ELSEVIER

Journal of Food Composition and Analysis 19 (2006) 669–675

JOURNAL OF FOOD COMPOSITION AND ANALYSIS

www.elsevier.com/locate/jfca

Original Article

Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts

Kriengsak Thaipong[a], Unaroj Boonprakob[a,*], Kevin Crosby[b], Luis Cisneros-Zevallos[c], David Hawkins Byrne[c]

[a]*Department of Horticulture, Kasetsart University, Kamphaengsaen Campus, Nakhon Pathom 73140, Thailand*
[b]*Department of Horticultural Sciences, Texas A&M Research & Extension Center, Weslaco, TX 78596, USA*
[c]*Department of Horticultural Sciences, Texas A&M University, College Station, TX 77843-2133, USA*

Received 28 March 2005; received in revised form 5 January 2006; accepted 10 January 2006

Abstract

Guava fruit extracts were analyzed for antioxidant activity measured in methanol extract (AOAM), antioxidant activity measured in dichloromethane extract (AOAD), ascorbic acid, total phenolics, and total carotenoids contents. The ABTS, DPPH, and FRAP assays were used for determining both AOAM and AOAD, whereas the ORAC was used for determining only AOAM. Averaged AOAM [μM Trolox equivalent (TE)/g fresh mass (FM)] were 31.1, 25.2, 26.1, and 21.3 as determined by the ABTS, DPPH, FRAP, and ORAC assays, respectively. Averaged AOAD (μM TE/g FM) were 0.44, 0.27, and 0.16 as determined by the ABTS, DPPH, and FRAP assays, respectively. AOAM determined by all assays were well correlated with ascorbic acid ($0.61 \leqslant r \leqslant 0.92$) and total phenolics ($0.81 \leqslant r \leqslant 0.97$) and also among themselves ($0.68 \leqslant r \leqslant 0.97$) but had negative correlation with total carotenoids ($-0.67 \leqslant r \leqslant -0.81$).

Keywords: Ascorbic acid; Phenolic; Carotenoid; *Psidium guajava* L.

1. Introduction

Natural antioxidants, particularly in fruits and vegetables have gained increasing interest among consumers and the scientific community because epidemiological studies have indicated that frequent consumption of natural antioxidants is associated with a lower risk of cardiovascular disease and cancer (Renaud et al., 1998; Temple, 2000). The defensive effects of natural antioxidants in fruits and vegetables are related to three major groups: vitamins, phenolics, and carotenoids. Ascorbic acid and phenolics are known as hydrophilic antioxidants, while carotenoids are known as lipophilic antioxidants (Halliwell, 1996).

Guava (*Psidium guajava* L.) fruit is considered a highly nutritious fruit because it contains a high level of ascorbic acid (50–300 mg/100 g fresh weight), which is three to six times higher than oranges. Red-fleshed Brazilian guava has several carotenoids such as phytofluene, β-carotene, β-cryptoxanthin, γ-carotene, lycopene, rubixanthin, cryptoflavin, lutein, and neochrome (Mercadante et al., 1999). Setiawan et al. (2001) reported that Indonesian guava is an excellent source of provitamin A carotenoids. Phenolic compounds such as myricetin and apigenin (Miean and Mohamed, 2001), ellagic acid, and anthocyanins (Misra and Seshadri, 1968) are also at high levels in guava fruits. Therefore, producing guava specially bred for higher levels of antioxidant compounds, is a realistic approach to increase dietary antioxidant intake. Evaluation in any plant-breeding program, however, has to deal with numerous plants, particularly at the early selection stage. Therefore, the assay for screening germplasm and hybrids should be simple, inexpensive, rapidly performed, and provide a high degree of precision.

Several assays have been frequently used to estimate antioxidant capacities in fresh fruits and vegetables and their products and foods for clinical studies including 2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS)

*Corresponding author. Tel.: 66 34 281084; fax: 66 34 281086.
E-mail address: unaroj.b@ku.ac.th (U. Boonprakob).

0889-1575/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.jfca.2006.01.003

(Leong and Shui, 2002; Miller and Rice-Evans, 1997), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Brand-Williams et al., 1995; Gil et al., 2002), ferric reducing antioxidant power (FRAP) (Benzie and Strain, 1999; Guo et al., 2003; Jimenez-Escrig et al., 2001), and the oxygen radical absorption capacity (ORAC) (Cao et al., 1993; Ou et al., 2001; Prior et al., 2003). The ORAC assay is said to be more relevant because it utilizes a biologically relevant radical source (Prior et al., 2003). These techniques have shown different results among crop species and across laboratories. Ou et al. (2002) reported no correlation of antioxidant activity between the FRAP and ORAC techniques among most of the 927 freeze-dried vegetable samples, whereas these methods revealed high correlation in blueberry fruit (Connor et al., 2002). Similarly, Awika et al. (2003) observed high correlation between ABTS, DPPH, and ORAC among sorghum and its products.

The aim of this research was to compare the efficiency of ABTS, DPPH, FRAP, and ORAC assays to estimate antioxidant activities and their correlations with ascorbic acid, total phenolics, and total carotenoids contents in guava fruit extracts.

2. Materials and methods

2.1. Plant materials

Guava fruits were harvested at maturity from one white-fleshed ('Allahabad Safeda') and three pink-fleshed ('Fan Retief', 'Ruby Supreme' and an advanced selection) clones at Weslaco, TX, USA with the cooperation of Dr. Kevin Crosby. Whole fruit was stored at −20 °C for 6 months before extraction.

2.2. Extractions

Fruit extracts for ascorbic acid analysis were obtained by homogenizing 3 g of guava tissue (pulp and peel) in 20 mL cold solution of 3% (w/v) oxalic acid plus 8% glacial acetic acid (v/v) until uniform consistency, using an Ultra-Turrax homogenizer (T25, Ika Works Inc., USA). The homogenates were centrifuged at 15,000 rpm at 4 °C for 10 min. The supernatants were recovered and ascorbic acid immediately measured.

Fruit extracts for total phenolics and antioxidant activity measured in methanol extract (AOAM) analysis were prepared using the method of Swain and Hillis (1959), with some modifications. Three grams of guava tissue were mixed with 25 mL methanol and homogenized using the Ultra-Turrax homogenizer. The homogenates were kept at 4 °C for 12 h and then centrifuged at 15,000 rpm for 20 min using a vacuum micro centrifuge (Beckman, J2-21, Beckman Instruments Inc., USA). The supernatants were recovered and stored at −20 °C until analysis. The pellet was re-dissolved with 20 mL dichloromethane and homogenized for antioxidant activity measured in dichloromethane extract (AOAD) analysis. The homogenates were centrifuged at 15,000 rpm for 20 min. The supernatants were recovered and stored at −20 °C until analysis. In general, methanol extraction and dichloromethane extraction are used for determining hydrophilic and lipophilic antioxidant activities (Arnao et al., 2001).

Fruit extracts for total carotenoids analysis were prepared by the method of Wilberg and Rodriguez-Amaya (1995), with some modifications. Three grams of guava tissue were mixed with 20 mL ethanol–hexane (1:1) solution containing 200 mg/L 2,6-di-ter-butyl-*p*-cresol to avoid carotenoid oxidation and then homogenized using the Ultra-Turrax homogenizer until uniform consistency. The homogenates were filtered using a Whatman No. 4 filter paper and re-extracted two or three times, depending on the clone, with 20 mL solvent. The extracts were washed three times with nanopure water. The supernatants were recovered and added with hexane to a final volume of 10 mL, and then stored at −20 °C until analysis.

2.3. Antioxidant determinations

Ascorbic acid content was determined using the 2, 6-dichlorophenol-indophenol titration method described in Association of Office Analytical Chemists (1996). 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 fresh mass.

Total phenolics content was determined by the Folin–Ciocalteu method, which was adapted from Swain and Hillis (1959). The 150 μL of extract, 2400 μL of nanopure water, and 150 μL of 0.25 N Folin–Ciocalteu reagent were combined in a plastic vial and then mixed well using a Vortex. The mixture was allowed to react for 3 min then 300 μL of 1 N Na_2CO_3 solution was added and mixed well. The solution was incubated at room temperature (23 °C) in the dark for 2 h. The absorbance was measured at 725 nm using a spectrophotometer (Hewlett Packard 8452A, Diode Array, USA) and the results were expressed in gallic acid equivalents (GAE; mg/100 g fresh mass) using a gallic acid (0–0.1 mg/mL) standard curve. Additional dilution was done if the absorbance value measured was over the linear range of the standard curve.

Total carotenoids content was determined by the spectrophotometric method at 470 nm, which was adapted from Talcott and Howard (1999) using a β-carotene (0.001–0.005 mg/mL) standard curve. The total carotenoids content was expressed based on β-carotene equivalents (β-carotene; mg/100 g fresh mass). Additional dilution was done if the absorbance value measured was over the linear range of the standard curve.

2.4. Antioxidant activity determinations

For ABTS assay, the procedure followed the method of Arnao et al. (2001) with some modifications. The stock solutions included 7.4 mM $ABTS^{\bullet+}$ solution and 2.6 mM

potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in the dark. The solution was then diluted by mixing 1 mL $ABTS^{\bullet+}$ solution with 60 mL methanol to obtain an absorbance of 1.1 ± 0.02 units at 734 nm using the spectrophotometer. Fresh $ABTS^{\bullet+}$ solution was prepared for each assay. Fruit extracts (150 μL) were allowed to react with 2850 μL of the $ABTS^{\bullet+}$ solution for 2 h in a dark condition. Then the absorbance was taken at 734 nm using the spectrophotometer. The standard curve was linear between 25 and 600 μM Trolox. Results are expressed in μM Trolox equivalents (TE)/g fresh mass. Additional dilution was needed if the ABTS value measured was over the linear range of the standard curve.

The DPPH assay was done according to the method of Brand-Williams et al. (1995) with some modifications. The stock solution was prepared by dissolving 24 mg DPPH with 100 mL methanol and then stored at −20 °C until needed. The working solution was obtained by mixing 10 mL stock solution with 45 mL methanol to obtain an absorbance of 1.1 ± 0.02 units at 515 nm using the spectrophotometer. Fruit extracts (150 μL) were allowed to react with 2850 μL of the DPPH solution for 24 h in the dark. Then the absorbance was taken at 515 nm. The standard curve was linear between 25 and 800 μM Trolox. Results are expressed in μM TE/g fresh mass. Additional dilution was needed if the DPPH value measured was over the linear range of the standard curve.

The FRAP assay was done according to 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 fresh working solution was prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ solution, and 2.5 mL $FeCl_3 \cdot 6H_2O$ solution and then warmed at 37 °C before using. Fruit extracts (150μL) were allowed to react with 2850 μL of the FRAP solution for 30 min in the dark condition. 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 are expressed in μM TE/g fresh mass. Additional dilution was needed if the FRAP value measured was over the linear range of the standard curve.

The ORAC procedure used an automated plate reader (KC4, Bio Tek, USA) with 96-well plates (Prior et al., 2003). Analyses were conducted in phosphate buffer pH 7.4 at 37 °C. Peroxyl radical was generated using 2, 2'-azobis (2-amidino-propane) dihydrochloride which was prepared fresh for each run. Fluorescein was used as the substrate. Fluorescence conditions were as follows: excitation at 485 nm and emission at 520 nm. The standard curve was linear between 0 and 50 μM Trolox. Results are expressed as μM TE/g fresh mass.

2.5. Statistical analysis

Each antioxidant activity assay was done three times from the same extract in order to determine their reproducibility. Analysis of variance was used to test any difference in antioxidant activities resulting from these methods. Duncan's new multiple range test was used to determine significant differences. Correlations among data obtained were calculated using Pearson's correlation coefficient (r).

3. Results and discussion

3.1. Ascorbic acid, total phenolic, and total carotenoid contents

The amount of ascorbic acid (AA), total phenolics (TPH), and total carotenoids expressed as β-carotene (BET) were significantly different among guava clones (Table 1). The AA was 378.6 mg/100 g in 'Allahabad Safeda' and ranged from 174.2 to 396.7 mg/100 g in the pink pulp clones. The TPH was 344.9 mg GAE/100 g in 'Allahabad Safeda' and ranged from 170.0 to 300.8 mg GAE/100 g in the pink pulp clones. The BET ranged from 0.78 to 2.93 mg/100 g in the pink pulp clones, while it was not present in the white pulp clone. Luximon-Ramma et al. (2003) have also reported that white pulp guavas had higher AA and TPH than pink pulp guavas in which the AA was 142.6 and 72.2 mg/100 g in white and pink pulp, respectively, and the TPH was 247.3 and 126.4 mg GAE/100 g in white and pink pulp, respectively. The AA, TPH, and BET contents in guavas were very high compared to other fruit crops. The ranges of AA contents (mg/100 g) were 4.8–13.2 in nectarines, 3.6–12.6 in peaches and 2.5–10.2 in plums (Gil et al., 2002), 19.0 in starfruit, 27.5 in pineapple, 60.5 in mango, 92.9 in papaya, 13.8 in litchi (Luximon-Ramma et al., 2003). The ranges of TPH contents (mg/100 g) were 14–102 in nectarines, 21–111 in peaches and 42–109 in plums (Gil et al., 2002), 142.9 in starfruit, 47.9 in pineapple, 56.0 in mango, 57.6 in papaya, 28.8 in litchi (Luximon-Ramma et al., 2003). The ranges of BET contents (mg/100 g) were 0.01–0.19 in nectarines, 0.01–0.26 in plums (Gil et al., 2002).

3.2. Reproducibility of ABTS, DPPH, FRAP, and ORAC assays

Antioxidant activities measured in methanol extract obtained using ABTS, DPPH, FRAP, and ORAC assays from a single extract were measured three times to test the reproducibility of the assays. The DPPH and FRAP assays showed no differences among determinations, while the ABTS and ORAC assays differed among runs (Table 2). All assays, however, had no genotype × time interaction, indicating that all techniques gave a comparable ranking of antioxidant activity among clones within each time of determination. Therefore, the DPPH and FRAP assays

Table 1
Ascorbic acid, total phenolics, and total carotenoids contents of four guava genotypes

Genotype[a]	Ascorbic acid[b]	Total phenolics[c]	Total carotenoids[d]
Allahabad Safeda	378.6±29.2 a[e]	344.9±33.6 a[e]	na
Fan Retief	396.7±25.0 a	300.8±12.7 b	1.59±0.12 b
Ruby Supreme	174.2±5.8 c	170.0±5.6 c	2.93±0.35 a
Advanced selection	258.9±49.9 b	270.6±2.9 b	0.78±0.16 c
P value	<0.01	<0.01	<0.01

na = not available.
[a]Fan Retief', 'Ruby Supreme', and advanced selection are pink pulp; 'Allahabad Safeda' is white pulp.
[b]Ascorbic acid expressed in mg/100 g fresh mass.
[c]Total phenolics content expressed in mg gallic acid equivalents/100 g fresh mass.
[d]Total carotenoids content expressed in mg β-carotene equivalents/100 g fresh mass.
[e]Mean separation within columns by Duncan's new multiple range test.

Table 2
ANOVA for antioxidant activity among three determinations of a single methanol extract by ABTS, DPPH, FRAP, and ORAC assays from four guava genotypes

Source	df	MS	*P*
ABTS			
Guava	3	410.95	<0.01
Repeatability	2	82.69	<0.01
Guava × Repeatability	6	11.73	0.27
Error	24	8.68	
DPPH			
Guava	3	393.37	<0.01
Repeatability	2	13.76	0.32
Guava × Repeatability	6	8.95	0.65
Error	24	12.76	
FRAP			
Guava	3	554.81	<0.01
Repeatability	2	2.35	0.45
Guava × Repeatability	6	0.81	0.94
Error	24	2.87	
ORAC			
Guava	3	85.54	<0.01
Repeatability	2	75.67	<0.01
Guava × Repeatability	6	4.56	0.56
Error	24	5.52	

could be used to determine antioxidant activity in guava as both showed high reproducibility. Working solutions of the DPPH, FRAP, and ORAC were used immediately after preparation while that of ABTS needed to be kept in the dark for 12 h to generate free radicals from the ABTS salt and then was used within 4 h (Awika et al., 2003; Arnao et al., 2001). Since the ABTS working solution was not always the same age, the activity of the solution to react with guava extracts might have been different among the determination times. For the ORAC, a 96-well plate machine (KC4, Bio Tek, USA) was used in this research. Reading value tended to be higher at the top than that at the bottom and also from the left than the right of the 96-well plates (data not shown). Prior et al. (2003) noted that a lower coefficient of variance (CV) is obtained using the 48-well format compared to the 96-well format. The 48-well plate data had a CV about 50% of the CV of the data generated in a 96-well plate. Therefore, the location of samples in the plate induced an increased error rate in the assays.

In terms of cost and time of running these methods, the main disadvantage of the ORAC technique is that it required the use of expensive equipment (Awika et al., 2003), whereas the other three methods required a simple machine, a spectrophotometer, which is commonly available in most laboratories. Another advantage of the ABTS and FRAP was that extracts reacted rapidly with ABTS (2 h) or ferric ion (30 min), respectively, whereas the DPPH reaction took much longer (24 h).

3.3. Antioxidant activity measured in methanol extract

The genotypes and the assays resulted in different antioxidant activity measured in methanol extract (AOAM) (Table 3). The white pulp clone, 'Allahabad Safeda', had the highest AOAM value (32.25 μM TE/g). The pink pulp clones had 28.45, 18.03, and 25.13 μM TE/g for 'Fan Retief', 'Ruby Supreme' and the advanced selection, respectively (Table 4). Higher level of AOAM in the white pulp clone was found in all assays as compared to the pink pulp clones due to its higher AA and TPH (Table 1). It, however, cannot be generalized that white pulp guava has a higher level of antioxidant activity than pink pulp guava because limited numbers of samples were studied in this research. There are three major pulp colored types: white, pink and maroon. Each consists of many genotypes, especially the white and pink pulp types. Therefore, more genotypes of all of the classes need to be measured for antioxidant activity to properly assess the variation of antioxidant activity among guava types.

The antioxidant activity as determined by ORAC assay of guavas (18.03–32.25 μM TE/g) was comparable to that of blueberries (13.9–45.9 μM TE/g) which contain an exceptionally high antioxidant activity (Prior et al., 1998).

Table 3
ANOVA for antioxidant activity by the ABTS, DPPH, FRAP, and ORAC assays based on methanol extraction from four guava genotypes

Source	df	MS	*P*
Guava	3	433.0	<0.01
Assay	3	192.9	<0.01
Guava × Assay	9	16.8	<0.01
Error	32	4.7	

Table 4
Antioxidant activity of guava fruit methanol extracts as determined by the ABTS, DPPH, FRAP, and ORAC assays from four guava genotypes

Genotype	Antioxidant activity (μM TE/g FM)				Genotypic mean ($P = 0.01$)
	ABTS	DPPH	FRAP	ORAC	
Allahabad Safeda	37.9±3.4	32.0±5.1	33.3±1.4	25.5±1.6	32.25.1 a
Fan Retief	34.4±2.1	27.7±1.7	30.4±1.2	21.0±2.4	28.45.6 b
Ruby Supreme	22.3±0.9	16.2±1.0	15.5±1.4	18.2±2.3	18.03.1 d
Advanced selection	29.6±2.3	24.9±0.5	25.3±1.1	20.5±1.8	25.13.7 c
Assay mean ($P = 0.01$)	31.1±6.8 a	25.2±6.7 b	26.1±7.8 b	21.3±3.1 c	

Therefore, guava is another fruit that has an exceptionally high antioxidant activity. The antioxidant activities obtained in the present study were very high compared to other fruit crops. Wang et al. (1996) reported the antioxidant activity of 12 fresh fruits (melon, pear, tomato, apple, banana, white and pink grape, pink grapefruit, orange, kiwi, plum, strawberry) ranging from less than 1 μM TE/g for melon up to 15 μM TE/g for strawberry.

The average AOAM values were 31.1, 25.2, 26.1 and 21.3 μM TE/g as determined by the ABTS, DPPH, FRAP, and ORAC assays, respectively (Table 4). The different AOAM levels obtained from the assays may reflect a relative difference in the ability of antioxidant compounds in the extracts to quench aqueous peroxyl radicals and to reduce $ABTS^{\bullet+}$, the DPPH free radical and ferric iron in in vitro systems. Although the interaction of guava and assay was significant for the AOAM, it only explained a small amount of the total variation as compared to either guava or assay (Table 3). The interaction of guava and assay, however, was not significant for the AOAM when the ORAC technique was excluded from the analysis (data not shown). These indicated that the other three techniques yielded comparable results between clones.

3.4. Antioxidant activity measured in dichloromethane extract

Only three assays, ABTS, DPPH, and FRAP, were used to measure AOAD. The ANOVA showed that AOAD level was significantly different among assays ($P < 0.01$) but not between guava clones, with no interaction between guavas and assays (Table 5). Means of the AOAD levels from the four guavas were 0.44, 0.27, and 0.16 μM TE/g as determined by the ABTS, DPPH, and FRAP assays, respectively (Table 6). The AOAD levels were very low: less than 2% of the total. Means of the AOAD levels from the four guavas as determined by the ABTS were 2 and 3 times of DPPH and FRAP assays, respectively (Table 6).

3.5. Correlations

Correlations between AOAM obtained from all assays, TPH and AA were positively high ($0.61 \leqslant r \leqslant 0.97$, $P < 0.05$), especially between AOAM based on FRAP assay and TPH ($r = 0.97$, $P < 0.01$) and AA ($r = 0.92$, $P < 0.01$) (Table 7). Most techniques, including these four, used for determining antioxidant activity, showed high correlation with TPH in different crops. It could be that phenolic compounds, which are known as hydrophilic antioxidants, are secondary metabolites that are most abundant in fruits (Macheix et al., 1990). Gil et al. (2002) found high correlation ($r > 0.9$, $P \leqslant 0.05$) between antioxidant activities as determined by DPPH or FRAP assays and TPH in nectarines, peaches and plums. Also, high correlation between TPH and antioxidant activity as determined by FRAP or electron spin resonance spectroscopy were reported in fruit juices (Gardner et al., 2000). Whereas, high correlation between antioxidant activity using any method and AA was likely to be found in only fruits that contain high AA such as orange (Gardner et al., 2000) and guava (Table 6). Gil et al. (2002) demonstrated that there was no correlation between AA and antioxidant activity as determined by DPPH or FRAP assays in nectarines, peaches and plums. The AA in nectarines (4.8–13.2 mg/100 g), peaches (3.6–12.6 mg/100 g), and plums (2.5–10.2 mg/100 g) were very low compared to guavas (174.2–396.7 mg/100 g). The high correlation between any of AOAM with TPH or AA in guava suggested that it was feasible to use TPH or AA to screen for AOAM. Both AA and TPH showed high positive correlation with AOAM as determined by all assays, which indicates that AA and TPH are important contributors to antioxidant activity in guava extracts. Although high phenolics may cause problems with browning in fruits, Ozoglu and Bayindirli (2002) reported that ascorbic acid can inhibit enzymic browning in apple juice. Therefore, guava fruits containing high amount of AOAM are less likely to experience flesh browning problem.

Table 5
ANOVA for antioxidant activity by the ABTS, DPPH, and FRAP assays based on dichloromethane extraction from four guava genotypes

Source	Df	MS	P
Guava	3	0.032	0.11
Assay	2	0.238	<0.01
Guava × Assay	6	0.023	0.18
Error	24	0.014	

Table 6
Antioxidant activities of guava fruit extracts as determined by the ABTS, DPPH, and FRAP assays based on dichloromethane extraction from four guava genotypes

Genotype	Antioxidant activity (μM TE/g FM)			Genotypic mean ($P = 0.11$)
	ABTS	DPPH	FRAP	
Allahabad Safeda	0.38±0.10	0.20±0.06	0.25±0.06	0.28±0.09
Fan Retief	0.42±0.06	0.35±0.28	0.12±0.01	0.30±0.16
Ruby Supreme	0.58±0.15	0.39±0.19	0.13±0.02	0.37±0.23
Advanced selection	0.38±0.10	0.15±0.04	0.13±0.02	0.22±0.14
Assay mean ($P = 0.01$)	0.44±0.10 a	0.27±0.12 b	0.16±0.06 c	

Table 7
Pearson's correlation coefficients of antioxidant activities, ascorbic acid, total phenolics, and total carotenoids content

Trait[a]	TPH	BET	AA	ABM	ABD	DPM	DPD	FRM	FRD
BET	−0.79**								
AA	0.89**	-0.50^{ns}							
ABM	0.97**	−0.67*	0.88**						
ABD	−0.58*	-0.53^{ns}	-0.44^{ns}	-0.55^{ns}					
DPM	0.86**	−0.81**	0.81**	0.85**	−0.66*				
DPD	-0.26^{ns}	0.52^{ns}	-0.12^{ns}	-0.26^{ns}	0.22^{ns}	-0.38^{ns}			
FRM	0.97**	−0.73*	0.92**	0.97**	−0.60*	0.92**	-0.32^{ns}		
FRD	0.56^{ns}	-0.05^{ns}	0.36^{ns}	0.57^{ns}	-0.29^{ns}	0.62*	-0.32^{ns}	0.54^{ns}	
ORM	0.81**	-0.57^{ns}	0.61*	0.82**	-0.21^{ns}	0.68*	-0.18^{ns}	0.74**	0.74**

[a]TPH = total phenolics, BET = β-carotene, AA = ascorbic acid, ABM = antioxidant activity measured in methanol extract based on ABTS assay, ABD = antioxidant activity measured in dichloromethane extract based on ABTS assay, DPM = antioxidant activity measured in methanol extract based on DPPH assay, DPD = antioxidant activity measured in dichloromethane extract based on DPPH assay, FRM = antioxidant activity measured in methanol extract based on FRAP assay, FRD = antioxidant activity measured in dichloromethane extract based on FRAP assay, and ORM = antioxidant activity measured in methanol extract based on ORAC assay.
^{ns} = non significant and *,** = significant at $P < 0.05$ or 0.01, respectively.

The AOAM based on ABTS, DPPH, and FRAP assays were negatively correlated with BET. Correlation between all pairs of AOAM assays were positively high ($0.68 \leqslant r \leqslant 0.97$, $P < 0.01$) indicating that guava extracts had comparable activities in all four assays. High correlation between these four techniques was also found in other crops. Connor et al. (2002) found high correlation among ORAC, FRAP, and methyl linoleate oxidation assays in blueberries. Awika et al. (2003) also found high correlation between ORAC, ABTS, and DPPH in sorghum and its products. There were no consistent correlations between assays to measure AOAM and AOAD nor were AOAD assays correlated among themselves. Correlations among AOAM based on ABTS, DPPH, FRAP, and ORAC assays were positively high and ranged between 0.68 and 0.97: the highest correlation was between ABTS and FRAP (0.97) and the lowest correlation was between DPPH and ORAC (0.68).

4. Conclusion

The ABTS, DPPH, FRAP, and ORAC assays gave comparable results for the antioxidant activity measured in methanol extract of guava fruit extracts. The FRAP technique showed high reproducibility, was simple, rapidly performed and showed the highest correlation with both ascorbic acid and total phenolics. Therefore, it would be an appropriate technique for determining antioxidant in guava fruit extract. Antioxidant activity measured in methanol extract may also be estimated indirectly by using ascorbic acid or total phenolics since they showed high correlation with all assays. Antioxidant activity measured in dichloromethane extract in guava fruit extract was low (2% of total) compared to antioxidant activity measured in methanol extract. Ascorbic acid and phenolics are the major contributors to antioxidant activity in guava fruit.

Acknowledgements

This research was supported financially by the Thailand Research Fund through the Royal Golden Jubilee Ph.D. Program (PHD/0203/2545) and a grant from the Graduate School of Kasetsart University, Thailand. The authors acknowledged L.F. Reyes and B. Surjadinata for technical assistance.

References

Arnao, M.B., Cano, A., Acosta, M., 2001. The hydrophilic and lipophilic contribution to total antioxidant activity. Food Chemistry 73, 239–244.

Association of Office Analytical Chemists, 1996. Official Methods of Analysis, 15th ed. George Banta, Washington, DC.

Awika, J.M., Rooney, L.W., Wu, X., Prior, R.L., Cisneros-Zevallos, L., 2003. Screening methods to measure antioxidant activity of sorghum (*Sorghum bicolor*) and sorghum products. Journal of Agricultural and Food Chemistry 51, 6657–6662.

Benzie, I.F.F., Strain, J.J., 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Analytical Biochemistry 239, 70–76.

Benzie, I.F.F., Strain, J.J., 1999. Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods in Enzymology 299, 15–27.

Brand-Williams, W., Cuvelier, M.E., Berset, C., 1995. Use of free radical method to evaluate antioxidant activity. Lebensmittel Wissenschaft und Technologie 28, 25–30.

Cao, G., Alessio, H.M., Cutler, R.G., 1993. Oxygen-radical absorbance capacity assay for antioxidants. Free Radical Biology and Medicine 14, 303–311.

Connor, A.M., Luby, J.J., Tong, C.B.S., 2002. Variability in antioxidant activity in blueberry and correlations among different antioxidant activity assays. Journal of the American Society for Horticultural Science 127, 238–244.

Gardner, P.T., White, T.A.C., McPhail, D.B., Duthie, G.G., 2000. The relative contributions of vitamin C, carotenoids and phenolics to the antioxidant potential of fruit juices. Food Chemistry 68, 471–474.

Gil, M.I., Tomas-Barberan, F.A., Hess-Pierce, B., Kader, A.A., 2002. Antioxidant capacities, phenolic compounds, carotenoids, and vitamin C contents of nectarine, peach, and plum cultivars from California. Journal of Agricultural and Food Chemistry 50, 4976–4982.

Guo, C., Yang, J., Wei, J., Li, Y., Xu, J., Jiang, Y., 2003. Antioxidant activities of peel, pulp and seed fractions of common fruits as determined by FRAP assay. Nutrition Research 23, 1719–1726.

Halliwell, B., 1996. Antioxidants in human health and disease. Annual Review of Nutrition 16, 33–50.

Jimenez-Escrig, A., Rincon, M., Pulido, R., Saura-Calixto, F., 2001. Guava fruit (*Psidium guajava* L.) as a new source of antioxidant dietary fiber. Journal of Agricultural and Food Chemistry 49, 5489–5493.

Leong, L.P., Shui, G., 2002. An investigation of antioxidant capacity of fruits in Singapore markets. Food Chemistry 76, 69–75.

Luximon-Ramma, A., Bahorun, T., Crozier, A., 2003. Antioxidant actions and phenolic and vitamin C contents of common Mauritian exotic fruits. Journal of the Science of Food and Agriculture 83, 496–502.

Macheix, J.J., Fleuriet, A., Billot, J., 1990. Fruit Phenolics. CRC Press, Boca Raton, FL.

Mercadante, A.Z., Steck, A., Pfander, H., 1999. Carotenoids from guava (*Psidium guajava* L): isolation and structure elucidation. Journal of Agricultural and Food Chemistry 47, 145–151.

Miean, K.H., Mohamed, S., 2001. Flavonoid (myricetin, quercetin, kaempferol, luteolin, and apigenin) content of edible tropical plants. Journal of Agricultural and Food Chemistry 49, 3106–3112.

Miller, N.J., Rice-Evans, C.A., 1997. Factors influencing the antioxidant activity determined by the $ABTS^{\bullet+}$ radical cation assay. Free Radical Research 26, 195–199.

Misra, K., Seshadri, T.R., 1968. Chemical components of the fruits of *Psidium guajava*. Phytochemistry 7, 641–645.

Ou, B., Hampsch-Woodill, M., Prior, R.L., 2001. Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. Journal of Agricultural and Food Chemistry 49, 4619–4626.

Ou, B., Huang, D., Hampsch-Woodill, M., Flanagan, J.A., Deemer, E.K., 2002. Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays: a comparative study. Journal of Agricultural and Food Chemistry 50, 3122–3128.

Ozoglu, H., Bayindirli, A., 2002. Inhibition of enzymic browning in cloudy apple juice with selected antibrowning reagents. Food Control 13, 213–221.

Prior, R.L., Cao, G., Matin, A., Sofic, E., McEwen, J., O'Brien, C., Lischner, N., Ehlenfeldt, M., Kalt, W., Krewer, G., Mainland, C.M., 1998. Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity, and variety of Vaccinium species. Journal of Agricultural and Food Chemistry 46, 2686–2693.

Prior, R.L., Hoang, H., Gu, L., Wu, X., Bacchiocca, M., Howard, L., Hampsch-Woodill, M., Huang, D., Ou, B., Jacob, R., 2003. Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity ($ORAC_{FL}$)) of plasma and other biological and food samples. Journal of Agricultural and Food Chemistry 51, 3273–3279.

Renaud, S.C., Gueguen, R., Schenker, J., d'Houtaud, A., 1998. Alcohol and mortality in middle-aged men from eastern France. Epidemiology 9, 184–188.

Setiawan, B., Sulaeman, A., Giraud, D.W., Driskell, J.A., 2001. Carotenoid content of selected Indonesian fruits. Journal of Food Composition and Analysis 14, 169–176.

Swain, T., Hillis, W.E., 1959. The phenolic constituents of *Prunus domestica* I—the quantitative analysis of phenolic constituents. Journal of Science of Food and Agriculture 10, 63–68.

Talcott, S.T., Howard, L.R., 1999. Phenolic autoxidation is responsible for color degradation in processed carrot puree. Journal of Agricultural and Food Chemistry 47, 2109–2115.

Temple, N.J., 2000. Antioxidants and disease: more questions than answers. Nutrition Research 20, 449–459.

Wang, H., Cao, G., Prior, R.L., 1996. Total antioxidant capacity of fruits. Journal of Agricultural and Food Chemistry 44, 701–705.

Wilberg, V.C., Rodriguez-Amaya, D.B., 1995. HPLC quantitation of major carotenoids of fresh and processed guava, mango and papaya. Lebensmittel Wissenschaft und Technologie 28, 474–480.