Guava Fruit (*Psidium guajava* L.) as a New Source of Antioxidant Dietary Fiber

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Guava (*Psidium guajava* L.) is a tropical fruit, widely consumed fresh and also processed (beverages, syrup, ice cream, and jams). Pulp and peel fractions were tested, and both showed high content of dietary fiber (48.55−49.42%) and extractable polyphenols (2.62−7.79%). The antioxidant activity of polyphenol compounds was studied, using three complementary methods: (i) free radical DPPH• scavenging, (ii) ferric reducing antioxidant power assay (FRAP), and (iii) inhibition of copper-catalyzed in vitro human low-density lipoprotein (LDL) oxidation. All fractions tested showed a remarkable antioxidant capacity, and this activity was correlated with the corresponding total phenolic content. A 1-g (dry matter) portion of peel contained DPPH• activity, FRAP activity, and inhibition of copper-induced in vitro LDL oxidation, equivalent to 43 mg, 116 mg, and 176 mg of Trolox, respectively. These results indicate that guava could be a suitable source of natural antioxidants. Peel and pulp could also be used to obtain antioxidant dietary fiber (AODF), a new item which combines in a single natural product the properties of dietary fiber and antioxidant compounds.

Keywords: *Guava; Psidium; tropical fruits; dietary fiber; polyphenols; antioxidant dietary fiber; radical scavenging; reducing ability; low-density lipoprotein oxidation*

INTRODUCTION

Epidemiological studies indicate that frequent consumption of fruits is associated with lower risk of stroke and cancer (*1*). However, the specific constituents responsible for this effect remain elusive. Dietary fiber (DF), and more recently antioxidant microconstituents of plant foods, are believed to play a significant role in the prevention of chronic and degenerative diseases.

DF intake in western countries is deficient, and dietary guidelines recommend an increase in consumption of DF-rich products such as fruits, vegetables, and cereals. At the same time, a good antioxidant level may also be important for human health. Vitamins C and E and some phytochemicals (phenolics and carotenoids) are effective free-radical scavengers. Recent literature suggests that plant food phytochemicals may have potential health beneficial effects (*2, 3*). Although the metabolic mechanics of phytochemicals are poorly understood, it has been suggested that a suitable intake of these compounds may prevent oxidative stress and derived metabolic disorders (*1*). Among bioactive compounds, polyphenols have shown potential health benefits, chiefly relating to antioxidant capacity. Polyphenols are the most abundant phytochemicals in our diets, and fruits are the main contributors (*4*).

The selection of suitable sources to provide new DF products with high antioxidant capacity derived from natural associated compounds could be an appropriate tool with which to achieve a better antioxidant status along with the recommended higher DF intakes. DF-rich foodstuffs and DF concentrates are popular in the food market, but the antioxidant capacities of these items are negligible. On the basis of previous studies on physiological and nutritional properties of polyphenols associated with DF, we have classified polyphenol compounds, according to solubility, into two categories: extractable (EPP) and nonextractable polyphenols (NEPP). The basic structure of EPP is flavan-3-ol and flavan-3,4-diol, whereas that of NEPP is condensed tannins. The properties of EPPs are related to soluble DF fraction, and the properties of NEPPs are related to the insoluble DF fraction (*5*). Recently, the concept of antioxidant dietary fiber (AODF) has been introduced. The main characteristics of this natural product are that they are rich in both DF and polyphenolic compounds (*6*).

Guava (*Psidium guajava*) is an important tropical fruit, mostly consumed fresh. The fruit is a berry, which consists of a fleshy pericarp and seed cavity with fleshy pulp and numerous small seeds. World production of guava was estimated at about 500,000 metric tons. Of the South American countries, Brazil, Colombia, Mexico, and Venezuela produce significant quantities of guava. The guava industry provides a variety of processed products: beverages, syrup, ice cream, jams, jellies, cheese, toffee, juice, wine, and dehydrated and canned products (*7*).

The aim of the present work was to evaluate the guava as a source of natural antioxidant compounds and antioxidant dietary fiber by analyzing its DF content and the antioxidant activity of associated bioactive compounds.

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Table 1. Dietary Fiber Constituents of *Psidium guajava* Fractions (% on Dry Matter)[a]

	Psidium guajava peel			Psidium guajava pulp		
	sDF	iDF	tDF	sDF	iDF	tDF
rhamnose	ND	0.49 ± 0.18	0.49 ± 0.18	ND	0.53 ± 0.02	0.53 ± 0.02
fucose	ND	0.27 ± 0.01	0.27 ± 0.01	ND	0.20 ± 0.01	0.20 ± 0.01
arabinose	0.75 ± 0.16	1.51 ± 0.34	2.26 ± 0.34	0.68 ± 0.09	1.21 ± 0.34	1.89 ± 0.34
xylose	ND	11.31 ±1.02	11.31 ± 1.02	ND	11.51 ± 1.06	11.51 ± 2.01
mannose	ND	0.52 ± 0.13	0.52 ± 0.13	ND	0.53 ± 0.11	0.53 ± 0.11
galactose	ND	0.59 ± 0.12	0.59 ± 0.12	ND	0.80 ± 0.13	0.80 ± 0.13
glucose	0.77 ± 0.20	11.29 ± 1.20	12.06 ± 1.10	0.87 ± 0.18	12.21 ± 1.25	13.08 ± 0.97
total NS	1.52 ± 0.27	25.98 ± 1.60	27.51 ± 1.60	1.55 ± 0.27	26.99 ± 2.25	28.54 ± 2.25
UA	0.30 ± 0.01	2.19 ± 0.10	2.49 ± 0.10	0.23 ± 0.00	2.22 ± 0.11	2.45 ± 0.11
KL	-	18.55 ±0.57	18.55 ± 0.57	-	18.47± 0.31	18.47 ± 0.31
DF	1.83 ± 0.27	46.72 ± 2.16	48.55 ± 2.16	1.77 ± 0.27	47.65 ± 2.25	49.42 ± 2.25

[a] Each value is the mean ± standard deviation of three replicate experiments. DF = dietary fiber; iDF = insoluble dietary fiber; KL = Klason lignin; ND = not determined; NS = neutral sugars; sDF =soluble dietary fiber; tDF = total dietary fiber; UA = uronic acids.

MATERIALS AND METHODS

Chemicals. Stable free radical DPPH• (2,2-diphenyl-1-picrylhydrazyl) and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a water-soluble analogue of vitamin E, were from Aldrich Co. (St Louis, MO). TPTZ (2,4,6-tri(2-pyridyl-s-triazine) was from Fluka Chemicals (Madrid, Spain). $FeCl_3 \cdot 6H_2O$, acetone, ethanol, and methanol were from Panreac Química S. A. (Madrid, Spain). Ethylenediamine tetraacetic acid (EDTA) and $CuCl_2 \cdot 2H_2O$ were from Sigma Co. (St Louis, MO). All reagents used were of analytical grade.

Samples. Two varieties of *Psidium* fruits, rosy and wild (*Psidium guajava* L. and *Psidium acutangulum* D.C., respectively), were obtained from the local market in Caracas. These were separated into peel and deseeded-pulp, and the fractions were freeze-dried immediately. They were then ground and stored until analysis.

Chemical Analysis. *Dietary Fiber*. The AOAC enzymatic−gravimetric method (*8*) was used, modified by using dialysis against water instead of ethanol precipitation of soluble dietary fiber (*9*) for dietary fiber analysis. After enzymatic hydrolysis of digestible components, insoluble dietary fiber (iDF) and soluble dietary fiber (sDF) fractions were separated and chemically hydrolyzed. The remaining residue was gravimetrically quantified as Klason lignin (KL). Constituent neutral sugars (NSs) and uronic acids (UAs) were quantified in the hydrolysates. UAs were quantified spectrophotometrically by the Scott method (*10*). NSs were determined by gas chromatography as alditol acetates (*11*).

Insoluble DF was calculated as (NS + UA + KL), and sDF was calculated as (NS + UA). Total DF (tDF) was calculated as (iDF + sDF).

Indigestible Fraction. In vitro determination of the indigestible fraction (IF) followed the procedure established at our laboratory (*12*). Briefly, after enzymatic treatments to remove digestible components, insoluble indigestible fraction (iIF) and soluble indigestible fraction (sIF) were obtained. The remaining residue was gravimetrically quantified as iIF, and sIF was measured with dinitrosalicylic acid. Total IF (tIF) was calculated as (iIF + sIF).

Extractable Polyphenols. EPPs were extracted from samples using aqueous−organic solvents. The extraction procedure is described elsewhere (*13*). The supernatants were combined, and total extractable phenols were estimated by the Folin−Ciocalteau method (*14*) using gallic acid as a standard and expressing the results as gallic acid equivalent (GAE).

Nonextractable Polyphenols. Residue obtained from the previous extractions was treated with 5% HCl in *n*-butanol (100 °C, 3 h) to determine nonextractable polyphenols (NEPPs). Condensed tannins were analyzed by reading the absorbance of anthocyanidin solutions at 555 nm (*15*). Carob pod (*Ceratonia siliqua*) condensed tannins (Nestec, Ltd., Vers-Chez-les Blanes, Switzerland) were used as standard.

Antioxidant Activity Assays. Supernatants containing EPP obtained as described above were used to determine the antioxidant capacity of vegetable extracts by three different methods.

DPPH• Free-Radical Scavenging Assay. The antioxidant activity of *Psidium* was measured in terms of radical scavenging ability, according to the DPPH• method (*16*). The parameter EC_{50}, which reflects 50% depletion of DPPH• free-radical, was expressed in terms of grams of *Psidium* equivalent per gram of DPPH• in the reaction medium. The time taken to reach the steady state at EC_{50} (T_{EC50}) was calculated. The antiradical efficiency (AE), a parameter defined by Sánchez-Moreno et al. (*16*) which combines both factors, was also calculated in order to recognize both effects of the above-mentioned parameters: $AE = 1/EC_{50}T_{EC50}$.

FRAP Assay. The antioxidant capacity of each sample was estimated according to the procedure described by Benzie et al. (*17*) with some modifications introduced in our laboratory. The readings at 30 min were selected for calculation of FRAP values (*18*). Methanolic solutions of known Trolox concentrations were used for calibration.

In vitro Copper-Induced Oxidation of Human Low-Density Lipoprotein Assay. Low-density lipoprotein (LDL) was obtained from Ramón y Cajal Hospital, Madrid, Spain. The plasma was collected from a patient with homozygous familial hypercholesterolemia. LDL was isolated following the procedure of Tani (*19*) as described in detail elsewhere (*20*).

In vitro LDL oxidation was performed by the procedure of Esterbauer et al. (*21*), as modified by Sánchez-Moreno et al. (*20*) to avoid the influence of LDL status. In this system, LDL hydroperoxidation was estimated on the basis of formation of conjugated dienes by measuring the change in absorption at 234 nm. The parameter CLT_{50} was calculated to measure the concentration of antioxidant that increases the lag time to 50% greater than that of the control.

Statistical Analysis. Results are expressed as mean values ± standard deviation. Comparison of means of three measurements, using a significance level of $p < 0.05$, was performed by one-way analysis of variance (ANOVA) using the Statgraphics Computer System, version 5.1.

RESULTS AND DISCUSSION

General composition of guava was 74−87% moisture, 0.8−1.5% protein, 4.2−11.1 total sugars, 2.0−7.2% crude fiber, 0.4−0.7% fat, and 0.5−1.0% ash. The composition of guava varies significantly with variety, stage of maturity, and season (*7*), but scarce information about specific physicochemical composition of guava is reported.

Dietary Fiber, Indigestible Fraction, and Polyphenols. The results of DF determinations in peel and pulp of *Psidium guajava* are shown in Table 1. The peel and pulp of guava showed a total DF content in the range 48.00−49.00 g/100 g of dry matter (dm) and therefore met the first requirement for consideration as an antioxidant dietary fiber (AODF) (*6*). The main fraction was iDF (96−97% of tDF) in both cases. Nahar

Table 2. Dietary Fiber and Indigestible Fraction Content of *Psidium* Fruits (% on Dry Matter)[a]

	DF[b]			IF[c]		
	iDF	sDF	tDF	iIF	sIF	tIF
Psidium guajava peel	46.72 ± 2.16	1.83 ± 0.27	48.55 ± 2.16	58.20 ± 0.56	3.43 ± 0.69	61.63 ± 0.69
Psidium guajava pulp	47.65 ±2.25	1.77 ± 0.27	49.42 ± 2.25	54.29 ± 2.4	3.20 ± 0.52	57.49 ± 2.4
Psidium acutangulum peel	50.16 ± 1.83	1.48 ± 0.08	51.52 ± 1.83	53.42 ± 0.08	4.10 ± 0.93	57.52 ± 0.93
Psidium acutangulum pulp	39.19 ± 0.97	1.53 ± 0.17	40.86 ± 0.97	40.40 ± 0.87	4.17 ± 0.53	44.57 ± 0.87

[a] Each value is the mean ± standard deviation of three replicate experiments. DF = dietary fiber; iDF = insoluble dietary fiber; IF = indigestible fraction; iIF = insoluble indigestible fiber; sDF = soluble dietary fiber; sIF = soluble indigestible fiber; tDF = total dietary fiber; tIF = total indigestible fiber. [b] Determined by the AOAC modified procedure (*9*). [c] Determined by in vitro determination of the indigestible fraction (*12*).

Table 3. Total Extractable Phenol Content (TEP) Expressed as Gallic Acid Equivalents (GAE), Free Radical DPPH• Scavenging Activity, and Ferric Reducing Antioxidant Power (FRAP) and Inhibition of Copper-Induced Oxidation of LDL in Two Fractions of the Fruit of *Psidium guajava*[a]

		DPPH•				
Psidium guajava	TE (g GAE kg^{-1} d m)	EC_{50} (g d m/g DPPH•)	T_{EC50} (minutes)	AE (1/ EC_{50} T_{EC50})	FRAP 30 min (μmol Trolox/ g d m)*	CLT_{50} (μg of d m mL^{-1})
peel	58.7 ± 4.0[a]	1.92 ± 0.08[a]	54.74 ± 2.05[a]	0.007 ±0.002[a]	462 ± 51[a]	1.65 ± 0.05[a]
pulp	26.3 ± 0.8[b]	3.70 ± 0.06[b]	30.75 ± 2.64[b]	0.009 ± 0.002[b]	238 ± 7[b]	2.75 ± 0.15[b]

[a] Each value is the mean ± standard deviation of three replicate experiments. Different prefix letters indicate significantly different values ($p < 0.05$; vertical comparison). For calculation see Methods section. AE = anti-radical efficiency; CLT_{50} = concentration of antioxidant that increases the lag time to 50% greater than that of the control; $EC_{50\%}$ = efficient concentration 50 (reflects 50% depletion of DPPH• free-radical); DF = dietary fiber; DM = dry matter; DPPH• = 2,2,-diphenyl-1-picrylhydrazyl; TEP = total extractable phenols; GAE = galic acid equivalents; FRAP = ferric reducing antioxidant power; T_{EC50} = time to reach the EC_{50}.

et al. (*22*) found a similar relative value for iDF (91% of tDF) in the edible portion of *Psidium guajava*. The minor fraction in the samples was sDF (3−4% of tDF). The NS profiles of the iDF and sDF fractions are shown in Table 1.

There was a considerable amount of KL, a residue with which various different compounds may be associated. Treatment with HCl/*n*-butanol showed a relatively high presence of condensed tannins in the iDF fraction of guava: 2.87 ± 0.30 and 1.68 ± 0.35% dm in peel and pulp, respectively. These NEPP contribute to the physiological properties of the IDF fraction, mainly increasing fat excretion (*5*).

Table 2 shows DF and IF contents in the analyzed guava fractions. The term IF has been recently proposed as an alternative to the DF measurement. The method to determine the IF of foods would comprise most components of vegetable foods that escape digestion and absorption in the small intestine and reach the colon, where they are susceptible to bacterial fermentation (*12*). As expected, IF values were higher than DF values in all cases, because of the presence of constituents other than nonstarch polysaccharides and lignin. Substantial amounts of resistant starch, resistant protein, and minor amounts of minerals may be expected in the insoluble residue. tIF in peel and pulp of guava was in the range 57.50−61.50 g/100 g dry matter (dm). iIF was the main fraction and sIF was the minor fraction in peel and pulp portions (94% and 5% of tIF, respectively).

Total extractable phenol content in the aqueous−organic extracts of pulp and peel portion of guava, estimated by the Folin−Ciocalteu method, is shown in Table 3. Both fractions, and especially the peel portion, contained high amounts of extractable soluble phenols and polyphenols.

Because of the high polyphenol and high dietary fiber content of guava, the antioxidant activity of soluble polyphenolic guava extracts was determined by three different methods.

Antioxidant Capacity. There are a number of factors that influence the effectiveness of antioxidants in complex heterogeneous foods and biological systems and in multiphase models. These include the lipid/aqueous phase partitioning properties of the antioxidants, the oxidation conditions, and the physical state of the oxidizable substrate (*23*). The influence of all relevant parameters cannot be evaluated using only one assay protocol, and so three systems were chosen to evaluate the antioxidant activity of the *Psidium* extracts. DPPH• and FRAP respectively measure the radical scavenging activity in organic systems and the total reduction power in aqueous systems. In addition, copper-induced oxidation of lipoprotein model was selected to measure the prevention of lipid peroxidation. The results of these procedures are discussed below.

Free Radical Scavenging Capacity. The values of the parameter EC_{50} are shown in Table 3. All the different fractions of guava tested had exceptionally high scavenging activity. Compared to pulp, guava peel presented lower EC_{50}, and higher total polyphenol content, on the basis of dry weight of the fruit portion.

Linear regression analyses of the scavenging of DPPH• by guava extracts showed a statistically significant correlation ($r = -0.928$; $p < 0.01$) between EC_{50} values and estimated total phenol content by Folin−Ciocalteu. A linear correlation between radical scavenging activity and polyphenolic extract has been reported in an extensive range of vegetables and fruits (*24*) and beverages (*25*), but not heretofore in guava. This correlation suggests that the contribution of phenolic compounds in this model is high. The guava extracts contain other antioxidants such as ascorbic acid, and carotenoids (*7*), but these compounds have shown less antioxidant potential in comparison with polyphenols in different models (*16*, *18*, *20*, *26*). T_{EC50} values of guava extracts showed lower kinetic behavior for peel fraction than for pulp fraction.

The free radical scavenging capacity activity equivalents of 1 g of the fractions of guava analyzed exceed the second requirement of the AODF (*6*): 1 g of guava peel and of guava pulp are equivalent to 104.1 mg and 54.0 mg of D,L-α-tocopherol, respectively.

Ferric Reducing Antioxidant Power. A useful way of viewing the interactions among various antioxi-

Table 4. Total Extractable Phenol Content (TEP), Condensed Tannins, Free Radical DPPH• Scavenging Activity, and Ferric Reducing Antioxidant Power (FRAP) and Inhibition of Copper-Induced Oxidation of LDL in Two Fractions of the Fruit of *Psidium acutangulum*[a]

	peel	pulp
TEP (g GAE kg^{-1} dm)	77.9 ± 3.0	26.2 ± 1.3
condensed tannins (% dm)	2.40 ± 0.08	1.06 ± 0.17
DPPH•(EC_{50}, g dm/g DPPH•)	2.62 ± 0.57	3.72 ± 0.42
FRAP (μmol Trolox/g dm)	392 ± 17	233 ± 8
LDL (CLT_{50}, μg of dm ml^{-1})	3.30 ± 0.20	8.50 ± 0.50

[a] Each value is the mean ± standard deviation of three replicate experiments. AE = anti-radical efficiency; CLT_{50} = concentration of antioxidant that increases the lag time to 50% greater than that of the control; $EC_{50\%}$ = efficient concentration 50 (reflects 50% depletion of DPPH• free-radical); DM = dry matter; DPPH• = 2,2,-diphenyl-1-picrylhydrazyl; TEP = total extractable phenols; GAE = galic acid equivalents; FRAP = ferric reducing antioxidant power; LDL = low-density lipoprotein; T_{EC50} = time to reach the EC_{50}.

dants is to take into account oxidation−reduction potentials (*27*). In simple terms, antioxidants can be described as reductants, and inactivation of oxidants by reductants can be described as redox reactions in which one reaction species (oxidant) is reduced at the expense of the oxidation of another (antioxidant) (*17*). The FRAP assay measures the antioxidant effect of any substance in the reaction medium as reducing ability. Antioxidant potential of the guava extracts was estimated from their ability to reduce TPTZ−Fe(III) complex to TPTZ−Fe-(II) complex. Antioxidant capacities of the different guava extracts tested varied considerably, although both values could be considered high (Table 3). Guava peel showed the highest FRAP antioxidant activity as with the DPPH• method. There was a high correlation between these two methods in guava extracts ($r = -0.627$; $p < 0.05$). Moreover, there was a noticeable correlation between the total extractable phenol content estimated by Folin−Ciocalteu method and FRAP values in guava ($r = 0.818$; $p < 0.01$). High correlation has also been reported with other substrates, e.g., tea (*28*).

Inhibition of In Vitro Copper-Induced Oxidation of LDL. In the present study, the antioxidative effect of guava peel and pulp extracts were studied in a system containing 50 μg protein mL^{-1} of human LDL which was oxidized in vitro by 5 μM of $CuCl_2$. In this system, LDL peroxidation was estimated in terms of the formation of conjugated dienes. It is clear from these experiments that the different portions of guava fruit tested prolonged the lag phase in a concentration-dependent manner. Table 3 shows the CLT_{50} index. The *Psidium guajava* peel extract exhibited high inhibition of in vitro copper-induced LDL oxidation.

Results of the LDL oxidation test suggest that guava extracts were an efficient antioxidant for LDL in vitro. Guava fruits may have potential health effects on the prevention of atherosclerosis development (*29*) and may be related to in vivo antioxidant activity of guava components.

The evaluated parameters showed that guava fruit is a good source of both natural antioxidants and antioxidant dietary fiber. The generation of these products at pilot plant scale has been recently undertaken by our group (*30*).

The same two portions of a wild variety of *Psidium* (*Psidium acutangulum*) were analyzed for dietary fiber content and indigestible fraction (Table 2). Polyphenol contents and antioxidant activity by three methods were also measured (Table 4). These values implicate that this variety could be also considered a suitable source of AODF.

CONCLUSIONS

Peel and pulp of *Psidium guajava* fruit presented high levels of dietary fiber, indigestible fraction, and phenolic compounds. There was a statistically significant correlation between estimated extractable phenol content and radical scavenging activity/ferric reducing power ($r = -0.928$, $p < 0.01$; and $r = 0.818$, $p < 0.01$, respectively). These bioactive compounds contributed significantly to the high antioxidant capacity of guava fruit as measured by these methods.

Very briefly, given that *Psidium* fruits are a vegetable food rich in dietary fiber with associated natural antioxidant components and a material suitable for AODF production, they could prove a useful dietary supplement.

ABBREVIATIONS USED

AE, anti-radical efficiency; AODF, antioxidant dietary fiber; CLT_{50}, concentration of antioxidant that increases the lag time to 50% greater than that of the control; $EC_{50\%}$, efficiency concentration 50; DF, dietary fiber; DM, dry matter; DPPH•, 2,2-diphenyl-1-picrylhydrazyl; EDTA, ethylenediaminetetraacetic; EPP, extractable polyphenols; FRAP, ferric reducing activity power; GAE, galic acid equivalents; iDF, insoluble dietary fiber; iIF, insoluble indigestible fraction; KL, Klason lignin; LDL, low-density lipoproteins; ND, not determined; NEPP, non extractable polyphenols; NS, neutral sugars; sDF, soluble dietary fiber; sIF, soluble indigestible fraction; tDF, total dietary fiber; tIF, total indigestible fraction; TE, Trolox equivalents; T_{EC50}, time to reach the efficiency concentration 50; TEP, total extractable phenol; TPTZ, 2,4,6-tri(2−pyridyl)-s-triazine; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; UA, uronic acids.

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