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Pineapple organic acid metabolism and accumulation during fruit development

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Abstract

Developmental changes in pineapple (*Ananas Comosus* (L.) Merrill) fruit acidity was determined for a 'Smooth Cayenne' high acid clone PRI#36-21 and a low acid clone PRI#63-555. The high acid clone gradually increased in fruit acidity from 1.4 meq/100 ml 6 weeks from flowering, and peaked a week before harvest at ca 10 meq/100 ml. In contrast, the low acid clone increased in acidity 6 to 8 weeks after flowering, peaked 15 weeks after flowering at ca. 9 meq per/100 ml and then sharply declined in 2 weeks to 6 meq/100 ml. The increased in total soluble solids (TSS) of the low acid clone began 6 weeks after flowering and for the high acid clone at 12 weeks after flowering. The increase in titratable fruit acidity (TA) paralleled the changes in the citric acid content of both clones. Citric acid content increased from less than 1 mg/g at 6 weeks after flowering to 6 to 7 mg/g, 9 weeks later. The malic acid concentration in both clones varied between 3 and 5 mg/g and showed no marked changes just before harvest. The developmental changes in fruit potassium were significantly correlated with fruit acidity and fruit total soluble solids in both the high and low acid clones. Developmental changes in acid-related enzymatic activities showed an increase in citrate synthase (EC 4.1.3.7) activity that occurred a week before harvest, coincided with the peak in citric acid in the high acid clone. An increase in aconitase (ACO, EC 4.2.1.3) activity was observed just before harvest as the decline in acidity occurred in the low acid clone. The activities of phosphoenolpyruvate carboxylase (PEPC, EC 4.1.1.31), malate dehydrogenase (MDH, EC 1.1.1.37) and malic enzyme (ME, EC 1.1.1.40) did not parallel any changes in fruit acidity. The results indicated that the change in pineapple fruit acidity during development was due to changes in citric acid content. The major difference in acid accumulation occurred in the low acid clone just before harvest when acidity declined by one-third. The activities of citrate synthase and aconitase possibly played a major role in pineapple fruit acidity changes.

Keywords: *Ananas comosus*; Fruit acidity; Total soluble solids; Potassium; Citrate synthase; Aconitase; Citrate; Malate

'1. Introduction

Fruit acidity and sweetness are two of the major factors that determine pineapple fruit eating quality. Other measures of fruit quality include shell color, fruit size and shape, aroma, crown size, crown to fruit ratio and the absence of disease and blemishes (Paull and Chen, 2003). Variation of pineapple fruit acidity and sweetness are associated with the pineapple clone used, fruit maturation and growing conditions (Singleton and Gortner, 1965; Py et al., 1987; Bartolome et al., 1995). Fruit acidity increases during pineapple fruit growth and as the fruit approaches maturity and starts to ripen, the acidity declines (Singleton and Gortner, 1965; Smith, 1988). Citric acid shows the greatest changes during fruit growth, increasing and then reaching a peak prior to ripening, whereas malic acid shows little change during development (Singleton and Gortner, 1965; Chan et al., 1973). Fruit sweetness gradually increases during the later stages of fruit growth (Bartholomew and Paull, 1986) with the sugar to acid ratio being recommended as a harvest index (Paull and Chen, 2003). However, citric acid alters sucrose perception (Schifferstein and Fritjers, 1990) and pineapple clones may have sufficient sugars but high citric acid may mask some of the sweetness perception. This masking of sucrose means that higher acid fruit may be perceived as being sour.

Low acid pineapple clones have been available for a number of years though high acid clones are preferred for canning. The low acid pineapple hybrid clones have become more important

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as interest in providing a year round supply of fruit for the fresh market has developed (Paull and Chen, 2003). The availability of cultivars or clones of other economically important fruits differing in acid content, has facilitated comparative studies of organic acid metabolism in apple (Beruter, 2004), peach (Genard et al., 1999; Moing et al., 1998a), citrus (Sadka et al., 2001) and grape (Diakou et al., 2000; Terrier et al., 2001).

The final organic acid content of fruit is determined by the net balance of acid synthesis, degradation, utilization and compartmentation (Laval-Martin et al., 1977; Ruffner et al., 1984; Muller et al., 1996; Yamaki, 1984). The enzymes potentially involved in fruit acid metabolism are citrate synthase (CS, EC 4.1.3.7), aconitase (ACO, EC 4.2.1.3) (Sadka et al., 2000a, 2001; Luo et al., 2003), phosphoenolpyruvate carboxylase (PEPC, EC 4.1.1.31), malate dehydrogenase (MDH, EC 1.1.1.37) and malic enzyme (ME, EC 1.1.1.40) (Hirai and Ueno, 1977; Moing et al., 2000; Diakou et al., 2000). MDH catalyzes the interconversion of malate and oxaloacetate in the cytoplasm. CS catalyzes the acetylation of oxaloacetate using acetyl-CoA to yield citrate that is isomerized by ACO to isocitrate. PEPC condenses phosphoenolpyruvate with bicarbonate to yield oxaloacetate and ME reduces and decarboxylates malate to pyruvate.

Although studies had been reported on the compositional changes in some pineapple fruit clones (Kermasha et al., 1987; Bartolome et al., 1995; Brat et al., 2004), information on fruit acid metabolism is limited. Potassium ion is thought to be involved in organic acid charge balancing (Lang, 1983) and potassium fertilization does increase pineapple titratable acidity (Py et al., 1987; Spironello et al., 2004), though potassium accumulation in pineapple fruit has not been reported in relation to fruit acidity. The objectives of this study were to examine developmental changes in pineapple fruit acidity and organic acid metabolism during fruit development. This background information is essential to undertake field studies on modifying fruit acidity to ensure a consistent year around supply of fruit with a uniform sugar to acid ratio. The activities of five enzymes possibly involved in acid metabolism (CS, ACO, PEPC, MDH and ME) were determined throughout fruit development. Knowledge of the key enzyme(s) in determining final fruit acidity would allow a decision to be made as to whether molecular modification of activity would be a viable possibility. Two 'Smooth Cayenne' clones were compared, clone PRI#36-21 is a high acid fruit developed for canning (Paull and Chen, 2003) and clone PRI#63-555 is a low acid fruit more suited to the fresh fruit market.

2. Materials and methods

2.1. Plant material

Pineapple fruit of PRI#36-21 clone (high acid) and PRI#63-555 clone (low acid) were harvested from adjacent fields at the Dole Fresh Fruit Co. plantation on the island of Oahu, Hawaii. Both clones were planted in 2003 March and a second planting of both clones occurred in 2003 August. The first planting was forced in 2003 August and the second planting 2004 February. Forcing of the planting in the adjacent fields occurred within a week of each other. No significant differences were found in the pattern of increase and magnitude of the increase in fruit weight, acidity and total soluble solids between the two different planting times for each clones. In the absence of significant differences, the average of the two harvest series was used for data presentation. Fifteen uniform fruit were randomly sampled throughout fruit development from the same uniform section of each field from 6 weeks after forcing (WAF) to the week of commercial harvest at 17 WAF. The fruit were transferred to the laboratory within 2 h.

2.2. Determinations of flesh total soluble solids (TSS) and titratable acidity (TA)

The juice samples were squeezed from pieces taken from opposite ends of a vertical fruit slice, centrifuged at 10,000 × *g* for 10 min and TSS and TA determination on the supernatant. TSS was determined by refractometry and TA by titration with 0.1 N NaOH to an endpoint of pH 8.3 and expressed as meq/100 ml juice.

2.3. Organic acid determination

A 2 ml aliquot of the above supernatant was diluted (1 to 5) with 95% ethanol and stored at −20 °C for HPLC analysis. The diluted juice for chromatography was prepared using a modification of the procedure of Paull et al. (1983) with separation achieved on a different type of organic acid column (Bio-Rad HPX-87H, 7.8 mm × 300 mm) with 0.05 N sulfuric acid as the solvent at a flow rate of 0.8 ml/min. The eluant was monitored at 210 nm and the peaks identified using pure organic acids and calibrated with solutions of known concentration.

2.4. Potassium determination

A 2 ml aliquot of the above alcoholic solution was air-dried and redissolved in deionized water (2 ml). Potassium was determined with an ion selective electrode (Candy K^+ Meter; Spectrum Technologies, Inc.) and potassium concentration expressed as mg/l relative to a potassium chloride standard.

2.5. Enzyme extraction

Crude enzymes were extracted from the fruit flesh by a modification of published methods (Jeffery et al., 1988; Knee and Finger, 1992; Diakou et al., 2000). Sections from four fruit were extracted at <4 °C as individual replicates by mixing 10 gm of tissue with 20 ml of extraction buffer (250 mM Tris–HCl (pH 7.5), 600 mM sucrose, 10 mM KCl, 10mM $MgSO_4$, 3 mM EDTA, 1% PVP-40, 1 mM PMSF, 0.05% mercaptoethanol). The mixture was filtered through a double layer of Miracloth and centrifuged at 1000 × *g*. The supernatant was collected and re-centrifuged at 20,000 × *g* for 20 min. Both supernatant and pellet were used for enzyme activities. The

supernatant was used as the cytosol portion to determine PEPC, MDH and ME.

For CS and ACO assays, the pellet was further extracted using a modified method of Iredale (1979) by first washing with 2 ml solution of 50 mM Tris–HCl (pH 7.5), 300 mM mannitol and 1 mM EDTA and centrifugation at 20,000 × *g* for 20 min. The mitochondria pellet was collected, mixed with 1 ml of buffer (50 mM Tris–HCl (pH 7.5), 1 mM EDTA) and then sonicated for 5 min. The solution was centrifuged at 20,000 × *g* for 10 min and the supernatant collected as the mitochondrial solution. All procedures were conducted at 4 °C.

2.6. Enzyme assays

Citrate synthase (CS) activity was determined by using Ellman's reagent (DTNB) following the procedures of Sadka et al. (2000b). The assay mixture consisted of 1 ml of 100 mM Tris–HCl (pH 7.5), 1 mM EDTA, 0.1 mM DTNB, 0.2 mM acetyl-CoA and 0.2 mM oxaloacetate. The reaction was started by adding the extracted enzyme and the increase in absorbance at 412 nm was monitored at 25 °C. Aconitase (ACO) activity was determined in a 1 ml mixture composed of 20 mM Tris–HCl (pH 7.5), 100 mM NaCl, and 200 μM *cis*-aconitate. The reaction was started by the addition of the extracted enzyme and the decline in absorbance at 240 nm was monitored at 25 °C (Hirai and Ueno, 1977). Phosphoenolpyruvate carboxylase (PEPC) activity was determined in a 1 ml mixture composed of 100 mM Tricine (pH 7.8), 2.5 mM $MgSO_4$, 0.25 mM EDTA, 2 mM DTT, 5 mM $NaHCO_3$, 0.2 mM NADH, 3 units of MDH and 2.2 mM PEP. The reaction was started by the addition of the extracted enzyme and the decline in absorbance at 340 nm was monitored at 25 °C (Diakou et al., 2000). Malate dehydrogenase (NAD-MDH) activity was determined in a 1 ml mixture composed of 50 mM MOPS (pH 8) and 0.4 mM NADH. The reaction was started by the addition of oxaloacetate (to 0.2 mM) and the rate of decline in absorbance at 340 nm was determined at 25 °C (Jeffery et al., 1988). Malic enzyme (NADP-ME) activity was determined in a 1 ml mixture composed of 100 mM MOPS (pH 7) with 0.5 mM NADP, 10 mM malate and 5 mM $MnCl_2$. The reaction was started by the addition of the extracted enzyme and the increase in absorbance at 340 nm was monitored at 25 °C (Knee and Finger, 1992). Protein was quantified using the dye-binding assay (Bradford, 1976) with bovine serum albumin as the standard.

3. Results

3.1. Developmental changes in fruit growth, and flesh total soluble solids and titratable acidity

During fruit growth and development, fruit weight of both clones increased from about 650 to 1900 g (Fig. 1A). The pattern of change in fruit weight was similar for both clones and both clones weigh about the same when harvested at 17 WAF.

The low acid clone had a higher TSS for most of the growth period (Fig. 1B) and was significantly higher at harvest. The pattern of TA changes for both clones also differed during fruit development (Fig. 1C). During the 6 to 15 WAF period the low acid clone had a greater TA than the high acid clone. The TA of the low acid clone remained constant from 14 to 16 WAF then declined 40% in the last week before harvest. In contrast, the high acid clone showed a gradual increase in TA and peaked at about 15 WAF at a higher acidity level than the low acid clone's peak. At harvest (17 WAF), the high acid clone's TA was about 70% higher than the low acid clone. The changes in juice pH agreed with the changes in TA. The low acid clone had a lower juice pH during 6 to 14 WAF period and then increased toward the harvest stage, whereas the high acid clone constantly declined. The TSS/TA ratio (as citric acid) for the high acid fruit declined during the from 6 WAF to 12 WAF, then remained stable (Fig. 1D), while in the low acid fruit the ratio declined till 12 WAF, then increased.

3.2. Developmental changes in organic acids

The citric acid content in the low acid clone increased earlier than in the high acid clone during fruit development, and peaked at 14 to 15 WAF and then declined sharply toward harvest (Fig. 2A). In contrast, the high acid clone continued to increase in citric acid content and peaked at 16 WAF, a week before harvest. The high acid clone was about 50% higher in citric acid content than the low acid clone at harvest. The

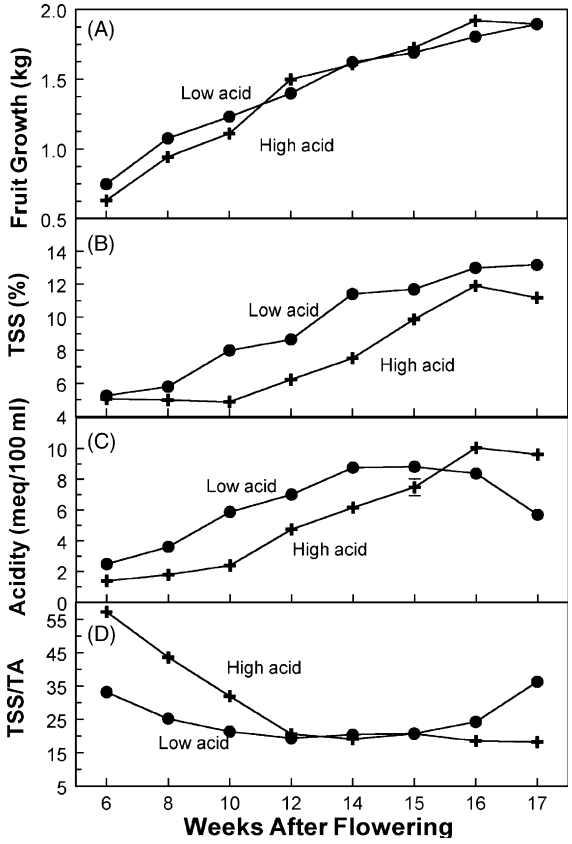

Fig. 1. Fruit growth (A) and developmental changes in pineapple fresh juice total soluble solids (TSS) (B), titratable acidity (C) and TSS to TA ratio (D) of the high acid (36-21) and the low acid (63-555) clones during fruit growth and development. Most of the standard errors bars were smaller in size than the symbol, mean ± S.E., $n = 10$.

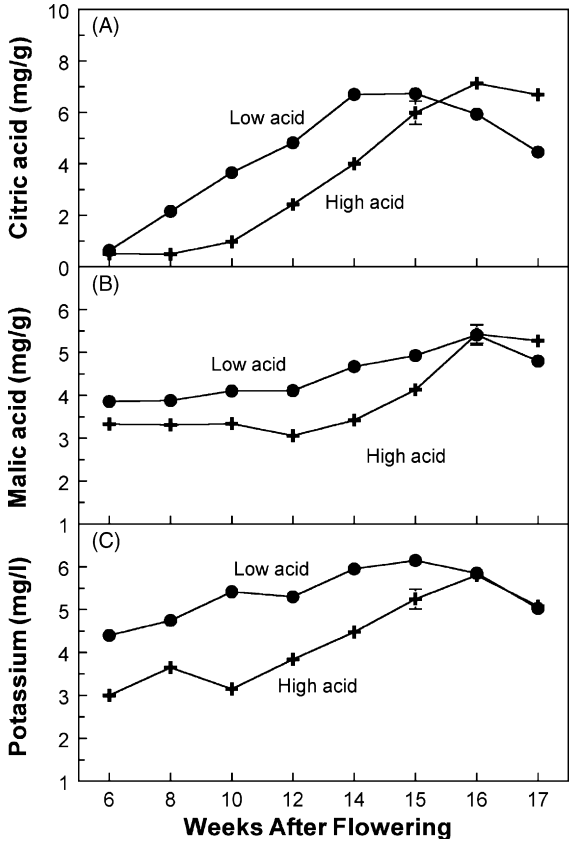

Fig. 2. Developmental changes in citric (A), malic (B) acids and potassium (C) of the high acid (36-21) and the low acid (63-555) clones during pineapple fruit growth and development. Most of the standard errors bars were smaller in size than symbol, mean ± S.E., $n = 10$.

pattern of change in fruit citric acid content was similar to that of TA.

Fruit malic acid content in both clones changed only slightly during fruit development (Fig. 2B). Prior to fruit harvest, the low acid clone was higher in malic acid content than the high acid clone. The malic acid in both clones increased and concurrently peaked at 16 WAF and had a similar concentration at harvest.

3.3. Flesh potassium changes

Potassium concentration increased during fruit growth and development in both clones (Fig. 2C). During early growth (6 to 16 WAF), the low acid clone fruit showed greater potassium content than in the high acid clone fruit and subsequently declined at harvest. The potassium content in the high acid clone increased and peaked at 16 WAF. The concentration in the last 2 weeks before harvest was similar for both clones.

The increase in potassium and acidity of the high acid clone were nearly parallel as the fruit approached maturity. The correlation between potassium content and TA was highly significant ($r = 0.94$) for the high acid clone (Table 1). The pattern of potassium content and TA in the low acid clone showed less similarity than in the high acid clone, however, the correlation between potassium and TA was still significant ($r = 0.72$). The correlation of potassium concentration and TSS

Table 1
Correlation analysis of fruit potassium concentration, titratable acidity and total sugar content in the high and low acid clones during fruit growth and development

Relationship	'High acid' clone	'Low acid' clone
Potassium - acidity	0.939***	0.718*
Potassium - total sugar	0.917***	0.793**
Acidity - total sugar	0.926***	0.427 ns

ns: no correlation ($p > 0.05$).
* Significant at $p = 0.05$.
** Significant at $p = 0.01$.
*** Significant at $p = 0.001$.

in the high and low acid clones were significant with correlation coefficients of $r = 0.92$ and 0.79, respectively.

3.4. Changes in acid-involved enzymatic activities

The pattern of changes in fruit CS activity were similar in both clones (Fig. 3A). The pattern showed a major peak at 16 WAF and then declined. The pattern of ACO activity in both clones were similar during 6 to 14 WAF period with a small peak at 10 WAF (Fig. 3B). At 16 WAF when both clones showed a peak in ACO activity. The low acid clone had a higher ACO activity than the high acid clone.

PEPC activity in the high acid clone was higher during the 6 to 12 WAF period (Fig. 4A), while in the low acid clone PEPC

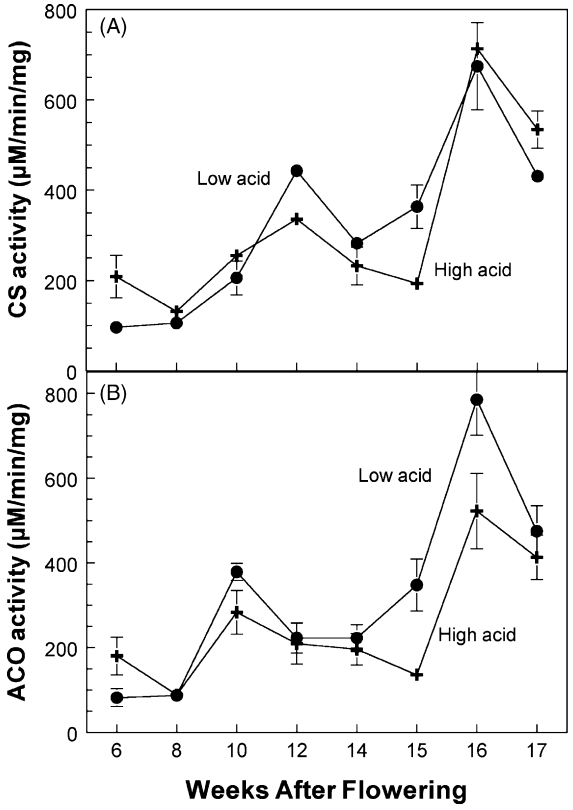

Fig. 3. Developmental changes in citrate synthase (CS) (A) and aconitase (ACO) (B) activities per mg protein of the high acid (36-21) and low acid (63-555) clones during pineapple fruit growth and development. Some of the standard errors bars were smaller in size than symbol, mean ± S.E., $n = 4$.

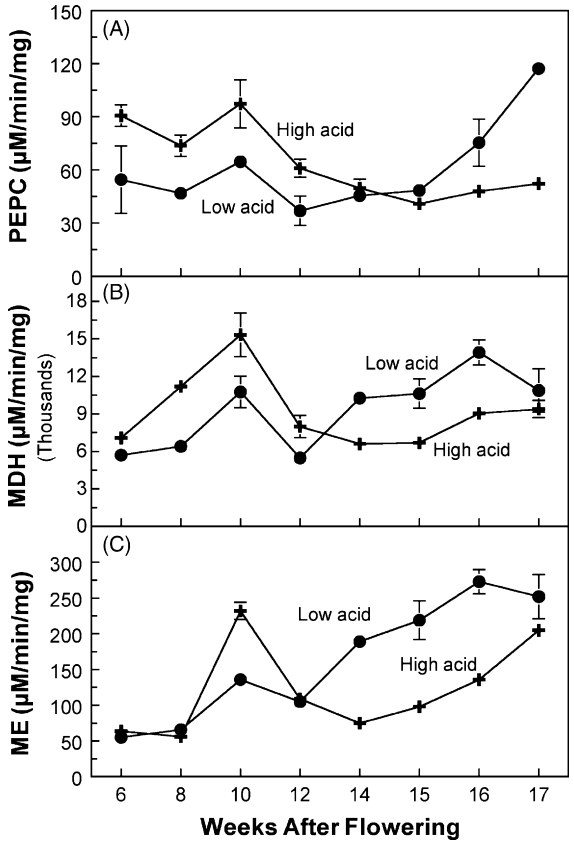

Fig. 4. Developmental changes in phosphoenolpyruvate carboxylase (PEPC) (A), malate dehydrogenase (MDH) (B) and malic enzyme (ME) (C) activities per mg proteinof the high acid (36-21) and low acid (63-555) clones during pineapple fruit growth and development. Some of the standard error bars were smaller in size than the symbol, mean ± S.E., $n = 4$.

activity increased dramatically as harvest approached. The high acid clone showed a higher MDH activity in the first half of fruit development whereas the low acid clone had a higher activity in the last half of fruit development (Fig. 4B). The ME activity in the low acid clone increased gradually throughout fruit development (Fig. 4C). In contrast, the high acid clone showed high level of ME activity at 10 WAF and during the harvest week.

4. Discussion

Pineapple fruit weight and that of the fruit's components increases in a sigmoid fashion once the inflorescence has been initiated (Sideris and Krauss, 1938; Singleton, 1965; Gortner et al., 1967; Teisson, 1973; Teisson and Pinaeu, 1982). Fruit cell division is completed prior to anthesis and all further development is the result of cell enlargement (Okimoto, 1948). In this study, fruit weight increased about three fold (Fig. 1A) and the final fruit weight was similarly for both clones and similar to that reported for other high acid 'Smooth Cayenne' clones (Bartolome et al., 1995; Tay, 1977).

Pineapple juice pH declines from 3.9 to 3.7 as fruit approach the full yellow stage (Teisson and Pinaeu, 1982), and increases as the fruit senesces, with TA showing the opposite trend (Singleton and Gortner, 1965; Teisson and Pinaeu, 1982). The same trend in pH was found in this study for both clones (data not shown) and TA (Fig. 1C). The juice titratable acidity (TA) of the high acid clone was 1.7 fold higher than that of the low acid clone at harvest (Fig. 1C). The change in TA in the high acid clone was similar to that reported for other 'Smooth Cayenne' clones used for canning (Singleton and Gortner, 1965). The higher initial TA (Fig. 1C) and citric acid (Fig. 2A) in the low acid clone and the rapid decline near harvest, has not been previously reported. The decline in TA and citric acid near harvest altered the harvest TSS/TA ratio (Fig. 1D). Commercially, TSS/TA ratio is regarded as the most reliable measure of fruit flavor. However, Singleton and Gortner (1965) found that TSS/TA ratio is only comparable for high acid canning-type fruit at the same developmental stage. A sweetness index (TSS/TA) of from 20 to 40 is recommended by Soler (1992), while Smith's (1988) data on TSS/TA ratio for a canning clone ranged from 8 to 23. This study showed that the TSS/TA ratio varied widely between two clones during fruit development and probably required different recommendations for TSS/TA ratio for the different clones. Using Soler (1992) desirable range for TSS/TA, the low acid clone had a more desirable ratio than the high acid clone (Fig. 1D). The high acid clone would be regarded as acidic in taste and the low acid clone sweet, however, the high ratio of 36 may be regarded as lacking sufficient acid and would have a blander sweet taste.

Pineapple fruit acidity is a result of the total nonvolatile acids that occurs as free organic acids (Chan et al., 1973; Teisson and Combres, 1979), mostly stored in the vacuoles of cells (Yamaki, 1984). The differences in the total amount of the major organic acids (Fig. 2A and B) in the high and low acid clones explained the changes in TA (Fig. 1C). At harvest, the high acid clone had 1.5 and 1.1 times more citric (Fig. 2A) and malic (Fig. 2B) acids than the low acid clone, respectively. This difference led to about a 60% lower TA in the low acid clone. Since both clones had similar contents of malic acid but markedly different TA, malic acid was not apparently a major cause for the differences in acidity. This lack of changes or difference in malic acid supports the conclusion that the change in TA paralleled the pattern of changes in citric acid content. Similar difference in organic acid concentrations between cultivars has been observed in peach (Etienne et al., 2002; Moing et al., 1998a), and sweet lime and sour lemon (Sadka et al., 2001). Three related hypotheses have been proposed to explain the difference in acidity. The hypothesis involves changes in the acid synthesis and activity, acid utilization and compartmentation into the vacuole (Knee and Finger, 1992; Moing et al., 1998b, 2000; Yamaki, 1984).

Potassium accumulation serves a role in tissue expansion growth and organic acid charge balance in the vacuole (Lang, 1983; Lobit et al., 2003). The fruit potassium concentration in the both pineapple clones increased during fruit development and peaked in the last few weeks before declining toward harvest (Fig. 2C). At most stages of fruit development, the patterns of changes in potassium (Fig. 2C) and TA (Fig. 1C) were parallel suggesting that changes in TA were associated with potassium possibly to balance vacuolar charge. Both clones also showed similar changes between potassium content

and TSS resulting in highly significant correlation between potassium and TSS (Table 1). These results agree with the many reports that have shown that pineapple potassium fertilization increases fruit TA and TSS (Marchal et al., 1981; Py et al., 1987; Spironello et al., 2004) possibly due to the promotion of sugar translocation to the fruit (Py et al., 1987). Like ripening grape berry, potassium may not drive tissue expansion at all stages of fruit development (Davies et al., 2006) and may serve a role of charge balancing in acid accumulating fruit. Moreover, potassium fertilization can affect pineapple shell color and fruit lodging resistance by increasing peduncle diameter (Py et al., 1987) and reducing internal browning (Soares et al., 2005).

CS is possibly a key enzyme that plays a significant role in citric acid synthesis due to a positive correlation between CS activity and fruit acidity in citrus (Wen et al., 2001). The patterns of changes in pineapple CS activity were similar between both clones, but seemed to be unrelated to the decline in acid content of the low acid clones at 17 WAF (Fig. 3A). The increase in CS activity at 16 WAF coincided with the peak of citric acid in the high acid clone. A similar relationship has been reported for sour lemon (Sadka et al., 2001) and some citrus varieties (Luo et al., 2003) where CS activity is induced early in fruit development and parallels the increase in acid content. However, sweet lime that accumulates less citric acid, shows a similar patterns of CS activity as sour lemon. In addition, arsenite that reduces CS expression and activity temporarily may not explain the reduction in citric acid content, detected a few weeks later. In the low acid pineapple clone fruit, a peak of CS activity occurred when the citric acid content declined (Fig. 2A). This indicated that CS was not a major factor regulating pineapple fruit acid accumulation during ripening. However, the lack of a relationship between CS activity and changes in citric acid levels does not imply that CS is not a contributor to general citrate accumulation. The difference in citric acid contents between the clones might be due to degradation by ACO as proposed by Sadka et al. (2001) for citrus.

The difference in ACO activity between the clones especially at the late stage of fruit development (Fig. 3B) could possibly explain the difference in citric acid content at harvest. The high acid clone showed lower ACO activity than the low acid clone at 16 WAF. The peak of ACO activity in the low acid clone was significantly greater than that in the high acid clone and this peak coincided with the decline in citric acid content in the low acid clone. This difference in ACO activities agreed with the finding from sour lime and sweet lemons where ACO activity declines earlier in sour lemon than in sweet lime (Sadka et al., 2000b). The result supports the hypothesis that metabolic reduction in ACO activity plays a role in citric acid accumulation (Bogin and Wallace, 1966).

The changes in enzymatic activities of PEPC, MDH and ME did not correlate with changes in citric or malic acids throughout fruit development (Fig. 4). This finding was in agreement with the difference in acid content of peach that also shows no correlation between acidity and *in vitro* PEPC, MDH and ME activities (Moing et al., 1998b, 2000). Gene expressions of those enzymes in peach showed similar patterns between the high and low acid cultivars and did not correlate with acid changes (Etienne et al., 2002). However, the difference in pineapple organic acid content could partially relate to PEPC, MDH and ME. The enzymes might account indirectly for fruit acid metabolism since their intermediate products such as OAA, malate and pyruvate could be transferred into mitochondria to support the TCA cycle.

The acid accumulation pattern shown by the low acid clone, a higher initial TA and citric acid content and a rapid decline near harvest altered the TSS/TA ratio at harvest. This change could be used commercially to adjust for seasonal variation in TSS/TA ratio and provide a more uniform product. Environmental and field management practices are known to alter pineapple fruit acid accumulation (Py et al., 1987; Paull and Chen, 2003), though data on low acid clones is limited. Potassium fertilization does play a role in fruit acidity (Py et al., 1987) with the response varying with soil type and environment. The fruit acidity accumulation response of low acid clones to field management practices needs to be determined as this will influence final fruit quality. The difference in pineapple ACO activity in the high and low acid pineapple clones could account for the difference in the citric acid accumulation, especially during the last few weeks of fruit development. CS and ACO gene expression and activities should be investigated further in other pineapple clones that vary in fruit acidity and in fruit that develop under different environmental conditions and field management practices that could affect pineapple fruit acidity.

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