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Original Research Paper

Effect of post harvest ripening on bioactive secondary metabolites and antioxidant activity in mango cv. Amrapali

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ABSTRACT

Mango possesses many bioactive phytonutrients at ripe stage which boost our immune system against many diseases. Post harvest ripening plays a major role in changes in those bioactive phytochemicals and their antioxidant activity. Hence, the present study was undertaken to assess the changes in bioactive phytonutrients and total antioxidant activity during ripening of mango cv. Amrapali. The fruits were analyzed for total antioxidants, total phenols, total flavonoids and total carotenoids from the day of harvest to its deterioration. Fruit peel and pulp color was measured with SPH850 spectrophotometer on the basis of the CIE LAB color system (L*, a* and b*). The results revealed that total phenols (36.11 to 66.53mg GAE 100g^{-1}), total flavonoids (14.33 to 34.67mg QE 100g^{-1}), total carotenoids (2.23 to 11.47mg 100g^{-1}) and total antioxidant (0.37 to 0.76 mmol Trolox 100g^{-1}) activity increased gradually from day one to ninth day after harvest and decreased slightly thereafter up to eleventh day of harvest except total carotenoids, which remained constant. Strong correlations between total phenols (0.94), total flavonoids (0.86) and total carotenoids (0.97) with total antioxidant activity were noticed. Positive relationship between total carotenoids and L*, a*, b* values in mango peel and pulp during ripening was also observed. It can be concluded that ripening affected the composition of bioactive phytonutrients and their antioxidant activity in mango andmaximum nutraceuticals contents were noticed from seven to nine days after harvest.

Key words: Antioxidant activity, bioactive phytonutrients, mango, ripening, total carotenoids

INTRODUCTION

Mango (*Mangifera indica* L.) is one of the most popular subtropical fruit which is well known for its nutritional quality due the rich source of many dietary antioxidants like ascorbic acid, carotenoids and phenolic compounds. India is the leading contributor in global mango production (around 40% of worlds production) followed by China, Kenya, Thailand, Indonesia, Pakistan, Mexico, Brazil, Bangladesh and Nigeria (Saxena and Gandhi, 2014). Indian Horticulture Database, 2014). Mango is a climacteric fruit and its ripening process occurs rapidly after harvest, depending on cultivar, stage of maturity at harvest, and postharvest conditions (V´azquez-Caicedo *et al*, 2004). Several biochemical changes occur during mango ripening, among which carotenoids biosynthesis is the most important one (V´azquez-Caicedo *et al*, 2005). Carotenoids have been noted as one of the most abundant micronutrients found in cancer-preventative foods (Cano and Ancos 1994). Carotenoids have very diverse roles in biological functions of animals and plants including provitamin A activity, antioxidant activity, cell communication and protection against photo-oxidative damage (Van de Berg *et al*, 2000). Carotenoids are mainly synthesized during fruit ripening through conversion of chlorophyll (Fennema 1996). The edible portion turns from pale yellow to deep or orange yellow during ripening due to the synthesis of carotenoid pigments, which are responsible for imparting yellow-orange color of mango mesocarp (V´azquez-Caicedo *et al*, 2005). Phenolic compounds possess anti-inflammatory, anti-microbial, cardio-protective activities and protect against neurodegenerative diseases and diabetes mellitus (Kris-Etherton *et al*, 2002; Scalbert *et al*, 2005). The most commonly occurring polyphenols in fruit include flavonoids and phenolic acids, which are capable of reducing the risks of cardiovascular diseases and atherosclerosis through the prevention of cellular oxidative damage (Kelly *et al*, 2001).

In the present study revealed that mango variety Amrapali had higher total carotenoids content compared to other commercial varieties. It contains approximately 2.5–3.0 times more β-carotene with deep orange red flesh. Measurement of color has earlier been correlated with carotenoids estimation in mango cultivars like Manila and Alphonso (V´azquez-Caicedo *et al*, 2005; Ornelas-Paz *et al*, 2008). The fruit color is the first visible quality attribute assessed by the consumer and critical determinant in the acceptance of the fresh mango fruit prior to consumption. Therefore, colorimetric value is an important parameter for ripe fruits. However, the changes in total carotenoids and total phenols during ripening of cultivar 'Amrapali' mango are unknown. Therefore, the present work was undertaken to evaluate the effect of ripening stage on total carotenoids content, total phenolic content and antioxidant activity of the cultivar 'Amrapali' and to work out the relationship of fruit color with total carotenoids content.

MATERIAL AND METHODS

Collection of fruit sample

Fully matured mango fruits were harvested from ICAR-CISH mango orchard. Fruits were selected for uniform size (200-300 g) and free from blemishes and defects. Fruits were washed with water to remove field heat and other dust particles adhered on the surface. Fruits are stored in CFB boxes for ripening grouped into 11 treatments (15 fruits for each treatment/box with 3 replications). Each day fifteen fruits/one box of fruits was analyzed for total antioxidants, total phenols, total flavonoids and total carotenoids from the day of harvest (1st day) to its deterioration (11th day). The fruits maintained acceptable quality up to 11 days at normal room temperature from the day of harvest. Therefore the analysis was carried out up to 11 days.

Color measurement

In each treatment three fruits were used for recording peel and pulp color. Fruit peel and pulp color was measured with SPH850 spectrophotometer (Colorlite, Germany) on the basis of the CIE LAB color system (L*, a* and b*). In this system L*, a* and b* describe a three dimensional space, where L* is the vertical axis and its value varies from 100 (perfect white) to zero (complete black). Values of a* and b* specify the green-red and blue-yellow axis, respectively. Peel color was longitudinally determined on three points of each flat side of the fruit and recorded for six points for each fruit. Pulp color was determined longitudinally at three equidistant points on each slice.

Chemical analysis

The content of total phenolics in mango pulp was estimated as per the method suggested by Singleton *et al*, (1999) using Folin-Ciocalteu reagent. The absorbance was recorded at 750 nm in a double beam UV-VIS spectrophotometer (Labomed INC., USA). Gallic acid was used to prepare the standard curve and result was expressed as mg gallic acid equivalent (GAE) 100 g^{-1} fresh weight.

The flavonoids concentration was determined colorimetrically according to the method reported by Dewanto *et al*, (2002). The analysis of total carotenoids was conducted with the help of a modified method of Ranganna (1997) using acetone and petroleum ether as extraction solvents.

Total antioxidant activity was analyzed by CUPRAC (cupric reducing antioxidant capacity) assay developed by Apak *et al*, (2004), which measures the copper (II) ion reducing ability of polyphenols, vitamin C and vitamin E.

Statistical analysis

Data were expressed as means standard deviation of three replications. ANOVA (SPSS version 16.0) and *Turkey's post hoc* test were used to determine the mean difference of different variables at different days after harvest. Pearson's correlation test was used to determine the correlation between different biochemical compounds and color values of pulp and peel. Relationship between color of peel and pulp on content of carotenoids was worked out by using regression.

RESULTS AND DISCUSSION

On the day of harvest the fruits were hard and green in color while pulp was light yellow in color. The fruits started ripening and attained consumable stage on fifth day after harvest with good pulp texture and peel color. The fruits are in good condition up to 9 days after harvest but in 10th and 11th day the firmness

of fruits become weak and black spots were visible on the surface. The fruits were analyzed for different biochemical compounds from the day of harvest to day of fruit deterioration. A significant increase in the contents of total phenols, total flavonoids and total carotenoids along with total antioxidant activity was noticed up to ninth day of ripening (**Table 1**). Thereafter these amounts decreased in tenth and eleventh day of ripening when the condition of fruits deteriorated except in total carotenoids content which remained constant. The content of total phenols increased from 36.11 to 66.53 mg GAE 100 g^{-1} during nine days of ripening and then decreased to 54.72 mg GAE 100 g^{-1} at eleventh day after harvest. Similarly, total flavonoids content increased gradually from 14.33 mg QE 100 g^{-1} on the day of harvest to 34.67 mg QE 100 g^{-1} on ninth day after harvest, when fruits were fully ripen, and then decreased to 28.33 mg QE 100 g^{-1} on eleventh day after harvest. However, the content of total carotenoids increased continuously during nine days of ripening (from 2.23 mg 100 g^{-1} at first day to 11.47 mg 100 g^{-1}at ninth day) and remained constant thereafter (Table 1). The antioxidant activity was minimum (0.37 µmolTrolox 100 g^{-1}) on the day of harvest and maximum on 9th day after harvest (0.76 µmolTrolox 100 g^{-1}). After 9th day of harvest the content was declined non - significantly (*P=0.01*). As the fruit starts ripening, antioxidant activity also increased up to complete ripening of fruit but started decreasing at over ripe stage.

Table 1. Effect of postharvest ripening on bioactive phytonutrients and antioxidants in mango cv. Amrapali

Days after harvest	Total antioxidants µmolTrolox100 g^{-1}	Total phenols mg GAE mg 100 g^{-1}	Total flavonoids mg 100 g^{-1}	Carotenoids mg 100 g^{-1}
1	0.37	36.11	14.33	2.23
2	0.39	38.47	22.33	4.37
3	0.43	41.39	24.33	5.77
4	0.49	47.08	24.67	6.80
5	0.53	48.89	25.00	8.40
6	0.58	58.75	26.00	9.00
7	0.69	62.22	27.33	10.87
8	0.74	64.03	28.67	11.43
9	0.76	66.53	34.67	11.47
10	0.73	56.67	28.67	11.47
11	0.71	54.72	28.33	11.47
CD (*P=0.01*)	0.01	1.72	1.20	0.11

An increase or no change of total phenolic content during ripening of mango cultivars Alphonso and Tommy Atkins have also been reported (Robles-Sánchez *et al*, 2009; Kim *et al*, 2009). The gradual increase in total soluble phenolics during ripening of mango might be due to the conversion of starch to simple soluble sugars by amylase enzyme as reported by Gil *et al*,(2000). The decline in total phenols at later stages of ripening might be related to the senescence or over-ripening of fruits as reported by Palafox-Carlos *et al* (2012). Another possible reason for the decrease in the total phenols content during over-ripening might be due to the increase in polyphenol oxidase (PPO) activity, which catalyses the oxidation of mono and di-phenols to *o*-quinones. Increase in PPO activity from harvest maturity to half-ripe stage and then decline has been reported in Banganpalli, Dashehari, Fazri and Langra varieties of mango (Selvaraj and Kumar 1989). Palafox-Carlos *et al*, (2012) have concluded that ripening does not affect the content of total flavonoids in mango (cv. Alphonso) since they were similar in fruits of all four ripeness stages. The increase in total carotenoids and its major constituent β-carotene during ripening has been reported in many mango varieties *viz.* Dashehari (Kalra and Tandon, 1983; Verma *et al*, 1986), Chausa, Neelam and Amrapali (Sahni and Khurdia, 1989), Keitt and Tommy Atkins (Mercadante and Rodriguez-Amaya, 1998). Saltveit (1999) has

observed that carotenoids biosynthesis increases in most mango varieties during ripening and is associated with the climacteric increase in respiration initiated by ethylene activity. The carotenoid composition in mango can be affected by many factors such as growth conditions, maturity, cultivar, geographical origin and processing conditions (Chen *et al*, 2007). Palafox-Carlos *et al* (2012) has also reported that in mango variety Alphonso, the antioxidant activity has increased up to ripening stage-3 (70 – 80% fruit is yellow in color) and decreased in ripening stage-4 (100% fruit is yellow in color). The authors concluded that total phenolics and total flavonoids contents had greater relationship with total antioxidant activity in mango during ripening. Similar observations regarding higher relationship between total phenolics and antioxidant activity have also been reported by Kim *et al,* (2009).

Colorimetric values

The L*, a* and b* values were recorded for pulp and peel colour up to 11 days after harvest (**Table 2**). L* is the measure of lightness, the positive values of a* are in direction of redness and positive values of b* are the vector of yellowness**.** The negative values of a* is towards greenness and negative values of b* depicts blueness (Higby, 1962). The results showed that L* (66.48) values of pulp was maximum at 1st day of harvest and it gradually decreased as the fruit ripens (43.85) but in peel L* values are increased up to complete ripening and little reduction was observed at over-ripe condition. The a* values were increased from day one (8.15 and -6.43) to 11 (19.67 and 10.12) in both peel and pulp, but negative values were observed up to 3 days in case of fruit peel due to greenness in the peel. The b* values were shown increasing trend in both the cases of pulp and peel from day one (39.38 and 15.25) to 11 (49.11 and 44.22) after harvest. It is the indication of increase in yellowness in both fruit peel and pulp, which symbolizes the synthesis of total carotenoids content. There is no significant difference among the treatments for the b* values of pulp.

Table 2. Effect of post harvest ripening on L*, a* and b* values of mango pulp and peel

Days after harvest	Pulp			Peel		
	L*	a*	b*	L*	a*	b*
1	66.48	8.15	39.38	44.00	-6.43	15.25
2	59.47	12.52	41.55	46.90	-6.05	16.36
3	49.50	15.35	42.84	51.73	-3.86	21.19
4	47.96	19.2	43.60	53.51	1.93	28.79
5	43.55	20.2	43.83	55.54	3.55	32.75
6	43.91	20.29	44.05	57.00	7.54	32.32
7	42.88	20.68	45.73	57.17	8.10	34.49
8	43.00	20.75	47.22	58.84	8.53	37.35
9	43.85	20.88	47.39	58.30	8.98	37.60
10	45.38	20.62	49.68	57.10	9.07	42.13
11	49.32	19.67	49.11	56.87	10.12	44.22
CD *(P=0.01)*	3.46	2.29	NS	4.39	2.05	5.09

Correlation matrix for different biochemical compounds and L*, a* and b* values of mango fruit peel and pulp was analyzed. Among the biochemical compounds analyzed, the total antioxidants have positive correlation with total carotenoids (0.973), total phenols (0.939) and total flavonoids (0.857). The correlation between total carotenoids with peel and pulp b* values were 0.96 and 0.94, which indicated that the higher the b* values in peel and pulp, the higher the total carotenoids content. Gradual increase in b* values during ethephon induced ripening of dusehari fruits due to the increase in carotenoids biosynthesis has also been reported by Gill *et al*, (2015).

Relationship between the total carotenoids content of fruit pulp on L*, a* and b* values of fruit peel and pulp on post-harvest ripening was also worked out (**Fig. 1**). The results revealed that the positive relationship was obtained for total carotenoids content

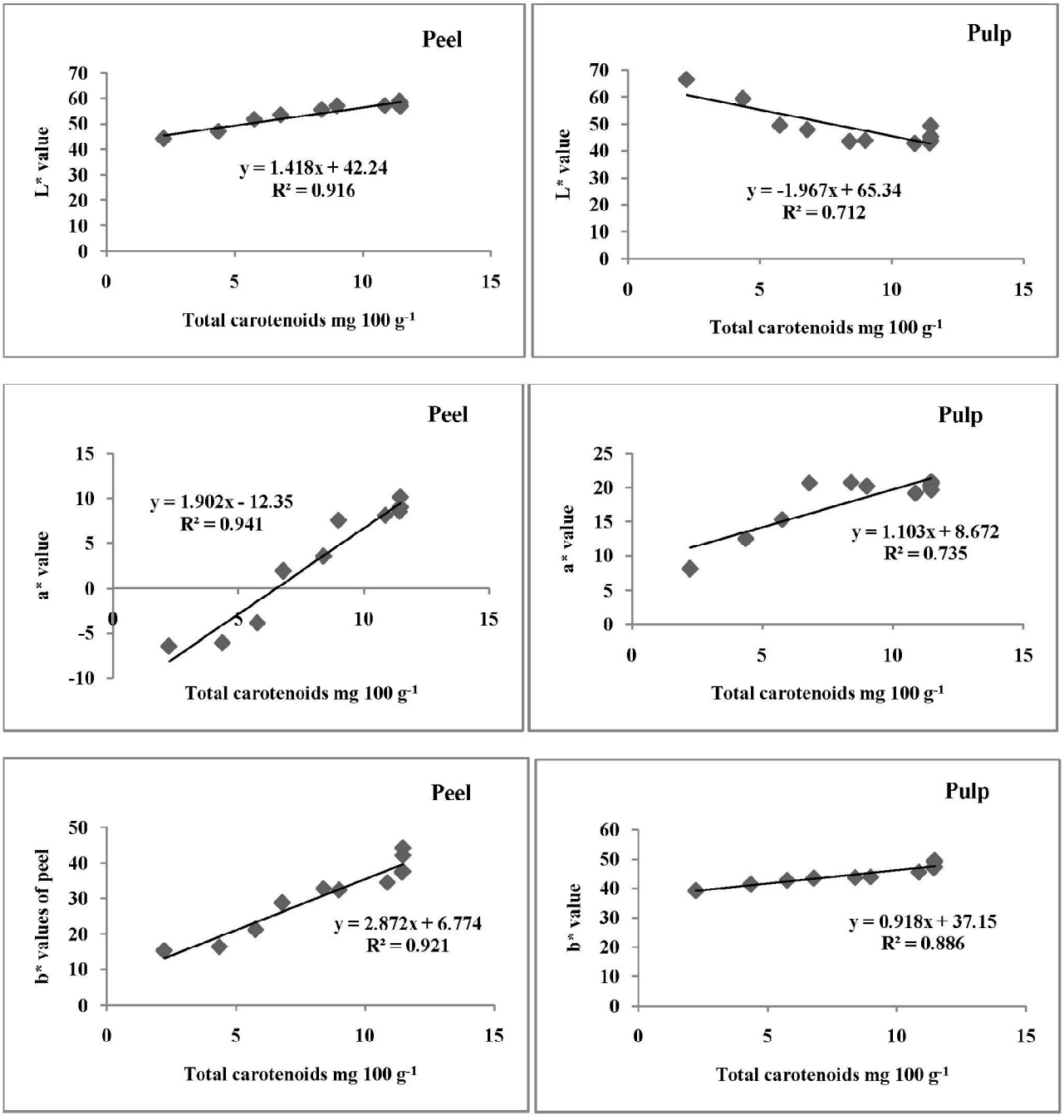

Fig. 1. Relationship between carotenoids and L*, a*, b* values of fruit peel and pulp during post harvest ripening of mango cv. Amrapali.

and peel L* (R^2 = 0.916), a* (R^2 = 0.941) and b* (R^2 = 0.92) values compared to pulp L* (R^2 = 0.712), a* (R^2 = 0.735) and b* (R^2 = 0.886) values. Fruit ripening processes influence the content of total carotenoids in cv. Amrapali and the fruits were best to consume within 9 days of ripening. The completely ripened fruits have higher amount of antioxidant activity, total phenols and total carotenoids contents as compared to mature and over-ripe fruits. The peel color is the best indicator for estimating total carotenoids content compared to pulp colour.

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