# ORGANIC ACID METABOLISM AND ACCUMULATION DURING PINEAPPLE FRUIT GROWTH AND DEVELOPMENT

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## ABSTRACT

Pineapple fruit quality is mainly determined by the balance of acid to sugar content. The acidity of 'Smooth Cayenne' fruit changes significantly during the few weeks before harvest. To investigate fruit acid accumulation and metabolism, fruit from clone 36-21 a high acid clone and clone 63-555 (D10) a low acid clone were compared during the 11 weeks of fruit growth and development before harvest. The developmental changes in fruit acidity and sugar content were different between the high and low acid clones. Fruit acidity in the low acid clone increased comparatively earlier, peaked and sharply declined just prior to harvest. In contrast, the high acid clone gradually increased in acidity, peaked at a week before harvest and then declined slightly. At harvest, the high acid clone had higher fruit acidity than the low acid clone. Developmental changes in fruit acidity resulted from changes in fruit citric acid concentration due to a high relationship between citric acid concentration and fruit acidity. The fruit malic acid concentration varied only slightly before harvest in both clones.

Developmental changes in the activities of acid related enzymes citrate synthase (CS), aconitase (ACO), phosphoenolpyruvate carboxylase (PEPC), malate dehydrogenase (MDH) and malic enzyme (ME) were determined during fruit growth and development. CS activity greatly increased a week before harvest and the increase was coincident with the peak in the citric acid content of the high acid clone. Increased ACO activity was coincident with a sharp reduction in organic acid in the low acid fruit just before harvest. The activities of PEPC, MDH and ME did not directly relate to the changes in fruit acidity. The changes in fruit potassium were significantly correlated with the changes in fruit acidity in both clones during fruit growth and development, although the potassium concentrations were similar between clones at harvest.

It was suggested that the acid accumulation in pineapple fruit during fruit growth and development was mainly due to changes in citric acid concentration. The activities of CS and ACO and the fruit potassium content participated in the regulation of pineapple fruit acid metabolism and accumulation.

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| ACO | Aconitase |
| CS | Citrate synthase |
| HPLC | High performance liquid chromatography |
| MDH | Malate dehydrogenase |
| ME | Malic enzyme |
| PEPC | Phosphoenolpyruvate carboxylase |
| TA | Titratable acidity |
| TSS | Total soluble solids |
| WBH | Weeks before commercial harvest |

# CHAPTER 1

## INTRODUCTION

Pineapple (*Ananas comosus* L. Merrill) is the third most important tropical fruit worldwide after banana and citrus (Rohrbach et al., 2003). Approximately 70% of fruit production is consumed in the country of production (Loeillet, 1997). Pineapple flavor is a major factor determining fruit quality and is influenced by environmental factors. In addition, fruit size, an important marketing criteria, correlates with plant mass at flower induction (Malezieux and Bartholomew, 2003).

Variation in fruit acidity and sugar content are associated with fruit maturation and seasonal development (Singleton and Gortner, 1965). Sugars and organic acids considerably influence pineapple fruit flavor. The main nonvolatile organic acids found in pineapple fruit are citric and malic acids (Chan et al., 1973). During fruit development, sugar and acid contents change especially during the last few weeks of development (Singleton and Gortner, 1965; Smith, 1988). Fruit acidity increases until close to the start of ripening, then declines (Bartholomew and Paull, 1986). The increase in citric acid is associated with fruit maturity and reaches a peak prior to ripening whereas malic acid shows little change (Singleton and Gortner, 1965). Flesh acidity also increases distally from the central core outwards, from 4 to 10 meq $100mL^{-1}$ and from bottom to the top of the fruit (Huet, 1958).

Fruit acidity is influenced by external and internal factors. Seasonal weather including temperature and irradiance plays a crucial role in determining fruit acidity. Plant potassium levels are also correlated with fruit acidity and sugar content (Py et al., 1987). Genetic diversity within species and between cultivars determines phenotypic expressions including fruit characteristics and significantly influences fruit acidity and sugars (Sripaoraya et al., 2001).

The availability of cultivars of citrus, grape, apple and peach that differ in fruit acidity has facilitated the comparison of acid accumulation and the biochemical pathways involved (Beruter, 2004; Diakou et al., 2000; Moing et al., 1998; Sadka et al., 2000). The enzymes possibly involved in fruit acid metabolism are citrate synthase (EC 4.1.3.7), aconitase (EC 4.2.1.3), phosphoenolpyruvate carboxylase (EC 4.1.1.31), malate dehydrogenase (EC 1.1.1.37) and malic enzyme (EC 1.1.1.40).

In pineapple, the typical canning 'Smooth Cayenne' including clone 36-21 is considered an acidic fruit that is undesirable for fresh market due to excessive acidity during the cool season (Pauli and Chen, 2003). The clones 63-555 (D10) and 73-114 (MG-3) are characterized as low acid clones due to low acid content when ripe. The difference in fruit acid between these clones allows a comparative study of acid metabolism and accumulation in pineapple fruit.

The objective of this study was to understand organic acid accumulation during pineapple fruit development, via the comparison of pineapple clones that differ in fruit acidity. In the first study, temporal changes in fruit growth and physicochemical constituents of developing fruit were monitored during the cool and warm seasons. The activities of five enzymes involved in fruit citric and malic acid metabolism were also determined throughout fruit development. Finally, the effects of potassium application on fruit potassium and on fruit acidity during development were studied.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Introduction

Pineapple is monocotyledon plant that belongs to the Bromeliaceae that has 56 genera (Coppens de'Beckenbrugge and Leal, 2003). In *Ananas* genus, *A. comosus* is the most important economic crop and can be divided into five cultivars based upon leaf and fruit characteristics. The five cultivars are 'Cayenne', 'Queen', 'Spanish', 'Pernambuco' and 'Mordilona'. The differentiation may be due to ecological isolation rather than genetic variation (Aradhya et al., 1994; Duval and Coppens d'Eeckenbrugge, 1993). All cultivars are inter-compatible and their hybrids are difficult to classify into a group (Sripaoraya et al., 2001).

Though approximately 70% of pineapple production in the world is consumed as fresh fruit in the country of origin, the remainder (30%) is canned and plays a significant role in international trade (Loeillet, 1997). Due to its superior characteristics, 'Smooth Cayenne' is the most widely grown cultivar throughout the tropical world. About 70% of the world production and 95% of the canned pineapple comes from the 'Smooth Cayenne' (Rohrbach et al., 2003). Many clones have been derived from 'Smooth Cayenne' with local selection for growing in specific areas (Bartholomew et al., 2003).

The world became aware of pineapple after Columbus' journey to the new world in 1493. During the 15$^{th}$ to 16$^{th}$ centuries, pineapple was spread by the Portuguese to Africa, India and later to China and Japan, and by the Spanish to Europe and the Philippines. By the 1800s, pineapple was widely cultivated in tropical countries. The fruit processing and canning makes pineapple well known throughout temperate countries (Rohrbach et al., 2003). During 1900 to 1965, Hawaii was the center of the

world in pineapple technology and processing. The pineapple production in Hawaii declined in the latter half of the 20th century as production in Thailand and the Philippines rose (Rohrbach et al., 2003). At the same time, production in Central America, principally Costa Rica, became important.

'Smooth Cayenne' is not as suitable for fresh fruit because of their higher acidity, especially in winter. Production switched to clones to serve the domestic fresh fruit demand (Paull, 1993; Sanewski and Scott, 2000). Del Monte in Hawaii selected low acid clones from material bred by the Pineapple Research Institute in Hawaii and grew them in Costa Rica (Malezieux, 2000). The 'Queen' group is an alternative that meets specific needs for a high quality fresh fruit but has low yield and fruit defects (Loeillet, 1996).

## 2.2 Pineapple fruit acid development

### 2.2.1 Fruit development

After the initiation of reproductive development, the pineapple inflorescence continues to develop pathenocarpically to the mature fruit stage without interruption (Bartholomew et al., 2003). The environment influences fruit formation and fruit quality (Py et al., 1987). The time from flower induction to fruit maturity varies with cultivars. 'Smooth Cayenne' and 'Pernambuco' take longer than 'Queen' and 'Spanish' to mature (Py et al., 1987). Flowering begins with florets at the base of the inflorescence upward in a spiral manner (Dull, 1971). Fruit growth as measured by mass, volume, flesh and fruit shell follows a sigmoid pattern (Sideris and Krauss, 1938) or slight linear, with the highest rate just before harvest (Dull, 1971). Many biochemical processes occur during the late stage of fruit growth and development leading to changes in the chemical properties as the fruit reaches maturity. The most dramatic changes in composition occurs 3 to 7 weeks before the half-yellow (ripe) stage (Dull, 1971).

### 2.2.2 Acid development

Fruit acidity is one of the important parameters influencing fruit quality, especially for processing (Abd Shukor et al., 1998). The developmental changes in fruit acidity can be divided into two stages (Gortner et al., 1967). In the first stage, 7 to 4 weeks before harvest, the fruit total acid concentration and total soluble solids begin to increase. The crown growth rate declines and the fruit is low in soluble nitrogen and pigments (Gortner et al., 1967).

In the second stage, from 4 weeks before harvest until harvest, the fruit changes greatly in its biochemical characteristics that results in the first signs of ripening such as increases in total sugar, carotenoid synthesis, chlorophyll break down and a reduction in the rate of increase in fruit weight and size (Gortner et al., 1967). Fruit acid content also increases in this stage, peaks a week before harvest at 0.8% (as citric acid) in the summer; 1.2% in the winter and slightly declines toward the ripe stage concurrent with a linear decline in juice pH from 5.5 to 3.3 followed by a slight increase (Singleton and Gortner, 1965). The flesh acidity increases distally from the central core outward to the periphery (Huet, 1958).

The major organic acids in pineapple fruit are citric and malic acids accounting for approximately 60 and 30% of the total organic acids, respectively (Chan et al., 1973). The change in citric acid concentration is associated with fruit development and maturation. Short-term environment factors such as solar radiation do not appear to affect the citric acid level (Singleton and Gortner, 1965). Change in malic acid concentration is not related to fruit maturation but inversely related to evapotranspiration, and ascorbic acid is positively correlated to solar radiation (Singleton and Gortner, 1965). The minor organic acids are malonic, glycolic, tartaric and galacturonic acids, and they vary little during fruit development (Chan et al., 1973).

### 2.2.3 Sugar development

No starch accumulates in pineapple fruit. The total soluble solids (TSS) in 'Smooth Cayenne' increases rapidly during the last six weeks before ripening from 4 to 16% (Singleton and Gortner, 1965). The major sugars in pineapple fruit are sucrose, glucose and fructose. The increase in three sugars starts to occur about 30 days before harvest and continues up to harvest (Dull, 1971). At the ripening stage, sucrose reaches a peak and then declines, in contrast, glucose and fructose appear to continue to increase (Singleton and Gortner, 1965).

### 2.2.4 Fruit at harvest

Various fruit components determine the acceptability for fresh consumption and canning. Generally, fruit acidity and sugar content are some of the major characteristics contributing to eating quality (Abd Shukor et al., 1998; Py et al., 1987).

'Smooth Cayenne' fruit at the ripe stage rapidly loses shell chlorophyll, the flesh pH reaches minimum and begins to rise and maximum total soluble solids occurs (Dull, 1971). At this stage, the flesh is composed of 1.0-3.2% glucose, fructose 0.6-2.3%, sucrose 5.9-12.0%, starch less than 0.002%, cellulose 0.43-0.54%, pectin 0.06-0.16% by dry weight. The mineral present are potassium 11-330, phosphorus 6-21, calcium 7-16, magnesium 11, nitrate 120 and sulfur 7 mg $100g^{-1}$ fresh weight (Dull, 1971).

Fruit titratable acidity at harvest is 0.6-1.62% as citric acid with citric acid being 0.32-1.22%, malic 0.1-0.47% and oxalic 0.005% on a fresh weight basis (Dull, 1971; Singleton and Gortner, 1965). Generally, consumers appreciate the increase in sweetness, to more than 16% for the fresh market. In contrast, the acidity must be within certain limits. Too little acid means a fruit has little flavor and little aroma. Too much

acid reduces the sensation of sweetness and is also unpleasant (Py et al., 1987). Refrigeration of the fruit results in a slight increase in acidity.

Fruit sugar content is mainly composed of sucrose, glucose and fructose and is reflected in the total soluble solids (TSS) value measured by refractometer (Dull, 1971). TSS is an important parameter highly correlated to eating quality (Abd Shukor et al., 1998). Though desirability for TSS varies from place to place, generally, a minimum TSS requirement is set between 10 to 14%; and higher than 14% is suitable for the fresh market (Chan et al., 2003; Smith, 1988). Difference in TSS appears among cultivars such as 'Smooth Cayenne' 13.6%, 'A25-34' 17%, 'D6-85' 16%, 'Mas Merah' 11.3% and 'Red Spanish' 16%, respectively (Lizana et al., 1990; Nayar et al., 1981).

The ratio of TSS and acidity (% of acid in meq $100mL^{-1}$) is used as a measurement of consumer flavor perception, and should preferably be greater than 1.0, or 1.3 at harvest if fruit is to be refrigerated (Py et al., 1987).

## 2.3 Factors effecting pineapple fruit acidity

### 2.3.1 Genetic variety

'Smooth Cayenne' has been selected for cultivation and grown for many years as the superior cultivar for canning and fresh market. It is widely grown In the tropics under the name 'Kew', 'Champaka', 'Sarawak' and 'Pattavia' (Aradhya et al., 1994; Chan et al., 2003; Sripaoraya et al., 2001). Breeding programs that aimed to develop new cultivars for the fresh market are still based on 'Smooth Cayenne' (Chan et al., 2003). 'Smooth Cayenne' is probably not the best for fresh market due to its higher fruit acidity (Paull, 1993), some hybrid clones derived from 'Smooth Cayenne' breeding programs have lower acidity and higher sugar being more desirable to the fresh market. The Pineapple

Research Institute of Hawaii (PRI) developed and released potential clones that have better fruit characters for the fresh fruit market.

Desirable clones from PRI include PRI 73-50 ('CO-2') and PRI 73-114 (MD-2) are a good examples of fresh market fruit with high sugar (15-18%), high ascorbic acid and low acidity (0.4-0.9 meq $100mL^{-1}$) (Chan et al., 2003). PRI 63-555 ('Monte Cristo' or 'D-10) is a clone with low acidity and lower sugar (less than 13%). 'FLHORAN41' has high sugar, acid and aroma (Brat et al., 2004).

### 2.3.2 Temperature

Temperature plays a significant role in fruit growth and development, and determines the duration of fruit development which can range from 150 to 300 days (Py et al., 1987). Fruit development takes longer with increases in latitude and altitude due to a reduction of air temperature. Grape and citrus grown at higher altitude locations or during the cool period are more acidic than if grown in warmer areas (Lakso and Kliewer, 1978; Richardson et al., 1997)

Temperature determines pineapple fruit acidity that ranges from 5 to 20 meq $100mL^{-1}$ (Py et al., 1987). Fruit acidity is negatively correlated with minimum temperature and irradiance (Chadha and Shikhamany, 1974). Pineapple harvested in summer have lower fruit total acidity (12.54 meq 100 $mL^{-1}$) than those from the winter (15.30 meq $100mL^{-1}$). Difference in acid content between seasons is due to difference in citric acid content (Chan et al., 1973). In Hawaii, fruit harvested in summer has 13.3 meq $100mL^{-1}$ (0.85% total acidity as citric acid), in winter 19.5 meq $100mL^{-1}$ (1.25% total acidity as citric acid) (Bartholomew, 2003). Sugar content increases with an increase in temperature (Collins, 1960) and especially with an increase in mean air temperature during the 2 to 3 weeks before harvest (Nightingale, 1942).

### 2.3.3 Irradiance

Irradiance indirectly influences air temperature and affects fruit growth and development (Sideris and Krauss, 1936). Low irradiance leads to low air temperature and an increase in fruit acidity. Partly shaded plants due to increased plant density affects photosynthesis and results in a reduction in fruit weight and total soluble solids (Bartholomew and Kadzimin, 1977). Reduction of irradiance to 66% during flowering to the harvest period leads to an increase in free acid from 6.3 to 15.5 meq $100mL^{-1}$ and ascorbic acid from 364 to 636 μM.

Fruit ascorbic acid content is directly correlated to total sunlight 2 to 3 week before harvest, but negatively correlated to malic acid content (Gortner, 1963; Singleton and Gortner, 1965). Fruit acidity increases from 0.74 to 1.4% with a 50% decline in light but does not effect sugar content (Hamner and Nightingale, 1946). Cloudiness prior to harvest can increase fruit acidity. Shading fruit with opaque paper 3 weeks before harvest increases juice total soluble solids and titratable acidity, but reduces fruit translucency (Chen and Paull, 2001).

### 2.3.4 Water

Water supply is an essential factor for plant growth and development. Although pineapple shows CAM metabolism which has very high water use efficiency, sufficient water supply is required for the proper functioning of all metabolic activity including organic acid metabolism and translocation of photosynthates (Sideris and Krauss, 1933). A slight water stress appears to be less effect on growth and development than excess water stress.

Water supply also plays a role in fruit acidity and sugar content. Water deficiency during pineapple fruit development leads to low fruit acidity and sugar content (Py et al.,

1987) due to a reduction in assimilate translocated into the fruit (Linford, 1933) as the peduncle withers. Irrigation or rainfall after drought results in an increase in fruit acidity and total soluble solids.

### 2.3.5 Plant nutrition and fertilizer

Except for potassium, mineral absorption generally ceases during fruit development. The level of mineral nutrients at floral differentiation affects fruit development (Py et al., 1987). Mineral nutrient supplied as a final dressing just before flower differentiation is very important for plant growth and fruit mass. Insufficient nutrition at the flower induction period results in reductions in fruit weight, sugar, acidity, flavor and an increase in peduncle length (Py et al., 1987).

The total uptake of nutrients does not reflect a plant's nutrient requirement. Pineapple can take up more than the requirement for the optimum growth and this is referred to as luxury consumption (Malezieux and Bartholomew, 2003). Nitrogen and potassium are the most important elements influencing fruit mass and quality.

**Nitrogen:** Plant growth and fruit weight are mainly determined by nitrogen supply (Py et al., 1987). Absorption of nitrogen and potassium is relatively low during the first four months after planting, thereafter, the absorption increases until the flower induction period. Nitrogen can be effectively applied until two months after floral differentiation and fruit weight can be slightly increase (Teisson and Pineau, 1982).

Absorption of nitrogen fertilizer as luxury consumption, during vegetative growth results in reductions in fruit acidity and total soluble solids, increases in diameter of core and peduncle, peduncle length, fruit fragility, translucency and tendency to lodge and sunburn (Py et al., 1987). Over nitrogen fertilization for canning fruit leads to excess juice nitrate (Chongpraditnun et al., 2000; Scott, 1994).

**Potassium:** Potassium plays an essential role in fruit composition and quality. Generally, potassium absorption seems to increase until about 5% in the D-leaf dry weight and results in two fold increase in fruit free acidity (Neog et al., 1995; Py et al., 1987; Su and Li, 1963). The increase in fruit acidity indicates that potassium participates in organic acid metabolism (Marchal et al., 1981; Sanford, 1968; Tapchoi, 1990). Fruit total soluble solids are also influenced by the amount of potassium applied during fruit growth and development (Abd Shukor et al., 1998). An increase in potassium to the plant results in improvements of fruit sugars, aroma, ascorbic acid, flavor, shell color, fruit firmness, peduncle diameter and resistance to lodging (Gonzalez-Tejera and Gandia-Diaz, 1976; Py et al., 1987; Sanford, 1968; Tay et al., 1968). Application of potassium chloride after planting prevents internal browning (Marchal et al., 1981; Nanayakkara et al., 1997; Soares et al., 2005) and reduces flesh marbling disease (Verawudh and Thongleung, 2001)

Generally, potassium application just before floral differentiation is the most effective application time. Application during fruit development is slightly less effective (Py et al., 1987).

**Phosphorus, chloride and calcium:** Though phosphate is a macro-element, it seems rarely deficient for pineapple growth and development. Excessive phosphorus results in a reductions in fruit sugar and acidity but an increase in ascorbic acid (Tay et al., 1968).

Chloride ion from potassium chloride probably has negative effects on fruit weight and development (Sanford, 1968). However, potassium chloride application can be used without negative effects in some locations (Tapchoi, 1990; Verawudh and Thongleung, 2001).

Calcium plays an important role in the control of translucency, and postharvest internal browning. Calcium can be effectively applied pre-harvest and post harvest

(Herath et al., 2003; Hewajulige et al., 2003; Selvarajah et al., 1998; Talukdar and Ahmed, 2002).

## 2.4 Fruit organic acids and metaboiism

### 2.4.1 Fruit organic acids

Organic acids are an essential component of fruit quality, attributing to fruit flavor in combination with sugars (Sweeney et al., 1970). Organic acids also play an essential role in the respiratory metabolism of plant cells. The pulp and peel of many fruit are rich in organic acids, however, the concentrations differ depending on fruit type and position in the fruit. The carboxylic group (-COOH) present in organic acids determines fruit acidic properties. Phenols and ascorbic acid that have no carboxyl group are minor constituents contributing to a fruit acid properties (Ulrich, 1970).

The carboxyl group means that most of organic acids are dissolved in the cell solution or, at high concentration, crystallized such as calcium oxalate. Free carboxyl groups function as weak acid and associate with potassium ion generating buffering system that stabilizes pH in the cells (Ulrich, 1970). Changes in acidity during fruit development and storage vary with fruit type, though generally, fruit acidity is lost during ripening and storage.

The most common and abundant acids in fruit are citric and malic acids (Romero Rodriguez et al., 1992). Fruit in which citric acid predominates are lemon, citrus, guava, pineapple, pomegranate, medlar, tomato, strawberry, and kiwi fruit (Al-Maiman and Ahmad, 2002; Chan et al., 1973; ElBulk et al., 1997; Glew et al., 2003; Karadeniz, 2004; Marsh et al., 2004; Moing and Renaud, 2001; Sakiyama and Stevens, 1976). Apple, apricot, banana, cherry, and peach are rich in malic acid (Romero Rodriguez et al.,

1992; Ulrich, 1970; Wang et al., 1993; Wu et al., 2005). Grape's predominant acid is tartaric acid (Soyer et al., 2003).

### 2.4.2 Fruit acid metaboiism

Citric and malic acids are synthesized in the fruit pulp from photosynthetic assimilates (Talukdar and Ahmed, 2002; Tucker, 1993). The levels of malic and citric acids in some fruit species such as grape, pummelo and apple appear to be regulated by single major gene (Cameron and Soost, 1977; Visser and Verhaegh, 1978; Yoshida, 1970). However, many enzymes may be associated with organic acid metabolism and its accumulation in fruit.

Organic acids generally originate from photosynthetic assimilates translocated from a photosynthetic source into the fruit. Upon arrival at the fruit, the photosynthate is metabolized to organic acids (Tucker, 1993), although small amount of organic acids can be translocated in vascular tissues (Lopez-Bucio et al., 2000; Ramakrishnan, 1971). Since citric and malic acids are the major organic acids in fruit, most studies have emphasized their metabolism and accumulation. Generally, intermediate precursors in organic metabolism are other organic acids or sugars (Ulrich, 1970), therefore organic acids can be synthesized from sugar and vise versus. The main pathway for synthesis is via the respiratory oxidation, and carboxylation or decarboxylation reactions with specific reactions associated with individual acids (Ulrich, 1970).

Fruit respiratory oxidation occurs mainly in the tricarboxylic acid (TCA) cycle (citric acid cycle or Kreb's cycle). This cycle takes place in the mitochondrial matrix and provides the major oxidation reactions for organic acid production. The TCA cycle is the principal source of ATP and many of the intermediate metabolites used within cells (Ulrich, 1970). Organic acids found in fruit also occur in TCA cycle, although their amounts may vary and often are at low concentrations. Citric and malic acids are found

in large concentrations in fruit cell vacuole whereas some acids such as iso-citric acid are found in very low amounts (Ulrich, 1970). Carboxylation and decarboxylation are other metabolic processes that provide organic acids to fruit. β-carboxylation occurs in cytosol and involves carbon dioxide fixation by the activity of phosphoenolpyruvate carboxylase (PEPC) yielding oxaloacetate (OAA). PEPC is possibly the main enzyme accounting for fruit acid synthesis (Clark and Wallace, 1966). Decarboxylation of malate occurs by malic enzyme to give pyruvate and carbon dioxide (Ulrich, 1970).

Organic acids, after synthesized, are subsequently transported and compartmented into vacuoles. The final organic acid content in fruit is determined by the balance of the acid synthesis, degradation or utilization and storage compartmentation in the vacuole (Brune et al., 1998; Laval-Martin et al., 1977; Muller et al., 1996)

### 2.4.3 Citrate metabolism

Citric acid is the first metabolite of the TCA cycle and involves the activity of citrate synthase (CS) that occurs in the fruit cell mitochondria (Sadka et al., 2001). The citrate synthase catalyzes the condensation of oxaloacetate (OAA), a four-carbon acid, with an acetyl-CoA, a two-carbon molecule, generating citrate, a six-carbon acid. Citrate is subsequently isomerized into isocitrate by aconitase (AC) activity. The isocitrate is then oxidized by NADP-dependent isocitrate dehydrogenase (NADP-IDH) to yield oxoglutarate (Popova and de Carvalho, 1998). Therefore, CS probably plays a key role in citric acid synthesis whereas ACO and NADP-IDH are involved in citric acid degradation.

**Citrate synthase (CS):** Citrate synthase (EC 4.1.3.7) has been found in many forms in higher plants. Mitochondrial, glyoxisomal and peroxisomal forms have been reported (Kato et al., 1995; LaCognata et al., 1996; Papke and Gerhardt, 1996). The mitochondrial CS is located in the mitochondrial matrix and plays a central role in

production of energy and other metabolites by catalyzing the condensation of acetyl CoA and OAA to generate citroyl CoA, which spontaneously hydrolyzes to form citrate and CoA molecules (Hill, 1997).

The CS protein purified from pea leaves is composed of a single subunit of 50 kDa (Unger and Vasconcelos, 1989). In citrus, the mitochondrial CS is encoded by a single gene *cit1* (Canel et al., 1996; Sadka et al., 2001) and a single gene is also found in carrot (Takita et al., 1999). The gene *cit1* is not allelic to *acitric* that is found in '2240' acidless pummelo which is thought to cause very low acidity in homozygous individuals (Soost and Cameron, 1961), and they do not co-segregate together (Canel et al., 1996; Fang et al., 1997).

**Aconitase (ACO):** Aconitase (EC 4.2.1.3) catalyzes the isomerization of citrate into isocitrate, via cis-aconitate intermediate (Hill, 1997). This enzyme is composed of a single subunit of 90 to 100 kDa containing an Fe-S cluster at the active site (De Beilis et al., 1993). ACO is found in two forms in plants; mitochondrial and cytosolic (Hayashi et al., 1995). Both forms show similar kinetic and physical properties (Brouquisse et al., 1987). Mitochondrial ACO participates in the TCA cycle whereas cytosolic ACO may be associated with the glyoxylate cycle (Courtois-Verniquet and Douce, 1993).

In the *Arabidopsis* genome, ACO is encoded by only one gene. It is possible that a single gene encodes both the mitochondrial and cytosolic isoforms (Peyret et al., 1995). In citrus, citrate metabolism is depended on both mitochondrial and cytosolic ACO activities (Sadka et al., 2000b). Cytosolic aconitase is homologous to the mammalian iron-regulated proteins (IRPs) which functions under optimum iron availability. When iron concentration is limited, cytosol ACO loses its activity and bind to its mRNA to play a role in iron homeostasis (Sadka et al., 2000b).

### 2.4.4 Malate metabolism

Malate plays a unique role in carbon metabolism. In CAM plants, malate accumulation is involved in carbon assimilation and acts as a major charge balancing in the vacuole. In fruit, the key enzyme in malic acid biosynthesis is cytosolic phosphoenolpyruvate carboxylase (PEPC) catalyzing carboxylation of phosphoenol-pyruvate (PEP) to yield oxaloacetate (OAA) and inorganic phosphate (O'Leary, 1982)). The OAA is reduced by NAD-dependent malate dehydrogenase (MDH) to generate malate. Both malate and oxaloacetate can enter into mitochondrial TCA to produce citrate and other metabolites. Malate degradation can also occur by cytosolic-NADP dependent malic enzyme (ME) (Ruffner, 1982) or the oxidation reaction of NAD-MDH in the reverse direction of MDH.

**Phosphoenolpyruvate carboxylase (PEPC):** Phosphoenolpyruvate carboxylase (EC 4.1.1.31) catalyzes the irreversible carboxylation of phosphoenolpyruvate to yield oxaloacetate and Pi in the presence of Mg and/or Mn. This enzyme is found widely in plants and microorganisms and plays a role in carbon and nitrogen metabolism (Vance, 1997b). PEPC is encoded by a small gene family. The expression of PEPC in ice plant is regulated by at least three genes (Vance, 1997a). The enzyme is a tetramer composting of subunits of 100 to 110 kDa. Many PEPC isoforms have been detected in both C4 and CAM plants (Cretin et al., 1991). The regulation of the enzyme is achieved by transcription and post-translation controls (Cretin et al., 1991).

PEPC post-translational regulation is achieved by phosphorylation of the protein via PEPC kinase (Chollet et al., 1996) and malate, glucose-6-phosphate and protein turnover (Jiao and Chollet, 1991). In C4 and CAM plants, the phosphorylated form is less sensitive to feedback inhibitor by malate (Bakrim et al., 1993). *In vivo* PEPC activity is strongly modified by cytosolic pH and malate concentration, and regulated via

phosphorylation via specific kinase (Chollet et al., 1996). In banana and peach, PEPC is very sensitive to inhibition by malate and low pH (Law and Plaxton, 1995; Moing et al., 2000).

**Malate dehydrogenase (MDH):** Malate dehydrogenase (EC 1.1.1.37) catalyzes the reduction of OAA to malate in cytosol. The reaction is coupled to cofactor oxidation/reduction and is important in cellular metabolism (Munoz et al., 2004). MDH is a ubiquitous enzyme in plants and participates in many catabolic and anabolic pathways (Gielt, 1992). There are many isoforms found in higher plant which differ in their localization and specificity for the cofactors NAD or NADH (Gielt, 1992). MDH showed a subunit molecular mass of 39.4 KDa. Maximum activity for OAA reduction and malate oxidation are at pH 7.0 and 7.2 to 8.4, respectively (Tripodi and Podesta, 2003). MDH from grape berries is not significant different in Km for OAA and malate in the forward or reverse directions of catalysis (Taureilles-Saurel et al., 1995b).

**Malic enzyme (ME):** NADP-linked malic enzyme (EC 1.1.1.40) is found only in plants and catalyzes the oxidative decarboxylation of malate to give pyruvate and carbon dioxide in the present of Mg or Mn (Goodenough et al., 1985). Other forms of ME (EC 1.1.1.38 and EC 1.1.1.39) are differentiated by their distribution, specificity for NAD or NADP and ability to decarboxylate malate (Edwards and Andreo, 1992).

NADP-ME composts of four subunits, each of molecular mass of 63 kD (Franke and Adams, 1992). This enzyme is restricted to the cytosol (Goodenough et al., 1985) and is present in every tissue (Knee et al., 1996). The enzyme has a general function in plant metabolism, probably not a specific role in fruit development or ripening (Knee et al., 1996). NADP-ME is a component of malate decarboxylation system that provides partial substrate for fruit respiration without glycolysis (Munoz et al., 2004). The increases in NADP-ME activity in many fruits are coincident with an increase in

respiration rate (Dilley, 1962; Edwards and Andreo, 1992). ME does seem to play an important role in malate remobilization during fruit maturation (Taureilles-Saurel et al., 1995a)

### 2.4.5 Acid metabolism in some fruit

**Citrus:** Citric acid is the major organic acid, and is approximately 90% of the total acid that accumulates in the pulp. The acid begins to accumulate early in fruit development, reaches a peak and then declines toward fruit maturation. Citrate synthase plays an important role in citric acid synthesis observed in citrus. For instance, CS activity in Satsuma mandarin and sour lemon shows a remarkable increase in activity prior to the increase in citric acid during the first half of fruit development (Hirai and Ueno, 1977; Sadka et al., 2001).

A reduction of citric acid synthesis caused by enzyme inhibition confirmed that CS plays a key role in citric acid synthesis. Arsenic compounds can reduce CS activity and CS transcript resulting in low citric acid synthesized (Sadka et al., 2000a; Vines and Oberbacher, 1965; Yamaki, 1990). These results confirmed that CS plays a major role in citric acid synthesis since blocking of CS activity results in a reduction in the amount of citric acid produced.

Nevertheless, when low acid or acidless fruit are compared with the normal acid fruit, the CS activity does not account for the low acid accumulated in those fruit. No differences were found in fruit CS activities between sweet lime and Satsuma mandarin during fruit development (Hirai and Ueno, 1977; Sadka et al., 2000a). CS transcripts and enzyme activity are similar between normal acid and mutant acidless pummelo fruit (Canel et al., 1996). This difference in fruit acidity between citrus cannot be accounted for by CS activity. Fruit acidity could be explained in terms of different regulatory

mechanisms being involved at different stages of fruit development rather than CS activity (Sadka et al., 2001).

ACO plays a role in citric acid metabolism by the regulation of acid degradation as opposed to CS activity. Bogin and Wallace (1966) found that sour lemon is higher in citramalate, a competitive inhibitor of aconitase, than sweet lime. The authors suggested that a reduction in mitochondrial aconitase activity may play a key role in citrate accumulation by creating a partial or complete block of the TCA cycle that leads to create a local increase in citric acid. This was supported by Sadka et al. (2000) who found that sour lemon is lower in mitochondrial ACO activity than sweet lime during fruit development. The cytosolic ACO activity remains stable toward the end of fruit maturation and it is suggested that the low activity of mitochondrial ACO played a role in acid accumulation whereas an increase in cytosolic ACO during the late stage of fruit development leads to decline in acid toward fruit maturation (Luo et al., 2003). Although PEPC activity in Satsuma mandarin increases before the increase in citric acid during the first half of fruit development (Hirai and Ueno, 1977), it cannot account for the difference in acidity in low acid fruit.

**Peach:** The predominant organic acids in peach are malic and citric acids. Malic acid accumulates during the first phase of rapid growth whereas citric acid accumulates during the second growth phase, before the onset of ripening (Moing et al., 1998b). PEPC activity, mRNA expression and protein concentration of high and low acid peaches showed that expression and activity paralleled the increase in acidity. This suggests that PEPC participated in organic acid accumulation in the normal acid fruit. However, it does not fully explain the difference in acidity between varieties (Moing et al., 2000). *In vitro* and *in vivo* activities of MDH and ME in high and low acid peach during

fruit development are not correlated with the difference in fruit acidity (Moing et al., 1998a).

Gene expression of CS, MDH, IDH are similar between high and low acid peaches. Their expression is stronger during fruit ripening than earlier phases of fruit development (Etienne et al., 2002). However, the expressions do not also correlated with the difference in acid content between varieties.

**Grape:** Malic acid is the most abundant organic acid in grape and it accumulates prior to the onset of ripening, and then declines toward maturity. During berry development, PEPC transcript is detected in the early stage when malic acid accumulates. MDH and ME transcripts peaked in the young berry, declined toward the onset of ripening, and then increased again during ripening when there is a large decline in acidity. It is suggested that malate metabolism in grape likely involves the regulation of all three enzymes. However, Diakou et al. (2000) found that protein level and PEPC activity is greater in low acid berry than in normal acid berry, and PEPC does not account for the low acid content in the low acid berry.

Nevertheless, there is no correlation between the changes in malic acid concentration and ME activity in fleshy tissue or mesocarp. It is suggested that fruit malate maybe regulated by acid compartmentation rather than enzyme availability. However, if malate storage in the vacuole is interrupted in low acid grape, cytosolic malate could be catabolized by ME before maturation (Gutierrezgranda and Morrison, 1992).

**Cherimoya:** In cherimoya, the increase in fruit titratable acidity during ripening is due to a higher activity of MDH rather than ME. The acidity seems to be controlled by the balance of malic synthesis and consumption (Munoz et al., 2004).

In summary, the regulation of fruit acid accumulation seems to be associated with acid synthesis and degradation and varies with a particular fruit at different stages of development. However, the major control step may vary from fruit species to species. Other mechanisms may also play a role in fruit acid accumulation.

### 2.4.6 Organic acid compartmentation

The organic acids after synthesis are transported and accumulated in the fruit vacuole. The accumulation of those acids is developmentally regulated by tonoplast permeability. Malate and citrate seem to cross the tonoplast by means of the same carrier (Rentsch and Martinoia, 1991).

The electrochemical gradient for $H^+$ to cross the tonoplast is created by two types of proton translocating pumps presenting in tonoplast of fruit cells (Oleski et al., 1987). The energetic uptake is driven by $H^+$-ATPase or $H^+$-pyrophosphatase (Martinoia and Rentsch, 1994) that pump protons into the vacuole (Muller et al., 1996). Therefore, it is possible that vacuolar $H^+$ATPase (V-ATPase; EC 3.6.1.3) and vacuolar $H^+$-pyrophosphatase (V-PPase; EC 3.6.1.1) may play a role in the storage of organic acids in vacuole (Maeshima, 2000; Ratajczak, 2000).

Moing et al. (1998a) suggested that low acid content in low acid peach is probably due to the interruption of malate compartmentation into the vacuole that limits malate accumulation in the mesocarp. The pattern of gene expression of $H^+$-ATPase and $H^+$-PPase is stronger during the acid accumulation stage and ripening stage, than earlier phases of fruit development. However, the expression between high and low acid peaches does not correlated with the changes in acids of the low acid peach (Etienne et al., 2002).

In citrus, pulp acidity is thought to be depended on citric acid accumulation in the vacuoles (Sadka et al., 2000b) and acidification of the vacuole by tonoplastic $H^+$-ATPase, creating proton influx and reduces the vacuolar pH to about 2.5 (Muller et al., 1996). However, the ability of tonoplast vesicles from acidless pummelo sac cells to corporate radioactive citrate is higher than vesicles from high acid fruit. The $^{14}C$-citrate uptake is stimulated by nitrate-sensitve ATP hydrolysis. No evidence exists for a defective acid transport at the tonoplast of acidless fruit and tonoplast transport functions normally in these fruit (Canel et al., 1995). The results indicated that the vacuolar transport plays a partial role in acid accumulation; however, it may not be a key factor.

## 2.5 Potassium and plants

Potassium is an essential cation for plant growth and development that plays a significant role in many biological processes such as photosynthesis, photosynthate translocation, maintenance of water potential and cell volume, hormone secretion, stomatal regulation, activation of enzymes and the reduction of excess uptake of ions such as sodium and iron in saline and flooded soils (Jones, 1998; Mengel and Kirkby, 2001).

### 2.5.1 Existence and regulation in plant

Potassium absorption by the plant exceeds its requirement, leading to luxury consumption (Jones, 1998). For instance, the potassium content in leaf tissue is approximately 1 to 5% of the dry weight whereas an adequate potassium is only 1.5 to 3%. Potassium concentration in plant tissue varies by tissue and localization; high concentrations are found in new leaves, petioles and stems.

Potassium in soil exists in various forms, as soluble-K, exchangeable-K on soil colloids and fixed-K in soil material. Availability of potassium in soil is influenced by the

clay fraction, the potassium dynamics in the rhizophere and potassium from the subsoil (Beringer, 1985). In contrast, potassium in plant exists only as a cation resulting in simplified extraction from the tissue (Jones, 1998). The external supply of potassium determines the potassium concentration in tissues and regulates plant growth (Leigh and Wyn Jones, 1984).

In plant cells, potassium ion is found in the cytosol and vacuole (Leigh, 2001). Cytosolic potassium concentration is about 80 mM and it is maintained at that level until extreme K deficiency occurs. Walker et al. (1998) reported that potassium concentration in barley root cytosol remained relatively constant at 75 to 83 mM. At this concentration, cytosolic potassium can play an essential role as an activator of biochemical processes, particularly protein synthesis (Leigh and Wyn Jones, 1984; Walker et al., 1998). To obtain high yield, potassium in the cell sap needs to be 150 to 200 mM depending on plant species, the extent of the root system and potassium content in the harvested products (Beringer, 1985).

In contrast, vacuolar potassium concentration is variable and directly related to external potassium supply. Since the vacuole occupies the greatest intracellular volume, change in vacuolar potassium are the major determinant of potassium concentration in the tissue (Leigh and Wyn Jones, 1984). The concentration can decline to very low level without severe potassium deficiency occurring (Asher and Ozanne, 1967; Leigh, 2001). Nevertheless, the high concentration of vacuolar potassium is maintained when potassium is over supplied (Asher and Ozanne, 1967).

### 2.5.2 Uptake and translocation

Potassium uptake occurs via passive and active transports at low concentration of potassium (<0.5 mM) in the outer solution (Cheeseman and Hanson, 1979). The rate

of potassium uptake is depended on the relative permeability of cell membrane that results from potassium channels located in the membrane (Mengel and Pfluger, 1972).

All known potassium channels are related members of a single protein family that are found in eukaryotes and prokaryotes. Their highly conserved sequence (called K+ channel signature sequence) forms a structural element, the selective filter, which prevents sodium ion but allows potassium ion to cross the membrane. This provides the specific selectivity for potassium over sodium ion (MacKinnon, 2003).

At the plasma membrane, potassium uptake under low external concentration is mediated by a high-affinity, $K^+$-selective, saturable mechanism (System 1). In *Arabidopsis* roots, this system 1 is probably an active $K^+$:$H^+$ symporter (Maathuis and Sanders, 1993). Under higher external potassium concentration, a second less selective mechanism (System 2) operates, at the tonoplast, and has active transporter functions to move potassium from cytosol to vacuole (Walker et al., 1996).

Potassium ion is abundant in the cytosol and phloem sap and can be translocated upward or downward throughout the entire plant generally toward younger tissues. The bulk of potassium translocation mainly occurs during the vegetative growth stage (Mengel and Kirkby, 1987).

### 2.5.3 Role in plants

Potassium ion plays a significant role on plant growth and development. It participates in many physiological and biochemical processes such as plant water regulation, enzyme activation, photosynthates transloation, cellular oxidative regulation etc (Cakmak, 2005). sufficient plant potassium supply leads to increases in yield and fruit quality (Dass and Srivastava, 1997; Morris et al., 1980; Quaggio et al., 2002).

**Water status:** Potassium plays a significant role in plant water status since water uptake into tissues or cells is the consequence of potassium uptake and concentration via osmotic maintenance (Mengel and Kirkby, 1987). The water potential of young leaf cells of *Phaseolus vulgaris* is directly related to the potassium content (Mengel and Arneke, 1982) which indicated that potassium is essential to maintain plant water potential. Moreover, potassium plays a significant role in stomatal opening and closing (Humble and Raschke, 1971) by regulating osmotic potential. During stomatal opening, potassium concentration of guard cells is greater than that of guard cells when the stomata are closed.

**Enzyme activation:** Potassium ion is the most effective biochemical activator and, as a cofactor, is superior to sodium ion. As a cofactor, it plays a significant role in activation of more than forty enzyme activities. Cytosol potassium concentration is responsible for activating enzymes present in the cytosol. Change in potassium concentration affects enzymatic reactions and metabolic processes. Tomato fruit grown under potassium deficiency showed a reduction of malate dehydrogenase activity, but increase in respiration during the climacteric period (Besford and Hobson, 1975).

**Photosynthesis:** Potassium plays an essential role in photosynthesis, although potassium does not directly participate in photosystem I or II. The role of potassium is in the regulation of carbon dioxide assimilation and also the reduction of carbon dioxide resistance in the mesophyll (Peoples and Koch, 1979). The ion functions as a enzyme activator of ribulose bisphosphate carboxylase (RuBP) in carbon dioxide fixation process (Demmig and Gimmler, 1983). Furthermore, potassium is directly associated with the photosynthetic electron transport chain.

Plants under potassium deficiency are extremely sensitive to light intensity that leads to chlorosis and necrosis due to photooxidative damage to the chloroplast

(Cakmak, 2005). The reduction of photosynthesis seems to be related to reduced stomatal conductance, increased mesophyll resistance and lower ribulose bisphosphate carboxylase activity (Peoples and Koch, 1979; Zhao et al., 2001).

**Translocation:** Potassium participates in assimilation and translocation of photosynthates due to its concentration at the sink and source (Addiscott, 1974). Potassium directly influences the promotion of phloem loading by depolarization of the plasma membrane (Malek and Baker, 1977) and enhances the translocation of new photosynthates and remobilization of stored materials (Hartt, 1970). A lack of K results in severe decrease in phloem export of sucrose from source leaves (Cakmak and Marschner, 1994). In wheat during spring, potassium increases the mobilization of proteins stored in stems and leaves and promotes translocation of the nitrogenous degradation compounds to the grains (Koch and Mengel, 1977).

**Drought resistance:** Drought stressed plants require large amount of potassium. This stress is apparently related to reduction of photosynthesis and carbohydrate metabolism (Jiang and Zhang, 2002). Under low potassium supply, drought stress induces greater production of reactive oxidation agents due to, at least, disturbance in stomatal opening, water relations, and reduced photosynthesis (Marschner, 1995; Sen Gupa and Berkowitz, 1987). Adequate levels of potassium are required to maintain photosynthesis and protect chloroplasts from oxidative damage (Cakmak, 2005).

**Salt stress:** Excessive soil salinity results from sodium chloride concentration and leads to a reduction of crop production due to ion toxicity and water deficiency (Zhu, 2001). Salt stress affects photosynthesis and causes oxidative stress by inducing water deficiency. Increase in potassium concentration in plant cells under salt stress condition may be necessary to minimize oxidative cell damage by, at least, reduction of reactive oxidation agents during photosynthesis (Shen et al., 2000).

**Iron toxicity stress:** Iron toxicity commonly occurs in wetland rice production and results in the loss of growth and yield (Neue et al., 1998). Iron toxicity symptoms are enhanced by potassium deficiency (Li et al., 2001). Increase in potassium supply can reduce iron concentration in leaves and improve plant growth due to enhancing root-oxidizing capacity for iron and preventing iron uptake (Neue et al., 1998).

### 2.5.4 Deficiency symptoms

Potassium deficiency symptoms are invisible in the initial stages. The symptoms develop from the reduction of plant growth and then, visibly, marginal chlorosis and then the development of necrosis, primarily at the leaf margin and between veins. In monocots, these necrotic lesions may form at the leaf tips and then extend toward the leaf base (Mengel and Kirkby, 1987).

Generally, the symptoms are initially observed from the mature leaves of the base of the plant due to remobilization of potassium toward younger leaves. Some potassium deficient plants are susceptible to lodging and sensitive to diseases (Bloom, 2002).

### 2.5.5 Potassium fertilizers

Muriate of potash or potassium chloride (KCl) is the most widely used and cheapest potassium source and it contains about 60% $K_2O$ (50% K). Other fertilizer forms are potassium sulfate ($K_2SO_4$), potassium nitrate ($KNO_3$) and potassium magnesium sulfate ($K_2SO_4.MgSO_4$). (Mengel and Kirkby, 1987).

Application of KCl to some crops may reduce growth and yield due to the crop sensitivity to high concentration of chloride ion. Some chlorophobic species include grape, fruit trees, cotton, tobacco, potato, tomato, strawberry, cucumber and onion (Mengel and Kirkby, 1987). The application of KCl or $K_2SO_4$ at rate 120-240 kg $ha^{-1}$ $K_2O$

on maize, sunflowers, potatoes, tomatoes, cabbage showed similar plant growth and development. It was indicated that at this rate, potassium chloride does not result in excessive chloride ion accumulation, and suggested that chloride-sensitive crops such as potatoes and tomatoes can be fertilized with potassium chloride due to its inexpensive cost (Milcheva et al., 1988).

# CHAPTER 3

# HYPOTHESES AND OBJECTIVES

## 3.1 Hypotheses

Fruit acidity is influenced by external and internal factors at different stages of fruit growth and development. The variation in fruit acidity at harvest is due to differences in fruit acid accumulation during pre-harvest period. The availability of high and low acid clones allows the investigation of differences in the pattern of changes in fruit acid content during fruit growth and development. Seasonal variation could possibly plays a role in fruit acid accumulation due to differences in temperature, irradiance and relative humidity and the crop can therefore be evaluated in different seasons.

Fruit acid synthesis, compartmentation and degradation play a role in fruit acid content. Differences in fruit acidity between cultivars may be due to difference in enzymatic activities during fruit development, especially the metabolism of citric acid, the major organic acid in pineapple fruit.

Since potassium plays a role in sugar transportation, differences in the pattern of change in fruit acidity and sugar content are possibly correlated with fruit potassium concentration. Increases in fruit potassium by potassium fertilizer application may increase fruit acidity and sugar contents.

## 3.2 Objectives

I. Determine the developmental changes in the high and low acid pineapple cultivars in fruit physical and chemical components and determine the seasonal influences on fruit growth and development.

II. Determine the metabolic activities of citrate synthase (CS, EC 4.1.3.7), aconitase (ACO, EC 4.2.1.3), 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) that could determine fruit acid contents between the high and low acid cultivars during fruit development.

III. Determine the pattern of changes in fruit potassium between the high and low acid cultivars, to correlated to fruit acidity and fruit sugar content, and determine the effect of potassium chloride fertilizer on fruit acidity.

# CHAPTER 4

# CHANGES OF LOW AND HIGH ACID PINEAPPLE FRUIT DURING GROWTH AND DEVELOPMENT

## 4.1 Introduction

Fruit acidity is one of the parameters that determines fruit quality at harvest and is due to acid metabolism and accumulation during fruit growth and development. A few economically acid fruits have been studied to understand factors that effect fruit acid developmental changes. In addition, the availability of cultivars or clones that differ in acid accumulation facilitated comparative studies of acid metabolism in apple (Beruter, 2004; Kabu and Chopra, 1988), peach (Genard et al., 1999; Moing et al., 1998), citrus (Sadka et al., 2001; Vu et al., 1995), and grape (Diakou et al., 2000; Terrier et al., 2001). The insights from this research provide a partial understanding of acid metabolism and accumulation and may apply to other fruits including pineapple.

'Smooth Cayenne' pineapple is one of the most important fruit consumed both fresh and processed (Chan et al., 2003). In Hawaii, pineapple requires 18 months to produce the first fruit on the plant crop (Bartholomew et al., 2002). After initiation of the reproductive organ, the inflorescence continues to develop to mature fruit stage without interruption (Bartholomew et al., 2003). Fruit growth as measured by mass and volume shows a sigmoid or slightly linear increase (Sideris 1938; Dull, 1971). Many biochemical processes occur during pineapple fruit growth that lead to physical and chemical changes associated with maturation. Sugar and acid contents change, especially in the last weeks of development (Gortner et al., 1967; Smith, 1988).

The acid content of the fruit increases and peaks at 0.8%, a week before harvest and declines toward the ripe stage (Singleton and Gortner, 1965), coincident with this

pattern is a linear decline in juice pH from 5.5 to 3.3, and the slightly increases just before harvest. Change in total soluble solid (TSS) shows a sigmoid curve and peaks about 30 days before the ripe stage at 14-15% (Singleton and Gortner, 1965). TSS/acid ratio slightly declines from 60 to 25 during the 10 to 2 weeks before harvest (Singleton and Gortner, 1965). Variations in acid and sugar contents are associated with seasonal changes (Singleton and Gortner, 1965). The major non-volatile organic acids in pineapple fruit are citric and malic acids which account for approximately 60 and 30% of the total organic acid, respectively (Chan et al., 1973).

Recently, developmental changes in pineapple fruit composition had been reported for 'Red Spanish' (Bartolome et al., 1995), the new hybrid FLHORAN41 (Brat et al., 2004), and 'Kew' (Kermasha et al., 1987). However, little information is available on acid metabolism and accumulation in pineapple fruit. The availability of pineapple clones with differences in acid content at harvest allowed us to investigate and compare acid accumulation during fruit growth of Dole clone 36-21 and 63-555 ('D10'), high and low acid clones, respectively.

The objective of this study was to compare changes in the fruit's physical and chemical components during fruit growth and development between the high and low acid clones. The understanding of acid temporal changes and accumulation in pineapple flesh should provide preliminary indication of differences in pineapple fruit acidity.

## 4.2 Materials and methods

### Fruit material

Uniform fruit of 36-21 and 63-555 ('D10') clones were harvested as high and low acid clones, respectively, from two crop production cycles. The fruit were harvested

from the Dole Fresh Fruit Co. plantation on the island of Ohau, Hawaii. In the first cycle, fruit were sampled between March and June and the second cycle between May and August, 2003. The two cycles were from fruit that developed during the cool and warm seasons, respectively. Fifteen uniform fruit were randomly sampled throughout fruit development, biweekly from 11 to 3 week before harvest (WBH) and weekly from 3 WBH to the week of commercial harvest (0 WBH) . The fruit were transferred to the laboratory within two hours. Five fruit of clone 73-50 ('D30'), another low acid fruit clone, were harvested in the few weeks before commercial harvest for comparison with 'D10' and '36-21'.

**Measurements of fruit physical characteristics**

**Fruit and crown weight**

The crown was removed from the whole fruit. The crown and the fruit (without crown) were weighed separately.

**Fruit size**

Fruit length was measured longitudinally from top to the base of fruit. Fruit diameter was determined at the equator of the fruit cut longitudinally.

**Measurements of fruit chemical characteristics**

**Flesh juice sample extraction**

Two pieces of flesh were removed from opposite side of the equatorial part of the fruit and squeezed and 20 mL juice collected. The juice was centrifuged at 10,000 g for 10 min. The juice pH, titratable acidity (TA), total soluble solids (TSS) and total sugar determined on the supernatant. A 2 mL aliquot of supernatant was diluted with 95% ethanol to 1/5 volume and stored at -20°C for quantification of organic acid by high performance liquid chromatography (HPLC).

**Juice pH and titratable acidity (TA)**

Juice pH and TA was determined by titration with 0.1 N NaOH at endpoint pH 8.3 (Radiometer ABU 80 auto-burette). Titratable acidity was expressed as meq $100mL^{-1}$ juice and percentage of acidity as citric acid.

**Total soluble solid (TSS)**

TSS of flesh juice was determined by refractometor.

**Total sugar**

Total sugar was analyzed according to the modified phenol-sulfuric acid method of (Dubois et al., 1956). A 1 mL aliquot of an one hundredth diluted juice was mixed with 25 µL of 80% phenol, 2.5 mL of concentrated sulfuric acid was added and mixed and allowed to stand for 10 min before mixing again before standing for another 30 min and absorbance measured at 485 nm. Total sugar was determined relative to a glucose standard.

**Organic acid quantification**

The frozen diluted juice supernatant was prepared for HPLC using a modification of the procedure of Paull et al. (1983). The alcoholic solution was air-dried and mixed with deionized water. A 20 µL aliquot of the solution was filtered though a 0.45 µm filter and degassed before injection into HPLC. The HPLC column was organic acid column (Bio-Rad HPX-87H, 7.8 x 300 mm). The solvent was 0.05 N sulfuric acid at a flow rate of 0.8 mL $min^{-1}$ and monitored at 210 nm. Peak area was measured and calculated relative to standard solution. A sample injection was a combination of three fruit samples.

### Weather data collection

A weather data logger was set up within 2 km of the sampled blocks to record the weather data throughout the crop production periods. The data recorded were maximum and minimum temperature, relative humidity and photosynthetic active radiation (PAR). The accumulation of daily thermal time (DTT) was calculated by the equation: DTT = TM - TB, where TM is the mean temperature and TB is the base temperature of 16°C (Malezieux et al., 2003).

## 4.3 Results

The data recorded were presented as cool and warm seasons and combined data.

### Fruit physicai changes during growth and deveiopment

### Fruit weight

From 11 weeks before harvest (11 WBH) to commercial harvest week (0 WBH), fruit weight of both clones increased from approximately 650 to 1900 g (Figure 4.1). The fruit weight at commercial harvest was not significant different between clones. The pattern of change in fruit weight was linear during 11 to 1 WBH period and was stable in the few weeks before harvest. During the early stage of fruit growth, fruit weight was significantly higher in low acid clone than in high acid clone. However, from 3 WBH until commercial harvest, fruit weight of two clones was not significantly different.

### Crown weight

Crown weight increased from about 50 to 290 g in the high acid clone and to 250 g in the low acid clone in the 11 to 0 WBH period (Figure 4.2). The crown weight of two clones was not significantly different during the early stage 11 to 7 WBH, thereafter the high acid clone increased in crown weight at a greater rate than in the low acid clone. Crown weight during the last two weeks before harvest did not increase in both clones.

**Fruit length and diameter**

Fruit length of both low and high acid clones increased from 13 cm at 11 WBH to 18 cm at commercial harvest with similar linear pattern of change (Figure 4.3). There was no significant difference between two clones. The diameter at the fruit equator of the low and high acid clones increased from 9 cm at 11 WBH to 13 cm at commercial harvest with a similar pattern during fruit development (Figure 4.4).

Both the high and low acid clones showed a similar pattern of fruit growth and development when characterized by fruit weight, length and diameter except crown weight.

**Fruit chemical changes during growth and development**

**Juice pH**

Flesh juice pH declined throughout fruit development with a different pattern being shown by the two clones (Figure 4.5). At 11 WBH, juice pH of the high acid clone was 4.5, versus 4.0 for the low acid clone. Juice pH of both clones then declined gradually toward maturity by 3 WBH, both clones had a juice pH of 3.3. Thereafter, juice pH of the high acid clone continued to decline to pH 3.1 at commercial harvest, while the pH of low acid clone increased to 3.7 at commercial harvest.

**Titratable acidity (TA)**

Juice TA of both clones differed in concentration and pattern of change during fruit development (Figure 4.6). From 11 to 2 WBH the low acid clone had significantly greater TA than the high acid clone and increased from about 2.4 and 1.4 meq $100mL^{-1}$ at 11 WBH to 8.8 and 7.5 meq $100mL^{-1}$ at 2 WBH, respectively. Subsequently, the TA of low acid clone rapidly declined to 5.7 meq $100mL^{-1}$ at commercial harvest. The high

acid clone TA continued to increase and plateau at 9.3 meq $100mL^{-1}$. At commercial harvest, the high acid clone's TA was about 70 % higher than the low acid clone.

**Total soluble solid (TSS)**

Both the high and low acid clones exhibited similar developmental pattern of TSS increase from 11 WBH (Figure 4.7). However, the low acid clone showed significantly greater TSS than the high acid clone from 9 WBH, while high acid clone increased just after 7 WBH. At commercial harvest, the TSS of the low acid clone was about 13% and that of the high acid clone of about 11%.

**Total sugar**

The patterns of total sugar changes in both clones were similar, increasing from 11 WBH (Figure 4.8). From 9 WBH until commercial harvest, total sugar of the low acid clone increased at a higher rate than that of the high acid clone. At commercial harvest, the total sugar of low and high acid clones was 107 and 94 mg $mL^{-1}$, respectively.

**Citric acid concentration**

Citric acid content in both high and low acid clones differed during fruit development (Figure 4.9). The citrate content in the low acid clone increased from 0.5 mg $g^{-1}$ at 11 WBH, peaked at 7 mg $g^{-1}$ of 3 WBH and, subsequently, sharply declined toward commercial harvest. The citrate content of high acid clone began to increase three weeks after that of low acid clone, peaked at 1 WBH and then slightly declined at the time of commercial harvest. At commercial harvest, the high acid clone had 50% more citrate than the low acid clone.

**Malic acid concentration**

Malic acid content in both clones changed slightly between 3.0 and 5.5 mg $g^{-1}$ during fruit development (Figure 4.10). During the early stage of fruit development (11 to

5 WBH), the low acid clone was higher in malate content than the high acid clone. Subsequently, the malic acid concentration in both clones increased and concurrently peaked at 1 WBH of 5.5 mg $g^{-1}$, and then declined toward commercial harvest. Near harvest, the malate content was not significant different between the high and low acid clones.

**Changes in fruit acidity of 'D30' pineapple fruit**

Fruit of 'D30', another low acid clone, was sampled during the five weeks before commercial harvest. The TA increased from 6 meq $100mL^{-1}$ at 5 WBH to peak of 10 meq $100mL^{-1}$ at 1 WBH and, thereafter, sharply declined (Figure 4.11). The pH dropped to minimum of 3.1 at 3 WBH and then increased toward harvest. TSS increased from 9.0 to 13.6% and total sugar from 54 to 116 mg $mL^{-1}$ during 5 to 0 WBH period.

**Seasonal effect on fruit changes**

**Fruit physical changes**

Fruit weight and fruit length of both clones in warm season were higher and increased earlier than those in cool season (Figure 4.1, 4.2, 4.3 and 4.4). However, as harvest approached, fruit were not significantly different between the two seasons. Crown weight and fruit diameter of both clones were significantly higher in warm season. At harvest, crown weight and fruit diameter in warm season crop was 80 and 13% higher than those in cool season crop, respectively.

**Fruit chemical changes**

Juice pH changes in both clones showed similar patterns between cool and warm season crops, declining from 11 to 3 WBH and then increasing toward commercial harvest (Figure 4.5). However, during 11 and 3 WBH period juice pH of fruit from the warm season crop was lower than that of the cool season crop. Titratable acidity of the

fruit from the warm season crop obviously increased earlier than fruit from the cool season crop (Figure 4.6). Although, the titratable acidity peak for the cool and warm season crops were concurrent at about 1 WBH, the fruit from the cool season crop had a higher peak than those from the warm season.

In the high acid clone, fruit citric and malic acid contents were similar between the warm and cool season fruit (Figure 4.9 and 4.10). Citrate accumulation began to increase 7 WBH and peaked at 1 WBH, while malate accumulation was constant from 11 to 3 WBH, then showed a small peak at 1 WBH.

In the low acid clone, fruit citric acid content was higher and increased earlier in the warm season fruit but peaked at the same time as in the cool season fruit. Malic acid contents of the warm season fruit showed a greater increase than fruit from the cool season 5 to 0 WBH.

Fruit from the cool and warm season were not significantly different in TSS and total sugar at commercial harvest, although in the warm season fruit appeared to increase earlier than in the cool season.

### 4.4 Discussion

Developmental changes in fruit morphological characteristics were similar between the low and high acid clones. From 11 WBH to harvest, fruit weight increased about 3 fold and fruit length and diameter about 1.4 fold (Figure 4.1, 4.3 and 4.4). All these changes in morphological characteristics agreed with previous reports for 'Smooth Cayanne' fruit (Bartolome et al., 1995; Chan et al., 2003). Little differences in crown weight occurred between these two clones. A similar pattern of increase in fruit weight, fruit length and fruit diameter occurred in these two clones indicative of the normality of

fruit growth and development in pineapple fruit (Dull, 1971). Generally, changes in external characteristics of the high and low acid clones were similar.

Difference in the juice pH and acidity at harvest confirmed the distinctiveness of the acid content in these two clones. The juice pH of the high acid clone was 0.45 points significantly lower than that of the low acid clone. The titratable acidity was 1.7 folds higher in the high acid clone than that of low acid clone. The low acid clone showed markedly less acidity in flesh and this trait appeared in 'D30', another low acid clone, that had a pH of 3.56 and 6.03 meq $100mL^{-1}$ titratable acidity.

Developmental changes of acid contents during fruit growth were different between these two clones. The pattern of juice pH in the high acid clone from 11 to 0 WBH declined gradually from 4.5 to 3.1 and was similar to that reported for 'Smooth Cayenne' (Singleton and Gortner, 1965). In contrast, the low acid clone's juice pH declined to 3 WBH and then significantly increased toward harvest. The different patterns of juice pH changes were concomitant with the pattern of titratable acidity between these two clones. At early stages of fruit development, the low acid clone was higher in acidity than the high acid clone until 3 WBH, thereafter significantly declined toward harvest. Although the low acid clone showed an earlier increase in acidity than the high acid clone, its peak in acidity was smaller and about 1 week earlier than in the high acid clone. In addition, the patterns of changes in juice pH and titratable acidity of 'D30' were similar to the low acid clone (D-10) (Figure 4.11).

These results showed that the low acid clone was lower in fruit acid content at harvest than the typical 'Smooth Cayenne' cultivars, including '36-21'. The high acidity in '36-21' is not considered desirable for the fresh fruit market (Chan et al., 2003; Paull and Chen, 2003) though preferred for canning (Paull, 1993).

The differences in the major fruit organic acid contents in the high and low acid clones agreed with the differences in the titratable acidity. At harvest, the high acid fruit had 1.5 and 1.1 times more citric and malic acid contents than the low acid fruit, respectively (Figure 4.9 and 4.10). This difference is attributed to about 60% lower acidity of the low acid fruit.

Since both clones had about equal malic acid content but marked different titratable acidity, malic acid did not apparently have a major influence on the differences in acidity. In contrast, there was a 1.5 times difference in citric acid content between the clones that could account for the difference in acidity. This conclusion was supported by the pattern of change in citric acid that paralleled the pattern of change in titratable acidity whereas the pattern for malic acid not paralleled the change.

Difference in organic acid concentration between cultivars has been observed in peach (Etienne et al., 2002; Moing et al., 1998), sweet lime and sour lemon (Sadka et al., 2001). Three related hypotheses have been proposed to explain the difference in acidity. The hypothesis involve changes in citrate and malate synthesis, catabolism and compartmentation (Knee and Finger, 1992; Moing et al., 2000; Moing et al., 1998).

Although the patterns of total soluble solid and total sugar in these two pineapple clones increased, those in the low acid clone increased earlier than in the high acid clone and resulted in a higher TSS and total sugar in the low acid clone at harvest. TSS/TA ratio (as citric acid) in both clones were different (Figure 4.12); in the high acid fruit the ratio declined from 11 to 5 WBH, then remained stable, while in the low acid fruit the ratio declined to 5 WBH, then increased. Singleton and Gortner (1965) suggested that TSS/TA ratio was only comparable for fruit at the same developmental stage. The fruit used in that study were high acid canning-type. This study showed that the TSS/TA ratio varied between clones and during fruit development.

Fruit from the two crop seasons showed smaller difference due to weather than clone (Figure 4.13 and Table 4.1). The maximum, minimum and mean temperature of cool season was 1 to 2 °C lower than that of warm season. However, it caused little differences in fruit growth and development. The warm season fruit did have higher crown weight and fruit diameter, but there were little difference in fruit weight and fruit length.

The patterns of juice pH and titratable acidity accumulation were slightly different between seasons although the fruit were not different at harvest (Figure 4.5 and 4.6). The high acid fruit showed consistent changes in juice pH, titratable acidity, citric and malic acids between the two seasons (Figure 4.9 and 4.10). In contrast, low acid fruit showed higher acid accumulated, especially of citric acid, during the early stage of growth in the warm season prior to a sharp decline. This suggests that acid accumulation of the low acid clone was sensitive to seasonal temperature, and acid accumulation in the high acid clone was highly associated with the stage of fruit development.

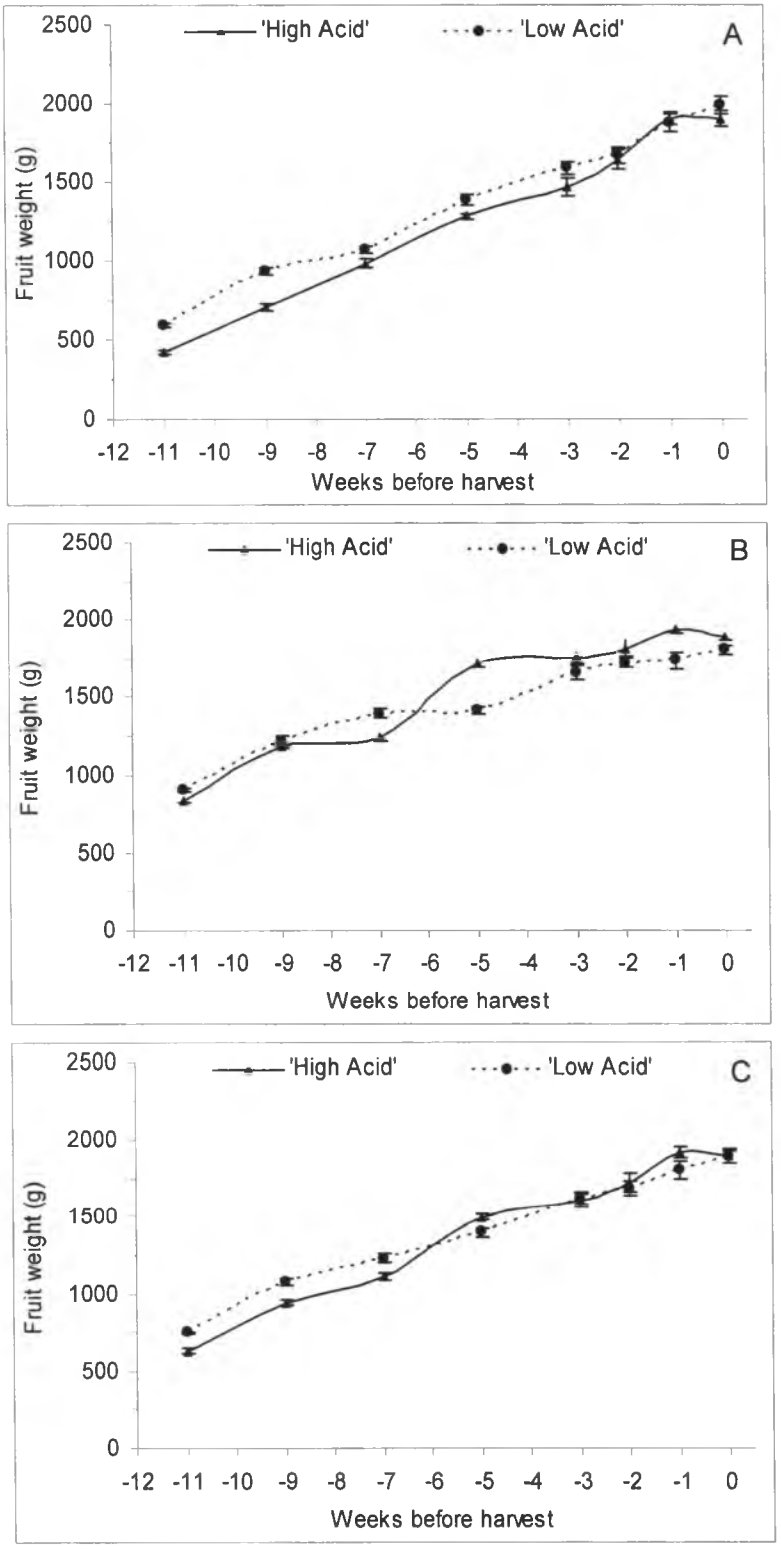



Figure 4.1. Changes in fruit weight (without crown) of pineapple 'High acid' (36-21) and 'Low acid' (63-555) during fruit development in cool season (A), warm season (B) and two seasons combined (C). Mean ± SE of 10 replicates.

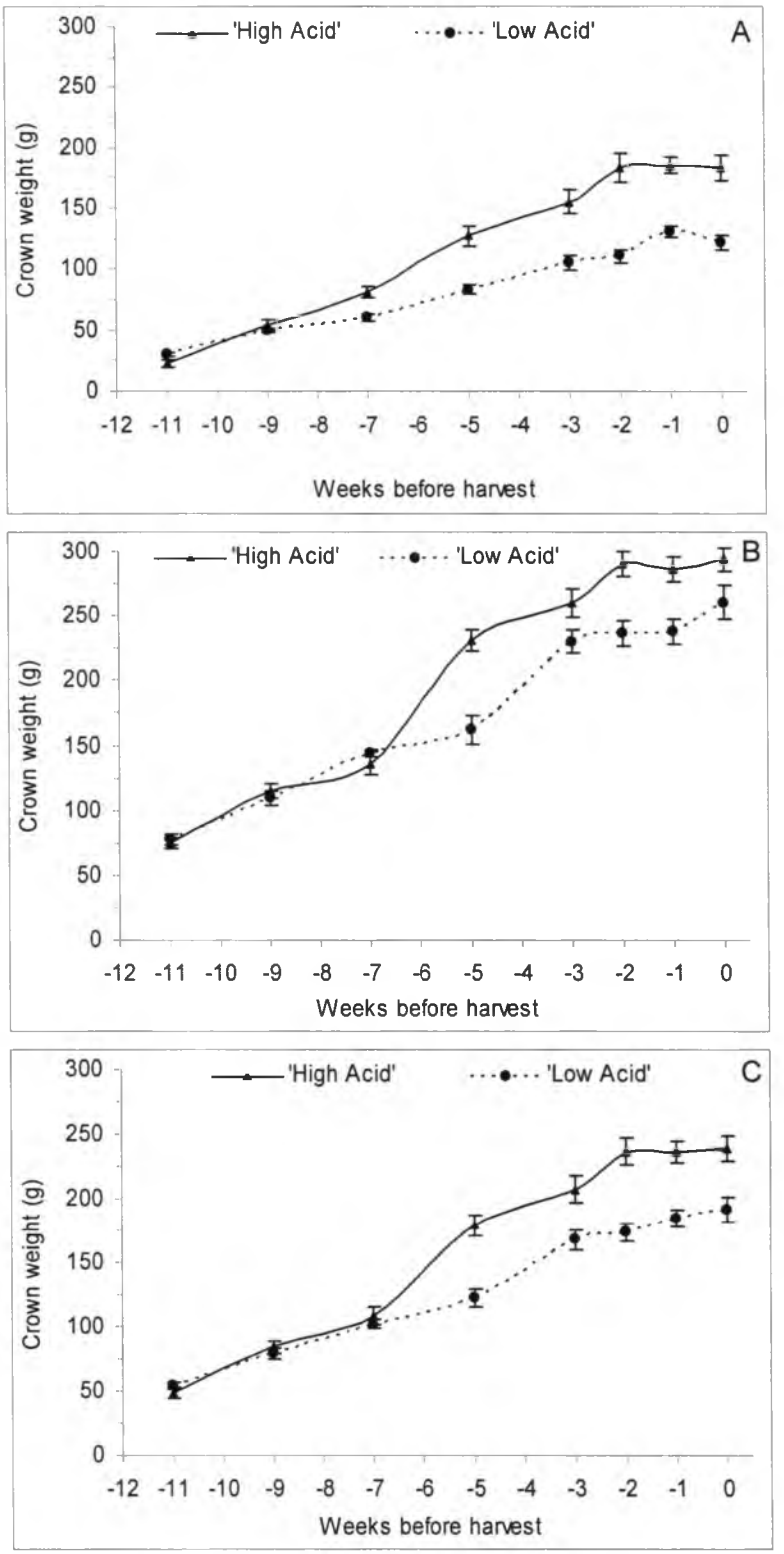



Figure 4.2. Changes in crown weight of pineapple 'High acid' (36-21) and 'Low acid' (63-555) during fruit development in cool season (A), warm season (B) and two seasons combined (C). Mean ± SE of 10 replicates.

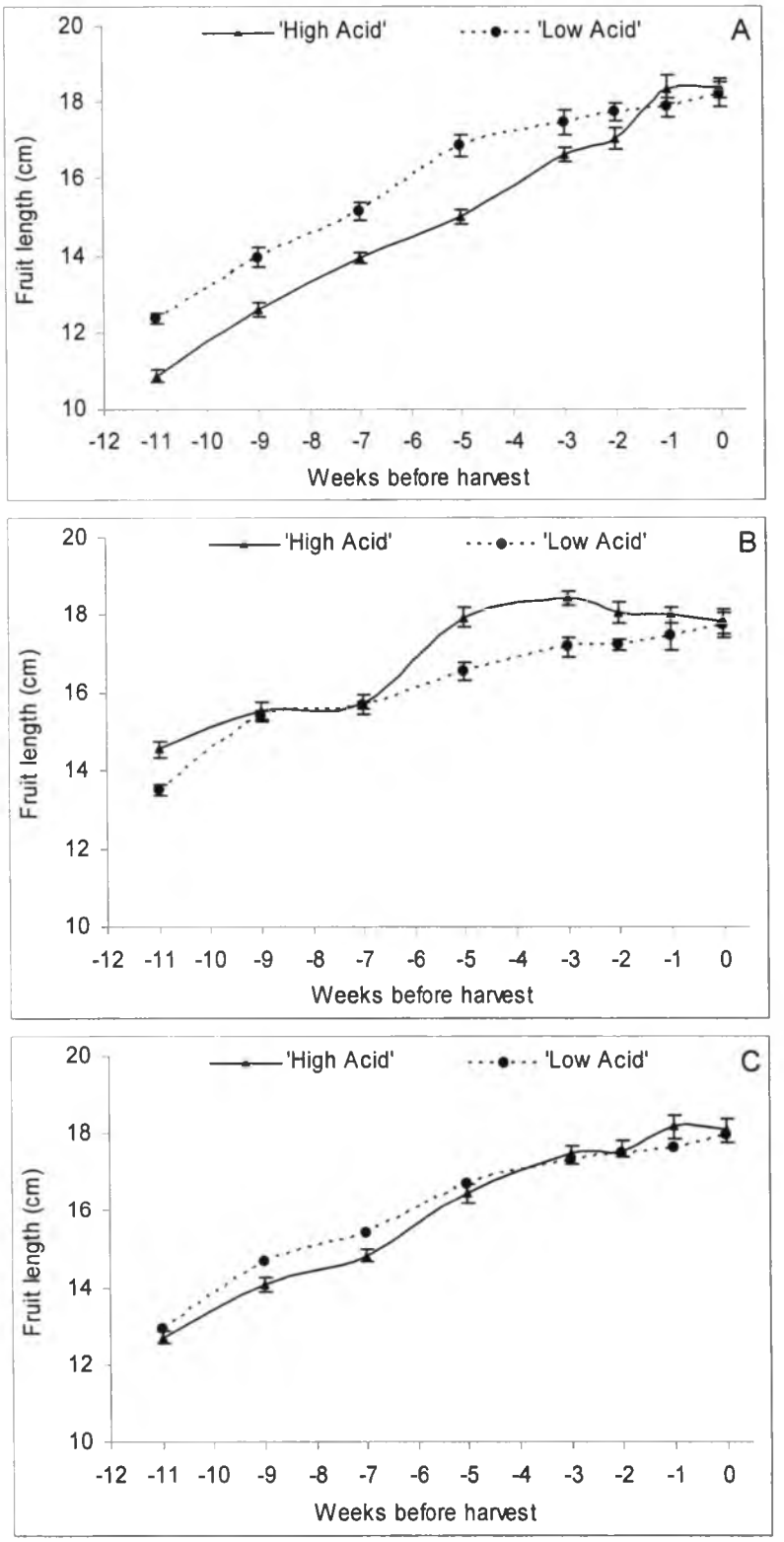



Figure 4.3. Changes in fruit length of pineapple 'High acid' (36-21) and 'Low acid' (63-555) during fruit development in cool season (A), warm season (B) and two seasons combined (C). Mean ± SE of 10 replicates.

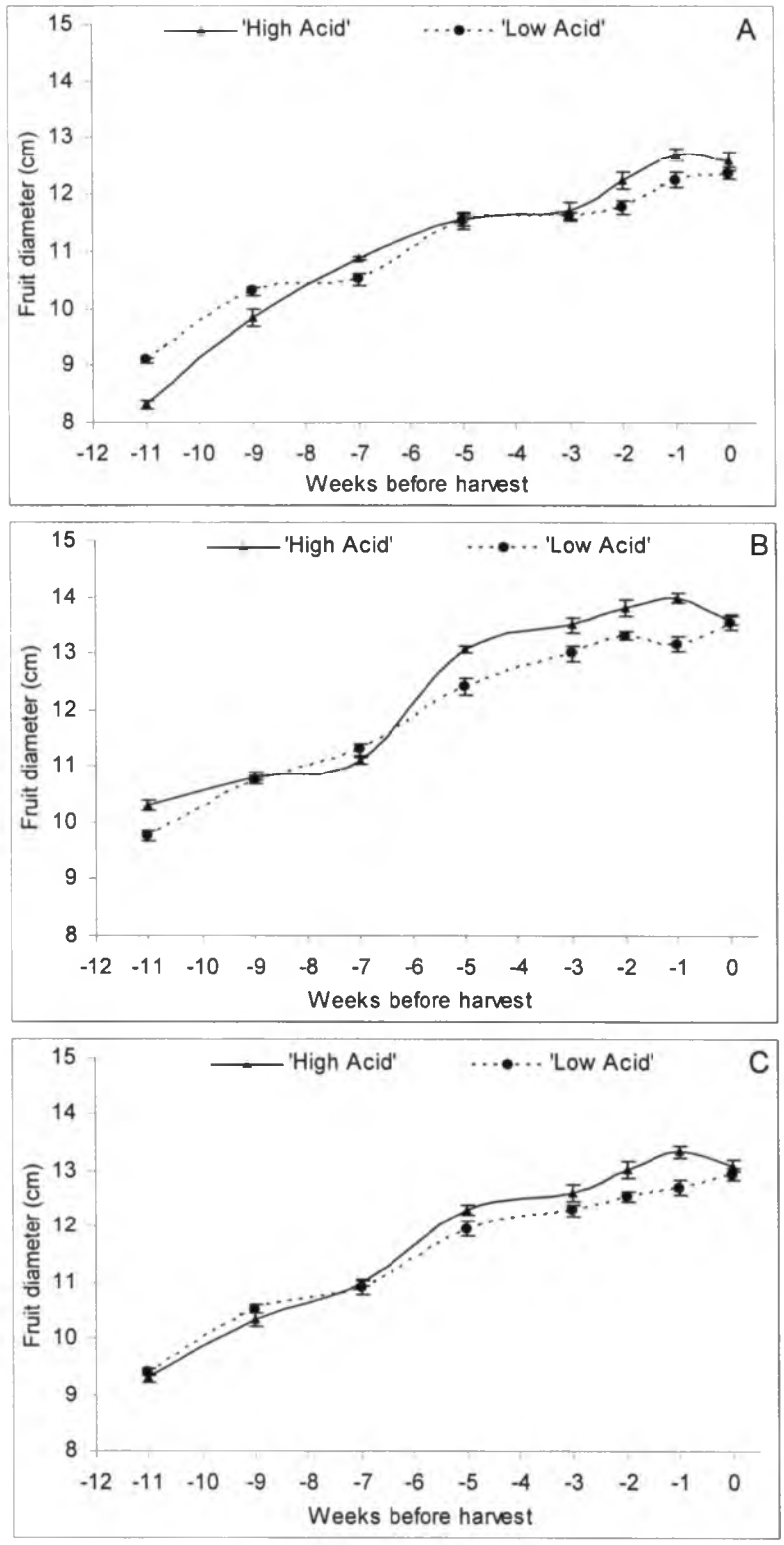



Figure 4.4. Changes in fruit diameter of pineapple 'High acid' (36-21) and 'Low acid' (63-555) during fruit development in cool season (A), warm season (B) and two seasons combined (C). Mean ± SE of 10 replicates.

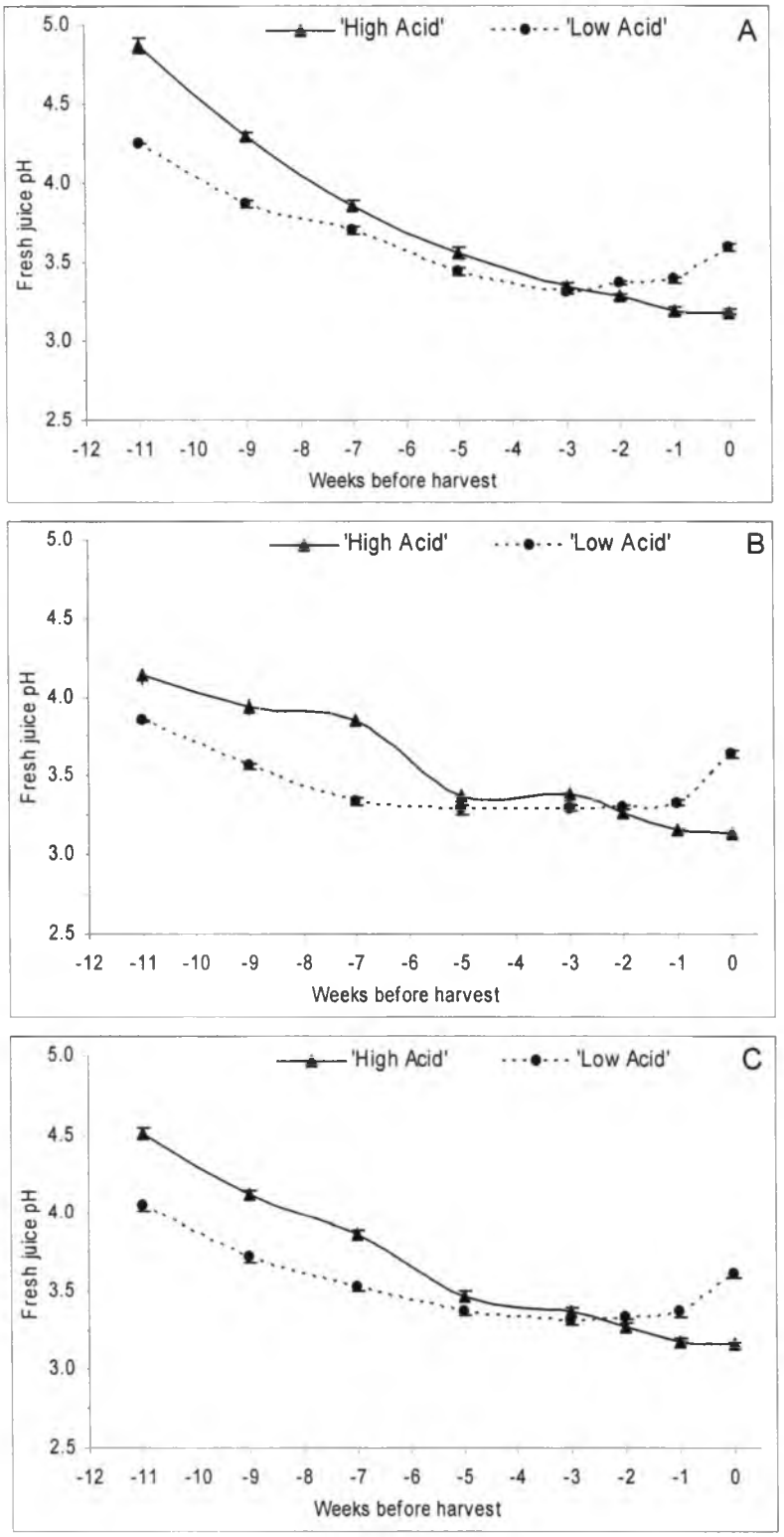



Figure 4.5. Changes in fresh juice pH of pineapple 'High acid' (36-21) and 'Low acid' (63-555) during fruit development in cool season (A), warm season (B) and two seasons combined (C). Mean ± SE of 10 replicates.

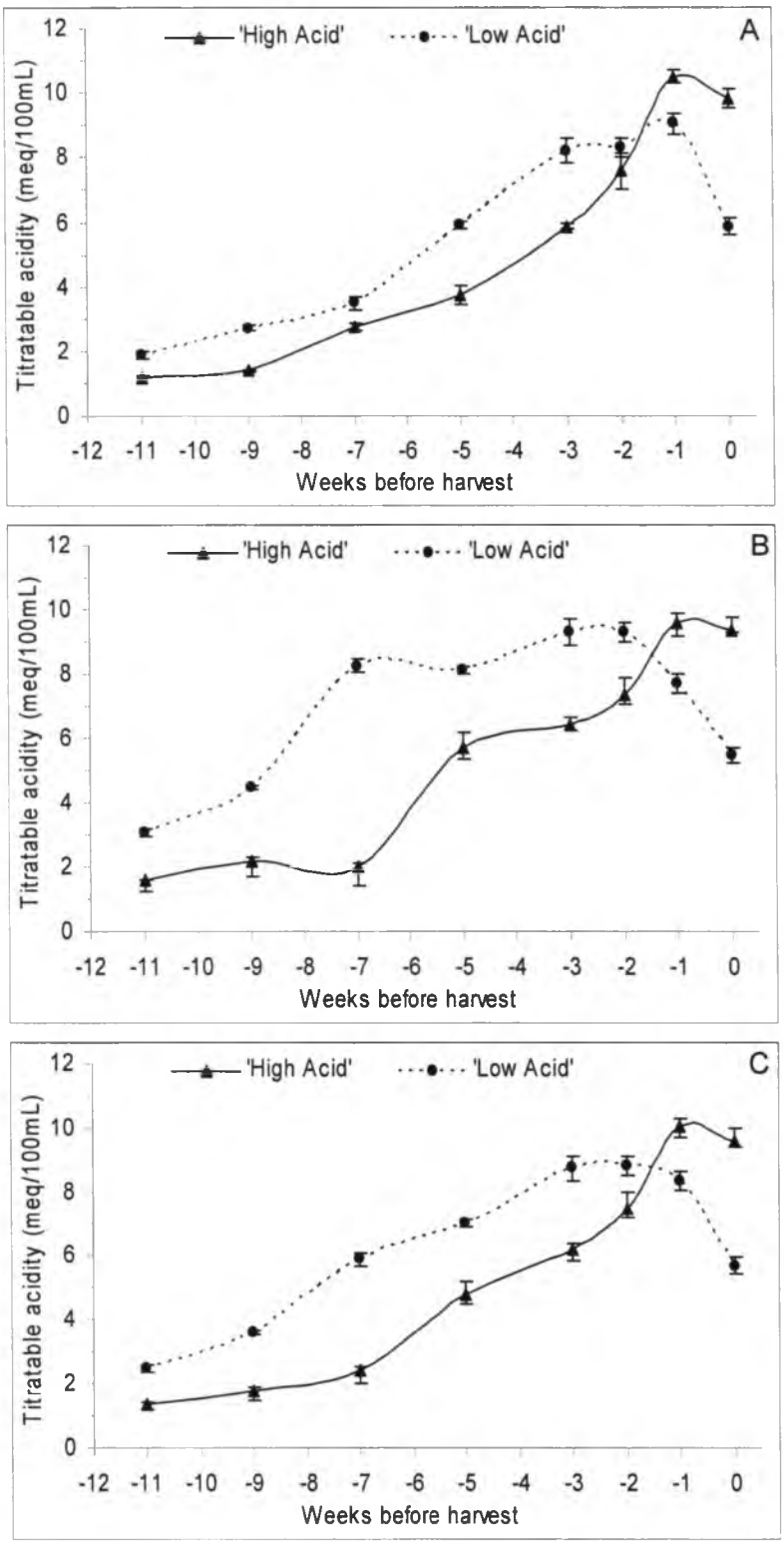



Figure 4.6. Changes in flesh titratable acidity of pineapple 'High acid' (36-21) and 'Low acid' (63-555) during fruit development in cool season (A), warm season (B) and two seasons combined (C). Mean ± SE of 10 replicates.

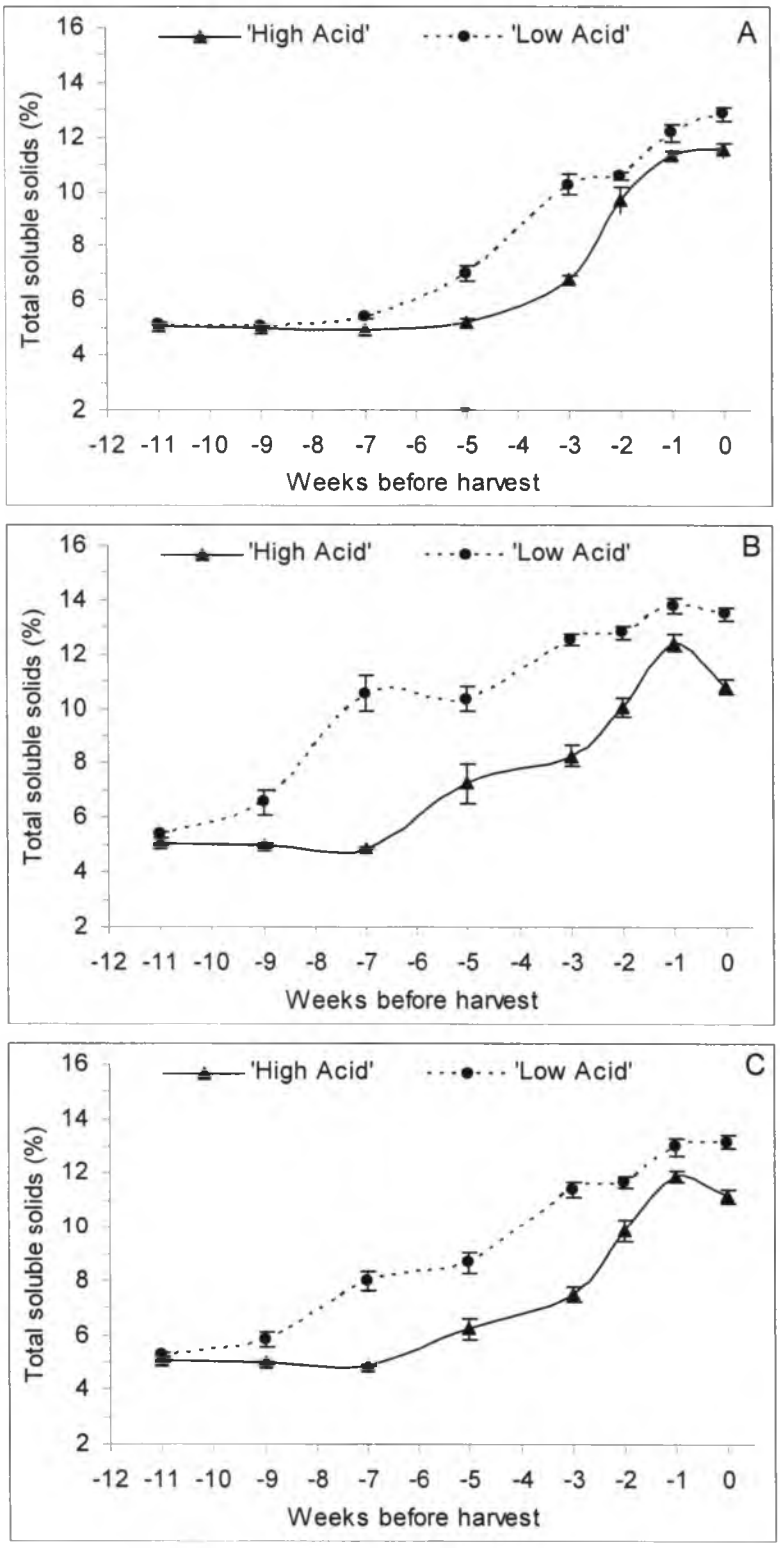



Figure 4.7. Changes in fresh total soluble solids of pineapple 'High acid' (36-21) and 'Low acid' (63-555) during fruit development in cool season (A), warm season (B) and two seasons combined (C). Mean ± SE of 10 replicates.

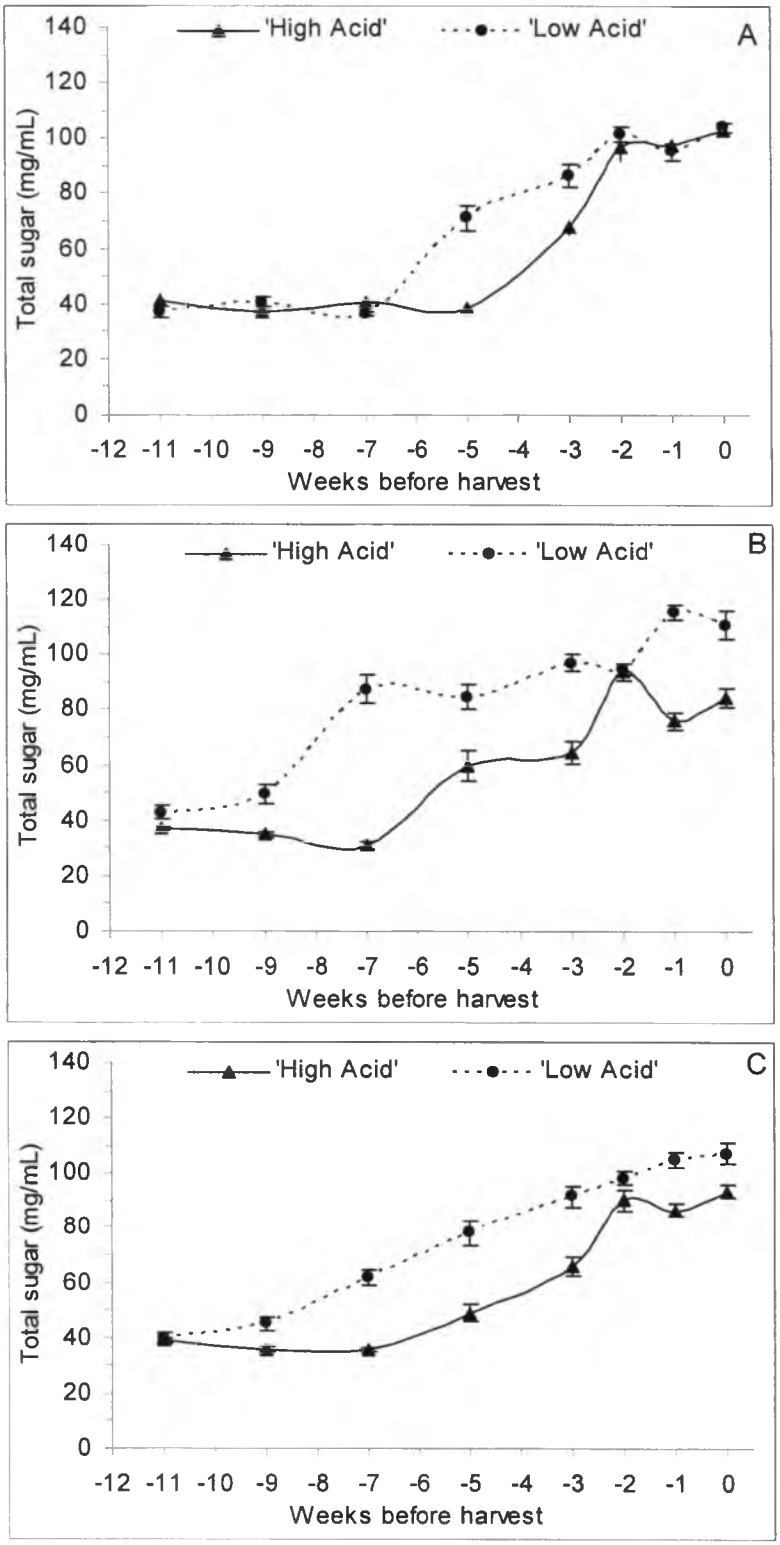



Figure 4.8. Changes in fresh total sugar of pineapple 'High acid' (36-21) and 'Low acid' (63-555) during fruit development in cool season (A), warm season (B) and two seasons combined (C). Mean ± SE of 10 replicates.

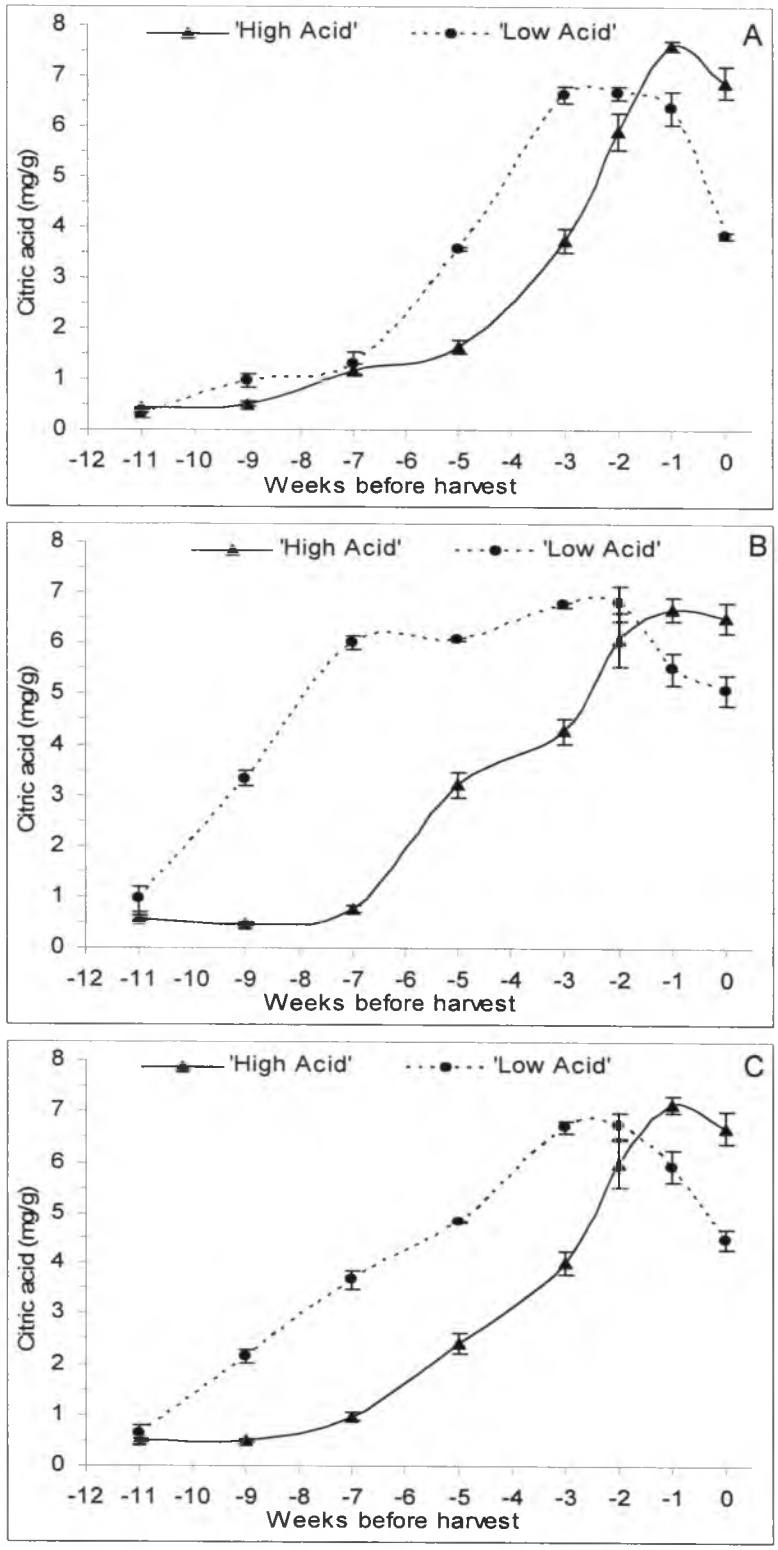



Figure 4.9. Changes in citric acid of pineapple 'High acid' (36-21) and 'Low acid' (63-555) during fruit development in cool season (A), warm season (B) and two seasons combined (C). Mean ± SE of 10 replicates.

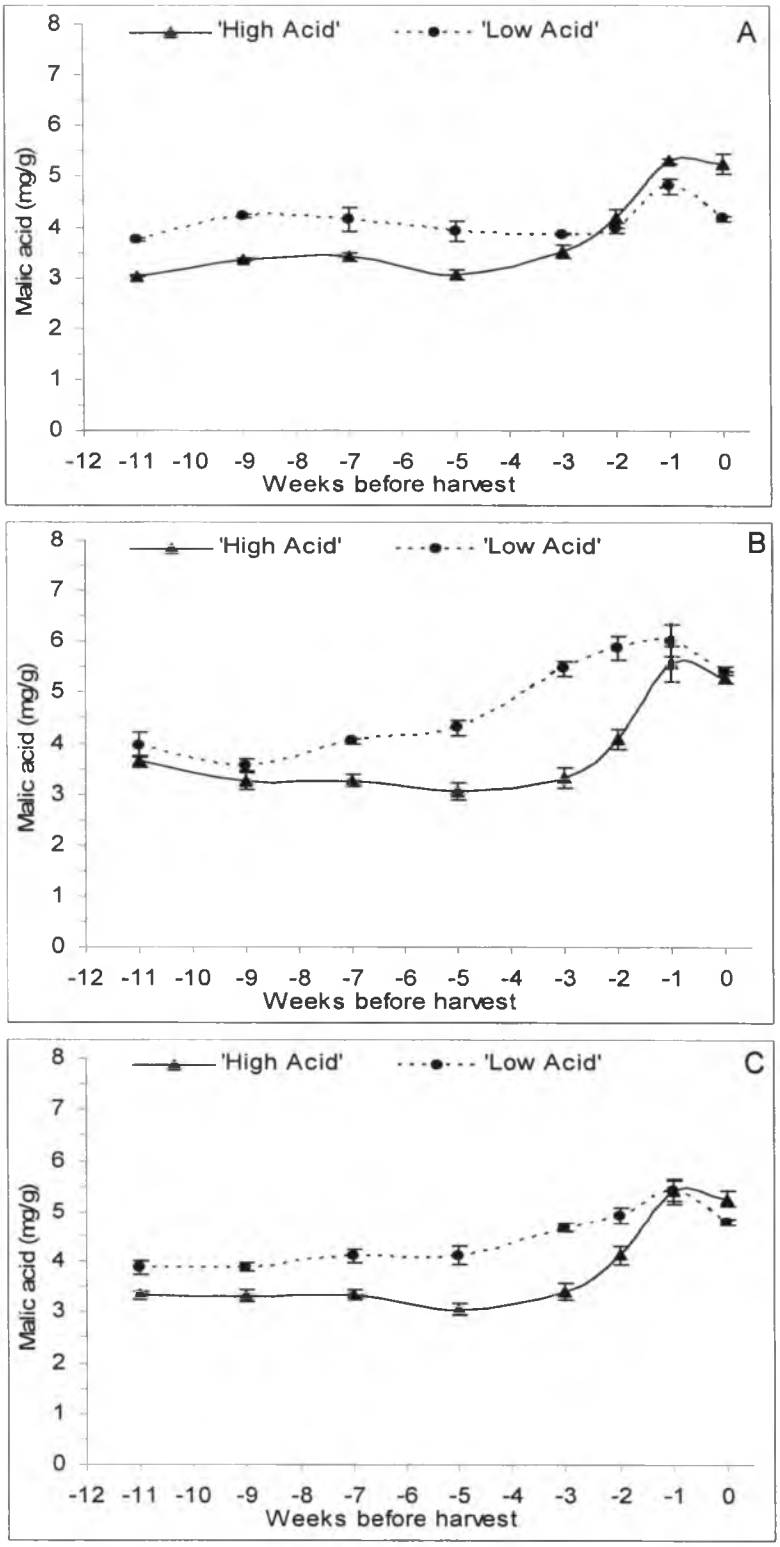



Figure 4.10. Changes in malic acid of pineapple 'High acid' (36-21) and 'Low acid' (63-555) during fruit development in cool season (A), warm season (B) and two seasons combined (C). Mean ± SE of 10 replicates.

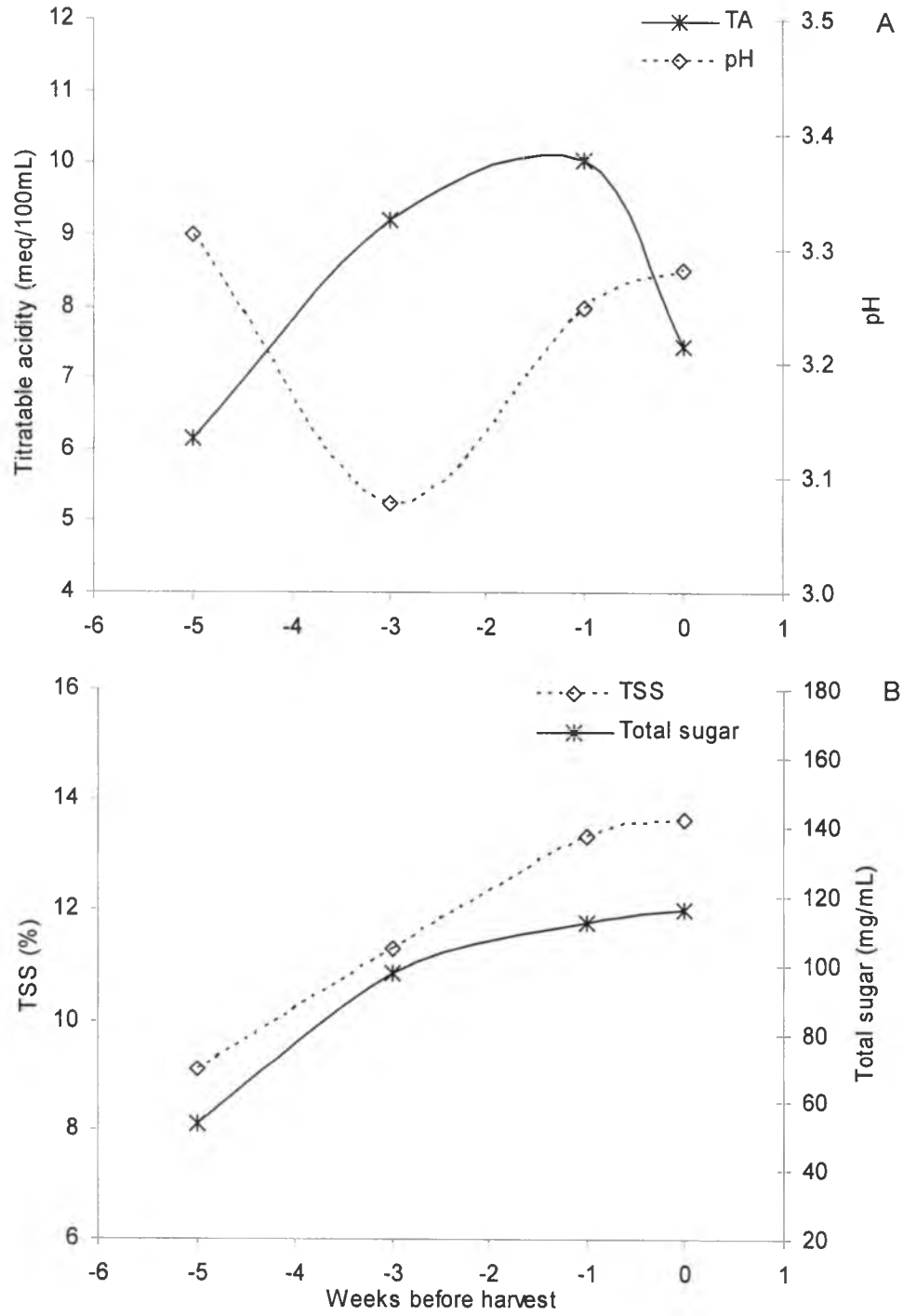



Figure 4.11. Developmental changes of the low acid cultivar 'D30' in titratable acidity and pH (A), total soluble solids and total sugar (B) during the 5 weeks before commercial harvest. Mean of 5 fruit.

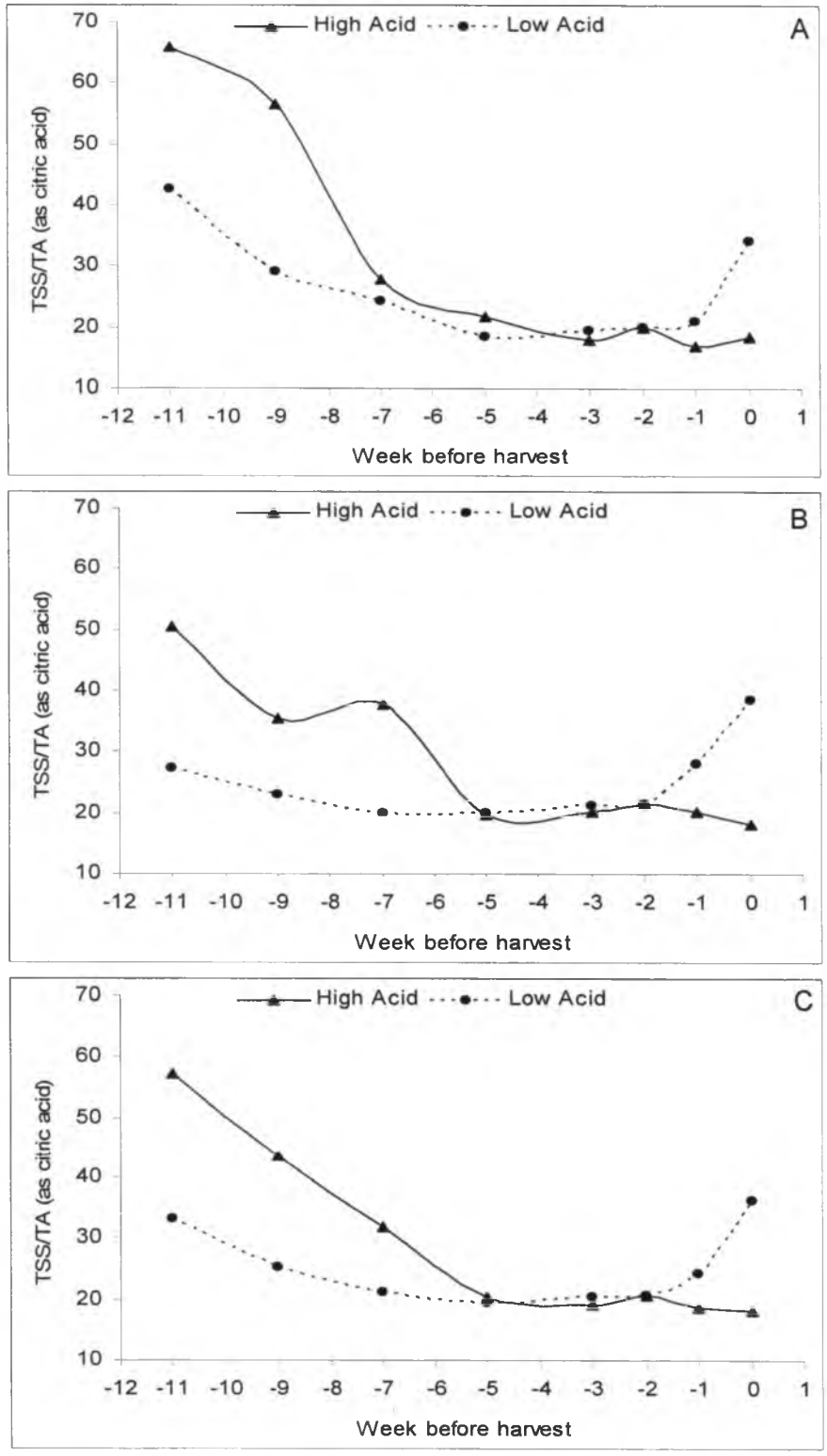



Figure 4.12. Changes in TSS/TA (as citric acid) of pineapple 'High acid' and 'Low acid' during fruit development in cool season (A), warm season (B) and two seasons combined (C).

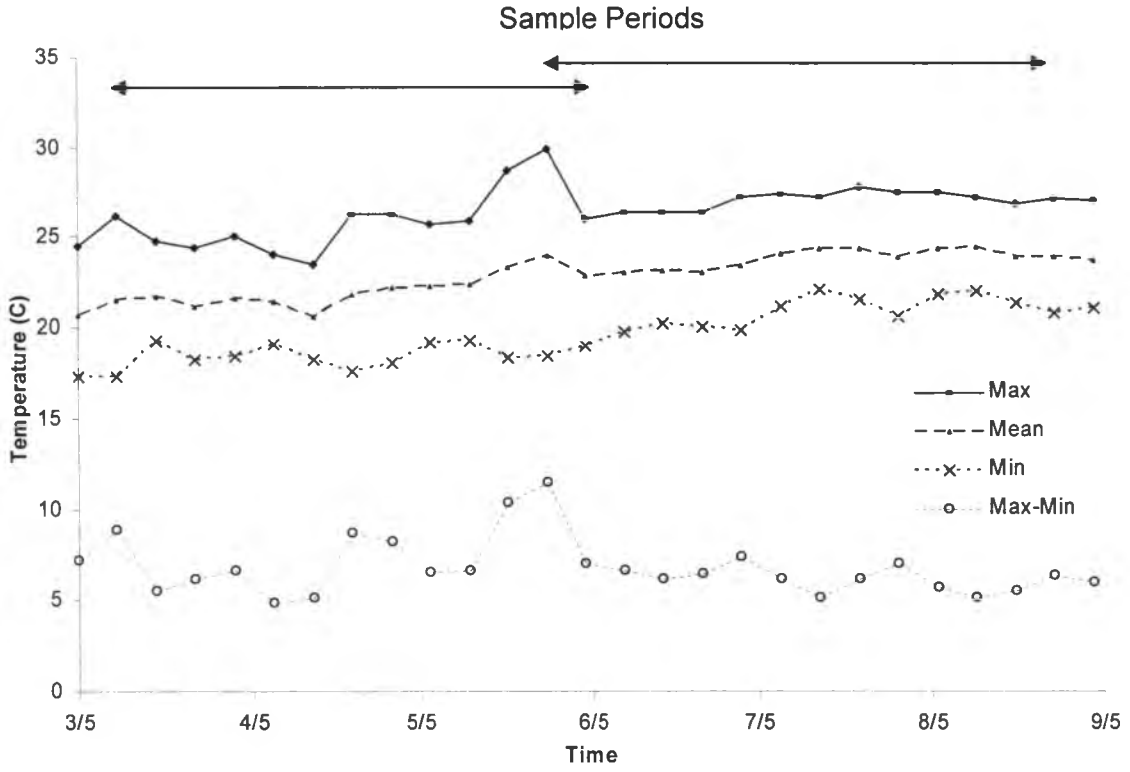



Figure 4.13. Maximum, mean, minimum and max - min temperature during pineapple fruit development.

Table 4.1. Temperature data during 11 weeks before commercial harvest of pineapple fruit

| | Cool season | Warm season |
|---|---|---|
| Collection date | 3/13 – 6/16 | 5/28 – 8/20 |
| Temperature (°C) | | |
| Maximum | 21-33 | 25-32 |
| Minimum | 16-21 | 17-23 |
| Mean | 20-25 | 22-25 |
| DTT | 472 | 598 |
| Relative humidity (%) | | |
| Maximum | 100 | 100 |
| Minimum | 22 | 42 |
| Mean | 77 | 74 |
| Photosynthetic Active Radiation (PAR) Daily mean ($\mu mol\ m^{-2}\ sec^{-1}$) | 545 | 470 |

# CHAPTER 5

# ENZYME ACTIVITIES DURING FRUIT DEVELOPMENT

## 5.1 Introduction

Organic acids and sugars in fruit are important components of fruit flavor and organoleptic quality. The predominant organic acids in pineapple fruit are citric and malic acids that accumulated during fruit growth and development. Mechanisms controlling organic acid accumulation in fruit have been studied in some fruits such as peach (Etienne et al., 2002; Moing et al., 2000; Moing et al., 1998b), grape (Diakou et al., 2000; Ruffner et al., 1984), and citrus (Sadka et al., 2000b; Sadka et al., 2001). The final organic acid content is determined by the net balance between their synthesis (Laval-Martin et al., 1977), mobilization (Ruffner et al., 1984) and vacuolar compartmentation (Muller et al., 1996).

The keys enzymes in citric acid synthesis are citrate synthase (EC 4.1.3.7), that catalyzes the combination of oxaloacetic acid (OAA) with acetyl-CoA to yield citric acid. Citric acid is isomerized by aconitase (EC 4.2.1.3) to isocitrate. These processes take place in the mitochondrial TCA cycle (Sadka et al., 2000).

Sadka et al. (2001) reported that the increases in citrate synthase activity in sour lemon parallels the increase of acid content, however, sweet lime showed a similar pattern. The difference between the acid content in sweet and sour fruit might not result from changes in citrate synthase activity. It was hypothesized that a metabolic block in aconitase activity plays a role in citrate accumulation (Bogin and Wallace, 1966). Recently, it was found that mitochondrial-aconitase plays a role in acid accumulation by different acid-containing citrus (Sadka et al., 2000b; Sadka et al., 2001).

Malic acid accumulation also involves enzymatic activities of synthesis, degradation and vacuolar storage. Phosphoenolpyruvate carboxylase (PEPC; EC 4.1.1.31) is a key enzyme for malic and citric acid metabolism. Cytosolic-PEPC catalyzes the carboxylaton of phosphoenol pyruvate (PEP) and carbonic acid to yield OAA (O'Leary, 1982). The OAA then is reduced by NAD-dependent malate dehydrogenase (MDH; EC 1.1.1.37) to produce malate. Malate can be degraded into pyruvate by catalyzing of cytosolic-NADP dependent malic enzyme (ME; EC 1.1.1.40) (Knee and Finger, 1992). These metabolic activities occur in the cytosol and are important to both malic and citric acid metabolism since malate and pyruvate from the cytosol can enter into mitochondrial TCA cycle (Roe et al., 1984).

Although a difference in fruit acid content between high and low acid pineapple have been observed, the organic acid metabolism in pineapple fruit during fruit development is still unknown. The objective of the present study was to investigate developmental changes in enzymatic activities of CS, ACO, PEPC, MDH and ME which might play a role in difference of fruit acid accumulation between the high and low acid clones, 36-21 and 63-555 (D-10) respectively.

## 5.2 Materials and methods

### Fruit material

Uniform pineapple fruit of 36-21 and 63-555 ('D10') clones were harvested as high and low acid cultivars, respectively. The fruit were harvested from the Dole Fresh Fruit Co. plantation on the island of Ohau, Hawaii between May and August, 2003. The fruit was sampled biweekly from 11 to 3 weeks before harvest (WBH) and weekly from 3 WBH to the week of commercial harvest. Fifteen uniform fruit were sampled and transferred to the laboratory within two hours.

## Organic acid analysis

Ten fruit from each cultivar were prepared for organic acid quantification and another four fruit were used for measurement of enzyme activity. To prepare samples for organic acid quantification, two pieces of flesh were removed from opposite side of the equatorial part of the fruit and squeezed and 20 mL juice collected. The juice was centrifuged at 10,000 g for 10 min. A 2 mL aliquot of supernatant was diluted with 95% ethanol to 1/5 by volume and stored at -20°C until quantification of organic acid by high performance liquid chromatography (HPLC).

The frozen diluted juice supernatant was prepared for HPLC as modified from Pauli et al. (1983). The alcoholic solution was air-dried and mixed with deionized water. A 20 µL aliquot of the solution was filtered though a 0.45 µm filter and degassed before injection into HPLC fitted with an organic acid column (Bio-Rad HPX-87H, 7.8x300 mm). The solvent was 0.05 N sulfuric acid at a flow rate of 0.8 mL $min^{-1}$ and monitored at 210 nm. Peak area was measured and calculated relative to a standard solution. A sample injection was a combination of three fruit.

## Enzyme activity

**Enzyme extraction:** Crude enzymes were extracted from flesh of four fruit from each cultivar by modified published methods (Diakou et al., 2000; Jeffery et al., 1988; Knee and Finger, 1992). Each fruit was extracted in replicate. The extraction solution was 250 mM Tris-HCl, 600 mM sucrose, 10 mM KCl, 10mM $MgSO_4$, 3 mM EDTA, 1% PVP-40, 1 mM, PMSF, 0.05% mercaptoethanol at pH 7.5. Two 10 g pieces of flesh were removed from opposite sides of the equatorial part of the fruit, added to 20 mL extraction buffer, and then homogenized at 4°C for about 1 min. The mixture was immediately filtered through two layers of miracloth and then centrifuged at 1,000 g for 10 min at 4°C. The supernatant was collected and centrifuged again at 20,000 g for 20 min. Both

supernatant and pellet were collected and kept at 4°C for further enzyme activity assays within 3 hours. The supernatant represent the cytosol portion was used for PEPC, MDH and ME assays. The pellets represent as the mitochondria portion was used for CS and ACO assays. The enzyme activity monitored by spectrophotometer were compared to a control, boiled enzyme.

**Enzyme extraction for CS and ACO assays:** The pellet from the previous extraction above was further conducted by modified method (Iredale, 1979). The pellet was washed with 2 mL solution of 50 mM Tris-HCl pH 7.5, 300 mM manitol and 1 mM EDTA, centrifuged at 20,000 g for 20 min and the mitochondria pellet was collected. A one mL buffer composed of 50 mM Tris-HCl pH 7.5 and 1 mM EDTA was added to the pellet, and then sonicated for 5 min. The solution was centrifuged at 20,000 g for 10 min, and the supernatant collected as a mitochondrial solution for CS and ACO assays. All processes were conducted at 4°C.

**Enzyme assays**

**Citrate synthase (CS) activity:** The assay was conducted using Ellman's reagent (DTNB) (Sadka et al., 2000a). The assay mixture was 1 mL total volume composed of 20-50 µL crude extract, 100 mM Tris-HCl, 1 mM EDTA, 0.1 mM DTNB, 0.2 mM acetyl-CoA and 0.2 mM oxaloacetate. The increase in absorbance at 412 nm at 25°C was measured.

**Aconitase (ACO) activity:** The assay mixture was 1 mL total volume composed of 20-50 µL crude extract, 20 mM Tris-HCl, pH 7.5, 100 mM NaCl, 200 µM cis-aconitate. The decline of absorbance at 240 nm was determined at 25°C (Hirai and Ueno, 1977).

**Phosphoenoi pyruvate carboxylase (PEPC) activity:** The assay mixture was 1 mL total volume composed of 20-50 µL crude extract, 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 decline of absorbance at 340 nm was determined at 25°C (Diakou et al., 2000).

**Malate dehydrogenase (NAD-MDH) activity:** The assay mixture was 1 mL total volume composed of 10 µL crude extract, 50 mM MOPS at pH 8, 0.4 mM NADH. The reaction was started by the addition of oxaloacetate (to 0.2 mM) to a total volume of 1 mL. The rate of decline of absorbance at 340 nm was determined at 25°C (Jeffery et al., 1988).

**Malic enzyme (NADP-ME) activity:** The assay mixture was 1 mL total volume composed of 20-50 µL crude extract, 100 mM MOPS (pH 7) with 0.5 mM NADP, 10 mM malate and 5 mM $MnCl_2$. The increase in absorbance at 340 nm was determined at 25°C (Knee and Finger, 1992).

**Protein measurement**

Protein was quantified from the crude extract following a Bradford assay (Bradford, 1976) with modification using Bio-Rad protein micro-assay dye reagent compared with a BSA standard.

## 5.3 Results

**Developmental changes of fruit citric and malic acid contents**

The low acid cultivar had a significantly greater citric acid content than the high acid fruit in the period 9 to 3 WBH, and it declined sharply in the three weeks before harvest (Figure 5.1). In contrast, the increase in citric acid in the high acid fruit occurred approximately four weeks after the low acid fruit and peaked at 1 WBH. The high and low acid fruit increased in citric acid content about 9 and 7 folds, respectively, between 11 to 0 WBH.

The changes in fruit malic acid content were less than the citric acid content (Figure 5.2). The malic acid content was again higher in the low acid fruit. The low acid fruit showed a gradual increase while the high acid fruit increased in the last few weeks. Both clones had peaked in acid concentration about 1 WBH. The changes in malic acid content between the high and low acid fruit from 11 WBH to harvest were about 1.5 and 1.4 folds, respectively.

**Developmental changes of CS**

Changes in fruit CS activity were similar between the high and low acid cultivars (Figure 5.3). During 11 to 3 WBH period the patterns showed a small peak at 5 WBH and declined, however a larger peak occurred at 1 WBH in both cultivars, thereafter declined toward harvest.

**Developmental changes of ACO**

The patterns of ACO activity between the high and low clones were similar during 11 to 3 WBH with a small peak at 7 WBH (Figure 5.4). A difference in activity occurred between the two clones where both cultivars showed a peak in ACO activity at 1 WBH. The low acid fruit showed a higher activity than the high acid fruit during a few weeks before harvest.

**Developmental changes of PEPC**

Changes in PEPC activity between cultivars were similar (Figure 5.5). The patterns of activity were parallel between 11 to 5 WBH with a higher PEPC activity in the high acid fruit. After 2 WBH the low acid fruit showed an increase of PEPC that was higher than high acid fruit.

### Developmental changes of MDH

The patterns of MDH activity between the high and low acid cultivars differed between the first half of fruit growth and the last half (Figure 5.6). During the early stage (11 to 5 WBH) the high acid fruit showed higher in MDH activity than the low acid fruit. In contrast, from 4 WBH to harvest the low acid fruit had a greater MDH activity than the high acid fruit.

### Developmental changes of ME

ME activity of both cultivars tended to slightly increase from 11 WBH to harvest (Figure 5.7). The low acid fruit showed a gradual increase in ME activity throughout fruit development whereas the high acid fruit showed a peak that exceeded the low acid fruit at 7 WBH, then declined and increased again toward harvest.

## 5.4 Discussion

The patterns of changes in CS activity were similar between both cultivars throughout fruit development and CS seemed unrelated to the differences in acid content between the high and low acid cultivars. The remarkable increase in CS activity at 1 WBH coincided with the peak of citric acid content in the high acid cultivar but not with the low acid clone. A similar result was reported for sour lemon (Sadka et al., 2001). In sour lemons, CS activity was induced early in fruit development and paralleled the increase in acid content. However, sweet lime showed similar patterns of CS activity as sour lemon. A peak of pineapple CS activity occurred at 1 WBH (Figure 5.3) whereas the fruit citric acid content in the low acid cultivar declined at that time (Figure 5.1). Luo et al. (2003) reported that there was no relationship between CS activity and the differences in acid content of six citrus fruit varieties.

CS is a key enzyme that plays a significant role in citric acid synthesis. This was supported by a positive correlation between CS activity and organic acid contents in citrus (Wen et al., 2001). The reduction of CS activity by arsenite application results in a decrease in citric acid content in satsuma mandarin and tangelo citrus fruit (Sadka et al., 2000a; Yamaki, 1990). This result might suggested that CS explained the citric acid synthesis in pineapple fruit. However, difference in citric acid content between the high and low acid fruit at harvest or during the early stage of fruit development might be due to compartmentation or degradation by aconitase as proposed by Sadka et al. (2001).

The ACO activity was different between the high and low acid pineapple cultivars at the late stage during fruit development (Figure 5.4). This difference might account for the difference in citric acid content between the high and low acid cultivars at harvest. The patterns of ACO activity between the high and low acid fruit in early stage of fruit development were similar, with a small peak at 7WBH. During the 2 to 1 WBH the patterns were different and the high acid cultivar showed a smaller increase in ACO activity than the low acid cultivar. At 1 WBH, ACO peak in the low acid fruit was significantly greater than that in the high acid fruit and this coincided with decline in citric acid content in the low acid fruit.

The role of aconitase in pineapple agrees with the result reported by Sadka et al. (2000b) for lime and lemons that ACO activity declined earlier in sour lemon than in sweet lime. Mytochondrial-aconitase activity plays a role in acid accumulation by isomerized degradation. Moreover, the degradation of citric acid in the late stage of citrus fruit development seemed to be accelerated by cytosolic-ACO activity (Luo et al., 2003). The result supports the hypothesis that metabolic reductions in ACO activity play a role in citric acid accumulation (Bogin and Wallace, 1966).

During the last few weeks before pineapple harvest, the difference in ACO activity in the high and low acid cultivars could account for the difference in citric acid accumulation. Although ACO activity could not account for the increase in citric acid content in the low acid fruit during the early stage of fruit growth, ACO may therefore play a partial role to regulate citric acid accumulation in pineapple fruit.

The pattern of changes in enzymatic activity of pineapple PEPC, MDH and ME did not correlate to the changes in citric or malic acids throughout fruit development. This finding was in agreement with the result reported for the difference in acid content of peach that is also not correlated with *in vitro* PEPC, MDH and ME activities (Moing et al., 2000; Moing et al., 1998a). Gene expressions of those enzymes showed the similar patterns between the high and low acid clones did not correlate with organic acid changes (Etienne et al., 2002). PEPC activity in grape berry was high in both acid and acidless berries (Diakou et al., 2000). The difference in pineapple organic acid content could be partially due to PEPC though the effect may be minor.

Compartmentation of organic acid was another possibility that could account for the low acid in fruit. Echeverria et al. (1997) reported that sweet lime had a lower capacity for $H^+$-retention in the vacuole. This lower capacity might result in a lower content in acid fruit compared to acid lime. However, in some low acid fruits the evidence for difference in organic acid uptake by the tonoplast vesicles is not found (Canel et al., 1995). Furthermore, PEPC, MDH and ME might account indirectly for fruit acid metabolism since their intermediate products such as OAA, malate and pyruvate could be transferred into mitochondria supporting the TCA cycle.

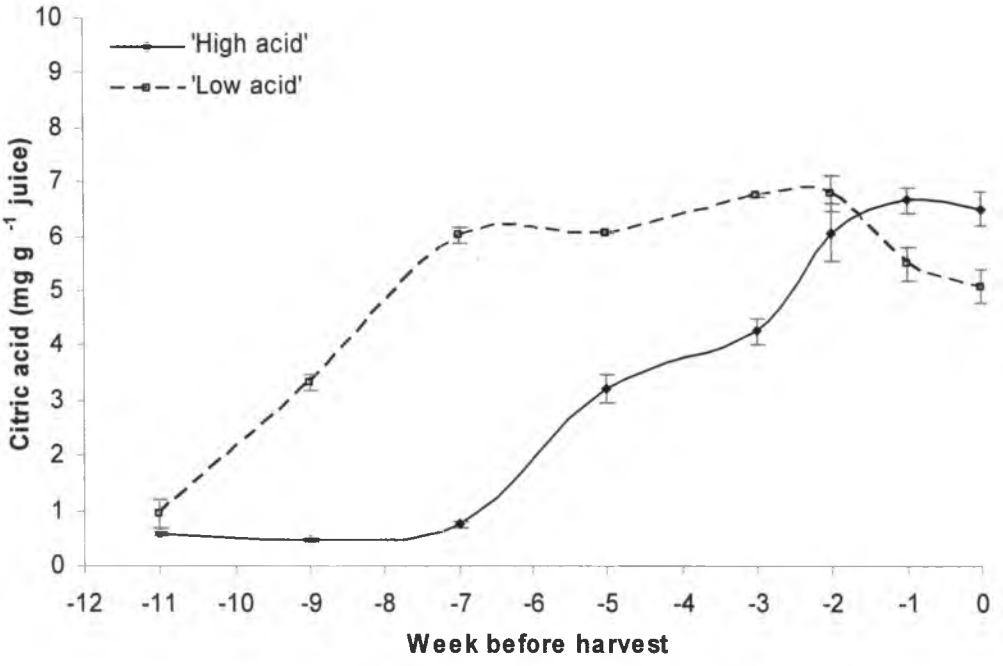



Figure 5.1. Changes in fruit citric acid concentration of pineapple 'High acid' (36-21) and 'Low acid' (63-555) during fruit growth and development. Fruit were sampled from 11 WBH to commercial harvest week. Means ± SE of 10 replicates.

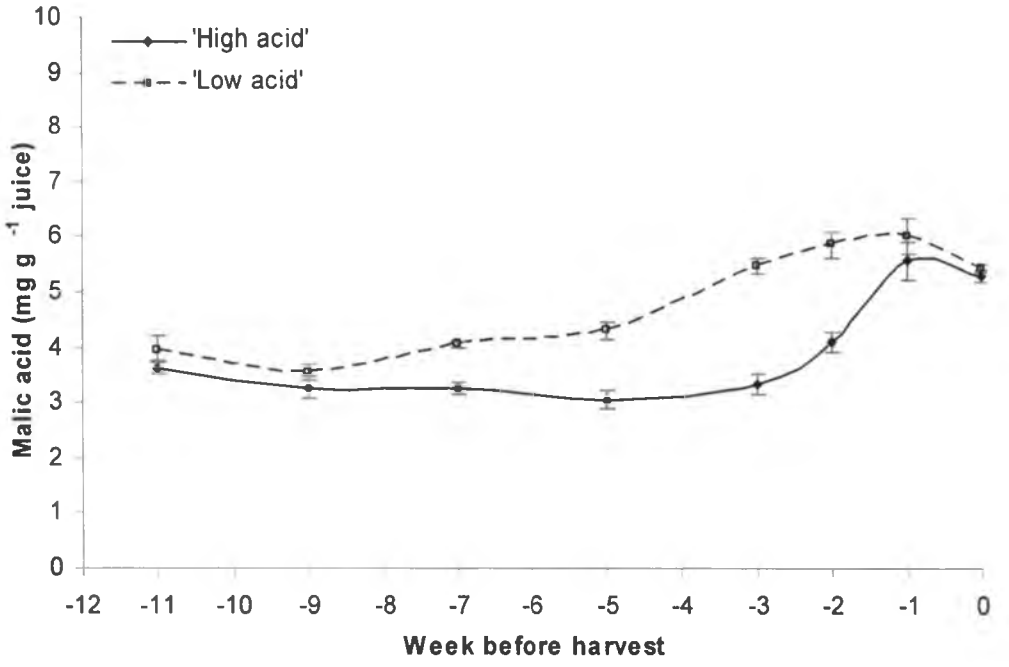



Figure 5.2. Changes in fruit malic acid concentration of pineapple 'High acid' (36-21) and 'Low acid' (63-555) during fruit growth and development. Fruit were sampled from 11 WBH to commercial harvest week. Means ± SE of 10 replicates.

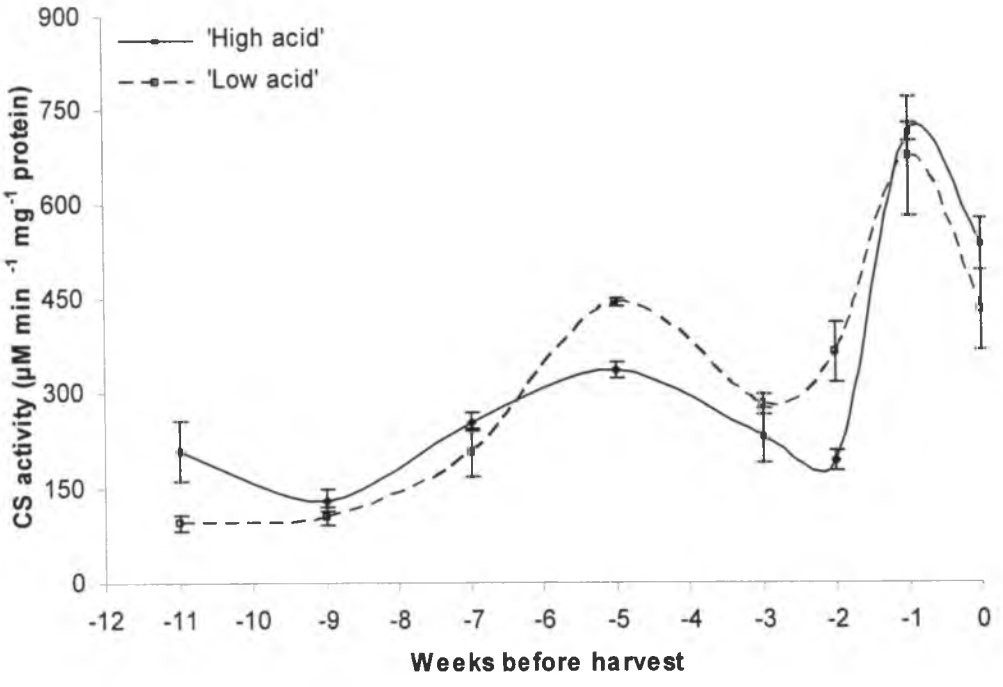



Figure 5.3. Changes in citrate synthase (CS) activity of pineapple 'High acid' (36-21) and 'Low acid' (63-555) during fruit growth and development. Fruit were sampled from 11 WBH to commercial harvest week. Means ± SE of four replicates.

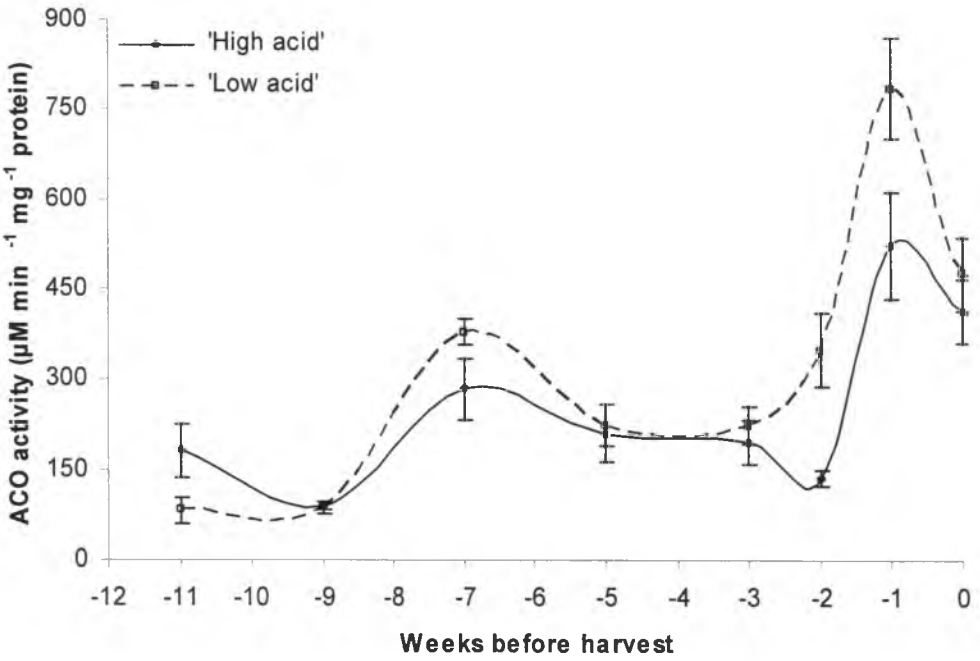



Figure 5.4. Changes in aconitase (ACO) activity of pineapple 'High acid' (36-21) and 'Low acid' (63-555) during fruit growth and development. Fruit were sampled from 11 WBH to commercial harvest week. Means ± SE of four replicates.

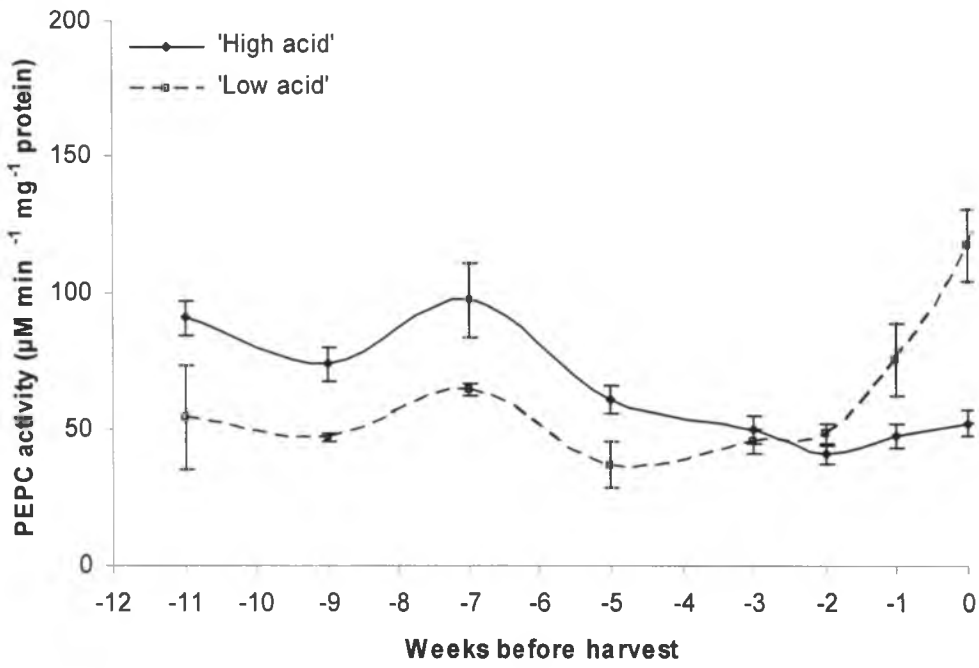



Figure 5.5. Changes in phosphoenolpyruvate carboxylase (PEPC) activity of pineapple 'High acid' (36-21) and 'Low acid' (63-555) during fruit growth and development. Fruit were sampled from 11 WBH to commercial harvest week. Means ± SE of four replicates.

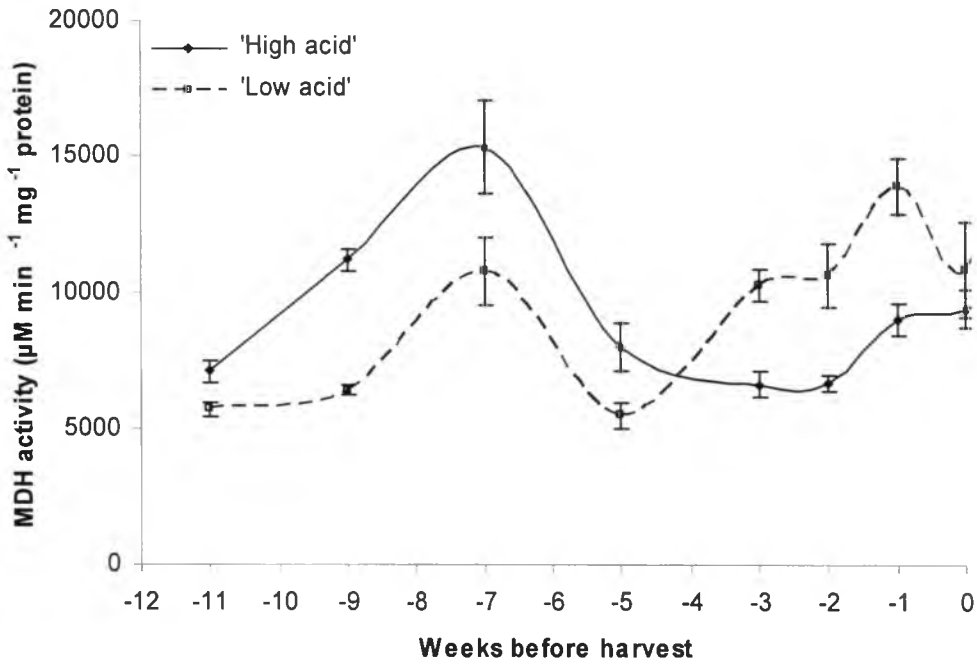



Figure 5.6. Changes in malate dehydrogenase (MDH) activity of pineapple 'High acid' (36-21) and 'Low acid' (63-555) during fruit growth and development. Fruit were sampled from 11 WBH to commercial harvest week. Means ± SE of four replicates.

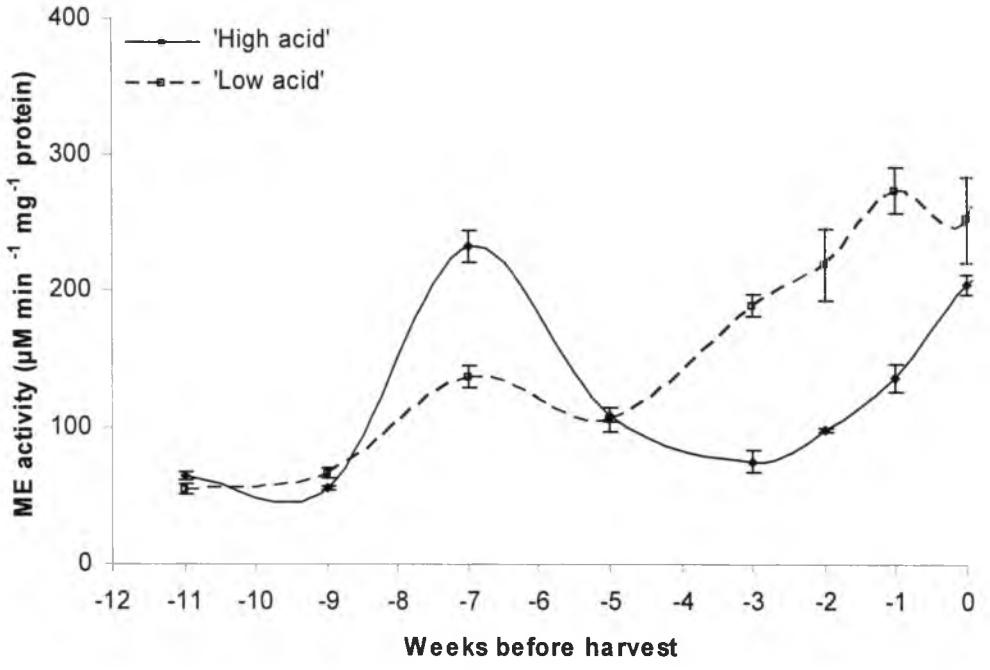



Figure 5.7. Changes in malic enzyme (ME) activity of pineapple 'High acid' (36-21) and 'Low acid' (63-555) during fruit growth and development. Fruit were sampled from 11 WBH to commercial harvest week. Means ± SE of four replicates.

# CHAPTER 6

# FRUIT POTASSIUM CONCENTRATION AND FRUIT ACIDITY

## 6.1 Introduction

Potassium exists in plants in an ionic form and makes up approximately 1% of the dry matter (Epstein, 1999). In pineapple, potassium and nitrogen are the most important elements that must be considered in the relation to plant and fruit growth (Py et al., 1987).

Potassium ion functions as a co-factor with more than 40 enzymes, is a primary cation used for osmotic adjustment in cell turgor and in maintaining electroneutrality (Epstein, 1999; Evans and Sorger, 1966). Potassium ion is also involved in sugar and organic acid transportation (Py et al., 1987). However, potassium can have antagonistic effects on the absorption of other cations such as calcium and magnesium.

In pineapple, an increase in potassium leads to an increase in total soluble solids, acidity, ascorbic acid, flesh firmness, aroma and flavor (Py et al., 1987; Spironello et al., 2004) whereas an increase of nitrogen reduces acidity and total soluble solids (Py et al., 1987; Velez-Ramos and Borges, 1995). Moreover, potassium can effect shell color and fruit lodging resistance by increasing peduncle diameter (Py et al., 1987) and a reduction of internal browning (Soares et al., 2005).

High rates of nitrogen and potassium fertilization are required to increase pineapple yield and quality, even in tropical peat soil (Hanafi and Razzaque, 2001). Nitrogen has a greater effect on fruit weight than potassium and normally, nitrogen and other minerals can be applied to the plants until flower induction. In contrast, potassium can be applied after flower induction since potassium continues to absorbed to improve fruit quality (Py et al., 1987).

The most commonly used potassium fertilizers are potassium sulfate and potassium chloride. The suitability of each form is still controversial (Hepton, 2003). The chloride form may have negative effect on fruit weight, maturation delay and total soluble solids but increase fruit acidity (Marchal et al., 1981; Sanford, 1968). Many have shown that potassium sulfate fertilizer is superior to potassium chloride at improving fruit quality (Hepton, 2003; Su and Li, 1963). However, in other areas, potassium chloride is used without adverse effect on yield and fruit quality (Hepton, 2003; Tapchoi, 1990). To increase fruit acidity, Py et al. (1987) recommended the application of potassium chloride as 25 to 33% of total potassium fertilizer prior to flower induction. In Thailand, Tapchoi (1990) reported that potassium chloride, nitrate and sulfate gave similar fruit yield, and no negative effects from the chloride form were found. However, potassium chloride treatment gave the highest fruit acidity (0.41%) and potassium sulfate the lowest (0.34%). A treatment of multiple applications of potassium chloride between the vegetative growth phase until 90 days after forcing reduced fruit marbling disease (Verawudh and Thongieung, 2001) caused by *Erwinia ananas* (Pegg, 1993).

Since potassium plays a significant role in pineapple fruit quality including fruit acidity, the difference in fruit acid content between the high and low acid clones may therefore be due to difference in fruit potassium concentration. The objectives of present studies were to investigate the developmental changes in fruit potassium concentration between the high and low acid clones and correlate potassium concentration, acidity and total soluble solid during fruit development. The effect of potassium chloride fertilizer on fruit quality was determined with clone 'D30' (73-50).

## 6.2 Materials and Methods

### Developmental changes in fruit potassium, titratable acidity and total sugar

### Plant materials

Uniform pineapple fruit of 36-21 and 63-555 (D10) clones were harvested as the high and low acid clones, respectively, between May and August, 2003. Ten fruit from each clone were sampled weekly throughout fruit development, (11 to 0 WBH), from the Dole Fresh Fruit Co. plantation on the island of Ohau, Hawaii. The fruit were transferred to the laboratory within two hours.

Two pieces of flesh from opposite sides at the fruit equator were squeezed and 20 mL of juice collected. The juice was centrifuged at 10,000 g for 10 min and the supernatant used for juice pH and titratable acidity (TA), total soluble solids (TSS) and total sugar determination as described in chapter 4. A 2 mL aliquot of supernatant was diluted with 95% ethanol to 1/5 volume and stored at -20°C for quantification of potassium concentration.

### Measurement of potassium concentration

The 2 mL alcoholic solution was air-dried and re-dissolved in deionized water (2 mL). The Cardy Potassium $K^+$ Meter (Spectrum Technologies, Inc.) was used to determine potassium concentration and expressed as ppm relative to 2000 ppm KCl standard.

### Effect of potassium chloride fertiiizer on fruit potassium and quality at harvest

'D30' pineapple fruit were sampled during the commercial harvest period from the plant crops grown at the Dole Fresh Fruit Co. plantation on the island of Ohau, Hawaii. The plant crop was treated with foliar potassium chloride at 0 and 123 kg $ha^{-1}$ during the mid-flowering stage. Standard cultural practice was used throughout crop

development. Both treatments were applied with 51 kg $ha^{-1}$ N, 0.3 kg $ha^{-1}$ $ZnSO_4$ and 5.5 kg $ha^{-1}$ $FeSO_4$. Ten fruit from each treatment were harvested and transferred to the laboratory within two hours. Sample preparation and measurements of potassium and fruit quality were performed as described above.

## 6.3 Results

### Developmental changes of fruit potassium concentration

Potassium concentration of pineapple fruit increased with fruit growth and development in both the high and low acid clones, from 3.53 to 5.08 and 4.40 to 5.03 mg $mL^{-1}$, respectively (Figure 6.1). In the early stage, 11 to 2 WBH, the low acid clone fruit showed significant greater potassium concentration than the high acid clone fruit. A peak of potassium content occurred in the low acid clone at 2 WBH, then subsequently declined at harvest week. The potassium content in the high acid clone increased and peaked at 1 WBH and thereafter declined to harvest week. The concentration in the last two weeks was not significant different between the high and low acid fruit.

### Potassium content and fruit titratable acidity

The change in patterns of flesh juice potassium concentration and titratable acidity (Figure 6.2, 6.3) showed that the increases of potassium and acidity in the high acid clone were nearly parallel as the fruit approached maturation. The correlation between potassium content and acidity was highly significant (r=0.94) (Table 6.1). The patterns of juice potassium content and titratable acidity in the low acid clone showed less similarity than the high acid clone, however, the correlation between potassium and juice acidity was still significant (r=0.72).

**Potassium and total sugar contents**

The change in pattern of flesh juice potassium concentration and total sugar content in the high and low acid clones (Figure 6.2, 6.3) were parallel as the fruit approached maturity. The correlation of potassium concentration and total sugar content in the high and low acid clones were significant with r=0.92 and 0.79, respectively (Table 6.1).

**Total sugar content and fruit titratable acidity**

The change in pattern of fruit acidity and total sugar content between the high and low acid clones were different. The high acid clone showed highly significant correlation between fruit acidity and total sugar content, r=0.93 (Table 6.1). In contrast, the low acid clone showed little correlation between acidity and total sugar, r=0.43.

**Potassium chloride fertilization on pineapple fruit quaiity at harvest**

The fruit of 'D30' pineapple treated with 123 kg $ha^{-1}$ foliar potassium chloride fertilizer at the mid-flowering were not significant different from untreated fruit (Table 6.2). The fruit had 1.09 mg $mL^{-1}$ potassium concentration, 6.73-6.74 meq 100 $mL^{-1}$ titratable acidity, 3.43 - 3.44 juice pH, 13.5 - 13.6 total soluble solid and 111 mg $mL^{-1}$ total sugar concentration.

## 6.4 Discussion

**Potassium concentration and fruit acidity and total sugar content**

The fruit potassium concentration in the high and low acid clones increased during fruit development and peaked in the last few weeks, then declined toward harvest. At the early stage of fruit development, the potassium patterns were very similar to the patterns of titratable acidity. The low acid clone fruit had greater potassium concentration and acidity than the high acid clone fruit. In addition, during the last few

weeks prior to harvest, the high and low acid clones showed a decline in potassium content that paralleled the decline in fruit acidity. This suggested that a change in fruit acidity was associated with the change in fruit potassium.

Changes in potassium concentration of the high and low acid clone fruit paralleled the changes of total sugar content and resulted in highly significant correlation between potassium and total sugar contents. This results agrees with many reports that showed that high potassium increases fruit acidity and total soluble solids (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).

**Potassium chloride fertilizer on pineapple fruit quality**

Fruit quality, as measured by titratable acidity, juice pH, total soluble solid and total sugar, from the potassium chloride treatment was not significantly different from the non-treated fruit due to the similarity in fruit potassium concentration between treatments. A single foliar application of potassium chloride at rate of 123 kg $ha^{-1}$ in this experiment had no effect on potassium concentration in the fruit. Tapchoi (1990) reported that multiple applications of potassium fertilizer increase fruit yield and quality. Split applications of potassium fertilizer (four times during vegetative growth and another two after forcing) with total 14 g/plant $K_2O$ increased fruit yield.

The effective application of potassium fertilizer ranges from 200 to 1000 kg $ha^{-1}$ in the potassium-limited soil (Hepton, 2003; Velez-Ramos and Borges, 1995). It was suggested that a single foliar application of potassium chloride at rate of 123 kg $ha^{-1}$ in this experiment was not sufficient to increase potassium concentration in the pineapple sufficiently to influence fruit quality. Moreover, in areas used for pineapple production that had not received renewed potassium fertilizer application for a long time might lead to inadequate soil potassium for plant growth.

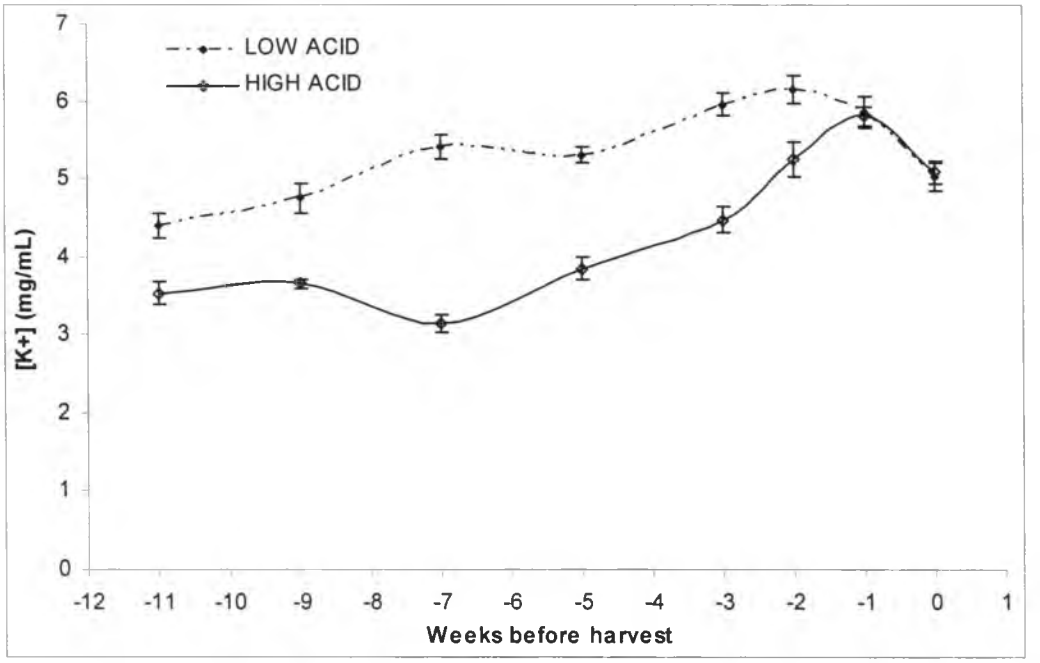
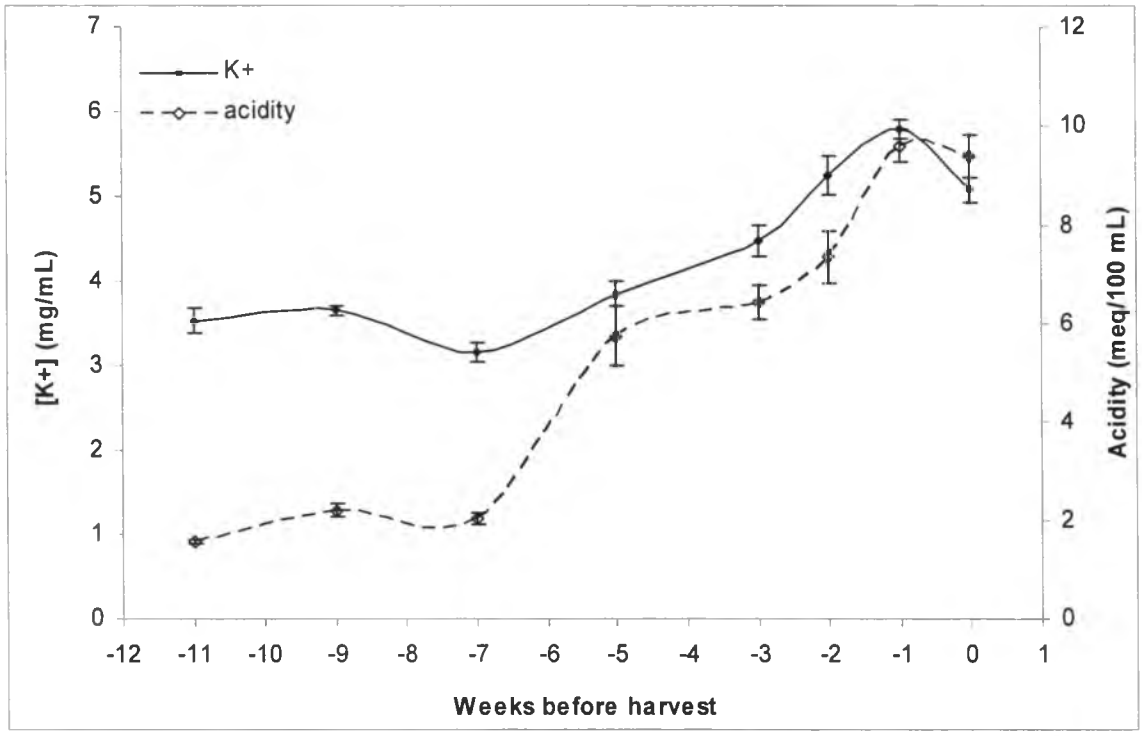



Figure 6.1. Changes in fruit potassium concentration of pineapple 'High acid' (36-21) and 'Low acid' (63-555) during fruit growth and development. Fruit were sampled from 11 WBH to commercial harvest week. Means ± SE of 10 replicates.



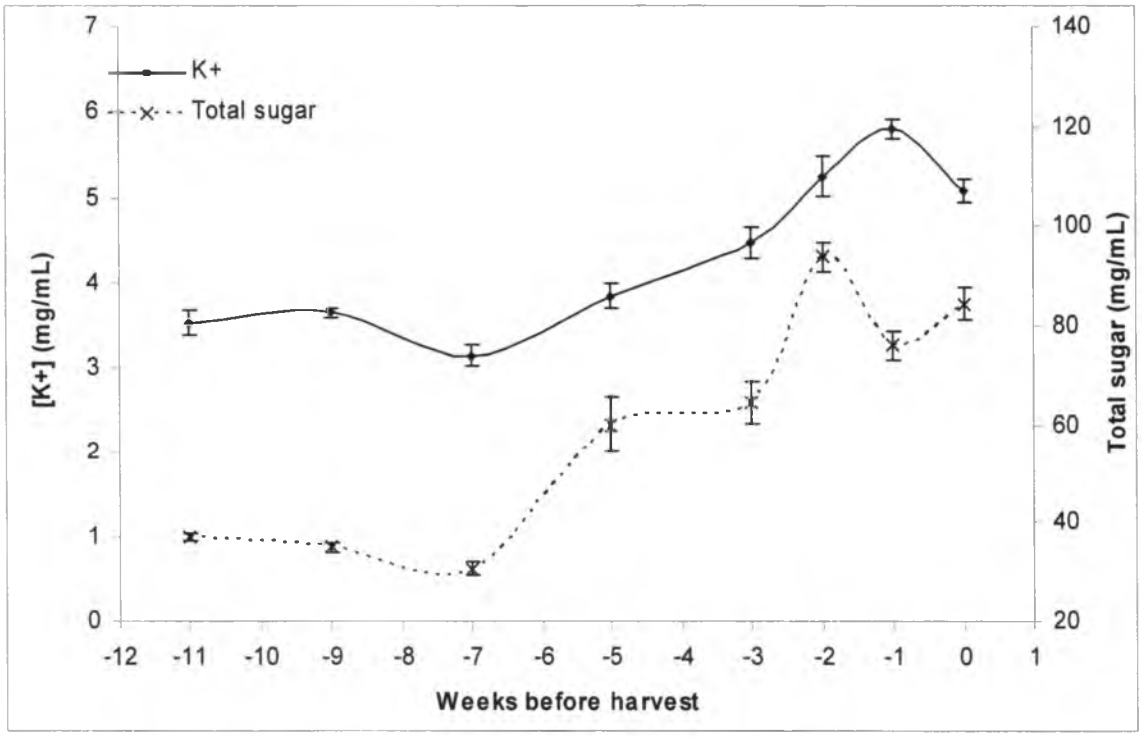




Figure 6.2. Changes in fruit potassium concentration, titratable acidity and total sugar of pineapple 'High acid' (36-21) during fruit growth and development. Fruit were sampled from 11 WBH to commercial harvest week. Means ± SE of 10 replicates.

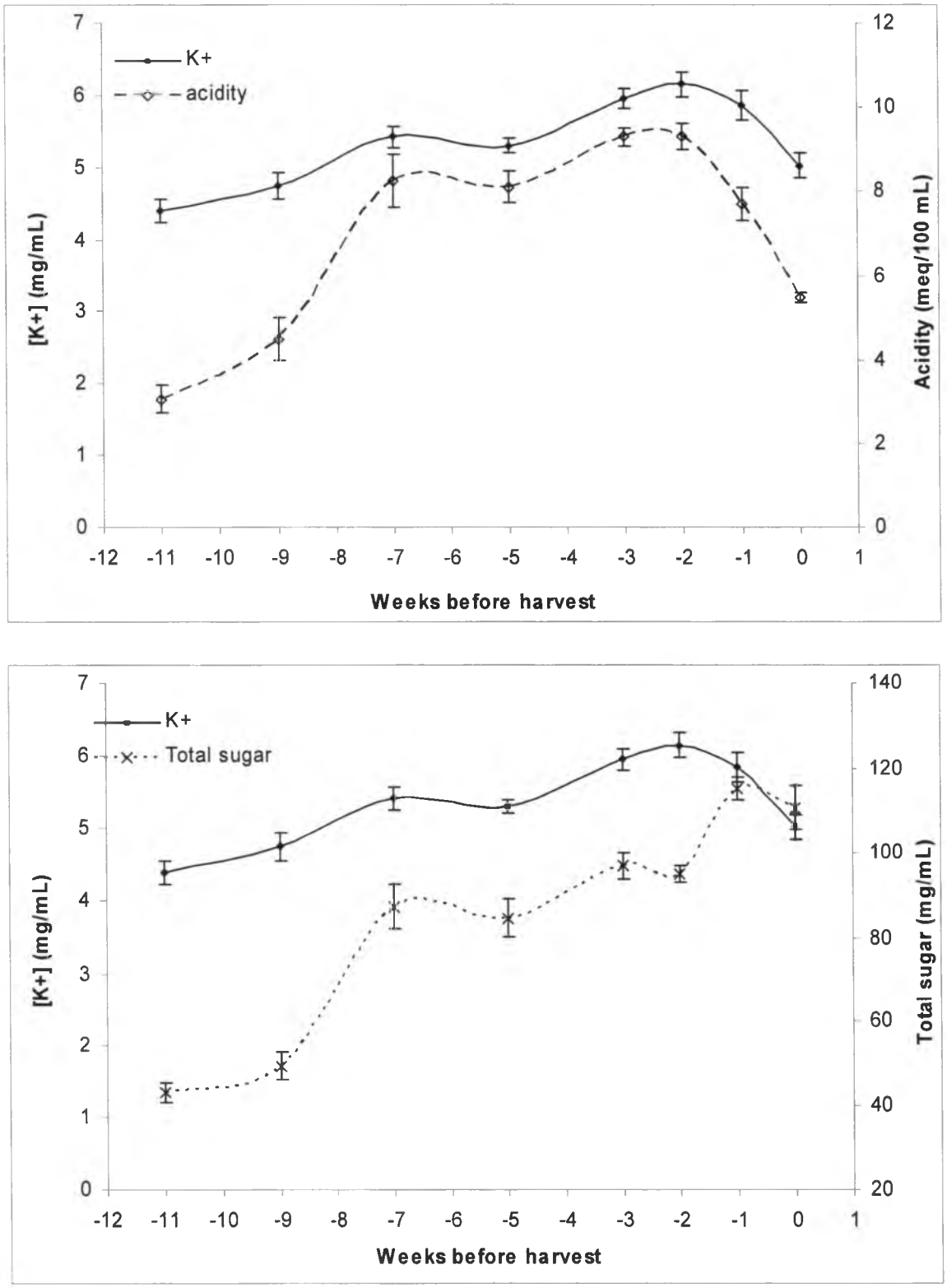



Figure 6.3. Changes in fruit potassium concentration, titratable acidity and total sugar of pineapple 'Low acid' (63-555) during fruit growth and development. Fruit were sampled from 11 WBH to commercial harvest week. Means ± SE of 10 replicates.

Table 6.1. Correlation analysis of fruit potassium concentration, titratable acidity and total sugar content in the high and low acid cultivars during fruit growth and development. N = 8 replicates, 10 fruit per replicate.

| Relationship | 'High acid' (36-21) | 'Low acid' (63-555) |
|---|---|---|
| Potassium – acidity | 0.939 *** | 0.718 * |
| Potassium – total sugar | 0.917 *** | 0.793 ** |
| Acidity – total sugar | 0.926 *** | 0.427 ns |

*, **, ***: significant correlation at p=0.05, 0.01, 0.001, respectively

ns: no correlation (p>0.05)

Table 6.2. Effects of potassium chloride application on 'D30' pineapple fruit quality at harvest. KCl was applied at rate of 123 kg $ha^{-1}$ during the mid-flowering stage. N=10 replicate.

| Fertilizer application | K+ (mg $mL^{-1}$) | TA (meq $100mL^{-1}$) | pH | TSS (%) | Total sugar (mg $mL^{-1}$) |
|---|---|---|---|---|---|
| Without KCl | 1.09 | 6.74 | 3.44 | 13.60 | 110.75 |
| With KCl | 1.09 | 6.73 | 3.43 | 13.50 | 110.58 |
| F-test | ns | ns | ns | ns | ns |
| CV(%) | 16.2 | 10.0 | 3.3 | 3.0 | 6.9 |

ns: Non-significant different between treatments ($p>0.05$).

# CHAPTER 7

# SUMMARY

## Morphological fruit growth and development

The developmental changes in fruit morphological characteristics were similar between the high and low acid clones. The fruit weight, fruit length and fruit diameter increased in parallel between these two clones. Thus, the fruit growth and development of these clones were similar to typical 'Smooth Cayenne' fruit (Bartolome et al., 1995; Singleton and Gortner, 1965)

## Developmental change of fruit acidity and total soluble solid

The developmental changes of fruit acidity and total soluble solids were different between the high and low acid clones. The increase in fruit titratable acidity in the low acid clone occurred earlier, peaked and then sharply declined a week prior to the high acid clone. In contrast, the high acid clone gradually increased in acidity and peaked at a week before harvest and then declined slightly. The changes in fruit titratable acidity were consistent with changes of juice pH. The increases in total soluble solid and total sugar were earlier in the low acid fruit than high acid fruit resulting in higher values at harvest. The results suggested that the low acid clone was lower in fruit acid content at harvest than the typical 'Smooth Cayenne' (Singleton and Gortner, 1965).

## Developmental fruit organic acid changes

The developmental changes in fruit citric acid concentration paralleled the changes in fruit titratable acidity that increased, peaked and then declined earlier in the low acid fruit than the high acid fruit. The fruit malic acid concentration varied only slightly before harvest in both clones. The change in citric acid content accounted for

the developmental change in fruit acidity and the difference between the high and low acid fruit.

**Role of citrate synthase (CS) on fruit citric acid synthesis**

The changes in CS activity were similar between the high and low acid clones throughout fruit development. The 3 folds increase in CS activity at a week before harvest coincided with the peak of citric acid content in the high acid clone. This result agrees with the changes in CS activity and organic acid contents in citrus (Sadka et al., 2001; Wen et al., 2001). The results suggest that CS played an essential role in citric acid synthesis in pineapple fruit. However, the difference in the low acid fruit acidity was not accounted by CS activity.

**Role of aconitase (ACO) on citric acid accumulation**

The changes in ACO activity in the high and low acid clones were significantly different during the last two weeks before harvest. The low acid fruit had greater in ACO activity than the high acid fruit and coincided with a sharp reduction of organic acid in the low acid fruit. This result agreed with the change in citrus fruit acidity (Sadka et al., 2000) and accounted for the difference in fruit acidity during the last stage of pineapple fruit development. ACO also played a role in pineapple fruit acid accumulation. The low acid fruit had a higher acid degradation rate than the high acid fruit just before harvest.

**Role of phosphoenolpyruvate carboxylase (PEPC), malate dehydrogenase (MDH) and malic enzyme (ME) on fruit acid metabolism**

The developmental changes in PEPC, MDH and ME activities were not directly correlated to the changes in citric or malic acids in either the high or low acid clone. This was similar to the enzyme activities in peach and grape berry (Diakou et al., 2000; Moing et al., 2000) in which the activities did not directly account for developmental change in

fruit acidity. However, pineapple fruit acid metabolism could be partially due to these enzyme activities though the effect may be minor due to an indirect participation in organic acid metabolism.

**Potassium concentration and fruit acidity**

The developmental changes in fruit potassium were significantly correlated with fruit acidity and fruit total sugar in both the high and low acid clones. The low acid fruit was higher in fruit potassium concentration than the high acid fruit that was coincident with higher in fruit acidity during the early stage of fruit development. This could suggest that a change in fruit acidity was associated with the change in fruit potassium concentration and the cause of the highly significant correlation, especially in the high acid fruit. However, fruit potassium concentration did not differ between both clones at harvest.

**Application of KCl fertilizer and pineapple fruit acidity**

A single foliar application of potassium chloride at rate of 123 kg $ha^{-1}$ during mid-flowering in this experiment had no effect on fruit potassium concentration, acidity and total sugar. The fruit potassium concentration at harvest was not different between KCl applied and non-applied treatments resulting in no difference in fruit acidity and total sugar between treatments. It was indicated that higher rate of application may lead to increase in fruit potassium resulting in an increase in fruit acidity.

## REFERENCES


Abd Shukor, A.R., A. Faridah, H. Abdullah, and Y.K. Chan. 1998. Pineapple, p. 137-190. In: P.E. Shaw, H.T. Chan, and S. Nagy (eds.). Tropical and Subtropical Fruits. AgScience, Inc., Auburndale, Florida.

Addiscott, T.M. 1974. Potassium in relation to transport of carbohydrate and ions in plants. Potassium Research and Agricultural Production. Proceedings of the 10th Congress of the International Potash Institute held in June: 205-220.

Al-Maiman, S.A.and D. Ahmad. 2002. Changes in physical and chemical properties during pomegranate (*Punica granatum* L.) fruit maturation. Food Chemistry. 76: 437-441.

Aradhya, M.K., F. Zee, and R.M. Manshardt. 1994. Isozyme Variation In cultivated and wild pineapple. Euphytica. 79: 87-99.

Asher, C.J.and P.G. Ozanne. 1967. Growth and potassium content of plants in solution cultures maintained at constant potassium concentrations. Soil Science. 103: 155-161.

Bakrim, N., J.L. Prioul, E. Deleens, J.P. Rocher, M. Arrio-Dupont, J. Vidal, P. Gadal, and R. Chollet. 1993. Regulatory phosphorylation of $C_4$ phosphoenolpyruvate carboxylase. Plant Physiology. 101: 891-897.

Bartholomew, D.P., E. Malezieux, G.M. Sanewski, and E. Sinclair. 2003. Inflorescence and Fruit Development and Yield, p.167-202. In: D.P. Bartholomew, R.E. Pauli, and K.G. rohrbach (eds.). The Pineapple: Botany, Production and Uses. CABI, New York.

Bartholomew, D.P., K.G. Rohrbach, and D.O. Evans. 2002. Pineapple cultivation in Hawaii. Fruits and Nuts. 7: 1-8.

Bartholomew, D.P.and R.E. Paull. 1986. Pineapple. CRC handbook of fruit set and development / edited by Shaul P. Monselise, p: 371-388.

Bartholomew, D.P.and S.B. Kadzimin. 1977. Pineapple. In: P.d.T. Alvim and T.T. Kozlowski (eds.). Ecophysiology of Tropical Crops. Academic Press, New York.

Bartolome, A.P., P. Ruperez, and C. Fuster. 1995. Pineapple fruit morphological characteristics, chemical composition and sensory analysis of Red Spanish and Smooth Cayenne cultivars. Food Chemistry. 53: 75-79.

Beringer, H. 1985. Potassium uptake as a function of its requirement and K supply. Journal of Potassium Research. 1: 1-16.

Beruter, J. 2004. Carbohydrate metabolism in two apple genotypes that differ in malate accumulation. Journal of Plant Physiology. 161: 1011-1029.

Besford, R.T.and G.E. Hobson. 1975. Effect of potassium nutrition on some enzymes from ripening *Lycopersicon esculentum* fruit. Phytochemistry. 14: 57-59.

Bloom, A.J. 2002. Mineral Nutrition. In: L. Taiz and E. Zeiger (eds.). Plant Physiology. Ainauer Associates, Inc, Massachusetts.

Bogin, E.and A. Wallace. 1966. Organic acid synthesis and accumulation in sweet and sour lemon fruit. Journal of the American Society for Horticultural Science. 89: 182-194.

Boulton, R. 1980. The relationships between total acidity, titratable acidity and pH in grape tissue. Vitis. 19: 113-120.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Analytical Biochemistry. 72: 248-254.

Brat, P., M. Reynes, J.M. Brillouet, L.N.T. Hoang, and A. Soler. 2004. Physicochemical characterization of a new pineapple hybrid (FLHORAN41 cv.). Journal of agricultural and food chemistry. 52: 6170-6177.

Brouquisse, R., M. Nishimura, J. Gaillard, and R. Douce. 1987. Characterization of a cytosolic aconitase in higher plant cells. Plant Physiology. 84: 1402-1407.

Brune, A., P. Gonzalez, R. Goren, U. Zehavi, and E. Echeverria. 1998. Citrate uptake into tonoplast vesicles from acid lime (*Citrus aurantifolia*) juice cells. Journal of Membrane Biology. 166: 197-203.

Cakmak, I. 2005. The role of potassium in alleviating detrimental effects of abiotic stresses in plants. Journal of Plant Nutrition and Soil Science. 168: 521-530.

Cakmak, I.and H. Marschner. 1994. Changes in phloem export of sucrose in leaves in response to phosphorus, potassium and magnesium deficiency in bean plants. Journal of Experimental Botany. 45: 1251-1257.

Cameron, J.W.and R.K. Soost. 1977. Acidity and total soluble solids in Citrus hybrids and advanced crosses involving acidless orange and acidless pummelo. Journal of the American Society for Horticultural Science. 120: 510-514.

Canel, C., J.N. Baileyserres, and M.L. Roose. 1995. In-Vitro [C-14] Citrate Uptake By Tonoplast Vesicles Of Acidless Citrus Juice Cells. Journal Of The American Society For Horticultural Science. 120: 510-514.

Canel, C., J.N. Bailey-Serres, and M.L. Roose. 1996. Molecular characterization of the mitochondrial citrate synthase gene of an acidless pummelo (*Citrus maxima*). Plant Molecular Biology. 31: 143-147.

Chadha, K.L.and S.D. Shikhamany. 1974. High density planting increases pineapple yields. Indian Journal of Horticulture. 18: 3-5.

Chan, H.T., Jr., E. Chenchin, and P. Vonnahme. 1973. Nonvolatile acids in pineapple juice. Journal of Agricultural and Food Chemistry. 21: 208-211.

Chan, Y.K., G.C. d'Eeckenbrugge, and G.M. Sanewski. 2003. Breeding and Variety Improvement, p. 33-55. In: D.P. Bartholomew, R.E. Pauli, and K.G. Rohrbach (eds.). The Pineapple: Botany, Production and Uses. CAB, Honolulu.

Cheeseman, J.M.and J.B. Hanson. 1979. Energy-linked potassium influx as related to cell potential in corn roots. Plant Physiology. 64: 842-845.

Chen, C.C.and R.E. Paull. 2001. Fruit temperature and crown removal on the occurrence of pineapple fruit translucency. Scientia horticulturae. 88.

Chollet, R., J. Vidal, and M.H. Oleary. 1996. Phosphoenolpyruvate carboxylase: a ubiquitous, highly regulated enzyme in plants. Annual Review of Plant Physiology And Plant Molecular Biology. 47: 273-298.

Chongpraditnun, P., P. Luksanawimol, P. Limsmuthchaiporn, and S. Vasunun. 2000. Effect of fertilizers on the content of nitrate in pineapple fruit. Acta Horticulturae: 217-220.

Clark, R.B.and A. Wallace. 1966. Dark $CO_2$ fixation in organic acid synthesis and accumulation in Citrus fruit vesicles. American Society for Horticultural Science. 83: 322-332.

Collins, J.L. 1960. The pineapple, botany, cultivation and utilization. Leonard Hill Ltd, London.

Collins, J.L. 1968. The Pineapple. Botany, Cultivation and Utilization. World Crop Books, London.

Coppens d'Eeckenbrugge, G. and F. Leal. 2003. Morphology, Anatomy and Taxonomy, p. 13-32. In: D.P. Bartholomew, R.E. Pauli, and K.G. Rohrbach (eds.). The Pineapple: Botay, Production and Uses. CABI, New York.

Courtois-Verniquet, F.and R. Douce. 1993. Lack of aconitase in glyoxysomes and peroxisomes. Biochemical Journal. 294: 103-107.

Cretin, C., S. Sonti, E. Keryer, L. Lepiniec, D. Tagu, J. Vidal, and P. Gadal. 1991. The phosphoenolpyruvate carboxylase gene family of Sorghum: promoter structures, amino acid sequences and expression of genes. Gene. 99: 87-94.

Dass, H.C.and A.K. Srivastava. 1997. Role of potassium in citrus nutrition: a review. Journal of Potassium Research. 13: 80-92.

De Bellis, L., R. Tsugeki, M. Alpi, and M. Nishimura. 1993. Purification and characterization of aconitase isoforms from etiolated pumpkin cotyledons. Physiologia plantarum. 88: 485-492.

Demmig, B.and H. Gimmler. 1983. Properties of the isolated intact chloroplast at cytoplasmic $K^+$ concentrations. Plant Physiology. 73: 169-174.

Diakou, P., L. Svanella, P. Raymond, J.P. Gaudillere, and A. Moing. 2000. Phosphoenolpyruvate carboxylase during grape berry development: protein level, enzyme activity and regulation. Australian Journal of Plant Physiology. 27: 221-229.

Dilley, D.R. 1962. Malic enzyme activity in apple fruit. Nature. 196: 387-388.

Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers, and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. Analytical Chemistry. 28: 350-356.

Dull, G.G. 1971. The Pineapple: General, p. 303-324. In: A.C. Hulme (ed.). The Biochemistry of Fruits and their Products. Academic Press, Norwich, England.

Duval, M.F. and G. Coppens d'Eeckenbrugge. 1993. Genetic variability in the genus Ananas. Acta Horticulturae. 334: 573-621.

Echeverria, E., P.C. Gonzalez, and A. Brune. 1997. Characterization of proton and sugar transport at the tonoplast of sweet lime (*Citrus limmetioides*) juice cells. Physiologia plantarum.

Edwards, G.E.and C.E. Andreo. 1992. NADP-malic enzyme from plants. Phyto-chemistry. 31: 1845-1857.

Elbulk, R.E., E.F.E. Babiker, and A.H. ElTinay. 1997. Changes in chemical composition of guava fruits during development and ripening. Food Chemistry. 59: 395-399.

Epstein, E. 1999. Silicon. Annual Reviews of Plant Physiology Plant Molecular Biology. 50: 641-664.

Etienne, C., A. Moing, E. Dirlewanger, P. Raymond, R. Monet, and C. Rothan. 2002. Isolation and characterization of six peach cDNAs encoding key proteins in organic acid metabolism and solute accumulation: involvement in regulating peach fruit acidity. Physiologia Plantarum. 114: 259-270.

Evans, H.J.and G.J. Sorger. 1966. Role of mineral elements with emphasis on the univalent cations. Annual Reviews of Plant Physiology. 17: 47-76.

Fang, D.Q., C.T. Federici, and M.L. Roose. 1997. Development of molecular markers linked to a gene controlling fruit acidity in citrus. Genome. 40: 841-849.

Franke, K.E.and D.O. Adams. 1992. Inhibition of malic enzyme from grape berries by sulfhydryl-reagents and oxalic-acid. American Journal Of Enology And Viticulture. 43: 153-158.

Genard, M., M. Reich, P. Lobit, and J. Besset. 1999. Correlations between sugar and acid content and peach growth. Journal Of Horticultural Science & Biotechnology. 74: 772-776.

Gielt, C. 1992. Malate dehydrogenase isoenzymes: cellular locations and role in the flow of metabolites between the cytoplasm and cell organelles. Bichemica et Biophysica Acta. 1100: 217-234.

Glew, R.H., F.A. Ayaz, C. Sanz, D.J. Vanderjagt, H.S. Huang, L.T. Chuang, and M. Strnad. 2003. Changes in sugars, organic acids and amino acids in medlar (*Mespilus germanica* L.) during fruit development and maturation. Food Chemistry. 83: 363-369.

Gonzalez-Tejera, E.and H. Gandia-Diaz. 1976. The effect of nitrogen and potassium fertilizers on the prodcutivity and quality of the pineapple cultivar Smooth Cayenne. 24$^{th}$ Annual Congress of the American Society for Horticultural Sciences, Tropical Region, Mayaquez Institute of Tropical Agriculture. p. 196-205.

Goodenough, P.W., I.M. Prosser, and K. Young. 1985. NADP-linked malic enzyme and malate metabolism in aging tomato fruit. Phytochemistry. 24: 1157-1162.

Gortner, W.A. 1963. A short term effect of weather on malic acid in pineapple fruit. Journal of Food Science. 28: 191-192.

Gortner, W.A., G.G. Dull, and B.H. Krauss. 1967. Fruit development, maturation, ripening and senescence: a biochemical basis for horticultural terminology. HortScience. 2: 141-144.

Gutierrezgranda, M.J.and J.C. Morrison. 1992. Solute distribution and malic enzyme-activity in developing grape berries. American Journal Of Enology And Viticulture. 43: 323-328.

Hamner, K.C.and G.T. Nightingale. 1946. Ascorbic content of pineapples as correlated with environmental factors and plant composition. Food Research. 11: 535-541.

Hanafi, M.M.and A.H.M. Razzaque. 2001. Effect of potassium on growth, yield and quality of pineapple in tropical peat. Fruits. 56: 45-49.

Hartt, C.E. 1970. Effect of potassium deficiency upon translocation of $^{14}C$ in detached blades of sugarcane. Plant Physiology. 45: 183-187.

Hayashi, M., L. De Bellis, A. Alpi, and M. Nishimura. 1995. Cytosolic aconitase participates in the glyoxylate cycle in etiolated pumpkin cotyledons. Plant And Cell Physiology. 36: 669-680.

Hepton, A. 2003. Cultural System, p. 109-142. In: D.P. Bartholomew, R.E. Pauli, and K.G. Rohrbach (eds.). The Pineapple: Botany, Production and Uses. CABI, Honolulu.

Herath, H.M.I., D.C. Bandara, and D. Banda. 2003. Effect of pre-harvest calcium fertilizer application on the control of internal browning development during the cold storage of pineapple 'Mauritius' (*Ananas comosus* (L.) Merr.). Journal of Horticultural Science & Biotechnology. 78: 762-767.

Hewajulige, I.G.N., R.S.W. Wijeratnam, R.L.C. Wijesundera, and M. Abeysekere. 2003. Fruit calcium concentration and chilling injury during low temperature storage of pineapple. Journal of The Science of Food and Agriculture. 83: 1451-1454.

Hill, S.A. 1997. Carbon metabolism in mitochondria, p. 181-199. In: D.T. Dennis, D.B. Layzell, D.D. Lefebvre, and D.H. Turpin (eds.). Plant Metabolism. Longman, England.

Hirai, M.and I. Ueno. 1977. Development of citrus fruits: fruit development and enzymatic changes in juice vesicle tissue. Plant and Cell Physiology. 18: 791-800.

Huet, R. 1958. La composition chimique de l' ananas. Fruits. 13: 187-197.

Humble, G.D.and K. Raschke. 1971. Stomatal opening quantitatively related to potassium transport. Plant Physiology. 48: 447-453.

Iredale, S.E. 1979. Properties of citrate synthase from *Pisum sativum* mitochondria. Phytochemistry. 18: 1057.

Jeffery, D., P.W. Goodenough, and P.D.J. Weitzman. 1988. Citrate synthase and malate-dehydrogenase from tomato fruit. Phytochemistry. 27: 41-44.

Jiang, M.Y.and J.H. Zhang. 2002. Involvement of plasma-membrane NADH oxidase in abscisic acid and water stress induced antioxidant defense in leaves of maize seedlings. Planta. 215: 1022-1030.

Jiao, J.A.and R. Chollet. 1991. Post-translational regulation of phosphoenolpyruvate carboxylase in $C_4$ and CAM plants. Plant Physiology. 95.

Jones, J.B. 1998. Plant Nutrition Manual. CRC Press, New York.

Kabu, K.L.and S.K. Chopra. 1988. Influence of pre-harvest spray of calcium and growth regulators on fruit acidity in Starking Delicious apple at various stages of development. Indian Journal of Plant Physiology. 31: 399-402.

Karadeniz, F. 2004. Main organic acid distribution of authentic citrus juices in Turkey. Turk J Agric For. 28: 267-271.

Kato, A., M. Hayashi, H. Mori, and M. Nishimura. 1995. Molecular characterization of a glyoxysomal citrate synthase that is synthesized as a precursor of higher molecular-mass in pumpkin. Plant Molecular Biology. 27: 377-390.

Kermasha, S., N.N. Barthakur, A. Inteaz, and N.K. Mohan. 1987. Changes in chemical composition of the Kew cultivar of pineapple fruit during development. Journal of the Science of Food and Agriculture. 39: 317-324.

Knee, M., F.L. Finger, and L.M. Lagrimini. 1996. Evidence for a cytosolic NADP-malic enzyme in tomato. Phytochemistry. 42: 11-16.

Knee, M.and F.L. Finger. 1992. NADP-malic enzyme and organic-acid levels in developing tomato fruits. Journal of the American society for horticultural science. 117: 799-801.

Koch, K.and K. Mengel. 1977. The effects of K on N utilization by spring wheat during grain formation. Agronomy journal. 69: 477-480.

Lacognata, U., V. Landschutze, L. Willmitzer, and B. MullerRober. 1996. Structure and expression of mitochondrial citrate synthases from higher plants. Plant And Cell Physiology. 37: 1022-1029.

Lakso, A.N.and W.M. Kliewer. 1978. The Influence of temperature on malic-acid metabolism in grape berries part 2 temperature responses of net dark carbon

dioxide fixation and malic-acid pools. American Journal of Enology and Viticulture. 29: 145-149.

Laval-Martin, D., J. Farineau, and J. Diamond. 1977. Light versus dark carbon metabolism in cherry tomato fruits. Plant Physiology. 60: 872-876.

Law, R.D.and W.C. Plaxton. 1995. Purification and characterization of a novel phosphoenolpyruvate carboxylase from banana fruit. Biochemical Journal. 307: 807-816.

Leigh, R.A. 2001. Potassium homeostasis and membrane transport. Journal of Plant Nutrition and Soil Science. 164: 193-198.

Leigh, R.A.and R.G. Wyn Jones. 1984. A hypothesis relating critical potassium concentrations for growth to the distribution and functions of this ion in the plant cell. New Phytol. 97: 1-13.

Li, H., X. Yang, and A. Luo. 2001. Ameliorating effect of potassium on iron toxicity in hybrid rice. journal of plant nutrition. 24: 1849-1860.

Linford, M.B. 1933. Fruit quality studies. II. Eye number and eye weight. Pineapple Quarterly. 3: 185-195.

Lizana, L.A., L.E. Luchsinger, and J.G. Luza. 1990. Evaluation of market quality of pineapple fruit imported to Chili. Acta Horticulturae. 269: 69-74.

Loeillet, D. 1996. 'Victoria' pineapple: a promising market. Fruitrop. 24: 7-9.

Loeillet, D. 1997. The world pineapple market: the importance of Europe. Acta Horticulturae. 425: 37-48.

Lopez-Bucio, J., M.F. Nieto-Jacobo, V. Ramirez-Rodriguez, and L. Herrera-Estrella. 2000. Organic acid metabolism in plants: from adaptive physiology to transgenic varieties for cultivation in extreme soils. Plant Science. 160: 1.

Luo, A., X. Yang, Y. Deng, C. Li, K. Xiang, and D. Li. 2003. Organic acid concentrations and the relative enzymatic changes during the development of citrus fruits. Scientia Agricultura Sinica. 36: 941-944.

Maathuis, F.J.M.and D. Sanders. 1993. Energization of potassium uptake in *Arabidopsis thaliana*. Planta. 191: 302-307.

MacKinnon, R. 2003. Potassium Channels. FEBS Letters. 555: 62-65.

Maeshima, M. 2000. Vacuolar $H^+$-pyrophosphatase. Biochimica Et Biophysica Acta-Biomembranes. 1465: 37-51.

Malek, F.and D.A. Baker. 1977. Proton co-transport of sugars in phloem loading. Planta. 135: 297-299.

Malezieux, E. 2000. Global network for pineapple research. Acta horticulturae: 35-47.

Malezieux, E.and D.P. Bartholomew. 2003. Plant Nutrition. In: D.P. Bartholomew, R.E. Paull, and K.G. Rohrbach (eds.). The Pineapple: Botany, production and Uses. CABI, New York.

Malezieux, E., F. Cote and D.P. Bartholomew. 2003. Crop Environment, Plant Growth and Physiology. In: D.P. Bartholomew, R.E. Paull, and K.G. Rohrbach (eds.). The Pineapple: Botany, production and Uses. CABI, New York.

Marchal, J., A. Pinon, and C. Teisson. 1981. Effects of the form of potassium fertilizer on pineapple quality in the Ivory Coast. Fruits. 36: 737-743.

Marschner, H. 1995. Mineral Nutrition of Higher Plants. Academic Press, San Diego.

Marsh, K., S. Attanayake, S. Walker, A. Gunson, H. Boldingh, and E. MacRae. 2004. Acidity and taste in kiwifruit. Postharvest Biology And Technology. 32: 159-168.

Martinoia, E.and D. Rentsch. 1994. Malate compartmentation - Responses to a complex metabolism. Annual Review of Plant Physiology and Plant Molecular Biology. 45: 447-467.

Matsumoto, A.and S. Shiraishi. 1981. Studies on the organic-acids of citrus-fruits.7. Seasonal-changes in organic-acid levels in Satsuma Mandarin fruit. Journal of the Japanese Society for Horticultural Science. 49: 519-522.

Mengel, K.and E.A. Kirkby. 1987. Principles of Plant Nutrition. International Potash Institute, Worblaufen-Bern.

Mengel, K.and E.A. Kirkby. 2001. Principles of Plant Nutrition. Kluwer Academic Publishers, Dordrecht.

Mengel, K.and R. Pfluger. 1972. The release of potassium and sodium from young excised roots of *Zea mays* under various efflux conditions. Plant Physiology. 49: 16-19.

Mengel, K.and W.W. Arneke. 1982. Effect of potassium on the water potential, the pressure potential, the osmotic potential and cell elongation in leaves of *Phaseolus vulgaris*. Physiologia plantarum. 54: 402-408.

Milcheva, M., M. Nikolova, V. Rankov, S. Dimitrov, and E. Yubel. 1988. Effect of forms of potassium fertilizers on chemical composition and quality of the produce of different crops. Rasteniev"dni Nauki. 25: 27-34.

Moing, A., C. Rothan, L. Svanella, D. Just, P. Diakou, P. Raymond, J.P. Gaudillere, and R. Monet. 2000. Role of phosphoenolpyruvate carboxylase in organic acid accumulation during peach fruit development. Physiologia Plantarum. 108: 1-10.

Moing, A., L. Svanella, D. Rolin, M. Gaudillere, J.P. Gaudillere, and R. Monet. 1998. Compositional changes during the fruit development of two peach cultivars differing in juice acidity. Journal of the American Society for Horticultural Science. 123: 770-775.

Moing, A., L. Svanella, R. Monet, C. Rothan, D. Just, P. Diakou, J.P. Gaudillère, and D. Rolin. 1998a. Organic acid metabolism during the fruit development of two peach cultivars. Acta Hort. 465: 425-432.

Moing, A.and C. Renaud. 2001. Biochemical changes during fruit development of four strawberry cultivars. Journal of the American Society for Horticultural Science. 126: 394-403.

Morris, J.R., D.L. Cawthon, and J.W. Fleming. 1980. Effects of high-rates of potassium fertilization on raw product quality and changes in pH and acidity during storage of Concord grape Juice. American Journal of Enology and Viticulture. 31: 323-328.

Muller, M.L., U. Irkens-Kiesecker, B. Rubinstein, and L. Taiz. 1996. On the mechanism of hyperacidification in lemon. The Journal of Biological Chemistry. 271: 1916-1924.

Munoz, T., M.T. Sanchez-Ballesta, J. Ruiz-Cabello, M.I. Escribano, and C. Merodio. 2004. The acid metabolism of Annona fruit during ripening. Journal of Horticultural Science & Biotechnology. 79: 472-478.

Nanayakkara, K.P.G.A., H.M.W. Herath, and Y.D.A. Senanayake. 1997. Effects of some pre-harvest treatments of potassium and hormones on fruit core tissue deterioration and internal browning of pineapple under cold storage. Tropical Agricultural Research. 9: 90-95.

Nayar, N.K., V. Mathew, and M. Aravindakshan. 1981. Studies on variations in pineapple (*Ananas comosus* L. Merr) from various morphological and nutritive characters. Indian Horticulture. 29: 81-86.

Neog, M., B.K. Chakrabarty, D.K. Gogoi, and K. Baruah. 1995. Effect of varying levels of NPK on growth, yield and fruit quality of ratoon pineapple cv. 'Kew'. Horticultural Journal. 8: 25-31.

Neue, H.U., C.D. Senadhira, and T. Setter. 1998. Strategies for dealing with micronutrient disorders and salinity in lowland rice systems. Field Crops Research. 56: 139-155.

Nightingale, C.T. 1942. Nitrate and carbohydrate reserves in relation to nitrogen nutrition of pineapple. Bot. Gaz. 103: 409-456.

O'Leary, M.H. 1982. Phosphoenolpyruvate carboxylase: an enzymologist's view. Annual Reviews of Plant Physiology. 33: 297-315.

Oleski, N., P. Mahdavi, G. Peiser, and A.B. Bennett. 1987. Transport-properties of the tomato fruit tonoplast.1. Identification and characterization of an anion-sensitive $H^+$-ATPase. Plant Physiology. 84: 993-996.

Papke, I.and B. Gerhardt. 1996. Demonstration of citrate synthase in non-glyoxysomal peroxisomes. Plant Science. 116: 131-140.

Paull, R.E. 1993. Postharvest handling of Smooth Cayenne pineapple in Hawaii for the fresh fruit market. Acta Horticulturae. 334: 273-285.

Paull, R.E., J. Deputy, and N.J. Chen. 1983. Changes in organic-acids, sugars, and headspace volatiles during fruit ripening of soursop (*Annona Muricata* L). Journal of the American Society for Horticultural Science. 108: 931-934.

Pauli, R.E.and C.C. Chen. 2003. Postharvest Physiology, Handling and Storage of Pineapple. In: D.P. Bartholomew, R.E. Pauli, and K.G. Rohrbach (eds.). The Pineapple: Botany, Production and Uses. CABI Pub., New York.

Pegg, K.G. 1993. Diseases, p. 21-29. In: R.H. Broadley, R.C. Wassman, and E. Sinclair (eds.). Pineapple: Pests and Disorders. Queensland Department of Primary Industries, Brisbane.

Peoples, T.R.and D.W. Koch. 1979. Role of potassium in carbon dioxide assimilation in *Medicago sativa* L. Plant Physiology. 63: 878-881.

Peyret, P., P. Parez, and M. Alric. 1995. Structure, genomic organization, and expression of the *Arabidopsis thaliana* aconitase gene. J Biol Chem. 270: 8131-8137.

Popova, T.N.and M. de Carvalho. 1998. Citrate and isocitrate in plant metabolism. Biochimica Et Biophysica Acta-Bioenergetics. 1364: 307-325.

Py, C., J.J. Lacoeuilhe, and C. Teisson. 1987. The Pineapple. Cultivation and Uses, Maisonneuve, Paris.

Quaggio, J.A., J.D. Mattos, H. Cantarella, E.L.E. Almeida, and S.A.B. Cardoso. 2002. Lemon yield and fruit quality affected by NPK fertilization. Scientia Horticulturae. 96: 151.

Ramakrishnan, C.V. 1971. Citric acid metabolism in the fruit tissues of *Citrus acida*. Curr. Sci. 5: 97-100.

Ratajczak, R. 2000. Structure, function and regulation of the plant vacuolar $H^+$-translocating ATPase. Biochimica Et Biophysica Acta-Biomembranes. 1465: 17-36.

Rentsch, D.and E. Martinoia. 1991. Citrate transport into mesophyll vacuolescomparison with malate-uptake activity. Planta. 184: 532-537.

Richardson, A.C., K.B. Marsh, and E.A. Macrae. 1997. Temperature effects on Satsuma mandarin fruit development. Journal of Horticultural Science. 72: 919-929.

Roe, B., P.L. Davis, and J.H. Bruemmer. 1984. Pyruvate metabolism during maturation of Hamlin oranges. Phytochemistry. 23: 713-717.

Rohrbach, K.G., F. Leal, and G.C. d'Eeckenbrugge. 2003. History, Distribution and World Production. In: D.P. Bartholomew, R.E. Paull, and K.G. Rohrbach (eds.). The Pineapple: Botany, Production and Uses. CABI Pub., New York.

Romero Rodriguez, M.A., M.L. Vazquez Oderiz, J. Lopez Hernandez, and J. Simal Lozano. 1992. Determination of vitamin C and organic acids in various fruits by HPLC. Journal of Chromatographic Science. 30: 433-436.

Ruffner, H.P., D. Possner, S. Brem, and D.M. Rast. 1984. The physiological role of malic enzyme in grape ripening. Planta. 160: 444-448.

Sadka, A., B. Artzi, L. Cohen, E. Dahan, D. Hasdai, E. Tagari, and Y. Erner. 2000a. Arsenite reduces acid content in citrus fruit, inhibits activity of citrate synthase but induces its gene expression. Journal of the American Society for Horticultural Science. 125: 288-293.

Sadka, A., E. Dahan, E. Or, M.L. Roose, K.B. Marsh, and L. Cohen. 2001. Comparative analysis of mitochondrial citrate synthase gene structure, transcript level and enzymatic activity in acidless and acid-containing Citrus varieties. Australian Journal of Plant Physiology. 28: 383-390.

Sadka, A., E. Dahan, E. Or, M.L. Roose, K.B. Marsh, and L. Cohen. 2001. Comparative analysis of mitochondrial citrate synthase gene structure, transcript level and

enzymatic activity in acidless and acid-containing Citrus varieties. Australian Journal of Plant Physiology. 28: 383-390.

Sadka, A., E. Dahan, L. Cohen, and K.B. Marsh. 2000. Aconitase activity and expression during the development of lemon fruit. Physiologia Plantarum. 108: 255-262.

Sakiyama, R.and M.A. Stevens. 1976. Organic acid accumulation in attached and detached tomato fruits. Journal of the American Society for Horticultural Science. 101: 394-396.

Sanewski, G.and C. Scott. 2000. The Australian pineapple industry. Acta horticulturae. 529: 53-55.

Sanford, W.G. 1968. Potassium builds pineapple quality. Better Crops with Plant Food. 1: 12-13.

Scott, C. 1994. Update of nitrate trials, Pineapple Field Day Notes, Queensland Fruit and Vegetable Growers, Queensland, pp. 9-21.

Selvarajah, S., H.M.W. Herath, and D.C. Bandara. 1998. Physiological effects of pre-heat treatment on pineapple fruit stored at low temperatures. Tropical Agricultural Research. 10: 417-419.

Sen Gupa, A.and G.A. Berkowitz. 1987. Osmotic adjustment, symplast volume, and non-stomatally mediated water stress inhibition of photosynthesis in wheat. plant Physiology. 85: 1040-1047.

Shen, W., K. Nada, and S. Tachibana. 2000. Involvement of polyamines in the chilling tolerance of cucumber cultivars. Plant Physiology. 124: 431-439.

Sideris, C.P.and B.H. Krauss. 1933. Physiological studies on the factors influencing the quality of pineapple fruits. I. Physiochemical variations in the tissues of ripe pineapple. Pineapple Quarterly. 3: 82-114.

Sideris, C.P.and B.H. Krauss. 1936. The classification and nomenclature of groups of pineapple leaves, section of leaves and section of stems based on morphological and anatomical differences. Pineapple Quarterly. 6: 135-147.

Sideris, C.P.and B.H. Krauss. 1938. Growth phenomena of pineapple fruits. Growth. 2: 181-196.

Singleton, V.L.and W.A. Gortner. 1965. Chemical and physical development of the pineapple fruit II. Carbohydrate and acid constituents. J Food Sci. 30: 19-23.

Smith, L.G. 1988. Indices of physiological maturity and eating quality in Smooth Cayenne pineapples. 1. Indices of physiological maturity. Queensland Journal of Agricultural and Animal Sciences. 45: 213-218.

Smith, L.G. 1988. Indices of physiological maturity and eating quality in Smooth Cayenne pineapples. 2. Indices of eating quality. Queensland Journal of Agricultural and Animal Sciences. 45: 219-228.

Soares, A.G., L.C. Trugo, N. Botrel, and L.F.D. Souza. 2005. Reduction of internal browning of pineapple fruit (*Ananas comusus* L.) by preharvest soil application of potassium. Postharvest Biology And Technology. 35: 201-207.

Soost, R.K.and J.W. Cameron. 1961. Contrasting effects of acid and nonacid pummelos on the acidity of hybrid citrus progenies. Hilgardia. 30: 351-357.

Soyer, Y., N. Koca, and F.k.a.a.e.t. Karadeniz. 2003. Organic acid profile of Turkish white grapes and grape juices. Journal of Food Composition & Analysis. 16: 629.

Spironello, A., J.A. Quaggio, L.A.J. Teixeira, P.R. Furlani, and J.M.M. Sigrist. 2004. Pineapple yield and fruit quality effected by NPK fertilization in a tropical soil. Revista Brasileira de Fruticultura. 26: 155-159.

Sripaoraya, S., N.W. Blackhall, R. Marchant, J.B. Power, K.C. Lowe, and M.R. Davey. 2001. Relationships in pineapple by random amplified polymorphic DNA (RAPD) analysis. Plant Breeding. 120: 265-267.

Su, N.R.and C.Y. Li. 1963. Comparison of two potassium salts as regards their effects on yield and quality of pineapple fruits. J Agric Ass China. 39: 31-32.

Sweeney, J.P., V.J. Chapman, and P.A. Hepner. 1970. Sugar, acid and flavor in fresh fruits. Journal of American Dietary Association. 57: 432-435.

Takita, E., H. Koyama, Y. Shirano, D. Shibata, and T. Hara. 1999. Structure and expression of the mitochondrial citrate synthase gene in carrot cells utilizing Al-phosphate. Soil Science and Plant Nutrition. 45: 197-205.

Talukdar, S.and F. Ahmed. 2002. Effect of pre-harvest chemical treatments on shelf life of pineapple fruit. Journal of Food Science and Technology. 39: 53-54.

Tapchoi, K. 1990. Effects of potassium forms on growth, yield, and fruit quality of pineapple, Kasetsart University, Bangkok.

Taureilles-Saurel, C., C.G. Romieu, J.P. Robin, and C. Flanzy. 1995a. Grape (*Vitis vinifera* L.) malate dehydrogenase. II. Characterization of the major mitochondrial and cytosolic isoforms and their role in ripening. American journal of enology and viticulture. 46: 29-36.

Tay, T.H., W.S. Chong, and Y.C. Wee. 1968. The nutritional requirements of pineapple (*Ananas comosus* (L.) Merr var. Singapore Spanish) on peat soil in Malaya. I.

Effect of nitrogen, phosphorus and potassium on yield, sugar and acid content of the fruit. Malaysian Agr J. 46: 458-468.

Teisson, C.and P. Pineau. 1982. Some data on the last stages of pineapple development. Fruits. 37: 741-748.

Terrier, N., F.X. Sauvage, A. Ageorges, and C. Romieu. 2001. Changes in acidity and in proton transport at the tonoplast of grape berries during development. Planta. 213: 20-28.

Tripodi, K.E.J.and F.E. Podesta. 2003. Purification and characterization of an NAD-dependent malate dehydrogenase from leaves of the crassulacean acid metabolism plant *Aptenia cordifolia*. Plant Physiology and Biochemistry. 41: 97.

Tucker, G.A. 1993. Introduction, p. 3-43. In: G. Seymour, J. Taylor, and G. Tucker (eds.). Biochemistry of Fruit Ripening. Chapman & Hall, London.

Ulrich, R. 1970. Organic Acids, p. 89-118. In: A.C. Hulme (ed.). The Biochemistry of Fruits and their Products. Academic Press, London.

Unger, E.A.and A.C. Vasconcelos. 1989. Purification and characterization of mitochondrial citrate synthase. Plant Physiology. 89: 719-723.

Vance, C.P. 1997a. The molecular biology of N metabolism, p. 449-477. In: D.T. Dennis, D.B. Layzell, D.D. Lefebvre, and D.H. Turpin (eds.). Plant Metabolism. Longman, England.

Velez-Ramos, A.and J. Borges. 1995. Foliar application of nitrogen, potassium and magnesium, and pineapple yield and quality. J Agric Univ PR. 79: 111-119.

Verawudh, J.and T. Thongleung. 2001. Effects of chloride on fruit acidity, fruit quality and yield of pineapple. Kasetsart University Report: 10.

Vines, H.and M. Oberbacher. 1965. Response of oxidation and phosphorylation in citrus mitochondria to arsenate. Nature. 206: 319-320.

Visser, T.and J.J. Verhaegh. 1978. Inheritance and selection of some fruit characters of apple 1. Inheritance of low and high acidity. Euphytica. 27: 753-760.

Vu, J.C.V., S.K. Gupta, G. Yelenosky, and M.S.B. Ku. 1995. Cold-induced changes in ribulose 1,5-bisphosphate carboxylase-oxygenase and phosphoenolpyruvate carboxylase in citrus. Environmental And Experimental Botany. 35: 25-31.

Walker, D.J., C.R. Black, and A.J. Miller. 1998. Role of cytosolic potassium and pH in the growth of barley roots. Plant Physiology. 118: 957-964.

Walker, D.J., R.A. Leigh, and A.J. Miller. 1996. Potassium homeostasis in vacuolate plant cells. Proc. Natl. Acad. Sci. USA. 93: 10510-10514.

Wang, T., E.E. Gonzalez, and J.M. Aselage. 1993. Organic acid changes during ripening of processing peaches. Journal of Food Science. 58: 631-632.

Wen, T., E. Xiong Qing, W. Zeng, and Y. Liu. 2001. Study on the change of organic acid synthetase activity during fruit development of navel orange (*Citrus sinensis* Osbeck). Journal of Sichuan Agricultural University. 19: 27-30.

Wu, B.H., B. Quilot, M. Genard, J. Kervella, and S.H. Li. 2005. Changes in sugar and organic acid concentrations during fruit maturation in peaches, *P. davidiana* and hybrids as analyzed by principal component analysis. Scientia Horticulturae. 103: 429-439.

Yamaki, Y.T. 1989. Variation in acidity and acid content in rind among citrus-fruits, and their relationship to fruit juice acidity. Journal of the Japanese Society for Horticultural Science. 57: 568-577.

Yamaki, Y.T. 1990. Effect of lead arsenate on citrate synthase activity in fruit pulp of Satsuma mandarin. Journal of the Japanese Society for Horticultural Science. 58: 899-906.

Yoshida, M. 1970. Genetic studies on the fruit quality of peach varieties I. Acidity. Bull Hortic Res Stn Jpn Series A. 9.

Zhao, D.L., D.M. Oosterhuis, and C.W. Bendnarz. 2001. Influences of potassium deficiency on photosynthesis, chlorophyll content, and chloroplast ultra structure of cotton plants. Photosyntetica. 39: 103-199.

Zhu, J.K. 2001. Plant salt tolerance. Trends in Plant Science. 6: 66-71.