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Antioxidant activity in pulp and peel of three mango varieties

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ABSTRACT

The aim of the present study was to estimate the content of total polyphenols and flavonoids and to investigate in-vitro antioxidant potential of methanolic extracts of peel and pulp in three Indian mango varieties. Antioxidant activity was assessed using [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)] ABTS+ assay, 2,2-diphenyl-1-picryl-hydrazyl (DPPH) assay, ferric-reducing ability of plasma (FRAP) assay, and phosphomolybdate assay for Total Antioxidant Capacity (TAC). Total phenolic and flavonoid content was also determined, and expressed in gallic acid equivalent (GAE) and quercetin equivalent (QE), respectively. Results of this study indicated that methanolic extracts of mango peel had significantly higher antioxidant activity compared to that of pulp (29.69 and 3.12), irrespective of the method or variety used. Free radical scavenging and antioxidant activity may be attributed to presence of phenolic (24.61mg GAE/g DM in the peel and 2.01mg GAE/g DM in the pulp) and flavonoid compounds (24.95mg QE/g DM in the peel and 16.15mg E/g DM in the pulp). Antioxidant activity determined by ABTS, DPPH and FRAP assays in mango peel was significantly higher than in the mango pulp (24.95 1.96mg TE /g DM, 23.68 versus 4.60mg BHA/g DM and 40.52 versus 2.781mg TE/g DM), respectively. Results for scavenging activity against DPPH were 96.18% for the peel and 23.86% for the pulp, while, free radical scavenging activity results using ABTS+ assay were 99.62% in the peel and 13.46% in the pulp. Our study justifies research in processing of mango peel into useful, functional food ingredients (powders or extracts).

Key words: Total polyphenols, flavonoids, bioactive compounds, edible waste, antioxidant activity

INTRODUCTION

The growing interest about potential health-promoting effects of antioxidants in everyday foods, combined with an assumption that a number of common, synthetic antioxidant preservatives may have harmful effects (Krishnakumar and Gordon, 1996) has led research and development to focus on the field of natural antioxidants. Natural antioxidants, particularly from fruits and vegetables, have gained increasing interest among both the consumer and the scientific research community, because, recent developments in epidemiological studies have indicated that frequent consumption of natural antioxidants is associated with lower risk of cardiovascular disease and cancer (Renaud *et al*, 1998; Temple, 2000).

Different assays have been introduced for measuring antioxidant capacity of foods and a variety of biological samples. The concept of antioxidant capacity first originated from chemistry, and was later adapted to biology, medicine, epidemiology and nutrition (Prior and Cao, 1999; Pellegrini *et al*, 2003; Floegel *et al*, 2011). It describes the ability of redox molecules in foods and biological systems to scavenge free radicals. Antioxidant capacity of any food is due to a mixture of various antioxidant compounds through different mechanisms; therefore, antioxidant capacity of any food product must be evaluated with a variety of methods (Pérez-Jiménez *et al*, 2008). In the recent years, a wide range of spectrophotometric assays has been adopted to measure antioxidant capacity of foods, the most popular being 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) and 1,1'-diphenyl-2-picrylhydrazyl (DPPH) assay, among others (such as oxygen radical absorbance capacity (ORAC) and ferric reducing ability of plasma (FRAP) assays) (Brand-Williams *et al*, 1995; van den Berg *et al*, 1999; Re *et al*, 1999; Ou *et al*, 2002; Kim *et al*, 2003; Thaipong *et al*, 2006). Most assays employ the same principle: a synthetic, coloured radical or redox-active compound is generated; thereafter, the ability of a biological sample to scavenge the radical or to reduce the redox-active compound is monitored by a spectrophotometer while applying an appropriate standard

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to quantify the antioxidant capacity. The most widely-used methods are ABTS and DPPH radicals (Kuskoski *et al*, 2005; Ali *et al*, 2008; Almeida *et al*, 2011).

Mango is a seasonal fruit processed into various products such as puree, nectar, leather, pickles, canned slices, etc., which have worldwide popularity (Loeillet, 1994). During the processing of mango, a huge amount of peel is generated and is considered a waste by-product. Also, its disposal is a major problem, causing environmental pollution. The peel constitutes about 15% to 20% of the whole mango fruit. Fresh mango-peel contains a number of valuable compounds such as polyphenols, carotenoids, enzymes and dietary fibres (Ajila *et al*, 2007a, b). Peels are a major by-product obtained during processing of various fruits, and these have been shown to be a good source of polyphenols, flavonoids, carotenoids, dietary fibres and other bioactive compounds that possess various beneficial effects on human health (Larrauri *et al*, 1996; Larrauri, 1999; Wolfe *et al*, 2003; Ajila *et al*, 2007a; Luthria, 2012). Some of these compounds exhibit good antioxidant property (Ajila *et al*, 2007b). Use of fruits such as mango, as a source of some phytochemicals (carotenoids, phenolics and flavonoids) is health-promoting as, the latter are, natural antioxidants (Saxena *et al*, 2009) by their action against free radicals generated by lipid peroxidation. Phenolics play an important role as aroma constituents in fruits (Saxena *et al*, 2009). Mango is a good source of many of these beneficial phytochemicals. Devising appropriate methods of utilization of this waste (mango peel) can help overcome some of the nutrition-security challenges in developing countries such as India, and help combat many diet-related diseases or to overcome malnutrition.

The aim of the present research was to compare the efficiency of ABTS, DPPH, FRAP and phosphomolybdate assays for estimating antioxidant activity in mango and their correlation with total phenolics and total flavonoids in the pulp and peel of three varieties.

MATERIAL AND METHODS

Reagents and standards

All the chemicals used in the study were of analytical grade. ABTS, (+)-catechin, DPPH, Folin–Ciocalteu's phenol reagent, gallic acid, Trolox, Quercitin and BHA were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Ascorbic acid was obtained from Fischer Scientific (Fair Lawn, NJ, USA). 2,2-Azo-bis (2-amidinopropane) dihydrochloride was purchased from Wako Chemicals Inc. (Richmond, VA, USA). Standard solutions were prepared with distilled deionized water obtained through Simplicity™ water purification system (Millipore, USA).

Sample collection

For this study, ripe mangoes of Alphonso, Kesar and Totapuri varieties were procured from the wholesale traders of UAS, Dharwad, Karnataka, India. Mangoes were washed in water and their peel was removed using a sharp knife. The underlying pulp was removed by gently scraping with the knife's blunt edge. The pulp was homogenized using a hand-held blender, whereas, the peel was cut into small pieces before both were dried using a cabinet drier maintained at 55±2R"C for 12 h. Following drying, the peel was ground to a fine powder, packed in a polyethylene bag and stored at -20R"C for further chemical analysis.

Sample extraction

Sample extraction was done in Department of Bioresource Engineering, Macdonald Campus, McGill University, Montreal, Canada. Approximately 1.5g of mango pulp and peel powders were transferred to 50ml graduated centrifuge tubes and mixed with 25ml methanol. The extraction was carried out by placing the tubes in an incubator shaker (Benchmark company) for 24h at 31^{o}C. Filtration and recuperation was done using Whatman No. 4 filter paper and methanol solution, making the final volume to 25ml and stored at -20^{o}C for further analysis.

Determination of antioxidant constituents

Total phenolics quantification

Total phenolic content in mango peel and pulp extracts was determined using Folin-Ciocalteu reagent (spectrophotometric method), using gallic acid as a standard. A slight modification was made in the method of Singleton and Rossi (1965) and Waterhouse (2002). Briefly, 320µl of the extract was mixed with 1280µl Folin Ciocalteu reagent, to which 800µl of 7.5% sodium carbonate solution was added along with 800µl deionized water. This solution was mixed well, incubated at 40^{o}C for 30 minutes and the absorbance was measured using the reagent blank at 765nm with a spectrophotometer (Ultraspec1000, Amersham Pharmacia Biotech, NJ, USA).

Total flavonoid quantification

Total flavonoid content in mango pulp and peel extracts was determined using the method developed by Zhishen *et al* (1999).

Antioxidant activity quantification

ABTS assay: (2, 2-azino-bis (3-ethylbenzothiazolin-6sulphonic acid):

For ABTS assay, methods of Arnao *et al* (2001) and Re *et al* (1999), with some modification, were followed. Fresh ABTS solution was prepared on the day of the experiment. Mango pulp or peel extract or standard Trolox solution (150µl) were allowed to react with 2850µl ABTS solution for 2h in the dark at room temperature. Then, absorbance was measured at 734nm using a spectrophotometer.

DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay:

DPPH assay was carried out as per Ohnishi *et al* (1994) with some modification. A solution of 0.1mM DPPH was prepared in 50ml methanol. The 270µl standard BHA (butylated hydroxyanisole) or mango pulp or peel extract were mixed with 1620µl DPPH solution and incubated for 20 minutes in the dark (covered with aluminium foil) and absorbance read at 517nm using a spectrophotometer (Ultraspec1000, Amersham Pharmacia Biotech, NJ, USA).

% Scavenging = $[(A_B-A_E) \times 100]/A_B$

where,

A_B is absorbance of the blank solution with DPPH, and A_E is absorbance of the extract solution with DPPH.

FRAP (Ferric reducing ability of plasma) assay

FRAP assay was done as per Benzie and Strain (1996), with some modification. To evaluate the antioxidant activity, mango pulp and peel extracts or Trolox standard (150µl) were allowed to react with 2850µl FRAP solution for 30 minutes at room temperature under dark conditions. Absorbance of the coloured solutions (ferrous tripyridyltriazine complex) was then read at 593nm using a spectrophotometer (Ultraspec 1000, Amersham Pharmacia Biotech, NJ, USA).

Statistical analysis

Each assay of antioxidant activity, total polyphenols, total flavonoids, TAC and Scavenging activity was made in triplicate in each sample extract to ensure reproducibility. Analysis of Variance (ANOVA) was used for testing any difference in antioxidant activity resulting from using these methods. Duncan's new multiple range test was used for determining significant difference. Correlations among the data obtained were calculated using Pearson's correlation coefficient. These statistical analyses were carried out using SPSS software, version 16.0.

RESULTS AND DISCUSSION

Antioxidant constituents

In this study, antioxidant activity of three popular mango varieties, viz., Alphonso, Kesar and Totapuri of South India, were compared using different, standard chemical-antioxidant activity protocols. Polyphenols and flavonoids are secondary metabolites in plants and are widely distributed in fruits and vegetables, beverages and plant-derived foods. Phenolic compounds and flavonoids are a major groups of compounds contributing to antioxidant activity in fruits, vegetables, cereals and other plant-based materials. These bioactive compounds are heat-sensitive or thermomobile, as, high temperature may cause their degradation and decomposition (Garau *et al,* 2007). In this study, 50°C was fixed as the maximum temperature for drying the samples to conserve these valuable bioactive compounds. Solvent extraction is the most common method used for extraction of bioactive compounds. Different solvent-extraction methods are used currently, of which hot water bath extraction (de Rijke *et al,* 2006; Søltoft *et al,* 2009), soxhlet extraction (Bhushan *et al,* 2008) and microwave extraction are the most commonly used for extraction of bioactive compounds.

Total polyphenols

Phenolic compounds are known, powerful chain-breaking antioxidants (Shahidi *et al,* 1994; Wanasundara and Shahidi, 1998; Shahidi and Wanasundara, 2002) and are very important plant constituents owing to their scavenging ability attributed to their hydroxyl groups (Hatano *et al,* 1992). Total polyphenol content in our study was significantly higher in mango peel (21.613mg GAE/g DM) compared to that in the mango pulp (2.013mg GAE/g DM) irrespective of the variety. Among varieties, in both peel and pulp, significant difference in total polyphenol content was observed (Table 1). Kesar peel and Alphonso pulp had the highest total polyphenol content at 35.144 and 2.249mg GAE/g DM, respectively. During the development of the mango fruit, total phenols have been found to be higher in the peel than in the flesh, at all the stages of fruit development (Lakshminarayana *et al,* 1970). Earlier, Larrauri *et al* (1996) reported total polyphenol content in aqueous methanol extract of ripe peel of 'Hayden' variety of mango to be 70mg/g. This value falls within the range reported in the present study. Similar results (54.64mg/g GAE) were reported by Ajila *et al* (2010a, b) stating that gallic acid, syringic acid, mangiferin, ellagic acid, gentisyl-protocatechuic acid and quercetin were the phenolic compounds present in ripe

mango peel. (Abdul Aziz *et al,* 2012) reported total phenolics content in ripe mango peel and pulp to be 70.20 and 14.57mg GAE/g DM, respectively. Mango peel extract was reported to contain 9mg GAE/g DM polyphenols (Gondi *et al,* 2014), 19.06mg GAE/g (Ashoush and Gadallah, 2011), 96.2mg GAE/g in Mango Peel Powder (MPP) (Ajila *et al,* 2008; Ajila and Prasada Rao, 2008; Ajila *et al,* 2010a). Phenolic content in mango has been reported to vary from 15.3 to 266mg GAE/100g fresh weight (FW) (Wu *et al,* 2004; Noratto *et al,* 2010). The slight variation reported in polyphenol content may be attributed to a difference in the variety, region or agroclimatic conditions. Total polyphenol content decreased with peel browning during cold storage (Chidtragool *et al,* 2011). Total polyphenol content in 'Langra' and 'Chausa' mango varieties was 116.80 and 122.60mg GAE/g DM, respectively (Sultana *et al,* 2012). Higher phenolics content can contribute potentially to improved antioxidant activity (Gonzalez Aguilar *et al,* 2008).

Table 1. Total polyphenolics and total flavonoids content in peel and pulp of three mango varieties

Part of mango	Variety	Total polyphenols (mg GAE/g DM)	Total flavonoids (mg QE/g DM)
Peel	Alphonso	23.919 ± 0.635[b]	25.519 ± 1.886[a]
	Kesar	35.144 ± 0.263[c]	34.897 ± 0.703[b]
	Totapuri	14.776 ± 0.442[a]	14.429 ± 0.228[ab]
	Mean	24.613 ± 8.844	24.948 ± 8.930
Pulp	Alphonso	2.249 ± 0.205[b]	13.870 ± 1.886[a]
	Kesar	1.975 ± 0.130[c]	9.084 ± 0.468[b]
	Totapuri	1.815 ± 0.134[a]	25.557 ± 1.286[ab]
	Mean	2.013 ± 0.235	16.150 ± 7.436
Grand Mean		13.313	19.333

	Sem±	CD	Sem±	CD
Portion	0.114	0.493**	0.359	1.550**
Variety	0.155	0.669**	0.486	NS
Portion x Variety	0.260	1.124**	0.818	3.533**

Note: Values are the mean of three replications; SEm: Standard error of mean; CD: Critical difference; AAE: Ascorbic acid Equivalent; GAE: Gallic acid equivalent; QE: Quercetin Equivalent, **Significant @ 1%; Values with the same superscript (a, b, c) in the same row are not significantly different *(p≤0.01).*

Total flavonoids

Flavonoids are capable of effectively scavenging reactive oxygen species because of their phenolic hydroxyl groups and are, therefore, considered to be potent antioxidants (Cao *et al,* 1997). Flavonoids have been demonstrated to have antioxidant activity and to exert a positive effect on prevention of cardiovascular disorders and diseases caused by free radicals (Yao *et al,* 2004). Besides, these also exhibit several other biological effects such as anti-inflammatory, anti-hepatotoxic, anti-ulcer, anti-allergic, anti-viral and anti-cancer activity (Umamaheswari and Chatterjee, 2008). Total flavonoid content was significantly higher in mango peel (24.948mg QE/ g DM) compared to that in the mango pulp (16.150mg QE/g DM), and, a significant difference was observed among varieties (Table 1). Total flavonoid content was significantly higher in 'Kesar' peel (34.897mg QE/g DM) and 'Alphonso' pulp (13.89mg QE/g DM). Similar results on total flavonoid content were reported by Abdul Aziz *et al* (2012) in ripe mango peel at 29.24mg QE/g DM, and the pulp at 5.43mg QE/g DM; whereas, Gondi *et al* (2014) reported 8.5mg QE/ g DM of flavonoids in the mango peel. Total flavonoid content in 'Langra' and 'Chausa' mango varietes was reported at 90.89 and 92.55mg CE/g DM (Sultana *et al,* 2012). Our results showed that flavonoid content in the peel was higher than in the pulp, in accordance with results of Li *et al* (2013).

Antioxidant activity

Table 2 shows antioxidant/ antiradical activity of methanolic extract prepared from the peel and pulp of three mango varieties. Peel from the three varieties showed variable, but high, antioxidant activity in the three assays tested (FRAP, DPPH and ABTS). Large variations in antioxidant activity were observed when the peel and pulp were tested separately. These variations were statistically significant *(p=0.01).* According to several authors, content of the antioxidant compounds and related antioxidant activity are particularly high in the peel of some fruits (Ajila *et al,* 2007a, b; Vieira *et al,* 2009). Variations in antioxidant activity between and within food groups are well-documented. Antioxidant activity exhibited a dose-dependent trend in all the assays used. In our work, the total antioxidant activity in mango peel, evaluated using FRAP assay, was significantly higher compared to ABTS or DPPH assays. On the other hand, the total antioxidant activity in mango pulp, evaluated with DPPH assay, was significantly higher than in the other two assays. Among the two mango-fruit parts studied, total antioxidant activity in the peel was significantly higher in all the assays evaluated (ABTS, DPPH, FRAP) compared to that in the pulp of the mango fruit. 'Kesar' variety had significantly higher antioxidant activity, irrespective of the antioxidant-activity assay used, or the part of the fruit, followed by that in 'Alphonso' and 'Totapuri'.

ABTS assay

Table 2 shows antioxidant activity in ABTS value measurements of methanolic extracts of the peel and pulp in three mango varieties. The overall ABTS value averaged 13.373mg TE/g DM, and ranged from 1.619 to 24.814mg

Table 2. Antioxidant activity as determined by the ABTS, DPPH and FRAP assays based on methanol extraction from peel and pulp of three mango varieties

Mango Varieties	Antioxidant activity									Mean C
	ABTS (mg TE/ g DM)			DPPH (mg BHA/g DM			FRAP (mg TE/ g DM)			
	Pulp	Peel	Mean	Pulp	Peel	Mean	Pulp	Peel	Mean	
Alphonso	1.62 ± 0.29	24.77 ± 0.10	13.19 ± 12.68	4.35 ± 0.28	23.73 ± 0.09	14.04 ± 10.61	2.99 ± 0.12	39.16 ± 1.48	21.08 ± 19.83	16.11 ± 14.17[B]
Kesar	1.73 ± 0.47	24.81 ± 0.04	13.27 ± 12.65	3.30 ± 0.25	23.79 ± 0.04	13.54 ± 11.22	2.26 ± 0.13	57.24 ± 1.41	29.75 ± 30.13	18.86 ± 20.35[A]
Totapuri	2.54 ± 0.13	24.76 ± 0.05	13.65 ± 12.17	6.17 ± 0.44	23.70 ± 0.03	14.93 ± 9.60	3.09 ± 0.51	25.15 ± 0.46	14.11 ± 12.09	14.23 ± 10.68[C]
Mean	1.96 ± 0.52	24.78 ± 0.07	13.37 ± 11.75	4.61 ± 1.29	23.74 ± 0.064	14.17 ± 9.88	2.78 ± 0.48	40.52 ± 13.97	21.65 ± 21.65	
Factor A		13.37 ±11.75[c]			14.17 ± 9.88[b]			21.65 ± 21.65[a]		
Factor B	Pulp	3.11 ± 1.39								
	Peel	29.68 ±11.01								
	SEm ±	CD								
Factor A	0.084	0.324**								
Factor B	0.062	0.240**								
Factor C	0.084	0.324**								
A x B	0.142	0.545**								
A x C	0.192	0.740**								
B x C	0.142	0.545**								
A x B x C	0.324	1.244**								

Note 1: Values are the mean of three replications; SEm: Standard error of mean; CD: Critical difference; ABTS-2: 2-azino-bis (3-3thylbenzthiazolin-6sulphonic) acid; DPPH- 2: 2-diphenyl-1-picrylhydrazyl; FRAP: Ferric Reducing Ability of Plasma, **Significant @ 1%. Values with the same superscript (a, b, c) in the same row are not significantly different *(p≤0.01)*; Values with the same superscript (A,B,C) in the same column are not significantly different *(p≤0.01)*; Factor A: Between assays (ABTD, DPPH, FRAP), Factor B: Between portions (Pulp, Peel); Factor C: Between varieties (Alphonso, Keasr, Totapuri)

TE/g DM. When the means of total antioxidant activity evaluated by ABTS assay were compared, mango peel had 24.782mg TE/g DM (99.128µmol TE/g DM) which was significant higher than in the mango pulp at 1.964mg TE/g DM (7.856µmol TE/g DM)). Among the three varieties studied, no significant difference was observed in antioxidant activity either the peel or the pulp as evaluated by ABTS assay. Le (2012) reported ABTS scavenging activity of dehydrated mango as varying from 46.7 to 73.8µmol TE/g DM. Antioxidant activity of dried mango pulp was reported as 27.1µmol/g db ascorbic acid equivalent, using ABTS assay (Soong and Barlow, 2004). Antioxidant property of dried mango samples varied from 50.7 to 103.8µmol TE/g db (Sogi *et al*, 2014). Values obtained in our study for antioxidant capacity as Trolox equivalent fall within a close range of previously reported results.

DPPH assay

DPPH antioxidant activity is shown in Table 2. DPPH values in methanolic extracts of the peel and pulp in three mango varieties varied. Overall DPPH value averaged 14.17mg BHA/g DM, and ranged from 3.29 to 23.73mg BHA/g DM. Antioxidant activity in DPPH assay in the mango peel (23.68mg BHA/g DM) was significantly higher than in the pulp (4.60mg BHA/g DM), irrespective of the variety. This significantly higher level of antioxidant activity in mango peel is attributed to a higher level of total polyphenols and total flavonoids, and is comparatively lower in the pulp (Table 1). Among the varieties tested, significant difference was not observed in peel or the pulp. Mango samples extracted from the peel part showed strong scavenging effects compared to the mango pulp. These results are in agreement with previous reports (Ajila *et al*, 2007, 2007a; Abdul Aziz *et al*, 2012) who reported mango peel and pulp as having antioxidant activity in DPPH assay of 43.30 and 9.82mg TE/g DM, respectively. Our results are in agreement with these findings. DPPH radical-scavenging activity varied from 36.5 to 52.0µmol TE/g DM in dried 'Tommy Atkins' mango flesh (Le, 2012). DPPH antioxidant values varied from 34 to 88.6µmol TE/g DM in dehydrated mango powder (Sogi *et al*, 2014).

FRAP assay

As with the other two assays, methanolic extracts from the peel and pulp of three mango varieties were tested and results presented in Table 3. FRAP antioxidant activity ranged from 2.258 ± 0.126 to 57.244 ±1.405 (mg TE/g DM), with an overall average of 21.650mg TE/g DM. The antioxidant activity in FRAP assay for mango peel (40.518 ± 13.973mg TE/g DM) was significantly higher than that in the pulp (2.781 ± 0.477mg TE/g DM). Among the different assays, FRAP indicated significantly higher antioxidant activity, with 21.650mg TE/g DM, compared to ABTS or DPPH (13.373mg TE/g DM and 14.172mg BHA/g DM, respectively).

Several authors have reported antioxidant activity by FRAP assay in different parts and varieties of mango. Abdul Aziz *et al* (2012) reported antioxidant activity using FRAP assay in mango peel and pulp as 65.92 and 15.30mg/g, respectively. Antioxidant compounds like the polyphenols may be more efficient as reducing agents for ferric iron, but will certainly not be effective in scavenging DPPH free-radicals (Wong *et al*, 2006). An inverse correlation was observed between peel-browning and total antioxidant capacity measured using FRAP assay (Chongchatuporn *et al*, 2013). FRAP values varied from 41 to 81µmol TE/g DM in dried mango powder (Sogi *et al*, 2014).

Radical scavenging activity

A free radical is an atom or molecule containing one or more unpaired electrons, making it highly reactive (Halliwell and Gutteridge, 1990; Halliwell *et al*, 1995). Free radicals such as trichloromethyl (CCl_3), superoxide (O_2), hydroxyl (HO), peroxyl (ROO), and nitric oxide (NO) are known to be produced metabolically in living organisms. In addition, some non-radical derivatives of the oxygen molecule [hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl)] can be generated in foods and in biological systems. All these reactive oxygen species (ROS) participate in a chain reaction of free radicals. Thus, tests on ability of a substance to scavenge radical species may be relevant in evaluating their antioxidant activity (Halliwell and Gutteridge, 1989; Halliwell and Gutteridge, 1990; Halliwell *et al*, 1995). Free radical scavenging activity in mango peel and pulp was 97.89 and 18.66%, respectively, irrespective of the variety or assay used by us for determining it (ABTS and DPPH), as presented in Table 3.

ABTS radical scavenging activity

ABTS activity was quantified in terms of percentage inhibition of ABTS+ radical cation by antioxidants in each sample. Significant variation was seen in percentage inhibition in mango peel and pulp (12.12 to 99.65% inhibition), as presented in Table 3. Overall inhibition of ABTS assay was 56.54%, whereas, mango peel had significantly higher free radical scavenging activity (99.62%) than mango pulp (13.46%), irrespective of the variety.

Table 3. Comparison of radical scavenging activity in three mango varieties in peel and pulp using two different assays

Portion of fruit	Variety	Scavenging activity (%)		
		ABTS	DPPH	Mean
Pulp	Alphonso	18.89 ± 1.20	12.59 ± 1.74	15.74 ± 3.70
	Kesar	22.86 ± 1.07	12.12 ± 1.07	17.49 ± 5.96
	Totapuri	29.83 ± 2.01	15.657± 0.67	22.74 ± 7.88
Mean of pulp		23.86 ± 4.97	13.26 ± 1.98	18.66 ± 6.49
Peel	Alphonso	99.65 ± 0.11	96.28 ± 0.05	97.97 ± 1.85
	Kesar	99.65 ± 0.06	96.22 ± 0.05	97.94 ± 1.88
	Totapuri	99.56 ± 0.06	96.03 ± 0.11	97.79 ± 1.94
Mean of peel		99.62 ± 0.08	96.18 ± 0.13	97.90 ± 1.78
Mean of assay		61.74 ± 44.35	57.42 ± 37.36	58.28 ± 40.46
Mean of variety		Alphonso	Kesar	Totapuri
		56.85 ± 43.03[b]	57.71 ± 42.22[b]	60.27 ± 39.58[a]

	SEm ±	CD
Assay	0.113	0.445**
Portion	0.126	0.500**
Variety	0.152	0.603**
Assay x Portion	0.232	0.916**
Assay x Variety	0.257	1.015**
Portion x Variety	0.257	1.015**
Assay x Portion x Variety	0.431	1.706**

Note : Values are the mean of three replications; SEm: Standard error of mean; CD: Critical difference; ABTS-2: 2'-azino-bis (3-3thylbenzthiazolin-6sulphonic) acid; DPPH-2: 2-diphenyl-1-picrylhydrazyl; *Significant @ 5%,** Significant @ 1%

Table 4. Pearson's correlation of antioxidant activities, total polyphenolics and flavonoid content

Trait	ABTS	DPPH	FRAP	TAC	TPP	TF1	TF2
ABTS	1	.997**	.897**	-.155	.887**	.787**	.589*
DPPH		1	.895**	-.208	.884**	.781**	.602**
FRAP			1	-.117	.999**	.909**	.793**
TAC				1	-.108	-.151	-.255
TPP					1	.912**	.796**
TF1						1	.694**
TF2							1

ABTS: 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid; DPPH: 1,1'-diphenyl-2-picrylhydrazyl; FRAP: ferric reducing antioxidant power; TAC-Total Antioxidant Capacity; TPP-Total Polyphenols, TF1 -Total Flavonoids method 1, TF2- Total Flavonoids method 2.
*,** Correlation significant at 0.05, 0.01 levels respectively (2-tailed)

DPPH radical scavenging activity

Radical scavenging ability of extracts measured by DPPH is an important indicator of the anti-oxidative activity. This is highly correlated with total phenolics content in a sample. Results obtained in our study reveal DPPH radical scavenging activity in mango peel to be significantly higher than in the pulp (Table 3). The increase observed in free radical scavenging activity is probably due to a presence of bioactive compounds or natural antioxidants in mango, which, in turn, is attributed to their hydrogen-donating ability (Ajila *et al,* 2008). DPPH radical inhibition activity in mango ranged from 18.89 to 96.28%, with an overall average of 60.02%. DPPH radical scavenging activity was significantly higher in mango peel compared to that in mango pulp, irrespective of the variety. Among the varieties tested, significant differences were observed, with 'Alphonso' and 'Kesar' showing significantly higher radical scavenging activity than 'Totapuri' variety, in both peel and the pulp. Mango peel powder extract in earlier studies has exhibited free radical scavenging activity of 79.6% (Ajila *et al,* 2008, 2010) and 93.89% (Ashoush and Gadallah, 2011).

Correlation between antioxidant activity and antioxidant constituent

Table 4 presents Pearson's correlation among the methods used, and, between the method and the antioxidant constituent. Significant and strong correlation is noticed. Total antioxidant capacity, total polyphenolics and total flavonoid content were strongly and, significantly and positively, correlated with the three different antioxidant activity assays: whereas, only total antioxidant capacity (TAC) was significantly and negatively correlated with all the three antioxidant activity assays, and with total polyphenolics and total flavonoids, as reported by others (Chun *et al,* 2003; Kim *et al,* 2003). These findings, taken together, suggest that total phenolics and flavonoids are major bioactive compounds that act as and perform antioxidant activity in these foods. However, this is presumably due not only to flavonoids, but also non-flavonoid phenolics. Phenolics, commonly found in fruits, have been reported as exhibiting antioxidant activity due to reactivity of the phenol moiety, and have the ability to scavenge free radicals via hydrogen donation or electron donation (Shahidi *et al,* 1992). A causative relationship has been demonstrated between total phenolic content and antioxidant activity (Jayaprakasha and Patil, 2007).

Among the different assays used for analysis of antioxidant activity (ABTS, DPPH and FRAP), results obtained from ABTS and DPPH assay were comparable. FRAP technique showed a high reproducibility, was simple, could be rapidly performed, and showed the highest correlation with total polyphenolics and flavonoids. Therefore, FRAP can be recommended as an appropriate technique for determining antioxidants in mango pulp and peel extracts. Similar results were reported earlier in guava fruit (Thaipong *et al,* 2006).

The peel is considered an edible tissue of the unripe mango fruit. Using unripe mango fruit with its peel, *chutneys* and pickles are prepared. On the other hand, peel of the ripe mango fruit, due to its leathery texture, is not too acceptable taste-wise; therefore, the peel is generally removed and discarded. Thus, in the food processing industry, mango peel ends up generally as a waste by-product. Our study revealed that methanolic extracts of mango peel had significantly higher antioxidant activity than the pulp, which is attributed to higher content of total polyphenols and flavonoids in the peel. Thus, mango peel is rich in bioactive compounds that represent a potential source of natural antioxidants. Mango peel powder, rich in bioactive compounds, can therefore be used as a sprinkle or incorporated into a variety of food preparations to enhance nutraceutical value of the food. Development and utilization of such functional and nutritional products can provide health benefits by preventing degenerative diseases.

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