

BIOACTIVE COMPOUNDS AND ANTIOXIDANT CAPACITY OF PINK PUMMELO (*CITRUS GRANDIS* (L.) OSBECK) CV. "THONG DEE" IN THAILAND

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ABSTRACT

The pink pummelos cv. "Thong Dee" (*Citrus grandis* (L.) Osbeck) at 80% maturity were harvested from Chiang Rai (CR), Nakhon Pathom (NP), Nakhon Nayok (NY), Prachin Buri (PB), and Nakhon Si Thammarat (NST) provinces for consuming in domestic market. The contents of vitamin C, total phenolic compounds and seven flavonoids were determined. The total antioxidant capacities were also determined by both the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and ferric reducing antioxidant power (FRAP) assay. The highest contents of vitamin C (74.63 mg/100 ml) and total phenolic compounds (94.93 mgGAE/100g fresh weight) were found in pummelos from NST and CR, respectively. Naringin was the major flavonoid compounds in pummelo and had the highest content in sample from NST (71.90 mg/100g fresh weight). The highest antioxidant capacities (DPPH-assay) were found in pummelo from NST and CR whereas there was no significant differences among samples in FRAP-assay. Overall, pummelo from NP had the lowest vitamin C (32.94 mg/100 ml), total phenolic compounds (54.87 mgGAE/100g fresh weight), and antioxidant capacity (DPPH-assay) (164.23 µmolTrolox/100g fresh weight).

Key words: Naringin, total phenolic compounds, vitamin C

INTRODUCTION

Natural antioxidant compounds are present in all parts of all higher plants. They have been found to protect against a variety of disorders, particularly cardiovascular diseases (Kurowska *et al.*, 2000) and some types of cancer (Vanamla *et al.*, 2006). Citrus fruits are important because of their nutritional and antioxidant properties. Beside ascorbic acid, citrus also contains flavonone glycosides, such as naringin which is the most important phenols in the water soluble fraction (Gil-Izquierdo *et al.*, 2001). Citrus flavonoid properties have been found to include anticancer, antiviral, antiinflammatory activities, effect on capillary fragility, and an ability to inhibit human platelet aggregation (Benavete-Garcia *et al.*, 1997).

One of the most important citrus fruits in Thailand is a pummelo (*Citrus grandis* (L.) Osbeck). Among pummelo cultivars grown in Thailand, the pummelo cv. "Thong Dee" has the highest yield (a half of total pummelo production). It is also widely consumed in Thailand. The main production areas include Chiang Rai (CR), Nakhon Pathom (NP), Nakhon Nayok (NY), Prachin Buri (PB), and Nakhon Si Thammarat (NST) provinces (Department of Agricultural Extension Thailand, 2008). "Thong Dee" cultivar is of the Thai group of pummelos. The fruit is very large and broadly obovoid to oblate, with a slightly depressed apex. The smooth light yellow rind is medium-thin and tightly adherent. The flesh is tinged pink and the flavor is good when grown under appropriate climatic

conditions. The pulp is juicy and easily separable from the membrane walls (Hodgson, 1967).

In recent years, the pummelo has received much attention because of its nutritional and antioxidant properties, especially flavonoids in Asia i.e. Thailand, China, Taiwan, and Japan (Wattanasiritham *et al.*, 2005; Xu *et al.*, 2008; Wang *et al.*, 2007 and Nagata *et al.*, 2006). In previous study, Wattanasiritham *et al.* (2005) reported that the naringin content of Thai pummelo was 26-58 mg/100g which varied on cultivar and cultivation area. Furthermore, the naringin content of “Thong Dee” cultivar from Nakhon Pathom province was 26.11 mg/100g. Generally, the quality and bioactive compounds of fruits and vegetables depend on environmental factors, production areas, and cultural practices (Vicente *et al.*, 2009). However, there has been no detailed research on bioactive compounds and antioxidant capacity of pummelo flesh cv. “Thong Dee” obtained from all cultivar regions in Thailand. Thus, the main objective of this study was to evaluate chemical properties, bioactive compounds and antioxidant capacities of the pummelos cv. “Thong Dee” with local grade (80% maturity) in Thailand.

MATERIALS AND METHODS

Sample collection

Pummelos cv. “Thong Dee” at an optimum maturity (80%) were harvested from 5 major cultivation areas in Thailand during September 2008. The collected provinces were Chiang Rai (CR); the northern part, Nakhon Pathom (NP) and Nakhon Nayok (NY); the middle part, Prachin Buri (PB); the eastern part, and Nakhon Si Thammarat (NST); the southern part. The pummelos were harvested from four orchards in each province and transported to the laboratory within 3 days after harvesting. Fruits were peeled and flesh samples were frozen at -20°C until analysis.

Reagents and flavonoid standards

6-hydroxy-2, 5, 7, 8-tetramethyl-2-carboxylic acid (Trolox), 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), ascorbic acid (AA) and Folin-Ciocalteu phenol reagent were purchased from Sigma-Aldrich (St. Louis, MO, USA). Naringin (naringenine-7-rhamnosidoglucoside, NAR) and hesperidin (hesperetin-7-rutinoside, HES) were purchased from ACROS (Geel, Belgium). Neohesperidin (hesperetin 7-neohesperidoside, NEH), kaempferol (3,5,7,4'-tetrahydroxyflavone, KAP), rutin trihydrate (RUT), apigenin (5,7,4'-trihydroxyflavone, APG) and quercetin dihydrate (3,5,7,3',4'-pentahydroxyflavone dehydrate, QUE) were purchased from Sigma-Aldrich (Missouri, USA). HPLC grade dimethyl formamide (DMF) and acetonitrile (ACN) were purchased from Fluka. HPLC grade methanol and TFA (trifluoroacetic acid), were supplied by Carlo Erba (Rodano, Italy). All other chemicals used were analytical grade.

Sample preparation

The juice sac (15 g) from each orchard was extracted and homogenized with 60 ml of distilled water for 5 min. The mixture was centrifuged (5,000 g) at 4°C for 15 min. The supernatants were diluted with distilled water at 10, 1 and 5 fold for total phenolic compounds analysis, DPPH-assay and FRAP-assay, respectively.

Determination of quality parameters

The pummelo flesh was determined with color measurement spectrophotometer (CIE LAB/color Quest XE, USA). L*, a*, b* CIE Chromaticity values were recorded by three replicates in each fruit. The flesh was squeezed by hand and the juice was filtered to remove pulp and seed. The

clarify juice was measured the total soluble solids (TSS) with a hand refractometer (Atago, Japan). Titratable acidity (TA) was characterized by titration of pummelo juice with 0.1 M sodium hydroxide and expressed as citric acid (% w/w).

Determination of vitamin C and total phenolic compounds

Ascorbic acid was determined by visual titration, using 2, 6-dichlorophenolindophenol (Rapisarda, 1996) and reported as mg ascorbic acid per 100 ml juices. The total phenolic compound was measured using Folin-Ciocalteu's reagent (Singleton and Rossi, 1965). The result was reported as milligram gallic acid equivalent (GAE) per 100 g flesh.

Determination of flavonoids

Flavonoids in fresh pulp samples were analyzed by the method of Mouly *et al.* (1998), with some modifications. The juice sac (10 g) from each orchard was homogenized (8,500 g for 1.5 min) with 20 ml of DMF and centrifuged at 8,500 g for 15min. The supernatant was filtered through syringe filter (0.45 µm, PTFE) and then injected into the high-performance liquid chromatography (HPLC) system by an auto-sampler. The flavonoids of HPLC analyses were carried out on an Alliance 2965 separations module (Waters) linked simultaneously to a photodiode array detector (PDA) 2996 (Waters). Separation of flavonoid compounds was performed using a stainless-steel column (250 x 4.6mm I.D.) packed with C18 Altima, 5 µm (Alltech, USA), equipped with a pre-column (7.5 x 4.6mm I.D.) A binary solvent system of ACN and water with 4% acetic acid was operated to start at 0% and end at 70% ACN concentration for a 70 min period. The flavonoid peaks were detected at 280 nm and were identified by matching spectra and retention times with commercial standards.

Determination of antioxidant capacities

The antioxidant capacities were evaluated by DPPH radical scavenging activity (Yen and Duh, 1994) and ferric reducing power activity (Yen and Chen, 1995). The DPPH-assay was expressed as micromole equivalents of Trolox (TE) per 100 grams of pummelo (fresh weight basis). The FRAP-assay was reported as micromole equivalents of ascorbic acid per 100 grams of sample (fresh weight basis).

Statistical analysis

The data were subjected to statistical analysis using the SAS® system for window (version 8.1). The average data of four replicates were presented with standard errors of means. Significant differences between production areas were tested by the general liner model (GLM). The means of pummelo samples were compared by Duncan Multiple Range Test (DMRT) at the 5% level of significance.

RESULTS AND DISCUSSION

The pummelos cv. "Thong Dee" were harvested at 80% maturity for the domestic market from Chiang Rai (CR), Nakhon Pathom (NP), Nakhon Nayok (NY), Prachin Buri (PB), and Nakhon Si Thammarat (NST) provinces. The quality parameters including TA, TSS, TSS/TA ratio, color values and some chemical compositions are shown in Table 1. The TA and TSS varied slightly among provinces ranging from 0.44-0.68% and 9.20-11.55%, respectively. The TSS/TA ratios varied significantly which the highest value was found in NP and the lowest value was found in CR province. The samples from NST province obviously showed the highest vitamin C content (74.63

mg/100ml) whereas the samples from CR showed the significantly greatest value (94.93 mgGAE/100g fresh weight) of the total phenolic content than those from NP, NY, PB and NST provinces.

The antioxidant capacities of local grade pummelos were evaluated using DPPH-radical scavenging assay (DPPH-assay) and ferric reducing antioxidant power assay (FRAP-assay). The ranges were from 164.23-257.02 $\mu\text{molTrolox}/100\text{g}$ fresh weight and 209.78-278.99 μmol ascorbic acid/100g fresh weight from DPPH-assay and FRAP-assay, respectively (Table 2). The sample collected from NP province which had the lowest DPPH-assay (164.23 $\mu\text{molTrolox}/100\text{g}$ fresh weight). The antioxidant capacity (FRAP- assay) of all sample were not significantly differences.

Table 1. Some physical and chemical compositions of the local grade pummelo cv. “Thong Dee”.

Analysis	Chiang Rai	Nakhon Pathom	Nakhon Nayok	Prachin Buri	Nokhon Si Thammarat
Titrateable acidity (%)	0.68 \pm 0.09 a	0.44 \pm 0.02c	0.53 \pm 0.04b	0.50 \pm 0.03bc	0.51 \pm 0.02bc
TSS (%)	9.20 \pm 0.28 c	11.55 \pm 0.34a	10.10 \pm 0.90b	10.67 \pm 0.65ab	10.55 \pm 0.52b
TSS:TA ratio	16.62 \pm 1.86 d	26.12 \pm 1.38a	18.87 \pm 1.23c	21.29 \pm 1.15b	20.55 \pm 1.01bc
L*	42.90 \pm 2.24a	21.94 \pm 1.29d	36.70 \pm 0.76b	33.92 \pm 1.49c	38.03 \pm 1.17b
a*	0.75 \pm 1.26c	7.15 \pm 0.47a	2.74 \pm 1.31b	1.54 \pm 0.50bc	1.84 \pm 0.76bc
b*	11.34 \pm 1.98c	33.84 \pm 3.98a	10.77 \pm 2.96c	18.11 \pm 3.42b	19.08 \pm 1.26b
Vitamin C (mg/100 ml)	53.43 \pm 6.50b	32.94 \pm 1.73c	49.50 \pm 4.23b	50.77 \pm 6.02b	74.63 \pm 2.20a
Total phenolic compounds (mgGAE/100g)	94.93 \pm 6.48a	54.87 \pm 12.62b	62.03 \pm 12.30b	51.93 \pm 17.07b	65.62 \pm 5.90b

¹ Different letters in the same row for DMRT test indicate significant differences (p \leq 0.05)

² Mean \pm SE obtain from analysis of four replicates (orchards)

Table 2. Total antioxidant capacity of the local grade pummelo cv. ‘Thong Dee’ ^{1,2}

Analysis	Chiang Rai	Nakhon Pathom	Nakhon Nayok	Prachin Buri	Nokhon Si Thammarat
DPPH-assay ³	257.02 \pm 19.84 a	164.23 \pm 12.41 b	213.42 \pm 21.99 ab	214.70 \pm 26.37 ab	250.91 \pm 22.08 a
FRAP-assay ⁴	235.58 \pm 19.69	209.78 \pm 24.78	268.78 \pm 40.02	243.45 \pm 41.34	278.99 \pm 14.95

¹ Different letters in the same row for DMRT test indicate significant differences (p \leq 0.05)

² Mean \pm SE obtain from analysis of four replicates (orchards)

³ Total antioxidant capacity (DPPH) expressed as μmol of Trolox (TE) per 100 gram of fresh weight

⁴ Total antioxidant capacity (FRAP) expressed as μmol of Ascorbic acid (AA) per 100 gram of fresh weight

Seven flavonoid compounds mainly found in pummelo flesh cv. “Thong Dee” were determined. Standards and typical chromatograms obtained from pummelo flesh are shown in Fig. 1a and b. Six flavonoids including hesperidin, neohesperidin, kaempferol, rutin, apigenin and quercetin were not detectable. Only naringin was found and showed the predominant content. The retention time (32.9 min) of naringin peak and spectra (λ max 233, 283, 328 nm) in all samples were similar to naringin standard. Interestingly, there were unknown peaks which cannot be identified. It might be

other flavonoids and/or bioactive compounds in pummelo flesh cv. “Thong Dee”. In this study, the highest naringin content was found in pummelo from NST (71.90 mg/100g) and the naringin contents in pummelo flesh cv. “Thong Dee” collected from other areas (CR, NP, NY and PB provinces) ranged from 25.37-38.77 mg/100g (Table 3).

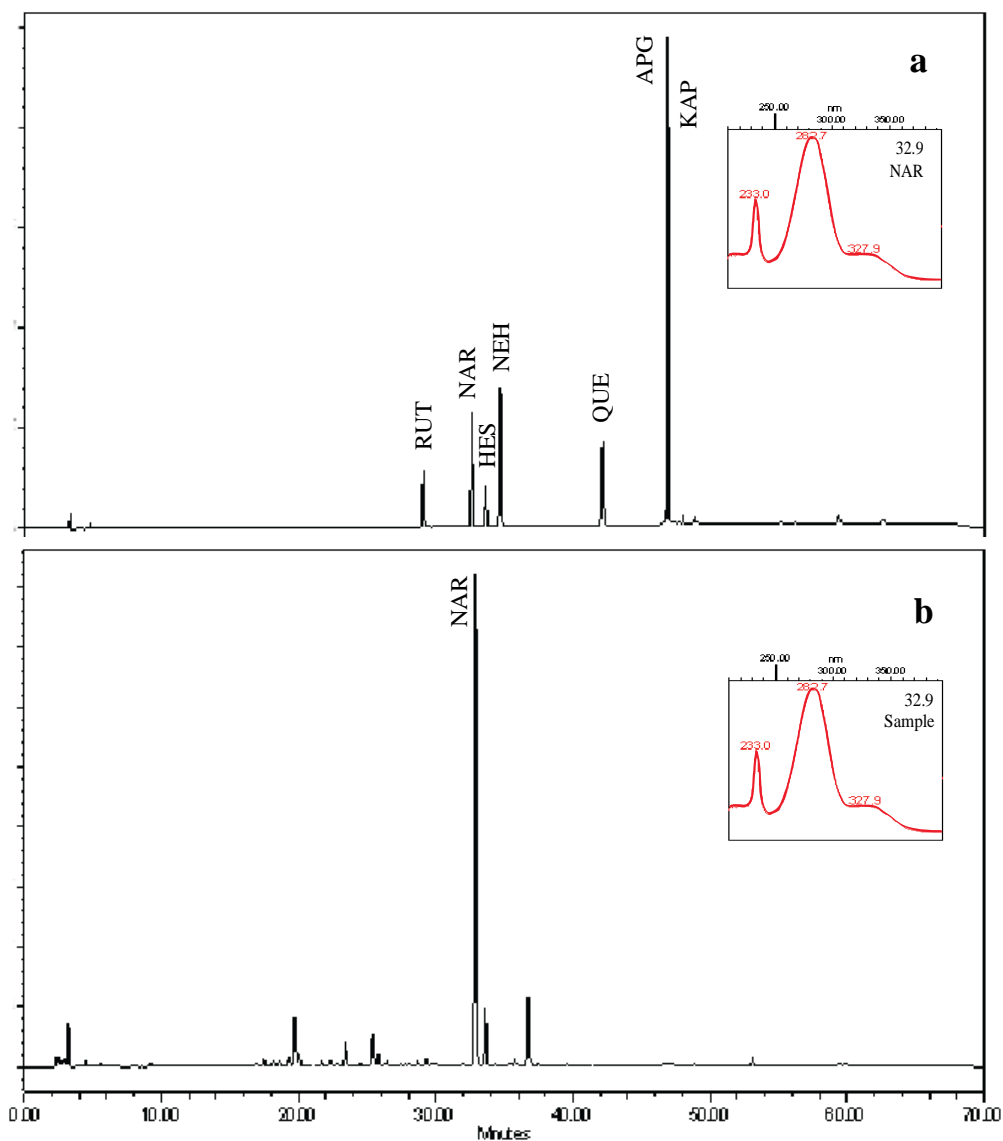


Fig. 1. HPLC chromatograms of flavonoid standards (a), RUT (20.6 μ g/ml), NAR (15.2 μ g/ml), HES(19.4 μ g/ml), NEH (15.2 μ g/ml), QUE (15.6 μ g/ml), APG (17.8 μ g/ml) and KAP (16.9 μ g/ml) and typical local grade pummelo flesh cv. “Thong Dee” (b).

Table 3 Naringin content (mg/100g) of the local grade pummelo cv. ‘Thong Dee’^{1,2}

Analysis	Chiang Rai	Nakhon Pathom	Nakhon Nayok	Prachin Buri	Nokhon Si Thammarat
Naringin	32.36±11.52b	32.20 ±5.36b	38.77 ±12.37b	25.37 ±7.43b	71.90 ±10.90a

¹ Different letters in the same row for DMRT test indicate significant differences ($p \leq 0.05$)

² Mean ± SE obtain from analysis of four replicates (orchards)

The flavonoid content in grapefruit from China, was found to be approximately 118.83 mg total flavonoids/100g. Naringin are the main component (62.43 mg/100g) followed by small amounts of neohesperidin, narirutin and hesperidin, respectively (Xu *et al.*, 2009). In Thailand, it has been reported that the major flavonoid found in pummelo cv. ‘Thong Dee’ flesh was naringin (26.11 mg/100g) but no maturity detail was indicated (Wattanasiritham *et al.*, 2005). This result could confirm our work that only naringin found in pummelo cv. ‘Thong Dee’ flesh and the naringin content was slightly higher than those previously reported in pummelo but lower than grapefruit from China, except the pummelo cultivated in Nokhon Si Thammarat province. This indicates that there was a variation in naringin content in the local grade pummelo cv. ‘Thong Dee’ grown in local cultivation areas. Some factors such as environment, production areas, cultivations, and cultural practices might cause the divergence. Therefore, pummelo cv. ‘Thong Dee’ from Nokhon Si Thammarat province may be considered as an excellent source of phytochemicals with have potential health benefits.

CONCLUSION

In this study, bioactive compounds and antioxidant capacities in pummelo cv. ‘Thong Dee’ from five major cultivation areas, namely Chiang Rai, Nakhon Pathom, Nakhon Nayok, Prachin Buri, and Nokhon Si Thammarat provinces, in Thailand were examined. Variations in chemical content were observed. Pummelos from Nokhon Si Thammarat and Chiang Rai provinces had the highest vitamin C and total phenolic compound content, respectively. Naringin is the most abundant flavonoid found in pummelo flesh, especially pummelo from Nokhon Si Thammarat province. However, pummelo from Nakhon Pathom province had the lowest vitamin C and total phenolic compound content, and DPPH antioxidant capacity.

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