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Development of internal browning during low temperature storage of pineapple cv. 'Trad-Srithong' fruit harvested at different times of the day

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Abstract

Pineapple plants cv. 'Trad-Srithong' (*Ananas comosus* L.) were found to exhibit crassulacean acid metabolism (CAM). The concentrations of organic acids were higher in mature crown and stem leaves harvested at 0600 than at 1200 h but there were no significant differences in acid concentrations among fruit harvested at these times. Fruit of 'Trad-Srithong' (Queen group) are highly sensitive to internal browning (IB), a form of chilling injury, when stored at <15 °C. Fruit were harvested at 0600 and 1200 h and stored at 8, 13 and 20 °C. IB developed in fruit stored at 8 and 13 °C after 10 days but no symptom developed in control fruit stored at 20 °C. Time of day, when the fruit were harvested had no effect on the development of IB in 'Trad-Srithong' fruit. TA increased in fruit during storage at all temperatures. Juice extracted from the pulp had higher TA and lower pH than the core tissue and symptoms of IB were more severe in the flesh surrounding the core. Ascorbic acid decreased late during storage period.

Key words: *Ananas comosus,* CAM plant, fruit acidity, harvesting time, black heart

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Introduction

Pineapples, one of the most important commercial fruits of Thailand, are consumed either fresh or processed. There is a world wide market for fresh pineapples but access to these markets is limited by their short storage life as the fruit are susceptible to fungal infections, development of internal browning (IB) and off flavours (Rohrbach and Johnson, 2003). IB is a form of chilling injury that develops when the fruit are stored at <15 °C (Nukulthornprakit and Siripanich, 2005; Youryon *et al.,* 2008). 'Trad-Srithong' pineapple (Queen group), popular for consumption as fresh fruit in Thailand, is more susceptible to IB than the commonly grown smooth cayenne cultivar (Nukulthornprakit and Siripanich, 2005; Weerahewa and Adikaram, 2005). IB develops initially in the flesh (pulp) around the core of pineapple fruit (Youryon *et al.,* 2008).

Pineapple is a member of the Bromeliaceae, which exhibit varying degrees of intensity of crassulacean acid metabolism (CAM). The stomata of mature leaves of CAM plants open at night and close during most of the day. Closing the stomata during the day minimizes water loss, but since H_2O vapour and CO_2 share the same diffusion pathway, then CO_2 must be taken up at night (Taiz and Zeiger, 1998). CAM plants fix CO_2 at night through β–carboxylation of phosphoenolpyruvate into organic acids, which are stored in the cell vacuole until the next day. Organic acids are removed from vacuoles during the day, decarboxylated, and the CO_2 released is incorporated into carbohydrates via the Calvin (C_3) pathway of photosynthesis (Keeley, 1981). Acid concentrations in pineapple leaves reach a high level about 2-4 h before the end of the dark period (Friend and Lydon, 1979). Titratable acidity (TA) gradually increases in pineapple fruit during development until maturity (Saradhuldhat and Paull, 2007). Pineapple fruit have a cluster of leaves on the top of the fruit called the 'crown' leaves, which may also accumulate acids at night. It is recommended practice to harvest fruits in early morning when fruit temperatures are low. However, if pineapple fruit also exhibit CAM metabolism then fruit acidity may be higher in the morning when the fruit are normally harvested. The aim of this research was to determine whether harvesting pineapples in early morning or at noon affects acid concentrations in the fruit and susceptibility to IB during low temperature storage.

Materials and methods

Plant materials and storage conditions: 'Trad-Srithong' pineapple fruit, from a commercial farm in Chonburi province in Eastern Thailand (13 °N, 100 °E and altitude 300 m) were harvested in December 2006 at commercial maturity (two rows of the fruitlets turning yellow). Fruits (1-1.2 kg) were harvested at 0600 and 1200 h. The crown leaves were removed and the peduncles were trimmed. Fruit were packed in black boxes and transported to the Postharvest Technology laboratory at King Mongkut's University of Technology Thonburi (PHT-KMUTT), Bangkok in a refrigerated truck at 25 °C (about 2 h). Fruit were placed in plastic baskets and stored at 8, 13, and 20 °C with 80-90% RH. IB was assessed in four fruit from each harvest x storage treatment at five-day intervals.

A supplementary experiment was conducted in February 2010 to confirm our findings in December 2006 that acid concentrations

fluctuate diurnally in crown and stem leaves but not in the fruit. Leaves and fruit were harvested at 0600 and 1200. The third leaves from the top of 5-7 stem shoots and crowns were collected and placed on ice at harvest pending measurement of titratable acidity (TA). Some fruits at harvest were cut in half longitudinally and juice was extracted from one half for measurement of TA. The remaining half fruits were placed in ice pending measurement of TA after transport to PHT-KMUTT. Other whole fruit were transported to PHT-KMUTT in a refrigerated truck at 25 °C.

Evaluation of internal browning (IB): Stored fruit were cut longitudinally and the severity of symptoms of IB was assessed according to the area of the cut surface affected (Teisson, 1979); 0 (no symptoms), 1 (<10 %), 2 (10-25 %), 3 (25-50 %), 4 (50-75%), 5 (> 75 %).

Analyses of pH, TA, total soluble solids (TSS) and ascorbic acid (AA): TA was measured in stem and crown leaves and fruit that were placed in ice immediately after harvest and in fruit during storage. Samples were cut from the middle of the leaf tissue (1 g), boiled in 30 mL of distilled water for 10 min, and then homogenised with an IKA Ultra-Turrax T25 (Germany). The homogenates were filtered with No.1 Whatman paper (adapted from Ota and Yamamoto, 1991; Nievola *et al.,* 2005). These water extracts were titrated with 0.1 N NaOH to an end point of pH 8.1 using an auto-titrator (Automatic titrator AUT-501 TOA, Japan). Juice was squeezed from the core tissue and from the flesh (pulp) tissue at the middle of the fruit for the determination of pH, TA, TSS, and AA from four replications (1 fruit/ rep). pH of the juice was measured using a Hanna pH 211 meter. TA was measured by titrating aliquots of juice with 0.1 N NaOH to an end point of pH 8.1 using an auto-titrator. The data are reported as percentages of malic acid. TSS was measured using a refractometer (pocket refractometer PAL-1, Japan) standardised at 20 °C.

Ascorbic acid (AA) was measured according to A.O.A.C. (1984). Five mL of metaphosphoric acid were mixed with 2 mL of fruit juice and then titrated with 2, 6-dicloroindophenol to the end point (distinct rose pink for ≥5s). Concentrations of AA in pineapple tissue were calculated as follows:

mg AA/mg = (X-B) x ((F/E) x (V/Y)

Where,
X = average volume (mL) used for sample titration,
B = average volume (mL) used for sample blank titration,
F = mg AA equivalent to 1.0 mL of indophenol standard solution, E = number of g mL, V = volume (mL) of initial assay solution,
Y = volume of sample aliquot titrated.

Statistical analysis: The data were subjected to ANOVA and the means were compared using least significant differences (LSD). Statistical analyses were conducted using SPSS Version 17.

Results and discussion

In the first experiment conducted in December 2006, organic acid concentrations were measured only in fruit harvested at 0600 and 1200 and since no significant differences were found among fruit harvested at these times doubts were raised whether plants of cv. 'Trad-Srithong' exhibit CAM. The supplementary experiment in February 2010 confirmed that this cultivar does express CAM in mature crown and stem leaves but not in fruit. Harvested stem and crown leaves had higher TA at 0600 than at 1200 but TA did not vary significantly among fruit harvested at these times (Table 1). It has been reported that organic acid concentrations in pineapple leaves reach a minimum about 2 h before the end of the light period and a maximum about 2 h before the end of the dark period (Friend and Lydon, 1997). However, as reviewed by Ritchie and Bunthawin (2010), pineapple is a facultative CAM plant and the level of expression of CAM varies between different cultivars and is also affected by the water status of the plants. The lack of an effect of harvest time on TA in fruit shows that CAM does not operate in the flesh of pineapple fruit and that organic acids are not translocated from the leaves to the fruit. The absence of CAM in the core and adjacent pulp tissue of the fruit is not surprising since chloroplasts and chlorophyll are confined to skin tissue of the individual fruitlets of this composite fruit.

Table 1. Effects of time of harvesting on TA (% malic acid) in fruit and leaves of 'Trad-Srithong' pineapple, February 2010

Treatment	Fruit at harvest	Same fruit after transport at 0 °C	Fruit after transport at 25 °C	F-test
Harvesting time (H)[1]				
0600 h	0.4±0.49	0.3±0.05	0.4±0.05	ns
1200 h	0.3±0.05	0.3±0.05	0.3±0.05	ns
F- test	ns	ns	ns	
Fruit part (F) [1]				
Core	0.2±0.01A	0.1±0.01B	0.2±0.02A	*
Pulp	0.3±0.02	0.5±0.02	0.5±0.02	ns
F- test	**	**	**	
H*F	ns	ns	ns	

Harvesting time (H) [1]	Stem leaves	Crown leaves	F-test
0600 h	1.0±0.04	0.2±0.04	**
1200 h	0.4±0.07	0.1±0.03	**
F- test	**	**	

[1] Means with different capital letters within the same row are significantly different.

ns: not significant. * Significantly different at $P \leq 0.05$. ** Significantly different at $P \leq 0.01$

In the December 2006 study, TA increased in fruit from both harvest times during storage, both in the core and the pulp tissue (Table 2). Increased TA was associated with a decrease in TSS, but pineapples harvested at 0600 apparently contained higher levels of TSS during storage (Table 3). The highest TA concentrations were measured in the pulp. Generally, TA decreases in harvested fruit of most species but TA was found to increase in stored pineapple fruit. This suggests that the fruit tissue assimilates CO_2 released by respiration, thus explaining the increase in TA and the decrease in TSS during storage. An increase in TA in limes during storage at low temperatures has been reported although lime is not a CAM plant (Ziena, 2000; Win *et al.,* 2006). Changes in pH in stored pineapples broadly paralleled the changes in TA with the fruit becoming more acidic during storage at all temperatures (Table 4).

Ascorbate content was higher in fruit harvested at 1200 than at 0600 (Table 5). With the exception of data for 10 days of storage there was a gradual decrease in AA in both core and pulp tissue. There were no significant differences in AA between core and pulp tissue (Table 5). IB did not develop in fruit stored at 20 °C but was first detected in fruit stored at 8 and 13 °C for 10 days and became more severe in fruit stored for 15 and 20 days. Fruit

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stored at 20 °C for 20 days deteriorated and turned unsaleable (over stored). However, harvest time had no effect on the severity of IB (Table 6). There was a large decrease in AA after 20 days of storage at 13 °C (Table 5). This may have been associated with the development of more severe IB in these fruit (Table 6). IB symptoms that developed in 'Trad-Srithong' during storage at 8 ºC were different to those that developed in fruit stored at 13 °C. The browning symptoms at 8 °C were tinted white-grey while the symptoms at 13 °C were dark brown. Furthermore, IB symptoms in fruit stored at 8 °C developed only around the core but the symptoms in fruit stored at 13 °C were severe around the core and in the adjacent pulp.

AA is an active antioxidant and anti-browning agent in living plant systems (Gonza´lez-Aguilar *et al.,* 2005; Altunkaya and Gökmen, 2009). However, this research did not reveal a role for AA in preventing internal browning in pineapple fruit since AA was higher in fruit harvested at 1200 compared to 0600 but time of day at harvest had no effect on IB. Furthermore, AA concentrations during storage at 20 °C were similar to those at the chilling temperatures 8 and 13 °C. No browning symptoms developed in fruit stored at 20 °C during 20 days of storage even though they became over ripe.

TA and juice pH were similar in fruit harvested at both times but TA increased and pH decreased during storage at both chilling and non chilling temperatures. Fruit harvested at 1200 had higher concentrations of AA than fruit harvested at 0600 but AA appeared to have no influence on the development of IB. Measurements of TA in stem and crown leaves at 0600 and 1200

Table 2. Changes in TA [Malic acid (%)] in juice of 'Trad-Srithong' pineapple fruit harvested in December 2006 at 0600 and 1200 h during storage at 8, 13 and 20 ºC for 20 days

Treatment	Malic acid (%)[1]					
	Days of storage					
	0	5	10	15	20	F-test
Harvesting time (H) [2]						
0600	0.4±0.02D	0.5±0.04C	0.6±0.049BC	0.7±0.04B	0.8±0.04A	**
1200	0.4±0.04C	0.5±0.03AB	0.6±0.04AB	0.6±0.03AB	0.7±0.03A	**
F- test	ns	ns	ns	**	*	
Fruit part (F) [2]						
Core	0.3±0.01D	0.4±0.01C	0.4±0.019C	0.5±0.03B	0.7±0.04A	**
Pulp	0.5±0.02D	0.7±0.01C	0.8±0.02B	0.7±0.01B	0.9±0.02A	**
F- test	**	**	**	**	**	
Temperature (T) [2]						
8 ºC	0.4±0.02C	0.5±0.04B	0.5±0.05bB	0.6±0.03bB	0.7±0.04bA	**
13 ºC	0.4±0.03D	0.5±0.05C	0.6±0.04aBC	0.7±0.03aAB	0.8±0.07aA	**
20 ºC	0.4±0.05C	0.5±0.04BC	0.6±0.07aAB	0.6±0.05bBC	0.8±0.03bA	**
F – test	ns	ns	**	**	*	
H*F	ns	ns	ns	ns	ns	
H*T	ns	ns	*	ns	**	
F*T	ns	ns	**	*	ns	
H*F*T	ns	*	ns	ns	*	

[1] Means with different lower case letters within the same column are significantly different. [2] Means with different capital letters within the same row are significantly different. ns: not significant. * Significantly different at $P \leq 0.05$. ** Significantly different at $P \leq 0.01$

Table 3. Changes in TSS in juice of 'Trad-Srithong' pineapple fruit harvested in December 2006 at 0600 and 1200 h during storage at 8, 13 and 20 ºC for 20 days

Treatment	TSS (°brix)					
	Days of storage					
	0	5	10	15	20	F-test
Harvesting time (H) [1]						
0600	16.0±0.41A	15.7±0.45A	13.6±0.48C	15.2±0.44AB	14.3±0.42BC	**
1200	13.3±0.43	14.7±0.48	13.0±0.49	13.8±0.54	13.1±0.41	ns
F- test	**	*	ns	**	*	
Fruit part (F) [1]						
Core	13.3±0.47	14.3±0.41	12.1±0.39	13.2±0.49	12.7±0.34	ns
Pulp	16.0±0.36A	16.1±0.45A	14.6±0.36B	15.7±0.36AB	14.7±0.39C	*
F- test	**	**	**	**	**	
Temperature (T) [1]						
8 ºC	14.1±0.60ABC	15.0±0.43AB	13.8±0.63BC	15.5±0.36A	12.9±0.41C	**
13 ºC	14.8±0.70	14.9±0.78	13.6±0.63	14.2±0.87	14.3±0.48	ns
20 ºC	15.1±0.64AB	15.8±0.49A	12.6±0.49	13.7±0.50	13.9±0.63BC	**
F – test	ns	ns	ns	ns	ns	
H*F	ns	ns	ns	ns	ns	
H*T	ns	**	ns	*	ns	
F*T	ns	ns	ns	ns	ns	
H*F*T	ns	ns	ns	ns	ns	

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Table 4. Changes in pH in juice of 'Trad-Srithong' pineapple fruit harvested in December 2006 at 0600 and 1200 h during storage at 8, 13 and 20 ºC for 20 days

Treatment	pH[1]					
	Days of storage					
	0	5	10	15	20	F-test
Harvesting time (H)[2]						
0600	3.7±0.03A	3.5±0.33B	3.4±0.02B	3.4±0.03B	3.2±0.03C	**
1200	3.6±0.02A	3.5±0.03B	3.3±0.01C	3.4±0.01C	3.2±0.02D	**
F- test	**	ns	**	ns	ns	
Fruit part (F)[2]						
Core	3.7±0.02A	3.5±0.03B	3.4±0.02C	3.4±0.03C	3.2±0.04D	**
Pulp	3.7±0.03A	3.4±0.02B	3.3±0.01C	3.4±0.01D	3.2±0.02D	**
F- test	ns	*	**	**	*	
Temperature (T)[2]						
8 ºC	3.7±0.04aA	3.5±0.01B	3.3±0.02bC	3.4±0.01bB	3.3±0.01aC	**
13 ºC	3.7±0.04aA	3.5±0.05B	3.3±0.02cC	3.3±0.02cC	3.0±0.03cD	**
20 ºC	3.6±0.04bA	3.5±0.03BC	3.4±0.02aC	3.5±0.02aB	3.2±0.02bD	**
F – test	*	ns	**	**	**	
H*F	*	ns	*	ns	ns	
H*T	ns	ns	*	**	*	
F*T	*	ns	*	ns	**	
H*F*T	**	ns	ns	ns	ns	

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Table 5. Changes in ascorbic acid (AA) concentrations in juice of 'Trad-Srithong' pineapple fruit harvested in December 2006 at 0600 and 1200 h during storage at 8, 13 and 20 ºC for 20 days

Treatment	AA (mg/100gFW) [1]					
	Days of storage					
	0	5	10	15	20	F-test
Harvesting time (H)[2]						
0600	9.0±0.23BC	9.7±0.36B	12.6±0.38A	8.6±0.75BC	7.0±1.10C	**
1200	11.6±0.21B	10.2±0.22B	13.9±0.38A	10.1±0.73B	6.8±1.15C	**
F- test	**	ns	**	ns	ns	
Fruit part (F)[2]						
Core	10.4±0.31B	10.6±0.17B	12.5±0.23A	9.0±0.80B	5.9±1.10C	**
Pulp	10.2±0.45B	9.3±0.32BC	14.1±0.45A	9.7±0.73BC	7.9±1.09C	**
F- test	ns	**	**	ns	*	
Temperature (T)[2]						
8 ºC	10.8±0.44aAB	10.7±0.22aAB	12.2±0.44bA	8.7±0.72C	10.6±0.64aB	**
13 ºC	9.9±0.60bB	9.5±0.45bB	13.6±0.39aA	8.7±1.13B	1.4±0.49bD	**
20 ºC	10.2±0.31bB	10.6±0.29bB	14.0±0.53aA	10.6±0.84B	8.7±0.98aB	**
F – test	*	**	**	ns	**	
H*F	*	**	**	ns	**	
H*T	*	**	ns	ns	ns	
F*T	*	ns	*	ns	ns	
H*F*T	ns	ns	ns	ns	ns	

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Table 6. Development of IB in 'Trad-Srithong' pineapple fruit harvested in December 2006 at 0600 and 1200 h during storage at 8, 13 and 20 ºC for 20 days

Treatment	Browning (scores) [1]					
	Days of storage					
	0	5	10	15	20	F-test
Harvesting time (H)[2]						
0600 h	0.0A	0.00A	0.8±0.26AB	1.6±0.47BC	2.2±0.61C	**
1200 h	0.0A	0.00A	0.8±0.26A	1.4±0.41B	2.2±0.61B	**
F- test	ns	ns	ns	ns	ns	
Temperature (T)[2]						
8 ºC	0.0A	0.0A	1.3b±0.21bB	1.8±0.16bC	2.6±0.21bD	**
13 ºC	0.0A	0.0A	1.3b±0.21bB	2.8±0.30cC	4.0±0.36cD	**
20 ºC	0.0	0.0	0.0a	0.0a	0.0a	ns
F – test	ns	ns	**	**	**	
H*T	ns	ns	ns	ns	ns	

[1] Means with different lower case letters within the same column are significantly different. [2] Means with different capital letters within the same row are significantly different.
ns: not significant. ** Significantly different at $P \leq 0.01$

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h confirmed that 'Trad-Srithong' is a CAM plant. IB in 'Trad-Srithong' pineapple is a form of chilling injury that develops at low storage temperatures. Neither the onset of IB nor its severity was affected by the time of day when the fruit were harvested.

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