



Changes in physico-chemical characteristics of bitter gourd (*Momordica charantia*) fruits harvested at different stages in controlled environments

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ABSTRACT

The present study was carried out during 2021–2022 at ICAR-Indian Agricultural Research Institute, New Delhi to evaluate the impact of different harvesting stages on quality and storage life of two bitter gourd (*Momordica charantia* L.) varieties (Pusa Rasdar and S-32) grown under polyhouse conditions. The experiment was laid out in a factorial randomized complete block design (FRCBD). Results showed that S-32 achieved the longest fruit length (19.87 cm) at 24 days after pollination (DAP), while Pusa Rasdar had the largest fruit diameter (6.23 cm) and highest fruit weight (153.22 g). Pusa Rasdar also recorded the highest fruit juice content (61.65%) at 24 DAP. However, S-32 exhibited greater chlorophyll levels, with the highest chlorophyll-a (10.12 µg/g FW), chlorophyll-b (4.56 µg/g FW), and total chlorophyll content (14.68 µg/g FW) at 16 DAP. The maximum total carotenoids content was recorded in S-32 (3.63 µg/g FW) at 24 days after pollination compared to Pusa Rasdar (2.36 µg/g FW). In S-32 lines, the highest saponin content (0.95 mg/g DW) and maximum charantin levels (0.39 mg/g DW) were observed at 16 DAP, highlighting their superior biochemical composition at this stage. Biochemical compounds peaked at 16 DAP and declined thereafter, indicating early harvest favours nutritional quality, while later stages improve physical traits. The findings revealed that S-32 lines exhibited better retention of quality attributes, such as saponin and charantin, than the Pusa Rasdar variety. Notably, S-32 lines superior over Pusa Rasdar in terms of physical and biochemical parameters full fill the commercial and nutritional needs of consumers.

Keywords: Charantin, Fruit length, Saponin, Total chlorophyll, Total carotenoids

Bitter gourd (*Momordica charantia* L.), is a popular vegetable in the Cucurbitaceae family (Satkar *et al.* 2013). It is cultivated annually in tropical and subtropical regions, thriving in temperatures between 24–27°C. Bitter gourd is extensively cultivated in Asia, South America, India, East Africa, the Middle East, and America for its edible parts, including immature and mature fruits, leaves, seeds, vines, and roots, which can be consumed both raw and cooked (Islam *et al.* 2011). According to the NHB database (2021–2022), India cultivates bitter gourd on 0.19 million hectares, yielding an annual production of 1.33 million tonnes and a productivity of 12.2 tonnes/ha. Bitter gourd is the top-ranking cucurbit due to its high nutritional value, being rich in essential vitamins and minerals, particularly vitamin A, vitamin C, iron, phosphorus, and calcium (Devi *et al.* 2019).

The increased inhibition of α -glucosidase and α -amylase in treated fruits may result from higher accumulation of saponins and charantin in stored bitter gourd, compounds known for their effectiveness in lowering blood glucose levels (Klomann *et al.* 2010).

Bitter gourd is commonly utilized in conventional medicine, particularly for managing diabetes, earning the title of "plant insulin" due to its effectiveness in lowering blood and urine sugar levels. The fruits of bitter gourd are also known for their cooling, stomachic, appetizing, carminative, antipyretic, antihelminthic, and vermifuge properties (Prajapati *et al.* 2021a). Charantin has been shown to improve blood sugar levels by enhancing glucose uptake and promoting glycogen synthesis in the liver, fat cells, and muscles. It also supports the repair and growth of insulin-secreting beta cells (Nagappan *et al.* 2018). The fruits of bitter gourd are not only used as vegetables but also said to possess antidiabetic property. This is mainly due to presence of charantin, a steroidal saponin which has reduced blood glucose level (Cuong *et al.* 2017). It is a rich source of phenolic compounds, which exhibit strong antioxidant properties (Budrat and Shotipruk 2008). While it is difficult to determine maturity solely by external features, the fruit's

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colour can provide an indication of the ideal harvest time. With the growing focus on improving dietary standards, bitter melon has gained popularity among consumers due to its rich nutritional content. However, its availability is limited by significant post-harvest losses and a short shelf life, typically lasting only 4–5 days under ambient conditions (Prajapati *et al.* 2021b).

Bitter melon is grown under protected conditions to ensure uniform crop performance, superior fruit quality, and to facilitate off-season production, which is often limited in open-field cultivation. This controlled environment minimized biotic and abiotic stresses, thereby allowing a more reliable assessment of harvesting stage effects on fruit quality and storage life (Prajapati *et al.* 2024). Although bitter melon is valued for its bioactive compounds such as saponin, charantin, and carotenoids, limited information is available on how these compounds fluctuate with fruit development under controlled environments. Most previous studies have focused on open-field conditions, leaving a gap in understanding the biochemical dynamics and quality traits of fruits produced under protected cultivation, where growth and stress factors differ significantly. Moreover, the relationship between harvest stage and retention of nutritional compounds has not been clearly established across varieties. Therefore, the present study aimed to evaluate the changes in physico-chemical characteristics of bitter melon fruits harvested at different developmental stages under controlled conditions. Specifically, the objectives were to compare physical parameters, chlorophyll content, and key bioactive compounds between two varieties, and to identify optimal harvest stages that maximize nutritional quality while maintaining desirable physical attributes.

MATERIALS AND METHODS

Experimental details: The present study was carried out during 2021–2022 at ICAR-Indian Agricultural Research Institute (28°35'N, 77°12'E and an elevation of 228.6 m amsl), New Delhi. The region experiences a semi-arid, subtropical climate, characterized by hot summers and cool winters. The sandy loam soil at the experimental site was found to have a slightly saline pH of 8.0, an electrical conductivity of 0.81 dS/m, and an organic content of 0.25%. The soil was also higher in phosphorus (45 kg/ha) and potassium (587 kg/ha) as analyzed by the Central Soil Testing Laboratory, ICAR-Indian Agricultural Research Institute. Bitter melon seeds for the Pusa Rasdar variety and the advanced breeding line Selection-32 were sourced from ICAR-Indian Agricultural Research Institute, New Delhi. The crop was raised under protected conditions with a total experimental area of 500 m². The experiment was laid out in a randomized complete block design (RCBD) with three replications. Both varieties were grown under controlled conditions and harvested at five stages post-pollination, viz. 8, 12, 16, 20, and 24 days and were analysed for physical and biochemical parameters on a fresh weight (FW) and dry weight (DW) basis.

Physical parameters

Fruit length, fruit diameter, fruit weight and juice yield (%): The study recorded fruit length (cm) at the horticultural mature stage by measuring each fruit from the stalk end to the style end. Fruit diameter (cm) was taken at the harvest time or physiological maturity, specifically measured at the widest point of each fruit, immature, tender fruits were harvested from selected plants, and the weight of each individual fruit was recorded. Fruit juice content (%) was evaluated at three-day intervals using the formula:

$$\text{Juice yield} = \frac{(\text{Juice weight}) \times 100}{\text{Fruit weight}}$$

Biochemical parameters

Chlorophyll and total carotenoids: Pigment extraction from fruit samples was performed using dimethyl sulphoxide (DMSO) in two stages, with samples incubated at 60°C to fully leach out colour. The pooled extract was then brought to 10 mL, and absorbance was recorded at 645 and 663 nm using a UV/Vis spectrophotometer (Prajapati *et al.* 2021a). Total carotene content in bitter melon was estimated by extracting carotenoids with acetone and petroleum ether, followed by purification using sodium alginate. The final extract was analyzed for total carotenoids using a spectrophotometer at 452 nm (Ahmad *et al.* 2024a).

$$\text{Chlorophyll a} \left(\mu\text{g g}^{-1} \text{FW} \right) = \frac{(12.7 \times \text{OD}_{663}) - (2.69 \times \text{OD}_{645}) \times V \times W}{1000}$$

$$\text{Chlorophyll b} \left(\mu\text{g/g FW} \right) = \frac{(22.9 \times \text{OD}_{645}) - (4.68 \times \text{OD}_{663}) \times V \times W}{1000}$$

$$\text{Total chlorophyll} \left(\mu\text{g/g FW} \right) = \frac{(20.2 \times \text{OD}_{645}) - (8.02 \times \text{OD}_{663}) \times V \times W}{1000}$$

$$\text{Total carotenoid} \left(\mu\text{g/g FW} \right) = \frac{(3.85 \times \text{OD}_{452}) \times \text{Volume made up} \times 100}{\text{Weight of sample (g)} \times 1000}$$

Saponin and charantin content: For saponin and charantin extraction, bitter melon samples were prepared and analyzed through HPLC (Habicht *et al.* 2011, Kim *et al.* 2014). For saponin, 2 g sample of freeze-dried material was extracted with sodium acetate, filtered, and treated with methanol, followed by sonication and hexane drying under vacuum. The purified extract was then mixed with acetonitrile-water (9:1), filtered, and analyzed via HPLC, with quantification based on a standard curve (0, 1000, 2000, and 3000 ppm). For charantin, a 1 g fruit powder sample was extracted using a Soxhlet apparatus with ethanol, purified through methanol-water and hexane, then dried under vacuum. The extract was filtered and analyzed in HPLC with acetonitrile-water (87:13) as the mobile phase, and charantin concentration was calculated using a standard curve based on peak areas (Fig. 1). Result of saponin and charantin content was expressed as mg/g DW.

Statistical analysis: The experiment was arranged using a factorial randomized complete block design (FRCBD). Data analysis was performed with the SAS software (version

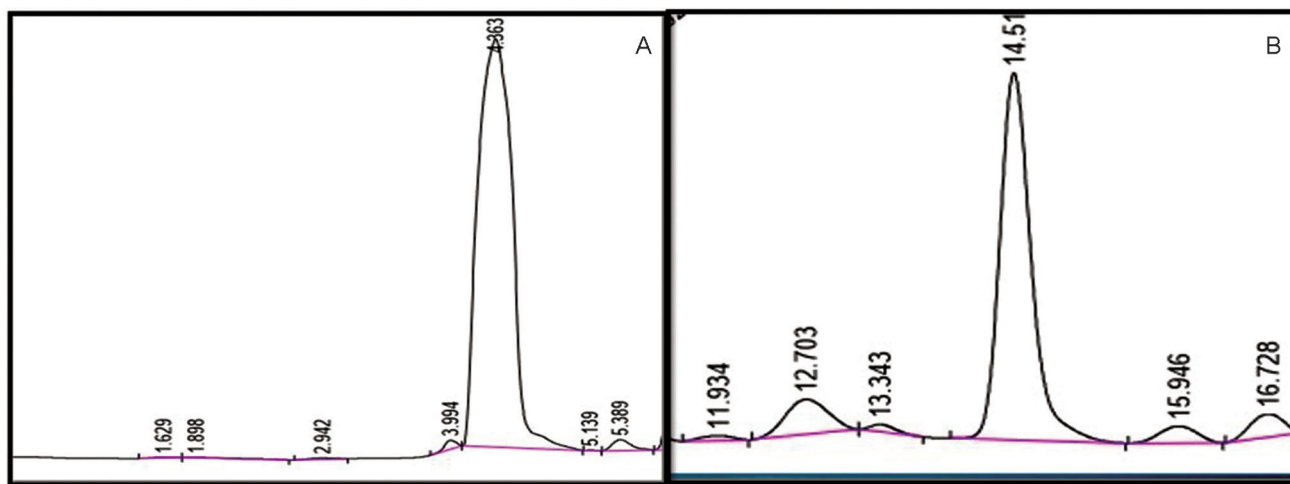


Fig. 1 Chromatogram of HPLC peaks of saponin and charantin content in bitter gourd. DAP, Days after pollination.

9.3, SAS Institute, Inc., USA). The values were computed and regarded as statistically significant ($p \leq 0.05$).

RESULTS AND DISCUSSION

Physical parameters

Fruit length, fruit diameter, fruit weight and juice yield:

The effects of five harvesting stages after pollination on various fruit characteristics of varieties Pusa Rasdar and Selection-32 were evaluated under controlled conditions (Table 1 and Fig. 2). The highest fruit length (cm) was observed at 24 days after pollination, measuring 19.87 cm for Selection-32 and 12.66 cm for Pusa Rasdar, with significant differences observed between the varieties and harvest days. At the same harvest stage, Pusa Rasdar exhibited the largest diameter at 6.23 cm, while Selection-32

measured 4.30 cm. Additionally, the fruit weight (g) also varied significantly, with Pusa Rasdar showing a range of 82.29–111.51 g compared to 50.47–66.53 g for Selection-32 between 8 and 12 days after pollination. By 24 days, the weight increased to 153.22 g for Pusa Rasdar and 112.27 g for Selection-32. Furthermore, juice content (%) was highest in Pusa Rasdar at 61.65 and 43.27% in Selection-32 at 24 days after pollination, with significant increases noted from 8–24 days, although the rise between 20 and 24 days was minimal for both varieties.

Physical parameters like fruit length and diameter, fruit weight and juice content increases with developmental phase of fruit growth. The variation in fruit length at different stage may be attributed to genotypic differences as reported by Khan *et al.* (2024). The fruits with maximum length were obtained at 24 days after pollination. Talukder *et al.* (2018) found significant difference among the cultivars like fruit length, fruit mass, diameter, and juice content in bitter gourd. Similar findings were reported in teal seed gourd under protected cultivation (Singh *et al.* 2015).

Chlorophyll and total carotenoids: This study examined the effects of five harvesting stages after pollination on chlorophyll content ($\mu\text{g/g FW}$) in two varieties, Pusa Rasdar and Selection-32, grown under protected environment. The chlorophyll levels in both varieties peaked at 16 days post-pollination (DPP), with a subsequent decline observed in the later stages. Maximum chlorophyll-a content was observed at 16 days after pollination (DAP), with Selection-32 recording 10.12 $\mu\text{g/g}$ and Pusa Rasdar 8.18 $\mu\text{g/g}$ (Table 2). Initial chlorophyll-a levels at 8 DPP were 8.12 $\mu\text{g/g}$ for Selection-32 and 6.12 $\mu\text{g/g}$ for Pusa Rasdar. Notably, chlorophyll-a content decreased after 16 days in both varieties. Similarly, chlorophyll-b content peaked at 16 DAP, reaching 4.56 $\mu\text{g/g}$ in Selection-32 and 3.46 $\mu\text{g/g}$ in Pusa Rasdar (Table 2). The lowest chlorophyll-b levels were noted at 8 DPP, with values of 2.50 $\mu\text{g/g}$ for Selection-32 and 1.95 $\mu\text{g/g}$ for Pusa Rasdar. Chlorophyll-b levels increased from 12–16 days but declined rapidly thereafter, particularly in Selection-32,



Fig. 2 Physical appearance of Selection-32 and Pusa Rasdar cultivar. DAP, Days after pollination.

Table 1 Effect of different harvesting stages fruit length, fruit diameter, fruit weight and juice content of bitter gourd (Pusa Rasdar and S-32) under protected structures

Days	Variety		Mean
	Pusa Rasdar	S-32	
Fruit length (cm)			
8-DAP	5.47	7.87	6.67
12-DAP	6.93	12.10	9.52
16-DAP	8.33	17.10	12.72
20-DAP	12.15	19.60	15.88
24-DAP	12.66	19.87	16.26
Mean	9.11	15.31	
	Variety	DAP	V × DAP
SEM	0.11	0.17	0.24
CD ($p=0.05$)	0.33	0.52	0.73
Fruit diameter (cm)			
8-DAP	1.83	1.13	1.48
12-DAP	2.81	1.87	2.34
16-DAP	4.73	3.47	4.10
20-DAP	5.89	3.97	4.93
24-DAP	6.23	4.30	5.27
Mean	4.30	2.95	
	Variety	DAP	V × DAP
SEM	0.03	0.04	0.06
CD ($p=0.05$)	0.08	0.13	0.19
Fruit weight (g)			
8-DAP	82.29	50.47	66.38
12-DAP	111.51	66.53	89.02
16-DAP	113.26	78.90	96.08
20-DAP	129.36	95.68	112.52
24-DAP	153.22	112.27	132.74
Mean	117.93	80.77	
	Variety	DAP	V × DAP
SEM	0.88	1.39	1.96
CD ($p=0.05$)	2.63	4.16	5.88
Juice content (%)			
8-DAP	19.06	11.33	15.20
12-DAP	32.87	17.27	25.07
16-DAP	42.47	33.20	37.83
20-DAP	60.36	41.81	51.09
24-DAP	61.65	43.27	52.46
Mean	43.28	29.38	
	Variety	DAP	V × DAP
SEM	0.34	0.53	0.75
CD ($p=0.05$)	1.00	1.59	2.24

DAP, Days after pollination.

Table 2 Effect of different harvesting stages chlorophyll-a, chlorophyll-b, saponin and charantin content of bitter gourd (Pusa Rasdar and S-32) under protected structures

Days	Variety		Mean
	Pusa Rasdar	S-32	
Chlorophyll-a ($\mu\text{g/g FW}$)			
8-DAP	6.12	8.12	7.12
12-DAP	7.22	8.18	7.70
16-DAP	8.18	10.12	9.15
20-DAP	6.25	4.52	5.39
24-DAP	3.20	2.6	2.68
Mean	6.19	6.70	
	Variety	DAP	V × DAP
SEM	0.015	0.024	0.035
CD ($p=0.05$)	0.046	0.073	0.104
Chlorophyll-b ($\mu\text{g/g FW}$)			
8-DAP	1.95	2.50	2.23
12-DAP	2.86	3.95	3.41
16-DAP	3.46	4.56	4.01
20-DAP	2.96	2.12	2.54
24-DAP	1.86	1.46	1.66
Mean	2.61	2.91	2.77
	Variety	DAP	V × DAP
SEM	0.016	0.025	0.035
CD ($p=0.05$)	0.047	0.075	0.106
Saponin (mg/g DW)			
8-DAP	0.027	0.040	0.033
12-DAP	0.097	0.18	0.14
16-DAP	0.12	0.95	0.53
20-DAP	0.59	0.69	0.93
24-DAP	0.19	0.24	0.37
Mean	0.26	0.32	
	Variety	DAP	V × DAP
SEM	0.006	0.010	0.014
CD ($p=0.05$)	0.019	0.030	0.043
Charantin (mg/g DW)			
8-DAP	0.22	0.29	0.25
12-DAP	0.31	0.37	0.34
16-DAP	0.28	0.39	0.34
20-DAP	0.24	0.35	0.29
24-DAP	0.20	0.32	0.26
Mean	0.252	0.348	
	Variety	DAP	V × DAP
SEM	0.003	0.004	0.006
CD ($p=0.05$)	0.008	0.013	0.019

DAP, Days after pollination.

which recorded a minimum of 1.46 $\mu\text{g/g}$ at 24 days. Total chlorophyll content also peaked at 16 DAP, with Selection-32 at 14.68 $\mu\text{g/g}$ and Pusa Rasdar at 11.64 $\mu\text{g/g}$ (Fig. 3). Both varieties exhibited the lowest total chlorophyll content at 24 DAP.

Chlorophyll is an important primary photosynthetic pigment in plants which captures light energy from sun. Total chlorophyll is composed of chlorophyll a (responsible for blue green colour) and chlorophyll b (responsible for yellow green colour). These forms of total chlorophyll coexist in plants at a ratio of 3:1 with chlorophyll-a being predominant (Ngamwonglumlert *et al.* 2017). The skin colour and variations in chlorophyll levels at different stages of harvest were examined. As the fruit matured, its skin colour transitioned from green to yellow, then to orange and orange-red, which correlated with a decline in chlorophyll-a, chlorophyll-b, and total chlorophyll content in bitter gourd. Fruits picked directly from the plant (unripe) with a dark green hue displayed changes in pigment composition during ripening, with a reduction in chlorophyll content. The fading of green colour and the emergence of yellow pigmentation were linked to a near-total depletion of chlorophyll and a rise in carotenoid levels, a pattern also observed by Prajapati *et al.* (2024).

Total carotenoids content in bitter gourd was recorded very less on 8 and 12 DAP because fruits were totally green in both varieties. It started to increase on 16 DAP, as fruits started to change colour (colour break) and carotenoids was recorded maximum on 20–24 DAP. The fruit of S-32 started ripening earlier than Pusa Rasdar. The recorded total carotenoids in S-32 on 24 DAP was 3.63 $\mu\text{g/g}$ FW while in Pusa Rasdar it was recorded 2.36 $\mu\text{g/g}$ FW (Fig. 4).

Carotenoids are fat-soluble pigments that are responsible for giving vibrant red, orange, and yellow hues and widely found in several fruit and vegetable. Carotenoids is widely divided into two major groups carotenes and xanthophylls. Carotenoid concentration increases as fruit or vegetables approaches ripening but during storage its concentration might reduce due to oxidation or isomerization of carotenoids as a result of its reaction with light, temperature, peroxides and enzymes (Ngamwonglumlert *et al.* 2017, Ahamad *et al.* 2025). Ripe bitter gourd fruits are abundant in carotenoid pigments, which give them yellow, orange, or

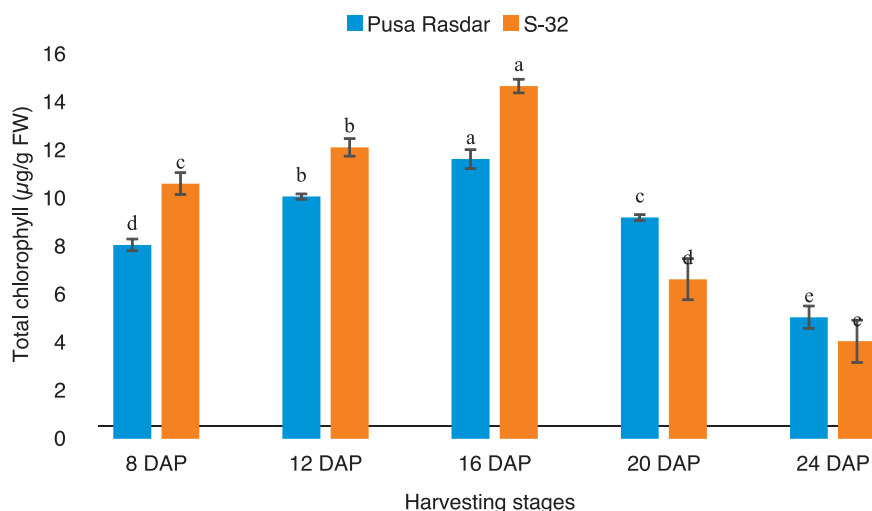


Fig. 3 Effect of different harvesting stages on total chlorophyll content of bitter gourd (Pusa Rasdar and S-32) under protected structures. Error bars specify standard deviation and alphabetic letters specify significant difference ($p \leq 0.05$). DAP, Days after pollination.

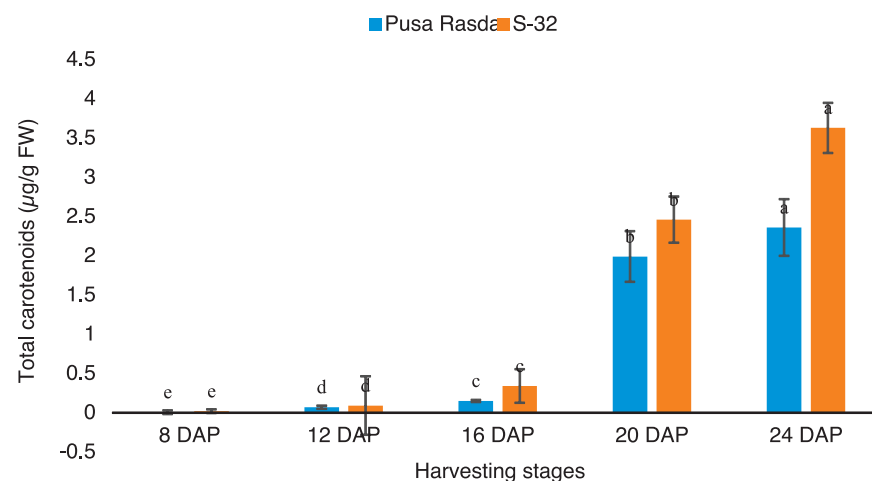


Fig. 4 Effect of different harvesting stages on total carotenoids content of bitter gourd (Pusa Rasdar and S-32) under protected structures. Error bars specify standard deviation and alphabetic letters specify significant difference ($p \leq 0.05$). DAP, Days after pollination.

orange-red colours. These fruits continue to ripen after being harvested, and a peak in ethylene production observed in the least mature fruits could suggest climacteric behaviour (Tran *et al.* 2017). This is likely because when fruits are harvested at a more advanced stage, the physical and biochemical changes in cell structure, such as cell division and enlargement, have already been completed. Typical signs of normal ripening include chlorophyll degradation, pigment accumulation, aroma development, loosening of the cell walls, and changes in texture (Bhumsaidon and Chamchong 2016, Ahamad *et al.* 2024b). At the colour break stage, the carotenoid content in the flesh of bitter melon fruits was very low. Therefore, harvesting at this early maturity stage, during the colour break, led to structural and molecular alteration not reaching their highest climacteric peak. As a result, there was a reduced accumulation of carotenoid pigments and aroma, incomplete degradation of chlorophyll,

and less red pigment formation, which ultimately caused the fruits to shrivel (Bhumsaidon and Chamchong 2016). Similar findings on effect of harvesting on carotenoids in bitter guard were presented by Tuan *et al.* (2011). Elevated carotenoid levels are associated with increased ethylene production and a higher respiration rate, which may occur due to bruising, wounding, water loss, and stress during fruit detachment (Nakano *et al.* 2003). According to Prajapati *et al.* (2021b), both treated and untreated bitter gourd fruits initially exhibited lower levels of total carotenoids, which then increased significantly by the 20th day of storage.

Saponin and charantin content: The influence of five different harvesting stages after pollination on saponin and charantin content (mg/g DW) in bitter gourd varieties Pusa Rasdar and Selection-32 was evaluated under polyhouse conditions (Table 2). Saponin levels were lowest at 8 DAP for both varieties. Following this initial stage, saponin content began to increase, peaking at 16 DAP in Selection-32, where it reached 0.95 mg/g. In Pusa Rasdar, the maximum saponin content of 0.59 mg/g was observed at 20 DAP. Notably, saponin levels in Selection-32 declined after 16 DAP, while those in Pusa Rasdar decreased following 20 DAP. Similarly, charantin content were also lowest at 8 DAP afterwards increased and recorded with a peak of 0.39 mg/g in Selection-32 at 16 DAP, while Pusa Rasdar showed a maximum of 0.31 mg/g at 12 DAP (Table 2).

Saponins are a major class of glycosides, consisting of an aglycone that can either be a triterpenoid or a spirosteroid. There are more than 98 triterpenoid saponin isolated from bitter gourd fruit. All these triterpenoid saponin has been classified into two groups, viz. cucurbitane type and oleanane type (Sun *et al.* 2020). Charantin, a cucurbitane-type triterpenoid, is naturally found in the leaves, fruits, and seeds of bitter gourd. Its antidiabetic potential has been reported due to its hypoglycemic nature. Basically, charantin is mixture of β -sitosteroyl glucoside and 5, 25-stigmasteryl glucoside and is synthesized from acetyl-CoA. 2,3-oxidosqualene and cycloartenol is known to be precursor of charantin and cycloartenol synthase is an enzyme responsible for its formation (Cuong *et al.* 2017). As consumer awareness of the connection between diet and health grows, people may increase their demand for bitter gourd varieties with higher phytonutrient content (Dhillon *et al.* 2016). The difference in the saponin and charantin contents of bitter gourd in this study and previous findings may be attributed to the variability of the tested cultivars, the diversity in the maturity groups used, and the agricultural conditions in the cultivated area (Jat *et al.* 2024). Furthermore, Cuong *et al.* (2017) observed that accumulation patterns of charantin coincide with the expression patterns of the McSE and McCAS1 genes, suggesting the involvement of these two genes in charantin biosynthesis in bitter gourd. The charantin content was higher in the leaves than in the fruits and was more pronounced at the young leaf stages (Lee *et al.* 2016). Similarly, the higher saponin and charantin content was observed by Prajapati *et al.* (2021a) in bitter gourd fruit.

The study compared various growth parameters, bioactive compounds, and chlorophyll content in two bitter gourd varieties, Selection-32 and Pusa Rasdar, under controlled polyhouse conditions. Findings indicate distinct differences in fruit characteristics, where Pusa Rasdar generally exhibited larger fruit size, weight, and juice content than Selection-32. Chlorophyll and bioactive compound analyses revealed that Selection-32 reached maximum levels of chlorophyll-a, chlorophyll-b, and total chlorophyll content, with peak concentrations observed at 16–20 DAP. The development of carotenoids, a key antioxidant compound, showed earlier ripening and higher content in Selection-32 compared to Pusa Rasdar, indicating varietal differences in the ripening process. Saponin and charantin levels, important bioactive compounds, also varied, with Selection-32 recorded higher saponin content at 16 DAP, while Pusa Rasdar peaked later. Overall, the findings emphasize that early harvesting, particularly at 16 DAP, enhances the nutritional and functional quality of bitter gourd, whereas later harvesting favours physical market attributes. The distinct biochemical trends across varieties suggest that Selection-32 is better suited for nutritional and functional food purposes, while Pusa Rasdar holds promise for fresh market preferences. These insights provide a basis for optimizing harvest timing and variety selection to balance nutritional quality with commercial traits in bitter gourd cultivation.

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