Physico-chemical Changes During Growth and Maturation of Tangerine Fruit cv. 'Sai Nam Phueng' and 'See Thong'

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ABSTRACT

Several tangerine cultivars are popular and commercially produced in Thailand. Despite this, no published data exists on the physico-chemical characteristics of the Thai cultivars, assessing their quality, throughout the various stages of fruit development. This study will assess the quality of the physico-chemical characteristics of two cultivars, 'Sai Nam Phueng' and 'See Thong', during growth and maturation.

Chlorophyll degradation and carotenoid accumulation in peel resulted in changing color from green to yellow-orange throughout fruit development of both cultivars. Carotenoid accumulation in juice resulted in changing color from yellow-orange to deep orange. Titratable acidity (TA) and citric acid content of both cultivars peaked at the early growth stage, then rapidly decreased during growth and slightly decreased during maturation. Malic acid and vitamin C content were very high at early growth stage, and then declined toward fruit maturation. Isocitric acid content gradually decreased during maturation. The total soluble solids (TSS), TSS/TA ratio, sucrose, glucose and fructose content rapidly increased during growth and slowly increased during maturation. During maturation, 'Sai Nam Phueng' had higher TA, TSS, citric acid, vitamin C, sucrose, glucose and fructose content and lower peel carotenoid, isocitric acid content, juice pH and TSS/TA ratio than 'See Thong'. Peel and juice colors, peel chlorophyll, juice carotenoid and malic acid content did not differ between the two cultivars during maturation.

Keywords: Tangerine fruit, Physico-chemical change, Pigment, Organic acid, Sugar

INTRODUCTION

Tangerine (*Citrus reticulata* Blanco), a non-climacteric subtropical fruit, is widely grown in many countries (Ting and Attaway, 1971). Mandarin and tangerine comprise about 20 percent of total world citrus production (Ladaniya, 2008). In Thailand, tangerines are a popular commercial fruit, primarily grown

in the north. Of the various cultivars grown commercially in Thailand, 'Sai Nam Phueng' has the highest value, followed by 'See Thong' (Office of Commercial Affairs Chiang Mai, 2008). 'Sai Nam Phueng' has a sweet/sour taste (high sugar and acid content) while 'See Thong' has a milder taste (lower sugar and acid content) (Roongruangsri et al., 2005).

Tangerine fruit are excellent sources of vitamins and minerals as well as supply an array of colors, flavors and textures to the pleasure of eating (Ahmad et al., 2006). This fruit is most commonly thought of as a good source of vitamin C (Ladaniya, 2008). The recommended daily intake (RDI) of vitamin C is 75 mg for women and 90 mg for men (FAO, 2006). This can be supplied by eating one medium-sized orange or tangerine a day, which can provide more than 70 mg of vitamin C (FAO, 2006). Tangerines also contain considerable amounts of sugars, organic acids, carotenoids, flavonoids and essential oil along with some minerals (Topuz et al., 2005).

The morphology, anatomy, physico-chemical and biochemical properties of tangerine fruit change throughout development (Gortner et al., 1967). The maturation of citrus fruit is associated with changes in peel color, juice composition and flavor (Iglesias et al., 2007). The declining acidity and increasing sugar content lead to the sweetening flavor of the fruit (Baldwin, 1993). The peel chlorophyll also starts to degrade while carotenoid biosynthesis increases, leading to peel color changing from green to yellow-orange (Mukhopadhyay, 2004).

Physico-chemical properties of fruits such as orange (Niu et al., 2008), mango (Aina, 1990), pomegranate (Al-Said et al., 2009), blackberry (Tosun et al., 2008) and kiwi (Zolfaghari et al., 2010) have been reported. Although several tangerine cultivars have been successfully grown and utilized in Thailand, there is no published data assessing the quality of their physico-chemical cha-racteristics throughout fruit development. Therefore, the objective of this study was to examine the changes in the physico-chemical properties of Thailand's two most popular tangerine fruit cultivars, 'Sai Nam Phueng' and 'See Thong', during growth and maturation.

MATERIALS AND METHODS

Fruit materials

Fruit was sourced from approximately 10-year old tangerine trees, cv. 'Sai Nam Phueng' and 'See Thong', grown at the Chiang Mai Thanathon Co., Ltd. in Chiang Mai Province in northern Thailand. On seven trees of each cultivar, young fruit of the same size (about 0.2 cm diameter at the equatorial axis) were tagged after full bloom and followed throughout fruit development. Fruit was picked starting 16 weeks after full bloom, and every 4 weeks thereafter until week 32, after which weekly until week 37. The growth period occurred 16 to 34 weeks after full bloom and the maturation period occurred 35 to 37 weeks after full bloom. 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 N_2 and stored at -70°C until analysis.

Measurement of weight of fruit

Fruit weight (gram) was determined by weighing individual fruit on an electronic analytical balance (Model PB3002-5, Mettler-Toledo, Switzerland).

Measurement of peel and juice color

Peel and juice colors of tangerine fruit were quantified objectively (lightness: peel L, chroma and hue angle values) using a colorimeter (ColorQuest XE, Hunterlab, Virginia) (McGuire, 1992). Ten fruits were randomly selected from each cultivar to measure the flavedo color. Tangerine juice color was analyzed through optical glass cuvettes of 12.5 mm path length backed with black cardboard reflectors of approximately 5% reflection in triplicate of each replication and averaged.

Determination of carotenoid

Carotenoid was extracted with hexane : acetone : ethanol in the ratio of 50:25:25 v/v from the peel powder (Wang et al., 2007) and the juice (Lee et al., 2001). All operations were conducted under subdued light to minimize oxidation of the carotenoids. All glassware was wrapped in aluminum foil to minimize exposure to light. All extracts were made and measured in triplicate for each replication and averaged. The total carotenoid content in peel and juice was measured at the absorbance of 450 nm with the extinction coefficient of 2,592 and expressed as β-carotene equivalents in $\mu g\ g^{-1}$ fresh weight for the peel and in $\mu g\ g^{-1}$ juice for the juice (John Scott, 2005).

Chlorophyll extraction and quantification

The chlorophyll a, b and total chlorophyll from the peel powder were extracted with 80% (v/v) acetone and determined by measuring the absorbance at 663 and 645 nm using Arnon's equations and expressing the calculated values in $\mu g\ g^{-1}$ fresh weight. The analysis was in triplicate for each replication and averaged (Rodrigo et al., 2003).

Measurement of TA and TSS

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

Assessment of organic acids

The juice (25 g) was homogenized with 25 ml of 4.5% (w/v) metaphosphoric acid solution. The liquid was centrifuged at 6,000 x g for 10 min at 4°C to remove the supernatant and then 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) (Agilent Model 1100 Series, Agilent Technologies, Inc., Waldbrom, Germany). Organic acids were separated by an ultra aqueous C18 (4.6×250mm, 5µm) column (Restek Corporation, Germany). Detection was done by visible wavelength detector (VWD) at 210 nm. Temperature of the column oven was set at 35°C. Mobile phase was 50 mM ortho-phosphoric acid (v/v) at a flow rate 0.5 ml/min for 30 min. A 20 µl of each sample was put into an injection loop with automatic system (Aarabi et al., 2008). Authentic standards of citric acid, malic acid, isocitric acid and ascorbic acid or vitamin C were prepared in 0.05% (w/v) metaphosphoric acid. HPLC samples were run in triplicate for each replication and averaged.

Determination of sugars

A 10 g of juice sample was diluted (1/5 dilution) with deionized water to adjust the final volume to 50 ml in a volumetric flask. The diluted supernatant was then filtered through 0.45 µm pore size nylon membrane filter. The filtrate was analyzed by HPLC with separation by a Zorbax carbohydrate column (4.6×150 mm, 5µm dp, Agilent Technologies, Waldbrown, Germany). Refractive index detector was connected in series and the temperature of the column oven was set at 30°C. Mobile phase used 25:75 of deionized water : acetonenitrile at a 1.4 ml/min flow rate and 10-12 min stop time. The 5 µl of the sample was directly injected into the HPLC (Cabálková and Chmeĺik, 2002). Sucrose, glucose and fructose of analytical grade were used as standards. The samples were run in triplicate for each replication and averaged.

Statistical analysis.

All data were analyzed by analysis of variance (ANOVA) using SPSS version 14 and the differences between sampling periods and cultivars were analyzed by a least significant difference (LSD) comparison. The level of significant difference was indicated with the following: $*P \leq 0.05$ for all comparisons.

RESULTS

Changes in fruit weight

The increases in fruit weight showed similar patterns between the two tangerine cultivars. The changes in fruit weight increased in a linear fashion during growth periods (16-34 weeks after full bloom) and remained constant during maturation periods (35-37 weeks after full bloom) (Table 1). There was no significant difference ($p>0.05$) in fruit weight between 'Sai Nam Phueng' and 'See Thong' cultivars.

Changes in peel tristimulus color and pigments

A change in peel color from green to yellow-orange was observed throughout fruit development in both cultivars. The changes in peel L, chroma and hue angle values showed similar patterns and did not significantly differ ($p>0.05$) between

both cultivars throughout fruit development. During growth, peel L, chroma and hue angle values of both cultivars remained almost constant 16-24 weeks after full bloom. Then, peel L and chroma values gradually increased and the hue angle value decreased significantly ($p \leq 0.05$) 28-34 weeks after full bloom. During maturation (35-37 weeks after full bloom), peel L and chroma values reached the highest levels and hue angle value decreased to the lowest levels (Table 1).

Table 1. Peel L, chroma and hue angle values during growth and maturation of two tangerine cultivars, 'Sai Num Phueng' and 'See Thong'.

Weeks after full bloom	Sai Num Phueng				See Thong			
	Weight (g)	L	chroma	hue angle	Weight (g)	L	chroma	hue angle
Growth								
16	18.03±1.35^{i}	39.19±0.76^{e}	17.79±0.96^{h}	116.31±0.34^{a}	15.97±0.90^{g}	38.50±1.24^{g}	17.05±2.05^{g}	110.43±0.3^{a}
24	57.55±1.46^{g}	39.50±0.45^{e}	24.66±0.93^{fg}	113.05±0.72^{a}	47.48±4.48^{f}	40.58±1.42^{fg}	22.01±1.13^{fg}	108.59±0.8^{a}
28	73.82±6.04^{f}	41.66±2.50^{e}	27.84±1.33^{f}	107.42±2.81^{b}	62.91±2.47^{e}	42.99±0.64^{f}	24.75±0.92^{f}	105.17±0.2^{b}
34	118.95±1.59^{c}	53.32±0.68^{c}	53.52±1.15^{c}	86.85±0.59^{d}	112.08±3.55^{b}	55.78±1.30^{c}	55.50±2.26^{c}	83.59±1.10^{d}
Maturation								
35	131.19±1.45^{b}	55.44±0.72^{b}	57.15±1.85^{bc}	80.69±1.22^{e}	121.46±5.28^{a}	57.78±1.34^{c}	60.80±1.57^{bc}	76.09±1.51^{e}
37	141.07±1.83^{a}	64.00±1.42^{a}	63.82±1.10^{a}	73.79±0.93^{f}	128.08±4.12^{a}	66.68±0.51^{a}	65.35±2.88^{ab}	71.71±0.69^{f}
$LSD_{0.05}$	8.50	5.31	5.52	4.86	11.36	3.28	6.10	4.10

Note: Means within the same column followed by different letters are significantly different at 95% ($P \leq 0.05$) level by Least Significant Difference (LSD) comparison. Data are mean values ±SD/SQRT.

Peel chlorophyll degradation and carotenoid accumulation were observed in both cultivars throughout fruit development. During growth (16-34 weeks after full bloom), peel chlorophyll a and b and total chlorophyll content of 'Sai Nam Phueng' cultivar were higher than 'See Thong' cultivar while total carotenoid content was not significantly different ($p > 0.05$). During maturation (35-37 weeks after full bloom), peel chlorophyll a and b and total chlorophyll content were not significantly different ($p > 0.05$). However, total carotenoid content of 'Sai Nam Phueng' cultivar was lower than 'See Thong' cultivar. Chlorophyll a, b and total chlorophyll content of both cultivars gradually decreased during growth and slowly decreased during maturation (Table 2). Total chlorophyll content of 'Sai Nam Phueng' and 'See Thong' cultivars decreased by 42 and 40% at 32 weeks after full bloom and by 94 and 95%, respectively, at commercial harvest 37 weeks after full bloom. Total carotenoid content of both cultivars continually increased during growth and reached the highest levels during maturation. Total carotenoid content of 'Sai Nam Phueng' and 'See Thong' cultivars increased by 43 and 47% at 32 weeks after full bloom and by 70 and 75%, respectively, at commercial harvest 37 weeks after full bloom.

Table 2. Peel total chlorophyll and total carotenoid content and juice total carotenoid content during growth and maturation of two tangerine cultivars, 'Sai Num Phueng' and 'See Thong'.

Weeks after full bloom	Sai Num Phueng			See Thong		
	Peel total chlorophyll (g g^{-1} FRESH WEIGHT)	Peel total carotenoid (g g^{-1} FRESH WEIGHT)	Juice total carotenoid (g g-1 juice)	Peel total chlorophyll (g g^{-1} FRESH WEIGHT)	Peel total carotenoid (g g^{-1} FRESH WEIGHT)	Juice total carotenoid (g g^{-1} juice)
Growth						
16	152.44±0.81^{a}	18.37±0.09^{g}	1.38±0.08^{h}	123.50±0.16^{a}	17.28±0.29^{h}	1.72±0.25^{f}
24	130.57±2.20^{b}	21.99±0.29^{fg}	5.16±0.11^{f}	115.78±0.09^{b}	24.35±0.03^{g}	5.19±0.26^{e}
28	110.42±0.69^{c}	25.57±0.15^{f}	9.07±0.16^{e}	91.04±0.51^{c}	26.89±0.51^{g}	9.12±0.66^{d}
34	29.04±0.91^{f}	42.87±0.11^{cd}	13.11±0.18^{c}	25.65±0.11^{f}	52.72±0.14^{d}	12.17±0.71^{bc}
Maturation						
35	19.33±0.77^{fg}	46.31±0.10^{c}	13.70±0.17^{c}	16.77±0.25^{g}	62.54±0.31^{c}	13.33±0.04^{b}
37	8.57±0.42^{g}	59.23±0.67^{b}	16.30±0.21^{a}	6.96±0.59^{h}	71.29±0.23^{ab}	15.83±0.36^{a}
$LSD_{0.05}$	6.29	4.88	0.96	3.07	5.96	2.26

Note: Means within the same column followed by different letters are significantly different at 95% (P≤0.05) level by Least Significant Difference (LSD) comparison. Data are mean values ±SD/SQRT.

Changes in juice tristimulus color and pigments

The change in juice color from yellow-orange to deep orange was recorded throughout development of both cultivars. Juice L, chroma and hue angle values did not significantly differ (p>0.05) among the two cultivars. During growth, juice L and hue angle values of both cultivars continually decreased and chroma value gradually increased. Thereafter, juice L, chroma and hue angle values remained almost constant during maturation (Table 3).

Throughout fruit development, juice carotenoid accumulation was observed and there was no significant difference (p>0.05) in juice total carotenoid content between 'Sai Nam Phueng' and 'See Thong' cultivars (Table 2). Juice total carotenoid content of 'Sai Nam Phueng' and 'See Thong' cultivars increased by 86 and 83% at 32 weeks after full bloom and by 91 and 89%, respectively, at commercial harvest 37 weeks after full bloom.

Table 3. Juice L, chroma and hue angle values during growth and maturation of two tangerine cultivars, 'Sai Num Phueng' and 'See Thong'.

Weeks after full bloom	Sai Num Phueng			See Thong		
Growth	L	chroma	hue angle	L	chroma	hue angle
16	53.65±2.01^{a}	15.08±0.54^{h}	95.64±1.14^{a}	52.56±0.90^{a}	14.41±0.56^{i}	98.67±0.17^{a}
24	49.71±0.35^{abc}	23.62±0.70^{f}	87.13±0.88^{b}	48.56±1.65^{bd}	22.97±0.65^{g}	87.91±0.06^{c}
28	47.92±0.89^{bcd}	28.41±0.45^{e}	85.13±0.27^{c}	47.34±0.10^{c}	27.68±0.25^{f}	84.25±0.11^{d}
34	43.96±2.97^{de}	37.42±0.35^{b}	78.72±0.26^{def}	42.44±0.16^{d}	36.19±0.12^{c}	80.78±0.10^{g}
Maturation						
35	43.78±1.20^{de}	37.97±0.06^{b}	77.55±0.48^{efg}	41.81±0.54^{d}	36.33±0.42^{c}	80.28±0.24^{g}
37	43.54±0.05^{e}	40.88±0.30^{a}	77.41±0.37^{fg}	41.56±0.90^{d}	37.24±0.11^{bc}	78.67±0.07^{i}
$LSD_{0.05}$	3.97	1.03	1.75	2.56	1.18	0.49

Note: Means within the same column followed by different letters are significantly different at 95% (P≤0.05) level by Least Significant Difference (LSD) comparison. Data are mean values ±SD/SQRT.

Changes in TA and organic acids

TA of both cultivars increased and reached a peak 16-24 weeks after full bloom. Then, TA rapidly decreased 28-34 weeks after full bloom and slightly decreased during maturation (35-37 weeks after full bloom). TA of 'Sai Nam Phueng' was significantly higher ($p \leq 0.05$) than 'See Thong' throughout fruit development (Table 4).

Citric acid content showed similar patterns in the two tangerine cultivars. During growth, the citric acid content of 'Sai Nam Phueng' and 'See Thong' cultivars increased 16-24 weeks after full bloom, peaking at week 24, and then sharply decreased 28-34 weeks after full bloom. Citric acid content slowly declined during maturation. Citric acid content of 'Sai Nam Phueng' was significantly greater ($p \leq 0.05$) than 'See Thong' throughout fruit development (Table 4).

Malic acid content of both cultivars was very high and significantly different ($p \leq 0.05$) at the early growth stage. Thereafter, malic acid content declined and remained almost constant at low levels and did not significantly differ ($p > 0.05$) for both cultivars during maturation (Table 4).

'Sai Nam Phueng' and 'See Thong' cultivars showed a similar pattern of isocitric acid content from fruit growth through maturation stages. Isocitric acid content increased and reached peak levels at 28 weeks after full bloom with 0.67 and 0.78 mg g^{-1} juice, respectively. This content then gradually decreased until 34 weeks after full bloom, after which it remained almost constant during maturation at 0.32 and 0.36 mg g^{-1} juice, respectively, at commercial harvest 37 weeks after full bloom. Isocitric acid content was slightly lower in 'Sai Nam Phueng' than 'See Thong' in all developmental stages (data not shown).

Vitamin C content, or total ascorbic acid content, of 'Sai Nam Phueng' and 'See Thong' cultivars were relatively high at early growth stage. Thereafter, it sharply decreased and then remained relatively constant at low levels during maturation. 'Sai Nam Phueng' had significantly higher ($p \leq 0.05$) vitamin C content (about 23%) than 'See Thong' during maturation and ripening (Table 4).

Table 4. TA, citric acid, malic acid and vitamin C content during growth and maturation of two tangerine cultivars, 'Sai Num Phueng' and 'See Thong'.

Weeks after full bloom	Sai Num Phueng				See Thong			
	TA (%)	Citric acid (mg g^{-1} juice)	Malic acid (mg g^{-1} juice)	Vitamin C (mg g^{-1} juice)	TA (%)	Citric acid (mg g^{-1} juice)	Malic acid (mg g^{-1} juice)	Vitamin C (mg g^{-1} juice)
Growth								
16	4.08 ± 0.04^{c}	32.20 ± 0.14^{c}	3.63 ± 0.14^{a}	0.57 ± 0.03^{a}	3.15 ± 0.05^{c}	26.03 ± 0.12^{c}	2.56 ± 0.05^{a}	0.54 ± 0.06^{a}
24	4.68 ± 0.02^{a}	45.51 ± 0.20^{a}	1.83 ± 0.03^{b}	0.22 ± 0.04^{h}	3.99 ± 0.09^{a}	38.09 ± 0.01^{a}	2.10 ± 0.01^{b}	0.33 ± 0.02^{f}
28	2.80 ± 0.02^{d}	31.50 ± 0.18^{c}	0.92 ± 0.06^{f}	0.24 ± 0.05^{g}	2.28 ± 0.06^{d}	25.11 ± 0.03^{d}	1.00 ± 0.04^{k}	0.38 ± 0.09^{c}
34	1.11 ± 0.06^{g}	11.18 ± 0.04^{f}	1.29 ± 0.05^{de}	0.38 ± 0.06^{d}	0.87 ± 0.02^{f}	9.15 ± 0.15^{g}	1.45 ± 0.05^{g}	0.35 ± 0.02^{e}
Maturation								
35	0.96 ± 0.01^{h}	9.82 ± 0.09^{g}	1.52 ± 0.01^{c}	0.38 ± 0.05^{d}	0.74 ± 0.02^{g}	8.48 ± 0.04^{h}	1.53 ± 0.04^{f}	0.32 ± 0.01^{g}
37	0.86 ± 0.02^{i}	9.12 ± 0.14^{h}	1.64 ± 0.01^{c}	0.34 ± 0.07^{e}	0.69 ± 0.01^{g}	6.52 ± 0.01^{i}	1.69 ± 0.02^{d}	0.26 ± 0.01^{i}
$LSD_{0.05}$	0.09	1.04	0.14	0.01	0.11	0.54	0.03	0.01

Note: Means within the same column followed by different letters are significantly different at 95% ($P \leq 0.05$) level by Least Significant Difference (LSD) comparison. Data are mean values ±SD/SQRT.

Changes in TSS, TSS/TA ratio and sugars

TSS of both cultivars increased during growth and maturation (16-34 weeks after full bloom). However the rate of increase is more rapid during maturation, with no significant difference between the two tangerine cultivars (Table 5).

The TSS/TA ratio during the early growth stage (16-24 weeks after full bloom) was low, 2.08 for 'Sai Nam Phueng' and 2.80 for 'See Thong'. This ratio gradually increased to 13.26 and 16.07, respectively, at commercial harvest 37 weeks after full bloom. TSS/TA ratio of 'Sai Nam Phueng' was significantly lower ($p \leq 0.05$) because of the relatively higher TA in comparison with 'See Thong' (data not shown).

The sucrose, glucose and fructose content of both cultivars gradually increased during growth, and then slowly increased with fruit maturation. The sucrose content was approximately double the glucose and fructose content (Table 5). Moreover, TSS correlated strongly with sucrose content, but did not correlate with glucose and fructose content. There was no significant difference ($p > 0.05$) in sucrose, glucose and fructose content between both cultivars during growth. Thereafter, sucrose, glucose and fructose in 'Sai Nam Phueng' fruit were significantly higher ($p \leq 0.05$) than 'See Thong' during maturation.

Table 5. TSS, citric acid and vitamin C content during growth and maturation of two tangerine cultivars, 'Sai Num Phueng' and 'See Thong'.

Weeks after full bloom	Sai Num Phueng				See Thong			
	TSS (%)	Sucrose (mg g^{-1} juice)	Glucose (mg g^{-1} juice)	Fructose (mg g^{-1} juice)	TSS (%)	Sucrose (mg g^{-1} juice)	Glucose (mg g^{-1} juice)	Fructose (mg g^{-1} juice)
Growth								
16	9.25±0.03^{i}	9.97±0.04^{k}	4.34±0.05^{k}	3.50±0.01^{k}	9.15±0.03^{f}	9.11±0.03^{i}	3.56±0.01^{j}	3.43±0.03^{i}
24	9.60±0.02^{h}	17.41±0.01^{i}	6.78±0.02^{j}	7.24±0.01^{j}	9.78±0.03^{e}	18.01±0.02^{g}	7.09±0.01^{h}	7.13±0.04^{g}
28	9.75±0.06^{g}	21.13±0.01^{h}	12.86±0.01^{h}	13.38±0.02^{h}	9.75±0.03^{e}	20.07±0.11^{f}	12.50±0.07^{g}	13.78±0.07^{f}
34	10.66±0.06^{d}	35.31±0.07^{e}	16.24±0.03^{f}	18.39±0.03^{f}	10.18±0.02^{d}	30.90±0.02^{e}	14.80±0.08^{d}	15.45±0.01^{e}
Maturation								
35	11.05±0.03^{c}	39.71±0.04^{d}	17.95±0.05^{d}	20.32±0.03^{d}	10.73±0.05^{c}	34.80±0.09^{c}	15.35±0.09^{d}	16.12±0.07^{d}
37	11.38±0.02^{b}	43.69±0.06^{b}	21.86±0.07^{a}	24.55±0.09^{a}	11.10±0.04^{b}	39.61±0.04^{a}	16.03±0.02^{b}	18.49±0.06^{b}
$LSD_{0.05}$	0.11	0.46	0.27	0.28	0.09	0.53	0.36	0.39

Note: Means within the same column followed by different letters are significantly different at 95% ($P \leq 0.05$) level by Least Significant Difference (LSD) comparison. Data are mean values ±SD/SQRT.

DISCUSSION

The changes in fruit weight of 'Sai Nam Phueng' and 'See Thong' cultivars agreed with the general morphological characteristics of citrus fruit. Citrus fruit matures on the tree for six to twelve months. The duration of fruit development varies according to cultivar, climate, etc. (Spiegel-Roy and Goldschmidt, 1996). Normally, mandarin and tangerine ripen 9 to 10 months after full bloom (Baldwin, 1993).

Peel chlorophyll degradation and carotenoid accumulation of 'Sai Nam Phueng' and 'See Thong' cultivars correlated with the decrease in hue angle value throughout fruit development. This resulted in color change from green to yellow-orange. Mandarin and orange are typical fruit that synthesize large amounts of carotenoids (Gross 1987). During the immature stage, chlorophylls predominate in the peel of all citrus fruit. During the maturation stage, carotenoids are rapidly synthesized in the chromoplast, accompanied by a simultaneous loss of chlorophyll. This is called natural color break or color development (Ladaniya, 2008). Color break of citrus fruit is the result of the chloroplast to chromoplast transition involving chlorophyll degradation and carotenoid accumulation. This effect is influenced by environmental, nutritional and hormonal control (Alós et al., 2006). In non-climacteric citrus fruit, ethylene plays the primary role of natural color break but is biosynthesized at low levels during the fruit maturation period (Katz et al., 2004). Sucrose accelerates color change in citrus peels, while nitrogen delays chromoplast formation (Iglesias et al., 2001).

Total carotenoid content is usually lower in the pulp than the peel of most citrus fruit (Gross, 1987), because of the lower expression of carotenoid biosynthetic genes in the pulp than the peel (Kato et al., 2004). The pulp of mature mandarin fruit accumulates unusually high amounts of carotenes (phytoene and phytofluene) and β-carotene (Xu et al., 2006).

Organic acids are usually used as respiratory substrates for synthesis of ATP in TCA cycle (Ting and Attaway, 1971) and also play an important role in the consumer perception of fruit flavor quality (Chen et al., 2009). In mandarin fruit, citric acid (75-80%) is the dominant organic acid while malic acid (15-20%) is the second most abundant (Baldwin, 1993). Isocitric acid is only present in very small amounts (Cancalon and Xu, 2002). In ripe fruit, TA is 0.9-1.3% for 'Sai Nam Phueng' and 0.5-0.7% for 'See Thong' cultivars (Roongruangsri et al., 2005).

The decreases in the citric and malic acid content of 'Sai Nam Phueng' and 'See Thong' cultivars observed here during maturation are 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 high value of isocitric acid content during fruit growth agree with that reported for Satsuma mandarin (Daito and Sato 1985), orange and grapefruit (Cancalon and Xu, 2002). The declines in organic acid content could be partly associated with the increase in fruit size and water content (Ting and Attaway, 1971). Organic acids might be used for energy production through cellular respiration (Purvis, 1983a) and the conversion of organic acids to sugar (glucose) through gluconeogenesis (Echeverria and Burns, 1989).

Vitamin C is the most abundant vitamin in citrus fruit, an important source of this vitamin (Iglesias et al., 2007). Vitamin C content in 'Sai Nam Phueng' and 'See Thong' cultivars decreased about 50% during maturation. Vitamin C is usually high in immature orange and grapefruit (Baldwin, 1993). The concentration of vitamin C decreases as fruit matures and increases in size Per-fruit basis, the vitamin C content usually decreases during citrus fruit maturation (Ting and

Attaway, 1971).

The principal sugars of citrus fruit are sucrose, glucose and fructose, although xylose is also present in trace amounts (Ting and Attaway, 1971). In ripened 'Sai Nam Phueng' and 'See Thong' cultivars, sucrose, glucose and fructose content were distributed in a ratio of approximately 2:1:1. A similar ratio was observed in 'Clementina' mandarin (Cercos et al., 2006) and 'Florida' orange (Villamiel et al., 1998).

Sugars are synthesized through the photosynthesis pathway in the leaf and translocated to the fruit (Taiz and Zeiger, 2002). Sugar translocation in citrus fruit is observed by using 14C-labeled compounds. Sucrose, which translocates from the leaf to the fruit, are converted to glucose and fructose in the fruit in juice vesicle cells (Echeverria, 1990). Therefore, most sugars in citrus fruit are stored in the form of sucrose. Other sugars, such as fructose and glucose, are stored in lesser amounts (Echeverria, 1990). The overall increase in the sugar content of 'Sai Nam Phueng' and 'See Thong' cultivars throughout fruit development observed here are in agreement with the general observation in most citrus fruit (Ladaniya, 2008), for example 'Clementina' mandarin (Cercos et al., 2006), Navel orange (YongZhong et al., 2007), and 'Okitsu Wase' and 'Silverhill' oranges (Daito and Sato, 1985), with the exception of acidic fruit like lemon and lime (Ladaniya, 2008). In 'Clementina' mandarin, the expression of genes involved in sucrose synthesis, namely, sucrose synthase and acid invertase increase during growth and strongly decrease in mature fruit (Cercos et al., 2006). In lemon, the increase in sugars might be due to the decline in organic acids, which are converted to sugars through gluconeogenesis (Echeverria and Burns, 1989).

The increase in TSS and the decrease in TA led to a gradual increase in the TSS/TA ratio in the 'Sai Nam Phueng' and 'See Thong' cultivars observed here. TSS/TA ratio is used as a guide of harvest indices in the citrus industry (Ladaniya, 2008), and to determine the relative sweetness of citrus juice (Ting and Attaway, 1971). A TSS/TA ratio ranging from 8 to 10 is generally accepted as a measure of minimum maturity, while a ratio of 10 to 16 is considered to be of acceptable quality for orange (Davis and Albrigo, 1994). When the fruit remains on the tree, TSS continues to increase and acid decreases until the fruit becomes overripe. When the TSS/TA ratio reaches 20 or more, the taste of citrus juice is too sweet and no acid is detected (Baldwin, 1993). TSS for tangerine fruit sold in the local market in Thailand averages 11-13% for 'Sai Nam Phueng' and 9-11% for 'See Thong'. The TSS/TA ratio was about 11-13% for 'Sai Nam Phueng' and 15-17% for 'See Thong' (Roongruangsri et al., 2005).

The physico-chemical changes observed here during growth and maturation were quite similar in both cultivars. The fruit weight and size increased in parallel between these two cultivars. Chlorophyll degradation and carotenoid accumulation in peel resulted in changing color from green to yellow-orange. Carotenoid accumulation in juice resulted in changing color from yellow-orange to deep orange. During maturation, peel and juice colors (L, chroma and hue angle values), peel chlorophyll content and juice carotenoid content did not differ between the two tangerine cultivars. Peel carotenoid content of 'Sai Nam Phueng'

cultivar was lower than 'See Thong' cultivar. TA and citric acid content of both cultivars increased to the maximum at early growth stage, then rapidly decreased and slightly decreased during maturation. Malic acid and vitamin C content were very high at the early growth stage, and then declined toward fruit maturation. Isocitric acid content gradually decreased during maturation. The TSS, TSS/TA ratio, sucrose, glucose and fructose content of both cultivars continually increased during growth and then slowly increased during maturation. 'Sai Nam Phueng' had higher TA, TSS, citric acid, vitamin C, sucrose, glucose and fructose content and lower juice pH, isocitric acid content and TSS/TA ratio than 'See Thong'. Malic acid content did not differ between these two cultivars.

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