ORIGINAL ARTICLE

Profiling of anthocyanins and carotenoids in fruit peel of different colored mango cultivars

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Abstract Mango cultivars are broadly categorized into green, yellow, and red types based on their peel colors. Anthocyanins and carotenoids are the most important pigments responsible for the color of fruits. The information available on the composition of pigments on mango peel was scanty, and the exact role of anthocyanins and carotenoids in imparting peel color was not clear. The present study was aimed at profiling anthocyanin and carotenoid pigments in the peels of green ('Langra', 'Amrapali', 'Hamlet' and 'Bombay No. 1'), yellow ('Arka Anmol', 'Lazzat Baksh', 'Peach' and 'Banganapalli') and red ('Tommy Atkins', 'Lalmuni', 'Gulabi' and 'Janardhan Pasand') colored mango cultivars through liquid chromatography and mass spectrometry at different stages of ripening. The analysis helped in the identification of eight carotenoids and 24 anthocyanins in mango peel. Higher levels of carotenoids were observed in yellow colored cultivars, and major compounds were β -carotene and

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violaxanthin. Further, the red colored types were found to possess the higher anthocyanin content than green and yellow types. The major groups of anthocyanin compounds include cyanidin, peonidin, petunidin, delphinidin and pelargonidin. During fruit ripening, a significant increase in carotenoids content, and no or marginal rise in anthocyanin content was observed in mango peels. The results, show the status of carotenoids and anthocyanins in mango peel of different colored cultivars and their developmental pattern during ripening.

Keywords Mangifera indica L. · Peel color · Anthocyanins - Carotenoids - UPLC - Fruit ripening

Introduction

Mango (Mangifera indica L.) is one of the most popular fruits. Apart from being consumed as fresh raw fruits, mangoes are extensively processed into various value-**Electronic supplementary material** The online version of this added products such as pulp, squash, candies, puree, nectar,

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pickles etc., making their cultivation a profitable proposition to the growers. Of the various quality indices, color of the fruits is the first and the foremost character, that attracts the consumers (Kayesh et al. [2013](#page-11-0)) and mangoes with attractive peel colors are popular in the markets.

Color development is one of the most important phenomena during fruit ripening. In case of mangoes, upon ripening, the peel may retain its green color, or may change to yellow or red, and, hence, the mango cultivars are mainly classified as green, yellow and red types. The role of pigments viz. carotenoids and anthocyanins, in fruit coloration is known.

Carotenoids are lipid soluble pigments, responsible for yellow, orange and red coloration. Mango peels are good sources of b-carotenes, provides vitamin A (Ribeiro et al. [2007\)](#page-11-0). The majority of the studies on mango carotenoids has been reported in pulp (Mercadante et al. [1997;](#page-11-0) Amotz and Fishler [1998](#page-10-0); Ribeiro et al. [2007](#page-11-0)) and only very few, in case of peel reported (Medlicott et al. [1986](#page-11-0)). Anthocyanins are water soluble pigments responsible for red, blue, and purple colors of fruits. They possess antioxidant properties contributing to their therapeutic values in the prevention of neuronal diseases, cardiovascular diseases, cancer, and diabetes in human beings (Rahman et al. [2008](#page-11-0); Giampieri et al. [2014](#page-11-0), [2018;](#page-11-0) Yang et al. [2017;](#page-11-0) Skates et al. [2018](#page-11-0)). Also, they serve as alternatives to artificial colorants (Kayesh et al. [2013](#page-11-0)).

Maximum diversity is available for colored mango fruits. Peel color and variation in pigments and their content with respect to color, needs to be examined and would help in utilization of mango peels. Keeping this in view, the present study was carried out to identify the anthocyanins and carotenoids and their content responsible for the color variation in mango peels.

Materials and methods

Plant materials

Mango cultivars with different fruit peel colors were selected for the present study, from the mango field gene bank of ICAR-Indian Institute of Horticultural Research, Bengaluru (Karnataka, India). The cultivars were categorized into three groups based on peel color, viz., green ('Langra', 'Amrapali', 'Hamlet' and 'Bombay No. 1'), yellow ('Arka Anmol', 'Lazzat Baksh', 'Peach' and 'Banganapalli') and red ('Tommy Atkins', 'Gulabi', 'Lalmuni' and 'Janardhan Pasand'). The fruits were harvested at a mature stage (S1) and allowed to ripe at ambient room temperature. During three stages of ripening S1, S2, S3 (fully ripe), fruits were peeled and the peels were separated

from the pulp. The peels of different colored fruits at different stages were sampled separately and frozen with liquid Nitrogen and stored at -80 °C until further analysis.

Care was taken to characterize peel sample for their uniformity, considering the influence of various factors like sunlight exposure, canopy position, and blushed/bicolor nature of the mango peel. For this purpose, fruits growing in all directions on the tree were tagged and the observations were taken for their maturity after fruit set. The peels of at least 10 fruits at each ripening stage was pooled and only colored part of the peel were used in order to have uniform samples for analysis. Mean maximum $(27.6 \degree C)$ and mean minimum (15.5 \degree C) temperatures were recorded during the period of the experiment.

Peel color evaluation

Peel colors of different colored mango cultivars at three ripening stages were evaluated using the 5th Edition of RHS (Royal Horticulture Society color charts 2007) by placing the colored leaves over both surfaces of fruits and recording the codes of color charts. The colors were observed for at least 20 fruits of each cultivar at three ripening stages separately. The new edition of the RHS color chart (5th edition) with a wider range of colors was used.

Mango peel carotenoid extraction and profiling by UPLC

The chlorophyll and carotenoid extraction was carried out according to the method described by Lichtenthaler [\(1987](#page-11-0)). The Acquity-UPLC system from Waters (Milford, MA, USA) with auto sampler injector and PDA detector with Acquity-UPLC-H-class withBEH-C18 column (1.7 µm) , 2.1×50 mm) guarded with BEH-C18 (1.7 μ m, 2.1×5 mm) column was used for the analysis. The mobile phase consisted of phase-A acetonitrile:methanol:ethyl acetate (53:7:40), and phase-B methanol at isocratic flow rate of 0.2 ml/min was used in the ratio of A:B (95:5) for 6 min with PDA scanning from 200 to 650 nm. A known quantity (1 ml) of hexane extract was evaporated to dryness, dissolved in the mobile phase, and filtered through 0.2 µm nylon filter prior to injection in UPLC for further analysis. Data was processed by MassLynx software and identification of each compound was done through assessment of characteristic absorption spectra in comparison with available literature (Supplementary Table 1). The quantification $(\mu g/g FW)$ of individual compounds was calculated from the peak area equivalent to standard β -carotene.

Anthocyanin extraction from mango peel and profiling by UPLC

Extraction of anthocyanins from mango peel was carried out as described by Shivashankara et al. ([2010\)](#page-11-0) with slight modifications. Peel $(5 g)$ was ground with 1% acidic methanol using pestle and mortar till clear extract was obtained and the volume was made up to a known quantity. Extract (500 μ l) was dried with a vacuum evaporator to remove methanol and hydrochloric acid, and later diluted with Milli O water. The extraction was again carried out by washing with 3 ml of ethyl acetate in a separating funnel. The extract was separated thrice wherein, the upper layer was discarded and the aqueous layer collected. The clear extract was evaporated under nitrogen flow and dissolved in 3 ml of mobile phase. The samples were then filtered through 0.2 µm nylon filter prior to injection in UPLC. All operations were carried out under dark conditions to avoid degradation of pigments.

An Acquity UPLC-H class coupled with TQD-MS/MS from Waters, USA with ESI source was used for anthocyanin estimation. The detection system allowed simultaneous detection at various wavelengths and MRM (Multiple reaction monitoring) for individual masses controlled by MassLynx software. The mobile phase consisted of an aqueous phase of 0.25% formic acid in water (A) and an organic phase of 0.25% formic acid in acetonitrile (B). The initial gradient composed of 100% aqueous phase, which was held for 0.5 min. At 10.0 min, the gradient was changed to 30% aqueous phase and 70% organic phase, which was held for 0.5 min, followed by a linear gradient up to 14.5 min with 100% aqueous phase and was held for 3.5 min for equilibrating before the next injection. The flow rate was set at 0.25 ml/min. The analytical column was BEHC18 (2.1 \times 50 mm, 1.7 µm) from Waters India Ltd., protected by a Vanguard BEH C18 (Waters, USA). The column temperature was maintained at 25° C during analysis. The sample injection volume was 2.0μ l. The anthocyanin eluted was monitored using a PDA detector, and the UPLC column effluent was pumped directly without any split into the TQD-MS/MS (Waters, USA) system, optimized for the anthocyanin analysis.

The MS/MS operational conditions were $ESI⁺$ ionization mode, cone voltage 4 V and RF lens 0.1 V. The gas flow for desolvation and the cone was set at 600 and 50 l/h. The collision energy for fragmentation of cyanidin was kept at 34 V. For identification of anthocyanins present in mango peel, each sample was run in full mass scan $(ESI⁺)$ and single ion reaction (SIR) mode to find out the most abundant mass. In the subsequent run, the individual masses were picked up and broken down to find out their most abundant fragments by manipulating collision energy. The compounds were identified as per their masses and fragment ions (Supplementary Fig. 1; Supplementary Fig. 2 and Supplementary Table 2). Accordingly, some of the abundant anthocyanin compounds (belonging to peonidin, cyanidin, pelargonidin, malvidin, delphinidin and petunidin) were noted through the SIR and chromatogram for each compound (Supplementary Fig. 2). Based on this, the parent mass of the individual compound and its daughter ions were compared with the reported data of anthocyanins (Supplementary Table 2) and quantification $(\mu g/100 \text{ g } FW)$ was done comparing with standard cyanidin contents.

Statistical analyses

The data were analyzed for variance (ANOVA) using AGRES statistical software version 3.01 (Pascal International Software Solutions, USA) following completely randomized design and the mean comparison was done by t test at 0.01% probability. Principle component analyses (PCA) were performed by software Past (Hammer et al. [2001](#page-11-0)) to interpret the relation of pigments and color of cultivars.

Results and discussion

Identification of mango peel carotenoids

In the present study, we have examined carotenoids in the peel of three different colored mango fruits during ripening. A total of 8 compounds was identified, information of which is summarized in Supplementary Table 1. The order in which compounds were identified are, unidentified compound (peak 1), all trans-violaxanthin butyrate (peak 2), violaxanthin (peak 3), luteoxanthin (peak 4), lutein (peak 5), zeaxanthin (peak 6), β -Carotene (peak 7) and *cis*- β -Carotene(peak 8). The major peaks were of β -carotene, cis- β -carotene, and violaxanthin isomers. Isomers of β carotene and violaxanthin were also reported in mango pulp (Cano and Ancos [1994;](#page-11-0) Wilberg and Rodriguez-Amaya [1995](#page-11-0); Mercadante et al. [1997;](#page-11-0) Mercadante and Rodriguez-Amaya [1998](#page-11-0); Amotz and Fishler [1998](#page-10-0); Setiawan et al. [2001;](#page-11-0) Pott et al. [2003;](#page-11-0) Chen et al. [2004](#page-11-0); Ornelas-Paz et al. [2007\)](#page-11-0). In our study, even mango peel was characterized by the abundant presence of these compounds (β -carotene, cis β -carotene and violaxanthin). However, carotenoids like mutataoxanthin, cryptoxanthin, auroxanthin, phytochrome etc., could not be identified here, which are reported in the pulp (Cano and Ancos [1994](#page-11-0); Mercadante et al. [1997\)](#page-11-0). In the present study, saponification was carried out in order to obtain pure carotenoids. However, saponification might result in degradation and loss of some carotenoids (Cano and Ancos [1994;](#page-11-0) OrnelasPaz et al. [2007](#page-11-0)) and, hence this might be the reason that these compounds were not recorded in this study.

The first peak with absorption spectra of 400 nm could not be identified even with latest available literature (Supplementary Table 1). John et al. ([1970\)](#page-11-0) reported an unidentified compound, presence of which was noted only in unripe 'Badami' mangoes and not in mature and ripe mangoes. We predict a possibility of this same compound reported by John et al. [\(1970](#page-11-0)), since the presence of this compound was recorded only in unripe peels in the present study.

Peel carotenoids composition and cultivar differences at the fully ripe stage

The total carotenoid content (Table 1) ranged from $0.74 \mu g/g$ (Janardhan Pasand) to $31.18 \mu g/g$ FW (Arka Anmol) in peels of different colored mango cultivars. The content reported in this study is in line with the values reported earlier for mango peels (Nordey et al. [2014](#page-11-0)). Among the yellow colored group, cultivar 'Arka Anmol' recorded high (31.18 µg/g FW) total carotenoids. However, cv. 'Banganapalli' recorded very low carotenoids $(1.55 \mu g)$ g FW) which is on par with red and green colored cultivars. Moreover, green colored 'Amrapali' and 'Lalmuni' were on par with two yellow colored 'Peach' and 'Lazzat Baksh' for carotenoids. Therefore, cultivar 'Arka Anmol' followed by 'Amrapali', 'Peach', 'Lazzat Baksh' and 'Lalmuni' had high carotenoid content. Other green colored ('Langra', 'Hamlet' and 'Bombay No. 1') and red colored ('Tommy Atkins' and 'Janardhan Pasand') cultivars showed low carotenoid contents.

An unknown compound at retention time of 0.73 min (Supplementary Table 1) was very less in peel of all colored cultivars except in green colored cv. 'Amrapali' (Table 1) in which $0.28 \mu g/g$ FW was observed at fully ripe stage. The presence of all trans-violaxanthin butyrate was higher in green and red types, compared to yellow types (Table 1). However, violaxanthin was higher in yellow types and 'Arka Anmol' showed significantly higher $(6.21 \mu g/g \text{FW})$ content when compared to others. The content of violaxanthin ranged from 0 to 6.21 μ g/g FW with cv. 'Arka Anmol' showing higher content followed by 'Lazzat Baksh', 'Peach', 'Amrapali', 'Tommy Atkins' and 'Lalmuni'. All *trans*-violaxanthin butyrate and violaxanthin levels were higher in green and yellow colored cultivars respectively. This suggests that the expression pattern differences within the isomers of the same compounds. Similarly, cultivar differences were observed in case of luteoxanthin (0–1.51 µg/g FW), lutein (0–1.41 µg/g FW) and zeaxanthin $(0-1.58 \text{ µg/g FW})$. A significant presence of β -carotene was evident in all the cultivars. The highest content of b-carotene was observed in yellow colored 'Arka Anmol' (13.01 μ g/gFW), which was 41.73% of the total carotenoids. In general, the contribution of β -carotenes in mango peel was from 24.52 to 98.06% for total carotenoids. It should be noted that cultivar 'Banganapalli' hardly produced any other carotenoids than β -carotene (98.06%). A similar pattern was observed for *cis* β -carotene with range from 0 to 7.47 μ g/g FW. There was no significant difference for zeaxanthin amongst the colored mango cultivars.

In mango pulp, *trans*-carotenes and xanthophylls are reported as major group of carotenoids (Wilberg and Rodriguez-Amaya [1995;](#page-11-0) Chen et al. [2004\)](#page-11-0). Now it appears

S. no.	Compounds	LAN	AMP	HAM	B _{N1}	AA	LZB	PEA	BGNP	TAM	LAL	GUL	JPM
	Unidentified	T _b	0.28_a	T _h	T _b	T _b	T _b	T _b	T _b	T _b	T _h	$\rm T_h$	T _b
2	<i>Trans</i> violaxanthin butyrate	1.03 _{bc}	4.71_a	$0.67_{\rm bc}$	0.04_c	T_c	0.24_c	0.26_c	T_c	T_c	1.65 _b	0.51_c	0.03_c
3	Violaxanthin	0.21_c	0.59 _b	0.23 _{hc}	0.12_c	6.21_a	1.42 _b	0.98 _{bc}	T_c	0.53_c	$0.52_{\rm bc}$	T_c	0.01_c
4	Luteoxanthin	0.13_{cd}	$0.45_{\rm bc}$	0.19 _{cd}	0.11_{cd}	1.51_a	$0.52_{\rm bc}$	0.74 _b	T_{d}	$0.49_{\rm bc}$	$0.36_{\rm bcd}$	0.11_{cd}	$0.02_{\rm d}$
5	Lutein	0.24_{cde}	$0.50_{\rm bede}$	0.53 _{hc}	T_e	1.41_a	$0.88_{\rm ab}$	0.66_{bc}	0.03_c	0.73 _{bc}	$0.43_{\rm bc}$	0.09_{de}	T_e
6	Zeaxanthin	0.19	0.35	т	т	1.58	T	0.55	T	0.62	0.37	0.24	T
τ	B-Carotene	$0.90_{\rm cd}$	3.24 _{bc}	1.59 _{cd}	4.94 _b		13.01_a 2.41_{cd}	5.52 _b	1.52_{cd}	2.05 _{cd}	$1.90_{\rm cd}$	0.38_{d}	0.68_{d}
8	Cis β -Carotene	0.47_a	2.09 _{bc}	1.02 _{cd}	0.00_d	7.47_a	1.35_{cd} 3.15_{bd}		T_{cd}	1.06 _{cd}	1.05_{cd}	0.22_{d}	0.00_{d}
	Total $(\mu g/g)$	3.17_c	12.19 _b	4.23_c	5.21_c	31.18_a	$6.85bc$ 11.87 _b		1.55_c	5.47_c	6.27 _{bc}	1.55_c	0.74_c
	β -Carotene $(\%)$	28.39	26.51	37.59	94.82	41.73	35.18	44.23	98.06	37.48	30.30	24.52	91.89
	Violaxanthin $(\%)$	39.11	43.48	20.56	3.07	19.92	24.23	10.44	0.0	9.69	34.60	32.90	5.40

Table 1 Carotenoid compounds (μ g/g FW) in the peel of different colored mango cultivars at fully ripe stage (S3)

Codes of different cultivars are: LAN ('Langra'), AMP ('Amrapali'), HAM ('Hamlet'), BN1 ('Bombay No. 1'), AA ('Arka Anmol'), LZB ('Lazzat Baksh'), PEA ('Peach'), BGNP ('Banganapalli''), TAM ('Tommy Atkins'), LAL ('Lalmuni'), GUL ('Gulabi') and JPM ('Janardhan Pasand'). Alphabets depict (subscript) the significant differences among the different colored cultivars. Similar alphabets represent no significant differences among the cultivars

that a similar pattern is present in case of mango peels. Here, we observed the differential pattern of carotenoids among the colored mango cultivars. In green and a few red colored cultivars, the level of violaxanthin and β -carotenes were on par with each other. However, in yellow colored types, content of β -carotene was significantly higher than violaxanthin (Table [1\)](#page-3-0). Mercadante et al. ([1997\)](#page-11-0) have evaluated 'Keitt' mango which was green in color with 30% red blush, recorded higher violaxanthin followed by b-carotene. Significantly higher violaxanthin was also observed in 'Arka Anmol' cultivar which is having little yellowish orange colored peel, indicating the importance of violaxanthin in giving reddish blush to the peel color in mangoes. These differences were also recorded by Amotz and Fishler ([1998\)](#page-10-0) and Setiawan et al. ([2001\)](#page-11-0), suggesting cultivar differences amongst the colored cultivars.

Accumulation of carotenoids in mango peel during ripening

The ripening of fruit is associated with the degradation of chlorophylls and accumulation of carotenoids and anthocyanins. The chloroplast is transformed to chromoplast resulting in yellow or red coloration. Carotenoid levels were found to increase during ripening (Fig. [1\)](#page-6-0). However, the increase was significantly high in yellow colored cultivars only. Except *trans*-violaxanthin the significant increase in other carotenoids were observed in cv. 'Arka Anmol'. β -Carotene content increased drastically (Fig. [1g](#page-6-0)) in other yellow colored cultivars also. This shows the increased carotenoid accumulation in peel during the ripening period. Similar pattern was observed in case of cultivars 'Keitt' and 'Tommy Atkins' (Mercadante and Rodriguez-Amaya [1998](#page-11-0)). In 'Tommy Atkins' increase in the levels of carotenoids during ripening has been reported (Medlicott et al. [1986](#page-11-0)).

Significant increase in total and individual carotenoids was observed in yellow colored cultivars during ripening, whereas little or no change was observed in case of other colored cultivars. Interestingly, the green colored ('Langra', 'Amrapali', 'Hamlet') and red colored cultivar 'Lalmuni' showed higher trans-violaxanthin content compared to other yellow types, which later decreased during the developmental stages, except in cultivars 'Amrapali' and 'Lalmuni', where it increased during ripening (Fig. [1c](#page-6-0)). The dominance of β -carotene and violaxanthin isomers was observed at fully ripe stage (Fig. [1g](#page-6-0), c). Among the yellow types cv. 'Arka Anmol' showed significantly higher content of b-carotene (twofold), violaxanthin (fourfold), zeaxanthin, and lutein compared to other cultivars. However, cv. 'Banganapalli' recorded much lower levels of carotenoids than the green and red cultivars.

Anthocyanin composition in fruit peel and cultivar differences at fully ripe stage

Highest total anthocyanin content (Table [2](#page-6-0)) was observed in red colored 'Tommy Atkins' $(228.2 \mu g/100 \text{ g FW})$ followed by 'Janardhan Pasand' (147.6 μ g/100 g FW), 'Lalmuni' (68.82 μ g/100 g FW) and 'Gulabi' (49.49 μ g/100 g FW). The moderate anthocyanin content (63.2 µg) 100 g FW) observed for yellow colored 'Arka Anmol' which was on par with red colored 'Gulabi'. Hence, it was observed that the total anthocyanin content in red colored cultivars was highest, followed by yellow, while the green cultivars had the least. Significant difference in anthocyanin content was observed amongst the cultivars as well as among compounds (Table [2\)](#page-6-0). Similarly, for total cyanidin, pelargonidin, delphinidin, malvidin, petunidin and peonidin content, significantly higher values were observed for 'Tommy Atkins' followed by 'Janardhan Pasand'. The presence of fairly good quantity of anthocyanins in the peel of 'Arka Anmol' indicates involvement of anthocyanin compounds other than carotenoids in color development. This may be the reason for the yellow orange color of the cv. 'Arka Anmol' instead of pure yellow color (Supplementary Table 3). It appears that shades of different colors are due to the presence of the various types of pigments in peels.

Anthocyanin contents have been the subject of debate, for a range of values have been reported in mango peels (Nordey et al. [2014](#page-11-0)). Total anthocyanin content ranged from 3600 to 5650 μ g/g in ripe stage, while in raw stage, it varied between 2030 and 3260 µg/g on dry weight basis in cultivars 'Raspuri' and 'Badami' (Ajila et al. [2007\)](#page-10-0). Total anthocyanin contents in the peels of some of the red colored mango cultivars ranged from 0.21 ('R2E2') to 3.71 ('Tommy Atkins') µg/g dry matter (Berardini et al. [2005a](#page-11-0)). In the present study, in cultivar 'Tommy Atkins', the anthocyanin content at the ripe stage was found to be $228 \mu g/100 \text{ g}$ FW, which was similar to the values mentioned by Berardini et al. ([2005b\)](#page-11-0), if expressed on dry weight basis. The extraction and measurement methods employed might determine the precise quantification (Nordey et al. [2014](#page-11-0)). Berardini et al. [\(2005b](#page-11-0)) concluded that peels are poor sources of anthocyanins. Mango peel is a by-product in processing and canning industry. The utilization of red colored peel would be profitable, when converted into valuable functional foods, as they possess antioxidant properties due to polyphenols, anthocyanins and carotenoids (Ajila et al. [2007\)](#page-10-0). The studies on other fruits like Blackberry fruits (Rubus adenotrichos) (Araya et al. [2017\)](#page-10-0), myrtle berry (Gilda et al. [2017\)](#page-11-0) and barberry (Mehdi et al. [2018](#page-11-0)) were recently undertaken showing the importance of anthocyanin content.

 $\begin{bmatrix} \frac{1}{2} & \frac{1}{2} \\ \frac{1}{2} & \frac{1}{2} \\ \frac{1}{2} & \frac{1}{2} \end{bmatrix}$

 $\overline{0}$

LAN AMP HAM BNI

Green

 $_{\rm A}$ A

Yellow Mango varieties

Ĥ.

LZB PEA BGNP TAM LAL GUL JPM

 $_{\rm Red}$

 \blacktriangleleft Fig. 1 Carotenoid contents (μ g/g FW) in peel as influenced by different cultivars at developmental stages. a Unidentified, **b** all *trans*violaxanthin butyrate, c violaxanthin, d luteoxanthin; e lutein, f zeaxanthin, $g \beta$ -carotene, h *cis-* β -carotene and i total carotenoids S1 (80% maturity), S2 (100% maturity) and S3 (fully ripen stage). Codes of different cultivars are: LAN ('Langra'), AMP ('Amrapali'), HAM ('Hamlet'), BN1 ('Bombay No. 1'), AA ('Arka Anmol'), LZB ('Lazzat Baksh'), PEA ('Peach'), BGNP ('Banganapalli''), TAM ('Tommy Atkins'), LAL ('Lalmuni'), GUL ('Gulabi') and JPM ('Janardhan Pasand'). Bars represent standard error of three replicates

In green colored cultivars, 'Langra', 'Hamlet' and 'Bombay No. 1', the compound cyanidin-3-O-monoglucoside was the major anthocyanin. In yellow colored 'Arka Anmol', the major compounds were cyanidin-3-Omonoglucoside, delphinidin-3-O-(6-O-acetyl)-glucoside, petunidin-3-O-glucoside, petunidin-3-O-acetylglucoside), which were almost similar to red colored types. In red types, 'Tommy Atkins' (cyanidin-3-O-monoglucoside, delphinidin-3-O-(6-O-acetyl)-glucoside and peonidin-3-O-

Table 2 Content of various anthocyanin compounds (μ g/100 g FW) in fruit peel of different colored cultivars identified at third stage *i.e.* fully ripe stage (S3)

C. no.	LAN	AMP	HAM	BN1	AA	LZB	PEA	BGNP	TAM	LAL	GUL	JPM
1	6.37 _c	1.71_{f}	1.86 _f	2.72_{f}	7.59_{ce}	3.18_{fe}	$1.9_{\rm 1f}$	$3.34_{\rm def}$	26.17_a	5.68 _{cd}	5.20_{cde}	14.08 _b
2	0.80_{bc}	0.72_{bc}	$0.13_{\rm ic}$	$0.61_{\rm bc}$	$0.34_{\rm bc}$	$0.55_{\rm bc}$	$0.87_{\rm bc}$	1.92 _b	1.83 _b	1.68_{bc}	0.43_{bc}	5.88_a
3	$0.44_{\rm cd}$	0.55_{cd}	T_d	$0.43_{\rm cd}$	1.08 _{bcd}	$0.42_{\rm cd}$	$0.51_{\rm cd}$	1.79_{bc}	3.36_a	0.58 _{cd}	$1.19_{\rm bcd}$	$2.34_{\rm ab}$
4	$1.31_{\rm ef}$	0.79_f	0.72_f	1.40_{cef}	3.08 _b	1.23_{ef}	$0.80_{\rm f}$	2.73_{cb}	9.96_a	2.88 _b	$2.28_{\rm cbe}$	6.47 _b
CT	$8.92_{\rm cdef}$	$3.76_{\rm fg}$	$2.71_{\rm g}$	$5.16_{\rm efg}$	$12.0_{\rm fc}$	$5.37_{\rm{defg}}$	4.08_{fg}	$9.78_{\rm cde}$	41.32_a	$10.83_{\rm cd}$	$9.10_{\rm cefd}$	$28.78_{\rm b}$
5	$1.69_{\rm de}$	$0.75_{\rm ef}$	0.04_f	1.46_e	3.03 _{bed}	$1.08_{\rm ef}$	$1.99_{\rm cde}$	1.28_{ef}	10.9_a	3.09 _{bc}	$1.94_{\rm cde}$	3.85 _b
6	4.64_a	3.91_a	1.71 _b	0.39 _{cd}	$0.21_{\rm cd}$	2.14 _b	$1.54_{\rm b}$	$0.48_{\rm cd}$	1.75 _b	3.90_a	1.18_{bc}	$\rm T_d$
7	$0.41_{\rm a}$	0.25_a	0.39_a	$\rm T_a$	0.08_a	0.45_a	0.39_a	0.09_a	$\rm T_a$	$0.70_{\rm a}$	0.43_a	0.22_a
8	1.60_{de}	0.81 _e	$0.30_{\rm e}$	$1.54_{\rm de}$	4.37 _c	1.37 _e	$0.98_{\rm e}$	$1.69_{\rm de}$	13.77_a	3.33_c	$3.07_{\rm cd}$	7.01 _b
PIT	8.34_{cd}	$5.73_{\rm def}$	2.45 _g	$3.47_{\rm fg}$	7.68_{de}	$5.04_{\rm efg}$	$4.90_{\rm efg}$	3.55_{fg}	26.68 _a	$11.03c$	$6.61_{\rm de}$	$22.6_{\rm b}$
9	1.17 _b	0.33_{fgh}	$0.81_{\rm cde}$	$0.24_{\rm gh}$	$0.18_{\rm gh}$	$0.57_{\rm def}$	$0.13_{\rm h}$	$0.21_{\rm gh}$	3.29_a	$0.88_{\rm bcd}$	$0.49_{\rm efg}$	$0.46_{\rm bc}$
10	2.29_e	$2.01_{\rm e}$	$\mathrm{T_{f}}$	$2.32_{\rm e}$	$5.84_{\rm c}$	1.72 _e	$1.88_{\rm e}$	$2.38_{\rm e}$	$20.8_{\rm a}$	4.13 _d	2.73 _e	$9.63b$
11	$2.86_{\rm c}$	1.81_{de}	$\rm T_{f}$	$2.06_{\rm de}$	2.15_d	$1.68_{\rm de}$	$1.74_{\rm de}$	$1.50_{\rm e}$	10.64_a	3.99 _b	1.97_{de}	$9.91_{\rm a}$
12	$0.38_{\rm bc}$	$0.44_{\rm bc}$	$0.38_{\rm bc}$	0.30 _c	$0.31_{\rm bc}$	$0.42_{\rm bc}$	$0.32_{\rm c}$	0.30 _c	1.46_a	0.59_{bc}	$0.42_{\rm bc}$	0.69 _b
DT	6.70_d	$4.58_{\rm ef}$	1.19_g	$4.91_{\rm ef}$	8.49 _c	$4.40_{\rm ef}$	4.07_f	$4.40_{\rm ef}$	36.2_a	9.59 _c	$5.60_{\rm de}$	20.68 _b
13	$0.61_{\rm cd}$	$0.66_{\rm cd}$	$\rm T_d$	$0.95_{\rm cd}$	$2.15_{\rm cd}$	$0.94_{\rm cd}$	$0.61_{\rm cd}$	1.36 _{cd}	$4.51_{\rm ab}$	$3.38_{\rm abc}$	$2.03_{\rm bcd}$	6.55_a
14	0.33_a	0.31_a	0.52_a	$0.31_{\rm a}$	0.45_a	0.36_a	0.25_a	0.28_a	1.33_a	0.62_a	0.39_a	1.20_a
15	$\rm T_h$	$1.21_{\rm g}$	0.33 _h	$1.74_{\rm ef}$	$2.21_{\rm de}$	1.29_{fg}	1.13 _g	$1.50_{\rm fg}$	11.3_a	3.89 _c	2.70 _d	5.72 _b
16	$\mathrm{T_{f}}$	$0.31_{\rm ef}$	$0.54_{\rm def}$	1.55_d	3.30 _c	$0.97_{\rm def}$	$0.90_{\rm def}$	1.29_{de}	10.59_a	$2.73_{\rm ec}$	$0.94_{\rm def}$	$6.76_{\rm b}$
MT	$0.94_{\rm f}$	$2.49_{\rm ef}$	1.39_f	$4.55_{\rm def}$	$8.11_{\rm cd}$	$3.56_{\rm def}$	$2.89_{\rm ef}$	4.44_{def}	$27.75_{\rm a}$	$10.63c$	$6.05_{\rm de}$	$20.23_{\rm b}$
17	0.43 _{gh}	$0.31_{\rm gh}$	$\rm T_h$	$1.06_{\rm de}$	1.93 _c	1.16_{de}	$0.79_{\rm def}$	$0.54_{\rm egh}$	6.44_a	$1.21_{\rm d}$	1.97 _c	$4.08_{\rm b}$
18	0.37 _c	$0.78_{\rm bc}$	0.38_c	$0.70_{\rm c}$	$0.83_{\rm bc}$	$0.45_{\rm c}$	0.53_c	$0.70_{\rm c}$	2.51_a	1.40 _b	$0.91_{\rm bc}$	2.03_a
19	0.50_d	0.92 _d	0.49 _d	0.73_d	2.04 _c	0.88 _d	0.71_d	1.01 _d	7.22_a	$1.80c$	$1.97_{\rm c}$	5.48 _b
20	$2.01e$	$1.65_{\rm ef}$	0.90 _f	$2.47_{\rm e}$	$4.74_{\rm cd}$	$1.97_{\rm ef}$	$1.73_{\rm ef}$	2.40 _e	19.28_a	5.45 _c	3.95 _d	13.20 _b
21	$0.67_{\rm fg}$	$0.70_{\rm fg}$	0.30 _g	$2.41_{\rm de}$	4.81_c	$1.09_{\rm efg}$	$1.65_{\rm efg}$	2.06 _{ef}	16.65_a	3.72_{cd}	3.64 _d	11.52_{bc}
PtT	3.99_{de}	4.37_{de}	$2.06_{\rm e}$	7.37_d	$14.36c$	$5.55d_{\rm e}$	5.41_{de}	6.71_d	52.09_a	13.57c	$12.44c$	$36.31_{\rm b}$
22	0.18_g	$0.49_{\rm efg}$	$0.28_{\rm fg}$	$0.84_{\rm ef}$	1.49 _{cd}	$1.04_{\rm de}$	$0.50_{\rm efg}$	$0.79_{\rm ef}$	$5.99_{\rm a}$	1.67 _c	$1.01_{\rm de}$	$4.58b$
23	2.38_{ef}	2.32_{ef}	0.48_f	$2.17_{\rm ef}$	7.19 _c	4.47 _{cde}	$2.38_{\rm ef}$	$3.00_{\rm def}$	24.83_a	5.64_{cd}	$5.62_{\rm cd}$	$16.55b$
24	4.23_d	$1.10_{\rm f}$	2.16_{ef}	$1.63_{\rm ef}$	3.88_d	2.03_{ef}	1.13_f	$1.77_{\rm ef}$	$13.37_{\rm a}$	5.87 _c	$3.07_{\rm de}$	$9.49b$
PeT	6.79_{de}	3.92 _e	$2.92_{\rm e}$	4.64 _e	$12.57_{\rm c}$	$7.53_{\rm de}$	$4.01_{\rm e}$	$5.56_{\rm de}$	$44.18_{\rm a}$	13.18_c	$9.70_{\rm cd}$	$30.61_{\rm b}$
GT	$35.67_{\rm ef}$	24.85_{fg}	12.71_g	$30.1_{\rm fg}$	63.2_{cd}	31.45_f	$25.3_{\rm fg}$	$34.44_{\rm ef}$	$228.2_{\rm a}$	$68.82_{\rm c}$	$49.49_{\rm d}$	147.6 _b

Codes of different cultivars are: LAN ('Langra'), AMP ('Amrapali'), HAM ('Hamlet'), BN1 ('Bombay No. 1'), AA ('Arka Anmol'), LZB ('Lazzat Baksh'), PEA ('Peach'), BGNP ('Banganapalli''), TAM ('Tommy Atkins'), LAL ('Lalmuni'), GUL ('Gulabi') and JPM ('Janardhan Pasand'). The lowercase alphabets represent the statistical analysis among the colored cultivars for each identified compounds.Similar or no representation of the alphabets means non-significant result

CT cyanidin total, PlT pelargonidin total, DT delphinidin total, MT malvidin total, PT petunidin total, PeT, peonidin total, T trace, GT grand total

Table 3 The comparison of the anthocyanins (μ g/100 g) contents in three peel colored groups (green, yellow and red) showing significant differences among them at fully ripe (colored) stage

The ANOVA was performed by taking the means of four cultivars under each group viz., green (content means of Langra, Amrapali, Hamlet and Bombay no. 1), yellow (content means Arka Anmol, Lazzat Baksh, Peach and Banganapalli') and red (content means Tommy Atkins, Lalmuni, Gulabi and Janardhan Pasand) for obtaining explicate results for individual contribution of anthocyanins for total anthocyanins and red coloration. The lowercase alphabets represent the statistical analysis among the colored cultivars for each identified compound. Similar or no representation of the alphabets means non-significant result

glucoside), 'Lalmuni' (cyanidin-3-O-monoglucoside, petunidin-3-O-glucoside, trans-peonidin-3-O-(6-O-p-coumaryl)-glucoside), Gulabi (peonidin-3-O-glucoside, cyanidin-3-O-monoglucoside) and 'Janardhan Pasand' (peonidin-3-O-glucoside, cyanidin-3-O-monoglucoside, pelargonidin 3-(acyl) glucoside, petunidin-3-O-glucoside and petunidin-3-O-acetylglucoside), cyanidin-3-Omonoglucoside and peonidin-3-O-glucoside were the common anthocyanins. Significant variations in anthocyanins have also been reported in different cultivars of grapes (Huang et al. [2009](#page-11-0)).

The results of composition of anthocyanins in peels of 12 colored cultivars (Table [2](#page-6-0)) belonging to green, yellow, and red groups showed significant differences amongst cultivars. However, the clear differences in the colored groups could not be obtained. Hence, a group of green, yellow and, red types was classified by taking an average of the contents of the four cultivars of each colored group (green, yellow and red) and comparison within groups were interpreted. The red color group had significantly higher anthocyanins, than yellow and green peel color (Table 3). Proctor and Creasy ([1969\)](#page-11-0) reported the presence of peonidin-3-O-galactoside in mango fruit, which was contradicted by Berardini et al. ([2005b\)](#page-11-0), who reported the presence of cyanidin 3-O-galactoside. In our study, the presence of both the compounds was observed. We could observe the equal contribution from both peonidin (10.6%) and cyanidin (10.3%). Differences in the relative proportions of cyanidin and delphinidin have been reported to be responsible for imparting diverse colors in kiwifruits (Montefiori et al. [2009](#page-11-0)). In mango peels also, the presence of these anthocyanins in different concentrations seems to be the cause of the variations in coloration. Further, the presence of chlorophyll and carotenoids also contribute to the variations seen in the color of fruits.

The Red mango fruit peel was characterized with the presence of the cyanidin, delphinidin, pelargonidin, petunidin, peonidin and malvidin. The composition was similar in the other colored groups, indicating the contribution of these compounds towards the total anthocyanin content in general in mango peel. But, significantly higher quantity of individual compounds in red types revealed improved accumulation of these compounds and their role in the development of color. Other two groups (green and yellow) had an almost similar quantity of individual compounds.

Developmental patterns of anthocyanins in mango peels during ripening

No significant variations with respect to anthocyanins were noticed during fruit maturation in red types (Fig. [2a](#page-9-0)–g). The profiling of anthocyanins carried out at three ripening stages did not show any significant variation [Supplement Table 4 (S1); Supplement Table 5 (S2) and Table 2 (S3)]. The drastic accumulation of anthocyanins during ripening was observed in most of the fruits. This accumulation, however, is not the key rule (Steyn [2009](#page-11-0)), as anthocyanins is known to surge and decline gradually during ripening and fruit development in pineapple and certain pear cultivars. In our results also, the decline in anthocyanins was observed in two red types, suggesting some intermediary role of other pigments. Presence of anthocyanins in the peel of green colored fruits indicates some masking effects by major chlorophyll pigments in peels. In yellow colored cv. 'Arka Anmol', color in the final stage was observed to be yellow-orange (supplementary Table 3), which explains the involvement of anthocyanins and carotenoids, resulting in the blending of colors. In red types, anthocyanin accumulation was almost complete before ripening, and it decreased during ripening in two cultivars.

Role of pigments in mango peel color

The results of the present study, revealed the significant differences exist among cultivars and also changes in pattern of carotenoid accumulation during fruit ripening.

However, there was not developmental changes in anthocyanin pigments during fruit ripening. Cultivars were selected for green, yellow and red peel colors. However, color parameters as evaluated by RHS color chart, showed seven groups, viz., green ('Amrapali'), yellow green ('Langra', 'Hamlet' and 'Bombay No. 1') yellow orange ('Arka Anmol'), yellow ('Lazzat Baksh', 'Peach' and 'Banganapalli'), greyed red ('Lalmuni'), greyed purple ('Gulabi'), and red ('Tommy Atkins' and 'Janardhan Pasand') (Supplementary Table 3). During ripening, the unclear relationship between the red peel color and anthocyanins indicate the role of other pigments in fruit peel color, and hence the principle component analysis (PCA) was done to find out the relationships between different colors and various pigments (chlorophylls, carotenoids and anthocyanins).

PCA analysis showed three clusters among mango cultivars used here, (Fig. [3](#page-10-0)) which indicated that green, yellow, and red colored cultivars fall under their respective colored clusters, except for cv. 'Banganapalli' which was clustered in the green colored cultivar group. Data on pigments like total chlorophyll, carotenoids and anthocyanins concentration (Supplementary Fig. 3) in different colored cultivars indicates that chlorophyll content in green colored cultivars i.e. 'Langra', 'Amrapali', 'Hamlet' and 'Bombay No. 1' $(6.3-27 \text{ µg}/100 \text{ g})$ were significantly higher. On the other hand, 'Gulabi' (red colored) and 'Banganapalli'' (yellow colored) was on par with green colored cultivars for chlorophyll content. In other cultivars, chlorophyll content was very low. As per the RHS color chart, yellow-green color was observed in green cultivars and this color might be due to the combination of chlorophyll and carotenoid pigments. 'Banganapalli', which is yellow colored, was found to have higher chlorophylls, but lower carotenoids amongst the yellow colored cultivars. The combination of red and green pigments (anthocyanins and chlorophylls respectively) might also contribute to the yellow color of the fruit in addition to the carotenoids in cv. 'Banganapalli'. In cv. 'Arka Anmol' which was grouped under yellow orange category had higher contents of both carotenoids and anthocyanins. In this cultivar, anthocyanin content was similar to that of red colored cultivars 'Lalmuni' and 'Gulabi'. These red colored cultivars also showed the combination of these pigments and hence might be the reason for the red/greyish/purplish colors. However, very high content of carotenoids in combination with high anthocyanins had given yellow-orange color to the fruits. Cultivar 'Arka Anmol' was selected as the yellow colored based on visual observation, but the RHS color chart (Supplementary Table 3) revealed its color as yellow orange at the fully ripened stage.

 \blacksquare S1 \blacksquare S2 \blacksquare S3 LAN AMP HAM BN1 AA LZB PEA BGNP TAM LAL GUL JPM Green Yellow Red Mango cultivars

Fig. 2 Variation in anthocyanins (µg/100 g FW) in peel of different colored mango cultivars at three developmental stages. a Total cyanidin, b total pelargonidin, c total delphinidin, d total malvidin, e total petunidin, f total peonidin, g total anthocyanins. S1 (80% maturity), S2 (100% maturity) and S3 (fully ripen stage). Codes of different cultivars are: LAN ('Langra'), AMP ('Amrapali'), HAM

('Hamlet'), BN1 ('Bombay No. 1'), AA ('Arka Anmol'), LZB ('Lazzat Baksh'), PEA ('Peach'), BGNP ('Banganapalli''), TAM ('Tommy Atkins'), LAL ('Lalmuni'), GUL ('Gulabi') and JPM ('Janardhan Pasand'). Bars represents standard errors of three replicates

Fig. 3 Principle component analysis (PCA) showing clusters for colored mango cultivars belonging to three color groups viz. green, yellow and red

Conclusion

 β -carotene, violaxanthin, and *cis*- β -carotene were the major carotenoids in the peels, which increased significantly during ripening. Concomitant presence of anthocyanin compounds belonging to cyanidin, peonidin, petunidin, delphinidin and pelargonidin groups was observed, and no significant differences in the content were observed during the ripening process. During ripening in peel, degradation of chlorophylls, development of carotenoids, and maintenance of anthocyanins was observed. Red colored cultivars exhibited very high content of anthocyanins, and carotenoids were higher in yellow peel colored cultivars. Reddish to orange blush in the peel color was observed to be due to the blending of anthocyanins and violaxnthin with β -carotene. The results of this study will help in understanding the development of color and the role of various pigments in mango peel. The information can be used for exploiting mango fruit peel as a source of anthocyanin and carotenoids.

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Author's contribution Observations of mango varieties and collection of mango peel samples: GRK and MRD; Design and Analysis of experiments: KVR, KSS and GRK; Analysis of Pigments and LCMS: GRK, TKR, GAG and PKC. All authors have contributed for preparation of manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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