



Dynamics of bioactive compounds and antioxidant activity in peel and pulp of loquat (*Eriobotrya japonica*) cultivars during fruit development

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Received: 27 November 2025; Accepted: 25 June 2026

ABSTRACT

Loquat [*Eriobotrya japonica* (Thunb) Lindl.] is an excellent source of phyto-chemical and natural antioxidants that prevents oxidative damage and reduces incidence of chronic diseases. An investigation was conducted to assess antioxidant properties in peel as well as pulp of three loquat cultivars (Tanaka, Golden Yellow and Saharanpur Special) during different developmental stages such as green stage at 60 days after fruit set (DAFS), colour break at 75 DAFS, yellow at 85 DAFS and orange stage at 92 DAFS during two consecutive years 2022 and 2023. The findings revealed that all antioxidant properties were higher in loquat peel as compared to pulp. Ascorbic acid, total phenolic, and flavonoid contents attained their highest levels at 60 DAFS (green stage) and gradually declined with advancing fruit maturity. Furthermore, at maturity compared to orange stage, yellow stage exhibited maximum values in both peel and pulp. Total carotenoid content was recorded maximum at orange stage in both peel and pulp while antioxidant activity was maximum at orange stage in pulp only. Cultivar Tanaka exhibited highest antioxidant activities (DPPH activity) while Saharanpur Special showed maximum total flavonoid and phenolic content both in peel and pulp at orange stage, and Golden Yellow exhibited maximum total carotenoids in both peel and pulp at orange stage. The peels of loquat cultivars were found to be rich source of bioactive components which can be helpful for nutraceutical industry and crop improvement programmes.

Keywords: Carotenoids, Days after fruit set, Fruit developmental stages, Phenolics

Regular consumption of phenolic compounds through human diet plays vital role in preventing oxidation-linked diseases like cancer, diabetes, heart diseases and neurodegenerative dysfunctions. Growing attention to human health and nutrition has spurred a higher need for foods that are abundant in essential nutrients (Contessa *et al.* 2013). A greater presence of phenolics indicates an elevated degree of antioxidant activity (Sagar *et al.* 2020). Loquat [*Eriobotrya japonica* (Thunb) Lindl.] fruit is valued for its nutraceutical potential because of phyto-chemicals such as phenolic compounds, carotenoids and flavonoids, suggesting its significance in medicinal applications as well as to create enhanced, value-added food products and culinary items (Bons 2023). The concentration of flavonoids and phenolic acids in loquat is 60% and 30%, respectively. These antioxidants play role in lowering an incidence of cancer, arthritis, heart diseases, brain dysfunction and early ageing (Bons and Bal 2017). Several researchers have reported various bioactive compounds that are likely associated with the health-relevant properties of loquat. Furthermore, loquat fruits are thought to have therapeutic properties,

which impart various biological functions to protect the body from oxidative damage, hypolipidemic, cytotoxic and hypoglycemic activities with anti-inflammatory, antiviral, anti-cancerous, anti-diabetic, anti-ageing properties, anti-mutagenic, anti-tumor (Liu *et al.* 2016, Sagar *et al.* 2020).

Phyto-chemical changes occur in fruits during the different fruit developmental stages. Potassium and zinc content demonstrate an increasing trend both in peel and pulp from 60–92 days after fruit set (DAFS) (Radhika and Bons 2024). Various studies have demonstrated that the peel of different loquat cultivars exhibits higher antioxidant activity and phenolic compounds compared to the pulp (Sagar *et al.* 2020, Dhiman *et al.* 2022). Owing to differences in their growing environments, loquat cultivars exhibit distinct phenolic compositions at different stages of fruit development (Rymbai *et al.* 2013; 2024).

Although several studies have characterised individual bioactive compounds in mature loquat fruit, comprehensive information regarding their dynamic changes in peel and pulp during successive stages of fruit development under subtropical Indian conditions remains limited. The roles of these bioactive compounds are critical for human health and plant defense mechanisms (Rymbai *et al.* 2013). Understanding these developmental changes is important for identifying the optimum harvest stage to maximise

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nutraceutical quality, as maturity stages significantly modulate bioactive constituents and antioxidant activity in fruit crops (Rymbai *et al.* 2024). Therefore, the present investigation was undertaken to evaluate the dynamics of major bioactive compounds and antioxidant activity in the peel and pulp of three loquat cultivars during fruit development stages.

MATERIALS AND METHODS

Fruit materials: The present study was carried out during 2022 and 2023 on five year old plants of three different loquat cultivars namely Tanaka, Golden Yellow and Saharanpur Special, grown in subtropical climate, at Punjab Agricultural University (PAU), Ludhiana (30°40'N, 74°44'E; at an elevation of 247 m amsl), Punjab. Uniform cultural practices were applied to all the plants as per 'Package of Practices for Fruit Crops' published by PAU, Ludhiana at spacing of 6.5 m × 6.5 m. For the collection of fruit samples, complete randomised block design (CRBD) was laid out. Three plants were selected and tagged in each cultivar. Healthy fruits with a uniform size were randomly harvested with a clipper at four developmental stages i.e. 60 DAFS (green stage), 75 DAFS (colour break stage), 85 DAFS (yellow stage) and 92 DAFS (orange stage) for analysis. Ten fruits per replication were collected, resulting in 30 fruits for determination of biochemical metabolites at each fruit developmental stages. Peel and pulp portions of loquat fruit was separated with the help of knife and used for analysis.

Preparation of methanolic extract of loquat peel and pulp: Uniform, healthy fruits were selected randomly with three replications. Loquat sample of 500 mg each of peel and pulp was mixed with 10 mL of 80% methanol and allowed to stand at room temperature for 2 h and then centrifuged for 20 min at 5,000 rpm in a cold centrifuge (Sigma 3-18KS). The mixture was filtered through Whatman no. 1 filter paper. The final filtrate volume was made to 10 mL with distilled water and extract was stored in amber coloured glass vials at 4°C. Analysis was conducted within 36 h after extraction. Different sample quantities were used according to the requirements of the respective standard protocols followed for each assay.

Estimation of ascorbic acid content: The ascorbic acid of loquat fruits was analysed by the standard protocol of AOAC (2005). For estimation, 2 mL of loquat juice was taken and 5 mL extracted solution of metaphosphoric acid-acetic acid was added to it and agitated with standard indophenol dye solution. The amount of dye used was recorded and ascorbic acid was estimated by using following formula and expressed as mg/100 g.

$$\text{Ascorbic acid content} = \frac{\text{Volume of juice taken} \times \text{Dye factor}}{\text{Volume of dye used}} \times 100$$

Estimation of total phenolic content: For total phenolic content (TPC), methanol was utilised as a solvent for extracting the bioactive compounds from various tissues of loquat fruits (peel as well as pulp) as it is a standard

solvent used for the extraction of antioxidants (Siddhuraju and Becker 2003). The phenolic content was determined by using Folin Ciocalteu reagents (FC) method as followed by Upadhyay *et al.* (2013). Sample extract (0.3 mL) was diluted with distilled water (1.5 mL) followed by adding FC reagent (0.5 mL). The content was vortexed and Na₂CO₃ saturated solution was added (10 mL), with the mixture incubated at 35°C for 30 min. Absorbance of the mixture was recorded at 750 nm using US-VIS 24 spectrophotometer and compared with gallic acid standard curve. The total phenolic content was expressed as mg of gallic acid equivalents (GAE) per gram of extract (peel and pulp).

$$\begin{aligned} \text{Total phenols (mg GAE/100 g)} = & \left[\frac{\text{Standard Conc}}{\text{Standard OD}} \times \text{sample OD} \right] \times \frac{1}{\text{Aliquot taken}} \times \\ & \frac{\text{Volume of methanol extract}}{\text{Wt. of sample taken for estimation}} \times \frac{100}{1000} \end{aligned}$$

Where OD, Optical density.

Estimation of antioxidant activity: DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was used for evaluating an antioxidant activity of samples (peel and pulp) as suggested by Cao *et al.* (2014). For this, 3.0 g of fruit peel (or) pulp was homogenised in 30 mL of methanol. This homogenate was centrifuged at 10,000 rpm for 15 min at 40°C. Supernatant of 100 µL was taken and mixed with 3 mL of 0.1 millimole DPPH methanol solution and then mixture was allowed to stand for 20 min at 25°C in the dark. US-VIS 24 spectrophotometer at 517 nm was used to measure the absorbance of the sample.

$$\text{DPPH radical scavenging } (\mu\text{mol/g}) = \frac{[(\text{Abs control} - \text{Abs sample})/\text{Abs control}] \times 100}{\text{Wt. of sample taken for estimation}}$$

Where Abs, Absorbance at 517 nm.

Estimation of total carotenoid content: Total carotenoid content (TCC) was determined by the method suggested by Kirk and Allen (1965). A 100 mg of loquat peel (or) pulp was homogenised completely in a pestle and mortar with 2.0 mL of acetone (80%) and centrifuged for 10 min at 3,000 rpm. Further, the solid materials (pellet) were separated from the liquid (supernatant) and a supernatant of clear green colour was collected into the test tubes. Pellet was titrated again and extracted with 2.0 mL of 80% acetone, recentrifuged and then two supernatants were pooled. Supernatant volume was then adjusted to 10 mL with acetone. The absorbance of this extract was read at 665, 645 and 480 nm.

$$\text{Total carotenoids } (\mu\text{g}/100\text{g}) = A_{480} + (0.114 A_{665}) - (0.638 A_{645}) \times V / (1000 \times W)$$

Where A, Absorbance; V, Total volume of extract (mL); W, Sample weight (g).

Estimation of total flavonoid content: Total flavonoid content (TFC) was determined according to the method suggested by Sharma *et al.* (2018). Loquat peel as well as pulp of 1 g were refluxed with 10 mL of hot methanol (80%) for one hour. The refluxed material was filtered and

the volume of filtrate was made to 10 mL with methanol. Known aliquot quantity of sample was taken and volume was made up to 5 mL with distilled water. After that, 0.3 mL of 5% Na₂CO₃ was added and after 5 min, 0.6 mL AlCl₃ was added. After 6 min, 2 mL of 1 M NaOH was added in 2 min. Then 2.1 mL of distilled water was added to make volume up to 100 mL. The absorbance of resulting pink colour was read at 510 nm against blank. The total concentration of flavonoid in the extract was determined as micro gram of rutin equivalents (RE).

$$\text{Total flavonoids (mg RE/100 g)} = \left(\frac{\text{Standard Conc.}}{\text{Standard OD}} \times \text{sample OD} \right) \times \frac{1}{\text{Aliquot taken}} \times \frac{\text{Volume of methanol extract}}{\text{Wt. of sample taken for estimation}} \times \frac{100}{1000}$$

Where OD, Optical density.

Statistical analysis: The data were analysed with three replications and the results were presented in term of their mean values. The difference among three loquat cultivars, represented by letters obtained through one-way analysis of variance and mean values were compared between two consecutive years. The *p* value of ≤ 0.05 was considered as statistically significant. Statistical analysis was carried by using SPSS 24 for Windows®.

RESULTS AND DISCUSSION

Ascorbic acid content: The data in Fig. 1 depicts that ascorbic acid content gradually decreased from 60 to 92 DAFS during both years (2022 and 2023) across all cultivars, and differ significantly at all the four developmental stages in both peel and pulp. The ascorbic acid content in the loquat peel ranged from 3.97–15.94 mg/100 g among all developmental stages. In case of pulp, it varied from 4.01–19.98 mg/100 g fresh weight (FW) among all developmental stages. Maximum ascorbic acid content was recorded in the peel as well as pulp of all cultivars at 60 DAFS and decreased as loquat fruit progressed towards maturity (Fig. 1). In both the peel and pulp, cultivar Golden Yellow

recorded the maximum ascorbic acid content during the developmental stages during both the years. The ascorbic acid content was notably higher in pulp in comparison to loquat peel.

The results are in agreement with findings of Pande and Akoh (2010) who reported ascorbic acid content in loquat fruit pulp and peel to be 6.00 and 5.3 mg/100 g FW, respectively at orange stage. They also noticed that pulp exhibited higher ascorbic acid content as compared to peel. Abu *et al.* (2005) also reported higher ascorbic acid content in pulp as compared to peel with ascorbic acid showed decreasing trend both in peel as well as pulp during fruit developmental stages in mango cultivars. Similarly, Ercisli *et al.* (2012) also reported that ascorbic acid content in loquat fruits varied from 4.17–8.33 mg/100 g FW at orange stage. The declining trend in ascorbic acid content might be attributed to conversion of ascorbic acid into 2-3-dioxy-L-gluconic acid by losing L-ascorbic acid during fruit development (Mapson 1970).

Total phenolic content: The TPC in peel as well as pulp showed decreasing trend from the mature green stage to full ripe orange stage (Fig. 2). Similar fluctuations in TPC across the developmental stages of loquat cultivars have been recorded by Deng *et al.* (2023). Higher TPC was observed in loquat peel in comparison to pulp. The findings were similar to that of Vieira *et al.* (2011) who also observed higher phenolic content in apple peel as compared to pulp. Significant differences were observed in TPC among all loquat cultivars at all developmental stages in both peel and pulp with variation in both years. The TPC of loquat peel ranged from 24.19–172.74 mg GAE /100 g FW, while in pulp, it varied from 12.61–112.41 mg GAE /100 g FW. Maximum TPC was recorded at 60 DAFS in both peel as well as pulp of loquat cultivars that declined with fruit maturity. Peel as well as pulp of loquat cultivar Golden Yellow recorded higher TPC at all the developmental stages during both years. The TPC was notably higher in the loquat peel during 2022 (Fig. 2A), whereas in 2023, the pulp reached its highest at the full ripe harvested stage (Fig. 2B). The declining trend of TPC in fruit peel as well

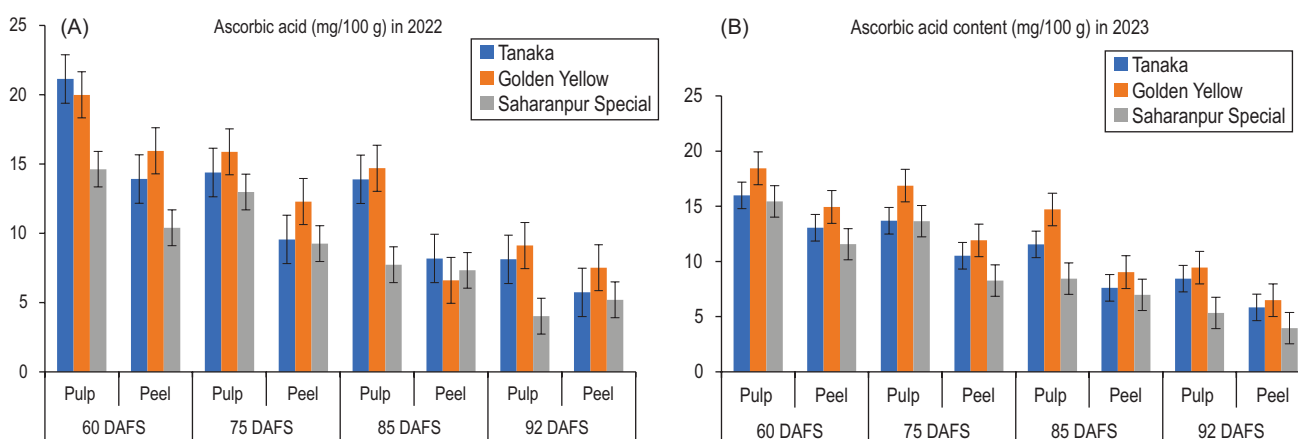


Fig. 1 Ascorbic acid content in peel and pulp of different loquat cultivars at various stages of fruit development. DAFS, Days after fruit set.

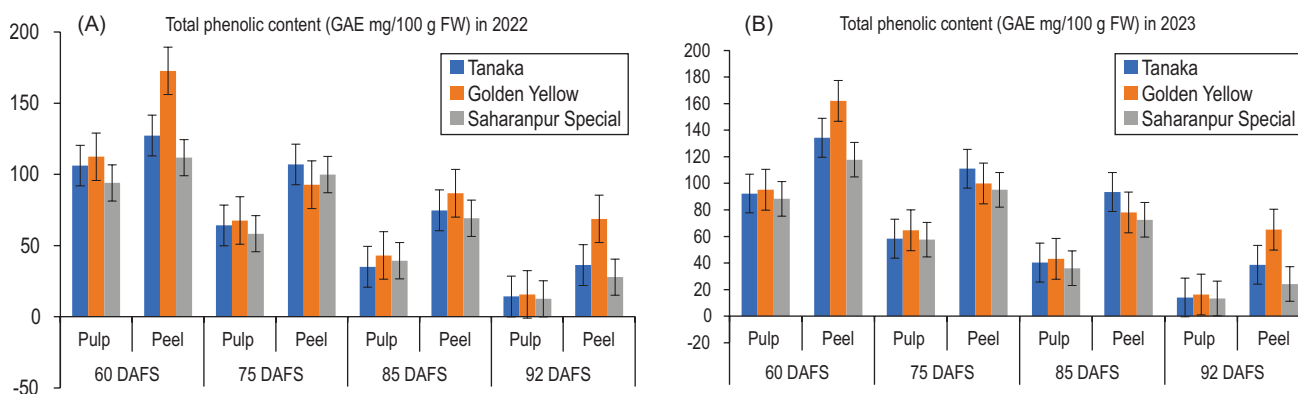


Fig. 2 Total phenolic content in peel and pulp of different loquat cultivars at various stages of fruit development. DAFS, Days after fruit set.

as pulp as loquat develops might be due to reduction of chlorophyll and synthesis of flavonoids (Rymbai *et al.* 2013; 2024). The progressive decline in TPC during fruit development might be attributed to reduced activity of the phenylpropanoid pathway, oxidative degradation of phenolic compounds by polyphenol oxidase and peroxidase and a dilution effect resulting from rapid fruit enlargement (Cai *et al.* 2019). Similar reductions in phenolic accumulation during ripening have been reported in various fruit crops by Rymbai *et al.* (2024).

The results were also similar with the findings of Rahimkhani *et al.* (2017) who reported total phenolic content in 20 different loquat genotypes that ranged from 13.15–31.65 mg GAE/100 g FW. In another study, the TPC of 11 loquat germplasm ranged from 18.5–26.3 mg GAE/100 g (Akbulut *et al.* 2017). Additionally, concentration of phenolic content varies with fruit developmental stages and cultivar type. The variation in total phenolic compounds in loquat fruits might be because of the difference in both environmental factors and genetic diversity (Pareek *et al.* 2014).

Total flavonoid content: The TPC of loquat cultivars differed significantly at all the four developmental stages in both peel as well as pulp (Fig. 3). The TPC showed decreasing trend with progression of fruit maturity both in loquat peel as well as pulp, during both the successive

years. Cultivar Saharanpur Special recorded the maximum TFC during the developmental stages in both peel and pulp during both the years. The total flavonoid content in peel ranged from 4.23–25.26 mg RE/100g FW while in pulp, it varied from 2.53–19.83 mg RE/100 g FW. The lowest TFC was recorded at 92 DAFS. The findings of the study were in accordance with Xue *et al.* (2022) who reported that total flavonoid content at orange stage ranged from 2.12–7.75 mg RE/100 g FW. Similarly, Rahimkhani *et al.* (2017) also evaluated TFC from 12 loquat genotypes at ripe stage that ranged from 0.35–8.79 mg quercetin/100 g FW. Loquat peel recorded maximum TFC as compared to pulp. Similar findings were reported by Pande and Alok (2010) who noticed maximum TFC in peel (2.4 mg RE/100 g FW) in comparison with pulp (1.9 mg RE/100 g FW). However, TFC in green stage was significantly ($p < 0.05$) higher as compared to orange stage. Difference in concentration of total flavonoids might be due to difference in the harvesting stage, species, cultivars and genotype variations (Sultan 2018).

Antioxidant activity: The DPPH activity in three loquat fruits varied from 0.73–9.40 $\mu\text{mol/g}$ FW during different fruit developmental stages (Fig. 4). During both the years, an antioxidant activity (DPPH) in loquat pulp was significantly ($p < 0.05$) higher during initial stages as compared to final stages in all three loquat cultivars. Fruit peel as well as

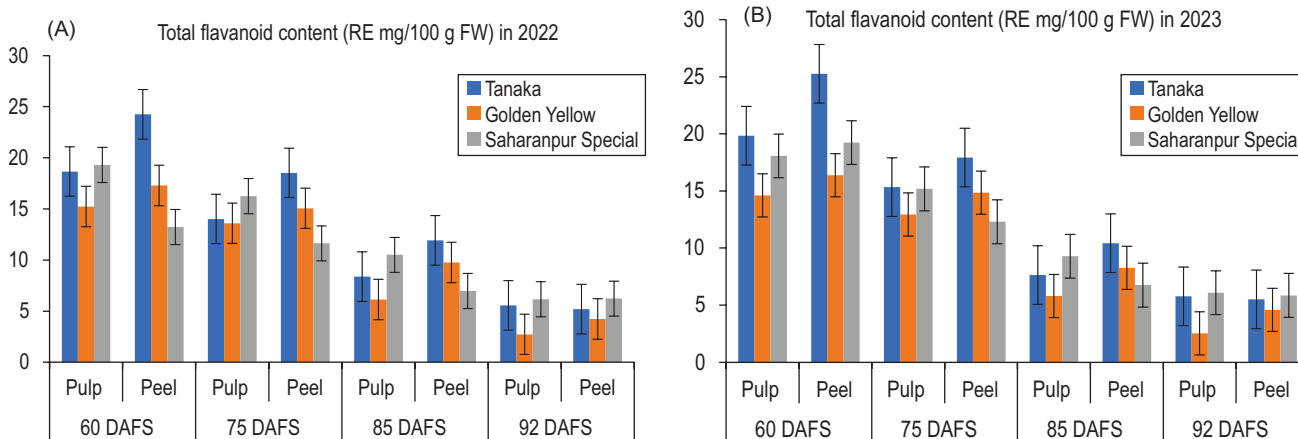


Fig. 3 Total flavanoid content in peel and pulp of different loquat cultivars at various stages of fruit development. DAFS, Days after fruit set.

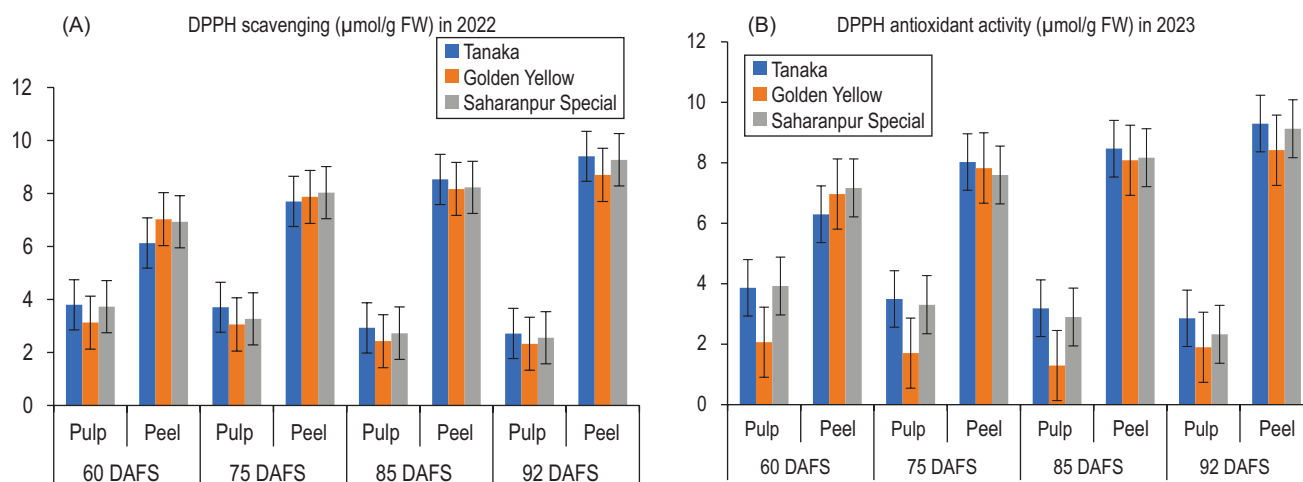


Fig. 4 Antioxidant activity (DPPH scavenging) in peel and pulp of different loquat cultivars at various stages of fruit development. DAFS, Days after fruit set.

pulp of cultivar Tanaka exhibited higher inhibiting activity than the peel and pulp of Golden Yellow and Saharanpur Special. Likewise, Zhen *et al.* (2013) noticed an increasing trend of antioxidant activities in the essential oils of *Citrus medica* L. var. *sarcodactylis* while progressing from green immature to yellow mature stage. Significant increasing trend in antioxidant activity of loquat peel and pulp was observed from 60 to 92 DAFS that ranged from 6.13–9.40 $\mu\text{mol/g FW}$ in fruit peel and 0.73–2.86 $\mu\text{mol/g FW}$ in pulp in all three loquat cultivars. These results are in line with findings of Xu *et al.* (2010) and Xu and Chen (2011) who observed antioxidant capacity of loquat fruit at orange stage varied from 1.85–3.23 $\mu\text{mol/g FW}$ and 1.45–4.24 $\mu\text{mol/g FW}$, respectively. An antioxidant activity was recorded maximum in peel as compared to pulp during both years. Similar changes in antioxidant activity of loquat peel as well as pulp was reported by Dhiman *et al.* (2022) and Xue *et al.* (2022). An increase in antioxidant activity might be attributed to an increased concentration of total carotenoid content. Hence, the variation in antioxidant activities depends on the cultivar as well as fruit developmental stages. Additionally, it also varies with respect to geographical

areas (Wang *et al.* 2006).

Total carotenoid content: During the progression of fruit development from 60 DAFS to 92 DAFS, significant increasing trend was observed in TCC among all the loquat cultivars during both successive years (Fig. 5). The total carotenoid content in peel varied from 777.6–7358.3 $\mu\text{g}/100 \text{ g FW}$ while in pulp, it ranged from 107.3–1516.6 $\mu\text{g}/100 \text{ g FW}$. Loquat peel exhibited maximum TCC in comparison to pulp. Maximum TCC was recorded in Golden Yellow.

The results are in accordance with findings of Ercisli *et al.* (2012) who observed TCC at final harvested stage that ranged from 3,890–7,850 $\mu\text{g}/100 \text{ g FW}$. Another study by Hadjipieri *et al.* (2017) revealed that TCC varied from 800–6,100 $\mu\text{g}/100 \text{ g FW}$ and 100–5,900 $\mu\text{g}/100 \text{ g FW}$ in peel and pulp, respectively. Zhang *et al.* (2016) also noticed that fresh weight of TCC in loquat fruit tissue (peel and pulp) increased progressively from mature stage to final ripe stage. The green colour of immature loquat fruit is attributed to chlorophyll, which undergoes degradation, leading to the fruit transformation into yellow or orange through the synthesis of carotenoids as the fruit ripens (Dhiman *et al.* 2022). It was found that major carotenoids in loquat fruit

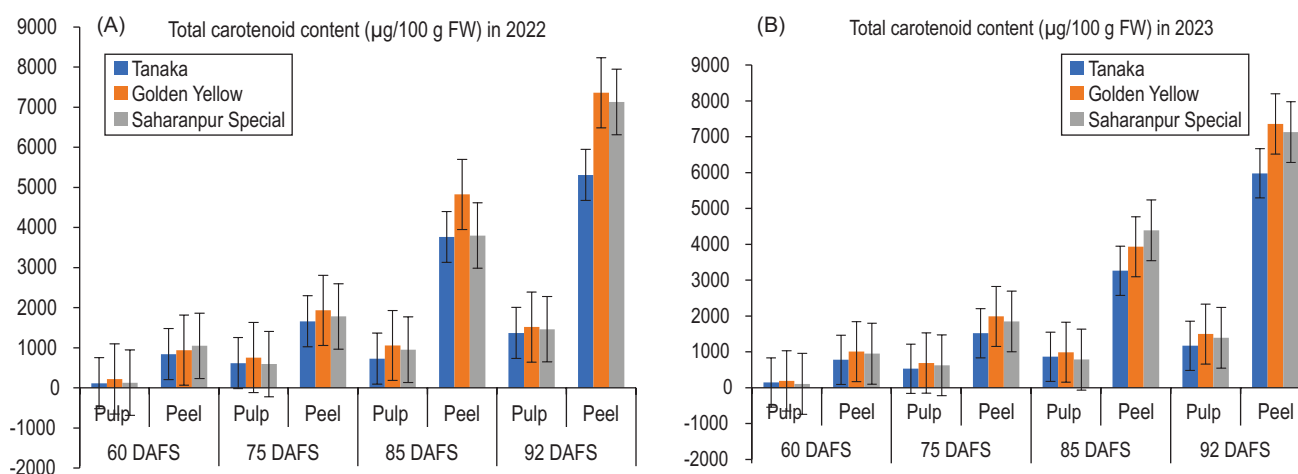


Fig. 5 Total carotenoid content in peel and pulp of different loquat cultivars at various stages of fruit development. DAFS, Days after fruit set.

peel were lutein and β -carotene while β -cryptoxanthin and β -carotene in pulp (Xu *et al.* 2010).

In conclusion, the study recorded a higher level of total carotenoids, antioxidant activity, phenolic content and flavonoids in the loquat fruit peel as compared to pulp except ascorbic acid content which showed maximum amount in loquat pulp than the peel. Ascorbic acid, total phenolic, and flavonoid contents attained their highest levels at 60 DAFS (green stage) and gradually declined with advancing fruit maturity. Furthermore, at maturity compared to orange stage, the yellow stage exhibited maximum in both peel and pulp. Total carotenoid content was recorded maximum at orange stage in both peel and pulp while antioxidant activity was recorded maximum at orange stage in only loquat pulp. Cultivar Tanaka exhibited highest antioxidant activities (DPPH activity) preferred for table purpose while Saharanpur Special showed maximum total flavonoid and phenolic content both in peel and pulp at orange stage. Golden yellow exhibited maximum total carotenoids in both peel and pulp at orange stage that can be recommended for nutraceutical purpose. The results of the present study may be helpful to determine the optimal fruit harvest stage to meet specific nutraceutical demand. It can further enhance crop improvement initiatives that aimed to develop new cultivars.

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