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Article in Indian Journal of Agricultural Sciences · October 2019



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## Antioxidants in ripe peel and pulp of twelve mango (Mangifera indica) cultivars

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Received: 07 September 2016; Accepted: 08 August 2019

### ABSTRACT

Mangoes are nutritionally rich subtropical fruits with unique flavour, fragrance, taste and health promoting compounds, making it one among new functional foods. Both peel and pulp contain many bioactive phytochemicals like phenolics, carotenoids, flavonoids, etc., which are proven antioxidants. The present study was carried out to profile these bioactive nutraceuticals in peel and pulp of 12 mango cultivars along with total antioxidant activity and L, a, and b values to identify the most beneficial health promoting cultivars. Total phenolics were estimated using Folin-Ciocalteu reagent, total flavonoids and total carotenoids by spectrophotometric methods and total antioxidant activity by CUPRAC (cupric reducing antioxidant capacity) assay. Colorimetric (L, a, and b) values were taken in a colorimeter. The data indicated significant variations in the contents of all three major phytochemicals in both peel and pulp of mango cultivars, where peel contained more nutraceuticals than pulp. The variation in total antioxidant activity in peel and pulp was also significant among the cultivars. It was noticed that total phenolics and total flavonoids contributed more towards total antioxidant activity as compared to total carotenoids. On the basis of the contents of total phenolics and total flavonoids along with total antioxidant activity, Neelum was found nutritionally richest cultivar followed by Mallika and Amrapali. Being rich in antioxidant principles, ripe mango peel can also be utilised for phenolics extraction through waste management technique and in phyto-pharmaceutical industry.

#### Key words: Antioxidants, Mango, L, a, b values, Peel, Pulp

Mango (Mangifera indica L.) is a nutritionally rich fruit with many health promoting qualities. Mango fruit (both peel and pulp) contains several bioactive principles, viz. polyphenols, flavonoids, carotenoids and ascorbic acid having different beneficial properties because of their antioxidant activities (Talcott et al. 2005, Ajila et al. 2007b). The action of phenolic compounds in fruit has drawn a lot of attention now-a-days due to their potential in preventing cancer and heart diseases (Kris-Etherton et al. 2002). These compounds are water soluble, easily oxidized in biological medium and act as antioxidants, protecting organisms against the oxidative stress (Bravo 1998). Flavonoids, a constituent of phenolic compounds, had been identified as flavonol O-and xanthone C-glycosides from mango (cv. Tommy Atkins) peels (Schieber et al. 2003). Among the carotenoid pigments present in fruits, β-carotene provides the highest vitamin A activity.

Mango is processed into several products like pulp,

puree, nectar, leather, pickles, canned dried slices, etc. to prolong its availability in the market. During processing, peel is produced as a major by-product (15-20% of the fruit) which is not generally utilised for any commercial purpose and discarded as waste. Interestingly, the peel fraction of some fruits like pomegranate contains higher antioxidant phytochemicals (Li et al. 2006). Mango peel also possesses a number of bioactive compounds like polyphenols, carotenoids, vitamins (C and E), enzymes and dietary fibre and exhibits good antioxidant properties (Ajila et al. 2007a). Berardini et al. (2005) have reported that mango peel contains much higher phenolic compounds than flesh. The literature on evaluation of antioxidant activity and its corresponding chemicals in popular mango varieties at edible ripe stage is very scanty. Hence the present study was carried out with the aim to evaluate the total phenolics, total flavonoids, total carotenoids and total antioxidant activity in ripe peel and pulp of twelve mango cultivars along with their L, a, b values to identify the most nutritious cultivar.

#### MATERIALS AND METHODS

The fruits of 12 mango cultivars (Neelum, Bangalora, Banganpalli, Amrapali, Mallika, Ambika, Arunika, Vanraj, Kesar, Kensington, Pairi and Lucknow Safeda) were

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harvested randomly at intermediary ripening stage from the Institute orchard located at Rehmankhera, Lucknow, India, during 2015. After washing under running tap water, the fruits were stored at room temperature (37–40°C) for uniform ripening. The peel and pulp of ten ripe fruits from each variety were separated and homogenised. Required quantity of homogenised peel and pulp samples was taken separately for the estimation of different biochemical components using quartering method.

The content of total phenolics in ripe mango peel and pulp was estimated using Folin-Ciocalteu reagent (Singleton *et al.* 1999) at 750 nm in an UV-VIS spectrophotometer (Labomed Inc., USA). The gallic acid was used to prepare the standard curve and result was expressed as mg gallic acid equivalent (GAE)/100 g fresh weight. Total flavonoids concentration was determined colorimetrically according to the method reported in literature (Dewanto *et al.* 2002) at 510 nm using quercetin as standard. It was expressed as mg quercetin equivalent (QE)/100g fresh weight. The analysis of total carotenoids was conducted with the help of a modified method of Ranganna (2000) using acetone and petroleum ether as extraction solvents. The optical density was read at 452 nm in an UV-VIS spectrophotometer.

The total antioxidant activity was measured by CUPRAC (cupric reducing antioxidant capacity) assay as suggested by Apak *et al.* (2004), which measured the copper (II) ion reducing ability of polyphenols, vitamin C and vitamin E. After centrifugation, the absorbance of the supernatant was read at 450 nm in the UV-VIS spectrophotometer (Decibel, Delhi, India). The units were expressed in µmol Trolox/g.

The fruit colour is the first visible quality attribute assessed by the consumer and is critical determinant in the acceptance of the fresh fruit (mangoes) prior to consumption. Therefore, colorimetric values are an important parameter for fresh ripe fruits. Pulp and peel colour of ripe mango fruits was estimated in SPH850 spectrophotometer (Colorlite, Germany) displaying colour in terms of L, a, and b values.

The experimental results were analysed statistically by using completely randomized design (CRD) with three replications each for peel and pulp of each cultivar. The means were compared using WASP (WebAgri Stat Package) software at 1% level of significance. Student's t-test was used for comparisons of means and calculation of LSD values at P $\leq$ 0.01.

#### **RESULTS AND DISCUSSION**

The evaluation of bioactive principles like total phenolics, total flavonoids and total carotenoids along with total antioxidant activity in mango peel and pulp was done to elucidate contribution of individual phytochemicals group towards antioxidant activity of fruit. The colorimetric (L, a, and b) values were estimated to understand the pigmentation pattern of ripe mango peel and pulp.

*Total phenolics in ripe mango peel and pulp:* Total phenolics in different varieties of ripe mango peel ranged between 278.75–379.17 mg GAE/100g, which is a significantly variable range (Table 1). Among the varieties,

Ambika had the highest amount of total phenolics in peel (379.17 mg GAE/100g) followed by Neelum (357.92 mg GAE/100g), whereas, Bangalora peel contained the lowest amount (278.75 mg GAE/100g). In contrast to peel, ripe mango pulp contained much lower total phenolics with a variation between 33.61-120.14 mg GAE/100g, which was also a significant one. In some varieties like Pairi and Banganpalli, it was almost 10 times lower in pulp than in peel (Table 1). Mallika contained the highest amount of total phenolics (120.14 mg GAE/100g) in pulp closely followed by Neelum (119.30 mg GAE/100g), while it was lowest in Pairi (33.61 mg GAE/100g) with second and third lowest in Banganpalli (38.61 mg GAE/100g) and Vanraj (40.56 mg GAE/100g), respectively. Except Mallika and Neelum, remaining varieties had 6-10 times lower amount of total phenolics than in peel. The data indicated that the variation in total phenolics in ripe mango peel and pulp depended on the variety. The range of the total phenolics content in 80% acetone extract of ripe mango peel has been reported between 55-100 mg/g depending on the variety studied (Raspuri and Badami) (Ajila et al. 2007b). Monaco et al. (2014) have also reported that peel of Palmer mango contained more polyphenols than pulp even after sanitization treatment with chlorinated and ozonated water. Berardini et al. (2005) have mentioned that mango peel possessed up to 4860 mg/ kg dry matter of total phenolics, while only traces could be detected in mango flesh. The content of total phenolics is higher in the mango peel than in pulp at any stage of fruit development as mentioned earlier (Lakshminarayana et al. 1970, Ueda et al. 2000). Higher phenolics content were also reported in pomegranate and apple peel (24.94 g GAE/100 g and 309 mg GAE/100 g) as compared to their pulp and flesh (Li et al. 2006, Drogoudi et al. 2008). Ribeiro et al. (2007) have reported that total phenolics content was variable among the four mango varieties, being highest in Uba pulp (around 200 mg GAE/100 g) and lowest in Tommy Atkins pulp (<50 mg GAE/100 g), which is also evidenced in the present study. The phenolics content was 174 mg GAE/100 g FW in ripe pulp of Ataulfo mango as reported by Palafox-Carlos et al. (2012).

Total flavonoids in peel and pulp of ripe mango fruit:

Table 1Variability for biochemical components in peel and pulp<br/>of twelve mango cultivars

Variety	Total phenolics (mg GAE/100g)*		Total flavonoids (mg QE/100g)*		Carot- enoids (mg/ 100g)*		Total antiox- idants (µmol- Trolox/g)*	
	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel
Neelum	119.30	357.92	9.73	310.07	2.89	5.06	1.04	4.20
B a n g a - lora	33.19	278.75	7.00	76.87	1.86	2.90	0.29	1.89
B a n g a - npalli	38.61	324.58	9.73	118.80	2.30	2.90	0.62	5.53
Vanraj	40.56	304.58	16.20	199.33	3.80	4.90	0.50	4.29
Amrapali	69.44	335.70	37.07	307.60	10.33	5.78	0.75	3.84

Similar to the total phenolics content in all the varieties under study, the total flavonoids content was also found much lower in mango pulp (10-30 times) than in mango peel (Table 1). Here also, the variation in the content of total flavonoids among the varieties was highly significant in both peel and pulp. In ripe mango peel, the highest flavonoids content (310.07 mg QE/100g) was recorded in Neelum followed by Ambika (300.93 mg QE/100g). Whereas in ripe mango pulp, the highest flavonoids content was noticed in Amrapali (37.07mg QE/100g) followed by Mallika (28.13 mg QE/100g). The total flavonoids content was recorded lowest in Bangalora (76.87 and 7.00 mg QE/100g in peel and pulp, respectively). Total flavonoids content was also noticed significantly higher in peel of Palmer variety (484.75 mg mangiferin/100 g) than in pulp (14.16 mg mangiferin/100 g) (Monaco et al. 2014). Present study also highlighted the presence of significantly higher total flavonoids in mango peel than in pulp. Marina and Noriham (2014) identified catechin, epicatechin and kaempferol as major flavonoids in peel of mango cv. Chokanan. In peel extract of Tommy Atkins, seven quercetin O-glycosides, one kaempferol O-glycoside and four xanthone C-glycosides were identified (Schieber et al. 2003). In flesh extract of five mango cultivars, mangiferin was identified as major flavonoid along with quercetin 3-O-galactoside and quercetin 3-O-glucoside (Berardini et al. 2005). Shivashankara et al. (2004) have identified quercetin and catechin as the main flavonoids present in Irwin mango.

Total carotenoids in ripe mango peel and pulp: The carotenoids contain  $\alpha$ -carotene,  $\beta$ -carotene and lycopene, and among them,  $\beta$ -carotene is the most prevalent one in mango. The variation in total carotenoids in ripe peel and pulp of 12 mango cultivars was evenly distributed and pulp of Amrapali, Kesar and Pairi possessed more carotenoids than peel of these cultivars. In rest of the cultivars, peel contained slightly more carotenoids than pulp (Table 1). In case of ripe mango peel, Ambika (8.96 mg/100g) contained highest total carotenoids closely followed by Arunika (8.06 mg/100g), both of them are coloured varieties. However, in case of ripe mango pulp, Amrapali possessed highest carotenoids (10.33 mg/100g) followed by Kesar (9.60 mg/100g). The lowest amount of total carotenoids was observed in peel (2.90 mg/100g) and pulp (1.86 mg/100g) of Bangalora variety which is pale vellow in colour when ripe. Though it is evenly distributed among varieties, a significant variation was noticed in both peel and pulp. Earlier, total carotenoids content of mango peel powder was reported highest in Chausa (6.09 mg/100 g), followed by Dashehari (5.12 mg/100 g) and Fazli (4.80 mg/100 g) (Nehra and Sharma 2012). The pulp of mango variety Haden reportedly contained significantly lower total carotenoids than the pulp of other three cultivars (Tommy Atkins, Palmer and Uba), while the content of  $\beta$ -carotene was significantly higher in pulp of Uba variety (Ribeiro et al. 2007). The total carotenoids content in acetone extracts of mango peel (raw and ripe) varied between 74-436 mg/g in Raspuri and Badami varieties and more carotenoids have been found in

ripe mango peel as compared to raw mango peel in both the varieties as reported earlier (Ajila *et al.* 2007b). The pulp of Palmer variety also contained more carotenoids than its peel irrespective of sanitization treatment (Monaco *et al.* 2014). In fruits of seven Mexican mango cultivars, Ornelas-Paz *et al.* (2007) have identified all-trans- $\beta$ -carotene and dibutyrates of all-trans-violaxanthin and 9-cis-violaxanthin as major carotenoid compounds.

Total antioxidant activity in ripe mango peel and pulp: The variation in total antioxidant activity among the cultivars followed similar pattern as that of total phenolics and total flavonoids contents, i.e. peel contained more antioxidant activity than pulp (Table 1). The highest total antioxidant activity in mango peel was recorded in Banganpalli (5.53 µmol Trolox/100g) followed by Kesar, Mallika, Vanraj, Neelum and Arunika (4.35, 4.34, 4.29 and 4.17 µmolTrolox/100g, respectively). In case of ripe mango pulp, Mallika had the highest total antioxidant activity (1.28 µmolTrolox/100g) followed by Neelum (1.04 µmolTrolox/100g). Ripe mango peel contained 3–10 fold higher antioxidant activity compared to ripe mango pulp depending on cultivars (Table 1). Higher antioxidant activity in peel than in pulp has also been reported in case of pomegranate (Li et al. 2006) and apple (Drogoudi et al. 2008). Among the two mango varieties tested, the antioxidant activity of Badami ripe peel extract (analyzed by reducing power and DPPH assay) was found more as compared to Raspuri ripe peel extract (Ajila et al. 2007b). Mango peel powder of Chausa (18.10%) showed highest radical scavenging activity followed by Dashehari (17.32%) and Fazli (17.02%) (Nehra and Sharma 2012). Marina and Noriham (2014) have reported that mango peel extract showed higher antioxidant activity than guava and papaya peel extracts.

The data indicated that the majority of antioxidant capacity in mangoes resided with the phenolics contents in fruit (whether in peel or in pulp). According to the recent reports, a highly positive relationship existed between the total phenolics content and antioxidant activity in many plant species (Chen and Yen 2007), which was also confirmed in the present study where higher positive correlation was observed between total phenolics content and antioxidant activity ( $R^2 = 0.799$  and  $R^2 = 0.008$  for peel and pulp, respectively). Mango varieties containing higher total phenolics content including total flavonoids, viz. Neelum, Ambika, Banganpalli, Arunika, Mallika and Kesar in peel showed greater antioxidant activity than other varieties. Mallika and Neelum contained higher phenolics in pulp also and so had better antioxidant activity or health promoting benefits in pulp when consumed ripe. As the flavonoids are also part of phenolic compounds, a positive correlation between total flavonoids content and total antioxidant activity was noticed ( $R^2 = 0.293$  and  $R^2 =$ 0.185 for pulp and peel, respectively). The antioxidant capacity of phenolic compounds is determined according to their chemical structure or due to their interaction with other antioxidants present in fruit (Palafox-Carlos et al.

2012). However, though the relationship between total carotenoids and total antioxidant activity was positive but the value was less ( $R^2 = 0.008$  and  $R^2 = 0.194$  for pulp and peel, respectively) and so it can be concluded that the total carotenoids contributed to a lesser extent towards antioxidant activity in ripe mango peel and pulp than total phenolics and total flavonoids.

Colorimetric values of ripe mango peel and pulp: The data on colorimetric values (L, a, and b) in peel and pulp of 12 mango varieties is presented in Table 2. 'L' value is the measurement of shining/luminosity that varies from zero (black) to 100 (white), positive 'a' values are in the direction of redness and negative 'a' values towards greenness, while positive values of 'b' indicate yellowness and negative values of 'b' indicate blueness. All 'L', 'a' and 'b' values in this study were found positive. Ripe peel of Kesar variety had the highest 'L' (62.04) and second highest 'b' values (38.10) indicating shining yellowness in colour. Banganpalli peel was also found highly yellowish in colour when ripe as evidenced by its highest 'b' value (38.42). In ripe peel, 'a' value was recorded highest (18.01) in Arunika followed by Ambika (11.30), which indicated red and purple colour in varieties. Highest 'a' (21.19) and 'b' (52.69) values of pulp were noticed in ripe fruits of Kesar mango, which also contained good amount of carotenoids (9.60 mg/100g) imparting attractive yellow colour to the pulp (Table 2). In case of colorimetric values, it has earlier been mentioned that positive values of 'a' and 'b' are attributed to the carotenoids present in mango pulp (Ribeiro et al. 2007). Ornelas-Paz et al. (2007) have reported that Ataulfo and Haden mango fruits had the highest 'a' values in flesh among the seven Mexican varieties and high 'a'

 Table 2
 Colorimetric value in peel and pulp of twelve ripe mango varieties

Variety	Colori	metric va peel*	alues of	Colorimetric values of pulp*			
	L	а	b	L	а	b	
Neelum	50.56	4.23	21.41	62.40	9.09	47.41	
Bangalora	50.39	2.75	25.28	49.33	9.04	33.98	
Banganpalli	55.56	2.29	38.42	57.64	8.59	41.53	
Vanraj	53.65	4.83	25.35	49.17	14.01	39.38	
Amrapali	54.43	2.14	26.11	45.77	20.03	42.99	
Ambika	45.15	11.30	26.31	45.34	10.92	38.69	
Kensington	56.23	2.05	27.41	55.78	9.78	45.24	
Mallika	58.75	2.61	27.15	45.05	12.62	40.22	
Kesar	62.04	4.65	38.10	47.04	21.19	52.69	
Arunika	44.27	18.01	28.72	50.42	20.23	43.80	
Pairi	50.46	0.48	32.73	45.67	12.67	43.00	
Lucknow Safeda	69.47	4.49	45.41	48.21	8.71	35.46	
LSD (P≤0.01)	11.50	8.58	14.67	8.15	4.06	7.14	

\* Average of three replications

values in flesh have been correlated with high  $\beta$ -carotene content. In the present investigation also higher positive values of 'a' and 'b' in the pulp of Amrapali and Kesar might be due to the presence of higher carotenoids in pulp of these two cultivars.

The present study revealed that all the mango cultivars contained appreciable amount of phenolics, flavonoids and carotenoids in fruit peel and pulp with marked variation among the cultivars. Consequently, total antioxidant activity also varied from cultivar to cultivar in ripe mango peel and pulp. All mango cultivars have represented a potential source of natural antioxidants but cultivars Neelum and Mallika can be considered as very rich source of dietary antioxidants. Besides these two cultivars, Amrapali, Kesar, Ambika and Arunika can also be considered nutritious from health point of view. Ripe mango peel of all the cultivars possessed higher antioxidant activity than ripe mango pulp and, therefore, can be utilized for the extraction of phenolic compounds by appropriate assayas well as in phyto-pharmaceutical industries.

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