

Comparison of antioxidant properties of pomelo [*Citrus Grandis* (L) Osbeck] varieties

¹Toh, J.J., ¹Khoo, H.E. and ^{2*}Azrina, A.

¹Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

²Laboratory of Halal Science Research, Halal Product Research Institute, Universiti Putra Malaysia, Selangor, Malaysia.

Article history

Received: 12 October 2012

Received in revised form:

14 January 2013

Accepted: 19 January 2013

Abstract

This study aimed to compare the antioxidant content and antioxidant capacity of pulp and peel of two varieties of pomelo fruit (Tambun White and Tambun Pink). Antioxidants including total phenolic content, total flavonoid content and ascorbic acid content were determined using Folin-Ciocalteu reagent assay, aluminium chloride colorimetric assay and AOAC method, respectively. Antioxidant capacity of pomelo pulp and peel was measured using ferric reducing antioxidant potential and trolox equivalent antioxidant capacity assays. The peels of both pomelo fruits had higher antioxidant content and capacity than their pulps. Moreover, the white variety of pomelo had higher antioxidant content and capacity compared to the pink counterpart. Trolox equivalent antioxidant capacity of the samples was positively high correlated with total phenolic content ($r = 0.978$) and total flavonoid content ($r = 0.959$), except for ascorbic acid. Therefore, pomelo peel from white variety possessed higher antioxidant properties and it is potentially rich sources of natural antioxidants.

Keywords

Antioxidant capacity

Ascorbic acid

Pomelo

Tambun White

Tambun Pink

Total phenolics

© All Rights Reserved

Introduction

Antioxidants are substances that known to delay or inhibit oxidation (Halliwell, 1995). Plant component especially fruit has antioxidant components that are able to reduce oxidative stress (Agudo *et al.*, 2007). Fruits are also important sources of vitamins, minerals and other kinds of phytochemicals. Bioactive compounds in fruit that having antioxidant properties are carotenoids, polyphenols, anthocyanins and vitamins (Dillard and German, 2000; Ness and Powles, 1997).

The cultivation of tropical fruits has bloomed since past few years back due to the attractive sensorial properties and antioxidants content of these fruits. Antioxidants in tropical fruits are including polyphenols, carotenoids, and vitamins that impart health benefits beyond basic nutrition (Yahia, 2010). Several tropical fruits such as ciku, guava, star fruit and salak are native to Southeast Asia and these fruits have high antioxidant activity (Leong and Shui, 2002). Beside these fruits, pomelo also has high antioxidants (Isabelle *et al.*, 2010).

Pomelo [*Citrus grandis* (L.) Osbeck] is one of the tropical fruit which native to Southeast Asia (Morton, 1987). In Malaysia, the fruit is called as *limau abong*, *limau betawi*, *limau bali*, *limau besar*, *limau bol*, *limau jambua* or Bali *lemon*. It is a large citrus fruit with a common name of pomelo or shaddock that

belongs to the family of Rutaceae (Morton, 1987; Scora, 1975). The fruit is widely grown in the states of Johor, Perak, Kedah, Melaka and Kelantan. There are few popular varieties of pomelo in Malaysia which known as Tambun (White or Pink), Shatian and Melomas. Tambun White pomelo is the most popular type due to its juiciness, sweetness and delicious taste.

Thai pomelo has been reported to contain high flavonoids as compared to other Thai fruits (Kongkachuichai *et al.*, 2010). Methanolic extracts of pomelo (*C. grandis*) from Taiwan are potential antioxidants (Wu *et al.*, 2011). Pomelo peel has also been traditionally used for beauty purposes. The peel of citrus fruit contained higher amount of antioxidant as compared to its pulp as the peel is to protect the antioxidants in the fruit from oxidation. Hence, it is recommended to consume fruit together with its peel rather than the flesh alone (Guo *et al.*, 2003; Abeysinghe *et al.*, 2007).

Excluding *C. grandis* (pomelo), some of the other citrus fruits had been determined for their phenolics content and antioxidant activities (Ghafar *et al.*, 2010). White and pink varieties of Malaysian pomelo might also have variation in total phenolics and ascorbic acid contents due to different in the color of pomelo pulp. Therefore, this study aims to determine the antioxidant properties of pomelo fruit, including its peel. Two different varieties of pomelo

*Corresponding author.

Email: azrina@medic.upm.edu.my

were compared for their phenolics content, ascorbic acid content and antioxidant capacity.

Materials and Methods

Sampling

In this study, two varieties of pomelo, Tambun White and Tambun Pink at commercial maturity stage were purchased from a pomelo farm located in Tambun, Perak, Malaysia. The fruit were randomly selected from the harvested fruit's bin as the farmer already harvested the fruit according to their schedule. All pomelo fruits were packed in paper bag and transported to laboratory on the same day in an ice-packed box (4°C).

Reagent and standards

Methanol was purchased from (Fisher Scientific, UK), while sodium acetate trihydrate, ferric trichloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), ferrous sulphate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) and hydrochloric acid (HCl) were purchased from Merck (Darmstadt, Germany). Sodium carbonate (Na_2CO_3), sodium nitrite, sodium hydroxide (NaOH), potassium persulphate, aluminum chloride hexahydrate ($\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$), metaphosphoric acid, 2, 6-dichlorophenol-indophenol (DCPIP), ABTS salt, trolox, 2,4,6-tripyridyl-1,3,5-s-triazine (TPTZ), gallic acid, quercetin and Folin-Ciocalteu reagent were purchased from Sigma Chemical Co (St. Louis, MO, USA).

Preparation of samples

Upon arrival at research laboratory, all fruits were washed under running tap water to remove dirt and air dried. Pomelo peel and pulp were separated manually with a sharp knife and cut into small pieces with all seeds removed. The pieces of peel and pulp were freeze-dried in a freeze dryer (Virtis, New York, USA). The freeze dried samples were ground into powder using a laboratory-scale grinder, sieved and stored at -20°C before further analysis. Sample preparation was performed in ambient temperature (25°C) without white light turn on.

Extraction of sample

The samples were extracted based on a method described by Chew *et al.* (2011) with slight modifications. Briefly, ground sample (1 g) was weighed and added with 20 ml of 80% methanol (v/v). The mixture was placed in a conical flask wrapped with an aluminum foil and shaken at 200 rpm at 30°C for 3 h on an orbital shaker (Unimax 1010, Heidolph, Germany). The extract was then filtered through a filter paper (Whatman No. 1) to obtain

a clear solution. This step was repeated twice. The filtrate was used for determination of total phenolics, ascorbic acid and antioxidant capacity.

Determination of moisture content

Moisture content of sample was determined using direct drying method. Sample was homogenized in a blender and mixed thoroughly. An empty aluminum dish together with its cover was dried in an oven at 105°C for 3 h. The dish was cooled in a desiccator and weighed. Then 10 g of homogenized sample was weighed and put into the dish and dried in an oven (105°C) for overnight. The difference between initial weight and constant weight after drying was taken as moisture lost. All samples were analyzed in triplicate and the result was expressed percentage of moisture in the sample.

Determination of total phenolic content

Total phenolic content (TPC) of the sample was determined by Folin-Ciocalteu assay, which was based on a method of Singleton and Rossi (1965) with modification by Ikram *et al.* (2009). Briefly, an aliquot (0.2 ml) of diluted extract was mixed with 1.5 ml of 10-fold diluted Folin-Ciocalteu reagent. The mixture was allowed to stand at room temperature for 5 min. Then, 1.5 ml of 6% sodium carbonate solution was added into the mixture. The mixture was homogenized and allowed to stand at room temperature for 90 min. The absorbance of the reacting mixture was measured at 725 nm against a blank using a spectrophotometer. All samples were analyzed in triplicate. A standard curve for quantification was plotted using gallic acid (0–0.2 mg/ml) and the results were expressed as mg gallic acid equivalent (GAE) per 100 g fresh weight (FW).

Determination of total flavonoid content

Total flavonoid content TFC of sample was determined based on aluminum chloride colorimetric assay as described by Liu *et al.* (2008). A known volume of diluted extract (2 ml) was mixed with 0.2 ml of 0.5% sodium nitrate and incubated for 5 min. Then, 0.2 ml of 10% aluminum chloride was added to the mixture and mixed well. After 6 min, 2 ml of 1M sodium hydroxide was added to the mixture. The mixture was made up to 5 ml with 80% methanol and stirred thoroughly. The absorbance of the mixture was measured at 510 nm versus blank using a spectrophotometer. Quercetin (0–0.2 mg/ml) was used to plot a calibration curve for quantification. The results of total flavonoids content were expressed as mg quercetin equivalents (QE) per 100 g FW (Chew *et al.*, 2011). All the samples was analyzed in

triplicate.

Determination of ascorbic acid content

The AOAC (Association of Official analytical Chemist) method (AOAC, 1991) was used in determination of ascorbic acid content in the sample. Briefly, 100 g of sample was mixed with 100 ml of 6% metaphosphoric acid and make up to 250 ml with 6% metaphosphoric acid. Then, 10 ml of the diluted sample was added and made up to 100 ml with 3% metaphosphoric acid. The diluted suspension was filtrated and 10 ml aliquot of the filtrate was pipetted into small conical flask. The filtrate was titrated immediately with a standard solution of DCPIP to a faint pink end which persists for 15 s. All samples were analyzed in triplicate.

Determination of ferric reducing antioxidant power (FRAP)

Ferric reducing antioxidant power (FRAP) assay was performed according to a method described by Benzie and Strain (1996). The FRAP reagent was consisted of 300 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl, and 20 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$. The FRAP reagent was prepared by mixing the acetate buffer, TPTZ solution, and $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ solution in proportion of 10:1:1 (v/v/v). Briefly, an aliquot of appropriately diluted sample (50 ml) was mixed with 3 ml of freshly prepared FRAP reagent and mixed thoroughly. The reaction mixture was incubated at 37°C for 30 min. Absorbance of the mixture was measured at 593 nm versus blank. Ferrous sulphate (0–1000 mM) was used to plot a calibration curve for quantification, and the results were expressed as mmol Fe^{2+} per 100 g FW. All samples were analyzed in triplicate.

Determination of trolox equivalent antioxidant capacity

Trolox equivalent antioxidant capacity (TEAC) assay that based on a method of Re *et al.* (1999) was used to measure the TEAC value of pomelo sample (Chew *et al.*, 2011). Briefly, ABTS salt was dissolved in water to a concentration of 7 mM. ABTS radical cation ($\text{ABTS}^{+\cdot}$) was produced by reacting ABTS solution with 2.45 mM potassium persulphate (final concentration). The reaction mixture was allowed to stand at room temperature in the dark for 12–16 h before used. $\text{ABTS}^{+\cdot}$ solution (1 ml) was diluted with 60 ml of 70% ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. An aliquot (0.1 ml) of appropriately diluted sample was mixed with 10 ml of diluted $\text{ABTS}^{+\cdot}$ solution and mixed thoroughly. After 6 min, absorbance of the reaction mixture was measured at 734 nm versus blank. Trolox (0–2000 nM) was used

to construct a standard curve for quantification and the results were expressed as mmol trolox equivalent (TE) per 100 g FW. All samples were analyzed in triplicate.

Statistical analysis

Data was reported as mean \pm standard deviation of triplicate determination. The SPSS Statistics for Windows version 19.0 was used to analyze the data. Analysis of variance (ANOVA) coupled with LSD test was conducted to identify the significant difference between samples ($p < 0.05$). Meanwhile, Pearson correlation test was conducted to determine the correlations between antioxidant content and antioxidant activity of the pulp and peel of Tambun White and Pink Pomelo.

Results and Discussion

Moisture content

In this study, moisture content was determined to obtain percentages of water in pomelo pulp and peel. Among the samples studied, Tambun White pulp had the highest moisture ($90.46 \pm 0.09\%$) followed by Tambun Pink pulp ($90.04 \pm 0.06\%$), Tambun Pink peel ($82.69 \pm 0.08\%$) and Tambun White peel ($79.63 \pm 0.02\%$). By comparing water contents of peels and pulps for both pomelo varieties, the peels contained about 10 % lesser moisture content than the pulps. Previous study on fruits commonly consumed in Thailand found that moisture contents of four different types of pomelo pulp (87.5% to 88.9%) were closer to the values found in this study (Kongkachuichai *et al.*, 2010).

Total phenolic content

Result shows that TPC of pomelo pulp and peel were in the range of 61.72 – 406.65 mg GAE/100 g FW (Table 1). The result also indicated that both varieties of pomelo peels exhibited almost 5-fold higher in total TPC as compared to the pulps. Besides that, Tambun White pomelo showed higher TPC compared to Tambun Pink pomelo for both peels and pulps.

TPC determined using Folin-Ciocalteu method relies on electron transfer reaction. According to Prior *et al.* (2005), Folin-Ciocalteu reagent can be reduced by the non phenolic substances such as ascorbic acid, fructose and sucrose. However, this method is acceptable if the analysis steps are following the original method (Singleton and Rossi, 1965) and gallic acid should be used as the reference standard. Nonetheless, many previous studies have used this method in determining total phenolic content; hence the results can be compared with other available

Table 1. Antioxidant properties of pomelo pulp and peel

Type of pomelo	Part of Pomelo	TPC (mg GAE)	TFC (mg QE)	AA (mg ascorbic acid)	FRAP [mmolFe (II)]	TEAC (mmolTE)
Tambun	Peel	406.65±1.85 ^a	356.95±2.49 ^a	10.66±1.42 ^a	1.01±0.08 ^{ab}	1.49±0.02 ^a
	Pulp	70.56±1.34 ^c	13.06±1.31 ^c	41.21±1.43 ^c	0.6±0.06 ^c	0.42±0.04 ^c
White	Peel	300.56±3.39 ^b	228.86±9.0 ^b	7.42±0.00 ^b	0.65±0.01 ^a	1.14±0.04 ^b
	Pulp	61.72±2.48 ^d	13.2±0.51 ^c	33.77±1.43 ^d	0.51±0.06 ^{bc}	0.3±0.01 ^d
Pink	Peel	406.65±1.85 ^a	356.95±2.49 ^a	10.66±1.42 ^a	1.01±0.08 ^{ab}	1.49±0.02 ^a
	Pulp	70.56±1.34 ^c	13.06±1.31 ^c	41.21±1.43 ^c	0.6±0.06 ^c	0.42±0.04 ^c

Values are expressed as mean ± standard deviation of triplicate measurement per 100 g fresh weight.

Values with different letters are significantly different at $p < 0.05$.

TPC, total phenolic content; TFC, total flavonoid content, AA, ascorbic acid; GAE, gallic acid equivalent, TE, trolox equivalent.

Table 2. Pearson correlation analysis of pomelo pulp and peel

	FRAP	TEAC
Total phenolic content	-0.574	0.978**
Total flavonoid content	-0.578*	0.959**
Ascorbic acid content	0.792**	-0.868**

** Correlations were significant at the 0.01 level (2-tailed).

* Correlations were significant at the 0.05 level (2-tailed).

data.

Pomelo pulp of six Taiwanese varieties had TPC ranged from 4.50 to 9.99 mg GAE/g dry weight (DW) (Wu *et al.*, 2011). Our finding showed that Malaysian pomelo pulps had TPC (6.2 and 7.39 mg GAE/g DW) within the range as determined in these Taiwanese varieties. Besides, Thai's pomelo had similar TPC as compared to Malaysian pomelo (Isabelle *et al.*, 2010).

TPC in the peels of Tambun White and Tambun Pink pomelo (4.07 mg GAE/g FW and 3.01 mg GAE/g FW, respectively) were comparable with the values reported in Mauritian pomelo of four varieties (3.0–5.5 mg GAE/g FW) (Ramful *et al.*, 2010). Wu *et al.* (2011) also reported that white pulp pomelo had the highest TPC (9.99 mg GAE/g DW) compared to pink pulp pomelo (4.5 mg GAE/g DW). Besides, Gorinstein *et al.* (2001) reported total phenolics in the peels of lemon, oranges and grapefruits were significantly higher than the pulps. Moreover, Bocco *et al.* (1998) indicated that fruit's peels are an important source of phenolics.

Generally, TPC of the studied samples were higher than the TPC determined by some of the previous studies. This is because phenolics are usually susceptible to different factors like acidic solution and high temperature during the extraction process. Drying of plant material at temperatures below 50°C has also yielded highest amount of TPC (Julkunen-Tiitto, 1985).

Total flavonoid content

Result shows that highest TFC was found in the peel of Tambun White (356.95 mg QE/100 g FW), followed by the peel of Tambun Pink (228.86 mg QE/100 g FW). Meanwhile, the lowest TFC was determined in both Tambun White and Tambun Pink pomelo pulps at 13.06 and 13.2 mg QE/100 g FW,

respectively (Table 1). In comparison to previous study, Ramful *et al.* (2011) reported four different types of pomelo pulp had TFC ranged from <0.4 to 0.97 mg QE/g FW, which the TFC were far higher than the TFC determined in the studied pomelo pulp. The study also found that different harvest time has led to a variation in TFC, where the Raining pomelo (<0.4 mg QE/g FW) harvested in August was lower than the pomelo harvested in (0.88 mg QE/g FW). Therefore, different harvest time might affect the flavonoids content in the pomelo.

Pomelo peels from Mauritian had TFC ranged from 1.6 to 4.1 mg QE/g FW (Ramful *et al.*, 2010), which are within the range of TFC found in both of the studied pomelo peels. Besides, the studied pomelo peels had TFC higher than the peels of Jaffa sweeties (a grapefruit hybrid) and white grapefruits (0.93 and 0.74 mg QE/g FW, respectively) (Gorinstein *et al.*, 2004). The relatively higher yield of flavonoids in pomelo fruit could be due to differences in variety and intense sunlight conditions (a characteristic feature of tropical fruits), where both of the factors can induce accumulation of flavonoids in the fruit (Li *et al.*, 1993).

An interesting review has highlighted the relationships between antioxidant activities and structures of flavonoid subclasses in citrus extracts. Scientific evidence suggests that glycosylation, O-methylation, and O-glycosylation may greatly influence the antioxidant potency of citrus flavonoids (Di Majo *et al.*, 2005). Besides, antioxidant activity decreases with glycosylation, but it is enhanced with hydroxylation and presence of C2–C3 double bond in conjugation with a 4-oxo function, whilst presence flavonoids in flavedo extracts may significantly the antioxidant activity of fruit's peel (Rice-Evans *et al.*, 1996).

Ascorbic acid content

Ascorbic acid contents in pomelo sample were ranged from 7.42 to 41.21 mg ascorbic acid equivalent/100 g FW, where Tambun White pulp > Tambun Pink pulp > Tambun White peel > Tambun Pink peel (Table 1). The results have shown that ascorbic acid content in the pulps of both pomelo varieties were significant higher ($p < 0.05$) than the peels.

These findings are consistent with the values of ascorbic acid found in nutrient composition tables of Malaysians Foods which stated that ascorbic acid content of edible portion of pomelo was 44.8 mg/100 g FW (Tee *et al.*, 1997). On the other hand, Ramful *et al.* (2010) found that ascorbic acid content of flavedo extracts of Mauritius pomelo (147.5 mg/100 g FW)

was ~15× richer than the studied pomelo peel.

Ascorbic acid contents in the samples were determined based on titration method as this is a simple and rapid method to determine vitamin C content (Loeffler and Pointing, 1942). This is crude estimation of vitamin C content in pomelo fruits. A wide variation of ascorbic acid content is commonly determined in fruits, especially different variety of citrus fruits (Lee and Kader, 2000). The wide variation of ascorbic acid content determined in this fruit may be due to climate conditions, cultural practices, stages of maturity during harvesting and harvesting method. Duration of sample storage is another factor of ascorbic acid degradation. Comparing between two different locations, an example for grapefruit grown in coastal area was generally contained more vitamin C than the fruit grown in the dessert areas of California and Arizona (Lee and Kader, 2000). Any mechanical injuries (bruising, surface abrasions and cuts) during harvesting may also accelerate vitamin C degradation (Mondy and Leja, 1986).

Ferric reducing antioxidant power

FRAP values of pomelo samples were ranged from 0.51 to 1.01 mmol Fe (II)/100 g FW (Table 1). FRAP values of the pulps of Tambun White and Tambun Pink pomelo were significantly higher ($p < 0.05$) than the peels of both pomelo varieties. Among the pomelo samples, the highest FRAP value (1.01 mmol Fe (II)/100 g FW) of Tambun White peel indicating higher reducing properties found in the extract compared to other sample extracts.

The FRAP values of Tambun White and Tambun Pink pulps were 0.6 and 0.51 mmol Fe (II)/100 g FW, respectively. The result is consistent with FRAP value of pomelo (0.633 mmol Fe (II)/100 g FW) from China (Fu *et al.*, 2011), but the FRAP values in the studied samples were higher than the FRAP value in pomelo pulp extract (0.39 mmol Fe (II)/100 g FW) as determined by Guo *et al.* (2003). On the other hand, the FRAP values of the peels of both pomelo varieties (1.01 and 0.65 mmol Fe (II)/100 g FW for Tambun White and Tambun Pink, respectively) were lower than the FRAP value of pomelo peel extract (1.84 mmol Fe (II)/100 g FW) determined by Guo *et al.*, (2003).

FRAP assay is typically used to measure total reducing capability of plant using antioxidants in a sample as reductants in a redox-linked colorimetric reaction. In other words, this assay is based on the ability of antioxidant to reduce ferric (III) ions to ferrous (II) ions (Benzie and Strain, 1996). FRAP assay is also sensitive to temperature, pH and oxidation. Although the reaction occurs for

quantification of possible antioxidants is rapid, but the preparation need extra caution in order to obtain desirable result. This assay is commonly used because it is relatively simple and easy to be standardized (Prior *et al.*, 2005).

Trolox equivalent antioxidant capacity

As shown in Table 1, Tambun White peel had the highest antioxidant activity (1.49 mmol TE/100 g FW), followed by Tambun Pink peel (1.14 mmol TE/100 g FW) and Tambun White pulp (0.42 mmol TE/100 g FW). The lowest TEAC value was found in Tambun Pink pulp (0.3 mmol TE/100 g FW). TEAC values of the peels of both pomelo varieties were higher than the pulps. The TEAC value of pulp and peel of Tambun White pomelo were higher than Tambun Pink. Similar trend was also found for TPC, ascorbic acid content and FRAP values, but not for TFC.

Previous study reported that TEAC value of pomelo pulp was 0.31 mmol TE/100 g FW, which is similar to TE value determined in the pulp of Tambun Pink pomelo (Fu *et al.*, 2011). TE values of the studied pomelo peels were also compared with the TE values of Thai's pomelo peels (0.46–2.1 mmol TE/100 g FW) (Contreras-Calderon *et al.*, 2011). Besides, TE values of the peel extracts of *C. sulcata* (Wang *et al.*, 2011) were lower than the peel extracts of the studied fruit.

TEAC assay has been used to determine the antioxidant activity in extracts of fruits. This assay measures the antioxidant ability of antioxidants in fruit to scavenge ABTS radical. The ABTS⁺ which generated using potassium persulfate are an excellent tool for determining antioxidant activity of hydrogen-donating antioxidants and chain-breaking antioxidants (Re *et al.*, 1999). ABTS⁺ assay can also be used to measure antioxidant activity of a broad range of substances.

Correlation between antioxidants and antioxidant capacity

Correlations between antioxidants (TPC, TFC and ascorbic acid) and antioxidant activities that analyzed using Pearson correlation analysis are tabulated in Table 2. There were significant correlations between FRAP values and antioxidants such as TFC and ascorbic acid. However no significant correlation was observed between FRAP values and TPC. Moderate high and negative correlation were found between FRAP values and TFC ($r = -0.578$). This indicates that flavonoids in pomelo samples may not the main bioactive compounds with potential reducing ability. Meanwhile, high and positive correlation was

observed between FRAP value and ascorbic acid ($r = 0.792$) implying that ascorbic acid has contributed to activity that tested using FRAP reagent.

A review article has stated that FRAP assay is poorly correlated to many other antioxidant analysed (Prior *et al.*, 2005). This observation is also consistent with a finding by Ramful *et al.* (2011) that TFC in fruits extract was poorly correlated with FRAP values ($r = 0.10$). Conversely, phenolics content was strongly correlated with antioxidant activity assessed by FRAP assay (Ramful *et al.*, 2010; Fu *et al.*, 2011).

As for ascorbic acid, previous studies have found no correlation between ascorbic acid contents and antioxidant activities of fruit extracts (Wang *et al.*, 1996; Zhang and Fang, 1990). On the other hand, ascorbic acid was the main contributor to antioxidant activity determined by FRAP assay (Abeyasinghe *et al.*, 2007). A study on pomelo extract has demonstrated that more than 80% of the FRAP values was contributed by ascorbic acid (Guo *et al.*, 2003). From the same study, other fruits such as apple, pomegranate and grape had contributed less than 20%. Thus the correlation between FRAP value and ascorbic acid found in fruit is varied.

In this study, Pearson correlation analysis also shows that there were significant correlations between TE values and antioxidants of TPC, TFC and ascorbic acid. Result shows that the TE values were highly and positively correlated with TPC and TFC ($r = 0.978$ and $r = 0.959$, respectively), which explaining the TE values were greatly influenced by phenolics and flavonoids contents in pomelo fruit. Meanwhile, TE values were high and negatively correlated with ascorbic acid content ($r = -0.868$) implying the TE values were not affected by the presence of ascorbic acid.

Ramful *et al.* (2010) reported that for Mauritian citrus fruit extracts, a high correlation was found between TPC and antioxidant capacity as determined by TEAC assay ($r = 0.920$). However, TFC was moderately high correlated with TE values ($r = 0.43$) (Ramful *et al.*, 2011). Besides that, Luximon-Ramma *et al.* (2003) have reported negative correlation between TEAC assay and vitamin C content in Mauritian exotic fruits. In contrast, ascorbic acid contents in citrus fruits and commercial orange juices were significantly contributed to the antioxidant activity (Sánchez-Moreno *et al.*, 2003)

Antioxidant capacity may not always strongly correlate with phenolic compounds. This is due to the correlation between TE values and antioxidants is depending on the types of fruit (Chang *et al.*, 2000). Obviously, the hydrophilic phase of the extract can greatly influence the TE value, while the hydrophobic

contribution was much lower (Scalzo *et al.*, 2005).

Conclusion

Tambun White pomelo is a potential functional food as it contained the highest antioxidants and antioxidant capacity compared to Tambun Pink pomelo. The antioxidant capacity determined in the pomelo fruit are not solely due to the phenolic compounds, but also contributed by ascorbic acid in the fruit. Data obtained from this study have provided a rough estimation of antioxidants content and antioxidant capacity in the pulp and peel of pomelo from two Tambun's varieties. Therefore, Tambun pomelo is one of the nutritious fruits with high antioxidant content.

Acknowledgements

Special thanks should go to the assistance of laboratory staffs from Department of Nutrition and Dietetics, UPM and Universiti Putra Malaysia for the use of laboratory facilities.

References

- Abeyasinghe, D. C., Li, X., Sun, C. D., Zhang, W. S., Zhou, C. H. and Chen, K. S. 2007. Bioactive compounds and antioxidant capacities in different edible tissues of citrus fruit of four species. *Food Chemistry* 104: 1338–1344.
- Agudo, A., Cabrera, L., Amiano, P., Ardanaz, E., Barricarte, A., Berenguer, T. and Gonzalez, C. A. 2007. Fruit and vegetable intakes, dietary antioxidant nutrients, and total mortality in Spanish adults: Findings from the Spanish cohort of the European Prospective Investigation into Cancer and Nutrition (EPIC-Spain). *American Journal of Clinical Nutrition* 85: 1634–1642.
- AOAC (Association of Official Analytical Chemists) 1990. Official methods of analysis of the Association of Official Analytical Chemists. 15th edn., p. 1058–1059, Arlington VA: Association of Official Analytical Chemists.
- Benzie, I. F. F. and Strain, J. J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. *Analytical Biochemistry* 239: 70–76.
- Bocco, A., Cuvelier, M. E., Richard, H. and Berset, C. 1998. Antioxidant activity and phenolic composition of citrus peel and seed extracts. *Journal of Agricultural and Food Chemistry* 4: 2123–2129.
- Chang, S., Tan, C., Frankel, E. N. and Barret, D. 2000. Low-density lipoprotein antioxidant activity of phenolic compounds and polyphenols oxidase activity in selected clingstone peach cultivars. *Journal of Agricultural and Food Chemistry* 48: 147–156.

- Chew, L. Y., Nagendra Prasad, K., Amin, I., Azrina, A. and Lau, C. Y. 2011. Nutritional composition and antioxidant properties of *Canarium odontophyllum* Miq. (dabai) fruits. *Journal of Food Composition and Analysis* 24: 670–677.
- Contreras-Calderon, J., Calderon-Jaimes, L., Guerra-Hernandez, E. and Garcia-Villanova, B. 2011. Antioxidant capacity, phenolic content and vitamin C in pulp, peel and seed from 24 exotic fruits from Colombia. *Food Research International* 44: 2047–2053.
- Dillard, C. J. and German, J. B. 2000. Phytochemicals: nutraceuticals and human health. *Journal of the Science of Food and Agriculture* 80: 1744–1756.
- Di Majo, D., Giammanco, M., La Guardia, M., Tripoli, E., Giammanco, S. and Finotti, E. 2005. Flavanones in citrus fruit: structure–antioxidant activity relationships. *Food Research International* 38: 1161–1166.
- Fu, L., Xu, B. T., Xu, X. R., Gan, R. Y., Zhang, Y., Xia, E. Q. and Li, H. B. 2011. Antioxidant capacities and total phenolic contents of 62 fruits. *Food Chemistry* 129: 345–350.
- Ghafar, M. F. A., Prasad, K. N., Weng, K. K. and Ismail, A. 2010. Flavonoid, hesperidine, total phenolic contents and antioxidant activities from Citrus species. *African Journal of Biotechnology* 9: 326–330.
- Gorinstein, S., Martin-Belloso, O., Park, Y. S., Haruenkit, R., Lojek, A., Číž, M., Caspi, A., Libman, I. and Trakhtenberg, S. 2001. Comparison of some biochemical characteristics of different citrus fruits. *Food Chemistry* 74: 309–315.
- Gorinstein, S., Cvikrová, M., Machackova, I., Haruenkit, R., Park, Y. S., Jung, S. T., Yamamoto, K., Ayala, A. L. M., Katrich, E. and Trakhtenberg, S. 2004. Characterization of antioxidant compounds in Jaffa sweeties and white grapefruits. *Food Chemistry* 84: 503–510.
- Guo, C. J., Yang, J. J., Wei, J. Y., Li, Y. F., Xu, J. and Jiang, Y. G. 2003. Antioxidant activities of peel, pulp and seed fractions of common fruits as determined by FRAP assay. *Nutrition Research* 23: 1719–1726.
- Halliwell, B. 1995. Antioxidant characterization: Methodology and mechanism. *Biochemical Pharmacology* 49: 1341–1348.
- Ikram, E. H. K., Eng, K. H., Jalil, A. M. M., Amin, I., Salma, I., Azrina, A. and Ruzaidi Azli, M. M. 2009. Antioxidant capacity and total phenolic content of Malaysian underutilized fruits. *Journal of Food Composition and Analysis* 22: 388–393.
- Isabelle, M., Lee, B. L., Lim, M. T., Koh, W. P., Huang, D. and Ong, C. H. 2010. Antioxidant activity and profiles of common fruits in Singapore. *Food Chemistry* 123: 77–84.
- Julkunen-Tiitto, R. 1985. Phenolic constituents in the leaves of northern willows, methods for the analysis of certain phenolics. *Journal of Agricultural and Food Chemistry* 33: 213–217.
- Kongkachuichai, R., Charoensiri, R. and Sungpuag, P. 2010. Carotenoids, flavonoids profiles and dietary fiber contents of fruits commonly consumed in Thailand. *International Journal of Food Science and Nutrition* 61: 536–548.
- Lee, S. K. and Kader, A. A. 2000. Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest Biology and Technology* 20: 207–220.
- Leong, L. P. and Shui, G. 2002. An investigation of antioxidant capacity of fruits in Singapore markets. *Food Chemistry* 76: 69–75.
- Li, J., Ou-Lee, T. M., Raba, R., Amundson, R. G. and Last, R. L. 1993. Arabidopsis flavonoid mutants are hypersensitive to UV-B irradiation. *Plant Cell* 5: 171–179.
- Liu, H., Qiu, N., Ding, H. and Yao, R. 2008. Polyphenols contents and antioxidant capacity of Chinese herbals suitable for medical or food uses. *Food Research International* 41: 363–370.
- Loeffler, H. J. and Pointing, J. D. 1942. Ascorbic acid: rapid determination in fresh, frozen, or dehydrated fruits and vegetables. *Industrial and Engineering Chemistry* 14: 846–849.
- Morton, J. F. 1987. Fruits of warm climates. Downloaded from <http://www.hort.purdue.edu/newcrop/morton/pummelo.html>.
- Mondy, N. I. and Leja, M. 1986. Effect of mechanical injury on the ascorbic acid content of potatoes. *Journal of Food Sciences* 51: 355–357.
- Ness, A. R. and Powles, J. W. 1997. Fruit and vegetables, and cardiovascular disease: a review. *International Journal of Epidemiology* 26: 1–13.
- Prior, R. L., Wu, X. L. and Schaich, K. 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry* 53: 4290–4302.
- Ramful, D., Bahorun, T., Bourdon, E., Tarnus, E. and Aruoma, O. I. 2010. Bioactive phenolics and antioxidant propensity of flavedo extracts of Mauritian citrus fruits: Potential prophylactic ingredients for functional foods application. *Toxicology* 278: 75–87.
- Ramful, D., Tarnus, E., Aruoma, O. I., Bourdon, E. and Bohorun, T. 2011. Polyphenol composition, vitamin C content and antioxidant capacity of Mauritian citrus fruit pulps. *Food Research International* 44: 2088–2099.
- Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. and Rice-Evans, C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine* 26: 1231–1237.
- Rice-Evans, C. A., Miller, N. J. and Paganga, G. 1996. Structure-antioxidant activity relationships of flavonoids and phenolics acids. *Free Radical Biology and Medicine* 20: 933–956.
- Sánchez-Moreno, C., Plaza, L., de Ancos, B. and Cano, M. P. 2003. Quantitative bioactive compounds assessment and their relative contribution to the antioxidant capacity of commercial orange juices. *Journal of the Science of Food and Agriculture* 83: 430–439.
- Scalzo, J., Politi, A., Pellegrini, N., Mezzetti, B. and

- Battino, M. 2005. Plant genotype affects total antioxidant capacity and phenolic contents in fruits. *Nutrition* 21: 207–213.
- Scora, R. W. 1975. On the history and origin of Citrus. *Bulletin of Torrey Botanical Club* 102: 369–375.
- Singleton, V.L. and Rossi Jr., J.A. 1965. Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. *American Journal of Enology and Viticulture* 16: 144–158.
- Tee, E. S., Noor, M. I., Azudin, M. N. and Idris, K. 1997. Nutrient composition of Malaysian food. 4th edn. Kuala Lumpur: Institute for Medical Research.
- Wang, A. Y., Zhou, M. Y. and Lin, W. C. 2011. Antioxidative and anti-inflammatory properties of *Citrus sulcata* extracts. *Food Chemistry* 124: 958–963.
- Wang, H., Cao, G. and Prior, R. L. 1996. Total antioxidant capacity of fruits. *Journal of Agricultural and Food Chemistry* 44: 701–705.
- Wu, S. E., Ng, C. C., Tzeng, W. C., Ho, K. C. and Shyu, Y. T. 2011. Functional antioxidant and tyrosinase inhibitory properties of extracts of Taiwanese pummelo (*Citrus grandis* Osbeck). *African Journal of Biotechnology* 10: 7668-7674.
- Yahia, E. M. 2010. The contribution of fruits and vegetables consumption to human health. In de la Rosa, L. A., Alvarez-Parilla, E. and Gonzalez-Aguilar, G. (Eds.). *Fruit and vegetable phytochemicals: Chemistry, nutritional and stability*, p. 3–52. Iowa: Wiley-Blackwell.
- Zhang, D. Z. and Fang, Y. Z. 1990. Observation on antioxidative activity of some vegetables and fruits. *Acta Nutrition Singapore* 12: 191–195.