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Original Article

Effects of fruit thinning on fruit drop, leaf carbohydrates concentration, fruit carbohydrates concentration, leaf nutrient concentration and fruit quality in Pummelo cultivar *Thong Dee*

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Abstract

The effects of fruit thinning on fruit drop, leaf carbohydrates concentration, fruit carbohydrates concentration, leaf nutrient concentration and fruit quality in pummelo cultivar *Thong Dee* growing in Nakhon Pathom province, Thailand, were studied during January-August 2013. The results showed that 50% fruit thinning by hand at 1 month after fruit set increased percent of fruit retention throughout fruit development. At 2 month after fruit set, 50% fruit thinning gave 62% of fruit retention, which were significantly higher than no thinning (36.60%), whereas, 50% fruit thinning gave 40% of fruit retention at 6 month after fruit set, which significantly higher than no thinning (20.2%). A significant difference in leaf carbohydrate concentration was found in 3-6 month after fruit set. At 3 months after fruit set, 50% fruit thinning gave significantly higher leaf carbohydrate concentration (81.80 $mg.g^{-1}$) than no thinning (73.56 $mg.g^{-1}$), whereas, at 6 month after fruit set, 50% fruit thinning gave significantly higher leaf carbohydrate concentration (128.92 $mg.g^{-1}$) than no thinning (102.90 $mg.g^{-1}$). Although, 50% fruit thinning gave no effect on peel and pulp dry weight including peel and pulp carbohydrates concentration during fruit development. For plant nutrient analysis, 50% fruit thinning gave significantly higher leaf nitrogen (N), phosphorus (P) and potassium (K) concentration than no thinning during 3-6 month after fruit set. However, 50% fruit thinning had no effect on fruit weight, fruit circumstance, fruit diameter, titratable acidity (TA) and total soluble solid (TSS). It was concluded that 50% fruit thinning increased percent of fruit retention and may have an effect on the accumulation of leaf carbohydrates, leaf N, leaf P and leaf K concentrations in pummelo cultivar *Thong Dee*.

Keywords: pummelo, fruit drop, fruit thinning, *Thong Dee*, leaf carbohydrates concentration

1. Introduction

Pummelo growers in Thailand have met pre-harvest fruit drop problems. Over the last 2-3 years this problem has become quite serious with growers in Thailand losing both production and income. A shortage of carbohydrates, imbalances in plant nutrition and plant hormone as well as insect and pathogen have all been reported as causes of the fruit drop in citrus species (Davies and Albrigo, 1998) and other fruit crops such as sweet cherry (Blanusa *et al.,* 2006) and apple (Ward, 2004). In favorable conditions fruit trees set more fruit than ideal. This phenomenon results in carbohydrate and plant nutrient competition among fruitlets and fruit drop will occur. Fruit thinning involves removing excess fruitlets after fruit set and natural dropping have occurred to improve fruit yield, fruit size and quality including fruit drop reduction. It is carried out on apples, pears, plums, peaches and nectarines. Many healthy fruit trees drop fruit naturally in early summer in what is known as the 'June drop'. Where a heavy crop has set, too many fruitlets may remain on the

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branches, resulting in a final crop of disappointingly small fruits. Deliberate thinning of the fruitlets produces better-sized, ripe and healthy fruits, albeit in smaller numbers. There are three types of thinning namely: hand, mechanical and chemical thinning.

Many researchers have reported that fruit thinning decreased fruit drop and increased carbohydrates availability for remaining fruitlets in citrus species and other fruit species (Ruiz *et al.,* 2001; Blanusa *et al.,* 2006; Beruter and Droz, 1991; Atkinson *et al.,* 2002). Spiegel-Roy and Goldschmidt (1996) reported that fruit drop in citrus trees consists of two successive abscission waves affecting flowers and developing fruitlets. The first wave massively induces flower and ovary abscission, whereas the second one reduces the number of growing fruitlets during the June drop. Moreover, a strong relationship between the carbohydrate amounts available for the fruitlets, especially soluble sugars, and their probability of abscission has been suggested (Iglesias *et al.,* 2003). Fruit thinning can reduce crop load and decrease carbohydrate and plant nutrient competition among fruitlets, which results in more fruit retention.

Pummelo, one of the citrus species in Thailand, has met pre-harvest fruit drop problem. Fruit thinning may be the solution to the problem. However, effects of fruit thinning on fruit drop and leaf carbohydrate concentration in pummelo have not been studied. Therefore, the objective of this study was to investigate the effects of fruit thinning of fruit drop and leaf carbohydrate concentration in pummelo cultivar *Thong Dee* growing in Nakhon Pathom province, Thailand.

2. Materials and Methods

2.1 Plant material

The research was conducted in the pummelo growing areas of Nakhon Pathom province, Thailand. Ten similar size (approximately 4 m in diameter) and age (8 years of age) of *Thong Dee* pummelo trees were selected to set up the experiment. Four branches in the same size (approximately 2 cm in diameter) from each pummelo tree were randomly selected around the canopy, two branches for 50% fruit thinning treatment and two branches for no thinning treatment. The initial fruit number before thinning of selected branches was 10 fruits per branch on average. Then, fruits were thinned 50% at 1 month after fruit set by hand. Normally, the pummelo fruitlets dropped approximately 40% at this stage of fruit growth, therefore the percent of fruit thinning in this study was more than the normal fruit drop occurred in the first month of pummelo fruit growth (Nartvaranant *et al.,* 2010).

The small, scarred, blemished and deformed fruits were firstly removed from the selected branches. Moreover, two or three fruit fused together were also removed from the selected branches to leave the remaining fruit with sufficient space. The research was conducted during January 2013 - August 2013.

2.2 Data collected

Twenty pummelo fruits from each of the 50% fruit thinning and no thinning treatments (one fruit per branch) were collected for fruit size measurement (fruit diameter and fruit circumstance) using vernier caliper. Percent of fruit retention was measured at 1, 2, 3, 4, 5 and 6 months after fruit set calculated by this formula: (number of retained fruit on the selected branches/number of fruits at the beginning)*100.

Ten pummelo fruits from each of the 50% fruit thinning and no thinning treatments were collected at harvesting time and taken to laboratory for fruit quality measurement as follow: fruit weight, fruit size, total soluble solid (TSS), titratable acidity (TA), peel and pulp carbohydrate concentration.

Twenty leaves from each of the 50 % fruit thinning and no thinning treatments (two leaves per tree) were collected for total non-structural carbohydrate concentration and twenty leaves from each treatment were also collected for plant nutrient concentration measurement once a month during 1-6 month after fruit set. The sampled leaves were collected from the pummelo leaves positioned near the selected fruit.

2.3 Total non-structural carbohydrates

Peel, pulp and 20 leaves from each of the 50% fruit thinning and no thinning treatment (two leaves per tree) were separated and taken for total nonstructural carbohydrates analysis using the Nelson's procedure. All the samples were analyzed for total TNC by washing the samples in tap water then rinsing in distilled water and finally drying in a hot air oven at 65°C for 72 hrs. After drying, the material was ground in a Wiley Mill and stored in a desiccator. Total non-structural carbohydrates were extracted with 0.2 NH_2SO_4 (Smith, 1969) and determined by the Nelson reducing sugar procedure method described by Hodge and Hofreiter (1962) at the Central Laboratory, Kasetsart University, Kamphaeng Saen Campus.

2.4 Plant nutrient concentration

Twenty leaves from each of the 50% fruit thinning and no thinning treatment (two leaves per tree) were separated and taken for plant nutrient concentration analysis. The plant material was dried and ground before plant nutrient analysis. From this dried material the nitrogen (N), phosphorus (P), potassium (K), concentration were determined. Levels of N were determined using the Microkjeldahl Method. P and K were extracted by HNO_3-$HClO_4$ (5:1) then the solution was left to cool down. Phosphorus in solution was determined calorimetrically by the Molybdate-vanadate yellow color method, whereas, K was determined using an atomic absorption spectrophotometer. All plant nutrient analysis was done at the Soil Plant and Agricultural Material Testing and Research Unit, Central Laboratory, Kasetsart University, Kamphaeng Saen Campus.

2.5 Statistical analysis

All the data were statistically tested by the dependent sample t-test method at the 95% confidential level. The randomized complete block design was employed for the experimental design.

3. Results

The effect of fruit thinning on percent of fruit retention in pummelo cultivar *Thong Dee* showed that 50% fruit thinning at 1 month after fruit set increased percent of fruit retention throughout fruit development. At 2 month after fruit set, 50% fruit thinning gave 62% of fruit retention, which was significantly higher than no thinning (36.60 %), whereas, 50% fruit thinning gave 40% of fruit retention at 6 month after fruit set, which significantly higher than no thinning (20%) (Table1). However, the percent of fruit retention in both 50% fruit thinning and no thinning continually decreased until 5 months after fruit set (Table 1).

Fruit diameter measurement indicated that 50% of fruit thinning did not have any effect on fruit size as there were no significant difference in fruit diameter between 50% fruit thinning and no thinning throughout fruit development (Table 2). Table 2 showed that 50% fruit thinning gave 12.9 cm fruit diameter which was no significantly different from no thinning (12.6 cm) at 6 month after fruit set.

The effect of fruit thinning on leaf carbohydrate concentration in pummelo was also studied. The results indicated that there was no significant difference in leaf carbohydrate concentration between fruit thinning and no thinning in the early stage of fruit development (1-2 months after fruit set). However, a significant difference in leaf carbohydrates concentration was found 3 - 6 months after fruit set (Figure 1). At 3 month after fruit set, 50% fruit thinning gave significant higher leaf carbohydrates concentration (81.80 $mg.g^{-1}$) than no thinning (73.56 $mg.g^{-1}$), whereas, at 6 month after fruit set, 50% fruit thinning gave significantly higher leaf carbohydrate concentration (128.92 $mg.g^{-1}$) than no thinning (102.90 $mg.g^{-1}$). However, 50% fruit thinning had no effect on peel and pulp dry weight or on peel and pulp carbohydrate concentration during fruit development (Figures 2 and 3).

However, the pummelo peel had the higher dry weight than the pummelo pulp in both 50% fruit thinning and no thinning, whereas, the pummelo peel had a lower carbohydrate concentration than the pummelo pulp (Figure 2 and 3).

Leaf nitrogen (N), phosphorus (P) and potassium (K) concentrations in pummelo cultivar *Thong Dee* were studied and found that 50% fruit thinning gave significantly higher

Table 1. Effect of fruit thinning on percent of fruit retention in pummelo cultivar *Thong Dee*. Data in parentheses presented number of fruit retention / total number of fruit at the beginning.

Fruit thinning	Month after fruit set					
	1[1/]	2	3	4	5	6
50 % Fruit thinning	100	62.00	40.0	40.0	40.0	40.0
	(5/5)	(3/5)	(2/5)	(2/5)	(2/5)	(2/5)
No fruit thinning	100	37.00	29.0	29.0	20.0	20.0
	(10/10)	(3.7/10)	(2.9/10)	(2.9/10)	(2/10)	(2/10)
t-Test		*	ns	ns	*	*

[1/] date of fruit thinning
* Significant difference at 95 % confidential interval analyzed by dependent sample t-test.
[ns] No significant difference at 95 % confidential interval analyzed by dependent sample t-test.

Table 2. Effect of fruit thinning on fruit diameter (cm) in pummelo cultivar *Thong Dee*.

Fruit thinning	Month after fruit set					
	1[1/]	2	3	4	5	6
50 % fruit thinning	1.90	4.93	9.90	11.00	12.25	12.90
No fruit thinning	2.10	5.71	10.60	11.85	12.00	12.60
t-Test	ns	ns	ns	ns	ns	ns

[1/] date of fruit thinning
* Significant difference at 95 % confidential interval analyzed by dependent sample t-test.
[ns] No significant difference at 95 % confidential interval analyzed by dependent sample t-test.

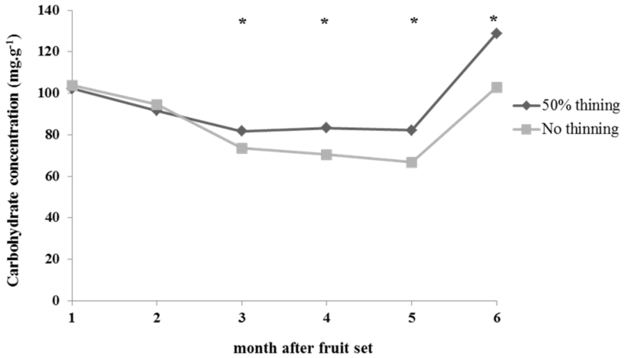

Figure 1. Effect of fruit thinning on leaf carbohydrate concentration during fruit development in pummelo cultivar *Thong Dee*. * presented the significant difference at 95 % confident interval analyzed by independent sample t-test.

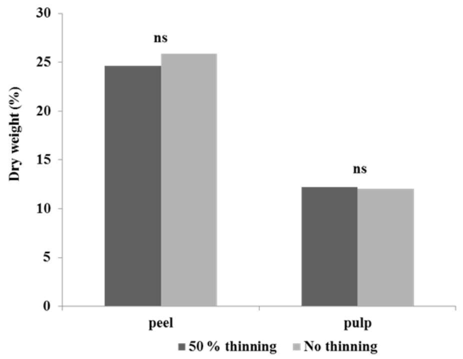

Figure 2. Effect of fruit thinning on percent of peel and pulp dry weight in pummelo cultivar *Thong Dee*. ns presented non-significant difference at 95% confident interval analyzed by dependent sample t-test.

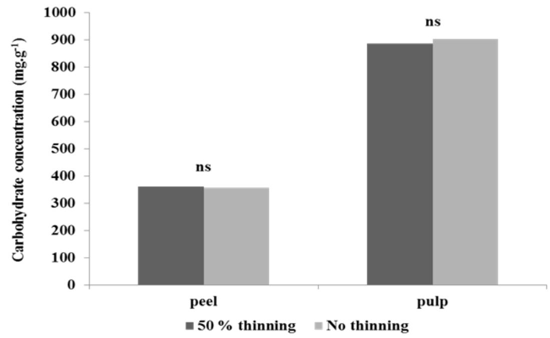

Figure 3. Effect of fruit thinning on peel and pulp carbohydrate concentration in pummelo cultivar *Thong Dee*. ns represented non-significant difference at 95 % confident interval analyzed by dependent sample t-test.

Table 3. Effect of fruit thinning on leaf nitrogen concentration (%) in pummelo cultivar *Thong Dee* during fruit development

Fruit thinning	Month after fruit set					
	1[1/]	2	3	4	5	6
50 % thinning	2.72	2.90	3.05	3.08	3.12	3.16
No thinning	2.70	2.64	2.70	2.80	2.84	2.82
t-Test	ns	ns	*	*	*	*

[1/] date of fruit thinning
* Significant difference at 95 % confidential interval analyzed by dependent sample t-test.
[ns] No significant difference at 95 % confidential interval analyzed by dependent sample t-test.

leaf N, P and K concentration than no thinning during 3-6 month after fruit set (Table 3-5). For leaf N concentration, 50% fruit thinning gave significantly higher leaf N concentration at 3, 4, 5 and 6 month after fruit set (3.05, 3.08, 3.12 and 3.16 %, respectively) than no thinning (2.70, 2.80, 2.84 and 2.82 %, respectively) (Table 3)

For leaf P concentration, there was a significant difference in leaf P concentration between 50% fruit thinning and no thinning at 3-6 month after fruit set. Leaf P concentration in 50% fruit thinning was 0.23, 0.24, 0.25 and 0.24 % at 3, 4, 5 and 6 months after fruit set, respectively, whereas leaf P concentration in no thinning was 0.19, 0.20, 0.21 and 0.20% at 3, 4, 5 and 6 month after fruit set, respectively (Table 4).

The result also showed that there was a significant difference in leaf K concentration between 50% fruit thinning and no thinning. 50% fruit thinning gave the higher leaf K concentration at 3, 4, 5 and 6 months after fruit set (2.94, 3.04, 3.08 and 3.23% respectively) than those in no thinning (2.20, 2.20, 2.10 and 2.32% respectively) (Table 5).

However, 50% fruit thinning had no effect on fruit

Table 4. Effect of fruit thinning on leaf phosphorus concentration (%) in pummelo cultivar *Thong Dee* during fruit development

Fruit thinning	Month after fruit set					
	1[1/]	2	3	4	5	6
50 % thinning	0.19	0.21	0.23	0.24	0.25	0.24
No thinning	0.18	0.20	0.19	0.20	0.21	0.20
t-Test	ns	ns	*	*	*	*

[1/] date of fruit thinning.
* Significant difference at 95 % confidential interval analyzed by dependent sample t-test.
[ns] No significant difference at 95 % confidential interval analyzed by dependent sample t-test.

Table 5. Effect of fruit thinning on leaf potassium concentration (%) in pummelo cultivar *Thong Dee* during fruit development

Fruit thinning	Month after fruit set					
	1[1/]	2	3	4	5	6
50 % thinning	2.23	2.34	2.94	3.04	3.08	3.21
No thinning	2.24	2.37	2.20	2.20	2.10	2.32
t-Test	ns	ns	*	*	*	*

[1/] date of fruit thinning.
* Significant difference at 95 % confidential interval analyzed by dependent sample t-test.
[ns] No significant difference at 95 % confidential interval analyzed by dependent sample t-test.

Table 6. Effect of fruit thinning on fruit weight, fruit circumstance, titratable acidity (TA) and total soluble solids (TSS) in pummelo cultivar *Thong Dee* at harvesting time

Fruit thinning	fruit weight (g)	fruit circumstance (cm)	titratable acidity (TA)	total soluble solids (TSS)
50 % thinning	821.30	41.59	0.56	12.38
No thinning	813.49	41.23	0.54	12.12
t-Test	ns	ns	ns	ns

[ns] No significant difference at 95 % confidential interval analyzed by dependent sample t-test.

weight, fruit circumstance, titratable acidity (TA) and total soluble solid (TSS) of pummelo fruit (Table 6).

4. Discussion

The 50% fruit thinning used in this experiment was more than normal fruit drop occurring in the first month of pummelo fruit growth. Normally, the percentage of fruit drop in pummelo was approximately 40% of the initial fruit number in the first month of fruit growth (Nartvaranant *et al.,* 2010). Thus, the 50% fruit thinning was selected to set up the experiment for this preliminary study about effect of fruit thinning on fruit drop in pummelo. This was in accordance with Ouma (2012), who reported that 50% fruit thinning was done in mandarin to improve fruit retention and fruit quality.

The results showed that 50% fruit thinning decreased fruit drop and increased percent of fruit retention compared to those in no thinning trees. However, 50% fruit thinning had no effect on fruit quality, fruit size, fruit dry weight and carbohydrate concentration in pummelo fruits. This indicated that pummelo fruits from the 50% fruit thinning and no thinning trees had no difference in fruit growth and development including carbohydrate utilization for fruit growth and development. However, 50% fruit thinning gave a higher percent of fruit retention and leaf carbohydrates concentration than no thinning. Thus, the high leaf carbohydrate concentration may result in high percent of fruit retention. It could be that fruit thinning reduce carbohydrates competition between pummelo fruits. This results in more carbohydrate availability for remaining fruits to grow and develop, which make the percent of fruit drop decreased and leaf carbohydrate concentration from fruit thinning branches higher than no thinning branches. It is also accordance with Guardiola and Garcia-Luis (2000), who reported that fruit thinning in citrus species increases fruit yield and fruit size. Iglesias *et al.* (2003) also indicated that increased carbohydrate availability to growing citrus fruitlets was associated with a decreased probability of abscission during fruit set, resulting in a greater number of fruits at the end of the growing periods. Moreover, Iglesias *et al.* (2006) revealed that the carbohydrates content may be a biochemical signal involved in the mechanisms controlling fruit abscission. Mehouachi *et al.* (1995) reported that the number of growing fruitlets that survive after June drop is mainly determined by nutritional factors such as photoassimilates. This phenomenon has been also described for other tree species such as apple (Beruter and Droz, 1991), pistachio (Nzima *et al.,* 1999) and cherry (Atkinson *et al.,* 2002).

There is evidence indicating the involvement of carbohydrates in fruit drop of citrus (Goldschmidt, 1999). Many data are supported by experiments modifying the sugar availability for growing fruitlets (Mehouachi *et al.,* 1995, Iglesias *et al.,* 2003). The reported results suggest that citrus possesses an inner mechanism that adjusts the number of developing fruitlets to carbon availability. Iglesias *et al,* (2006) found that girdling reduced fruit abscission in Satsuma mandarin and increased sugar content. Therefore, the conditions that induced higher sugar level or carbohydrates content can decrease fruit abscission or fruit drop rate. Pummelo fruit thinning in this experiment may be the condition inducing the high carbohydrates content (Figure 1) so that the percent of fruit drop reduced. This is in accordance with Goldschmidt and Koch (1996), who found that fruit thinning may decrease fruitlet abscission by increasing the availability of carbon among the remaining fruit. Previous experiments of many researchers together with our results in this study show that increased carbohydrate availability to growing citrus fruitlets including pummelo fruitlets was associated with a decreased probability of abscission during fruit set, resulting in a greater number of fruits at the end of the growing periods. Gillaspy *et al.* (1993) revealed that citrus fruit abscission is dependent on environmental, nutritional and hormonal factors. Moreover, several studies on source-sink imbalances in citrus species support the hypothesis that competition for photoassimilates among developing fruitlets regulates fruit abscission. Effects of defoliation, girdling, defruiting or fruit thinning have provided indirect evidence for the role of carbohydrates in various physiological processes related to fruit growth (Goldschmidt and Koch, 1996).

Nutritional factors are one of the limiting factors for fruit set in citrus and other fruit species. From this result, 50% fruit thinning gave the higher leaf N, P and K concentration than no thinning. This may the result in a higher percentage of fruit retention due to higher nutrient concentration available for the remaining pummelo fruitlets development as 50% fruit thinning reduced the nutrient competition among pummelo fruitlets. This is similar to the results of Zarei *et al.* (2013), who reported that leaf mineral concentration increased in response to fruit thinning by chemical means. Moreover, Moatshe *et al.* (2011) indicated that BA can be used as a chemical thinning agent to reduce crop load and improve the morula tree mineral uptake and mineral partitioning to the leaves and fruits. Generally fruiting reduces growth of all parts of the plant but with a greater effect on the roots (Forshey, 1986). This reduces development, strength and activity of the roots and hence reduces the supply of reserves and essential growth regulators such as water, plant growth regulators and the mineral elements. Therefore, due to the sufficient supply of assimilates in response to fruit thinning application, the root activity improves and hence promotes the translocation of minerals to the sinks such as pummelo leaves. However, Nartvaranant (2010) found that the suitable leaf N, P and K concentrations for pummelo production were 2.7-2.9 %, 0.18-0.21% and 2.4-3.5%, respectively. The leaf N, P and K concentrations found in this study seem to be the suitable level for those nutrients in both treatments. Therefore, it could be that plant nutrients may not be the major limiting factor in fruit retention in this condition.

There was no significant difference in fruit size between 50% fruit thinning and no thinning treatment but 50% fruit thinning gave higher percent of fruit retention than no thinning. Thus, farmers who make fruit thinning may have opportunity to gain more profit compared to those who make no thinning. Moreover, fruit thinning also reduces alternate bearing in the following year. Leaving too much fruit on a tree can lead to alternate bearing (a cycle in which the tree bears excessively in one year and little the next year) or limb breaking (Ingels *et al.,* 2002), due to shortage in carbohydrates and plant nutrients supply for fruit set and development in the following year. Fruit thinning can reduce crop load and supply enough carbohydrates and plant nutrient for fruit set and development in the following year. The evidences from this study indicates that 50% fruit thinning gave a higher leaf carbohydrate concentration, leaf N concentration, leaf P concentration and leaf K concentration than no thinning.

In this study, the difference in percentage of fruit retention caused no difference in fruit quality, which in against simple logic from basic knowledge that lower number of fruit on tree normally lead to higher quality of fruit. This may be explained by the consideration in fruit number retention per branch as 40% fruit retention of 50% fruit thinning and 20% fruit retention of no fruit thinning gave the same number of fruit retention/branch (2 fruits per branch) (table 1). Thus, the same number of fruit retained on the branch might result in no difference in fruit quality and fruit carbohydrates between the two treatments. Although fruit thinning had no effect on fruit quality in this study, pummelo fruit quality from both 50% fruit thinning and no thinning trees met the standard of Thai pummelo. The standard of Thai pummelo requires 8 °brix of TSS, 400 g of fruit weight, 100 mm of fruit diameter and 313 mm of fruit circumstance for the minimum level of pummelo fruit quality (The National Bureau of Agricultural Commodity and Food Standards, 2007), and all of pummelo fruits in this study met these standard levels (Table 6).

5. Conclusions

It is concluded that 50% fruit thinning increases percent fruit retention at 5-6 months after fruit set, and 50% fruit thinning also increases leaf carbohydrate concentration, leaf N concentration, leaf P concentration and leaf K concentration at 3-6 month after fruit set. However, there was no significant difference in pummelo fruit quality at harvesting time between 50% fruit thinning and no thinning.

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