

Physiological changes in 'Pusa Manohari' and 'Amrapali' mangoes: on-tree vs off-tree

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ABSTRACT

Physiological changes were studied in 'Pusa Manohari' and 'Amrapali' mangoes during on-tree and off-tree ripening. On-tree, sugar levels remained relatively stable between 9 and 11°Brix, respiration was slow (with 'Pusa Manohari' exhibiting a slightly higher respiration rate compared to 'Amrapali', ranging from 200-190 mg CO₂ kg⁻¹ hr⁻¹ for PM and 150-140 mg CO₂ kg⁻¹ h⁻¹ for AP), and softening gradual, suggesting the tree regulates ripening. In contrast to the stable on-tree behavior of both the cultivars of the mangoes, off-tree, sugar levels increased significantly (7-25°Brix), respiration accelerated, and rapid softening was recorded. Later harvests ripened faster off-tree. On-tree firmness decreased gradually in 'Pusa Manohari' from 100 to 70 N in 58 days, and 'Amrapali' from 90 to 70 N in 48 days. Off-tree, both cultivars softened rapidly to approximately 20 N within 20 days. Respiration rates remained consistent across harvests for 'Pusa Manohari' off-tree. Pulp color exhibited lower lightness (higher L* values) compared to peel color. The study revealed a flexible harvest window, with earlier harvests potentially extending shelf life. Both cultivars exhibited similar trends in on-tree and off-tree ripening.

Key words: Harvesting, fruit firmness, respiration rate, colour, ripening.

INTRODUCTION

Mango (Mangifera indica) is a major fruit crop of India, with numerous prominent varieties cultivated in various regions. Mango is a climacteric fruit, characterized by a significant rise in respiration rate during ripening. Enhancing post-harvest shelf life while maintaining fruit quality is of paramount importance for this valuable crop. The "right time" for mango harvest refers to a stage where the fruits have not yet commenced ripening on the tree but are also not overly immature. Harvesting mangoes that are already ripening on the tree can compromise their post-harvest shelf life. Conversely, harvesting immature mangoes can negatively impact postharvest ripening and diminish their eating quality. To effectively research ripening inhibition and storage studies, precise information on the optimum harvest time and duration (from the start of flowering or fruit set) is crucial for mangoes. Unfortunately, determining the "right" time/stage to harvest mangoes for a specific purpose remains challenging due to several factors like the lack of universal maturity indices (Abu et al., 1; Shah et al., 10). There is no single

set of universally agreed-upon maturity indices for mangoes. Ripening development varies considerably among different mango cultivars. The mango fruit may not ripen significantly pre-harvest (on-tree), possibly due to the presence of hormonal, ripening-inhibiting substances originating from the vegetative parts (Burg and Burg, 4). Karuna *et al.* (11) highlighted the fact that mangoes harvested according to commercial practices often exhibit inconsistent maturity levels due to the continued growth of the cymose panicle. Consequently, harvesting fruits of uniform maturity can be commercially impractical.

'Pusa Manohari' and 'Amrapali' are two prominent mango cultivars in North India. 'Pusa Manohari,' a hybrid of 'Amrapali' and 'Lal Sundari,' is medium-sized (approximately 223-250 g) with a greenish-yellow peel and reddish shoulders. Its pulp is yellowish-orange, less fibrous, and has a total soluble solids (TSS) content of around 20-22 °Brix (Jayachandran et al., 8)'Amrapali,' a hybrid of 'Dasheri' and 'Neelum,' is a small to medium-sized fruit (approximately 100-300 g). It has a light greenish-yellow peel and deep orange-red pulp. It is less fibrous and has a TSS content of around 23-25 °Brix (Meena and Asrey, 15). Existing literature provides limited information regarding the optimal harvest time or maturity indices for 'Pusa Manohari' and 'Amrapali'. While previous research has measured parameters such as TSS, firmness, density, respiration rate (RR), and peel/pulp

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colour at harvest for various mango cultivars, it does not provide specific guidance on the "right time" for harvesting these cultivars for ripening inhibition and storage. This variation in ripening indices is supported by previously reported research. (Jha *et al.*, 9; Karuna *et al.*, 11; Nadeem *et al.*, 18; Kour *et al.*, 12; Muiruri *et al.*, 17; Begum *et al.*, 2; Bhuiyan *et al.*, 3; Wang *et al.*, 20).

Due to the lack of specific information on the "right time" or "correct maturity indices" for harvesting 'Pusa Manohari' and 'Amrapali' mangoes for ripening inhibition and storage, this study was undertaken. The study aimed to determine the maturity indices of 'Pusa Manohari' and 'Amrapali' mangoes by harvesting fruits for 58 and 49 days, respectively, starting 103 and 112 days after flowering. This approach allowed for the monitoring of the progression of various maturity indices in both on-tree and off-tree mangoes.

MATERIALS AND METHODS

The cvs. 'Pusa Manohari' and 'Amrapali' mangoes were selected for this study. Five mango trees of each cultivar were selected with good vigour and similar crown size, approximately 20 years of age, located at ICAR-Indian Agricultural Research Institute, New Delhi, and labelled as 'experimental trees. The first sampling (initial harvest) was conducted 103 days after flowering (7 June 2023) for Pusa Manohari and 112 days after flowering (16 June 2023) for Amrapali, respectively (Table 1). At the initial stage, the fruits had fully developed cheeks and outgrown shoulders. Mango fruits were harvested in the early morning hours using a mango fruit picker (a long wooden stick with a blade and shade net bag fixed at the end) from selected experimental trees; the stems were trimmed to 2 cm. and then fruits were transported immediately to the laboratory. In the laboratory, fruits were immersed in water $(27 \pm 5^{\circ}C)$

Table 1. On-tree and off-tree mangoes - harvest schedule and storage duration.

Name	Julian Days		Julian Days		
	(Pusa Manohari)		(Amrapali)		
On-tree	158		167		
	Harvested every 3		Harvested every 3		
	days for 58 days		days for 49 days		
Off-tree	Storage duration		Storage duration		
	(days)		(days)		
Harvest 1	164	18	167	15	
Harvest 2	174	18	186	10	
Harvest 3	180	16	205	11	
Harvest 4	200	10	216	4	

for an hour to rapidly remove field heat and achieve ambient temperature in the laboratory. Afterwards, fruits were sorted for uniform size and freedom from mechanical damage and air-dried for 20 minutes. Each mango was then labeled, weighed using an analytical balance (SF-400C digital scale, Baijnath Premnath, India) and randomly assigned to various experiments described in the following sections.

To assess on tree fruit development during maturation on the tree, mangoes ('Pusa Manohari' and 'Amrapali') were monitored in situ. Approximately 3-5 mangoes per cultivar were harvested every 2-3 days for 58 days ('Pusa Manohari') and 49 days ('Amrapali'), commencing 103 and 112 days after flowering, respectively. Fruit firmness, TSS, respiration rate, and pulp and peel colour were evaluated as outlined in the following section on harvested mangoes within 4-5 hours of collection. Field heat removal procedures are described following section. Ambient temperature and relative humidity (RH) were monitored using data loggers to record wet and dry bulb temperatures (Chopra et al., 5). Average ambient conditions were $30 \pm 5^{\circ}$ C and $50 \pm 5^{\circ}$ RH. To investigate off-tree maturity characteristics, 'Pusa Manohari' and 'Amrapali' mangoes were harvested at four stages: early season (harvest 1), mid-season (harvest 2), late season (harvest 3), and very late season (harvest 4). Six replicates of each cultivar were stored under ambient conditions (Table 1). Each stage's harvest timing (days after flowering) is detailed in Table 1. Every 4-5 days, six mangoes per replicate were removed from storage and evaluated for fruit firmness, TSS, respiration rate, and pulp and peel color. A total of 62 'Pusa Manohari' and 40 'Amrapali' mangoes were included in the off-tree experiments.

The quality attribute includes TSS were determined using a hand refractometer (Erma, Tokyo, Japan; 0-32°Brix, 0.2°Brix resolution) as per (Karuna et al., 11). Juice from the middle portion of unripe mangoes was extracted using a pestle and mortar. For ripe mangoes, juice was obtained from squeezed pulp. TSS was measured using 2-3 drops of clear juice at 27°C and expressed in °Brix. Fruit firmness was measured at the equatorial region using a penetrometer (Model GY-3, Erma, India; 12 kg/ cm² capacity, 5-mm diameter probe). The force (N) required to penetrate the peel 1.5 cm was recorded (Jha et al., 9). The respiration rate was measured using a handheld infrared gas analyzer (CEM GD-3803, CEM Instruments, India; 0-9999 μ L L⁻¹ CO₂). The mango was placed in an 8-L airtight container with the analyzer. CO₂ emission was recorded at 7-minute intervals for 15 minutes and calculated as mg CO₂ kg⁻¹ hr⁻¹. Pulp and peel colour were measured using a colourimeter (AMT 599 Mini scan XE plus, USA). The instrument was calibrated with white and black tiles. Colour values were recorded as L^* , a^* , and b^* . L^* represents lightness (0-100, black to white), while a^* and b^* represent colour coordinates (green to red and blue to yellow, respectively). Measurements were taken at the equator of each fruit.

Three replicates were conducted, each using a different source tree. For each replicate and parameter, the rate of change was determined using linear curve fitting. Respiration rate data were fitted to a 3rd degree polynomial. The day of maximum respiration rate and the corresponding days postharvest were identified. When the maximum occurred between analysis dates, values were extrapolated. Data presented in the figures represent the average of the three replicates.

RESULTS AND DISCUSSION

Maturity indices (TSS, firmness, colour, and respiration rate) for on-tree 'Pusa Manohari' and 'Amrapali' mangoes at various days after flowering (DAFL) are presented in Table 2. TSS in mangoes primarily comprise soluble sugars, organic acids, vitamins, and pigments. 'Pusa Manohari' and 'Amrapali' mangoes showed minimal changes in TSS during ontree maturation. TSS remained consistently around 10 °Brix, indicating that these fruits require further ripening processes before becoming suitable for consumption. While fruit size increased during this period, a slight decline in TSS was observed (Fig. 1A and 1B). This is likely attributed to factors such as increased fruit size, starch conversion, and variations in tree vigour, fruit location, and sun exposure. In contrast, off-tree mangoes exhibited a significant increase in TSS during storage under ambient conditions. This increase was directly proportional to storage duration, as evident in the linear trends in Fig. 1A and 1B. Notably, later harvests (Harvest 2, 3, and 4) demonstrated a faster rate of TSS increase compared to the earliest harvest (Harvest 1). Late-harvested mangoes (Harvest 4) showed the most rapid increase in TSS, suggesting a shorter post-harvest shelf life under ambient conditions. These mangoes may benefit from ripening inhibition treatments to extend shelf life and maintain desirable quality attributes (TSS, firmness, colour). An increase in TSS is due to the hydrolysis of starch into soluble sugars such as sucrose, glucose, and fructose (Hossain et al., 7; Bhuiyan et al., 3; Karuna et al., 11) which did not happen so rapidly on-tree. Ripe mangoes are considered good to excellent by consumers, mostly mangoes with a TSS of 18 to 22 °Brix (Markoo et al., 14). However, the reported TSS ranges in various studies vary, spanning from as low as 8°Brix to as high as 25°Brix (Silva et al., 6; Begum et al., 2). These variations highlight the diverse preferences and quality

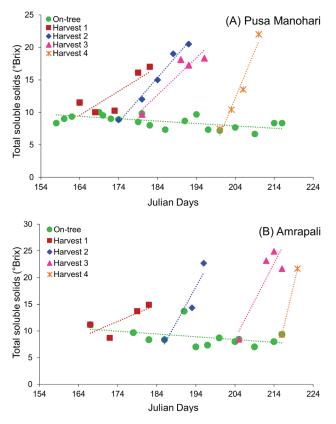


Fig. 1. Total Soluble Solids (TSS) changes in (A) 'Pusa Manohari' and (B) 'Amrapali' mangoes: On-tree maturation over 58 and 49 days, and during post-harvest storage of (Harvest 1 to Harvest 4 mangoes) under ambient conditions.

standards associated with mangoes across different regions and consumer groups.

Fruit firmness is primarily determined by cell wall composition, including moisture content, cellulose, hemicellulose, pectin, lignin, and glycoproteins. It is a crucial maturity/quality indicator in mangoes. Mango firmness can vary across the fruit. At harvest, the top portion is generally firmer than the bottom. Fruit firmness decreased gradually in on-tree mangoes. 'Pusa Manohari' firmness declined from an average of 100 to 70 N over 58 days, while 'Amrapali' firmness decreased from 90 to 70 N over 48 days (Fig. 2A, 2B). 'Pusa Manohari' exhibited a slightly higher rate of firmness reduction. On-tree mangoes maintained acceptable firmness for both local consumption and distant market transportation. Firmness of off-tree mango fruits decreased rapidly in both cultivars within approximately 20 days of harvest, reaching around 20 N. 'Pusa Manohari' showed a significantly slower firmness decline in harvests 1, 2, and 3 compared to harvest 4. Similarly, 'Amrapali' firmness declined more slowly in harvests 1 and 2 compared to harvests 3 and

Variety	DAFL	TSS, ºBrix	Firmness, N	L*	a*	b*	RR, mg CO ₂
	(Julian days)			(Peel color)	(Peel color)	(Peel color)	kg ⁻¹ hr ⁻¹
	158	8.3±0.12	82±0.06	55.79±0.79	-18.67±0.49	28.53±0.45	126.82±12.76
	160	9.7±0.17	101±6.98	56.94±1.36	-17.70±0.51	29.00±2.37	185.40±14.89
	162	9.0±0.12	105±1.78	53.31±0.18	-18.40±0.03	29.67±5.14	185.21±17.45
	164	12.2±0.34	94±0.60	51.1±0.64	-15.53±0.65	25.20±0.71	191.40±15.76
	169	10.0±0.24	90±0.02	47.25±0.27	-17.84±0.90	26.92±2.11	135.71±23.65
	170	10.1±0.24	97±0.37	53.49±0.32	-17.76±0.38	22.38±0.09	148.39±11.98
	172	9.6±0.35	92±4.73	49.94±4.41	-18.40±0.05	25.47±3.21	120.88±15.89
· 	174	9.0±0.39	80±6.21	51.59±1.61	-16.57±1.22	22.85±2.31	161.56±20.21
har	179	9.9±0.24	96±3.96	50.55±0.07	-17.82±0.65	22.44±0.47	280.24±19.76
anc	180	10.0±0.16	58±0.33	50.84±0.02	-13.99±1.81	20.72±0.33	150.14±20.12
Pusa Manohari	182	8.8±0.56	87±0.19	55.79±3.79	-17.40±4.20	24.50±1.92	116.98±15.24
snc	186	7.9±0.34	84±0.62	53.28±0.74	-15.14±0.18	24.44±0.32	197.06±14.32
ш.	191	9.0±0.16	87±0.41	56.04±0.41	-17.26±0.98	25.20±0.50	135.14±10.43
Amrapali	194	10.4±0.05	87±0.16	55.76±1.22	-17.44±0.56	21.78±2.63	100.08±20.21
	197	7.8±0.24	64±0.88	54.04±0.89	-16.65±0.06	25.50±0.18	146.71±22.32
	200	7.5±0.37	64±0.09	49.15±0.31	-14.30±0.27	25.27±0.11	197.14±25.43
	204	8.0±0.12	59±7.11	52.53±3.65	-16.56±1.01	25.84±2.56	121.72±16.76
	209	7.8±0.48	64±3.55	51.87±0.47	-15.59±0.68	33.47±5.40	145.58±18.65
	214	8.2±0.34	62±3.55	53.24±1.18	-15.81±0.64	21.49±3.70	107.79±23.43
	216	8.8±0.54	57±4.89	51.58±0.56	-16.70±0.69	26.72±2.67	195.97±17.54
	167	11.2±0.64	95±1.60	48.47±2.69	-19.55±0.22	23.48±1.12	145.81±11.91
	178	9.7±0.24	79±5.05	49.88±0.39	-18.07±1.20	18.68±0.08	120.38±12.56
	182	8.3±0.47	87±0.41	46.76±0.09	-18.92±0.61	21.39±0.83	110.91±11.67
	186	8.3±0.94	79±9.48	51.00±0.97	-17.81±0.46	16.45±0.92	194.96±8.97
	191	13.7±2.59	49±0.67	49.65±0.53	-18.01±1.01	19.48±0.74	139.34±10.32
	194	7.0±1.41	84±0.46	51.95±0.50	-17.99±1.26	19.79±0.96	107.40±11.56
	197	7.3±0.47	82±±0.81	48.47±0.68	-18.41±0.72	18.85±0.49	89.30±26.54
	200	8.7±0.94	65±0.06	50.64±0.15	-18.07±0.96	19.68±0.53	114.15±21.67
	204	8.0±0.71	75±0.81	50.87±0.31	-19.31±0.62	22.03±0.34	88.50±19.54
	205	8.4±0.17	70±2.16	47.55±1.94	-18.04±0.19	21.90±1.01	196.81±20.45
	209	7.0±1.41	77±0.46	48.14±1.75	-17.95±0.42	16.73±0.49	95.60±29.33
	214	8.0±0.71	67±0.34	49.40±0.61	-18.60±0.60	19.38±0.05	81.70±10.45
	216	9.3±2.36	68±1.18	48.61±0.65	-11.85±8.69	22.02±3.09	127.31±11.34

Table 2. Evaluation of maturity indices for on-tree 'Pusa Manohari' and 'Amrapali' mangoes.

Results are expressed as mean ± standard deviation

4 (Fig. 2A, 2B). Reported firmness values in literature vary widely (110-19 N) across studies (Silva *et al.*, 6; Begum *et al.*, 2) The firmness loss reflects ripening processes, such as cell wall degradation and softening of tissues (Begum *et al.*, 2).

During maturation and ripening, mangoes undergo physical and biochemical changes, including cell wall hydration and increased intercellular spaces. Enzymes catalyze the breakdown of starch and non-starch components (cellulose, hemicellulose, pectin, lignin), contributing to fruit softening. Starch conversion to sugars is more prominent during ripening than on-tree maturation. On-tree, softening is primarily influenced by changes in non-starch components, which provide structural integrity to the fruit.

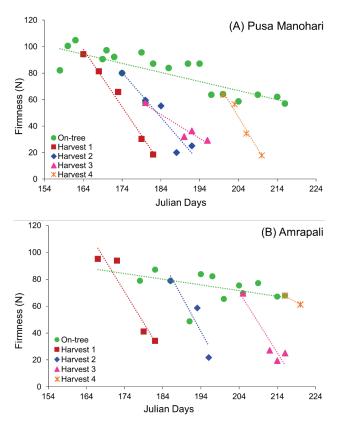


Fig. 2. Firmness changes in (A) 'Pusa Manohari' and (B) 'Amrapali' mangoes: On-tree maturation over 58 and 49 days, and during post-harvest storage of (Harvest 1 to Harvest 4 mangoes) under ambient conditions.

On-tree mangoes, while attached to the tree, undergo aerobic respiration. Respiration rate (RR) was measured on harvested "on-tree mangoes", maintaining aerobic conditions. Aerobic respiration involves the breakdown of carbohydrates, lipids, and organic acids into glucose, which is subsequently converted into carbon dioxide (CO₂), water, and energy. A significant portion of this energy is released as heat, known as the heat of respiration. RR reflects the rate of sugar and stored resource utilization within the fruit and significantly influences fruit physiology. Fruit respiration is crucial for various physiological processes, including development, ripening, enzyme synthesis, pigment formation, aroma and flavour development, and the loss of astringency. On-tree mangoes exhibited a relatively stable RR over 58 days for 'Pusa Manohari' (PM) and 49 days for 'Amrapali' (AP), with a minor difference of approximately 10 mg CO₂ kg⁻¹ hr⁻¹ between the two cultivars. No significant surge in RR was observed during this on-tree period. Figures 3A and 3B demonstrate a similar RR trend for both cultivars, with values ranging from 200-190 mg CO₂ kg⁻¹ hr⁻¹ for PM and 150-140 mg CO₂ kg⁻¹ hr⁻¹ for AP, mangoes, respectively.

For off-tree mangoes, 'Pusa Manohari' showed no significant difference in RR across harvests 1-4. However, 'Amrapali' exhibited a faster rate of RR increase in harvests 1, 2, and 3 compared to harvest 4 (Fig. 3A, 3B). These findings align with previous research demonstrating an increase in mango respiration rate post-harvest (Begum et al., 2; Nadeem et al., 18; Silva et al., 6). This post-harvest increase in RR is a natural phenomenon that contributes to the development of sweetness, fruit softening, and characteristic flavour and aroma, enhancing fruit guality for consumption and processing. The observed increase in RR for harvests 1 and 4 in off-tree 'Pusa Manohari' as well as Amrapali mangoes suggests climacteric behaviour. This indicates that mangoes from harvests 1-4 were within the maturity stage and would undergo proper ripening post-harvest. Previous studies have reported a wide range of RR values in mangoes, from 5.37 to 23.77 mg CO₂ kg⁻¹ hr⁻¹ (Begum et al., 2), 248.72 to 348.38 mg CO₂ kg⁻¹ hr⁻¹ (Karuna et al., 14), and 36.92 to 74.24 mg CO₂ kg⁻¹ hr⁻¹ (Silva et al., 6), highlighting the variability in RR

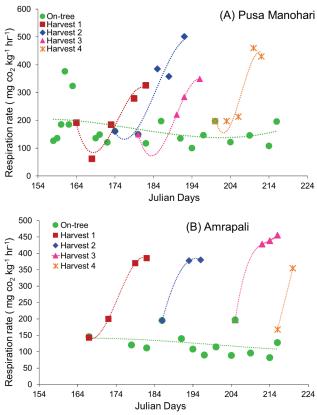


Fig. 3. Respiration rate changes in on-tree and off-tree (A) 'Pusa Manohari' and (B) 'Amrapali' mangoes: Ontree maturation over 58 and 49 days, and during post-harvest storage of (Harvest 1 to Harvest 4 mangoes) under ambient conditions.

across cultivars and experimental conditions. Further research is warranted to investigate chemical and physical treatments for ripening inhibition and shelflife extension. This includes exploring appropriate storage conditions and implementing effective ripening inhibition/delaying techniques.

Pulp colour exhibited lower lightness (elevated L^{*} values) compared to peel colour (lesser L^{*} values) for both 'Pusa Manohari' and 'Amrapali' mangoes (Fig. 4A). In the case of on-tree mangoes, peel lightness increased with time, while pulp lightness decreased for both the cultivars. This suggests the development of carotenoids in the peel during maturation (Nambi *et al.*, 19; Kour *et al.*, 12; Begum *et al.*, 2).

An increase in 'a*' values was observed in both peel (-20 to -15) and pulp (-5 to 5) for on-tree mangoes, indicating the development of reddish pigments and a reduction in green pigments (Fig. 4C). The 'b*' values remained relatively constant in the peel for both cultivars during on-tree maturation. However, pulp 'b*' values significantly increased from 30 to 65, indicating the development of yellow pigmentation in the pulp with maturity. Positive 'b*' values are indicative of increased yellowness.

A study conducted by Nambi et al. (19) demonstrated that the maturation of Alphonso and Banganapalli mangoes was accompanied by a decrease in pulp lightness (L*), attributable to the internal colour transition from white to yellow. Specifically, the L* value of the pulp decreased from 76.96 to 56.38 for Alphonso mangoes and from 91.24 to 73.28 for Banganapalli mangoes. In contrast, the L* value of the peel increased, rising from 49.21 to 64.55 for Alphonso mangoes and from 51.70 to 70.51 for Banganapalli mangoes. Peel a* values significantly increased during maturation, rising from -9.80 to 29.07 in Alphonso and from -10.95 to 16.45 in Banganapalli mangoes. Similarly, peel b* values also exhibited a substantial increase, progressing from 32.46 to 62.15 in Alphonso and from 27.74 to 48.81 in Banganapalli. Kour et al. (12) reported that the L* value for the pulp of Dussehri mango decreased from 80.16 to 63.196. In contrast, the a* value increased from 12.98 to 22.29, and the b* value rose from 48.68 to 65.81.

Similarly, Begum *et al.* (2) observed that for the Gopalbhog mango, the L* value of the peel diminished from 55.82 to 44.42. The a* value of the peel increased from -10.75 to 2.64, while the b* value went up from 27.10 to 32.42.

This study examined the changes in key quality parameters (TSS, respiration rate, firmness, and colour) during on-tree maturation and post-harvest ripening/storage of 'Pusa Manohari' and 'Amrapali' mangoes. On-tree, TSS remained relatively stable, while respiration rates were low and steady.

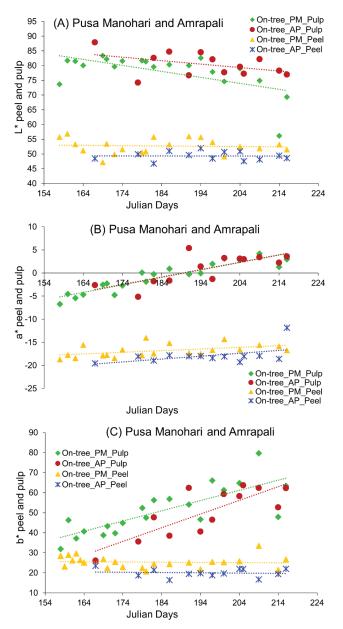


Fig. 4. Peel and pulp colour changes in on-tree mangoes during ripening (58 and 49 days, respectively).

Firmness gradually decreased, and colour changes indicated carotenoid development in the peel and the emergence of reddish and yellow pigments in both the peel and pulp. These findings suggest that consistent with the work of (Burg and Burg., 4; Morris *et al.*, 16), mangoes may possess inherent mechanisms, possibly involving hormonal inhibitors, that suppress ripening while attached to the tree. Post-harvest, TSS increased significantly, respiration rates increased, and firmness declined rapidly. This indicates that mangoes undergo more rapid ripening processes after harvest. The rate of these changes varied among harvest timings, with later harvests generally showing faster increases in TSS and respiration rates and more rapid firmness decline.

This study suggests that the "harvest window" for 'Pusa Manohari' (58 days) and 'Amrapali' (49 days) is relatively long, allowing for flexibility in harvest timing. However, earlier harvests within this window may exhibit better post-harvest storage quality compared to later harvests. These findings provide valuable insights into the physiological and biochemical changes occurring during mango ripening. This information can be used to optimize harvest timing, develop effective post-harvest handling and storage strategies, and potentially identify ripening inhibitors to extend shelf life. Further research is necessary to investigate the impact of different harvest timings and storage conditions on the sensory quality and consumer acceptance of these mangoes.

AUTHORS' CONTRIBUTION

Methodology, Investigation, Analysis, Writing-original draft, conceptualization (NSG); Conceptualization, Investigation, Analysis, Instrumentation, Supervision, Writing-editing, and review (SC); Conceptualization, Investigation, Analysis, Supervision, Writing-editing, and review (DD); Supervision, Review (PKS); Supervision, Review (RAP); Supervision, Raw Material, Guidance (MS); Analysis (MR); Methodology, Analysis (SN); Supervision, Validation (RB).

DECLARATION

The authors declare no conflict of interest.

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