Effect of zinc on fruit quality of pummelo (*Citrus grandis* (*L.*) Osbeck) cv. Tubtim Sayam

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Abstract

Effect of zinc on the fruit quality of pummelo cv. Tubtim Sayam was carried out between 2013 – 2014 at a pummelo orchard in Sub-district Klong Noy, Pak Panang District, Nakhon Si Thammarat Province. The objectives of the study were to examine the factors that affect the physical aspects of the fruit quality such as weight, width, height, and peel thickness as well as those affecting its chemical aspects such as total soluble solids, acid, pH, vitamin C, sugar, limonin, anthocyanine, locopene, Chrolophyll and β-Carotene. The study of fruit quality in relation to application of zinc at 4 rates (0, 12, 36, 60 g/tree) found that the Tubtim Sayam pummelo trees receiving zinc at 36 g/tree rate gave the highest of fruit weight and fruit height as 1,497.28 g and 17.61cm respectively and show significant differences. Chemically, the fruit from the tree receiving zinc at 36 g/tree rate had the highest amount of vitamin C (58.14 mg/100ml), each of which had the following significant quality: The trees receiving zinc at 60 g/tree rate yielded fruits which had the highest level of sucrose, glucose, fructose, and total sugar (123.14 g/l; 47.22 g/l; 18.06 g/l; and 188.43 g/l, respectively). The fruits from the trees receiving no zinc had the highest level of limonin (2.36 mg/l) which was significantly different from the level below 1.99 mg/l of those receiving zinc at all rate. The 36 g/tree rate resulted in the highest amount of chlorophyll A (0.03 mg/100 ml); the 12 g/tree rate the highest amount of chlorophyll B (0.03 mg/100 ml); and the 60 g/tree rate the highest amount of anthocyanin (9.58 mg/100 ml). However, the application of all zinc rates had no effect on lycopene and β-carotene in pummelo pulps.

Keywords: Zinc, Fruit Quality, Pummelo, Tubtim Sayam

1. Introduction

1.1 Background and Importance of the Research

Tubtim Sayam pummelo, which originated in Yarang District, Pattani Province, has been spread to Pak Panang District, Nakhon Si Thammarat Province. Its outstanding characteristics include big dark green leaves with many soft hairs on both sides, round fruits with a small knot on the top of each, yellowish green smooth skin with dense tiny oil glands, light pink thin peel, tight rows of small juicy pulps in colors ranging from dark pink to cooked-shrimp red, and sour-sweet taste (Maneepong, 2013; Kaewtubtim & Issarakraisila, 2011; Promotion and Development, 2009). Tubtim Sayam pummelo was originally grown in a small orchard with other cultivars such as Khaw Thong Dee, Roti, and Baan Yha as well as other kinds of fruit trees like mangosteens, durians, longkong and rambutans. The quality of Tubtim Sayam fruits grown in Pattani areas this way is generally low, possibly due to soil types and inappropriate cultivations. Its pulps are not of dark pink color and have a bitter taste. On the other hand, the Tubtim Sayam pummelo trees planted in new vernacular orchards as a single fruit crop in Pak Panang District, Nakhon Si Thammarat Province, with different soil type and environments, have produced fruits of a higher quality than those grown in Pattani (Maneepong, 2013). The areas for growing Tubtim Sayam pummelo commercially in Pak Panang District have been expanded rapidly in recent years (Maneepong, 2013). The price of Tubtim Sayam fruits is also much higher than prices of the other cultivars (Promotion and Development, 2009).

The study of the nutrient concentration in the experimented soil for growing the Tubtim Sayam pummeloes in Pak Panang District of the Nakon Si Thammarat Province and their leaves revealed that the most important problem was the lack of zinc both in the soil and leaves when compared to their appropriate levels (Maneepong, 2009; Wongchana, 2010). The reason could be that there was high concentration of phosphorous in the soil, resulting in little dissolution of zinc (Osotsapa, 2000; Barrow, 1987). Furthermore, the farmers' repeated use of a great amount of phosphorous increased the level of this nutrient in the soil while reducing zinc even more (Sethpakdee, 2001; Kaewtubtim, 1996). Researcher observed that the Kwao Thongdee pummeloes which were grown in the same experiment plot displayed a serious deficiency of zinc in their leaves although the Tubtim Sayam pummeloes did not showed visible signs of this insufficiency due to this cultivar 's better resistance. The addition of zinc increased the Tubtim Sayam pummelo production according to the Law of the Minimum because zinc helped synthesize trytophan (Pendials, 1992; Salisbury and Ross, 1992; Tsui, 1948) which was precursor in synthesizing indole-3-acetic acid, IAA (Smith et al.,1979; Skoog, 1940) which was component of enzymes (Ali et al., 2014; Osotsapa, 2000; Coleman, 1992; Pendials, 1992) functioning in metabolism process of protein (Zhang & Brown, 1999; Kathryn et al., 1996; Albrigo & Young, 1981; Johnson & Simons, 1979; Prask & Plocks, 1971; Arora & Singh, 1970).which would increase inflorescences, flowers, fruit setting and growth (Srivastava & Singh, 2005; Domingo et al., 1992; Sahota and Arora, 1981; Prask & Plocks, 1971). There have been studies which report that the increasing of zinc influence to the quantity and quality of citrus (Damrongrak, 2007; Storey & Treeby, 2000; Quaggio et al., 2000; Maust & Williamson, 1991; Dasberg, 1987; Dasberg et al., 1983; Legaz et al., 1982; Sahota & Arora, 1981; Moss et al, 1972).

1.2 Research Objective

Therefore, in addition to the examination of the effects of zinc on the fruit quality, zinc was sprayed at different levels onto the five-year-old trees of Tubtim Sayam pummeloes in order to study the reproductive growth as same as there have been studies which report that the zinc addition influence of the quality of citrus.

2. Methodology

2.1 Plant material

The experiment was conducted on the pummelo cv. Tubtim Sayam trees of 5 years old in Pak Panang District, Nakhon Si Thammarat Province, during June 2013 to May 2014. The study was conducted in Completely Randomized Design (CRD) with 4 replications for each of the four treatments as T1) pummelo trees receiving zinc rate at zinc 0 g/tree, T2) pummelo trees receiving zinc rate at zinc 12 g/tree, T3) pummelo trees receiving zinc rate zinc 36 g/tree, and T4) pummelo trees receiving zinc rate at zinc 60 g/tree.

2.2 Plant growth measurement

Growth parameters were evaluated as fruit weight (g), fruit width (cm.), fruit height (cm.) and peel thickness (cm.). The fruit weight was measured with a digital. The fruit width, fruit height and peel thickness were measured using a caliper.

2.3 TSS, TA, pH and vitamin C measurement

TSS was determined with hand refractometer. Total acid (TA) was titrated with sodium hydroxide to the end point pH 8.2 using a Microprocessor-based. The volume of sodium hydroxide was used to calculate the percentage of total acid by Boland (1995). pH was determined with pH-meter and vitamin C was recorded by putting 2 ml of the pummelo juice into a beaker with 5 ml of extracting solution (metaphosphoric acidacetic acid solution; titrating it with standardized dye solution (50 mg of dichloroindophenol sodium salt in 50 ml of distilled water and 42 mg of sodium bicarbonate) of ascorbic acid, concentration calculation according to AOAC (2000) method.

2.4 Sugar type and limonin measurement

Sugar contents (glucose, fructose, sucrose and total sugar) were measured using the high performance liquid chromatography (HPLC) system that consists of the Refractive Index detector, Sugar SH1011 (Shodex size 8.0 mm ID x 300 nm) column, filter with cellulose acetate syringe filter 0.20 μm, diameter 13 mm placed in Vial Size 2 ml. The mobile phase consisted of 0.04 N of sulfuric acid, the method was in accordance with AOAC (2000). Limonin contents were measured using the high performance liquid chromatography (HPLC) system that consisted of Variable Wavelength detector 210 nm, Hypersil ODS 4.0 x 250 mm column, flow rate 1.0 ml/min, injection volume 20 μL. The mobile phase consisted of acetone nitrite/methanol/water (31.8/22.7/45.5 v/v), the method according to AOAC (2000).

2.5 Pulp color measurement

Pigment contents (chlorophyll A, B, β-carotene, lycopene) in the pulp of the Tubtim Sayam pummelo were studied by Simple method (Nagata & Yamashita, 1992). All pigments in samples (1 g of peel or pulp) were extracted with acetone and hexane (4:6) at once, then optical density of the supernatant at 453 nm, 505 nm, 645 nm and 663 nm were measured by spectrophotometer at the same time. Anthocyanin contents of the pummelo pulp measured by pH-differential (Giusti & Wrolstad, 2005) were reported in mg of cyaniding-3-glucoside/100 g fresh weight basis).

2.6 Statistical analysis

The experiment was conducted with four treatments and four replications. The analysis of variance (ANOVA) was carried out on experimental data and in the case of significance. Means were compared by Duncan's new Multiple Range Test (DMRT) by SPSS Software.

3. Findings and Discussion

The quality of the Tubtim Sayam pummelo fruits harvested 28 weeks after the flowers bloomed, it was found that those from T3 had the highest level of fruit weight and fruit height (1,497.28 g and 17.61 cm, respectively) (Table 1) according to the law of the minimum. The analysis of zinc in the leaves before the experiment showed that its concentration was a little higher than its appropriate level (23.16 mg/kg,>20 mg/kg: data not presented). However, when this appropriate level is compared with that of other cultivars, its level was very low. For example, Shogun's level was 25-100 mg/kg (Damrongrak, 2007), tangerine's was 25-49 mg/kg (Davies and Albrigo, 1994), orange group's was 25-100 mg/kg (Alva et al., 2008; Storey & Treeby, 2000) and Navel oranges' was 25-100 mg/kg (Chapman, 1965). When zinc was sprayed onto the trees at the rate of 36 mg/kg per tree, the leaves had the most appropriate level of concentration (67.09 mg/kg: data not presented) when compared with other rates of the treatments. Applying zinc at the appropriate rate helped increase the production because zinc helped build auxin hormones (Srivastava & Singh, 2005; Kathryn et al., 1996; Domingo et al., 1992; Skoog, 1940). This finding agreed with the study of Sahota and Arora (1981), who found that spraying zinc sulphate onto Hamlin mandarin increased the growth of the trees, the fruit quantity, fruit weight from 130 g to 140 g, and increased flowers initiation. Besides, the addition reduced the dropping of fruits because this nutrient built and stimulated the operation of auxin hormones (Srivastava & Singh, 2005; Domingo et al., 1992; Smith et al., 1979; Tsui, 1948), built and developed the operation of DNA and protein (Osotsapa, 2000; Coleman, 1992), and controlled as well as maintain the status of tissue cells in preventing excessive entry of ions in the soil into plant cells (Sharma et al., 2006; Prask & Plocks, 1971).

Furthermore, this experiment found that the application of zinc at any rate did not have effects on fruit width and peel thickness which obviously increased when nitrogen was added (Dasberg et al., 1983; Damrongrak, 2007). All these because zinc played roles in metabolic process of carbon, and constituted the structures of many enzymes (Sharma et al., 2006; Marschner, 1995; Colemen, 1992; Pendials, 1992) which involved in metabolic process of protein (Kathryn et al., 1996; Johnson and Simons, 1979; Prask and Plocks, 1971), resulting in zinc's effects on bio-chemical quality than on physical aspect.

The addition of zinc in T2, T3, and T4 did not have effect on TSS, TA, and pH (Table 2). This result was different from the reports of Wongchana (2010) and Razeto and Salas (1986), who found that the addition of zinc increased TSS, and the studies on many kinds of fruits which reported that boron, iron and zinc increased TSS, and nitrogen as well as manganese increased acid while phosphorous and calcium reduced their sourness (Ali et al., 2014; Tariq et al., 2007; Obreza, 1990).

The trees of T3 had the highest level of Vitamin C (58.14 mg/100 ml) compared with those in other treatments (48.21-54.36 mg/100 ml) (Table 2). The concentration of Vitamin C in the Tubtim Sayam pummeloes was rather high when compared with that in oranges or other fruits such as oranges (30-70 mg/100 ml), grapefruits (45 mg/100 ml), Mandarin (50 mg/100 ml), lemmon (60 mg/100 ml), lime (30 mg/100 ml) (Kimball, 1999), guava (184 mg/100 g), Litchi 72 mg/100 g and strawberry (57 mg/100 g (Salunkhe et al., 1991). There was a tendency that the application of zinc helped increase Vitamin C (Navarro et al., 2010). The finding agreed with this experiment which found that the rate of zinc at 36 g/ tree was the most appropriate in making the Tubtim Sayam pummelo tress have the highest concentration of Vitamin C. This study also discovered that potassium, boron, copper, iron, and manganese helped increase Vitamin C (Hassan et al., 2010) while phosphorous decreased it. However, the increase or decrease of Vitamin C in plants may also depend on genetics and environment (Nyanga et al., 2013; Barros et al., 2012; Lee & Kader, 2000).

Compared with the pummeloes in other treatments, those from T4 had the most concentration of sucrose, glucose, fructose, and total sugar (123.14, 47.22, 18.06, and 188.43 g/l, respectively) (Table 3). This finding agreed with the report of Razeto and Salas (1986), who found that zinc increased TSS in Valencia oranges from 7.0 degrees brix to 8.4 degrees brix. Although not all soluble solid is sugar, most of its component is sugar. Therefore, the increase of TSS should also increase TS (Navarro et al., 2010). Zinc increased sugar in pummeloes because it decreased two enzymes which are related to metabolism of carbohydrate: 1) fructose-1,6-biphosphate fructose-6-phosphatase quickens the fructose-1,6-biphosphate fructose-6-phosphate 2) aldolase quickened the division of fructose-1,6-biphosphate into compound which had 3 atoms of carbon (Osotsapa, 2000; Epstein and Bloom, 2005).

Compared with the pummeloes in other treatments which had limonin below 1.99 mg/l, those from T1 had the highest level (2.36 mg/l) (Table 3). So far there has not yet been a report on the mechanism in which zinc reduces limonin. At this point it is assumed that the reduction was due to the functioning of enzymes because zinc is a component of more than 300 enzymes (Sharma, 2006; Marschner, 1995; Coleman, 1992). There might be one of these which prevented the work of limonoate D-ring lactone hydrolase enzyme that changed limonoate D-ring lactone found in internal peel, segment dividers, and seeds into bitter limonin.

Table 1 Fruit weight, fruit width, fruit height, and peel thickness of Tubtim Sayam pummelo after receive zinc different rate

treatment	fruit weight (g)	fruit width (cm.)	fruit height (cm.)	peel thickness (cm.)
T1	1,183.71±212.59 b	14.70±0.84 a	15.63±1.05 bc	1.00±0.17 a
T2	1,334.23±96.88 ab	15.16±0.55 a	16.77±0.64 ab	1.06±0.06 a
T3	1,497.28±94.07 a	15.60±0.45 a	17.61±0.80 a	1.22±0.08 a
T4	1,147.49±85.41 b	13.69±0.70 b	15.08±0.91 c	1.14±0.09 a
CV.	10.31	4.43	5.31	9.53
F-test	**	*	*	ns

[1] Mean within column with different alphabets differ significantly at P<0.05

Table 2 TSS, TA, TSS/TA, pH, and vitamin C of Tubtim Sayam pummelo after receive zinc different rate

treatment	TSS (brix)	TA (%)	TSS/TA ratio	pH	vitamin C (mg/100 ml)
T1	11.3±0.2 a	0.43±0.1 a	26.3±0.0 b	4.00±0.09 a	48.2±0.00 b
T2	11.2±0.6 a	0.33±0.1 a	33.9±0.0 a	4.12±0.14 a	48.6±0.00 b
T3	11.8±0.3 a	0.43±0.0 a	27.4±0.0 b	4.01±0.01 a	58.1±0.00 a
T4	11.4±0.3 a	0.43±0.0 a	26.5±0.0 b	4.14±0.03 a	54.4±0.00 ab
CV.	3.45	15.62	11.26	2.06	2.32
F-test	ns	ns	*	ns	**

[1] Mean within column with different alphabets differ significantly at P<0.05

Table 3 Sugar type and limonin of Tubtim Sayam pummelo after receive zinc different rate

treatment	sucrose (g/l)	glucose (g/l)	fructose (g/l)	total sugar (g/l)	limonin (mg/l)
T1	104.67±12.99 b	39.95±5.38 ab	13.51±1.55 b	158.13±19.93 a	2.36±0.23 a
T2	98.80±14.48 b	38.15±5.37 b	13.93±2.33 b	150.88±22.18 a	1.99±0.08 b
T3	114.08±2.39 ab	42.82±1.90 ab	14.19±2.62 b	171.08±6.88 a	1.99±0.01 b
T4	123.14±11.61 a	47.22±5.63 a	18.06±2.73 a	188.43±19.96 a	1.99±0.02 b
CV.	10.34	11.49	15.78	10.93	5.48
F-test	**	**	**	ns	**

[1] Mean within column with different alphabets differ significantly at P<0.05

Through the simple method it was found that the fruits from T3 had the highest level of chlorophyll A in their pulp (0.03 mg/100 ml), those from T2 had the highest level of chlorophyll B (0.03 mg/100 ml), and those from T4 had the highest level of anthocyanin (9.58 mg/100 ml) (Table 4). It was also found that the application of zinc at any rates had no effect on lycopene and β-Carotene (Table 4).

The mixture of many pigments resulted in the color of the Tubtim Sayam pulp. The red color ranging from deep red to bluish red was caused by anthocyanin the most and lycopene the next. The yellow color was influenced by β-Carotene but hidden by the green color of chlorophyll (Figure 1). When the fruits were fully ripe, they lost the chlorophyll and revealed other colors (Louis et al., 2010; Siriphanich, 2003; Lee, 2002; Mazza & Miniati, 1993). At this stage the fruits also produced phenylalanine ammonialyase (PAL) for synthesizing pigments and such organic components as phenolic, flavonoid, tannin, lignin, and anthocyanin. Then the fruits began to show their colors according to their kinds and cultivars (Louis et al., 2010; Rodrigo & Zacarias, 2006; Ortuno et al., 1997).

Because zinc has an important role in building and working of many hundreds of enzymes (Mengel et al., 2001), it can be assumed that in the pummeloes from T4, zinc stimulated PAL in producing anthocyanin the most (9.58 mg/100 ml) while in those from T3 the enzyme produced chlorophyll A the most (0.03 mg/100 ml), and in those from T2 the enzyme produced chlorophyll B the most (0.03 mg/100 ml). Besides, the proportion of zinc to sulphur also had a strong influence on producing certain colors because the two nutrients have a synergistic effect on colors. The addition of one or the other nutrient will result in a different synthesis of pigments (Osotsapa, 2009; Rapisarda et al., 2008; Rodrigo & Zacarias, 2006; Skrede & Wrolstad, 2002; Skrede et al., 1992; Timberlake, 1988).

The reason why application of zinc at any rate had no effect on the quantity of lycopene and β-Carotene in the pummelo pulp was that the two pigments are in the group, which needs geranylgeranyl pyrophosphate synthase (GGPP) in the synthesis (Lima et al., 2005). However, because the molecgules of carotenoids combine with phospholipid in thylakoid membrane of plastid, there is only a little change in citruses (Tripoli et al., 2007; Siriphanich, 2003).

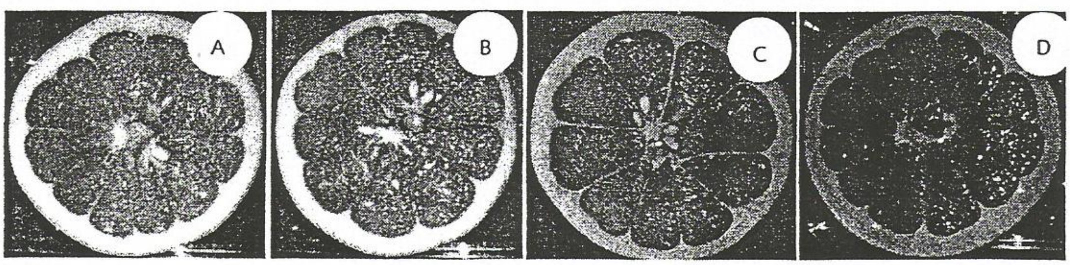

Figure 1 Pulp and peel of Tubtim Sayam pummelo after receive zinc different rate T1(A), T2(B), T3(C), and T4(D)

Table 4 Pigment in pulp of Tubtim Sayam pummelo after receive zinc different rate

treatment	chlorophyll A (mg/100 ml)	chlorophyll B (mg/100 ml)	lycopene (mg/100 ml)	β-carotene (mg/100 ml)	anthocyanin (mg/100 ml)
T1	0.01 ± 0.00^{c}	0.02 ± 0.00^{b}	2.17 ± 0.24^{a}	0.37 ± 0.01^{a}	1.13 ± 0.11^{c}
T2	0.01 ± 0.00^{bc}	0.03 ± 0.00^{a}	2.45 ± 0.00^{a}	0.41 ± 0.02^{a}	2.13 ± 0.15^{bc}
T3	0.03 ± 0.00^{a}	0.01 ± 0.00^{c}	2.48 ± 0.06^{a}	0.38 ± 0.04^{a}	4.38 ± 0.23^{b}
T4	0.02 ± 0.00^{b}	0.01 ± 0.00^{c}	2.81 ± 0.76^{a}	0.42 ± 0.02^{a}	9.58 ± 3.36^{a}
CV.	9.13	9.37	12.28	16.17	14.28

[1] Mean within column with different alphabets differ significantly at P<0.05

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6. References

Albrigo, L.G., & Young, R.H. (1981). Phloem zinc accumulation in citrus trees affected with blight. *HortScience. 16*, 158-160.

Ali, A., Perveen, S., Shah, S.N.M., Zhang, Z., Wahid, F., Shah, M., Bibi, S., & Majid, A. (2014). Effect of foliar application of micronutrients on fruit quality of peach. *American Journal of Plant Science, 5*, 1258-1264.

Alva, A.K., Mattos, D., & Quaggio, J.A. (2008). Advances in nitrogen fertigation of citrus. *Journal of Crop Improvement. 22*, 121-146.

AOAC, (2000). *Official Methods of Analysis of AOAC International :Secs. 993.13* (17 th ed). Gaithersburg, MD: AOAC International.

Arora, J. S. & Singh, J.R. (1970). Some effects of foliar spray of zinc sulphate on growth, yield and fruit quality of guava (*Psidium guajava* L.). *Japanese Society for Horticultural Science. 39*, 207-211.

Barrow, N.J. (1987). The effect of phosphate on zinc sorption by a soil. *Journal of Soil Science. 38*, 453–459.

Barros, H.R.M., Ferreira, T.A.P.C., & Genovese, M.I. (2012). Antioxidant capacity and mineral content of pulp and peel from commercial cultivars of citrus from Brazil. *Food Chemistry. 134*, 1892-1898.

Boland, F.E. (1995). Acidity (titratable) of fruit products. In (Cunniff, P. Ed), *Official Methods of Analysis of the Association Official Analytical Chemist International* (16 th ed., pp 2). Virginia: AOAC international.

Chapman, H.D. (1965).'The mineral nutrition of citrus, In *The Citrus Industry Volume2(Editer by W. Reuther, L.D. Batchelor and H.J. Webber), Revised Edition of Division of Agricultural Science*, Berkeley: University of California.

Coleman, J.E. (1992). Zinc proteins: enzymes, storage proteins, transcription factors, and replication proteins. *Annual Review of Biochemistry. 61*, 610-617.

Damrongrak, I. (2007). Nutrient concentration of nitrogen phosphorous and potassium in leaf of shogun orange. *Journal of Rajabhat Yala University. 2*, 56-71.

Dasberg, S. (1987). Nitrogen fertilization in citrus orchards. *Plant Soil. 100*, 1–9.

Dasberg, S., Bielorai, H., & Erner, J. (1983). Nitrogen fertigation of Shamouti oranges. *Plant Soil. 75*, 41–51.

Davies, F., & Albrigo, L.G. (1994). *Citrus*. Wallingford: CAB International.

Domingo, A.L., Nagamoto, Y., Tamai, M., & Takaki, H. (1992). Free tryptophan and indoleacetic acid in zinc-deficient radish shoots. *Soil Science and Plant Nutrition. 38*, 261-267.

Epstein, E. and A.J. Bloom, (2005). *Mineral Nutrient of plants: principles and Perspectives.* California : Sinauer Associate.

Giusti, M.M., & Wrolstad, R.E. (2005). Characterization and measurement of anthocyanins by UV-Visible spectroscopy, pp. 19-31. In *Handbook of Food Analytical Chemistry*. New Jersey: Wiley-Interscience Hoboken.

Hassan, H.S.A., Sarrwy, S.M.A., & Mostafa, E.A.M. (2010). Effect of foliar spraying with liquid organic fertilizer, some micronutrients and gibberellins on leaf minerals content, fruit set, yield, and fruit quality of Hollywood plum trees. *Agriculture and Biology Journal of North America. 1*, 638-643.

Johnson, A.D., & Simons, J.G. (1979). Diagnostic indices of zinc deficiency in tropical legumes. *Journal of Plant Nutrition. 1*, 123-149.

Kaewtubtim, M. (1996). *Floral biology and pollination of pummelo cv. Hom Hat Yai.* (Master's thesis, Prince of Songkla University, Thailand).

Kaewtubtim, M., & Issarakraisila, M. (2011). *Effects of nitrogen and zinc on fruit quality of pummelo cv. Tubtim Sayam*. In Commission on Higher Education Congress IV University Staff Development Consortium (Page 44), Chanburi: Commission on Higher Education.

Kathryn, C.T, Albrigo, L.G., & Christine, D.C. (1996). Purification of a Zn-binding phloem protein with sequence identity to chitin-binding proteins. *Plant Physiology. 110*, 657-664.

Kimball, D.A. (1999). *Citrus processing: a complete guide*. Gaithersburg, Maryland: Aspen Publishers.

Lee, S.H. (2002). Characterization of major anthocyanins and the color of red-fleshed Budd Blood orange. *Journal of Agricultural and Food Chemistry. 50*, 1243-1246.

Lee, S.K., & Kader, A.A. (2000). Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest Biology & Technology, 20*, 207-220.

Legaz, F., Primo-Millo, E., Primo-Yufera, E., Gil, C., & Rubio, J.L. (1982). Nitrogen fertilization in citrus I. Absorption and distribution of nitrogen in calamondin trees during flowering, fruit set and initial fruit development periods. *Plant and Soil. 66*, 339-351.

Lima, A.L.G., Krieger, N., & Bon, E.P.S. (2005). Streptomyces drozdowiczii cellulose production using agro-industrial by products and its potential use in the detergent and textile industries. *Enzyme and Microbial Technology, 37*, 272-277.

Louis, C.L., Ai. I., Salma, H., Jube B., & Madduri, V. R. (2010). Assessment of lycopene content of fresh tomato and tomato products in the United Emirates. *Journal of Food, Agricultural and Environment. 8*, 142-147.

Maneepong, S. (2013). *Nutrient management for the production of quality Pummelo*. Nakorn Si Thammarat: Walailak University.

Maneepong, S. (2009). Nutritional requirements and growth of pummelo. In The 8th National Horticultural Congress (Page 280), Chiang Mai: Maejo University.

Marschner, H. (1995). *Mineral nutrition of higher plants*. New York: Academic Press.

Maust, B.E., & Williamson, J.G. (1991). Nitrogen rate effect on growth of containerized citrus nursery plants. *HortScience*. 26, 751-752.

Mazza, G., & Miniati, E. (1993). Legumes, pp. 249-257. In *Anthocyanins in Fruits, Vegetable, and Grains*. Florida: CRC Press.

Mengel, K., Kirkby, E.A., Kosegarten, H., & Appel, T. (2001). *Principles of plant nutrition*. Dordrecht, The Netherlands: Kluwer Academic Publishers.

Moss, G.I., Steer, B.T., & Kriedemann, P.E. (1972). The regulatory role of inflorescence leaves in fruit-setting by sweet orange (*Citrus sinensis*). *Plant Physiology. 27*, 432-438.

Nagata, M., & Yamashita, I. (1992). Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruit. *Nippon Shokuhin Kogyo Gakkaishi. 39*, 925-928.

Navarro, J.M., Perez-Perez, J.G., Romero, P., & Botia, P. 2010. Analysis of the changes in mandarin fruit, produced by deficit irrigation treatments. *Food Chemistry. 119*, 1591-1596.

Nyanga, L.K., Gadaga, T.H., Nout, M.J.R., Smid, E.J., Boekhout, T., & Zwietering, M.H. (2013). Nutritive value of masau fruits from Zambezi Valley in Zimbabwe. *Food Chemistry. 138*, 168-172.

Obreza, T.A. (1990). Young 'Hamlin' orange tree fertilizer response in southwest Florida. *Proceedings of the Florida State Horticultural Society.* 103,12–16.

Ortuno, A., Reynaodo, I., Fuster, M.D., Botia, J., Puig, D.G., Sabater, F., Lidon, A.G., Porras, I., & Rio, J.A.D. (1997). Citrus cultivars with high flavonoid contents in the fruits. *Scientia Horticulturae. 68*, 231-236.

Osotsapa, Y. (2000). *Plant Nutrient*. Bangkok: Kasetsart University.

Pendials, A.K. (1992). *Trace element in soil and plant*. Boca Raton: CRC Press.

Prask, J.A., & Plocks, D.J. (1971). A role of zinc in the structural integrity of the cytoplasmic ribosomes of *Euglena gracilis*. *Plant Physiology. 48*, 150-155.

Promotion and development, (2009). *Planting and maintainace of pummel cv. Tubtim Sayam.* Nakhon Si Thammarat province agricultural office. Department of Agriculture, Ministry of Agriculture and Cooperatives.

Quaggio, J.A., Mattos, J.D., Cantarella, H., Almeida, E.L.E., & Cardoso, S.A.B. (2000). Lemon Yield Fruit Quality Affected by NPK Fertilization. *Scientia Horticulturae. 96*, 151-162.

Rapisarda, P.L., Bianco, M., Pannuzzo, P., & Timpanaro, N. (2008). Effect of cold storage on vitamin C, phenolics and antioxidant activity of five orange genotypes. *Postharvest Biology and Technology. 49*, 348-354.

Razeto, B., & Salas, A. (1986). Magnesium, manganese and zinc sprays on orange trees. *Foliar Fertilization Developments in Plant and Soil Sciences. 22*, 255-270.

Rodrigo, M.J., & Zacarias, L. (2006). Effect of postharvest ethylene treatment on carotenoid accumulation and expression of carotenoid biosynthetic genes in the flavedo of orange fruit. *Postharvest Biology and Technology. 43*, 14-22.

Sahota, G.S., & Arora, J.S. (1981). Effect of N and Zn on 'Hamlin' sweet orange (*Citrus sinensis* Osbeck) *Journal Japan Society Horticultural Science. 50*, 281-286.

Salisbury, F.B., & Ross, C.W. (1992). *Plant physiology*. 4th ed. Belmont: Wadsworth Publishing.

Salunkhe, D.K., Bolin, H.R., & Reddy, N.R. (1991). *Storage, processing, and nutritional quality of fruits and vegetable. Volume I. Fresh fruits and vegetables.* American : CRC Press.

Sethpakdee, R. (2001). Physiology and physiological disorders in citrus. In *Science Training Orange: Current options for the future*. (Page 3/1-3/21). Bangkok: Office of Extension and Training of Kasetsart University.

Sharma, R.R., Singh, R., & Saxena, S.K. (2006). Characteristics of citrus fruit in relation to granulation. *Scientia Horticulturae. 111*, 91-96.

Siriphanich, J. (2003). *Physiology and postharvest technology of fruits and vegetables*. Bangkok : Kasetsart University.

Skoog, F. (1940). Relationship between zinc and auxin in the growth of higher plant. *American Journal of Botany. 27*, 939-951.

Skrede, G., & Wrolstad, R.E. (2002). Flavanoids from berries and grapes. In *Functional Foods V.2.* Boca Ration: CRC press.

Skrede, G., Wrolstad, R.E., Lea, P., & Enersen, G. (1992). Color stability of strawberry and blackcurrant syrups. *Journal of Food Science. 57*, 172-177.

Smith, M.W., Storey, J.B., & Westfall, P.N. (1979). The influence of two methods of foliar application zinc and adjuvant solution on leatlet zinc concentration in pecan trees. *HortScience. 14*, 718-719.

Srivastava, A.K., & Singh, S. (2005). Zinc nutrition, a global concern for sustainable citrus production. *Journal of Sustainable Agriculture. 25*, 5-42.

Storey, R., & Treeby, M.T. (2000). Seasonal changes in nutrient concentrations of Navel orange fruit. *Scientia Horticulturae. 84*, 67-82.

Tariq, M., Sharif, M., Shah, Z., & Khan, R. (2007). Effect of foliar application of micronutrients on the yield and quality of sweet orange. *Pakistan Journal of Biological Sciences. 10*, 1823-1828.

Timberlake, C.F. (1988). The biological properties of anthocyanin compounds. *NATCOL Quarterly Bulletin*. *1*, 4-15.

Tripoli, E., Guardia, M.L., Giammaco, S., Majo, D.D., & Giammaco, M. (2007). Citrus Flavonoids: Molecular structure, biological activity and nutritional properties. *Food Chemistry*. *104*, 466-479.

Tsui, C. (1948). The role of zinc in auxin synthesis in the tomato plant. *American Journal of Botany*. *35*, 172-179.

Wongchana, B. (2010). *Effects of nitrogen and zinc on growth, photosynthesis, yield and quality of pummelo cv. Kao-thongdee*. (Ph.D. Thesis, Walailak University, Thailand).

Zhang, Q., & Brown, P.H. (1999). The mechanism of foliar Zinc absorption in Pistachio and Walnut. *Journal of the American Society for Horticultural Science*. 124, 312-317.