

Research Article

Mango Peel: A Potential Source of Bioactive Compounds and Phytochemicals

Tokas J^{1*}, Punia H¹, Baloda S² and Sheokand RN³¹Department of Biochemistry, CCS Haryana Agricultural University, India²Department of Horticulture, CCS Haryana Agricultural University, India³Department of Mathematics & Statistics, CCS Haryana Agricultural University, India***Corresponding author:** Tokas J, Department of Biochemistry, College of Basic Sciences & Humanities, CCS Haryana Agricultural University, India**Received:** January 13, 2020; **Accepted:** February 05, 2020; **Published:** February 12, 2020**Abstract**

Mango, a highly perishable fruit generates huge amount of wastes during postharvest handling procedures. To exploit the potential value of mango peel, the present study was conducted on two mango varieties viz. Amrapali and Dasherri, which showed that the moisture content ranged from 70-80 per cent and was more in ripe mango peels. The fat, carbohydrate and crude fibre content ranged from 2.00-2.56, 12.20-25.12 and 3.80-8.40 per cent and were significantly higher in ripe peels. The raw peel had high superoxide dismutase, catalase and peroxidase activities. However, the unripe mango peel exhibited good phenolic content and antioxidant potential as depicted by DPPH and FRAP assay. The results of the present study showed that mango peels may be used as a potential rich source of natural antioxidants and can be exploited as an ingredient for functional food products with improved nutritional value.

Keywords: Antioxidative enzymes; Bioactive compounds; By-product; Mango peel; Phytochemicals

Introduction

Mango (*Mangifera indica L.*) belongs to the family *Anacardiaceous* and is among the major important tropical edible fruits [1]. India is one of the world's leading mango fruit producers. Mango has characteristic succulence, exotic flavour, sweet taste and attractive colours. They have several health beneficial properties i.e. good source of vitamin A, carotenoids, flavonoids, which makes it a food with good bioactive potential that aids in prevention of degenerative diseases, including cardiovascular diseases, diabetes and cancer [2,3]. Mango being a seasonal fruit is processed into various products such as pickles, chutney, puree, nectar, canned slices, etc. During mango processing, by-products like kernel and peel are produced which have disposal problem and is thus becomes a source of pollution. Fresh fruit accounts for around 15-20% of the mango peel and has potential of being a functional ingredient in food products [4]. Food manufacturing industries produce 39% waste in developed countries. Being a by-product, little used for processed foods, it contains relative proportion of bioactive and rational use of these compounds can reduce environmental impacts in addition to health benefits.

Mango waste has high residual phenolic levels, which could damage the environment by inhibiting polyphenols seed germination activities. Therefore, the industry's waste treatment costs have increased. Animal feeding is the most common application of these mango wastes, although they can be processed to obtain more valuable products. Juice, wine, vinegar and good quality pectin's have been produced from peels. Peels have been found to be rich source of carotenoids, phenolic compounds and other different bioactive compounds, which have various positive health benefits [1]. The extraction of the beneficial compounds from these wastes can be a cost effective approach. The maceration of the fruit peel either in distilled beverages or ethanol is one of the best ways to obtain processed foods which holds good extraction of aromatic and bioactive compounds

in bulk from raw material [5]. Addition of pectinase enzyme during the extraction process can optimize the technique and facilitate the release of bioactive and phenolic compounds in a better way which also changes the phenolic profile of raw fruit [6,7]. *Mangiferin*, a natural bioactive compound found in mango peel has attracted the attention of research groups for cancer treatment.

Mango parts (stem bark, leaves, and pulp) have various biomedical applications, such as antioxidative and free radical scavenging [8], anticancer [9], anti-diabetic, anti-microbial, immunomodulatory, analgesic, and anti-inflammatory activities. Peel has been found to be a good source of phytochemicals, such as polyphenols, carotenoids, vitamin E, vitamin C [10] and exhibits good antioxidant properties. They also lower the risk of cataracts, Alzheimer's disease, cancer, and Parkinson's disease [11].

Polyphenol profiles of mango by-product including peel have been reported using HPLC-MS analysis. Polyphenol content of peel was reported to be more than that of flesh. The functional and chemical properties of processed mango flour have been investigated. Nonetheless, very few studies on the chemical composition of the mango peel, antioxidants and functional properties to assess the possible use of unripe and ripe mango peel in food formulation are available. Therefore, the objective of this analysis was to determine the chemical components, antioxidants, bioactive compounds and functional properties in ripe and unripe mango peels of the two Indian mango varieties, viz. Amrapali and Dasherri, with the aim of exploiting the potential value of their peels.

Material and Methods**Materials**

Present investigation was carried out in two varieties of mango (*Mangifera indica L.*) fruit viz. Dasherri and Amarpali procured from the orchards of Department of Horticulture, Chaudhary Charan

Singh Haryana Agricultural University, Hisar. All the chemicals & biochemicals used during the study were purchased either from Sigma Chemical Co. (St. Louis MD, USA), Hi Media or SRL (Sisco Research Laboratory Pvt. Ltd., India) and were of high purity. Some fruits were air dried and put in the cardboard box lined with newspaper and allowed to ripen to get ripe peels. A sharp knife was used to remove the mango peel and a blunt knife to remove the underlying pulp.

Total phenolics in mango peel extract

Mango peel was homogenized in 80% acetone and the clear solution obtained after centrifugation for 15 min. at 10,000g was subjected to total polyphenols estimation using the method of Swain & Hillis, et al. [12]. The Folin – Ciocalteu reagent was used to calculate phenols spectrophotometrically. Briefly, 0.1 ml of mango peel extract was added into 0.9 ml of deionized water plus 1 ml of Folin-Ciocalteu reagent and 10 ml of 7 percent Na_2CO_3 solution after five minutes of reaction and volume was made upto 25 ml with deionized distilled water. After 90 minutes in the dark, phenols were estimated at 750 nm. The result has been shown to be mg of GAE (Gallic Acid Equivalents) per gram fresh weight. Gallic acid was used as a standard.

Determination of total carotenoids

The total carotenoid concentration was determined by the Litchenthaler, et al. [13] process, based on the following formula and expressed as microgram (μg) per gram of the extract.

$$\text{Chlorophyll a} = 12.25 A_{663} - 2.79 A_{646}$$

$$\text{Chlorophyll b} = 21.50 A_{646} - 5.10 A_{663}$$

$$\text{Total carotenoids} = \frac{1000A_{470} - 1.82\text{Chl.a} - 85.02\text{Chl.b}}{198} \mu\text{g/g}$$

Antioxidant activity by DPPH

DPPH radical scavenging activity was estimated by the method of Brand-Williams, Cuvelier & Berset, et al. [14]. To 0.1 mL of each extract, 0.1 mL of a 500 μM methanol solution aliquot was added and vigorously shaken. For 40 minutes, the tubes are put in the dark at 27°C. The samples have been measured at 515 nm as changes in the absorbance. Radical antioxidant activity has been expressed in milligrams per gram. Trolox Equivalents (TE) was used as standard.

FRAP assay

Ferric Reducing Antioxidant Power (FRAP) assay was estimated by the method described by Benzie and Strain [15]. This test is focused on the quantification of antioxidant's scavenging capacity. The activity was expressed milligram per gram as Trolox Equivalents (TE).

Estimation of total carbohydrate content

The total carbohydrate content was determined by method of Dubois, Gilles, Hamilton, Robers & Smith, et al. [16]. The mango peel was first hydrolyzed using 6N HCl at 100°C for up to 6 hours. Reducing sugars were determined by Nelson [17] as modified by Somogyi [17]. Non-reducing sugars were estimated by subtracting the reducing sugars from the total sugars.

Biochemical analysis

Total Soluble Solids (TSS) were estimated by using Abbe' refractometer. The pulp of fruit was taken in muslin cloth and its juice was extracted which was placed on refractometer at lower end

of scale. The readings were taken in terms of °Brix.

The total Titrable Acidity (TA) was determined by the titrametric method [18]. 20 gram of pulp was macerated in a pestle and mortar by adding water. The extract was taken in a conical flask and titrated against 0.1 NaOH using 1% phenolphthalein as an indicator. The appearance of light pink colour was considered as end point. The acidity of fruit was expressed on percent (%) basis.

$$\text{Total acidity(\%)} = \frac{\text{Titervalue} \times \text{Normalityofalkali} \times \text{vol.made} \times \text{Equivalentwt.ofacid}}{\text{vol.ofsampletaken} \times \text{Wt.ofsample} \times 1000} \times 100$$

Proximate composition analysis

The fresh peel obtained was analysed for moisture, ash, fat, and crude fibre content [19]. micro-Kjeldhal method was used for the estimation of total nitrogen content. It was converted to protein by multiplying 6.25.

Activities of antioxidative enzymes

The enzymes were extracted by macerating 2 g of fresh peel with 5 ml of ice cold extraction medium (0.1 M Tris-HCl buffer, pH 7.5) in a pre-chilled pestle and mortar using acid washed sand as abrasive. The homogenate was filtered through four layers of cheese cloth and the filtrate was centrifuged at 10,000 rpm for 20 min in a refrigerated centrifuge at 4°C. The supernatant was carefully decanted and used as crude enzyme preparation to assay superoxide dismutase, ascorbate peroxidase, peroxidase and catalase activities.

Superoxide Dismutase (SOD) determination: SOD activity in mango peel extracts was determined by Beauchamp and Fridovich [20] method using 3 ml reaction mixture containing 50 mM Tris-HCl (pH 7.8), 14 mM L-methionine, 60 μM NBT, 3 μM riboflavin, 0.1 mM EDTA and 0.1 ml of enzyme extract. The absorbance was recorded at 560 nm. One SOD unit was defined as the amount of enzyme required to inhibit the photoreduction of one μmol of NBT.

Catalase (CAT) determination: Catalase activity was measured by Sinha [21] method with some modifications. A reaction mixture containing 0.1 M potassium phosphate buffer (pH 7.0), 0.2 M H_2O_2 , 50 μl of enzyme extract and 5% potassium dichromate: acetic acid (1:3) solution was prepared and absorbance was recorded at 570 nm using dichromate: acetate solution as blank. One CAT unit was defined as amount of enzyme required to consume one μmol of H_2O_2 min^{-1} .

Peroxidase (POX) determination: POX was assayed by Rao, et al. [22] method using 3 ml reaction mixture containing 0.1 M Tris HCl buffer (pH 7.0), 1% guaiacol, 0.1 ml of enzyme extract and 100 mM H_2O_2 . Absorbance was recorded at 470 nm at 15s interval up to 3 min. One unit of peroxidase activity was defined as the amount of enzyme required to oxidize one nmol of guaiacol min^{-1} ml^{-1} .

Ascorbate Peroxidase (APX) determination: Ascorbate peroxidase was assayed by the method of Nakano and Asada [23] using a 3 ml reaction mixture containing 95 mM potassium phosphate buffer (pH 7.0), 0.5 mM L-ascorbate, 0.5 mM H_2O_2 and 50 μl of enzyme extract. The decrease in absorbance at 290 nm was recorded spectrophotometrically for 2 min against reagent blank, which corresponded to the oxidation of ascorbic acid. One enzyme unit was expressed as amount of enzyme required to oxidise one nmol of ascorbate min^{-1} .

Table 1: The bioactive compounds in mango peel.

Mango Variety	Total phenolic content (mg GAE/g)	Total carotenoids ($\mu\text{g/g}$)	Ascorbic acid (mg/100g)	Tocopherol ($\mu\text{g/g}$)
Amarpali Raw	112.4 \pm 1.95 ^d	789.4 \pm 13.67 ^a	184.7 \pm 3.20 ^b	236.9 \pm 4.10 ^b
Amarpali Ripe	93.2 \pm 1.61 ^b	2077.5 \pm 35.99 ^d	328.7 \pm 5.69 ^d	370.5 \pm 6.12 ^c
Dasherri Raw	103.5 \pm 2.74 ^c	881.7 \pm 23.33 ^b	163.3 \pm 4.32 ^a	225.6 \pm 5.97 ^a
Dasherri Ripe	85.2 \pm 1.70 ^a	1958.2 \pm 29.16 ^c	298.0 \pm 5.96 ^c	386.5 \pm 7.73 ^d

Lipoxygenase (LOX) determination: LOX was assayed spectrophotometrically at 234 nm by the method of Catherine et al. [24]. The reaction mixture (3.0 ml) contained 2.93 ml of potassium phosphate buffer (0.1 M, 6.2 pH), 20 μl of 30 mM linoleic acid in ethanol and 50 μl of enzyme extract. The reaction was started by the addition of enzyme and increase in absorbance at 234 nm was noted at room temperature for 3 min against buffer blank. One unit of LOX was defined as amount of enzyme required to produce 1 nmol of conjugated dienes min^{-1} .

Polyphenol oxidase estimation: Polyphenol Oxidase (PPO) activity was assayed by the method of Saby, et al. [25]. 1 ml of reaction mixture contained enzyme extract (diluted), 0.5 M catechol (0.1 ml) in sodium phosphate buffer (50 mM, pH 7.0). One unit of activity for polyphenol oxidase was defined as the amount of enzyme, which produced an increase of one absorbance per minute at 460 nm.

Protease and amylase determination

Amylase and protease activities in peel extracts were estimated by the method described by Saby, et al. [25]. The activity of protease was measured using the reagent azocasein (25 mg/ml) and Tris-HCl buffer (50 mM) of pH 8.0) as a buffer. An increase of one absorption per minute at 440 nm was considered to be 1 activity unit. Amylase was estimated in sodium acetate buffer (50 mM, pH 4.6) using 1 percent gelatinized soluble starch solution as a substrate for amylase. A unit of enzyme activity has been described as the equivalent of one l mole maltose released per minute.

Estimation of ascorbic acid and tocopherol

Ascorbic acid (Vitamin C) was estimated by the method of Mukherjee and Choudhuri [26], which was based on the reduction of 2, 4-dinitrophenyl hydrazine. To the properly diluted aliquot of 0.1 ml, 1.9 ml of distilled water, 1 ml of 2% 2, 4 - dinitrophenyl hydrazine (in acidic medium) and one drop of 10 % thiourea (in 70% ethanol) were added, the contents were mixed thoroughly and kept in boiling water bath for 15 min. then cooled to room temperature, 2 ml of 80% (v/v) H₂SO₄ was added to the mixture at 0°C (in an ice bath). The absorbance was read at 530 nm. The quantity of ascorbic acid was determined from the standard curve of ascorbic acid (10-100 μg).

Tocopherol (Vitamin E) was estimated by the method of Joshi and Desai [27]. The quantity of Vitamin E was determined from α -Tocopherol and expressed as $\mu\text{g/g}$.

Statistical analyses

All the analyzed data were expressed as mean values \pm SD. The experimental design was completely randomized design, each sample with three replicates used for analysis. The comparison between mean values were analysed by using post-hoc Duncan's new multiple-range test at $P\leq 0.05$ level using a IBM SPSS program, Version 23.0, statistical package for Windows (SPSS, Chicago, USA).

Results and Discussions

Total phenolic content in mango peel extract

The total phenolic content in the mango peel extract was determined by Folin-Ciocalteu method, which is widely used for quantitative analysis of polyphenols. It measures the Folin-Ciocalteu reduction by formation of blue coloured complex in alkaline solution. Mango peels contained a significant amount of polyphenols. The total phenolic content pertaining to mango varieties viz. Dasherri and Amarpali in the mango peel are shown in Table 1. A significant difference was observed at raw and ripe stage. The raw Amarpali (112.4 mg GAE/g) and Dasherri (103.5 mg GAE/g) peel had higher phenolic content. While after ripening, phenolic content decreased in Amarpali (93.2 mg GAE/g) and Dasherri (85.2 mg GAE/g). These results are in agreement with the results obtained by Balasundram, et al. [28] and Garau, et al. [29] who observed the higher phenolic concentration in the fruit peels like apple, mango and peach. Ajila, et al. [8] observed that total phenolics in various Indian mango varieties at different ripening stages (raw and ripe) varied from 55 to 109 milligram/gram peel. Sellamuthu, et al. [3] observed phenolic content ranged from 30,000 to 76,430 $\mu\text{g}/100$ in different mango varieties. Similar findings have been reported by Ajila, et al. [10]; Coelho, et al. [4]. Tunchaiyaphum, et al. [6] used Subcritical Water Extraction (SCW) method in mango peel for phenolic compounds extraction and they found higher amount than Soxhlet extraction technique.

Total carotenoids in mango peel

The carotenoids are lipid-soluble pigments, which cause most fruits and vegetables to have a bright yellow colour. They act as free radical scavengers and protects from oxidative damage. Table 1 shows total carotenoids in mango peel. A significant difference was observed in total carotenoid content at both stages. At ripe stage, fruits had higher values of total carotenoids (Amarpali-2077.5 and Dasherri 1958.2 $\mu\text{g/g}$) and raw fruit had lower values (Amarpali-789.4 and Dasherri 881.7 $\mu\text{g/g}$) (Table 1). During ripening of mango, the carotenoid content increased significantly which increased the intensity of colour in mango fruit. The intensity of the colour depends on the level and fractions of chlorophyll pigments. These results are in accordance with Ajila, et al. [8] and Ajila, et al. [10]. In raw fruit, carotenoids are present along with chlorophyll pigments which affect the fruit colour. As the mango fruit ripens, the orange yellow colour appears as a result of chlorophyll breakdown and displays carotenoid pigmentation in plastids which are an important component of fruit maturation High intake of carotenoids rich diet also prevents from various degenerative diseases like sunburn-induced skin damage cataracts, and cancer.

Vitamin C and Vitamin E assay

Ascorbic acid is an important antioxidant and nutrient quality factor which destroys reactive oxygen species. Raw peels had higher

Table 2: Proximate composition (%) and chemical analysis of mango peel.

Mango Varieties	Moisture (%)	Protein content (%)	Crude Fibre content (%)	Fat content (%)	Ash content (%)	Total carbohydrates (%)	Reducing sugar (%)	Non-Reducing sugar (%)	TSS (°Brix)	Titratable acidity (%)
Amarpali Raw	66.6±1.15 ^a	1.9±0.0 ^b	3.8±0.10 ^a	2.6±0.05 ^c	2.7±0.07 ^c	18.2±0.10 ^a	1.2±0.01 ^a	9.2±0.03 ^a	7.2±0.12 ^a	1.6±0.04 ^c
Amarpali Ripe	74.2±1.28 ^c	2.1±0.0 ^c	8.4±0.17 ^d	2.2±0.03 ^b	1.5±0.03 ^a	26.6±0.15 ^c	9.5±0.06 ^d	26.0±0.26 ^c	10.3±0.18 ^c	0.6±0.01 ^a
Dasherri Raw	68.9±1.20 ^b	1.7±0.0 ^a	4.0±0.11 ^b	2.2±0.0 ^b	3.3±0.09 ^d	19.3±0.11 ^b	2.2±0.03 ^b	11.1±0.11 ^b	9.5±0.17 ^b	1.8±0.05 ^d
Dasherri Ripe	76.7±1.33 ^d	2.3±0.0 ^d	6.7±0.13 ^c	1.4±0.0 ^a	1.7±0.03 ^b	30.8±0.18 ^d	8.6±0.09 ^c	27.6±0.30 ^d	10.7±0.18 ^d	0.8±0.02 ^b

Table 3: Antioxidative enzyme activities (U mg⁻¹ protein) in mango peel.

Mango Variety	SOD	CAT	POX	APX	LOX	PPO
Amarpali Raw	71.6±0.42 ^d	167.3±2.58 ^d	29.4±0.46 ^c	7.8±0.09 ^d	849.3±8.58 ^d	25.8±0.68 ^a
Amarpali Ripe	64.3±0.38 ^c	152.2±2.35 ^b	12.4±0.19 ^a	3.0±0.03 ^b	801.7±8.10 ^b	70.4±0.70 ^c
Dasherri Raw	51.6±0.30 ^a	159.3±2.46 ^c	33.5±0.52 ^d	4.4±0.05 ^c	803.1±3.57 ^c	30.5±0.81 ^b
Dasherri Ripe	62.1±0.36 ^b	140.1±2.17 ^a	14.1±0.22 ^b	1.3±0.02 ^a	742.5±6.85 ^a	75.5±0.75 ^d

Table 4: Digestive enzymes (U mg⁻¹ protein) of mango peel.

Mango Variety	Amylase	Proteases
Amarpali Raw	1.2±0.02 ^a	10031.3±173.75 ^a
Amarpali Ripe	2.5±0.05 ^c	11054.7±191.47 ^c
Dasherri Raw	1.3±0.02 ^b	11021.3±190.90 ^b
Dasherri Ripe	2.9±0.06 ^d	11134.4±192.85 ^d

ascorbic acid, (264.66, 163.33mg/100g) than ripe peels (328.66, 298.0 mg/100g) in Amarpali and Dasherri varieties, respectively (Table 1). During storage and processing of fruits, ascorbic acid is one of the sensitive nutrients, which degrade owing to oxidation. Gomez and Lajolo [30] investigated the ascorbic acid metabolism in guava and mango fruits. It was observed that the ascorbic acid content of mango fruits decreased a lot from early stage of development to physiological maturation and after harvest it did not changed significantly. The decrease in ascorbic acid from green to ripe stage might be due to the oxidative destruction by ascorbic acid oxidase. Tocopherol content found to be higher in the ripe peel and lower in raw mango peels. It varied between 236.9-386.5 µg/g (Table 1). Previously, Burns, et al. [31] and Ajila, et al. [8] examined the α-tocopherol presence in the ripe mango pulp. Nevertheless, there are few studies of mango peel regarding the quality of vitamin E. All analyzed data are expressed as mean±SD. Values on fresh weight basis of expressed. Values at each stage followed by different letters in superscripts are significantly different (P≤0.05).

Antioxidant potential of mango peel

DPPH and FRAP assay have been used to determine the antioxidant behavior of the mango peel. DPPH is a free radical with greater stability and its radical scavenging activity constitutes a major indicator of the antioxidant potential that is strongly associated with total phenolics content of the sample. Figure 1a shows radical scavenging activity of the raw and ripe mango peels. Extraction of peel resulted in increase in antioxidant capacity. The degree of discoloration indicated the radical scavenging activity of the mango peel extract due to the hydrogen donating ability of antioxidants [32]. Higher values were observed in raw peels (75.3 mg/g in Amarpali and 72.4 mg/g in Dasherri) than ripe mango peels (63.4, 60.6 mg/g in Amarpali and Dasherri, respectively). These findings are in

harmony with those obtained by Ribeiro, et al. [2] and Ajila, et al. [8] who documented mango peels with active scavenging activity of free radicals to show strong antioxidant activity. Carotenoids and polyphenols may increase the free-radical scavenging activity. Apple and grape pomace too exhibited antioxidant activity as reported by Ajila, et al. [10] found that IC50 value of ripe mango peel was 67 µg. Soong, et al. [33] reported that mango peel had relative high phenolic content and potent antioxidant activity. Therefore, the extraction of antioxidant compounds from the peel allows the fruit to be fully exploited. Figure 1b depicts the FRAP assay. The FRAP activity of mango peel was considerably higher in raw peels (85.5, 81.9 mg/g in Amarpali and Dasherri, respectively) and less in ripe peels (75.8, 72.6 mg/g in Amarpali and Dasherri, respectively). Our results are consistent with the observations of Saura-Calixto and Goni [34], which have shown that fruit, vegetables and nuts have higher reduction power and antioxidant capacities compared to cereal products. Green/raw fruit peel exhibited strong reducing power and this might be attributed to the bioactive components presence, for example, flavonoids and polyphenols which are abundantly present in unripe mango. The pattern shown in the FRAP test was identical to the findings reported in the radical scavenging test for DPPH. But, the Ferric Reducing Antioxidant Power (TEACFRAP) represented in terms of Trolox's antioxidant equivalent potential was comparatively higher than with those reported for Trolox's antioxidant equivalent potential for Trolox's 1,1-Diphenyl-2-Picrylhydrazyl radical scavenging equivalent antioxidant potential (TEACDPPH).

Proximate composition analysis

The total protein, moisture, ash, fibre, and fat are given in Table 2. Results showed mango peel had higher moisture content at both maturity stages (ripe and raw). The moisture content was found higher in ripe peels (Amarpali-74.2 and Dasherri-76.7%) as compared with raw peels (Amarpali-66.6 and Dasherri-68.9%). Moisture levels increased during ripening processes and are in accordance with the Offem & Thomas report [35]. The increase in the sugar concentration during the ripening process might be due to the change of the osmotic pressure and the movement of water from peel to pulp. Raw peels had more ash (Amarpali-2.7 and Dasherri -3.3%) than ripe peels (Amarpali-1.5 and Dasherri-1.7%). The protein content followed the similar trend, more in raw peels than ripe peels.

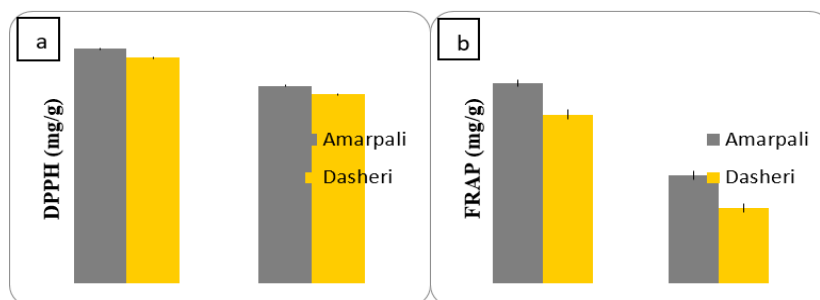


Figure 1: A DPPH (a) and FRAP (b) antioxidant activity in mango raw and ripe mango peels.

The crude fibre was more in ripe mango peel (Amarpali-8.4 and Dasherri-6.7%) than raw peels (Amarpali-3.8 and Dasherri-4.0%). The increase of overall dietary fiber content may be due to the formation of resistance to digestion enzymes, resistant starch and cross-linking protein/polysaccharides. These results are supported by the findings of Ajila, et al. [8]; Vergara-Valencia et al. who reported that a rich food fiber source is mango peel. The use of mango peel in functional healthy foods may reflect an alternative approach for individuals with special calories requirements [10]. The values of fat content were higher in raw peels (Amarpali-2.6 and Dasherri-2.2%) and lower in ripe peels (Amarpali-2.2 and Dasherri-1.4%). These results are also in agreement with Abdalla, Darwish, Ayad & El-Hamahmy [36]. The protein content was 1.9, 1.7% in raw peels and 2.1, 2.3% in ripe Amarpali and Dasherri peels, respectively. The findings of Ajila, et al. [8]; Vergara-Valencia et al. (2007); Ajila, et al. [10]; Abdullah, et al. [9] were near identical to the values reported in the present study.

Estimation of total carbohydrate content

Table 2 shows the total carbohydrate content in mango peel. Total sugars in raw and ripe mango peels were 18.2, 19.3, 26.6, and 30.8% in Amarpali and Dasherri, respectively. This might be attributed to reduction in starch levels, as ripening process progressed; starch is hydrolysed to sugar [37]. Raw mango peels possessed higher values of reducing sugars as compared to the ripe peels. Reducing sugars refers to the amount of retrogradation products of starch and sugar, which are not hydrolyzed and taken into the small intestine but fermented by microflora in the colon. Reducing and non-reducing sugars in raw peel were lower 1.2, 2.2, 9.5, 8.6% in Amarpali and Dasherri, respectively, while ripe mango peels had higher values of reducing and non-reducing sugars viz. 9.2, 11.1, 26.0 and 27.6% in Amarpali and Dasherri, respectively. This might be due to the dietary fibres in the raw fruit. Reducing sugars significantly increases the quantity of indigestible material that can easily move across the colon and also affects the bacterial growth, colonic fermentation, fecal bulking and energy value of food. Ajila, et al. [10] reported similar results. However, the total sugar content recorded in mango dietary fiber is relatively below the value. All analyzed data are expressed as mean \pm SD. Values on fresh weight basis of expressed. Values at each stage followed by different letters in superscripts are significantly different ($P \leq 0.05$).

Biochemical analysis of mango peel

The results of the chemical analysis of the mango peel are presented in Table 2. The total soluble solids were higher in ripe peels

(Amarpali-7.2 and Dasherri -9.5°Brix) than raw (Amarpali-10.3 and Dasherri -10.7 °Brix) peels. The raw and ripe mango peels produced different titrable acidity. At ripe stage, mango peel showed lower values (Amarpali-0.6 and Dasherri-0.85%) than raw peels (Amarpali-1.6 and Dasherri -1.8%). Increased values of titrable acidity were due to higher sugar/acidity ratio. The higher values of titrable acidity at ripe stage can be explained by the higher solubility of organic acids present in peels after extraction, as malic acid and citric acid [38]. The higher titrable acidity favors good taste balance [4]. All analyzed data are expressed as mean \pm SD. Values on fresh weight basis of expressed. Values at each stage followed by different letters in superscripts are significantly different ($P \leq 0.05$).

Mango peel enzymes

Antioxidative enzymes protect the fruits against oxidative damage and reduce the ROS concentration. Ripening stage significantly affected the activities of SOD, CAT, POX, and APX enzymes. Mango peel had potent antioxidant activity as depicted in Table 3. A significant difference was observed between the antioxidative enzyme activity of raw and ripe mango peels. Progressive decrease in enzyme activity was observed during ripening stage. SOD, a metalloenzyme removes superoxide anion radical and protects cells from oxidation of proteins, lipids, and DNA [38]. SOD activity was found higher in unripe mango peel (Amarpali-71.6 and Dasherri-51.6 U mg⁻¹ protein) as compared to ripe peels (Amarpali-64.3 and Dasherri-62.1 U mg⁻¹ protein). Huang, et al. [39] also reported that SOD activity decreased as ripening of sweet orange progressed. Catalase is a major antioxidant enzyme that removes the bulk of cellular H₂O₂. Catalase activity was higher in unripe mango peels (Amarpali-167.3 and Dasherri-159.3 U mg⁻¹ protein) compared to ripe peel (Amarpali-152.2 and Dasherri-140.1 U mg⁻¹ protein). Increased activity of CAT at raw stage has also been reported in mango, papaya, apple, pear, and grapes fruits. Peroxidases are important defensive enzymes that provide protection against a number of biotic and abiotic stresses. Similarly, POX and APX activities followed the similar trend, being higher in raw peel. Higher activities of CAT and POX might be attributed to CAT which decomposes H₂O₂ to oxygen and water while POX dismutates H₂O₂ by oxidation of phenolics. Our results also confirm the findings of Palafox-Carlos, et al. [40] who found that as ripening period progressed, antioxidant activity declined. In guava, antioxidant activity was highest at raw stage and decreased as ripening progressed. Such type of variation in the behaviour of antioxidative enzymes might be due to differences in the activities of their isoenzymes. LOX activity was also higher in raw peels

(Amarpali-849.3 and Dasher-803.1 U mg⁻¹ protein) and lower in ripe peels (Amarpali-801.1 and Dasher-742.5 U mg⁻¹ protein). PPO is involved in fruit ripening process and oxidative browning. PPO activity ranged from 25.8 and 75.5 U mg⁻¹ protein (Table 3). Prabha and Patwardhan [41] observed more PPO activity in ripe peels. A variety of fruits, such as mango, apple and ananas, are widely studied. Prabha and Patwardhan [41] observed that ripe peels had more PPO activity. A wide range of fruits has been studied, like mango, apple and pineapple.

Extracts from mango peel also demonstrated good amylase and protease enzyme activities. The protease activity in mature peels was substantially better than raw peel (Table 4). The protease activity was higher in ripe peels (Amarpali-11054.7 and Dasher-11134.4 U mg⁻¹ protein) and lower in raw peels (Amarpali-10031.13 and Dasher-11021.3 U mg⁻¹ protein). During the senescence stage, protease catabolise proteins which results in increase in free amino acids and amides and ultimately affects the protein turn over [8]. Amylase content ranged from 1.2-2.9 U mg⁻¹ protein in mango varieties (Table 4). During ripening stage, starch accumulated at raw stage is mobilized, resulting in fruit softening. Activity of α -amylase increased two times during mango ripening and 3-4 times increase is also reported by [42]. All analyzed data are expressed as mean \pm SD. Values on fresh weight basis of expressed. Values at each stage followed by different letters in superscripts are significantly different ($P\leq 0.05$).

Conclusion

The observations of this study indicated that mango peels contained a significant amount of bioactive compounds and could be used as a potential source of functional food ingredient. The raw mango peel represents a rich and active source of dietary fibres, phytochemicals with health-promoting properties and could be further processed in developing health food products of therapeutic importance.

References

1. AACC. Approved methods of the AACC (10th edn.). St. Paul MN: American Association of Cereal Chemists (methods 08-01, 30-25, 44-15A, 46-10, 54-10, 54-21). 2000.
2. Abdalla AE, Darwish SM, Ayad EH, El-Hamahmy R.M. Egyptian mango by-product. Compositional quality of mango seed kernel. *Food Chemistry*. 2007; 103: 1134-1140.
3. Abdullah ASH, Mohammed AS, Abdullah R, Mirghani MES, Qubaisi MA. Cytotoxic effects of *Mangifera indica* L. kernel extract on human breast cancer (MCF-7 and MDA-MB-231 cell lines) and bioactive constituents in the crude extract. *BMC Complementary and Alternative Medicine*. 2014; 14: 1-10.
4. Ajila CM, Aalami M, Leelavathi K, Prasada Rao UJS. Mango peel powder: A potential source of antioxidant and dietary fiber in macaroni preparations. *Innovative Food Science and Emerging Technologies*. 2010; 11: 219-224.
5. Ajila CM, Bhat SG, Prasada Rao UJS. Valuable components of raw and ripe peels from two Indian mango varieties *Food Chemistry*. 2007; 102: 1006-1011.
6. Ajila CM, Leelavathi K, Prasada Rao UJS. Improvement of dietary fiber content and antioxidant properties in soft dough biscuits with the incorporation of mango peel powder. *Journal of Cereal Science*. 2008; 48: 319-326.
7. AOAC, Official Methods of Analysis, 13th ed. Association of Official Analytical Chemists, Washington, DC, USA. 1980.
8. Balasundram N, Sundram K, Samman S. Phenolic compounds in plants and agri-industrial by-products: antioxidant activity, occurrence, and potential uses. *Food Chemistry*. 2006; 99: 191-203.
9. Beauchamp I, Fridovich I. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. *Annals of Biochemistry*. 1971; 44: 276-287.
10. Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power" the FRAP assay. *Analytical Biochemistry*. 1996; 239: 70-76.
11. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. *Lebensmittel Wissenschaft und Technology*. 1995; 28: 25-30.
12. Burns J, Fraser PD, Bramley PM. Identification and quantification of carotenoids, tocopherols and chlorophylls in commonly consumed fruits and vegetables. *Phytochemistry*. 2003; 62: 939-947.
13. Catherine NSPS, Perez-Gilabert M, Vander-Hijden HTWM, Veldink GA, Vliegthart JFG. Purification, product characterization and kinetic properties of soluble tomato lipoxygenase. *Plant Physiology & Biochemistry*. 1998; 36: 657-663.
14. Coelho EM, Azevedo LC, Correa LC, Bordignon-Luiz MT, Lima MDS. Phenolic profile, organic acids and antioxidant activity of frozen pulp and juice of the jambolan (*Syzygium cumini*). *Journal of Food Biochemistry*. 2016; 40: 211-219.
15. Coelho EM, de Souza MEO, Corrêa LC, Viana AC, de Azevêdo LC, dos Santos Lima M. Bioactive Compounds and Antioxidant Activity of Mango Peel Liqueurs (*Mangifera indica* L.) Produced by Different Methods of Maceration. *Antioxidants*. 2019; 8: 102.
16. Dubois M, Gilles KA, Hamilton JJ, Rubress PA, Smith F. Colorimetric method for determination of sugar and related substances. *Analytical Chemistry*. 1956; 28: 350-356.
17. Garau, MC, Simal S, Rosell C, Femenia A. Effect of air-drying temperature on physico-chemical properties of dietary fibre and antioxidant capacity of orange (*Citrus aurantium* v. *Canoneta*) byproducts. *Food Chemistry*. 2007; 104: 1014-1024.
18. Gomez MLP, Lajolo FM. Ascorbic acid metabolism in fruits: activity of enzymes involved in synthesis and degradation during ripening in mango and guava. *Journal of the Science of Food and Agriculture*. 2008; 88: 756-762.
19. Huang R, Xia R, Hu L, Lu Y, Wang M. Antioxidant activity and oxygen scavenging system in orange pulp during fruit ripening and maturation. *Scientia Horticulturae*. 2007; 113: 166-172.
20. Joshi PN, Desai RG. A method for the estimation of Tocopherols in blood plasma. *Indian Journal of Medical Research*. 1952; 40: 277-287.
21. Kumar K Yadav, AN Kumar V, Vyas P, Dhaliwal H S. Food waste: a potential bioresource for extraction of nutraceuticals and bioactive compounds. *Bioresources & Bioprocessing*. 2017; 4: 18.
22. Litchenthaler HK. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Methods in Enzymology*. 1987; 148: 350-383.
23. López-Cobo A, Verardo V, Diaz-de-Cerio E, Segura-Carretero A, Fernández-Gutiérrez A, Gómez-Caravaca AM. Use of HPLC- and GC-QTOF to determine hydrophilic and lipophilic phenols in mango fruit (*Mangifera indica* L.) and its by-products. *Food Research International*. 2017; 100: 423-434.
24. Ma B, Yuan Y, Gao M, Li C, Ogutu C, Li M, Ma F. Determination of predominant organic acid components in *Malus* species: correlation with apple domestication. *Metabolites*. 2018; 8: 74.
25. Majumdar G, Modi VV, Palejwala VA. Effect of plant growth regulators on mango ripening. *Indian Journal of Experimental Botany*. 1981; 19: 885-886.
26. Mukherjee SP, Choudhuri MA. Implications of water stress-induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in *Vigna* seedlings. *Physiologia Plantarum*. 1983; 58: 166-170.
27. Nakano Y, Asada K. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiology*. 1981; 22: 867-880.

28. Nelson N. A photometric adaptation of the Somogyi method for determination of glucose. *Journal of Biological Chemistry*. 1944; 153: 375-380.
29. Offem, JO, Thomas OO. Chemical changes in relation to mode and degree of maturation of plantain (*Musa paradisiaca*) and banana (*Musa sapientum*) fruits. *Food Research International*. 1993; 26: 187-193.
30. Palafox-Carlos H, Yahia E, Islas-Osuna MA, Gutierrez-Martinez P, Robles-Sánchez M, González-Aguilar GA. Effect of ripeness stage of mango fruit (*Mangifera indica* L, cv. Ataulfo) on physiological parameters and antioxidant activity. *Sci. Hortic*. 2012; 135: 7-1.
31. Powers KM, Oberle LW, Domann, FE. The adventure of superoxide dismutase in health and diseases: superoxide in the balance. In: Valacchi, G., Davis, P.A. (eds.), *Oxidants in Biology*. Springer. 2008; 183-196.
32. Prabha TN, Patwardhan MV. Endogenously oxidisable polyphenols of mango, sapota and banana. *Acta Alimentaria*. 1986; 15: 123-126.
33. Rao MV, Paliyath G, Ormrod DP. UV-B and ozone-induced biochemical changes in antioxidant enzymes of *Arabidopsis thaliana*. *Plant Physiology*. 1996; 110: 125-136.
34. Ribeiro SMR, Barbosa LCA, Queiroz JH, Knödler M, Schieber A. Phenolic compounds and antioxidant capacity of Brazilian mango (*Mangifera indica* L.) varieties. *Food Chemistry*. 2008; 110: 620-626.
35. Saby John K, Bhat SG, Prasad Rao UJS. Biochemical characterization of sap (latex) of a few Indian mango varieties. *Phytochemistry*. 2003; 62: 13-19.
36. Saura-Calixto F, Goñi I. Antioxidant capacity of the Spanish Mediterranean diet. *Food Chemistry*. 2006; 94: 442-447.
37. Sellamuthu PS, Denoy GI, Sivakumar D, Polenta GA, Soundy P. Comparison of the contents of bioactive compounds and quality parameters in selected mango cultivars. *Journal of Food Quality*. 2013; 36: 394-402.
38. Sinha AK. Colorimetric assay of catalase. *Annals of Biochemistry*. 1972; 47: 389-394.
39. Soong YY, Barlow PJ, Perera CO. A cocktail of phytonutrients: identification of polyphenols, phytosterols and tocopherols from mango (*Mangifera indica* L.). In IFT annual meeting, July 12-16, Las Vegas. 2004.
40. Summo C, Trani A, Faccia M, Caponio F, Gambacorta G. Volatiles and acceptability of liqueurs from kumquat and grapefruit. *Italian Journal of Food Science*. 2016; 28: 258-270.
41. Swain T, Hillis WE. The phenolic constituents of *Prunus domestica*: the quantitative analysis of phenolic constituents. *Journal of the Science of Food and Agriculture*. 1959; 10: 63-68.
42. Tunchaiyaphum S, Eshtiaghi MN, Yoswathana N. Extraction of bioactive compounds from mango peels using green technology. *International Journal of Chemical Engineering and Applications*. 2013; 4: 194-198.