Changing in TSS, TA and Sugar Contents and Sucrose Synthase Activity in Ethephon-Treated 'Pattavia' Pineapple Fruit

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ABSTRACT

Exogenous ethylene increases endogenous ethylene which plays a crucial role on ripening in climacteric fruits. Although pineapple is a non-climacteric fruit, ethylene released from ethephon is effectively used to hasten the harvesting period. Effects from the use of a high concentration of ethephon on eating quality, fruit size and the reduction in harvesting period have been reported. In this paper, the effect of a low concentration of ethephon on pineapple fruit quality and sucrose synthase (SuSy) activity was investigated. Field experiment was arranged in split plot design. In the main plot, two levels of ethephon concentrations, i.e. 0 and 500 mg/l, were used by spraying at 110 days after forcing (DAF) fruits. The sub plot was harvesting time, i.e. 5 times of one-week intervals from 124 to 152 DAF. We found that the total soluble solid (TSS) was significantly increased in most of harvesting-treated fruits while the titratable acid (TA) was significantly increased at 131 DAF of harvesting-treated fruits. Only at 131 DAF harvesting time, the glucose content and SuSy activity of ethephon-treated fruits were significantly reduced and return to the control level afterward. However, ethephon had no effect on the fructose and sucrose contents at all harvesting times. In conclusion, fruit quality with shortening of harvesting time could be improved by applying 500 mg/l ethephon at 110 DAF since TSS content which is one of the parameter predicting eating quality of pineapple was increased without decreasing fruit quality.

Key words: 'Pattavia' pineapple, ethephon, total soluble solid (TSS), titratable acidity (TA), sucrose synthase

INTRODUCTION

Ethephon is one of the most effective inflorescence forcing agents in pineapple [*Ananas comosus* L. (Merr.)] that is widely used presently (Bartholomew *et al*., 2003). Its function is to stimulate the respiration rate of fruit while chlorophyll remains in shell (Dull *et al.*, 1967). Moreover, it accelerates the ripening process and concentrates the harvest peak (Chalermglin, 1979; Smith, 1991). In other non-climacteric fruit such as pepper, exogenous ethylene promotes and increases a cellulase activity (Ferrarese *et al*., 1995).

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To achieve the high fruit quality, high total soluble solid (TSS) at the range of 12-14% and relatively low titratable acidity (TA) of citric acid at the range of 0.4-0.6% in pineapple flesh are recommended for pineapple production in Thailand (Thongtham, 1983). Though TSS and TA are eating quality prediction parameters, TSS is the only parameter suitable as a year-round index (Bartolome *et al*., 1995). Bartolome *et al*. (1996) found that TSS in pineapples was positively correlated with total sugars. Beside reflecting fruit quality, TA also indicates the sourness. In pineapples, TA is reported as citric acid, not malic acid. It varies primarily with fruit developmental stages but does not relatively respond to short-term environmental changes, while the malic acid varies with environmental changes especially the light (Singleton and Gortner, 1965).

Many factors including ethephon have affected pineapple fruit quality (Bartholomew *et al.*, 2003). An application time and the quantity of ethephon have influences on the quality of fruit. Too early application causes the reduction in size and weight of crown and fruit, whereas low TSS and high TA contents are also found (Audinay, 1970; Chalermglin, 1979). TSS is highly correlated with test-panel eating quality (Smith, 1988) and with total sugars (Bartoleme *et al*., 1996). In pineapple fruits, fructose, sucrose, and glucose play important roles in flavor characteristics and are major sugars which vary according to the stage of fruit development. Sucrose content is lowest in the flesh during the early stage of fruit growth but rapidly increases at 6 weeks before harvest and becomes predominant in mature fruit. In the early stage, glucose is slightly higher than fructose and remains relatively constant through development while fructose slightly increases at 2 weeks before harvest (Chen and Paull, 2000). The changes in total sugar contents are affected by the developmental stage of fruits, climates, and varieties (Bartoleme *et al*., 1996), nevertheless the change of each sugar

content in ethephon-treated pineapple fruits has not been reported.

In a sink organ, sugar accumulation is related to the presence of sucrose metabolizing enzymes. One of them is sucrose synthase (SuSy) (Taiz and Zeiger, 1998) which reversibly converts sucrose and UDP to fructose and UDP-glucose. SuSy is important in cell metabolism not only in sink strength (Nguyen-Quoc and Foyer, 2001) but also in cell wall synthesis (Nakai *et al*., 1999; Ruan *et al*., 2003), and starch synthesis (D/Aoust *et al*., 1999). Furthermore, it accumulates sucrose in edible tissue of satsuma mandarin fruit (Komatsu *et al*., 2002) and saves ATP in glycolysis pathway (Huber and Azakawa, 1986). Chen and Paull (2000) reported that in pineapple fruits SuSy activity was higher at young stage, lower at 6 weeks before harvest, and then constant till harvesting time. The change of SuSy activity in ethephon treated fruit has also not been reported. The objective of this work is to answer the question if ethephon could increase TSS, TA, sugar content and SuSy activity in pineapple fruit.

MATERIALS AND METHODS

Plant and fruit materials

Field-grown 'Pattavia' pineapple [*Ananas comosus* L. (Merr.) cv. smooth cayenne] planted at Sam Praya district, Petchburi Province, Thailand, were used. Forcing of pineapple inflorescence was done in the evening of November 18, 2002, by spraying 50 ml of 250 mg/l ethephon (a.i. 48% w/v) including 3% (w/v) urea on shoot. The experimental design used in this study was split plot design. Main plot was ethephon concentration of 0 and 500 mg/l by spraying 50 ml volume per fruit at the age of 110 days after forcing (DAF). Pineapple fruit at this age is pointed-eyes stage 3 according to the Dole Company, Thailand, which is the last stage of pointed-eyes pineapple (immature) and thereafter the eyes will become flatted. Sub-plot was

harvesting time which started from 124 DAF until 152 DAF. Three replications, 8 fruits each, were analyzed.

Fruit samples were brought to laboratory and cut transversely into 3 sections after the size and weight of crowns and fruits were measured. Only the flesh of the middle section was used in this study. A half of the flesh was crushed and the juice was then used for determination of TSS and TA. The other half, sliced into small pieces, was used for the determination of the sugar content and sucrose synthase activity. These sliced fleshes of 8 fruits were pooled together as one of three replications at each harvesting time. The tissues were then frozen immediately in liquid nitrogen and stored at -80°C until use.

Soluble sugar content

TSS was determined from extracted juice using hand sugar refractometer. Soluble sugars in the form of sucrose, fructose and glucose were extracted following the method of Chen and Paull (2000). After extraction, the solution was filtered through a 0.45 mm filter, and 20 ml was injected and analyzed with HPLC by using a Waters 2690 Separation Model instrumented with a Waters 410 Differential Refractometer detector, employing a Sugar-PAK I (Waters Associates, Milford, USA) column of stainless steel (300 mm length $\times$ 6.5 mm internal diameters).   The eluting buffer was 0.1 mM calcium EDTA and the flow rate was 0.5 ml/min. Experiments were performed at 90°C. Soluble sugars were quantified by comparing the peak areas with external sucrose, glucose and fructose standard solutions (Sigma Co., Ltd.).

Titratable acidity

TA was analyzed from extracted juice after the determination of TSS contents and reported as citric acid according to AOAC (1990). Sucrose synthase determination

Sucrose synthase (SuSy) in frozen flesh tissue was extracted as described by Chen and

Paull (2000). The extracted solution was desalted by Hitrap® Desalting column (Amersham Biosciences) and 50 µl of desalted mixture was used to determine the enzymatic activity in synthesis direction according to the method of Hubbard *et al.* (1989), as modified by Chen and Paull (2000).

Statistical analysis

All data were analyzed the variance (ANOVA) using statistical analysis software of IRRISTAT version 93-3.

RESULTS AND DISCUSSION

The last harvesting time in this study (152 DAF) was planned to coincide with commercial harvesting time. The commercial harvesting index for cannery fruit industry is apparent when fruits reach full-size and the shell color at the basal portion starts to change. The effects of ethephon and harvesting time on fruit quality, sugar content and SuSy activity after treating at 110 DAF are shown in Table 1. Ethephon concentration did not reduce the size and weight of the crowns and fruits. The crowns and fruits continued to develop after the treatment and the crowns reached a full-size one week (138 DAF) before the fruits did (145 DAF). Maximum growth of the crowns indicated that the fruits were nearly ready for harvest (Paull and Reyes, 1996). The concentration of ethephon plays a significant role in increasing the mean of TSS contents (11.02° Brix) when compared with the mean of untreated fruits (8.90°Brix). The mean of TA and sugar contents including SuSy activity did not change, compared with untreated fruits.   The harvesting time at 145 DAF provided the highest TA, TSS and sucrose contents of 0.62% citric acid, 12.16° Brix and 54.12 g/kg FW, respectively (P<0.01). These indicated that the quality of fruit changes during fruit development and TSS were related to sucrose more than glucose and fructose as reported

Table 1 Effects of ethephon concentrations and harvesting times on fruit quality, sugar content and sucrose synthase activity after treated at 110 days after forcing (DAF).

	Crown			Fruit			TA[1]	TSS[2]	Flesh			SuSy activity
	Width	Length	Weight	Width	Length	Weight	(%citric acid)	(°Brix)	Sucrose	Glucose	Fructose	(mmole/h/ g FW)
	(cm)		(g)	(cm)		(g)			(g/kg FW)			
Ethephon concentration												
0 mg/l	12.6	12.6	140.8	11.0	14.0	882.4	0.54	8.90b	31.07	14.78	11.61	2.374
500 mg/l	11.6	11.1	127.1	11.3	14.1	922.9	0.59	11.02a	43.20	13.42	11.40	1.717
Harvesting time												
124 DAF	11.4b	9.8b	124.8	11.5	14.2	846.2b	0.42c	8.08b	20.90d	14.46	10.86	2.297
131 DAF	11.3b	10.4b	114.4	10.7	13.4	760.4b	0.52b	8.78b	23.46cd	13.96	10.43	1.984
138 DAF	13.5a	13.4a	139.4	10.8	13.8	849.8b	0.58ab	8.48b	37.08bc	15.22	12.02	1.964
145 DAF	12.2ab	13.0a	143.3	11.8	15.0	1149.8a	0.62a	12.16a	54.12a	14.50	13.08	2.127
152 DAF	12.1ab	12.7a	147.9	11.1	13.8	906.7b	0.67a	12.30a	50.13ab	12.34	11.12	1.864
Ethephon concentration	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns
Harvesting time	*	**	ns	ns	ns	**	**	**	**	ns	ns	ns
Ethephon concentration X Harvesting time	ns	ns	ns	ns	ns	ns	*	*	ns	*	ns	*

Mean followed by the same letter within the same column are not significantly different at the 5% level according to LSD. Symbols * and ** indicate significance at the 0.05 and 0.01 levels analyzed by DMRT, ns indicates no significant.

[1] TA = Trirtatable acidity
[2] TSS = Total soluble solid

by Chen and Paull (2000). Figure 1 also showed that sucrose content was low in immature fruit and the highest content was achieved at 145 DAF while glucose and fructose contents were relatively constant during fruit growth as reported by Chen and Paull (2000).

The interaction of ethephon concentration with harvesting time significantly affected TA, TSS and glucose contents at P<0.05 (Table 1). Comparing between the treatments of ethephon concentration at 0 and 500 mg/l at each harvesting time, it was found that almost all TSS of treated fruits were significantly higher than those of the control (Figure 2B). However only treated fruits harvested at 131 DAF had TA content higher (Figure 2A), but glucose content (Figure 2C) and SuSy activity were lower (Figure 2D) than those of the untreated fruits. The high concentration of TSS in harvested fruits treated at 131 DAF was affected by high TA rather than sugar content because TSS does not represent only the sugar content but also the contents of organic acids,

soluble pectins and other dissolved substances which have different refractive indices from water (Holcroft and Kader, 1999). This is the reason why a direct measurement of sugar concentration by HPLC is carried out. From our results (Figure 2A, 2B), ethephon affected the TA and glucose contents of treated fruits in a few weeks after ethephon application because ethephon is an unstable substance which can be easily degraded by high temperature and high pH in cytoplasm (Bartholomew *et al.*, 2003). Changing in TA and glucose contents in pineapples may also be resulted from a high respiration rate which is induced by ethephon (Dull *et al.*, 1967). This is due to the use of glucose as a first glycolytic substance in a respiratory pathway (Taiz and Zeiger, 1998) which enhances organic acid contents (Ulrich, 1970). High respiration rate also causes high oxygen admission in tissue and this may be the other reason for increasing TA.

It was also found that the TSS contents of harvested fruits treated at 145 and 152 DAF
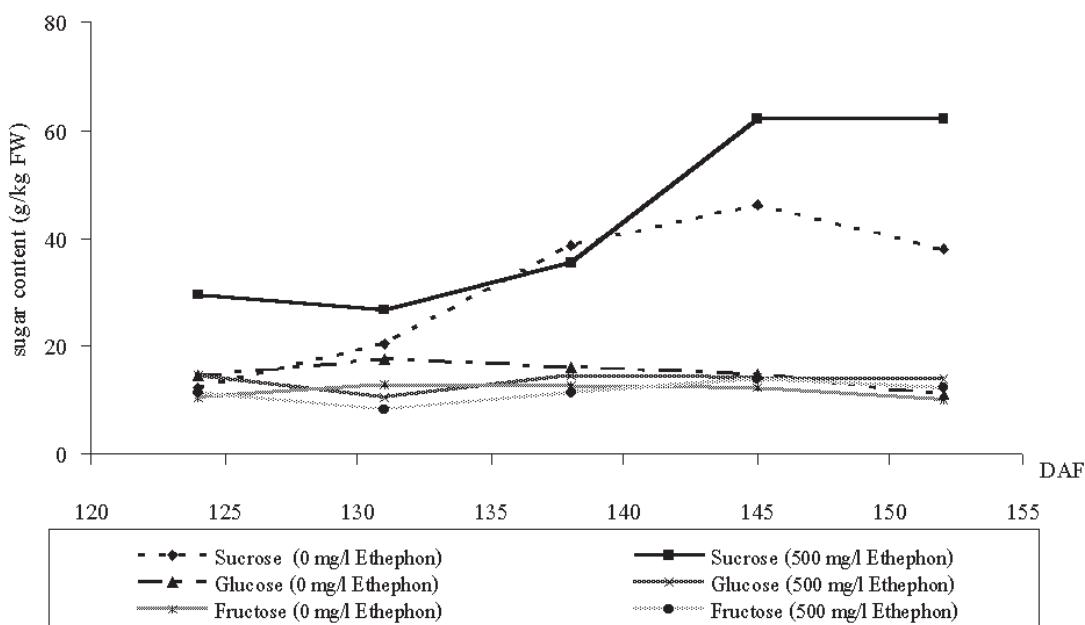
Figure 1 Sucrose, glucose and fructose contents in pineapple fruits after treated with 0 and 500 mg/l ethephon at 110 days after forcing (DAF).

were higher than that of the untreated fruits (Figure 2B). The exogenous ethylene which was suggested to increase the lipoxygenase activity by Yu *et al.* (2003) might change the permeability of the membrane and cause the increase of TSS in these mature fruits. From the results on high TSS (13.53°Brix) and TA (0.6% citric acid) contents measured at 145 DAF, the treated fruits which are in the range of high eating-quality fruit (Bartholomew *et al.*, 2003) could be harvested one week earlier. Chalermglin (1979) also reported that after applying 1,500 mg/l of ethephon at 112 DAF, the treated fruits could be harvested 11 days

earlier than those of the control. However, TA was found to be inereased in treated fruits while fruit size was reduced and TSS was unchanged. This study indicates that the application of 500 mg/l ethephon to 110 DAF fruits hastened the harvesting time without reducing fruit quality.

Figure 2 also showed SuSy activities which were affected by a significant interaction between ethephon concentration and harvesting time. When harvested at 131 DAF, the SuSy activity of the treated fruits was significantly lower than that of the untreated fruits. Chen and Paull (2000) suggested that the low SuSy activity in
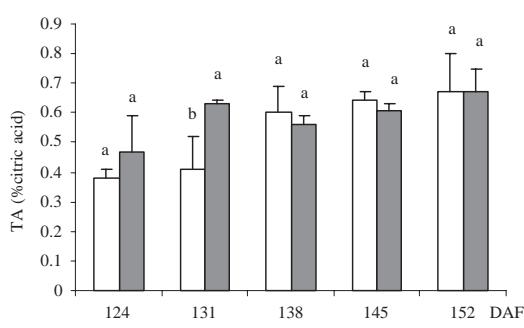
(A)
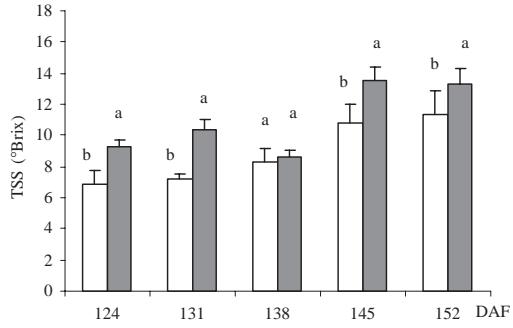
(B)
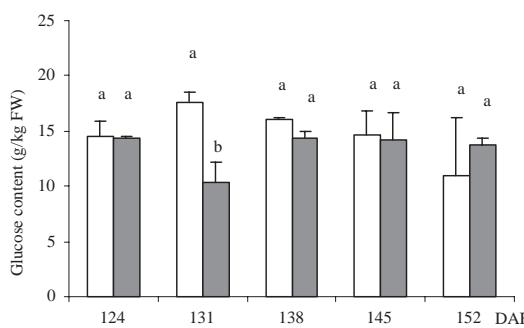
(C)
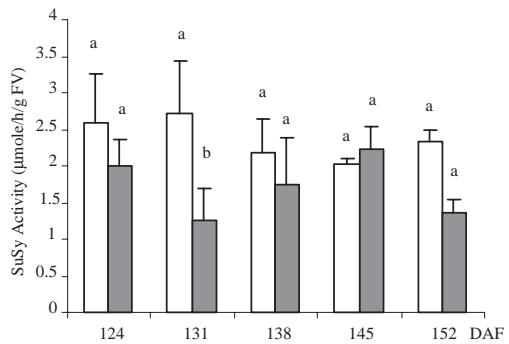
(D)

Figure 2 Changes in tritratable acidity (TA) (A), total soluble solid (TSS) (B) and glucose contents (C) and sucrose synthase activity (D) in pineapple fruits flesh at various harvesting times after treated with 500 mg/l ethephon (▨) and without ethephon (▢) at 110 days after forcing (DAF). Error bars represent standard error of the means of three replications. Bars with the same letter assigned are not significantly different at 0.05 probability level.

pineapple fruit allowed the accumulation of sucrose. However, we found that the low SuSy activity in harvested fruits treated at 131 did not enhance the sucrose accumulation (no significant interaction of sucrose was found, Table 1). Therefore, the SuSy activity was not related to the accumulation of sucrose in pineapples which is in contrast to the activity in non-climacteric, satsuma mandarin fruits (Komatsu *et al*., 2002). The decrease of SuSy activity of harvested fruits treated at 131 DAF might be resulted from the increase in respiration rate which increases the amount of ATP in cells. Therefore, SuSy activity which involves in energy-saving pathway of glycolysis (Huber and Akazawa, 1986) should be decreased. SuSy is an important enzyme for synthesizing UDP-glucose, the cellulose precursor (Nakai *et al*., 1999). Thus, exogenous ethylene enhances a cellulase activity (Ferrarese *et al*., 1995) which leads to high production of UDP-glucose that may act as a negative feedback to the SuSy activity. The exact mechanisms of the SuSy activity as well as the effect of ethylene on SuSy activity have still not been well-defined.

CONCLUSION

We conclude that the ethephon at the rate of 500 mg/l spraying at 110 DAF could increase TSS in pineapple fruit, but not TA, sugar contents and SuSy activity, and the treated fruits could be harvested at 145 DAF without the decrease of fruit size and weight.

ACKNOWLEDGEMENTS

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