

Changes in Organic Acid Contents and Related Metabolic Enzyme Activities at Different Stages of Growth of Two Tangerine Cultivars

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Abstract

Citric acid accumulation and decline and its related enzymes were studied at different stages of growth of two tangerine cultivars, 'Sai Num Phueng' and 'See Thong'. Citric acid content increased to the peak at early growth stage (16-24 weeks after full bloom, WAF), then decreased rapidly for 28-34 WAF and slightly decreased during maturation (35-37 WAF). 'Sai Num Phueng' had higher citric acid content than 'See Thong'. The activities of enzymes citrate synthase in mitochondrial fraction, aconitase in both mitochondrial and cytosolic fractions and NADP-isocitrate dehydrogenase (IDH) in cytosolic fraction were determined. The changes in these three enzyme activities followed similar patterns in both cultivars. For 16-24 WAF, citrate synthase activity increased and aconitase activity decreased in mitochondrial fraction, which accelerated the increase in citric acid content of both cultivars. Therefore, citrate synthase and aconitase enzymes in mitochondria were essential for citric acid accumulation at early growth stage. During 28-34 WAF, aconitase and NADP-IDH activities in cytosolic fraction were induced with the concomitant decrease of citric acid content. Thus, aconitase in cytosol played a key role in the reduction of citric acid. NADP-IDH could be consisted with aconitase for enhancement the reduction of citric acid in cytosol. Aconitase activity could account for the difference in citric acid content between 'Sai Num Phueng' and 'See Thong'. It was also found that the level of citrate synthase activity was quite similar between the two cultivars but aconitase activity was lower in 'Sai Num Phueng' than in 'See Thong'.

Keywords: tangerine fruit, citric acid, citrate synthase, aconitase, NADP-isocitrate dehydrogenase

1. Introduction

Tangerine fruit (*Citrus reticulata* Blanco) is a non-climacteric subtropical fruit of high commercial value in the citrus fruit market (Ting & Attaway, 1971). It comprised about 20 percent of the total of world citrus output and China leads its production (Ladaniya, 2008). In Thailand, tangerine was providing 80 percent of the national fruit production. 'Sai Num Phueng' was the major cultivar followed by 'See Thong' (Office of Commercial Affairs Chiang mai, 2008).

Organic acids played an important role in fruit taste, flavor and organoleptic quality (Lobit et al., 2003). In mandarin, citric acid was the major organic acid, accounting for 75-80% of the total organic acids while malic acid was the next most abundant (Baldwin, 1993). Since the flavor quality of citrus fruit was influenced by citric acid (Iglesias et al., 2007), citric acid metabolism, resulting in citric acid accumulation and reduction, was very interesting from the point of view of physiology and commercial value (Ladaniya, 2008). High or low citric acid content often reduced the citrus fruit quality, but the moderate concentration of citric acid could make the fruit more palatable (Iglesias et al., 2007).

Citric acid synthesis took place in the mitochondria of juice sac cells, and was part of the tricarboxylic acid (TCA) cycle (Sadka et al., 2001). The six-carbon citric acid was formed when citrate synthase condensed the two-carbon molecule acetyl-CoA with the four-carbon molecule oxaloacetic acid (Ting & Attaway, 1971). Citric acid was either transported to vacuoles, or degraded by aconitase catalysis of the reversible isomerization of isocitric acid in the cytosol (Sadka et al., 2000a). The enzymes potentially involved in fruit

citric acid accumulation and reduction included citrate synthase (EC 2.3.3.1) (Sadka et al., 2001), aconitase (EC 4.2.1.3) (Sadka et al., 2000a) and NADP-IDH (EC 1.1.1.42) (Sadka et al., 2000b).

Organic acid metabolism has been studied in other fruits, such as sweet lime and sour lemon (Sadka et al., 2000a), pineapple (Saradhudhat & Paull, 2007), loquat (Chen et al., 2009), melon (Tang et al., 2010) and grape (Sweetman et al., 2009). The data was notably missing any information on the tangerine fruit. Moreover, the regulation and control for citric acid in citrus fruit was not clearly clarified and understood (Sadka et al., 2000a). The objective of this study was to determine the role of citric acid metabolizing enzymes in the control of citric acid accumulation and reduction at different stages of growth of two tangerine cultivars, "Sai Num Phueng" and "See Thong".

2. Materials and Methods

2.1 Fruit Materials

Fruit from tangerine trees cv. 'Sai Num Phueng' and 'See Thong', approximately 10 years old, grown at the Chiang Mai Thanathon Co., Ltd., located at Chiang Mai Province, Northern Thailand, were used. In both tangerine cultivars, the young fruit of the same size (about 0.2 cm diameter at the equatorial axis) were tagged after full bloom of seven trees for each cultivar and followed throughout fruit development. The tangerine fruit were began to pick at 16 WAF and continued every 4 weeks until 32 WAF, after which fruit were harvested every week until 37 WAF. At each sampling, 12-18 tagged fruits of each cultivar were collected and divided into three replicates with 4-6 fruits per replicate depending on the fruit size. Each fruit was carefully dissected into pulp and peel. Then, the pooled pulp and peel of each replication were immediately frozen in liquid N₂ and stored at -70°C until analysis.

2.2 Measurement of Titratable Acidity (TA) and Total Soluble Solids (TSS)

TA was determined by titration of 5 ml of juice with 0.1 M NaOH until pH 8.1 and expressed as percentage of citric acid per 100 g juice (AOAC, 2005). TSS was determined using a digital refractometer (Model PR-101, ATAGO, Japan) (AOAC, 2005). TA and TSS were determined in triplicate for each replication and averaged.

2.3 Assessment of Organic Acids

The juice (25 g) was homogenized with 25 ml of 4.5% (w/v) metaphosphoric acid solution and filtered through a 0.45 µm pore size nylon membrane filter (Polydera et al., 2005). The filtrate was analyzed for organic acids by high-performance liquid chromatography (HPLC) (Model 1100 Series, Agilent, Germany), equipped with C18 column (4.6×250mm, 5µm, Restek, Germany) and visible wavelength detector (VWD) at 210 nm. Column temperature was 35°C and 50 mM *ortho*-phosphoric acid was used as mobile phase at a flow rate 0.5 ml/min (Aarabi et al., 2008). Standards of citric acid and malic acid were prepared in 0.05% (w/v) metaphosphoric acid. HPLC samples were run in triplicate for each replication and averaged.

2.4 Fractionation of Juice Sac Cells and Enzyme Assays

Juice vesicles of tangerine fruit were fractionated into cytosolic or soluble fraction and a crude fraction enriched with mitochondria as described by Sadka et al. (2000a) with slight modifications. Citrate synthase activity was determined by using Ellman's reagent [5,5'-dithiobis-(2-nitrobenzoic acid), DTNB]. The assay mixture was 1,500 µl and total volume composed of 0.2 mM acetyl-CoA, 0.5 mM oxaloacetate and 0.1 mM DTNB in 50 mM Tris-HCl (pH 7.8). The reaction was started by adding 20-50 µl of the extracted enzyme. The reaction product thionitrobenzoic acid (TNB) was the absorbing substance with intense absorption at 412 nm, 30°C (Kuznetsov et al., 2006). Aconitase activity was determined using 1,500 µl, mixture composed of 40 mM Tris-HCl (pH 7.5), 100 mM NaCl and 0.2 mM *cis*-aconitic acid. The reaction was started by adding 50-100 µl of the extracted enzyme, and the declining of *cis*-aconitic acid was followed by measuring the absorbance at 240 nm, 25°C (Hirai & Ueno, 1977). NADP-IDH activity was performed in 1,500 µl reaction mixture composed of 25 mM Tris-HCl (pH 7.5), 0.5 mM DL-isocitric acid, 1 mM β-NADP and 5 mM MgSO₄. The reaction was started by adding 20-50 µl of the extracted enzyme. The recorded increase in production of NADPH at absorbance 340 nm, 37°C was carried out (Kubo et al., 2002). All three enzymes activities were determined in duplicate for each replication and averaged. The protein content of the fractionated extracts were quantified according to the dye-binding assay at the absorbance of 595 nm (Bradford, 1976) in triplicate for each replication and averaged.

2.5 Statistical Analysis

All data were analyzed by analysis of variance (ANOVA) using SPSS version 14 and the differences between harvesting periods and cultivars were analyzed by a Least Significant Difference (LSD) comparison. The level of significant difference was indicated with the following: P<0.05 for all comparisons.

3. Results and Discussion

3.1 Changes in Fruit Size and Weight

The increases in fruit size (diameter at the equatorial axis) and weight showed similar patterns between the two tangerine cultivars (Figure 1A-B). The changes in fruit size and weight increased in a linear fashion during growth periods (16-34 WAF) and remained constant during maturation periods (35-37 WAF). There was no significantly different ($p>0.05$) for either size or weight between ‘Sai Num Phueng’ and ‘See Thong’ cultivars. The changes in fruit size and weight agreed with the general morphological characteristics of citrus fruit (Spiegel-Roy & Goldschmidt, 1996). The duration of fruit development varied according to cultivar, climate, etc. (Baldwin, 1993). Normally, mandarin and tangerine ripen 9 to 10 months after anthesis (Baldwin, 1993).

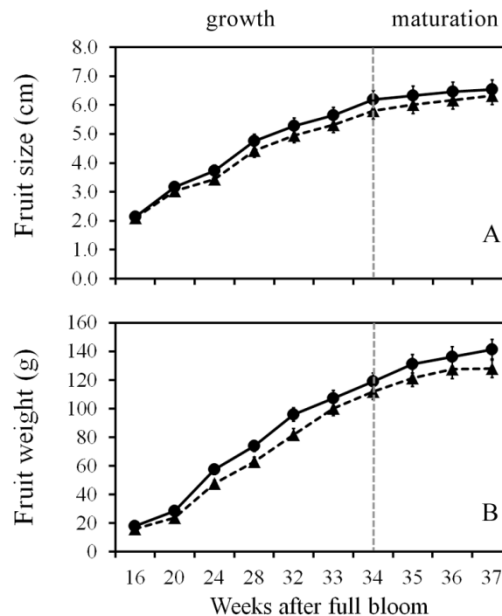


Figure 1. Changes in fruit size (A) and weight (B) during growth and maturation of two tangerine cultivars, ‘Sai Num Phueng’ (●) and ‘See Thong’ (▲)

3.2 Changes in TSS, TA and Organic Acids

TSS of both cultivars continually increased during growth (16-34 WAF) and then slowly increased during maturation (35-38 WAF). At commercial harvest 37 WAF, TSS was 11.38% for ‘Sai Num Phueng’ and 11.10% for ‘See Thong’. There was no significant difference ($p>0.05$) in TSS between the two tangerine cultivars during growth. Then, TSS of ‘Sai Num Phueng’ became higher than ‘See Thong’ during maturation (Figure 2A).

TA of both cultivars increased and reached a peak for 16-24 WAF with 4.68 and 3.99% of citric acid per 100 g juice, respectively at 24 WAF. Then, TA rapidly decreased for 28-34 WAF and slightly decreased during maturation (35-37 WAF). At commercial harvest 37 WAF, TA of ‘Sai Num Phueng’ and ‘See Thong’ was 0.86 and 0.69%, respectively. TA of ‘Sai Num Phueng’ was significantly higher ($p<0.05$) than ‘See Thong’ throughout fruit development (Figure 2B).

Citric acid content showed similar patterns of the two tangerine cultivars. During growth, citric acid content of ‘Sai Num Phueng’ and ‘See Thong’ cultivars increased for 16-24 WAF which reached the highest peaks at 45.51 and 38.09 mg g⁻¹ juice, respectively at 24 WAF, and then sharply decreased for 28-34 WAF. This content slowly declined during maturation to 9.12 and 6.52 mg g⁻¹ juice, respectively at commercial harvest 37 WAF. Citric acid content of ‘Sai Num Phueng’ was significantly greater ($p<0.05$) than that of ‘See Thong’ throughout fruit development (Figure 2C, Table 1 and 2).

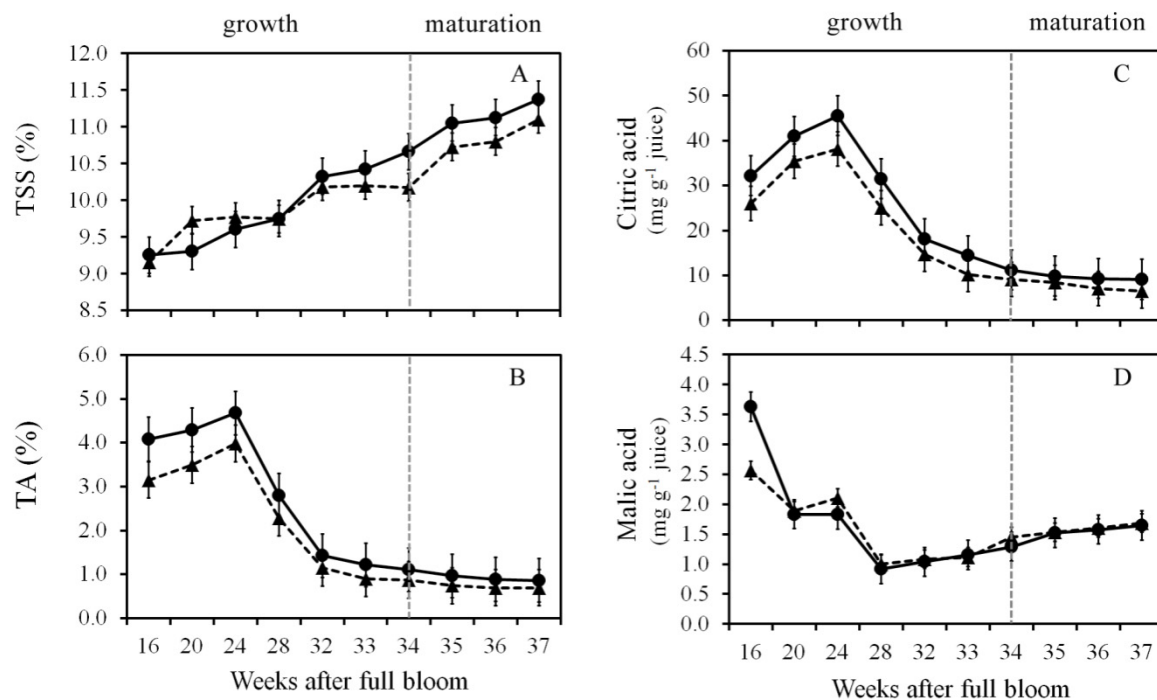


Figure 2. Changes in TSS (A), TA (B), citric acid content (C) and malic acid content (D) during growth and maturation of two tangerine cultivars, 'Sai Num Phueng' (●) and 'See Thong' (▲)

Table 1. Changes in enzyme activities in juice vesicle during growth and maturation of tangerine fruit, 'Sai Num Phueng' cultivar

'Sai Num Phueng'	Citric acid (mg g ⁻¹ juice)		Enzyme activity(unit mg ⁻¹ protein)			
	Stages	WAF	Mitochondrial citrate synthase	Mitochondrial aconitase	Cytosolic aconitase	Cytosolic NADP-IDH
Growth	16	32.20±0.14 ^c	4.17±0.15 ^{bc}	4.75±0.23 ^a	1.70±0.07 ^h	0.15±0.07 ^f
	20	40.95±0.04 ^b	4.31±0.12 ^{bc}	4.35±0.20 ^b	2.63±0.10 ^{fg}	0.16±0.02 ^f
	24	45.51±0.20 ^a	5.32±0.21 ^a	2.06±0.15 ^c	4.59±0.29 ^d	0.66±0.03 ^d
	28	31.50±0.18 ^c	4.64±0.11 ^{bc}	0.85±0.23 ^d	11.56±0.12 ^b	1.26±0.09 ^a
	32	18.10±0.13 ^d	4.15±0.19 ^{cd}	0.77±0.16 ^{de}	13.44±0.22 ^a	1.26±0.13 ^a
	33	14.40±0.01 ^e	3.75±0.03 ^{de}	0.74±0.10 ^{de}	13.49±0.20 ^a	1.12±0.15 ^b
	34	11.18±0.04 ^f	3.71±0.09 ^e	0.70±0.04 ^e	12.42±0.24 ^b	0.82±0.06 ^c
Maturation	35	9.82±0.09 ^g	3.51±0.13 ^e	0.81±0.12 ^e	5.99±0.15 ^c	0.60±0.04 ^d
	36	9.31±0.19 ^h	2.64±0.07 ^f	0.77±0.26 ^e	3.77±0.16 ^{de}	0.42±0.02 ^e
	37	9.12±0.14 ^h	2.82±0.07 ^f	0.78±0.06 ^e	3.21±0.19 ^{ef}	0.24±0.04 ^f
LSD _{0.05}		1.04	0.44	0.40	0.88	0.13
C.V. (%)		2.97	6.98	15.12	7.66	11.73

As shown in Table 1, means within the same column followed by different letters are significantly different at $P \leq 0.05$ level by Least Significant Difference (LSD) comparison. Data are mean values \pm SD/SQRT.

Table 2. Changes in enzyme activities in juice vesicle during growth and maturation of tangerine fruit, 'See Thong' cultivar

'See Thong'		Citric acid (mg g ⁻¹ juice)	Enzyme activity(unit mg ⁻¹ protein)			
Stages	WAF		Mitochondrial citrate synthase	Mitochondrial aconitase	Cytosolic aconitase	Cytosolic NADP-IDH
Growth	16	26.03±0.12 ^c	4.16±0.13 ^c	5.09±0.08 ^a	2.63±0.29 ^{fg}	0.23±0.06 ^f
	20	35.44±0.14 ^b	4.83±0.07 ^b	4.64±0.09 ^b	2.86±0.39 ^{fg}	0.25±0.02 ^f
	24	38.09±0.01 ^a	5.24±0.06 ^a	2.73±0.10 ^c	6.14±0.32 ^c	1.04±0.04 ^c
	28	25.11±0.03 ^d	4.79±0.09 ^b	1.11±0.15 ^d	16.45±0.31 ^{ab}	1.30±0.06 ^b
	32	14.73±0.02 ^e	4.10±0.04 ^{cd}	0.96±0.03 ^{de}	17.93±0.60 ^{ab}	1.56±0.17 ^a
	33	10.23±0.05 ^f	3.86±0.10 ^d	0.87±0.18 ^e	15.84±0.40 ^{bc}	1.52±0.06 ^a
	34	9.15±0.15 ^g	3.95±0.07 ^{cd}	0.81±0.06 ^e	13.93±0.36 ^c	1.04±0.02 ^c
Maturation	35	8.48±0.04 ^h	3.11±0.17 ^e	0.83±0.02 ^e	8.83±0.25 ^d	0.80±0.05 ^d
	36	7.05±0.01 ⁱ	3.03±0.02 ^e	0.81±0.09 ^e	4.36±0.55 ^{ef}	0.43±0.03 ^e
	37	6.52±0.01 ⁱ	2.93±0.01 ^e	0.83±0.14 ^e	3.43±0.40 ^{fg}	0.25±0.04 ^f
LSD _{0.05}		0.54	0.30	0.22	0.96	0.13
C.V.(%)		1.90	1.52	7.33	13.81	10.01

As shown in Table 2, means within the same column followed by different letters are significantly different at P≤0.05 level by Least Significant Difference (LSD) comparison. Data are mean values ±SD/SQRT.

Malic acid content of both cultivars was very high and significantly different ($p \leq 0.05$) at early growth stage with 3.63 mg g⁻¹ juice for 'Sai Num Phueng' and 2.56 mg g⁻¹ juice for 'See Thong' at 16 WAF. Thereafter, this content declined and remained almost constant at low levels and did not significantly differ ($p > 0.05$) for both cultivars during maturation (1.52-1.69 mg g⁻¹ juice) (Figure 2D).

Organic acids were usually used as respiratory substrates for synthesis of ATP in TCA cycle (Ting & Attaway, 1971) and also played an important role in the consumer perception of fruit flavor quality (Chen et al., 2009). The decreases in citric and malic acid contents of both tangerine cultivars during maturation were in agreement with the general observation in almost citrus fruit (Ladaniya, 2008) such as Navel orange (Iglesias et al., 2007) and 'Clementina' mandarin (Cercos et al., 2006). The declines in organic acid contents could be partly associated with the increases in fruit size and water content (Ting & Attaway, 1971). It might be due to the used for energy production through cellular respiration (Purvis, 1983) and the conversion of organic acids to sugar (glucose) through gluconeogenesis (Echeverria & Burns, 1989).

3.3 Changes in Activities of Citric Acid Metabolizing Enzymes

Citrate synthase activity was detected only in mitochondrial fraction. Aconitase activity was detected in both mitochondrial and cytosolic fractions. NADP-isocitrate dehydrogenase (NADP-IDH) activity was detected in cytosolic fraction. The changes in mitochondrial citrate synthase activity patterns in both tangerine cultivars were almost similar throughout fruit development (Figure 3A). At early growth stage (16-24 WAF), mitochondrial citrate synthase activity of 'Sai Num Phueng' and 'See Thong' cultivars increased and reached their peaks at 24 WAF about 5.32 and 5.24 unit mg⁻¹ protein, respectively. Such pattern accelerated the increase in citric acid content. Subsequently, the same activity continually decreased toward fruit maturation (28-37 WAF) to about 2.82 and 2.93 unit mg⁻¹ protein, respectively at commercial harvest 37 WAF. These paralleled the decrease in citric acid content (Tables 1 and 2).

The changes in aconitase activity in mitochondrial fraction showed similar patterns between 'Sai Num Phueng' and 'See Thong' cultivars throughout fruit development (Figure 3B). At early growth stage (16-24 WAF), mitochondrial aconitase activity of both cultivars significantly decreased ($p \leq 0.05$) which coincided with the high level of citric acid content. Subsequently, the same activity remained relatively constant at the low level for 28-37 WAF. At 16 WAF, 'Sai Num Phueng' and 'See Thong' cultivars had quite high level of mitochondrial

aconitase activity about 4.75 and 5.09 unit mg^{-1} protein, respectively. Thereafter, at 24 WAF, this activity decreased about two folds to 2.06 and 2.73 unit mg^{-1} protein, respectively (Table 1 and 2).

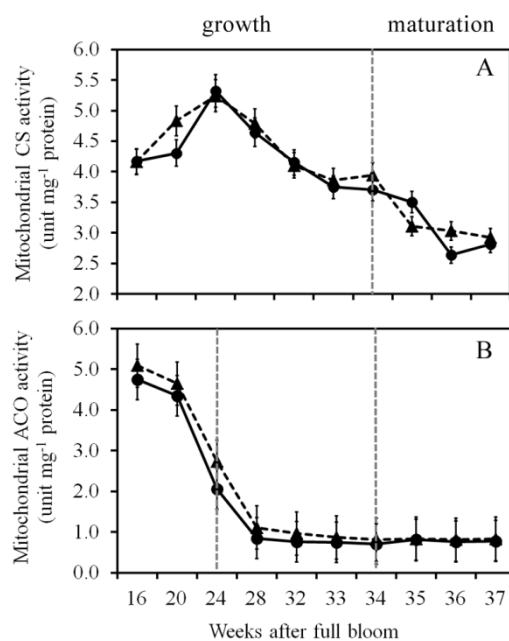


Figure 3. Changes in activities of citrate synthase (CS, A) and aconitase (ACO, B) in mitochondrial fraction during growth and maturation of two tangerine cultivars, 'Sai Num Phueng' (●) and 'See Thong' (▲)

Aconitase and NADP-IDH activities in cytosolic fraction showed similar patterns between 'Sai Num Phueng' and 'See Thong' cultivars throughout fruit development (Figure 4A-B). Cytosolic aconitase and NADP-IDH activities of both cultivars were relatively low at early growth stage (16-24 WAF). Thereafter, these activities dramatically increased and remained constant at relatively high levels for 28-34 WAF and were accompanied with the reduction in citric acid content. When the fruit entered the maturation stage (35-37 WAF), these activities sharply decreased about three folds between week 34th to 35th, then remained relatively constant at low levels until commercial harvest at 37 WAF while citric acid content slightly decreased. At 24 WAF, 'Sai Num Phueng' and 'See Thong' cultivars had cytosolic aconitase activity about 4.59 and 6.14 unit mg^{-1} protein, and cytosolic NADP-IDH activity about 0.66 and 1.04 unit mg^{-1} protein, respectively. At 28 WAF, cytosolic aconitase activity increased about three folds to 11.56 and 16.45 unit mg^{-1} protein, respectively and cytosolic NADP-IDH activity increased about two folds to 1.26 and 1.30 unit mg^{-1} protein, respectively (Tables 1 and 2).

Citrate synthase activity was detected only in mitochondria fraction, which similar to the reports in sour lemon and 'Shamouti' orange (Sadka et al., 2001). Aconitase activity was detected in both mitochondria and cytosolic fractions. The similar result was observed in sour lemon and sweet lime (Sadka et al., 2000a; Sadka et al., 2000b). NADP-IDH activities were detected in cytosolic fraction, which agreed to the report in sour lemon (Sadka et al., 2000b). In citrus fruit, citric acid was synthesized in the juice sac cells which took place in the mitochondria [through the TCA cycle] (Ramakrishnan, 1971). The synthesis occurred when citrate synthase condensed acetyl-CoA with oxaloacetic acid to yield citric acid (Sadka et al., 2001). The citric acid was then translocated to accumulate in the vacuoles of juice sac cells, where it reached high concentrations (Echeverria & Valich, 1988). In present study, the induction of citrate synthase activity and the reduction of aconitase activity in mitochondrial fraction, which accelerated the increase in citric acid content at early growth stage (16-24 WAF) of the two tangerine cultivars. Thus, it could be concluded that citrate synthase and aconitase enzymes in the mitochondria were essential in citric acid accumulation at early growth stage of tangerine fruit. The increase in citrate synthase activity was important for citric acid synthesis and its accumulation in citrus fruit (Kubo et al., 2002). Sadka et al. (2000a) reported the same that in young sour lemon, the decrease of aconitase activity in mitochondria played a direct role in citric acid accumulation.

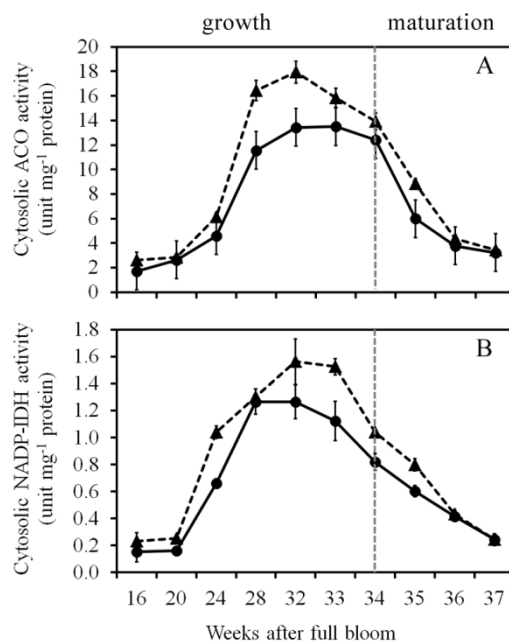


Figure 4. Changes in activities of aconitase (A) and NADP-IDH (B) in cytosolic fraction during growth and maturation of two tangerine cultivars, 'Sai Num Phueng' (●) and 'See Thong' (▲)

When citric acid declined in lemon fruit, it was mobilized from the vacuole to the cytosol and aconitase activity was responsible for the conversion of citric acid into isocitric acid in the cytosol (Sadka et al., 2000a). Isocitric acid was then oxidized by NADP-IDH to yield α -ketoglutaric acid in the cytosol (Sadka et al., 2000b). The results found that the dramatic increase of aconitase activity in cytosolic fraction which accompanied the reduction in citric acid content of both tangerine cultivars for 28-34 WAF. During maturation (35-37 WAF), the same enzymes activity decreased to the low level, which coincided with the slight decrease in citric acid content. NADP-IDH activity increased paralleled with the increase in aconitase activity in cytosolic fraction of two tangerine cultivars. Therefore, enzyme aconitase in the cytosol likely played a key role in the reduction of citric acid during growth and maturation of tangerine fruit. NADP-IDH could be consisted with aconitase for enhancement the reduction of citric acid in the cytosol. This was agree with the report for Satsuma mandarin (Hirai & Ueno, 1977), sour lemon (Sadka et al., 2000a) and lemon (*Citrus limon* L. Burm) (Luo et al., 2003). In the immature Satsuma mandarin, aconitase activity rapidly increased about four folds, while citric acid sharply declined (Hirai & Ueno, 1977). Sadka et al. (2000a) reported the same result that the re-induction of aconitase activity in cytosol preceded citric acid decline toward maturation of sour lemon. NADP-IDH played a role in glutamic acid synthesis from 2-oxoglutaric acid in the cytosol of plant cells (Chen et al., 2009). Sadka et al. (2000b) reported that, NADP-IDH activity increased in the cytosol, while citric acid started to decrease in lemon and suggested that the conversion of citric acid to glutamic acid via isocitric acid and 2-oxoglutaric acid might be a continuous process, which took place in the cytosol.

3.4 A comparison of Enzyme Activities from 'Sai Num Phueng' and 'See Thong' Cultivars

There was no significant difference ($p > 0.05$) in citrate synthase activity in mitochondria fraction between both cultivars throughout fruit development (Figure 3A). While citric acid content of 'Sai Num Phueng' was higher than that of 'See Thong' (Figure 2C). At early growth stage (16-24 WAF), Sai Num Phueng' cultivar had significantly lower ($p \leq 0.05$) aconitase activity in mitochondria fraction than 'See Thong' cultivar. Thereafter, there was no difference in the same activity between the two tangerine cultivars (Figure 3B). Aconitase and NADP-IDH activities in cytosolic fraction of 'Sai Num Phueng' were significantly lower ($p \leq 0.05$) in comparison with 'See Thong' cultivar for 16-35 WAF. Then, these activities in cytosolic fraction did not differ for both cultivars (Figure 4A-B, Tables 1 and 2). Sadka et al. (2000a) suggested that aconitase activity was decreased only in sour lemon but not in sweet lime, and this contributed to the different level of citric acid accumulation. However, the comparison in NADP-IDH activity between citrus cultivars has never been reported. Sadka et al. (2001) suggested that the difference in acid accumulation between acid-less and acid-containing fruits might not be attributed to the changes in the activity of citrate synthase. Therefore, the difference in the level of aconitase

activity could account for the difference in citric acid content between the two tangerine cultivars. However, citrate synthase might not have a relationship for citric acid content in different tangerine cultivar.

4. Conclusion

The results in this study can be concluded that citrate synthase and aconitase enzymes in the mitochondria were essential in citric acid accumulation at early growth stage of tangerine fruit. Aconitase in the cytosol played a key role in the reduction of citric acid during growth and maturation. NADP-IDH could be consisted with aconitase for enhancement the reduction of citric acid content in the cytosol. Aconitase could account for the difference in citric acid content between 'Sai Num Phueng' and 'See Thong' cultivars.

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