

## Total Antioxidant Activity and Fiber Content of Select Florida-Grown Tropical Fruits

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Fourteen tropical fruits from south Florida (red guava, white guava, carambola, red pitaya (red dragon), white pitaya (white dragon), mamey sapote, sapodilla, lychee, longan, green mango, ripe mango, green papaya, and ripe papaya) were evaluated for antioxidant activity, total soluble phenolics (TSP), total ascorbic acid (TAA), total dietary fiber (TDF), and pectin. ORAC (oxygen radical absorbance capacity) and DPPH (1,1-diphenyl-2-picrylhydrazyl, radical scavenging activity) assays were used to determine antioxidant activity. The TSP, ORAC, and DPPH ranged from 205.4 to 2316.7 g gallic acid equiv/g puree, <0.1 to 16.7  $\mu$ mol Trolox equiv/g puree, and 2.1 to 620.2  $\mu$ g gallic acid equiv/g puree, respectively. The TAA, TDF, and pectin ranged from 7.5 to 188.8 mg/100 g, 0.9 to 7.2 g/100 g, and 0.20 to 1.04 g/100 g, respectively. The antioxidant activities, TSP, TAA, TDF, and pectin were influenced by cultivar (papaya, guava, and dragon fruit) and ripening stage (papaya and/or mango). Antioxidant activity showed high correlations with levels of TSP compounds ( $r = 0.96$ ) but low correlations with levels of ascorbic acid ( $r = 0.35$  and  $0.23$  for ORAC and DPPH data, respectively). The antioxidant activities evaluated by both ORAC and DPPH showed similar trends where red guava and carambola exhibited the highest and sapodilla and green papaya exhibited the lowest levels. Guava and mamey sapote exhibited the highest TDF and pectin levels. Many of the tropical fruits were shown to contain an abundance of hydrolyzable tannins, ellagic acid conjugates, and flavone glycosides. Preliminary descriptions are given of the phenols in red/white pitaya (dragonfruit), lychee, and mamey sapote, these fruit being thus far uncharacterized in the literature.

**KEYWORDS:** ORAC; DPPH; total phenolic; ascorbic acid; galacturonic acid

### INTRODUCTION

Limited information on the nutritional value of tropical fruits, especially the more exotic species, is available. There are reports for various Asian (1, 2) and African (3) varieties, which are often different from those grown in Florida, analyzed by different methods and cultivated under different conditions. In this study, select tropical fruits from south Florida were analyzed for components that could be beneficial to human health using standard methods.

The function of natural antioxidants and dietary fiber in foods and biological systems has received much attention. Fruits and vegetables play a significant role in the human diet providing protection against cellular damage caused by exposure to high levels of free radicals (4–6), while also aiding digestion (7, 8).

This is attributed to the fact that these foods provide an optimal mix of antioxidants such as vitamin C and E, polyphenols, carotenoids (9–11), and complex carbohydrates (7).

Recently, several methods have been developed to measure total antioxidant activity, based on different reaction mechanisms such as Trolox equivalent antioxidant capacity (TEAC) (12), oxygen radical absorbance capacity (ORAC) (13), ferric ion reducing antioxidant parameter (FRAP) (14, 15), 1,1-diphenyl-2-picrylhydrazyl (DPPH) (15), and total radical trapping antioxidant parameter (TRAP) (16). On the basis of chemical principles, the ORAC assay is closely related to biological functions of chain-breaking antioxidants (17) and has been used extensively to evaluate antioxidant activity in fruits and vegetables (18–22), and thus, it is useful for comparison of data to other studies. The DPPH assay is also a simple and accurate method for measuring antioxidant levels of fruit (2, 15) and was used along with the ORAC assay for comparison of the peroxy and DPPH radicals for determination of antioxidant activity.

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Phenolic compounds account for a major portion of the antioxidants in many plants (23). Ascorbic acid (AA) is also abundant in many fruits, and it is believed that the role of AA in disease prevention is due to its ability to scavenge free radicals in biological systems (24). It has been reported that the contributions of phenolic compounds to antioxidant activities were much greater than those of vitamin C (18). Therefore, total soluble phenolics (TSP) and total ascorbic acid (TAA) were also assayed to determine if they contributed to antioxidant activity.

Dietary fibers in foods are also beneficial for good health. Physiological impacts of insufficient dietary fiber intake are constipation, increased risk of coronary heart disease, and increased fluctuation of blood glucose and insulin levels (7, 25). Including fruits and vegetables in the human diet may be beneficial, based on their dietary fiber content, with regard to some cancers (8, 26). The National Research Council (27) set Dietary Reference Intakes for the first time for dietary fiber determining that "adequate intakes" (AI) for dietary fiber be based on 14 g dietary fiber per 1,000 calories. The Food and Drug Administration (FDA) (28) set a daily reference value (DRV) on food labels for fiber at 25 g for a 2,000-calorie diet. Five grams or more fiber per serving is considered a significant amount.

Dietary fiber is largely composed of complex carbohydrates that are somewhat resistant to digestion. One major component of soluble fibers is pectin, which is largely composed of uronic acid residues such as galacturonic acid. Pectin and other soluble polysaccharides may undergo some metabolism in the small intestine and especially in the large intestine through bacterial enzymes, converting it to products that contribute to maintaining the colonic microflora, which is beneficial to digestion (8, 29, 30). Insoluble fiber like cellulose, found in plant cell walls, can aid in waste and toxin removal through several mechanisms (8).

The objective of this study was to obtain nutritional information for Florida-grown tropical fruits (red guava, white guava, carambola, red dragonfruit, white dragonfruit, mamey sapote, sapodilla, lychee, longan, mango, and papaya) in terms of antioxidant activity, TSP, TAA, total dietary fiber (TDF), and pectin. Because different ethnic groups prefer different maturity stages of some fruits, like mango and papaya, both ripe and green stages were assayed. In addition, qualitative analyses by high-pressure liquid chromatography with a photodiode array mass spectrometry (HPLC-PDA-MS) of the phenolic constituents in selected tropical fruit were conducted, especially in those where no analyses had been so far reported.

## MATERIALS AND METHODS

**Fruit.** Fourteen different tropical fruits from south Florida including red guava (*Psidium guajava* L., cv. Sardina), white guava (*Psidium guajava* L., Thai cultivar), carambola (*Averrhoa carambola* L., cv. Arkin), red pitaya (red dragon fruit, *Hylocereus* sp., cv. Red Jaina), white pitaya (white dragon fruit, *Hylocereus* sp., cv. David Bowie), mamey sapote (*Pouteria sapota*, cv. Pantin), sapodilla (*Achras (manilkara) zapota*, cv. Brown Sugar), lychee (*Litchi chinensis*, cv. Mauritius), longan (*Dimocarpus longana*, cv. Kohala), green and ripe mango (*Mangifera indica*, cv. Keitt), green papaya (*Carica papaya*, cv. Exp. 15, a variety that is produced for the green papaya market), and ripe papaya (*Carica papaya*, cv. Red Lady, a variety that is produced for the ripe papaya market) were obtained from Florida tropical fruit growers. A composite of at least 10 fruits were combined per each of three replicate samples. The edible portion of the fruit was cut, flash frozen in liquid nitrogen, and kept at  $-20^{\circ}\text{C}$  until analysis.

**Extraction and Analysis of TSP.** Fruit puree (20 g) was homogenized with 80 mL of methanol (100%) in an explosion proof blender

(Waring commercial) for 1 min and filtered through filter paper (No. 1, Whatman Inc.). The residue was re-extracted and filtered. The extracts were combined and concentrated to a volume of 40 mL using a rotary evaporator under partial vacuum at  $40^{\circ}\text{C}$ . Three replicates of initial fruit puree were extracted. Measurement of TSP was performed with the Folin-Ciocalteu assay as described by Sellappan et al. (31) with minor modifications using a 96-well microplate reader (Power Wave 340 microplate reader; KC4 version 3.01 software, BioTek Industries). The results were calculated on the basis of the standard curve for gallic acid and expressed as micrograms gallic acid equivalents per gram fresh weight.

**Measurement of DPPH Radical Scavenging Activity.** The stable organic nitrogen radical DPPH is commercially available, and assay time may vary from 10–20 min up to about 6 h (15, 32–34). Measurement of DPPH radical scavenging activity was performed as described by Manthey (35) with some modifications. The assay was performed in a 96-well microplate reader (Power Wave 340 microplate reader with KC4 version 3.01 software, BioTek Industries), and the reduction of absorbance at 517 nm was monitored at 0 min and every 5 min until the reaction reached a plateau. The remaining DPPH at completion of the reaction was determined and quantified as the DPPH radical scavenging activity using a gallic acid standard curve. The DPPH radical scavenging activity was expressed as micrograms gallic acid equivalents per gram fresh weight.

**Measurement of ORAC.** The ORAC assay was initially developed by Cao et al. (36) and improved by Ou et al. (37). Antioxidant activity was determined using the ORAC assay as described by Talcott et al. (38) for a 96-well microplate reader. Fluorescence loss was monitored on a Molecular Devices Fmax (Sunnyvale, CA) 96-well fluorescent microplate reader following appropriate dilution of each isolate, and data are expressed in micromole Trolox equivalents per gram fresh weight.

**Acid Hydrolysis of Phenolic Glycosides.** Glycosidic linkages were hydrolyzed as described previously (39) with minor modification. Methanolic extracts (50 mL) of fruit puree were hydrolyzed by refluxing for 1 h in 50 mL of 2 N hydrochloric acid. The solutions were cooled and extracted with chloroform to recover the hydrolyzed flavonoid aglycons. The chloroform extracts were concentrated by vacuum rotary evaporator for analysis by HPLC-MS.

**Qualitative Analysis of Phenols in Fruit Pulp.** Phenolic constituents in tropical fruit have previously been analyzed by HPLC-PDA and HPLC with an electrospray ionization (ESI) mass spectrometer (40–43). The phenolic compounds in the current study were analyzed in the fruit pulp with a Waters (Milford, MA) Alliance high-pressure liquid chromatographer, equipped with a Waters 996 PDA detector and a Waters/Micromass ZQ single-quadrupole mass spectrometer. Separation of the phenols was accomplished on a  $250 \times 4.6$  mm i.d. RP-Amide C16 (Supelco) column, with multistep linear water/acetonitrile/2% formic acid gradients at flow rates of 0.75 mL/min. Initial solvent conditions were 85:10:5 (water/acetonitrile/2% formic acid), which increased in linear gradients to 81:14:5 in 15 min, to 77:18:5 at 20 min, to 70:25:5 at 30 min, to 40:55:5 at 55 min, and to 0:95:5 at 67 min. The solvents were then held isocratic at 0:95:5 for 13 min. The chromatograms were recorded at 285 and 320 nm. PDA detection was monitored between 230 and 600 nm. Data handling was done with MassLynx software version 3.5 (Micromass, Division of Waters Corp., Beverly, MA). The postcolumn split to the PDA and mass ZQ detector was 10:1. MS parameters were as follows: ionization mode, ESI+; capillary voltage, 3.0 kV; extractor voltage, 5 V; source temperature,  $100^{\circ}\text{C}$ ; desolvation temperature,  $225^{\circ}\text{C}$ ; desolvation  $\text{N}_2$  flow, 465 L/h; cone  $\text{N}_2$  flow, 70 L/h; scan range,  $m/z$  150–900; scan rate, 1 scan/s; and cone voltages, 20, 40, and 60 eV. Ellagic acid, quercetin, and kaempferol glycosides and the hydroxycinnamates were tentatively identified on the basis of the molecular weights of aglycon fragment ions recorded by the ESI-MS and previously reported UV spectra (44, 45).

**Determination of TAA.** The TAA was assayed as previously described with some modification (46). The fruit puree (25 g) was blended with 25 mL of 0.05 N  $\text{H}_3\text{PO}_4$  for 3 min. The slurry was centrifuged, and the supernatant was collected and made to 50 mL with the extracting solvent. The extract was purified by passing 3 mL through

a disposable C18 Sep-Pak cartridge (Waters Associates), preconditioned by flushing with methanol (2 mL) followed by deionized (DI) water (5 mL), and a 0.45  $\mu\text{m}$  Millipore filter prior to injection. The AA and dehydroascorbic acid (DHAA) were determined by using HPLC with an organic acid column (OA-1000, 9  $\mu\text{m}$ , 300 mm  $\times$  6.5 mm, Alltech Associates, Inc.). An autosampler (Perkin-Elmer Series 200) with an isocratic pump was used. The column was equilibrated with the mobile phase 0.01 N  $\text{H}_2\text{SO}_4$ . The flow rate and temperature were 0.2 mL/min and 35  $^\circ\text{C}$ , respectively, with 45 min total run time. Detection of the acids was performed at 215, 260, and 295 nm using a PDA detector scanning from 200–500 nm (ThermoFinnigan Spectra System P4000). EZChrom software (Agilent Technologies) was used for data integration and quantification. The quantification of the acids was determined by comparing the peak areas with AA and DHAA standards at five levels by linear regression at all three wavelengths. Although AA absorbs UV light strongly at  $\lambda_{\text{max}} = 245$  nm, direct spectrophotometric analysis is precluded by the many other chromophores found in some of the fruits. DHAA absorbs only weakly at its  $\lambda_{\text{max}} = 300$  nm. Detection of both AA and DHAA was performed at 260 and 295 nm (carambola, dragon fruit, longan, lychee, and mamey sapote), respectively, and in some fruits DHAA was read at 260 nm (guava, mango, and papaya) to avoid interference. In other fruits detection of DHAA was performed at 215 and 260 nm (sapodilla), obtaining the difference in absorbance between 215 and 260, because interfering carboxylic acids absorb at 215 nm. The principle of differential spectrophotometry is frequently utilized in clinical studies for quantification in the presence of interfering compounds (46, 47).

**Sample Preparation for Pectin Content.** The sample preparation method was according to Theander (48) with some modification. Triplicate 500 mg dry fruit puree samples were transferred into 50 mL polypropylene centrifuge tubes (Beckman Instruments, Inc.). Acetate buffer (5 mL) was added at pH 5.0 along with 40 L of  $\alpha$ -amylase (heat stable  $\alpha$ -amylase from *Bacillus amyloliquefaciens*, Sigma-Aldrich, Inc.). The solution was mixed, placed in a boiling water bath for 1 h, and cooled to 40  $^\circ\text{C}$ . A 500  $\mu\text{L}$  amyloglucosidase solution (from *Aspergillus niger*) was added, and tubes were then incubated overnight in a 60  $^\circ\text{C}$  water bath equipped with shaker (shaker water bath Gallenkamp model BKS-350). Tubes were again cooled, 21.0 mL of absolute ethanol was added, and the tubes were mixed using a Vortex mixer. Tubes were refrigerated for 1 h at 4  $^\circ\text{C}$  and then centrifuged 10 min at 5000 rpm (Avanti J-E centrifuge, Beckman Coulter) or until a clear supernatant liquid was obtained. The supernatant was discarded, and the pellet washed twice by suspending and recentrifuging with 20 mL of 80% ethanol and then twice with 15 mL of acetone. The resulting residue was allowed to dry overnight in a 40  $^\circ\text{C}$  oven and then was dispersed with 3 mL of 12 M  $\text{H}_2\text{SO}_4$ . The mixture was incubated in a 30  $^\circ\text{C}$  water bath for 1 h, stirring occasionally. The solution was hydrolyzed in an autoclave for 1.15 h at 121  $^\circ\text{C}$  and filtered through a glass-fritted crucible, and the residue was washed with 10 mL of DI water. Filtrates were brought to a volume of 100 mL with DI water at room temperature. The hydrolyzed solution was then passed through a Sep-Pak C-18 (Waters Corp.) column to remove pigments and stored at 4  $^\circ\text{C}$  until galacturonic acid analysis.

**Galacturonic Acid Determination.** The determination of galacturonic acid in the hydrolyzed samples was optimized from the original method of Scott (49) by Luzio (50) using a microplate reader (Power Wave 340 microplate reader with KC4 version 3.01 software, BioTek Industries). The determination was performed in triplicate for each of the hydrolyzed samples, therefore nine replicates were obtained per fruit type. Three hundred microliters of sample was added with DI water to bring the volume up to 700  $\mu\text{L}$ . Three milliliters of concentrated sulfuric acid (96.2% Baker Analyzed 9681-33, J.T. Baker, Inc.) containing 0.1% NaCl was added to each tube and immediately vortexed for 15 s. Each tube was then immediately placed on ice and allowed to cool before transferring to a solution basin. From each solution basin, 240  $\mu\text{L}$  was pipetted into individual wells of a microplate, which had been preheated to 75  $^\circ\text{C}$  in the block heater (VWR Standard Heat Block-13259-032). The plate was then heated for 20 min at 75  $^\circ\text{C}$ , removed, cooled in a water bath at room temperature for 20 min, and then read at 450 nm using a microplate reader for background absorbance. Forty microliters of 3,5-dimethylphenol (DMP) solution (0.2 g of DMP,

Sigma-Aldrich, in 100 mL of glacial acetic acid, Fisher Chemical) was then added to each well, the microplate was shaken for 35 s on a microplate shaker (Cole Parmer), and then the wells were read at 450 nm using the microplate reader. A galacturonic acid standard curve was performed in duplicate. To separate test tubes, 0, 100, 200, 300, 400 and 500  $\mu\text{L}$  of a 0.02% galacturonic acid solution was added along with DI water to bring the total volume up to 700  $\mu\text{L}$  in each tube. The assay was performed, and when the pectin content was determined on the basis of calibration with galacturonic acid, an adjustment factor of 0.81 was used in the calculation (48).

**TDF Assay.** The assay was based on the method published in the 16th Edition of the *Official Methods of Analysis* of the Association of Official Analytical Chemists (AOAC) (51) using the total dietary fiber assay kit from Sigma-Aldrich (TDF 100A).

**Statistical Analysis.** Correlations and 95% confidence intervals were determined using Excel (Microsoft, Inc).

## RESULTS AND DISCUSSION

**Antioxidant Activity Assays.** There are many assays using different substrates, reaction kinetics, and analytical methods to evaluate antioxidant activity (12–16). For this reason the data obtained by different researchers are often difficult to compare and interpret. Nevertheless, antioxidant activity of fruit has been extensively reported using the ORAC method (18–22). An advantage of the ORAC assay is that the peroxy radical used in the assay is the predominant free radical found due to lipid oxidation in biological systems (52, 53). Because antioxidant assays are based on different reaction mechanisms, antioxidant activity using free radical DPPH was also employed as a comparison to ORAC.

**TSP, TAA, and Antioxidant Activity.** The selected Florida tropical fruits had widely different levels of TSP, TAA, and antioxidant activity. The TSP, TAA, ORAC, and DPPH ranged from 205.4 to 2316.7  $\mu\text{g}$  gallic acid equiv/g puree, 7.5 to 188.8 mg/100 g puree, less than 0.1 to 16.7  $\mu\text{mol}$  Trolox equiv/g puree, and 2.1 to 620.2  $\mu\text{g}$  gallic acid equiv/g puree, respectively (Table 1).

The antioxidant activities evaluated by both ORAC and DPPH showed similar trends with a correlation coefficient of  $r = 0.91$  (Table 1 and Figure 1A). Similar trends were observed between the 2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid (ABTS) and the DPPH assay ( $r = 0.90$ ) in fruits (2) and between FRAP and ORAC in beet ( $r = 0.96$ ) and carrot ( $r = 0.78$ ), but there is little or no relationship among these assays in other vegetables (e.g., green pepper, red pepper, and cauliflower) (17). Although a significant correlation between ORAC and DPPH was observed in the present study, differences in the ranking order of antioxidant activity of fruit by ORAC and DPPH assays were also observed due to the response variations in the antioxidant constituents of the fruit to the radical sources (peroxy radical or DPPH radical) in the assays. These results imply that a correlation may partly result from a similar reaction mechanism, but it is not always in complete agreement due to the different radicals in the respective assays and diverse group of antioxidants found in different fruits.

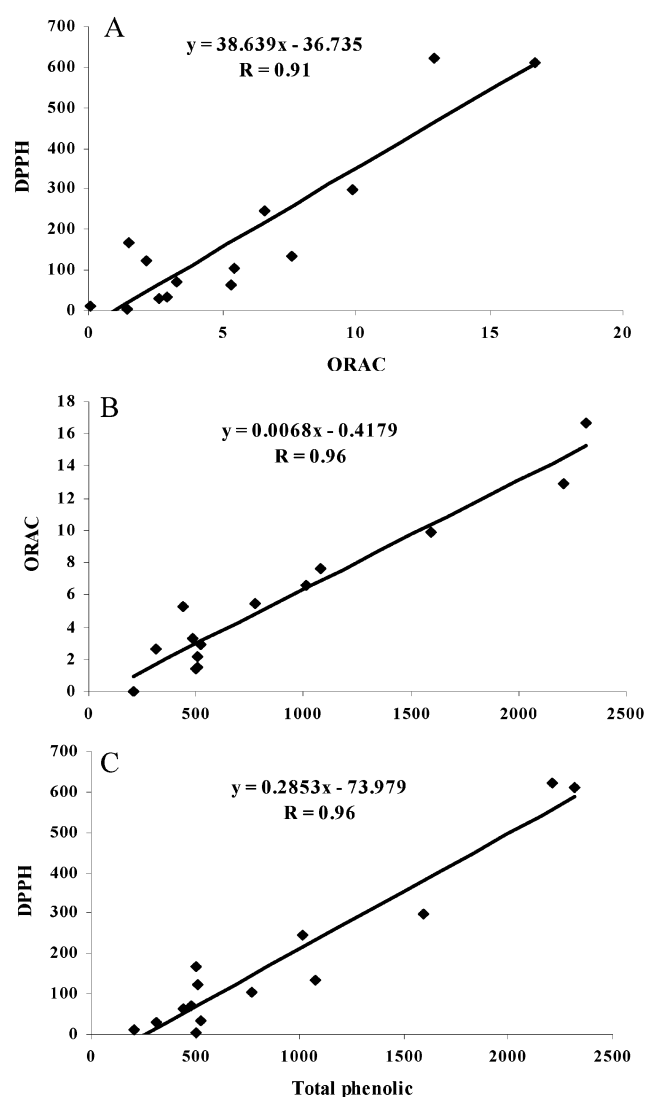
Carambola and red guava had the highest antioxidant activity of the selected Florida fruit while sapodilla and green papaya (cv. Exp. 15) had the lowest (Table 1). Leong and Shui (2) reported similar results showing carambola and guava having high antioxidant activity among 27 fruits studied in Singapore markets except that sapodilla (unripe) was ranked highest in antioxidant activity in that study. The present study investigated ripe sapodilla, because unripe fruit are considered unsuitable for consumption due to their astringent taste. The astringency of the unripe sapodilla is likely due to the high tannin content,



**Table 1.** Moisture (%), TSP, TAA, and Antioxidant Activity by ORAC and DPPH for Selected Florida Grown Tropical Fruits<sup>a</sup>

fruit	% moisture	TSP ( $\mu\text{g GA/g puree}$ )	TAA ( $\text{mg}/100 \text{ g puree}$ )	ORAC ( $\mu\text{M TE/g puree}$ )	DPPH ( $\mu\text{g GA/g puree}$ )
red guava	85.3	2316.7 $\pm$ 167.6	122.3 $\pm$ 15.0	16.7 $\pm$ 0.6	609.3 $\pm$ 31.9
carambola	91.4	2207.7 $\pm$ 156.7	16.9 $\pm$ 1.6	12.9 $\pm$ 1.0	620.2 $\pm$ 40.9
white guava	87.1	1589.3 $\pm$ 75.4	201. $\pm$ 17.4	9.9 $\pm$ 0.7	298.6 $\pm$ 22.6
red dragon	83.6	1075.8 $\pm$ 71.7	55.8 $\pm$ 2.0	7.6 $\pm$ 0.1	134.1 $\pm$ 30.1
mamey sapote	64.5	1010.5 $\pm$ 40.2	7.5 $\pm$ 2.1	6.6 $\pm$ 0.3	247.1 $\pm$ 18.3
lychee	85.1	770.1 $\pm$ 30.1	8.1 $\pm$ 1.5	5.4 $\pm$ 0.2	103.8 $\pm$ 13.8
white dragon	84.7	523.4 $\pm$ 33.6	13.0 $\pm$ 1.5	3.0 $\pm$ 0.2	34.7 $\pm$ 7.3
ripe mango	82.9	508.9 $\pm$ 29.4	92.8 $\pm$ 2.5	2.2 $\pm$ 0.1	123.7 $\pm$ 12.3
green mango	84.9	505.8 $\pm$ 51.8	29.8 $\pm$ 7.4	1.5 $\pm$ 0.2	167.5 $\pm$ 13.4
sapodilla	74.5	501.8 $\pm$ 39.3	11.9 $\pm$ 1.8	1.4 $\pm$ 0.1	2.1 $\pm$ 0.2
longan	82.6	481.9 $\pm$ 37.4	14.0 $\pm$ 0.5	3.3 $\pm$ 0.1	69.6 $\pm$ 19.7
ripe papaya (cv. Red Lady)	89.3	442.2 $\pm$ 29.7	153.8 $\pm$ 12.1	5.3 $\pm$ 0.3	65.1 $\pm$ 15.8
green papaya (cv. Red Lady)	92.4	311.1 $\pm$ 18.9	56.7 $\pm$ 3.5	2.6 $\pm$ 0.2	29.7 $\pm$ 5.4
green papaya (cv. Exp. 15)	93.9	205.4 $\pm$ 35.8	57.2 $\pm$ 1.3	<0.1	10.4 $\pm$ 1.6

<sup>a</sup> Data for TSP, AA, ORAC, and DPPH are  $\pm$ 95% confidence interval.



**Figure 1.** (A) Correlation between ORAC assay-derived antioxidant activity and DPPH assay-derived antioxidant activity; (B) correlation between total phenolic data and antioxidant activity by ORAC assay; and (C) correlation between total phenolic data and antioxidant activity by DPPH assay.

which decreases with ripening and contributes appreciably to the antioxidant activity of the fruit (54). Indeed, this was confirmed by analysis of the phenolic composition (Table 2,

discussed below). Both ripe papaya and mango exhibited higher antioxidant activity (ORAC for both fruit, DPPH for papaya only) and TSP compared to their green counterparts, perhaps due to the increase in TAA and carotenoids as the fruits ripened. Jimenez-Escrig et al. (55) showed that the DPPH radical can be scavenged by carotenoids. Red and yellow peppers showed higher carotenoid content than green peppers, and red peppers exhibited a higher level of DPPH radical scavenging activity compared to green and yellow peppers (56). Likewise, TAA was higher in ripe mango and papaya than in the respective green fruits. This is in agreement with values reported by Salunkhe and Desai (57) for papaya, but in that study TAA was higher in green than in ripe mango. However, because the carotenoids are not water soluble, they may not react significantly in the polar antioxidant assays used in this study. There may be other compounds synthesized during ripening that increase antioxidant activity in ripened fruit.

There were significant correlations ( $r = 0.96$  by both ORAC and DPPH) between antioxidant activity and TSP (Figure 1B,C), suggesting that phenolic compounds were likely significant contributors to antioxidant activity in the fruit extracts. Red guava and carambola, which exhibited the highest antioxidant activities, also contained high levels of TSP, and while guava was highest also in TAA, carambola was relatively low (Table 1). Some studies have demonstrated a linear correlation between total phenolic content and antioxidant activity by ORAC in fruits and vegetables (18–22), but TSP and antioxidant activity is not correlated across all types of foods (22). The higher antioxidant activity of red compared to white dragon fruit is likely due to the red pigment, betalaines, which has antioxidant activity (58, 59).

The phenolic compositions of tropical fruit have been widely studied (see footnotes to Table 2). Table 2 includes data for fruit analyzed in this study. HPLC-PDA-MS analyses of aqueous methanolic extracts of the pulp of the Florida fruit were consistent with the reported phenols (see footnotes to Table 2). Carambola and red and white guava were particularly rich in hydrolyzable tannins, and for guava there was also ellagic and gallic acid conjugates, as well as flavone glycosides. Similar analyses of the phenols in red and white dragonfruit and in mamey sapote, all of which are uncharacterized in the literature, provided evidence of an abundance of hydroxycinnamates in the former and a flavone glycoside among numerous unidentified phenols in the latter. Our HPLC-MS analysis of lychee pulp showed a number of flavone glycosides, consisting mainly of quercetin and kaempferol. The phenolic compositions of

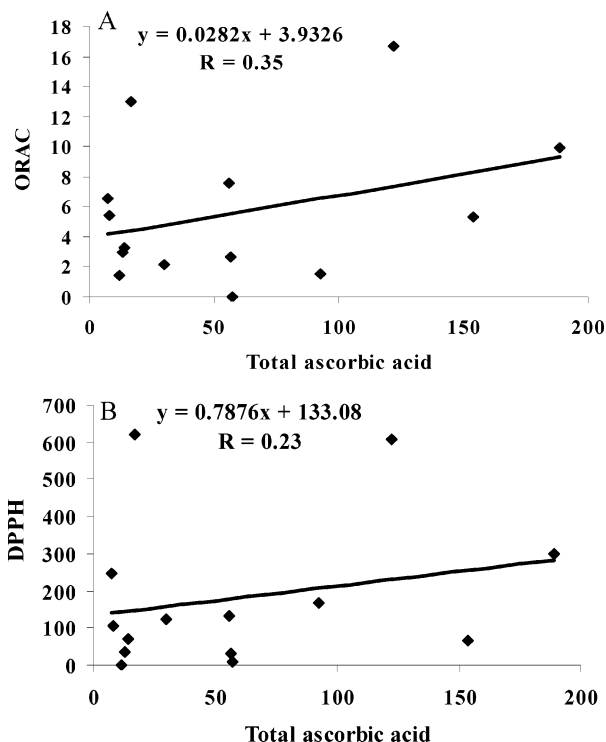
**Table 2.** Phenolic Components of Florida Tropical Fruit Pulp Detected by HPLC-PDA-MS

fruit (pulp)	phenols
red/white guava <sup>a</sup>	ellagic acid, flavone (quercetin) glycosides, gallic acid conjugates
carambola <sup>b</sup>	catechin, proanthocyanidin dimer and trimer conjugates
red/white dragonfruit <sup>c</sup>	hydroxycinnamates
mamey sapote <sup>d</sup>	flavone glycoside
lychee <sup>e</sup>	flavone (quercetin and kaempferol) glycosides
green/ripe mango <sup>f</sup>	mangiferin, gallotannins (tetramers to nonamers)
sapodilla <sup>g</sup>	catechin conjugates
longan <sup>h</sup>	ellagic acid conjugates, flavone (quercetin and kaempferol) glycosides
green/ripe papaya <sup>i</sup>	catechin conjugates

<sup>a</sup> No hydroxycinnamates were detected by HPLC-PDA analysis. Ellagic acid conjugates and quercetin glycosides were detected by comparisons of characteristic UV and MS spectra of quercetin and ellagic acid standards. Literature cited phenols include quercetin, gallic acid, ellagic acid, and guaijaverin-3-arabinoide (66). <sup>b</sup> No flavones or hydroxycinnamates were detected by HPLC-PDA analysis of hydrolyzed and nonhydrolyzed extracts. Spectral comparisons were made using published UV spectra of flavones (39) and hydroxycinnamates (44, 45). Proanthocyanidin conjugates were detected as previously reported (43). Literature cited phenols include catechin, epicatechin, and gallic acid (43). <sup>c</sup> The majority of phenols detected by HPLC-PDA analysis exhibited spectra suggestive of hydroxycinnamates. HPLC-PDA analysis of acid hydrolyzed extracts provided evidence of ferulic acid and other minor hydroxycinnamic acids. Spectral comparisons were made using published UV spectra of hydroxycinnamates (44, 45). No literature cited phenols. <sup>d</sup> No hydroxycinnamates or gallotannins were detected by HPLC-PDA. Spectral comparisons were made using published UV spectra of hydroxycinnamates (44, 45). No literature cited phenols. <sup>e</sup> At least seven flavone glycosides were detected by HPLC-PDA-MS analysis. Negligible hydroxycinnamates and hydrolyzable tannins were detected. No literature cited phenols for lychee pulp. <sup>f</sup> Galloyltannins were detected and characterized by negative ESI using conditions previously described by Berardini et al. (67). HPLC-PDA-MS analysis of acid hydrolyzed of extracts provided detection of quercetin and mangiferin. Literature cited phenols include gallic acid, gallotannins, quercetin, mangiferin, and kaempferol (40, 67). <sup>g</sup> Catechin conjugates were detected by HPLC-PDA analysis. Negligible flavones or hydroxycinnamates were detected. Spectral comparisons were made using published UV spectra of flavones (39) and hydroxycinnamates (44, 45). No galloyltannins were detected by HPLC-PDA-MS using negative ESI (67). Literature cited phenols include catechin, methyl chlorogenate, dihydromyricetin, epicatechin, gallic acid, quercetin, myricetin, and galocatechin (42, 54). <sup>h</sup> Ellagic acid-pentose conjugates and six flavone mono-, di-, and trisaccharides were detected by HPLC-PDA-MS analysis. HPLC-PDA-MS analysis of acid hydrolyzed extracts and flavone standards provided evidence of quercetin and kaempferol. Literature cited phenols include gallic acid, ellagic acid, and corilagin (60). <sup>i</sup> Catechin conjugates were detected by HPLC-PDA analysis. Negligible flavones or hydroxycinnamates were detected. Spectral comparisons were made using published UV spectra of flavones (39) and hydroxycinnamates (44, 45). No galloyltannins detected by HPLC-PDA-MS using negative ESI (67). Literature cited phenols include catechin and chlorogenic acid (68, 69).

mango, sapodilla, and longan pulp have been previously reported to contain hydrolyzable tannins and conjugated hydroxycinnamic, ellagic, and other phenolic acids (40, 42, 60). In this study, mangiferin and galloyltannins, catechin and ellagic acid conjugates, and flavone glycosides were detected in mango, sapodilla, and longan pulp, respectively. HPLC-PDA-MS analyses of ripe and green papaya showed few candidate phenols, other than catechin conjugates, which is consistent with the small number of compounds thus far reported for these fruit (68, 69).

Use of TAA, which includes DHAA and AA, is valid assuming that there is little DHAA in fresh fruit and that fruit

**Figure 2.** (A) Correlation of AA data with ORAC-derived antioxidant activity and (B) correlation of AA data with DPPH-derived antioxidant activity.

vitamin C is present mostly as AA. Only AA contributes to antioxidant activity, however, and in preparation of fruit material, some AA was oxidized to DHAA. Most of the TAA data were generally in agreement with literature values (57, 61–63) (Table 1). For the remaining fruit, correlation coefficients between TAA and the total antioxidant activity by ORAC and DPPH assays was not significant ( $r = 0.35$  and  $0.23$ , Figure 2A,B, respectively), especially compared to the correlation of phenolics with antioxidant activity (Figure 1B,C). Carambola exhibited high TSP, ORAC, and DPPH values yet relatively low levels of TAA compared to guava. This would indicate that carambola is likely to be more beneficial to health than would be indicated from TAA values alone. Wang et al. (18) reported that the contribution of AA to ORAC activity of a fruit was usually less than 15% (based on the AA content and ORAC activity of  $1 \mu\text{mol}$  AA being equivalent to  $0.52 \mu\text{mol}$  of Trolox) (36), except for kiwi fruit and honeydew melon. Leong and Shui (2) reported that the TAA of fruits contribution to scavenge ABTS' varied greatly among species (from 0.06% to 70.2%) but fruits with a high antioxidant activity are more likely to have a low percentage contribution from TAA. Nevertheless, just because there was not a good correlation between TAA and antioxidant activity does not mean that TAA was not a major source of the actual antioxidant activity. There is possible interaction of compounds in the fruit extract (including phenolic compounds) with the AA resulting in its not reacting fully with the radicals in the respective assays. On the other hand, according to the literature values (Table 3), TSP does not always result in high ORAC values, for example, cantaloupe, pepper, banana, and kiwi fruit (22). This is probably due to the type of the phenolic compounds in the TSP. Determination of TSP and TAA contribution could theoretically be made by multiple regression (64); however, the extracts of TSP and TAA were prepared separately. In addition, TSP values may be over-inflated because they can include some AA and even reducing sugars. It has been reported that AA and metals such as iron or

**Table 3.** Antioxidant Activity (ORAC) and Total Phenolics of Other Fruits and Vegetables<sup>a</sup>

fruit	ORAC (literature) ( $\mu$ M TE/g puree)	TSP (literature) ( $\mu$ g GA/g puree)
cucumber <sup>b</sup>	1.1	240
mango <sup>d</sup>		560
cantaloupe <sup>b</sup>	3	1240
tomato <sup>b</sup>	3.1	800
papaya <sup>d</sup>		576
celery <sup>b</sup>	5.3	560
lychee <sup>d</sup>		288
pepper <sup>b</sup>	5.4–9.6	2710–5660
onion <sup>b</sup>	5.9–11.3	740–1260
nectarine <sup>b</sup>	7.2	1070
banana <sup>b</sup>	8.1	2310
kiwi fruit <sup>b</sup>	8.9	2780
guava <sup>c,d</sup>		1600, <sup>c</sup> 2473 <sup>d</sup>
grape, green <sup>b</sup>	11.2	1450
carrot <sup>b</sup>	11.6	1250
grape, red <sup>b</sup>	12.6	1750
carambola <sup>c,d</sup>		1260, <sup>c</sup> 2099 <sup>d</sup>
broccoli <sup>b</sup>	14.8	3370
grapefruit, red <sup>b</sup>	15.1	2140
guava <sup>c,d</sup>		1240, <sup>c</sup> 1264 <sup>d</sup>
orange <sup>b</sup>	17.85	3370
spinach <sup>b</sup>	22.2	2170
apple <sup>b</sup>	25.7–38.6	2110–3410
strawberry <sup>b</sup>	35.4	3680
raspberry <sup>b</sup>	47.6	5040
blueberries <sup>b</sup>	61.8–92.1	5310–7950

<sup>a</sup> Cultivar names given if known. Fruits are ranked from low to high ORAC values. <sup>b</sup> Ref 22. <sup>c</sup> Ref 70. <sup>d</sup> Ref 3.

**Table 4.** Pectin and TDF of Selected Florida-Grown Tropical Fruits<sup>a</sup>

fruit	% moisture	TDF (g/100 g fruit)	pectin (g/100 g fruit)
guava (red)	85.3	7.2 ± 0.0	1.04 ± 0.02
mamey sapote	64.5	6.1 ± 0.0	0.77 ± 0.02
sapodilla	74.5	4.4 ± 0.1	0.35 ± 0.01
guava (white)	87.1	4.0 ± 0.1	0.77 ± 0.01
dragon (red)	83.6	3.2 ± 0.1	0.27 ± 0.01
papaya	93.9	2.1 ± 0.0	0.60 ± 0.02
(green, cv. Exp.15)			
papaya	92.4	1.8 ± 0.0	0.51 ± 0.01
(green, cv. Red Lady)			
mango (green)	84.9	1.6 ± 0.0	0.48 ± 0.01
lychee	85.1	1.6 ± 0.0	0.48 ± 0.01
papaya	89.3	1.5 ± 0.1	0.49 ± 0.01
(ripe, cv. Red Lady)			
mango (ripe)	82.9	1.4 ± 0.0	0.51 ± 0.01
carambola	91.4	1.3 ± 0.0	0.27 ± 0.01
dragon (white)	84.7	1.1 ± 0.0	0.12 ± 0.00
longan	82.6	0.9 ± 0.0	0.20 ± 0.00

<sup>a</sup> Data are ±95% confidence interval.

copper can create de novo radicals in the system (65) and that concentration of vitamin C alone exhibited significant linear correlation with ORAC values (36). In any case, these models and data dispel the assumption that foods must have high vitamin C content to have significant antioxidant activity. For example, compare longan and carambola which have TAA levels of 14.0 and 16.9 mg/100 g. Although their TAA levels are similar, carambola has about 4 times the ORAC value and about 10 times the DPPH value.

**Comparison of Antioxidant Data to Literature Values.** Because ORAC had been extensively used to evaluate antioxidant activity of fruits and vegetables, the data from this study were compared to published ORAC values. We selected ORAC studies that used fluorescein (22) as the fluorescent probe rather

**Table 5.** Pectin and TDF of Other Fruit<sup>a</sup>

fruit	TDF (g/100 g) (literature)	fruit	pectin (g/100 g) (literature)
longan <sup>b</sup>	0.19		
pineapple <sup>c</sup>	0.54		
mango <sup>b</sup>	0.86		
cantaloupe <sup>b</sup>	0.88		
grape <sup>b</sup>	0.88	grapes <sup>d</sup>	0.70–0.80
green mango (cv. Kaew) <sup>c</sup>	1.27		
lychee <sup>e</sup>	1.32		
pineapple <sup>f</sup>	1.42		
persimmon <sup>c</sup>	1.48		
peaches <sup>f</sup>	1.53		
grapefruit <sup>f</sup>	1.63	grapefruit (cv. Marsh) <sup>d</sup>	0.65
rambutan <sup>c</sup>	1.64		
papaya <sup>f</sup>	1.79		
mango <sup>f</sup>	1.79		
mango <sup>e</sup>	1.82		
strawberries <sup>f</sup>	1.99		
dragon fruit <sup>b</sup>	2.14		
lychee (cv. Hong Hua) <sup>c</sup>	2.20		
orange <sup>f</sup>	2.39	oranges <sup>d</sup>	0.57
apple <sup>f</sup>	2.39	apple (cv. Golden Delicious) <sup>d</sup>	0.25–0.63
blueberries <sup>f</sup>	2.41		
banana <sup>f</sup>	2.60	banana (ripening, cv. Williams) <sup>d</sup>	0.44–1.02
guava <sup>b</sup>	2.70		
carambola <sup>f</sup>	2.78		
mamey sapote <sup>e</sup>	3.00		
pear <sup>f</sup>	3.07		
ripe mango (cv. Keaw) <sup>c</sup>	3.10		
blackberries <sup>f</sup>	5.28		
sapodilla <sup>e</sup>	5.31		
guava <sup>e</sup>	5.39		
guava (cv. Klom Sali) <sup>c</sup>	5.60		
raspberries <sup>f</sup>	6.50		
cherries (cv. Lambert and Bing) <sup>d</sup>			0.44–1.02

<sup>a</sup> Cultivar names given if known. Fruits are ranked from low to high TDF values. <sup>b</sup> Ref 71. <sup>c</sup> Ref 72. <sup>d</sup> Ref 74. <sup>e</sup> Ref 73. <sup>f</sup> Ref 61.

than  $\beta$ -phycoerythrin, which gave lower values in earlier ORAC assays (37) (Table 3). Guava, carambola, red dragon fruit, mamey sapote, lychee, and ripe papaya ORAC values were higher than or similar to published ORAC values for other common fruits and vegetables, including cucumber, cantaloupe, tomato, celery, pepper, nectarine, banana, kiwi fruit, and some varieties of onion. Carambola and guava ORAC values also were higher than or similar to ORAC values for grape, carrot, broccoli, red grapefruit, and orange (Table 3). The TSP values for guava and carambola also compared favorably with those of red grapefruit, spinach, and some varieties of apple and were higher than many of the other fruits.

**TDF and Pectin.** The selected Florida tropical fruits had widely different levels of TDF and pectin. The TDF and pectin ranged from 0.9 to 7.2 g/100 g and 0.2 to 1.04 g/100 g, respectively (Table 4). Red guava again (as with antioxidant activity) was highest in TDF and pectin followed by mamey, sapodilla, and white guava for TDF, while white guava exhibited pectin levels similar to those in mamey sapote. Green papaya and green mango had more TDF than their ripe counterparts,



although ripe mango had slightly more pectin than green mango. The variety of papaya grown for the green (unripe) market that is preferred by certain ethnic groups (Exp. 15), had higher levels of TDF and pectin than the green or ripe stage for the papaya variety produced for the ripe market (Red Lady). Red dragon fruit exhibited higher TDF and pectin than did white dragon fruit.

**Comparison of Fiber Data with Literature Values.** Tropical fruit TDF and pectin values of some tropical fruits were higher than the reported values for many other common fruits (Table 5). Red guava had TDF values that were higher than all other common fruit literature values except cherries, and the mamey sapote TDF was higher than all but those of the cherries and raspberries. Red dragon fruit, white guava, and sapodilla all had higher TDF values than most common fruits with the exception of the above and blackberries. Ripe mango, papaya, lychee, guava, and mamey sapote all had pectin levels that were comparable to what literature values are available for grape, orange, apple, and banana, for which the latter two fruit have a wide range of published values (Table 5).

**Conclusion.** Overall, the data from this study indicate that consumption of Florida tropical fruit varieties may deliver healthful benefits by supplying natural antioxidants and dietary fiber that are protective against cellular damage, while improving digestion and maintaining blood sugar levels. Guava would appear to be an especially wholesome fruit, with high antioxidant activity, phenolics, TAA, TDF, and pectin (16.7  $\mu\text{M TE/g}$ , 2316.7  $\mu\text{g GA/g}$ , 122.3–188.8 mg/100 g, 7.2 g/100 g, and 1.04 g/100 g, respectively) compared to other fruits tested as well as those in the literature (Tables 1 and 3–5). Carambola appears also to have healthful benefits, being very high in antioxidants and phenolics (12.9  $\mu\text{M TE/g}$  and 2207.7  $\mu\text{g GA/g}$ , respectively), as well as mamey sapote, being high in TDF and pectin (6.1 and 0.77 g/100 g, respectively) compared to most fruits. Red dragon fruit and sapodilla were likewise elevated in TDF, and red dragon fruit was also high in antioxidant activity compared to other fruits and vegetables. As for preferences for ripe or green mango and papaya by different ethnic groups, it is interesting to know that consuming mango and papaya at the ripe stage would be preferable, according to this study, for antioxidant protection and vitamin C, but green mango and papaya offer more fiber.

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