid; **RH**, relative humidity; **MDA**, malondialdehyde; **TBA**, thiobarbituric acid; **TCA**, trichloroacetic acid; **TSS**, total soluble solid; **TA**, titratable acidity.

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the water content for Murcott tangor stored at 15 °C for 56 days (Chien et al., 2007). Waxing, acting as semipermeable barriers, may be an effective method to alleviate chilling injury (Meng et al., 2008; Ahmed et al., 2009). However, only two studies reported the application of waxing of pineapple fruits during cold storage, with emphasis on chilling injury symptoms, but less attention to coating-induced quality alterations and their physiological responses in the fruits (Paul and Rohrbach, 1985; Wijeratnam et al., 2006).

Meanwhile, the main pineapple cultivar grown in the world is 'Smooth Cayenne', and in China, the most popular cultivar is 'Comte de Paris', accounting for more than 80% of the total pineapple production (Li et al., 2011). However, few data exist extending storage life of the cultivar 'Comte de Paris'. The objective of this study was to find a new method for extending storage life of pineapple cultivar 'Comte de Paris' and to investigate the method of how to alleviate chilling injury and maintain fruit quality during cold storage. Thus, this study was performed to comprehensively investigate the effects of application of a novel wax formulation on the changes in several parameters related to quality and life extend of pineapple fruits (weight loss, firmness, color saturation, total soluble solids (TSS), soluble sugars, titratable acidity (TA), ascorbic acid (AsA) and soluble proteins) and on their physiological activities including the changes in relative leakage rate, malondialdehyde (MDA) and membrane integrity in response to cold storage of the chilling sensitive pineapple fruits. The results obtained from this study indicated that Sta-Fresh 2952 wax treatment is more effective in alleviating chilling injury and in maintaining the quality of these pineapple fruits.

#### **MATERIALS AND METHODS**

#### Plant material and experimental design

Pineapple fruits (A. comosus cv. Comte de Paris) (at about 3 matured stages) were selected on the basis of the uniformity of the color and size from a commercial grower in Zhanjiang, China. The stage of ripeness was determined by visual assessment of the shell (Selvarajah et al., 2001). The scale ranges from 0 to 5: 0, all eyes are totally green; 1, < 20% of the eyes are predominantly yellow; 2, 20 to 40% of the eyes are tinged with yellow; 3, up to 65% of the eyes are predominantly yellow; 4, 65 to 90% of the eyes are fully yellow; 5, > 90% of the eyes are fully yellow and no more than 20% of the eyes are reddish orange. All fruits were cleaned and soaked in 0.05% (w/v) Iprodione solution (Kuaida, Jiangsu, China) for 2 min to eliminate potential microbes. Afterwards, the treated fruits were divided into seven groups; each group (10 fruits) was placed in a clean plastic box. The fruits of one group were dipped in water (as control). The fruits of six groups were respectively treated with Sta-Fresh 2952 (FMC) wax solution at 30, 60 and 90 g/l and Sta-Fresh 7055 (FMC) wax solutions at 40, 80 and 120 g/l. After being air dried, the samples were placed in polyethylene bags (0.04 mm), stored at 90% relative humidity (RH) and 7℃ for 21 day and transferred to 25°C for 3 day to simulate shelf conditions for chilling injury and quality evaluation. Five fruits from each box were randomly sampled to determine quality characteristics of fruits after storage periods and then the optimal treatment condition was chosen.

Forty (40) fruits were treated with the optimal treatment and 40 fruits were dipped in water (as control). All fruits were stored at  $7\,^{\circ}\mathrm{C}$  and some samples were taken at intervals. After 21 day storage at  $7\,^{\circ}\mathrm{C}$ , the residual samples were transferred to 25\,^{\circ}\mathrm{C} for three days storage. Five fruits from each box were randomly sampled every 7 days in cold storage and each day in room temperature (25\,^{\circ}\mathrm{C}) storage to determine physical and biochemical changes during storage periods.

#### Chilling injury index (CI) determination

The fruits were cut longitudinally in half and the CI was determined. For each fruit, CI intensity was scored from 0 to 5 according to the percentage of flesh affected (0, free from CI; 1 to 5: 10, 10 to 25, 25 to 50, 50 to 75 and >75% of the flesh discolored, respectively) (Selvarajah et al., 2001). The average CI was calculated for each group of fruit.

#### **Firmness**

Fruit firmness was measured at six equational regions of the flesh of each fruit at different storage times, using a penetrometer (Instron5542) fitted with a 8 mm diameterflat probe and results were expressed as N.

#### Color assessment

Readings with colorimeter were randomly taken at six different locations on each pineapple fruit, using a total of five fruits from each group. Color factors Chroma (C) and Hue angle (H) values of each fruit were directly read with a CR-300 colorimeter (Minolta, Ramsey Corp. NY) (Nunes et al., 1995).

#### Soluble sugar and titratable acidity

Soluble sugar was measured by phenol-sulfuric acid method described previously (Masuko et al., 2005). Titratable acidity was determined by titrating fruit juice to pH 8.1 with 0.1 mol  $\Gamma^1$  NaOH. The results were expressed as mmol of hydrogenion concentration per 100 g of fresh weight (Jin et al., 2009).

#### Total soluble solid

Total soluble solid in the extracted juice of fruits was measured by a refractometer (ATAGO (Brix = 0 to 32%)) and the results were expressed as %Brix (Cheour et al., 1991).

#### Ascorbic acid

The ascorbic acid of pineapple fruits was measured by 2, 6-dichlorophenolindophenol titration according to the China National Analysis Standard (GB/T 6195-1986). Briefly, flesh (10 g) from each fruit was immediately homogenized in 50 ml of 0.02 g/ml oxalic acid solution and then centrifuged at  $15000 \times g$  at  $4 \, ^{\circ}$ C for 15 min. 10 ml supernatant was titrated to a permanent pink color with 0.1% 2, 6-dichlorophenolindophenol titration. Ascorbic acid concentration was calculated according to the titration volume of 2, 6-dichlorophenolindophenol and expressed as milligram per ml.

#### Weight loss

Fruit weight was measured just after harvest and at the different

**Table 1.** Effects of different wax treatments on chilling injury index, firmness, color saturation, contents of soluble sugars and titratable acidity of pineapple fruits after 21 day storage at 7 °C followed 3 day storage at 25 °C.

Treatment type (g/l)	Chilling index	Firmness (N)	Color saturation (Chroma)	Soluble sugar (µg.g <sup>-1</sup> )	Titratable acidity (mmol.100 g <sup>-1</sup> )
Control	3.8 <sup>a</sup>	14.61 <sup>c</sup>	24.89 <sup>b</sup>	94.45 <sup>b</sup>	13.19 <sup>a</sup>
Sta-Fresh 2952 (30)	2.3 <sup>b</sup>	18.22 <sup>a</sup>	28.07 <sup>a</sup>	95.73 <sup>ab</sup>	11.92 <sup>b</sup>
Sta-Fresh 2952 (60)	1.7 <sup>c</sup>	17.39 <sup>ab</sup>	29.20 <sup>a</sup>	98.07 <sup>a</sup>	10.04 <sup>c</sup>
Sta-Fresh 2952 (90)	2.1 <sup>bc</sup>	15.55 <sup>bc</sup>	28.02 <sup>a</sup>	97.10 <sup>ab</sup>	10.12 <sup>c</sup>
Sta-Fresh 7055 (40)	2.6 <sup>b</sup>	16.45 <sup>bc</sup>	26.56 <sup>ab</sup>	100.44 <sup>a</sup>	12.53 <sup>ab</sup>
Sta-Fresh 7055 (80)	2.5 <sup>b</sup>	18.30 <sup>a</sup>	26.22 <sup>ab</sup>	81.12 <sup>c</sup>	10.72 <sup>c</sup>
Sta-Fresh 7055 (120)	2.7 <sup>b</sup>	18.33ª	28.67 <sup>a</sup>	91.57 <sup>b</sup>	10.34 <sup>c</sup>

Means in the same column followed by different letters differs (P < 0.05) according to the least significant difference multiple range test.

sampling dates. The result was expressed as percentage of weight loss relative to the initial value (taken as 100%) (Sato-Zamora et al., 2005).

#### Relative leakage rate and malondialdehyde (MDA)

Membrane permeability, expressed by relative leakage rate was determined according to the method of Zheng and Tian (2006). MDA content was determined by the thiobarbituric acid (TBA) reaction (Dipierro and Leonardis, 1997).

#### **Protein content**

Protein content in the enzyme extracts was determined according to the Bradford (1976) method, using bovine serum albumin as a standard.

#### Statistical analysis

All data were at least repeated once and analyzed statistically by ANOVA and mean differences estimated by Duncan's new multiple range test (DMRT) (P < 0.05) using SPSS Version 16.0 (SPSS Inc., Chicago, IL, USA).

#### **RESULTS**

## Effect of different wax treatments on chilling injury index, firmness, color saturation, contents of soluble sugar and titratable acidity of pineapple fruits

After being stored at  $7^{\circ}$ C for 21 day and transferred to  $25^{\circ}$ C for 3 day, the pineapple fruits treated with wax exhibited significantly (P < 0.05) lower levels of chilling injury index (CI) than that of the control (Table 1), suggesting that wax may prevent chilling injury. The index in fruits of the  $2^{th}$  treatment was the lowest in all samples (Table 1). Titratable acidity (TA) levels in wax-treatments were lower than those in control (Table 1). There were significant differences in the levels of soluble sugar between wax-treatments and control (Table 1). Flesh firmness in wax-treatments was higher than that in control (Table 1), indicating that wax slowed down fruit softening

process as well as pectin degradation. The color saturation for the wax-treatments was significantly higher than that in control (P < 0.05) and the highest color saturation of fruits was in the  $2^{th}$  treated fruits (Table 1). Therefore, a lower chilling index in the  $2^{th}$  treated fruits was observed. The  $2^{th}$  treatment was the optimal treatment.

#### Chilling injury index

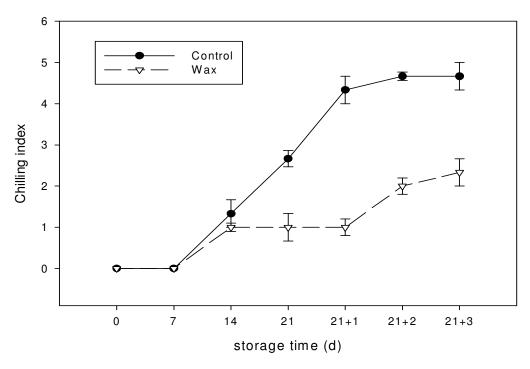
Symptoms of chilling injury (CI), including internal browning and flesh mealiness were assessed visually. As shown in Figure 1, the CI of the fruits in control was higher than that in wax-treatment and the CI of the wax-treatment was significantly lower than that in control (P < 0.05) after the  $14^{\rm th}$  day of storage. The results indicated that wax treatment could significantly decrease internal browning and flesh mealiness symptoms of pineapple fruits.

#### **Firmness**

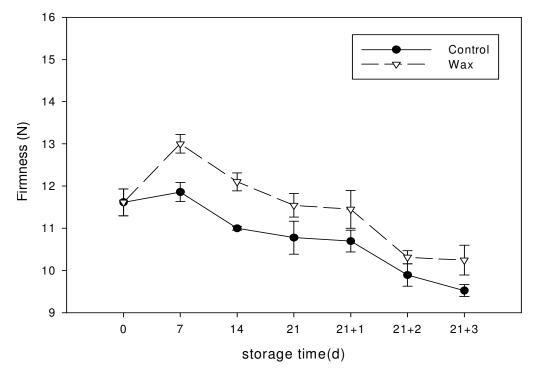
Loss of firmness is one of the main factors limiting quality and the postharvest shelf-life of fruits and vegetables. Figure 2 shows that the firmness in control fruits decreased gradually along with increased storage time. Throughout storage, the loss of firmness of control was significantly greater than that of wax-treatment (Figure 2), indicating that wax delayed the decline of firmness.

#### Color

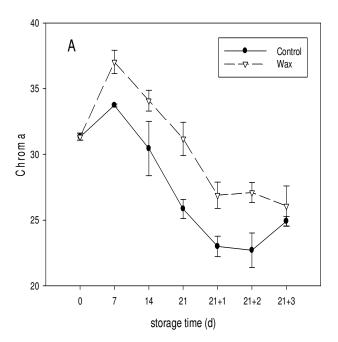
The color change of pineapple flesh can serve as an indicator of chilling injury and fruit quality. As shown in Figure 3a, during color development, the chroma increased slightly from harvest to the 7<sup>th</sup> day of storage and then decreased sharply up to the 22<sup>th</sup> day. It is clear that wax significantly reduced the decrease of the chroma during the storage period compared to that of the control



**Figure 1.** Effects of Sta-Fresh 2952 wax (60 g/l concentration) treatment on chilling index of pineapple fruits during storage at  $7^{\circ}$ C for 21 day and then transferred to 25 °C for 3 day. "0-21" represent 21 day storage at  $7^{\circ}$ C and "21+ n" (n= 1, 2, 3) mean 21 day storage at  $7^{\circ}$ C followed by n day storage at 25 °C. Values are the mean of five replicates and error bars represent the standard deviation.



**Figure 2.** Effects of Sta-Fresh 2952 wax (60 g/l concentration) treatment on firmness of pineapple fruits during storage at  $7^{\circ}$ C for 21 day and then transferred to  $25^{\circ}$ C for 3 day. "0-21" represent 21 day storage at  $7^{\circ}$ C and "21+ n" (n= 1, 2, 3) mean 21 day storage at  $7^{\circ}$ C followed by n day storage at  $25^{\circ}$ C. Values are the mean of five replicates and error bars represent the standard deviation.



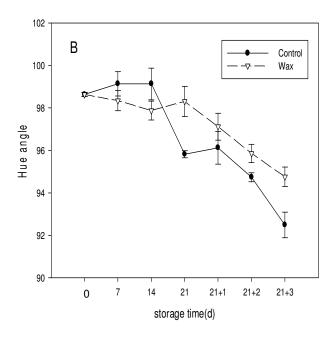


Figure 3. Effects of Sta-Fresh 2952 wax (60 g/l concentration) treatment on chroma (A) and hue angle (B) of pineapple fruits during storage at 7 °C for 21 day and then transferred to 25 °C for 3 day. "0-21" represent 21 day storage at 7 °C and "21+ n" (n= 1, 2, 3) mean 21 day storage at 7 °C followed by n day storage at 25 °C. Values are the mean of five replicates and error bars represent the standard deviation.

(P < 0.05). Chroma is the intensity or purity of the hue and it shows the brightness of fruits. Therefore, wax treatment diminished the color brightness in comparison with control.

Figure 3b shows that after 3 day of storage, hue angle was higher in wax-treatment than in control and higher hue angle showed less browning and better quality based on Nunes et al. (1995), who reported less browning in fruits with higher hue angle. The effect of wax may be due to decreased respiration rate, which prevents fruit senescence during storage.

#### Changes in soluble sugars and titratable acidity (TA)

As shown in Figure 4a, levels of soluble sugars of pineapple fruits were significantly affected by waxing, with wax-treatment having higher levels of sugars than control during most of days of storage. The soluble sugar content was increased in wax-treatment by 13.94 and 29.32%, respectively at the 21<sup>th</sup> and the 24<sup>th</sup> day of storage, when compared with that in control.

For TA, a steady increase was observed in both control and wax-treatment during cold storage time. After 21 day of storage, TA contents of all fruits decreased at the later storage time (Figure 4b). The wax treatment reduced TA by approximately 6 and 5% compared with the control at 14 and 21 day, respectively. These results indicated that wax treatment could reduce TA content of fruits in cold storage.

#### Changes in total soluble solid (TSS)

As shown in Figure 5, TSS of the control rapidly increased on day 22 and then fell toward the end of storage period. The changes in TSS content were more slowly in wax-treatment than in control. The wax-treatment tends to maintain significantly (P < 0.05) lower levels of TSS than compared with that in the control during most days of storage (days 14, 21, 22 and 24).

#### Ascorbic acid (AsA)

As shown in Figure 6, the AsA content in the control decreased during the first 14 days and increased gradually in the 3<sup>th</sup> week and then decreased again in the last three days of storage. The AsA content of wax-treatment decreased during the first 22 days of storage and increased gradually with prolonged storage time. Throughout the storage period, there were significant differences between control and wax-treatment (P < 0.05). The AsA content in wax-treatment was 146% higher than that in the control on the 24<sup>th</sup> day of storage.

#### Changes in weight loss

Fruit weight loss is mainly associated with respiration and moisture evaporation through the skin. Coatings act as barriers, thereby restricting water transfer and protecting

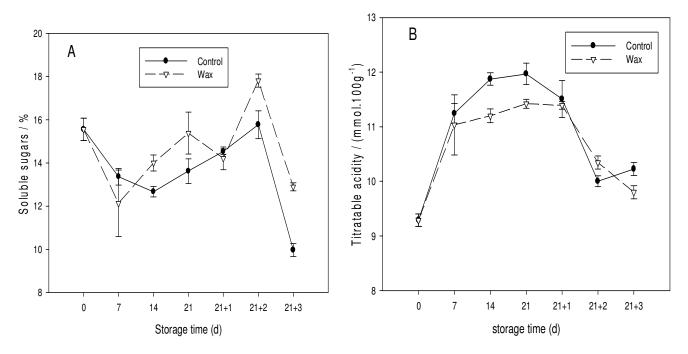
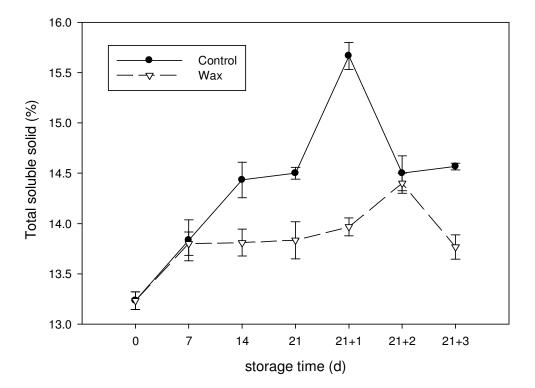
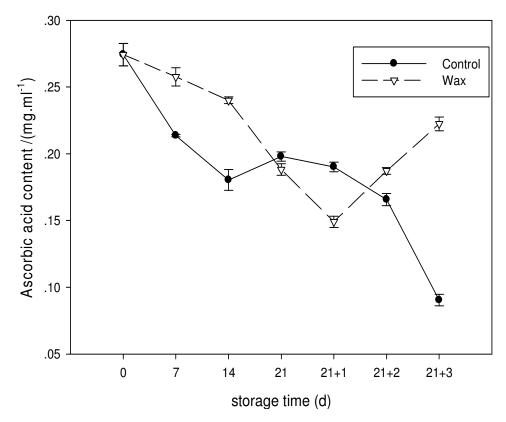


Figure 4. Effects of Sta-Fresh 2952 wax (60 g/l concentration) treatment on soluble sugars (A) and titratable acidity (B) of pineapple fruits during storage at  $7^{\circ}$ C for 21 day and then transferred to 25  $^{\circ}$ C for 3 day. "0-21" represent 21 day storage at  $7^{\circ}$ C and "21+ n" (n= 1, 2, 3) mean 21 day storage at  $7^{\circ}$ C followed by n day storage at 25  $^{\circ}$ C. Values are the mean of five replicates and error bars represent the standard deviation.



**Figure 5.** Effects of Sta-Fresh 2952 wax (60 g/l concentration) treatment on total soluble solid of pineapple fruits during storage at  $7^{\circ}$ C for 21 day and then transferred to 25  $^{\circ}$ C for 3 day. "0-21" represent 21 day storage at  $7^{\circ}$ C and "21+ n" (n= 1, 2, 3) mean 21 day storage at  $7^{\circ}$ C followed by n day storage at 25  $^{\circ}$ C. Values are the mean of five replicates and error bars represent the standard deviation.



**Figure 6.** Effects of Sta-Fresh 2952 wax (60 g/l concentration) treatment on ascorbic acid of pineapple fruits during storage at  $7^{\circ}$ C for 21 day and then transferred to  $25^{\circ}$ C for 3 day. "0-21" represent 21 day storage at  $7^{\circ}$ C and "21+ n" (n= 1, 2, 3) mean 21 day storage at  $7^{\circ}$ C followed by n day storage at  $25^{\circ}$ C. Values are the mean of five replicates and error bars represent the standard deviation.

fruit skin from mechanical injuries, as well as sealing small wounds and thus, delaying dehydration (Hernandezmunoz et al., 2008). As shown in Figure 7, while the weight losses of both control and wax-treatment increased continuously with storage time (P < 0.05), the weight loss of control was significantly greater than that of wax-treatment on the  $7^{th}$  and  $14^{th}$  day of storage. At the end of the storage, the control showed 3.1% loss in weight, whereas the weight losses of the wax-treatment were 2.6%.

## Changes in relative leakage rate and malondialdehyde (MDA) content

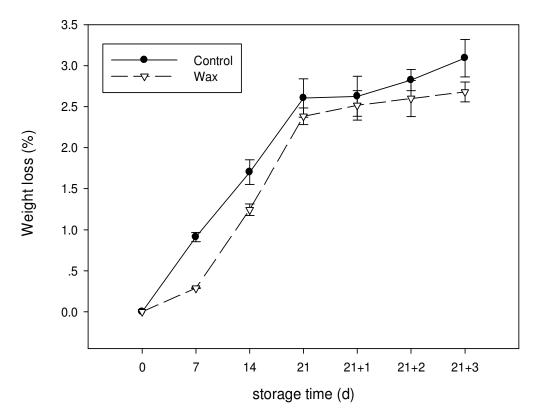
Relative leakage rate has been used an indicator of injury degree of fruits (Sun et al., 2010). As shown in Figure 8a, the relative leakage rate of both control and wax-treatment was increased continuously during the first 21 days of storage and then decreased continuously during the last 3 days of storage. After 21 days of storage, the relative leakage rate of all samples decreased, which suggested that pineapple fruits might repair the injury of cell membrane by themselves. The wax-treated fruits

exhibited significantly (P < 0.05) lower levels of relative leakage rate compared with that in the control during the first 23 days of storage.

As shown in Figure 8b, MDA content in both control and wax-treatment showed the same trend of relative leakage rate during the first 21 days of storage and then decreased during the last 3 days of storage. Wax coatings significantly reduced the MDA content of pineapple fruits during the cold storage period compared with the control (P < 0.05).

#### Soluble protein content

Soluble protein content in both control and wax-treatment decreased gradually during the whole storage period (Figure 9). The control had a significant decrease in soluble protein content after 21 day storage at 7°C followed by 1 day storage at 25°C, however, the soluble protein content of wax-treatment decreased slightly on the same day (Figure 9). Wax treatment markedly inhibited the decline of soluble proteins, thus, maintaining significantly (P < 0.05) higher levels of soluble protein throughout the storage period compared with the control



**Figure 7.** Effects of Sta-Fresh 2952 wax (60 g/l concentration) treatment on percentage weight loss of pineapple fruits during storage at 7°C for 21 day and then transferred to 25°C for 3 day. "0-21" represent 21 day storage at 7°C and "21+ n" (n= 1, 2, 3) mean 21 day storage at 7°C followed by n day storage at 25°C. Values are the mean of five replicates and error bars represent the standard deviation.

(Figure 9).

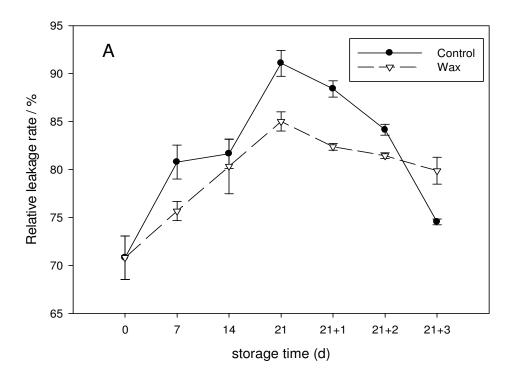
#### **DISCUSSION**

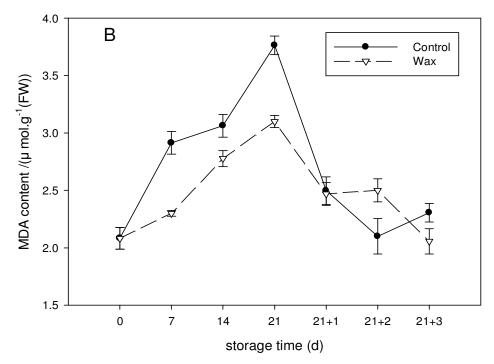
## Effects of different wax treatments on quality of pineapple fruits in cold storage

The application of wax effectively maintained quality attributes and extended postharvest life of some fruits (Baldwin et al., 1999; Hernandezmunoz et al., 2008; Dang et al., 2010). This study examined the effects of two different types of wax treatment on the quality and physiological changes of harvested pineapple fruits. The results showed that both types of wax treatment can effectively alleviate chilling injury of pineapple fruits and improve their quality after cold storage and it is in agreement with those reported in an earlier study, which has shown that fruits treated with chitosan scored superior quality compared with untreated fruits after cold storage (Wijeratnam et al., 2006). The chilling injury indices of fruits are significantly affected by wax treatment, with wax-treated fruits having a lower index than that of the control. Reuck et al. (2009) have also

reported that coating was effective in alleviating chilling injury of litchi fruit. Wax treatment kept firmness of pineapple fruits in a higher level than that in the control and this is similar to that observed by Martinezromero (2005). Fruit acidity and sweetness are two major factors that determine eating taste and quality of pineapple fruits. Wax treatment decreased significantly TA content of fruits, while it increased soluble sugars content after cold storage. This observation is similar to that reported by Sun et al. (2010), who observed that litchi fruits treated with chitosan coating tend to maintain significantly higher levels of sugars compared with the control fruits. Meanwhile, Eum et al. (2009) reported that the coatings of plum fruits slowed the TA changes. Together, these observations indicated that wax treatment not only could alleviate chilling injury, but also improve quality of pineapple in cold storage.

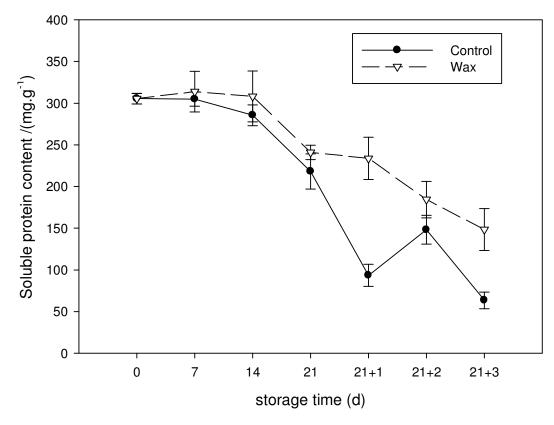
The effects of wax on fruits are associated with the source and concentration of wax, mode of wax application, types of fruits and storage conditions (Hernandezmunoz et al., 2008). The results presented herein indicated that Sta-Fresh 2952 wax treatment (60 g/l concentration) was more effective than the other treatments in alleviating chilling injury index and retarding





**Figure 8.** Effects of Sta-Fresh 2952 wax (60 g/l concentration) treatment on relative leakage rate (A) and MDA content (B) of pineapple fruits during storage at 7°C for 21 day and then transferred to 25°C for 3 day. "0-21" represent 21 day storage at 7°C and "21+ n" (n= 1, 2, 3) mean 21 day storage at 7°C followed by n day storage at 25°C. Values are the mean of five replicates and error bars represent the standard deviation.

change of color saturation, the most important factor related to chilling injury in pineapple fruits. Similarly, other studies also observed that the beneficial effects of wax on other types of fruits were enhanced with increasing the wax concentrations (Hernandezmunoz et al., 2008; Zhu et al., 2008).



**Figure 9.** Effects of Sta-Fresh 2952 wax (60 g/l concentration) treatment on soluble protein content of pineapple fruits during storage at  $7^{\circ}$ C for 21 day and then transferred to  $25^{\circ}$ C for 3 day. "0-21" represent 21 day storage at  $7^{\circ}$ C and "21+ n" (n= 1, 2, 3) mean 21 day storage at  $7^{\circ}$ C followed by n day storage at  $25^{\circ}$ C. Values are the mean of five replicates and error bars represent the standard deviation.

### Effect of wax on physiological responses of pineapple in cold storage

The second important observation made in this study is that wax treatment can maintain the cell membrane integrity of harvested pineapple. Cell membrane is the first barrier that separates cells from their environment and also is a primary target for damage (Cantin et al., 2010). Maintenance of membrane integrity at low temperature has been reported to be important in the resistance to chilling temperature (Antunes and Sfakiotakis, 2008; Wongsheree et al., 2009). It has been reported that the relative electrolyte leakage and MDA content, the proven excellent indicators of cell membrane damage, were gradually increased in fruits with storage time (Cantin et al., 2010; Rui et al., 2010). The results presented herein have shown that the increases in electrolyte leakage and MDA content during cold storage, which indicate the membrane deterioration in pineapple fruits under low temperature stress, were effectively inhibited by wax treatment. Cao et al. (2009) reported that the development of CI in loquat fruits was accompanied by loss of membrane integrity, increases in electrolyte leakage and MDA content. Also, enhanced tolerance to CI by treatment with 1-methylcyclopropene or with hot water was associated with the inhibition of membrane deterioration. The electrolyte leakage and MDA content of all samples decreased gradually after cold storage, suggesting that membrane integrity of pineapple fruits were recovered at room temperature. Similarly, Tsantili et al. (2010) reported that increases in total antioxidant activity that could be at least partially attributed to synthesis of anthocyanins occurred more or less concomitantly with an apparent juice recovery at elevated temperature.

The decrease in AsA level was associated with a reduced capability of preventing oxidative damage and with the incidence of physiological disorders during storage (Lin et al., 2008). Wax treatment kept AsA in a higher level in wax-treat fruits than in control, similar to that reported by Dang et al. (2010), who found that the content of AsA of CA-coated fruits was higher than that of the control. Furthermore, soluble protein levels were higher in wax-treatment than in control. Increasing the soluble proteins in plant could bound more moisture and reduce osmotic stress injury in adversity (Heuer, 2003). This may account for the less weight loss in wax-treatment as compared to the control.

In summary, this study indicates that wax treatment of pineapple fruits could be a potentially useful method to alleviate chilling injury associated with the major changes of cell membrane as indicated by altering electrolyte leakage, MDA content and AsA and to maintain their quality by retarding dehydration, providing a selective barrier to moisture loss and gas exchange, which lead to reduction of general metabolism rate, improvement of textural quality and maintenance of fruit quality.

The maintenance of quality and the extension of shelf life of pineapple fruits by waxing during cold storage demonstrated herein suggest that Sta-Fresh 2952 wax could be a potential application as a new effective method for commercial storage and marketing of pineapple fruits and perhaps, other types of fruits.

#### **ACKNOWLEDGEMENTS**

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