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Antioxidant Content and Free Radical Scavenging Ability of Fresh Red Pummelo [*Citrus grandis* (L.) Osbeck] Juice and Freeze-Dried Products

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The antioxidative phytochemicals in various fruits and vegetables are widely recognized for their role in scavenging free radicals, which are involved in the etiology of many chronic diseases. Colored fruits are especially considered a quality trait that correlates with their nutritional values and health benefits. The specific aim of this study was to investigate the antioxidants in the juice and freeze-dried flesh and peel of red pummelo and their ability to scavenge free radicals and compare them with those in white pummelo juice. The total phenolic content of red pummelo juice extracted by methanol (8.3 mg/mL) was found to be significantly higher than that of white pummelo juice (5.6 mg/mL). The carotenoid content of red pummelo juice was also significantly higher than that in white pummelo juice. The contents of vitamin C and δ-tocopherol in red pummelo juice were 472 and 0.35 μg/mL, respectively. The ability of the antioxidants found in red pummelo juice to scavenge radicals were found by methanol extraction to approximate that of BHA and vitamin C with a rapid rate in a kinetic model. The ability of methanol extracts of freeze-dried peel and flesh from red pummelo to scavenge these radicals was 20−40% that of BHA and vitamin C effects. Fresh red pummelo juice is an excellent source of antioxidant compounds and exhibited great efficiency in scavenging different forms of free radicals including DPPH, superoxide anion, and hydrogen peroxide radicals.

KEYWORDS: Red pummelo; scavenging ability; antioxidant; fresh, freeze-dried

INTRODUCTION

Natural antioxidant phytochemicals are present in all parts of all higher plants. They have been found to protect against a variety of disorders, particularly cardiovascular diseases (*1*, *2*) and some types of cancer (*3*). Previous studies have shown that a reduced risk of coronary disease is associated with a high dietary intake of fruits and vegetables, legumes, nuts, and whole grains (*4*−*6*). A high intake of fruits and vegetables has also been linked to reduced cancer risk in many epidemiological studies. Block et al. (*7*) reviewed approximately 200 studies of cancer and fruit and vegetable intake and found a statistically significant protective effect. Prior et al. (*8*) reviewed the various available analytical methods for measuring antioxidant activity. Multiple methods are recommended for measuring antioxidant properties of food materials to better reflect their potential protective effects.

Citrus fruits are rich sources of antioxidant nutrients. The major source of antioxidant capacity is not vitamin C or dietary fiber, but other antioxidant compounds (*9*). In Asian countries, citrus fruits, such as lime (*Citrus microcarpa* and *Citrus aurantifolia*), lemon (*Citrus limon*), and pummelo (*Citrus grandis*) are widely available and regularly consumed as whole fruits or fruit juices and preserved snacks. The pummelo, the largest of all citrus fruits, is a favorite at the mid-autumn Moon Festival for Chinese families. Traditionally, people eat white pummelo during this festival. Recently, the red pummelo has been widely cultivated and promoted in southern Taiwan. We speculated that the red pummelo may have more antioxidant compounds than the white variety, because lycopene, carotenoid, and anthocyanins have been reported among the colorful red-orange cultivars (Moro, Tarocco, and Sanguinello varieties) (*10*). However, the characteristic properties and antioxidant potential of red pummelo have not been reported. Therefore, the objective of this study was to assess the antioxidant properties and activity of fresh juice and freeze-dried flesh and peel from the red pummelo.

MATERIALS AND METHODS

Preparation of Pummelo Samples. Red pummelo [RP; *Citrus grandis* (L.) Osbeck] peel and edible flesh were collected and freeze-dried. The freeze-dried samples were ground, and the powder was put in a zipper bag and stored in the refrigerator until analysis. RP and

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Table 1. Composition of Freeze-Dried Red Pummelo Flesh and Peel[a]

pummelo/part[b]	moisture (%)	ash (%)	crude fiber (%)	crude protein (%)	crude fat (%)	total carbohydrate (%)
FRPF	21.1	3.2 a	2.1 a	0.23	1.5 a	71.9 b
FRPP	15.6	4.7 b	4.3 b	0.28	14.7 b	60.4 a

[a] Values are means of triplicate analyses and expressed as percent of freeze-dried red pummelo. Means with different letters within a column are significantly different ($p < 0.05$). Total carbohydrate was calculated by difference (sum of protein, fat, water, and ash subtracted from total weight). [b] Abbreviations: FRPF, freeze-dried red pummelo flesh; FRPP, freeze-dried red pummelo peel.

white pummelo (WP) juices were obtained with a domestic squeezer and stored at −20°C until use. No food preservatives were added.

Characterization of Freeze-Dried Red Pummelo. The contents of moisture, crude protein, crude fat, ash, and crude fiber of freeze-dried RP peel and flesh were analyzed according to AOAC methods 15.950.02, 15.976.05, 15.920.39, 15955.03, and 15.962.09, respectively (*11*). The results were expressed as percentages of freeze-dried weight.

Preparation of Methanol or Water Extracts of Juice. Phenolic compounds in RP or WP juice (10 mL) were extracted with 100 mL of methanol or water in a water bath with an automatic shaker at room temperature for 8 h. The methanol or water extracts were concentrated on a rotary evaporator and adjusted to a volume of 10 mL with methanol or water and further analyzed for antioxidant components and activity or total phenolic compound, respectively.

Preparation of Methanol Extracts of Freeze-Dried Product. One gram of freeze-dried ground powder of RP flesh or peel was extracted with 100 mL of methanol in a water bath with an automatic shaker at room temperature for 24 h. Immediately after extraction, the extracts of RP flesh and peel were vacuum filtered using hydrophilic polypropylene membrane filters with 0.45 μm diameter pores. RP flesh or peel clear extracts were concentrated on a rotary evaporator and adjusted to a volume of 10 mL with methanol for further analysis.

Total Phenolic Content and Antioxidant Profile. The pummelo juice and freeze-dried methanol extracts were analyzed for total phenolic content according to the Folin−Ciocalteu colorimetric method (*12*). Briefly, 7 mL of water, 0.1 mL of sample, and 0.5 mL of Folin−Ciocalteu reagent were added to a 25 mL volumetric flask. The mixture was allowed to stand for 3 min at room temperature. Next, 2 mL of a 20% sodium carbonate solution was added and heated at 100 °C for 3 min. The sample aliquots were filtered through a Whatman filter with 0.45 μm diameter pores prior to the determination of total phenols using a spectrophotometer (DU 530, Beckman, Fullerton, CA) at 750 nm. Gallic acid was used as a standard, and the total phenolic content was expressed as milligrams per milliliter. Carotenoid was determined according to the method of Yang et al. (*13*). Juice and freeze-dried powder samples were stirred vigorously in 80% acetone to dissolve the material. After solvent extraction, the mixture was allowed to stand for phase separation. The carotenoid in the lipid-soluble fraction was measured with a UV−vis spectrophotometer (DU 530, Beckman) at 663.6, 646.6, 628, 590, and 440.5 nm. Flavonoids and anthocyanins in the extracted fraction were determined using the methods of Geissman (*14*) and Mancinelli (*15*), respectively. Total flavonoids were measured using a Beckman spectrophotometer at 540 nm. Total anthocyanins were determined by measuring the absorbance at 530 and 657 nm. All three results were expressed as micrograms per gram or micrograms per milliliter of sample.

HPLC Analysis of Ascorbic Acid. The HPLC method of Rose and Nahrwold (*16*) was modified slightly for vitamin C analysis. Briefly, 1 mL of pummelo juice or 0.1 g of freeze-dried RP product was added to 1 mL of 10% metaphosphoric acid. This mixture was vortexed briefly and centrifuged for 10 min at 1000*g* at 4 °C (Sigma 3K12, Laborzentrifugen). The supernatant was passed through a Millipore filter (0.22 μm) and injected (20 μL) onto a reverse-phase HPLC column (Hypersil HS C18 250 × 4.6 mm, 5 μm, Thermo Quest). With a Jasco PU-1580 pump (Jasco, Tokyo, Japan), the isocratic elution flow rate was set at 0.7 mL/min with 1-pentane sulfuric acid sodium salt solution (0.871 g/L) at pH 3.1. The wavelength of the UV detector (UV-1575, Jasco, Tokyo, Japan) was 254 nm. One milliliter of RP or WP juice in an Eppendorf tube was subjected to heat treatment in a water bath for 1 min at either 50, 60, 70, or 80 °C and cooled for 5 min in an ice−water bath before HPLC measurements.

Table 2. Content of Total Phenolic Compounds of Extracts from Fresh Pummelo Juice and Freeze-Dried Pummelo[a]

	juice (mg/mL)		freeze-dried (mg/g)	
extract	RP	WP	FRPF	FRPP
water	1.26 ± 0.3	1.19 ± 0.4	ND	ND
methanol	8.26 ± 1.2 b	5.62 ± 0.5 a	12.3 ± 1.5 a	22 ± 1.2 b

[a] Abbreviations: RP, red pummelo; WP, white pummelo; FRPF, freeze-dried red pummelo flesh; FRPP, freeze-dried red pummelo peel; ND, not determined. Means with different letters in the same treatment within a row are significantly different ($p < 0.05$).

HPLC Analysis of Vitamin E. The α- and δ-tocopherol concentrations in juice and freeze-dried powder samples were analyzed after saponification with KOH followed by extraction with diisopropyl ether and HPLC with fluorometric detection for vitamin E (*17*). Separation of α- and δ-tocopherol was performed on a reverse-phase HPLC column (Hypersil HS C18 250 × 4.6 mm 5 μm, Thermo Quest). The mobile phase was composed of methanol and water (95:5, v/v), and the flow rate was 1.0 mL/min. The wavelength of the UV detector (UV-1575, Jasco, Tokyo, Japan) was 292 nm.

Measurement of Radical Scavenging Activity. *DPPH Radical Scavenging Activity*. The scavenging of DPPH radicals was measured using a method modified from that of Shimada et al. (*18*). Briefly, 1 mL of 0.2 mM DPPH methanol solution was added to 1 mL of pummelo juice in methanol (0.4 mL/mL methanol) or freeze-dried methanol extract (0.4 mg/mL methanol). The absorbance was measured for 4 min after the initial mixing. The decrease of the absorbance at 571 nm was calculated and plotted as a function of the concentration of the extract and fresh juice. The same concentrations of BHA and vitamin C (0.4 mg/mL methanol) were used as references.

Hydrogen Peroxide Scavenging Activity. The method of Pick and Keisari (*19*) was modified for determining the scavenging hydrogen peroxide radicals. A portion of 0.4 mL of hydrogen peroxide solution was added to 0.6 mL of horseradish peroxide−phenol red (0.5 mg/mL; HRPase 1 mL; 7.5 mM phenol red, 2 mL) and finally added to 1 mL of the pummelo juice in methanol (0.4 mL/mL methanol) or freeze-dried methanol extract (0.4 mg/mL methanol). The absorbance was monitored for 4 min after the initial mixing. The decrease of the absorbance at 610 nm was used to calculate the percentage scavenging effect of pummelo samples. The same concentrations of BHA and vitamin C (0.4 mg/mL methanol) were used as references.

Superoxide Anion Radical Assay. The superoxide radicals were generated from xanthine−xanthine oxidase, and radical scavenging activity was determined using a method modified from that of Noro et al. (*20*). Briefly, 1.5 mL of phosphoric acid buffer was added to 0.5 mL of 0.04 unit xanthine oxidase and 1 mL of 0.5 mM xanthine solution and allowed to stand for 3 min. To the mixture was added 1 mL of pummelo juice in methanol (0.4 mL/mL methanol) or freeze-dried methanol extract (0.4 mg/mL methanol). The absorbance was measured for 4 min after the initial mixing. The decrease of the absorbance at 295 nm was used to calculate the superoxide anion radical scavenging activity of the pummelo sample. The same concentrations of BHA and vitamin C (0.4 mg/mL methanol) were used as references.

Statistical Analysis. All experiments were conducted in triplicate. The data were subjected to one-way ANOVA. Significant differences among treatment means were tested using Duncan's multiple-range test ($p < 0.05$).

RESULTS

Characterization of Freeze-Dried Pummelo Flesh and Peel. A high content of total carbohydrate was found in both freeze-dried RP flesh and peel (**Table 1**). The peel had

Table 3. Contents of Phytochemicals in Fresh and Freeze-Dried Pummelo[a]

	juice (μg/mL)		freeze-dried (μg/g)	
	RP	WP	FRPF	FRPP
carotenoids	1.73 ± 0.5 b	0.34 ± 0.1 a	24.75 ± 2 a	40.8 ± 2.5 b
flavonoids	1.24 ± 0.2	1.29 ± 0.3	0.55 ± 0.15 a	4.56 ± 1.5 b
anthocyanin	0.94 ± 0.2	1.09 ± 0.1	0.67 ± 0.15 a	6.56 ± 0.8 b

[a] Abbreviations: RP, red pummelo; WP, white pummelo; FRPF, freeze-dried red pummelo flesh; FRPP, freeze-dried red pummelo peel. Means with different letters in the same treatment within a row are significantly different ($p < 0.05$).

Table 4. Content of Antioxidant Vitamins in Fresh and Freeze-Dried Pummelo[a]

	juice (μg/mL)		freeze-dried (μg/g)	
	RP	WP	FRPF	FRPP
α-tocopherol	ND	ND	ND	ND
δ-tocopherol	0.35	0.33	1.7	2.0
vitamin C	472 ± 12 a	353 ± 81 b	155.7 ± 4 a	91 ± 3 b

[a] Abbreviations: RP, red pummelo; WP, white pummelo; FRPF, freeze-dried red pummelo flesh; FRPP, freeze-dried red pummelo peel; ND, not detectable. Means with different letters in the same treatment within a row are significantly different ($p < 0.05$).

significantly higher fat, ash, and crude fiber content than the flesh. The protein content of flesh and peel were similar.

Total Phenolic Content and Antioxidant Profile. The total phenolic compounds from two different extract fractions of juice and freeze-dried products were expressed as milligrams of gallic acid equivalent per milliliter and milligrams, respectively (**Table 2**). The content of total phenolic compounds in the methanol extract of fresh RP juice was significantly higher (8.26 mg/mL) than that from the WP (5.62 mg/mL). The methanol extract of RP juice had the highest phenolic content (8.26 ± 1.2 mg/mL), followed by the methanol extract of WP juice (5.26 ± 0.5 mg/ mL), the water extract of RP juice (1.26 ± 0.3 mg/mL), and the water extract of WP juice (1.19 ± 0.4 mg/mL). The RP juice had a higher level of carotenoids than the WP, but there was no significant difference in the levels of flavonoids and anthocyanins between fresh RP and WP juices (**Table 3**). RP peel had significantly higher total phenolic content (22 mg/g) than flesh (12.3 mg/g) (**Table 2**), as well as carotenoid, flavonoid, and anthocyanin levels (**Table 3**).

Antioxidant Vitamins. α-Tocopherol was not detectable in any pummelo samples, whereas the contents of δ-tocopherol were similar in the juices of both RP and WP and in both the flesh and peel of freeze-dried RP (**Table 4**). The RP juice contained much higher levels of vitamin C than the WP juice. As expected, the RP peel contained less vitamin C than the flesh. Vitamin C content decreased as a result of a heating treatment in both RP and WP juice from 370 to 220 μg/mL and from 320 to 160 μg/mL, respectively (**Figure 1**). The RP juice retained more (60%) vitamin C than the WP juice (50%) after heating at 80 °C for 1 min.

Measurement of Radical Scavenging Activity. The DPPH scavenging abilities of RP and WP juices were the same as for the standards BHT and vitamin C at the same concentrations (0.4 mL/mL). The DPPH scavenging abilities of both RP peel and RP flesh were only approximately 20% that of the standards vitamin C and BHT. Similar results were obtained for scavenging both superoxide anions (**Figure 3**) and hydrogen peroxide radicals (**Figure 4**).

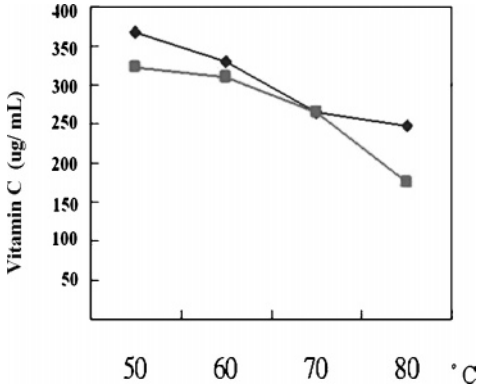

Figure 1. Content of vitamin C in fresh red (◆) and white (■) pummelo juice heated at different temperatures for 1 min.

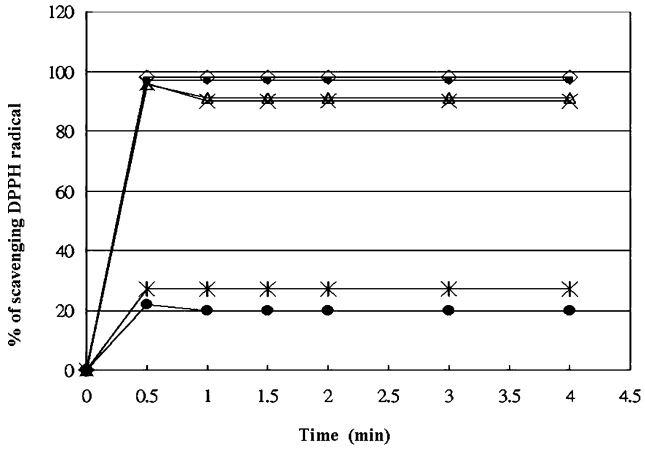

Figure 2. Kinetics of DPPH scavenging effects of butylated hydroxytoluene (0.4 mg/mL, ◇), red pummelo juice (0.4 mL/mL, △), white pummelo juice (0.4 mL/mL, ×), freeze-dried red pummelo flesh (0.4 mg/mL, ∗), and freeze-dried red pummelo peel (0.4 mg/mL, ●) extracted with methanol and vitamin C (0.4 mg/mL, ■) dissolved in water at 4 min.

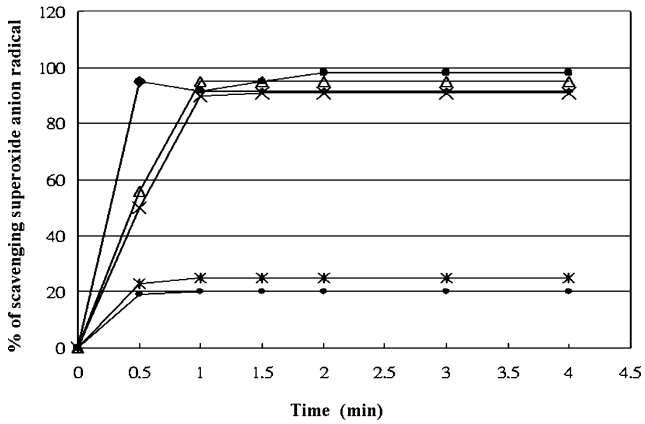

Figure 3. Kinetics of superoxide anion scavenging effects of butylated hydroxytoluene (0.4 mg/mL, ◇), red pummelo juice (0.4 mL/mL, △), white pummelo juice (0.4 mL/mL, ×), freeze-dried red pummelo flesh (0.4 mg/mL, ∗), and freeze-dried red pummelo peel (0.4 mg/mL, ●) extracted with methanol and vitamin C (0.4 mg/mL, ■) dissolved in water at 4 min.

DISCUSSION

Red pummelo is a new variety of fruit being promoted in Taiwan. This paper represents one of only a few reports on its composition, particularly with regard to the phytochemical content of red pummelo juice and fruit including its peel. In recent years there has been an inreasing focus on the components of food and their relative contributions to antioxidant potential

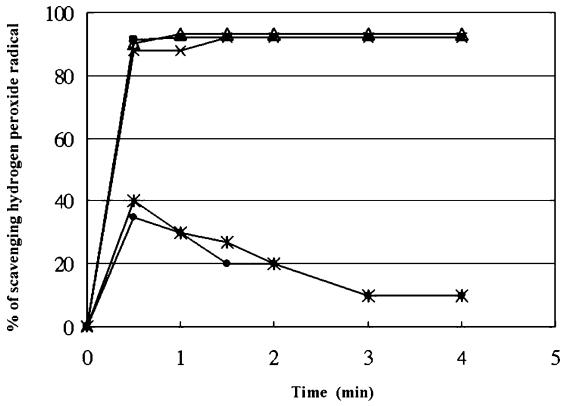

Figure 4. Kinetics of H_2O_2 scavenging effects of red pummelo juice (0.4 mL/mL, △), white pummelo juice (0.4 mL/mL, ×), freeze-dried red pummelo flesh (0.4 mg/mL, ∗), and freeze-dried red pummelo peel (0.4 mg/mL, ●) extracted with methanol and vitamin C (0.4 mg/mL, ■) dissolved in water at 4 min.

effects. The total phenolic content has been shown to be the main bioactive component for health benefits. The correlation between the total phenolic content and the total antioxidant potential of fruit juices is very high ($R^2 = 0.94$) (*21*). Other studies have also shown that increased levels of total phenolic content bring increased antioxidant ability (*10*). In our study, the total phenolic content in RP juice was similar to that of other citrus juices reported by Rapisarda et al. (*22*). Furthermore, RP juice had significantly higher total phenolic content than the WP juice, indicating that RP juice has a higher antioxidant ability than WP juice. Carotenoids, yellow and orange coloring pigments, are lipid-soluble antioxidants capable of inhibiting singlet oxygen in biological systems (*23*). The typical red color of the red-orange peel and pulp is due to these pigments. The content of carotenoids in RP, which is also responsible for its characteristic color, was significantly higher than that found in WP. Anthocyanins are a class of flavonoids and a large family of polyphenolic compounds synthesized by plants. It has been reported that anthocyanins and other flavonoids in fruits may be responsible for the antioxidant capacity (*24*). In our study, anthocyanins and flavonoids were not significantly different between fresh RP and WP juice.

Vitamin C is a powerful antioxidant found in many fruits. A strong positive relationship between the amount of vitamin C, but not of total phenolics and bioflavonoids, and total antioxidant activity has been reported (*25*). We found that RP juice contained much more vitamin C than the white variety. This result indicated that RP juice had a higher total antioxidant activity than WP juice. However, vitamin C is unstable to heat. In this study, we found that less (40%) vitamin C in RP juice was destroyed than in WP juice (50%) after heating at 80 °C for 1 min. Tocopherol is known to be heat-stable and is present in numerous fruits, including citrus fruits. Small amounts of δ-tocopherol in RP and WP juice were not significantly different from each other. δ-Tocopherols appeared in lower concentrations than other antioxidants of the pummelo fruit, although still within the ranges found in most fruits and vegetables (*26*). α-Tocopherol, the most abundant tocopherol in pear flesh (*27*), was not detectable in pummelo.

Freeze-drying is one of the dehydration techniques used to manufacture high-value fruit snacks in the market. The compositions of freeze-dried RP flesh (FRPF) and peel (FRPP) were compared, and the results showed that the FRPP had significantly higher fat, ash, and fiber content than the flesh. The fat content of FRPP (14.7%) can be extracted and purified to a product like other citrus peel oils functioning as a natural flavor, anxiolytic, cancer-preventive, and antimicrobial substance (*28*, *29*). The results of this study showed that FRPP was high in crude fiber, and the content of dietary fiber in peel would be higher, as well. These results are in accordance with other reports that fruit peels are good sources of dietary fiber (*30*). In the methanol extracts, the total phenolic contents of both FRPP (22 mg/g) and FRPF (12 mg/g) were higher than that of black raspberry (1.53 mg/g dry weight) reported by Wang and Lin (*31*). Our finding the FRPP had a higher total phenolic content than that in the FRPF was consistent with that of another study that citrus peels are good sources of phenolic compounds (*32*). The FRPP contained almost twice as much carotenoids as FRPF, further showing that carotenoids are mostly located in the skin of fruits and vegetables (*33*). Vitamin C in FRPF was significantly higher than that in FRPP. These results were similar to the results for orange and grapefruit showing higher vitamin C content in the flesh than in the peel (*34*). Only small amounts of δ-tocopherol were found in FRPP and FRPF without any significant differences.

Each antioxidant vitamin has a unique free radical scavenging mechanism. Vitamin C, a water-soluble ketoacetone, plays an important role in the suppression of superoxide radicals by blocking catecholamine autoxidation (*35*). α-Tocopherol is lipid soluble and can block peroxyl-mediated chain reactions. The radical scavenging ability of flavonoids and phenolics can reduce peroxyl radicals through their properties as electron or hydrogen atom donors (*36*). Pietta et al. (*37*) suggested that the radical scavenging activity of flavonoids depends on the structure and substituents of the heterocyclic rings and the B ring. It is well-known that reactive oxygen species (ROS) accelerate lipid peroxidation and the basic cause of many diseases, including superoxide anion, hydrogen peroxide, etc. DPPH, a stable free radical, is widely used as a model system to investigate the scavenging activity of natural compounds such as phenolic compounds, anthocyanins, or crude mixtures such as ethanol extracts. Brand et al. (*38*) showed that the scavenging rate of free radicals can be divided into fast, medium, and slow. Usually, DPPH radical scavenging activity is detected after 30 min of reaction. The scavenging ability within a short reaction time is not known. Most free radicals are reactive and short-lived, and in order to monitor their reactions, it has been suggested that the fast kinetic method be used to obtain a better understanding of the antioxidant ability (*39*). Hence, the activities of anti-DPPH, anti-hydrogen peroxide, and anti-superoxide anion radicals of the red pummelo were determined.

Recent studies reported that citrus and noncitrus fruits (e.g., blueberries) are the most powerful antioxidant foods (*40*, *41*), and citrus peel has antioxidant ability (*42*). In our study the radical scavenging activities of RP and WP juices at the concentration of 0.4 mL/mL were similar to those of BHA and vitamin C at the concentration of 0.4 mg/mL. All antiradical activity tests conducted in this study showed that juices had higher levels of activity than freeze-dried peels and flesh. These results were similar to those of others (*43*) who found that fresh juice contains high concentrations of bioactive substances (mainly antioxidant compounds) and possesses high antioxidant ability. Red pummelo peel was found to contain higher phytochemical content than its flesh, although its radical scavenging activity was almost the same. In this study, the vitamin C content of RP or WP juice was significantly higher than that in its freeze-dried products. Thus, the radical scavenging ability of juice can be attributed to vitamin C in addition to other natural bioactive compounds, although the phenolics in

the fruits were found to be more active antioxidants than vitamin C in the bioactive substances (*44*). In addition, there a study has reported that freeze-dried treatment significantly reduced bioactive compounds (lycopene, *d*-limonene, and myrcene content) of grapefruit (*45*). This can explain the lower antioxidant activity in the freeze-dried products than in the fresh juice in our study. Therefore, our results indicated that RP juice exhibited great efficiency in scavenging DPPH and reactive oxygen radicals (superoxide anion and hydrogen peroxide) with a rapid rate.

In conclusion, improved knowledge of the composition, analysis, and properties of red pummelo peel might assist in the promotion and utilization of this fruit. Red pummelo juice is an excellent source of antioxidants including vitamin C, total phenolics, and carotenoids and exhibits an excellent scavenging ability for different forms of free radicals including DPPH, superoxide anion, and hydrogen peroxide free radicals, similar to the abilities exhibited by BHA and vitamin C. Freeze-dried flesh and peel of red pummelo are good sources of total phenol and δ-tocopherol. The vitamin C and other natural bioactive compounds in red pummelo juice may be responsible for its high antiradical activity.

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