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Total Polyphenol Content and Antioxidant Properties in Different Tissues of Seven Pomelo (*Citrus grandis* (L.) Osbeck) Cultivars

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ABSTRACT

Pomelo (*citrus grandis* (L.) Osbeck) is one of the popular Thai fruits, which is well known for having high antioxidant properties. The total polyphenol content and antioxidant properties (1,1 diphenyl-1-picrylhydrazyl, DPPH) and ferric reducing antioxidant power (FRAP)) were determined in seven of the many pomelo cultivars growing in Thailand—namely, Kao Numpueng (KNP), Thong Dee (TD), Kao Paen (KP), Kao Yai (KY), Tha Knoi (TK), Pattavee (PV) and Kao Tanggwa (KTG). Different parts of the fruit tissue consisting of flavedo, albedo, segment membranes and seeds, were tested. The results indicated that the total polyphenol content was highest in the seeds in all cultivars (3,108.78–4,957.97 $\mu g.g^{-1}$), while for the other parts, the ranking in decreasing order was albedo (1,176.58–3,384.81 $\mu g.g^{-1}$), flavedo, (1,096.27–2,163.63 $\mu g.g^{-1}$) and segment membranes (825.68–2,266.66 $\mu g.g^{-1}$), respectively. The antioxidant properties (DPPH) were highest in the seeds, while for the other parts, the ranking in decreasing order was albedo, flavedo and segment membranes. By cultivar, Thong Dee (TD) produced the highest total polyphenol content and antioxidant properties (DPPH and FRAP) in the flavedo and seeds. Tha Knoi (TK) had the highest total polyphenol content and antioxidant properties (DPPH and FRAP) in the albedo and segment membranes. Kao Tanggwa (KTG) had the highest antioxidant properties (DPPH) in the seeds. A linear relationship between the total polyphenol content and the DPPH has a coefficient of determination (R^2) of 0.702, and for FRAP the R^2 was 0.659. Thus, edible tissues of pomelo could be a source of bioactive compounds which is high in antioxidant properties and suitable for industrial processing.

Keywords: pomelo, antioxidant properties, edible tissues, citrus fruit

INTRODUCTION

Pomelo (*Citrus grandis* (L.) Osbeck) is the largest citrus fruit (Hodgson, 1967). Many cultivars are grown in Thailand and can be divided into two groups according to whether they are colored white or pink, with Tong Dee and Tha Knoi being in the pink group while the white group includes Kao Yai, Kao Paen, Kao Nampheung, Kao Tanggwa, Kao Hom, Kao Phuang and Pattavee (Pichaiyongvongdee and Haruenkit, 2009). Citrus fruits have been recognized as a good source of health-promoting compounds including carotenoids, flavonoids, linonoids and

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fiber (Yu *et al*., 2005). Citrus fruit extracts are also found to have active anti-inflammatory, anti-tumor, anti-fungal and blood clot inhibition properties (Middleton and Kandaswami, 1994; Yehoshua *et al.,* 1995). The health benefits of citrus fruit have been attributed mainly to the presence of antioxidant activity; moreover, the total polyphenol content was reported to be the major antioxidant of citrus fruits (Rapisarda *et al*., 1999; Sun *et al.,* 2002). According to Abeysinghe *et al.* (2007), there are effects associated with different edible tissue parts such as the segment membrane, juice sacs and segment. They found the segment membrane contained a higher amount of antioxidant than the juice sacs and segment. Gorinstein *et al*. (2001) reported that the peel of citrus fruit had a good total radical antioxidant potential (ferric reducing antioxidant power, FRAP) and similar results were reported by Kamran *et al.* (2009). The evaluation of the antioxidant capacity of food, fruit and vegetables has received much attention due to the potential synergistic action of the bioactive compounds found in them (Zulueta *et al.,* 2007). Health-promoting compounds including flavonoids and linonoids in pomelo were reported (Pichaiyongvongdee and Haruenkit, 2009).

However the contents of polyphenol and antioxidant potential of different Thai pomelo cultivar have not been reported. The objectives of this study were to determine the total polyphenol content and antioxidant properties in different tissues collected from seven Thai pomelo cultivars. The results are aimed at promoting the pomelo industry worldwide, to provide information of the health-promoting compounds found in Thai pomelo fruits, and to present high levels of citrus as a major source of antioxidant in the diet.

MATERIALS AND METHODS

Materials

Samples of the seven pomelo cultivars (*Citrus grandis* (L.) Osbeck) were collected from orchards in five provinces. They were harvested at commercial maturity stage (8 mth). The cultivars were Thong Dee (TD), Kao Paen (KP) and Kao Nampheung (KNP) from Nakhon Pathom province; Kao Yai (KY) from Samut Songkhram province; Kao Tanggwa (KTG) from Chainat province; Pattavee (PV) from Nakhon Si Thammarat province and Tha Khoi (TK) from Phichit province.

Chemicals

Standard 2, 2-diphenyl-1-1-picrylhydrazl (DPPH), trolox and gallic acid monohydrated were purchased from Sigma-Aldrich Chemical Co., St Louis MO, USA. Other common reagents were of analytical grade.

Preparation of samples

The whole fruits were weighed and divided into four different parts—flavedo, albedo, segment membranes and seeds. First, each tissue part was separately homogeneously blended in a blender (Model A 327 R7; Moulinex; France) and then dried at -40 °C in a freeze dryer (model LyoPro 3000; Heto; Allerød, Denmark.) for 12–15 hr (to a moisture content less than 10%). Next, they were ground in a mill and then the milled fiber was separated using sieves for particle size analysis. The separated particles were smaller than 150 µm (mesh 100), vacuum packed and kept in a freezer at -20 °C for further analysis.

Preparation of extract for determination of total polyphenol content and antioxidant activity assays

Each tissue was defatted using Soxtech (Soxtec 2050; Hoganas, Sweden) prior to the analysis of antioxidant activity as described by Reddy *et al.* (2005). A sample (2g) was extracted with 20 mL of 95% ethanol (volume per volume, v/v), refluxed for 30 min at 80 °C and then the extract was filtered through Whatman No.4 filter paper and the filtered extract was stored in an amber-colored bottle at-4 °C for further analysis (Pinsirodom and Changnoi, 2002).

Determination of total polyphenol content

The total polyphenol content in the tissue of pomelo extract was determined using the Folin-Ciocalteu method (Singleton *et al*., 1999). The sample (0.5 ml) was added to 2 mL of 10% Folin-Ciocalteu reagent. After 5 min, 5% Na_2CO_3 reagent (1.6 mL) was added to the mixture and allowed to stand for 30 min before measurement. The absorbance was measured at 760 nm using an ultraviolet-visible spectrophotometer (UV-1601; Shimadzu Corp.; Tokyo, Japan). The total polyphenol was expressed as milligrams of gallic acid equivalent per 100 milligrams fresh weight (GAE per 100 mg FW), which is a common standard compound, and by reference to a standard curve (y = 0.007x; correlation coefficient, R^2 = 0.999).

Determination of antioxidant properties using a free radical scavenging assay (1,1 diphenyl-1-picrylhydrazyl)

The free radical scavenging DPPH method was used according to Shyu and Hwang (2002). An amount of 0.6 mL of 0.8 mM solution of DPPH, followed by 0.1 mL of the sample was adjusted to 6 mL final volume using ethanol. The absorbance was read at 517 nm after 30 min of initial mixing. The same concentration of methanol (6 mL) was used as the control. The inhibitory percentage of DPPH was calculated using Equation 1:

$$\% \text{ inhibition} = [A0–A1/A0] \times 100 \quad (1)$$

where A0 is the absorbance of the control and A1 is the absorbance in the presence of the sample.

Ferric reducing antioxidant power assay

The FRAP assay method was according to Benzie and Strain (1999). The FRAP reagent was composed of 0.1 M acetate buffer (pH 3.6), 40 mM TPTZ and 20 mM ferric chloride at the ratio of 10:1:1 by volume. The 0.1 mL of sample was added to 3 mL reagent, the absorbance was read at 593 nm and the reaction was monitored for 8 min. The result was expressed as milligrams of trolox equivalent per 100 milliliters fresh weight (TE per 100 mL FW) by reference to a standard curve for trolox (y = 0.0318x; R^2 = 0.998).

Statistical analysis

All experiments were performed in duplicate and the mean values with standard deviations were recorded. The statistical software program SPSS (version 16, SPSS Inc.; Chicago, IL, USA) was used to perform all statistical calculations.

RESULTS AND DISCUSSION

Distribution of total polyphenol content in pomelo tissues

The total polyphenol content of the different parts of pomelo tissue (flavedo, albedo, segment membranes and seeds) were evaluated on a dry weight basis. The results indicated that the total polyphenol content was highest in the seeds in all cultivars (3,108.78–4,957.97 $\mu g.g^{-1}$) and was the greatest in TD followed by KP, KNP, PV and KTG in decreasing amounts. For the other parts, the ranking in decreasing order was: albedo (1,176.58–3,384.81 $\mu g.g^{-1}$) and was the greatest in TK followed by TD, PV, KNP, KY, KTG and KP; flavedo (1,096.27–2,163.63 $\mu g.g^{-1}$) and was the greatest in TD followed by TK, KNP, KTG, KY, PV and KP; segment membranes (825.68–2,266.66 $\mu g.g^{-1}$) and it was the greatest in TK followed by TD, KTG, PV, KP, KY and KNP, respectively. (Figure 1 and Table 1). The findings of Abeysinghe *et al*. (2007) provided similar results for the distribution of the total polyphenol content and antioxidant capacity in different edible tissues of mandarin being greater in the segment membrane than in the segment. Wongpaisanrit (2006) reported that the extracts from tangerine seeds potentially showed greater antioxidant properties than the peel extracts.

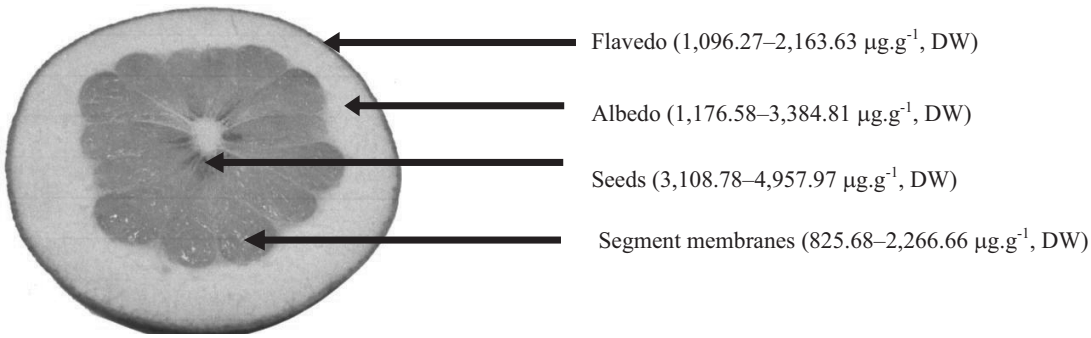

Figure 1 Cross section of pomelo showing distribution of total polyphenol content on a dry weight (DW) basis.

Table 1 Content of the total polyphenol and antioxidant properties of different pomelo fruit tissues from seven cultivars. Each value is mean±SD.

Cultivar	Fruit tissue	Total polyphenol ($\mu g.g^{-1}$, DW)	Antioxidant properties (DW)	
			DPPH(%)	FRAP(mg TE per 100mL)
Thong Dee (TD)	Flavedo	2,163.63±180.66	70.14± 2.48	1,227.13±15.95
	Albedo	2,718.48±167.66	66.07±1.73	1,567.78±11.97
	Segment membranes	1,825.06±86.610	42.96±5.42	837.46±5.64
	Seeds	4,957.97±121.28	80.92±0.66	2,431.31±18.14
Tha Knoi (TK)	Flavedo	2,056.16±195.90	61.28±2.43	1098.07± 9.18
	Albedo	3,384.81±155.91	69.18±2.63	2933.50±22.05
	Segment membranes	2,266.66±216.40	45.02±3.25	1354.79±10.08
	Seeds	ND	ND	ND
Kao Nampheung (KNP)	Flavedo	2,014.08±153.68	56.42±6.05	1107.40±8.65
	Albedo	1,416.43±101.02	63.02±3.16	562.69±7.01
	Segment membranes	825.68±84.06	38.36±2.19	294.89±7.26
	Seeds	4,195.74±434.96	81.34±0.47	1,951.82±19.74
Kao Yai (KY)	Flavedo	1,854.42±130.65	54.41±4.59	786.93±5.23
	Albedo	1,362.60±98.84	61.23±3.49	392.39±5.12
	Segment membranes	1,030.41±93.180	40.12±1.79	655.77± 6.01
	Seeds	ND	ND	ND
Kao Tanggwa (KTG)	Flavedo	1,895.75±251.92	53.54±5.54	565.40±6.19
	Albedo	1,209.08±97.780	62.26±2.23	385.72±4.61
	Segment membranes	1,144.97±25.350	41.33±3.32	366.39±5.40
	Seeds	3,108.78±284.91	81.56±1.32	924.23±5.20
Pattavee (PV)	Flavedo	1,581.04±179.32	47.83±2.99	285.11±3.50
	Albedo	2,005.10±232.18	63.61±3.06	1,503.83±15.04
	Segment membranes	1,100.05±80.440	40.49±3.24	630.97±7.68
	Seeds	3,114.05±65.240	79.97±0.62	928.75±5.09
Kao Paen (KP)	Flavedo	1,096.27±66.780	47.03±2.98	350.81±3.81
	Albedo	1,176.58±62.880	63.86±6.50	185.10±3.06
	Segment membranes	1,058.14±46.390	36.24±2.57	304.72±3.96
	Seeds	4,584.51±873.41	85.34±1.15	1,453.63±10.33

DW = Dry weight; DPPH = 1,1 Diphenyl-1-picrylhydrazyl; FRAP = Ferric reducing antioxidant power; ND = Not detected.

Distribution of antioxidant properties in pomelo tissues

The antioxidant properties in fruit can be measured by many methods according to the type of antioxidation, with at least two methods being DPPH and FRAP (Shyu and Hwang, 2002; Benzie and Strain, 1999). The DPPH assay measures the radical scavenging activity expressed as an inhibitory percentage. The FRAP assay can evaluate antioxidant activities in a relatively short time compared with other methods (Benzie and Strain, 1999). Reduction of the ferric tripyridyl triazine (Fe^{+3}) complex to the ferrous form (Fe^{+2}) which has an intense blue color occurs at a low pH and can be monitored by measuring the change in absorption at 593 nm. (Benzie and Strain, 1999). Each fruit part (flavedo, albedo, segment membranes and seeds) was analyzed for the total polyphenol content, DPPH and FRAP as shown in Table 1.

The antioxidant properties of different parts of pomelo tissue (flavedo, albedo, segment membranes and seeds) were evaluated. The results indicated that the antioxidant properties measured by DPPH assay were highest in the seeds in all cultivars (79.97–85.34%) with the most in KP followed by KTG, KNP, TD and PV. For the other parts, the ranking in decreasing order was: albedo (61.23–69.18%) with the most in TK followed by TD, KP, PV, KNP, KTG and KY; flavedo (47.03–70.14%) with the most in TD followed by TK, KNP, KY, KTG, PV and KP;. segment membranes (36.24–45.02%) with the most in TK followed by TD, KTG, PV, KY, KNP and KP, respectively. Cuddadarangavvanahally *et al.* (2008) reported that antioxidants are believed to intercept the free chain of oxidation and to donate hydrogen from the phenolic hydroxyl groups, thereby forming a stable end product, which does not initiate. The data obtained revealed that the fractions isolated from citrus fruit are free radical scavengers and primary antioxidants that react with the DPPH radical, which may be attributed to its proton donating ability. On the other hand, Jayaprakasha *et al.* (2007) reported that the antioxidant activity of the citrus fractions was ascribed to their hydrogen donating ability.

The antioxidant properties measured by FRAP were highest in the seeds in all cultivars (924.23–2,431.31 mg TE per 100 mL) with the most in TD followed by the KNP KP, PV and KTG. For the other parts, the ranking in decreasing order was: albedo (185.10–2,933.50 mg TE per 100 mL) with the most in TK followed by TD, PV, KNP, KY, KTG and KP; segment membranes (294.89–1,354.79 mg TE per 100 mL) with the most in TK followed by TD, KY, PV, KTG, KP and KNP; and in flavedo (285.11–1,227.13 mg TE per 100 mL) with the most in TK followed by TD, KNP, KY, KTG, KP and PV, respectively.

Among the seven pomelo cultivars, Tha Knoi and Thong Dee had the highest total polyphenol content and antioxidant properties in the albedo and segment membranes. Pichaiyongvongdee and Haruenkit (2009) also reported that pink pomelo juice (TK and TD) had better antioxidant capacity than the white varieties (KNP, KY, PV, KP and KTG). Tsai *et al*. (2007) reported that pink pomelo juice had a higher total polyphenol content and antioxidant ability than white pomelo juice due to its pigments. The carotenoid content in pink pomelo was also responsible for its characteristic color and was significantly higher than that found in white pomelo.

The correlation between the total polyphenol content and antioxidant properties was very high as shown in Figures 2 and 3. A linear relationship existed between the total polyphenol contents and DPPH (correlation coefficient, $R^2 = 0.702$) was greater than with FRAP ($R^2 = 0.659$). The data showed that a high total polyphenol content increases the antioxidant properties. Total polyphenols were reported to be the major antioxidant of citrus fruit (Sun *et al.,* 2002). Proteggente *et al.* (2003) reported that increasing the total polyphenol content also increased the antioxidant efficacy in fruit. Furthermore,

Pichaiyongvongdee and Haruenkit (2009) reported that the antioxidant properties measured by DPPH and FRAP assay gave good correlations with the total polyphenol content in pomelo juice. The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples (Lee *et al*., 2003). Theppakorn and Chaiwong (2009) also studied the antioxidant capacities of pomelo cultivar "Thong dee" which were evaluated using DPPH assay and FRAP assay.

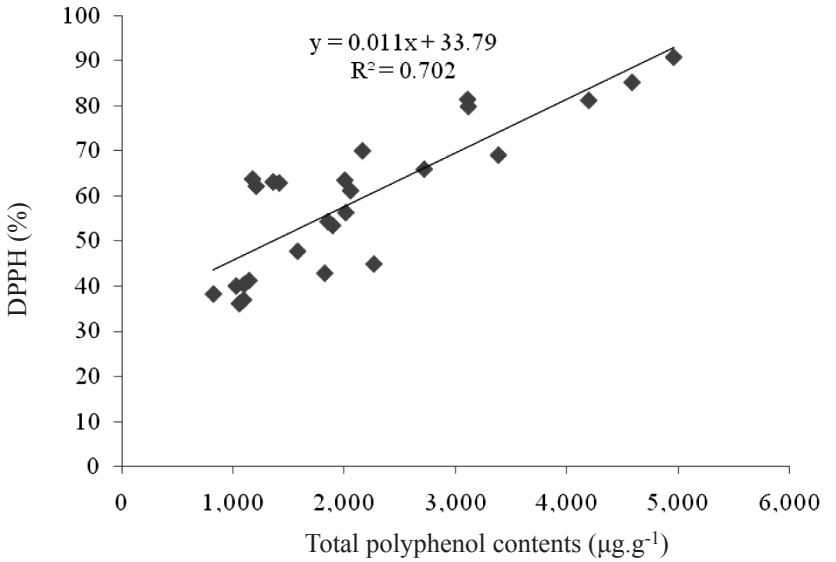

Figure 2 Correlation between the total polyphenol contents and antioxidant properties (DPPH = 1,1 Diphenyl-1-picrylhydrazyl; R^2 = Correlation coefficient).

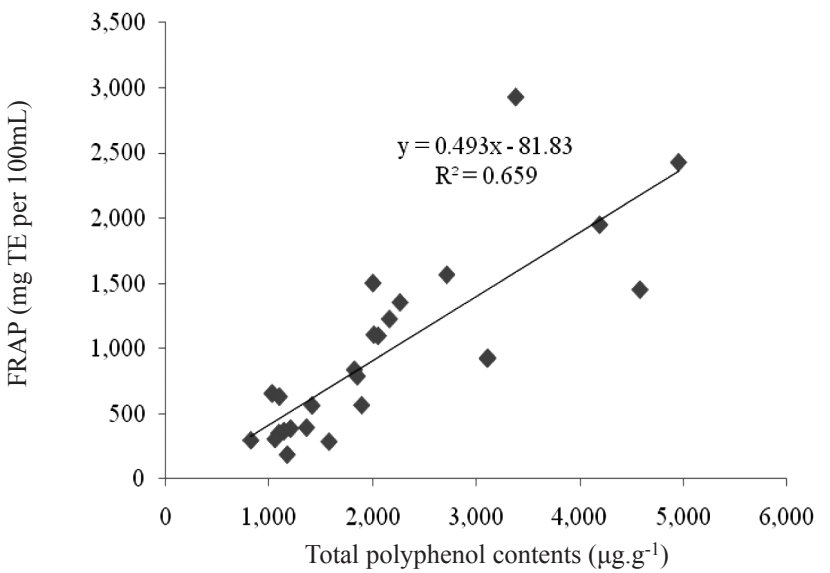

Figure 3 Correlation between the total polyphenol contents and antioxidant properties (FRAP = Ferric reducing antioxidant power; TE = trolox equivalent; R^2 = Correlation coefficient).

CONCLUSION

The highest total polyphenol content and antioxidant properties (DPPH and FRAP) of pomelo parts were found in the seeds and then in the following order: albedo, flavedo and segment membranes. The total polyphenol and the antioxidant properties found in TK and TD (pink cultivars) were higher than in PV, KY, KTG and KNP (white cultivars). The antioxidant properties of pink cultivars were higher than the white cultivars due to the pigments in the tissue, while the quality and bioactive compounds of the tissue depend on the growing region, environment, nutrition and cultivar color. It has been reported that the total polyphenols were the major antioxidant of citrus fruit (Sun *et al*., 2002) and the high total polyphenol content increases antioxidant capacity as measured by DPPH and FRAP (Proteggente *et al*., 2003; Pichaiyongvongdee and Haruenkit, 2009)

However, the current study suggested that the total polyphenols dominated the antioxidant properties (DPPH and FRAP) of the pomelo fruit. Generally, the seeds had higher antioxidant properties than other parts of the fruit (flavedo, albedo and segment membranes). Further study of the seeds may provide more information to assist in choosing drug products and the use of pomelo albedo in process dietary fiber powder will be further explored.

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