HYDROPHILIC AND LIPOPHILIC ANTIOXIDANT ACTIVITIES OF GUAVA FRUITS

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Abstract. The objectives of this study were to evaluate the hydrophilic antioxidant activity (AOAH) and the lipophilic antioxidant activity (AOAL); and their correlations with vitamin C, and total phenolic and β-carotene contents in fresh guava fruits of one white flesh clone ('Allahabad Safeda') and three pink flesh clones ('Fan Retief', 'Ruby Supreme,' and an advanced selection). A ferric reducing antioxidant power assay was used to estimate both AOAH and AOAL from methanol and dichloromethane extracts, respectively. The white flesh clone, 'Allahabad Safeda,' showed higher levels of both AOAH [33.3 μM Trolox equivalents (TE)/g fresh weight (FW)] and AOAL (0.25 μM TE/g FW) than the pink flesh clones that ranged from 15.5 to 30.4 and from 0.12 to 0.13 μM TE/g FW for AOAH and AOAL, respectively. The AOAH was positively correlated with vitamin C (r = 0.92, $p < 0.01$) and total phenolic (r = 0.97, $p < 0.01$) but was negatively correlated with β-carotene (r = -0.73, p = 0.03). The AOAL was not correlated with these antioxidants.

INTRODUCTION

Natural antioxidants, particularly in fruits and vegetables, have been of increasing interest to both consumers and scientists, such as epidemiologists, food scientists, chemists, and plant scientists because epidemiological studies have indicated that frequent consumption of natural antioxidants is associated with a lower risk of cardiovascular diseases and cancers (Renaud *et al*, 1998; Temple, 2000). The defensive effects of the natural antioxidants in fruits and vegetables are related to the three major groups: vitamins, especially vitamin C; phenolics; and carotenoids, especially β-carotene (Klein and Kurilich, 2000). Vitamin C and phenolics are known as hydrophilic antioxidants, while carotenoids are known as lipophilic antioxidants.

Guava fruit (*Psidium guajava* L.) contains a high level of antioxidant compounds, such as vitamin C (50-300 mg/100 g fresh weight, which is higher than that in an orange by three to six times) (Nakasone and Puall, 1998); carotenoids, such as β-carotene and lycopene (Mercadante *et al*, 1999); and phenolic compounds, such as ellagic acid and anthocyanin (Misra and Seshadri, 1968).

The aims of this research were to estimate hydrophilic and lipophilic antioxidant activities and their correlations with total phenolic, vitamin C, and total carotenoid contents in guava fruits.

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MATERIALS AND METHODS

Guava fruits from one and three of white and pink flesh cultivars, respectively, were harvested at Weslaco, Texas, USA.

Extractions

Fruit extracts for total phenolic content and hydrophilic antioxidant activity analyses were prepared following the method of Swain and Hillis (1959), with some modifications. Three grams of guava tissue (flesh and peel) was mixed with 25 ml methanol (MeOH) and homogenized using an Ultra-Turrax homogenizer (T25, Ika Works, USA). The homogenates were kept at 4°C for 12 hours and then centrifuged at 15,000 rpm for 20 minutes using a vacuum microcentrifuge (Beckman, J2-21, Beckman Instruments, USA). The supernatant was recovered and stored at -20°C until assayed. The pellet was redissolved with 20 ml of dichloromethane and homogenized for lipophilic antioxidant activity analysis. The homogenates were centrifuged at 15,000 rpm for 20 minutes. The supernatant was recovered and stored at -20°C until assayed.

Fruit extracts for vitamin C analysis were obtained by homogenizing three grams of guava tissue (flesh and peel) in 20 ml cold solution of 3% (w/v) oxalic acid, and 8% glacial acetic acid (v/v) in water until uniform consistency was achieved using the Ultra-Turrax homogenizer. The homogenates were centrifuged at 15,000 rpm at 4°C for 10 minutes. The supernatant was recovered and measured for vitamin C immediately.

Fruit extracts for carotenoid analysis were prepared following the method of Wilberg and Bodriguez-Amaya (1995), with some modifications. Three grams of guava tissue (flesh and peel) was mixed with 20 ml of ethanol-hexane (1:1) solution that contained 200 mg/l 2,6-di-ter-butyl-*p*-cresol to avoid carotenoid oxidation and then homogenized using the Ultra-Turrax homogenizer until there was uniform consistency. The homogenate was filtered using a Whatman No 4 and re-extracted three times with 20 ml of solvent. The extract was washed tree times with nanopure water. The supernatant was recovered and added with hexane to a final volume of 10 ml; then stored at -20°C until assayed.

Antioxidant activity determinations

The ferric reducing antioxidant power (FRAP) assay was used to determine both hydrophilic and lipophilic antioxidant activities. The procedure followed the method of Benzie and Strain (1996), with some modifications. The stock solutions included 300 mM acetate buffer (3.1 g $C_2H_3NaO_2 \cdot 3H_2O$ and 16 ml $C_2H_4O_2$), pH 3.6; 10 mM TPTZ (2, 4, 6-tripyridyl-*s*-triazine) solution in 40 mM HCl; and 20 mM $FeCl_3 \cdot 6H_2O$ solution. The working solution was freshly prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ solution, and 2.5 ml $FeCl_3 \cdot 6H_2O$ solution; then warmed at 37ºC before use. Fruit extracts (150 µl) were allowed to react with 2,850 µl of the FRAP solution for 30 minutes in dark conditions. Readings of the colored product [ferrous tripyridyltriazine complex] were then taken at 593 nm. The standard curve was linear, between 25 and 800 µM Trolox. Results were expressed in µM Trolox equivalents (TE)/g fresh weight (FW). Adequate dilution was needed if the FRAP value measured was over the linear range of the standard curve.

Antioxidant determinations

Total phenolic content was determined by the Folin-Ciocalteu method, which was adapted from Swain and Hillis (1959). This method combined 150 µl of extract, 2,400 µl of nanopure water, and 150 µl of 0.25 N Folin-Ciocalteu reagent in a plastic vial; then mixed well with a Vortex. Then the mixture was allowed to react for 3 minutes; 300 µl of 1 N Na_2CO_3 solution was then added and mixed well. The solution was incubated at room temperature (23ºC), in the dark, for 2 hours. The absorbance was taken at 725 nm using a spectrophotometer. The results were expressed in gallic acid equivalents (GAE; mg/100 g FW) using a gallic acid (0-0.1 mg/ml) standard curve. Adequate dilution was needed if the absorbance value measured was over the linear range of the standard curve.

Ascorbic acid content was determined using the 2, 6-dichlorophenol-indophenol titration method (Association of Office Analytical Chemists, 1990), with some modifications. L-ascorbic acid was used to prepare a standard solution (1 mg/ml). The ascorbic acid concentration was calculated by comparison with the standard and expressed as mg/100 g FW.

Total carotenoid content was determined by the spectrophotometric method at 470 nm, which was adapted from Talcott and Howard (1999), using a β-carotene (0.001-0.004 mg/ml) standard curve. The total carotenoid content was expressed based on β-carotene equivalents (β-carotene; mg/100 g FW). Adequate dilution was needed if the absorbance value measured was over the linear range of the standard curve.

Statistical analysis.

F-test and Duncan's new multiple range test were used to test statistically different significance. Pearson's correlation coefficient was used to determine correlations between antioxidant activities and compounds.

RESULTS

The white flesh clone ('Allahabad Safeda') contained a higher level of hydrophilic antioxidant activity (33.3 µM TE/g FW) and total phenolic content (345 GAE/g FW) when compared with the pink flesh clones; ranging from 15.5 to 30.4 µM TE/g FW and 170 to 301 GAE/g FW, respectively. A pink flesh clone ('Fan Retief') had a higher level of vitamin C content (397 mg/100 g FW) when compared with the white flesh clone (379 mg/100 g FW) (Table 1). However, the white flesh clone had a higher level of vitamin C content compared with the other pink flesh clones; at 174 and 259 mg for 'Ruby Supreme' and advanced selection, respectively. From the results, it could be concluded that the amount of antioxidant compounds and activities level depends on the cultivars and types. The correlations of hydrophilic antioxidant activity with total phenolic ($r = 0.97$) and with vitamin C ($r = 0.92$) contents were relatively positively high (Table 2).

DISCUSSION

Among fresh fruit, blueberries have exceptionally high antioxidant activity, as determined by oxygen radical absorbance capacity (ORAC) that ranged from 13.9 to 45.9 µM TE/g FW (Prior *et al*, 1998). A survey of 12 other fruits (Wang *et al*, 1996) demonstrated antioxidant activity ranging from less than 1 µM TE/g FW for melon (*Cucumis melo* L.) up to 15 µM TE/g FW for strawberry. Guava, therefore, is another fruit

that has an exceptionally high antioxidant activity, which ranged from 15.5 to 33.3 μM TE/g FW of the cultivars used in the present study (Table 1). Guava fruits also showed relatively high phenolic and vitamin C contents (Table 1), which are known as the two major natural hydrophilic antioxidants. Some epidemiological studies suggest that antioxidant consumption can positively influence health. Persons with high plasma vitamin C levels may have a decreased risk of types of cataracts (Jacques *et al*, 1988). Diets high in flavonoid antioxidants have been associated with lower rates of mortality from coronary heart disease (Hertog *et al*, 1997). Thus, regular consumption of guava fruits could be beneficial to human health, such as reducing the risks of cardiovascular diseases and cancers.

The white flesh clone also had a higher level of lipophilic antioxidant activity than the pink flesh clones, although it contained the lowest concentration of β-carotene (Table 1), which is known to be the major lipophilic antioxidant. It could be that white flesh guava contains other lipophilic antioxidants, such as lycopene. They, however, may not be of much importance for a contribution to the total antioxidant activity because it was very apparent that the hydrophilic antioxidant activity accounted more than 99% of the total antioxidant activity (Table 1). The major antioxidant activity obtained by ORAC assays of sorghum and sorghum products were also hydrophilic antioxidant activity (Awika *et al*, 2003).

A high correlation between hydrophilic antioxidant activity and phenolic contents has also been reported in several fruit crops, such as blueberry (Connor *et al*, 2002), and peach and plum (Gil *et al*, 2002). Phenolic and vitamin C contents, on the other hand, could be major contributors to the antioxidant activity in guava fruits. The major contributors to the antioxidant potential depend on plant species and their products. Gardner *et al* (2000) has shown that vitamin C was the major contributor to the antioxidant potential of only beverages derived from citrus fruit. There was no correlation between lipophilic antioxidant activity and other traits, although for β-carotene, Gardner *et al* (2000) has reported that both total

Table 1
Antioxidant activities and compounds of four guava clones.

Cultivar	Flesh color	AOAH[z] (% of total)	AOAL[y] (% of total)	TPC[x]	VTC[w]	BET[t]
Allahabad Safeda	White	33.3 a[f] (99.25)	0.25 a (0.75)	345 a	379 a	na
Fan Retief	Pink	30.4 b (99.60)	0.12 b (0.40)	301 b	397 a	1.6 b
Ruby Supreme	Pink	15.5 d (99.19)	0.13 b (0.81)	170 c	174 c	2.9 a
Advanced Selection	Pink	25.3 c (99.49)	0.13 b (0.51)	271 b	259 b	0.8 c
p		< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

[z]AOAH = hydrophilic antioxidant activity (μM TE/g^{-1} FW); [y]AOAL = lipophilic antioxidant activity (μM TE/g^{-1} FW); [x]TPC = total phenolic content (GAE; mg/100 g FW); [w]VTC = Vitamin C content (mg/100 g FW); [t]BET = β-carotene content (mg/100 g FW); [f]Mean separation within columns by Duncan's new multiple range test, $p < 0.01$; na = not available.

Table 2
Pearson's correlation coefficients among antioxidant activities, total phenolic, vitamin C, and β-carotene contents.

Trait[z]	AOAH	AOAL	TPC	VTC
AOAL	0.54[ns]			
TPC	0.97[b]	0.56[ns]		
VTC	0.92[b]	0.36[ns]	0.89[b]	
BET	-0.73[a]	-0.05[ns]	-0.79[b]	-0.50[ns]

[z]AOAH = hydrophilic antioxidant activity (μM TE/g^{-1} FW); AOAL = lipophilic antioxidant activity (μM TE/g^{-1} FW); TPC = total phenolic content (GAE; mg/100 g FW); VTC = Vitamin C content (mg/100 g FW); BET = β-carotene content (mg/100 g FW); [ns] = non-significant, [a,b] = significant at $p \leq 0.05$ and 0.01, respectively.

phenolic and vitamin C concentrations were strongly correlated with the hydrophilic antioxidant activity of several fruit juices, but not β-carotene. It could be that β-carotene is not the major lipophilic antioxidant in guava fruits. The high correlation of hydrophilic antioxidant activity with total phenolic and vitamin C contents indicated that it was feasible to use the total phenolic content, or vitamin C content, as an indicator to determine the hydrophilic antioxidant activity.

In conclusion, Both white and pink flesh guavas showed high hydrophilic antioxidant activity and compounds for phenolic and vitamin C indicated that regular consumption of guava might be beneficial to health. That hydrophilic antioxidant activity, the major activity, had high correlations with both total phenolic and vitamin C indicated that the use of the total phenolic or vitamin C content to determine antioxidant activity level in guava fruit was feasible. Phenolic and vitamin C are the major contributors to the antioxidant activity of guava fruits, while the contribution of carotenoid is negligible.

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