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RESEARCH ARTICLE

Maturity stages modulate fruit quality, bioactive constituents, and antioxidant activity of *Prunus jenkinsii*

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Abstract Researchers and consumers are highly interested in wild edible fruits around the world. *Prunus jenkinsii* grows in the wild and produces edible fruits, substantially contributing to the health, food, and livelihood of the people living in the eastern Himalayas, India. Although this species is extremely valuable, research on it is negligible. In this study, we investigated the changes in biochemical and functional properties at four different stages of fruit growth, including the immature, mature, ripe, and over-ripe stages, and determined the best stages for harvesting. Our findings showed that the fruit at the ripe stage had the highest fruit weight (6.57 ± 0.04 g), pulp content (43.33 ± 1.34%), total soluble solids (15.04 ± 1.14 °Brix), carbohydrate content (34.74 ± 0.08 mg glucose/g fw), phenols (552.41 ± 11.63 mg GAE/100 g fw), flavonoids (153.81 ± 3.44 mg QE/100 g fw), anthocyanins (313.03 ± 11.79 mg cyd-3-glu eq./100 g fw), and FRAP value (1374.54 ± 92.47 mM $FeSO_4$ eq./100 g fw). These characteristics and their corresponding values increased from the immature stage to the ripe stage; then, they decreased in the over-ripe stage. The number of fruits, firmness, acidity, and ascorbic acid content decreased after ripening. The a^* value of the peel changed abruptly from green colour (a^*, −9.44 ± 0.59) at the immature stage to red at the mature stage (a^*, 3.13 ± 1.03), and then, to reddish-purple (a^*, 5.36 ± 0.76 and b^* value, −1.82 ± 0.30) at the ripe stage and dark purple at the over-ripe stage. Therefore, the stages of fruit maturity strongly influenced the biochemical and antioxidant properties of the fruits and also indicated the ideal harvesting period for obtaining fruits of optimum quality.

Keywords Maturity · Harvesting indices · Underutilized fruits · Pigmentation · Endangered species · Functional foods

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Introduction

Plant genetic resources can help in resolving global food security challenges. They occur as plant genetic resources in different parts of the world, including temperate, subtropical, and tropical areas, and increase the utility of plants in agricultural activities. Wild edible fruits are important genetic resources in horticulture. They contribute to biodiversity as they include various genotypes. The high diversity of wild edible fruits offers numerous opportunities to researchers and breeders (Abanoz et al. 2022; Ari et al. 2022; Bozhuyuk et al. 2022; Dawadi et al. 2022).

Prunus jenkinsii Hook. f. & Thomson is an important wild edible fruit-bearing species that belongs to the family Rosaceae and is found in the hilly regions of the eastern Himalayas in India. This species is locally known as "*Satew*" in Pnar of Meghalaya. The species is synonymous with *Cerasus jenkinsii* (Hook. f. & Thomson) Ohle and *Laurocerasus jenkinsii* (Hook. f. & Thomson) Browicz. It is native to the foothills of the eastern Himalayas, including the Khasi and Jaintia Hills, which are an important part of the Indo-Myanmar biodiversity hotspot. The diverse agro-climatic conditions of this region provide a great opportunity for the evolution and development of different wild edible fruit species (Rymbai et al. 2016a). Therefore, this region is a reservoir of several crop species, including wild relatives, which grow naturally in the forests and are also grown in the backyards of the local tribes (Rymbai et al. 2023). The researchers found that *P. jenkinsii* prefers well-drained, loamy, and moist soil that is slightly acidic. The trees grow rapidly, forming the top canopy, and occasionally, the sub-canopy of moist subtropical forests (Balakrishnan 1981). The plants are found at altitudes of 1000 to 1600 m asl. This species is rarely cultivated and is generally found in the forest, hills, and ravines, as well as, in public places and backyards. Therefore, the species are wild or semi-cultivated. The species distribution is limited to the eastern Himalayas, particularly the hills of Khasi and Jaintia in India. It is also found in Assam (Lakhimpur and Sivasagar), Andhra Pradesh, northern India, and is rarely found in the northern Eastern Ghats of India (Rao et al. 2016). Globally, this species is also found in eastern Asia, southern China (southwestern Yunnan), Bhutan, Bangladesh, and northern Myanmar (Fern 2023). The ripe fruits are eaten raw and have a sweet and sour taste. Traditionally, the fruits are immersed in sugar syrup and dried under direct sunlight for a week. The sun-dried fruits are very sweet and ready for consumption; they are locally known as "Satew pdem". A dark reddish-purple dye can also be developed using the fruit.

Over many generations, this fruit has been a key dietary, healthcare, and livelihood component of the community. This could be due to the rich nutraceutical, antioxidant, and nutritional properties of these fruits. The antioxidant properties of polyphenols and their additions, as well as, the synergistic interactions with related bioactive components protect the cellular system from oxidative damage, which reduces oxidative stress (Wolfe and Liu 2008). Fruit-derived antioxidant compounds are also suitable alternatives to synthetic antioxidants due to their safety and applicability in the cosmetic, pharmaceutical, and food industries (Rymbai et al. 2023).

Research on the biochemical and antioxidant properties and production of several wild edible fruit crops, such as *Prunus nepalensis* Ser., *Elaeagnus latifolia* L., *E. pyriformis* Hook. f., *Myrica esculenta* Buch. -Ham. ex D. Don., *Myrica nagi* Thunb., *Pyrus pashia* Buch. -Ham. ex D. Don., *Docynia indica* (Wall.) Decne., *Baccaurea sapida* (Roxb.) Müll. Arg., and *Haematocarpus validus* (Miers) Bakh. f. ex Forman, found in the eastern Himalayan region, has been reported (Rymbai et al. 2016a, b, 2017, 2019, 2020, 2022, 2023). Although *P. jenkinsii* has wide applicability, no study has investigated the physicochemical and nutraceutical properties of this species. Additionally, the species is categorized as endangered (Indian Biodiversity Portal 2023). Therefore, in this study, we determined the changes in the biochemical and functional properties of *P. jenkinsii* at different stages of growth. Our findings might help in deciding the optimum harvesting stage and provide a deeper understanding of the potential functional food sources, higher consumption, and conservation of this fruit. Hence, this study might provide valuable and novel information on the economic, nutritional, and health conditions of the most vulnerable tribal communities of the eastern Himalayas and other suitable agro-climatic regions of the world.

Materials and methods

Plant description

A typical individual of *P. jenkinsii* is an evergreen, medium-sized tree that can reach a height of 25 m or more. The branchlets are reddish brown and blackish brown, glabrous, and contain sparse suborbicular small lenticels. Winter buds are reddish brown, ovoid, 2–4 mm long, and glabrous. The leaves turn yellow before falling; thus, the leaves are either green or yellow, depending on the time of the year. Leaves are compound with digitates (3–5 foliolate), stipules (1.5 cm long), petioles (2–8 cm long), and lamina (0.6–2.4 × 0.3–1.2 cm). The leaves are obovate, cuneate at the base, rounded at the apex, and obtusely serrated along the margins. The undersides of the leaves are thin, pubescent, and glabrous above. The petioles are 5–10 mm long and glabrous, and the leaf blades range from oblong to rarely obovate-oblong (8–16 × 2.5–5 cm). The petioles are herbaceous to thinly leathery, and both surfaces are glabrous. The petiole is abaxially paler, usually with a pair of purplish black basal nectaries near the margin and adaxially shining. The base is broadly cuneate. The margin is sparsely and shallowly acicularly serrated or sometimes entire near the base or basally from the middle, and the apex is shortly acuminate to caudate. The secondary veins (10–14) on either side of the mid-vein are abaxially raised and adaxially slightly prominent to somewhat impressed. The leaves are about 18 cm in length and 4 cm in diameter. They are simple, with a lanceolate shape; serrate leaf margin, reticulate venation; aristate leaf apex, and acute leaf base. The flowers appear in October, and the fruits ripen around February–April. The flowers are white, 1–2 cm long, and occur in terminal cymose panicles. The inflorescence type is racemes and is axillary, solitary, and 5–9 cm in size. The inflorescence rachis is sparsely pubescent. Bracts are 2–2.5 mm and slightly pubescent; basal is usually sterile, dark, hard, and tridentate at the apex. The pedicels are 2–3 mm long and sparsely pubescent. The hypanthium is 1.5–2 mm in size, shortly campanulate, and slightly pubescent on the outside. Sepals are 1–2 mm long, ovate-triangular, slightly pubescent at the outside, and ciliate at the margin. The petals are yellow. Achenes are small (1 mm), ellipsoid-globose, glabrous, and wrinkled. The petals are white, suborbicular, 2–3 mm in diameter, and glabrous. There are 20–30 stamens, which are 4–5 mm in size and glabrous. The ovary is glabrous. The style can range from being slightly longer to nearly as long as the stamens. The drupe is 1.7–2 × 1.4–1.6 cm in size, dark brown, broadly ellipsoid to obovoid-globose, glabrous, tapering toward the base and apex, but the apex is more obtuse. The endocarp is thick and hard and prominently coarsely reticulately rugose. Flowers are hermaphrodites and are pollinated by insects. The fruit is a drupe, dark brown or reddish-purple, and ovoid or obovoid in shape.

Plant materials and experimental site

The fruits of *P. jenkinsii* were harvested at four different development stages, including immature (105 days after full bloom, DAF), mature (120 DAF), ripe (130 DAF), and over-ripe (140 DAF) (Fig. 1), from a backyard orchard in Ummulong village, West Jaintia Hills District, Meghalaya, India (25° 19.05′ N, 92° 08.34′ E, alt 1200 m asl). The fruits were collected from 10 randomly selected trees and pooled together to form a composite sample for the study. The collected fruits were analyzed for different biochemical and functional characteristics at the ICAR Research Complex for the North Eastern Hill Region, Umiam, Meghalaya, India, between 2020 and 2022.

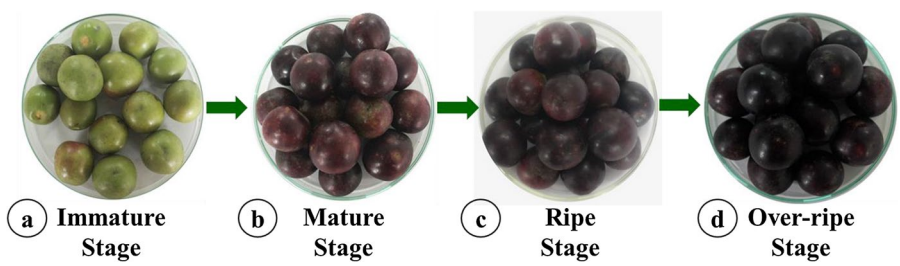


Fig. 1 Morphological characteristics of *Prunus jenkinsii* at different stages of maturity

Physical characteristics

To analyze the physical and biochemical characteristics, 25 fruits were used at each maturity stage. The harvested fruits from four developmental stages were rinsed with distilled water, wiped with tissue paper, and kept at room temperature for 10 min to dry off the adhering water before analysis. The parameters, i.e., the weight of the fruits and seeds, were determined using a precision electronic balance (Model: VWR Model LA 214i) and expressed in grams (g). The length and diameter of the fruits were measured using a digital Vernier caliper (Model: Insize Code 1108–150) and expressed in millimeters (mm). The pulp recovery percentage was estimated using the following formula:

$$Pulp\ recovery\% = \frac{Pulp\ weight(g)}{Fruit\ weight(g)} \times 100$$

The fruit yield per tree was determined by counting the number of fruits per tree at harvest and multiplying it by the fruit weight, expressed in kg per tree. Fruit firmness was measured using a Shimadzu EZ test with a trigger point of 0.049 N and a plunger (2 mm in diameter) set to pierce at a depth of 1 mm. The firmness of the fruits was measured along with their peel, as the peel is also edible. Readings were taken from three parts (i.e., the base, middle, and apex) of each fruit, and the average value was recorded in Newton (N).

Determination of biochemical properties

The total soluble solids (TSS) were measured by direct readings of the juice using a digital hand-held refractometer (HI 96801), with results expressed in °Brix. The measurements were taken from three positions of the fruit. Titratable acidity (TA) was determined based on the method described by Nielsen (2017). The contents of ascorbic acid, reducing sugar, and total carbohydrates were estimated as described below.

Ascorbic acid

The content of ascorbic acid was determined following the method described by Mehta et al. (2018). About 1 g of the sample was extracted with 20 mL of 3% (w/v) metaphosphoric acid, followed by shaking at 300 rpm for 30 min. The extract was centrifuged at 4000 rpm for 10 min. The supernatant was collected and used for further analysis. To 1 mL of the sample extract, 3 mL of 0.2 mM 2,6-Dichlorophenolindophenol (DCPIP) was added and measured immediately after mixing for 15 s at 515 nm using a UV–visible spectrophotometer (Shimadzu model UV-1720). The results were expressed in mg ascorbic acid per 100 g fresh weight (mg/100 g fresh weight, fw).

Reducing sugar and total carbohydrates

The fruit extract was prepared following the method described by Chow and Landhäussera (2004). To 50 mg of the sample paste, 5 mL of 80% ethanol was added. The samples were boiled in a water bath at 95 °C for 10 min. The tubes were then centrifuged at 2500 rpm for 5 min, and the supernatant was collected. The pellet was used to repeat the extraction steps two more times. The supernatants obtained from the three extractions were pooled and used for the sugar analysis.

The content of reducing sugars was estimated following the method described by Miller (1959). To 1 mL of the sample taken in a test tube, 3 mL of distilled water was added. Then, 3 mL of 3,5-dinitrosalicylic acid (DNS) solution was added to each test tube. The tubes were placed in a water bath containing boiling water for 15 min. While the tubes were still warm, 1 mL of 40% potassium sodium tartrate was added and cooled to room temperature. The absorbance of the samples was measured at 540 nm using a UV–visible spectrophotometer (Shimadzu model UV-1720). A series of standards using glucose (0–1 mg/mL) was run, and a standard curve was plotted.

The total carbohydrate content was determined following the method described by Dubois et al. (1956). To 1 mL of the sample taken in a test tube, 1 mL of distilled water was added. Then, 1 mL of 5% phenol and 5 mL of concentrated H_2SO_4 were added. Next, sulfuric acid was added quickly to all test tubes. The test tubes were placed in a water bath for 20 min at room temperature for color development. The absorption was measured at 490 nm in a UV–visible spectrophotometer (Shimadzu model UV-1720). A series of standards was run using glucose (0–100 µg/mL), and a standard curve was plotted.

Determination of functional attributes and antioxidant activity

The fruit extract was prepared following the procedures described by Rymbai et al. (2023). About 5 g of sample (pulp) was ground with 10 mL of methanol to fine particles. The samples were incubated for 1 h at room temperature with continuous magnetic stirring at 200 rpm. After 1 h, the samples were centrifuged at 1000*g* for 20 min. The supernatant was collected and stored at −20 °C until further analysis. The aliquot was used for assessing the total phenolic content, total monomeric anthocyanin content, total flavonoids, DPPH-free radical scavenging capacity, and the FRAP reducing power as described below.

Total phenolic content

The crude extracts were estimated for the TPC using the Folin–Ciocalteu procedure following the method described by Singleton and Rossi (1965). About 1 mL of the extract was mixed with 2 mL of Folin–Ciocalteu reagent (1:10 v/v distilled water). After 10 min, 1.6 mL (7.5%) of sodium carbonate was added. The mixture was vortexed for 15 s and left undisturbed for 30 min at room temperature for color development. The absorption was measured at 743 nm using a UV–visible spectrophotometer (Shimadzu model UV-1720). The concentration of polyphenols in the samples was estimated from a standard curve of gallic acid, and the total phenolic content was expressed as gallic acid equivalents (GAE) in mg GAE/100 g fw.

Total monomeric anthocyanin content

The total monomeric anthocyanin content was determined following the procedure described by Giusti and Wrolstad (2001) and Lako et al. (2007). About 0.4 mL of the extracted solution was mixed with 3.6 mL of the corresponding buffer. A buffer containing 0.025 M potassium chloride (pH = 1) and a buffer containing 0.4 M sodium acetate (pH 4.5) were added. The absorbance of each solution was recorded against a blank in a cuvette with a path length of 1 cm at 510 nm and 700 nm using a UV–Vis spectrophotometer. Total monomeric anthocyanin pigment concentration was expressed as cyanidin-3-glucoside equivalents as follows:

$$\text{Anthocyanin Pigment (mg cyd} - 3 - \text{glu eq./100 g fw)} = \frac{A \times MW \times DF \times 1000}{\varepsilon \times l}$$

where $A = (A_{510nm} - A_{700nm})$ pH 1.0 $- (A_{510nm} - A_{700nm})$ pH 4.5; MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside (cyd-3-glu); DF = dilution factor established in D; l = path-length in cm; ε = 26,900 molar extinction coefficients for cyd-3-glu; and 1000 = factor for conversion from g to mg.

Total flavonoid content

The total flavonoid content of extracts was estimated using the aluminum chloride ($AlCl_3$) colorimetric assay as described by Zhishen et al. (1999). About 0.3 mL of 5% $NaNO_2$ was added to 1 mL of extract. After 5 min, 0.3 mL of 10% $AlCl_3.6H_2O$ was added and incubated for 5 min. Then, 2 mL of NaOH (1 M) was added, and the final volume of the solution was adjusted to 5 mL with distilled water. After 15 min of incubation, the mixture turned pink and the absorbance was measured at 510 nm (UV–visible spectrophotometer, Shimadzu model UV-1720). The total flavonoid content was expressed as mg quercetin equivalent per gram (mg QE/100 g fw).

DPPH free radical scavenging activity

The free radical scavenging activity of the fruit extracts was estimated using the DPPH (1, 1-diphenyl-2- picrylhydrazyl) method (Shen et al. 2010). Ascorbic acid was used as a reference standard. About 100 µL of the aliquot was transferred to test tubes, to which 3.9 mL of a freshly prepared solution of DPPH (0.1 mM DPPH in methanol) was added. The mixtures were then thoroughly mixed and kept undisturbed for 30 min. The absorbance was measured at 517 nm (UV–visible spectrophotometer, Shimadzu model UV-1720). The percentage scavenging activity of DPPH was calculated using the following formula:

$$DPPH\ Scavenging\ activity(\%) = \frac{Ac - At}{Ac} \times 100$$

here Ac indicates the absorbance of the control reaction, and At indicates the absorbance of the sample of the extracts. The antioxidant activity of the extract was expressed as IC_{50} (the concentration of the fruit sample required to decrease the absorption at 517 nm by 50%). The IC_{50} value was expressed as milligrams of extract per mL that inhibited the formation of DPPH radicals by 50%.

FRAP reducing power

The FRAP assay was performed following the method described by Wetchakul et al. (2019). The FRAP reagent was prepared by mixing 25 mL of acetate buffer (300 mM acetate buffer, pH 3.6), 2.5 mL of TPTZ solution (10 mM TPTZ in 40 mM HCl), and 2.5 mL of 20 mM $FeCl_3.6H_2O$ solution (10:1:1). To 0.01 mL of extract taken in test tubes, 2.99 mL of the FRAP reagent was added. The samples were allowed to react with the FRAP solution in the dark for 30 min. The absorbance of the colored product (ferrous tripyridyl triazine complex) was checked at 593 nm using a UV–visible spectrophotometer (Shimadzu model UV-1720). The FRAP values were expressed as micromoles of $FeSO_4$ equivalents ($FeSO_4$ eq.) per gram of the sample using the standard curve constructed for different concentrations of ferrous sulfate.

Color measurement

The color of the peel and pulp of immature, mature, and ripened fruits was measured using a Color Hunter meter (HunterLab Color Quest XE). The instrument was calibrated using black and white tiles. The value was expressed as L* values, indicating lightness (black, L* = 0 and white, L* = 100), a* values, indicating redness-greenness (red: a* = 100 and green: a* = − 100), and b* values, indicating yellowness-blueness (yellow: b* = 100 and blue: b* = − 100). The measurements were replicated thrice for each sample. The measurements were performed at the base, middle, and apex of the fruits at an equidistant space under the aperture of the color meter.

Statistical analysis

The replicated (three of each parameter) data were analyzed using Statistical Package for Social Sciences (IBM SPSS) (Version 25.0), and the data were presented as the mean ± standard deviation using one-way ANOVA ($p < 0.05$) and Tukey's HSD (honestly significant difference) test. The relationship between antioxidant compounds and antioxidant activity was analyzed using the 'pairs.panels' package in R Studio (version 4.3.1).

Results and discussion

Physical changes

The physical characteristics of the fruits at different stages of maturity showed significant changes (Table 1). The number of fruits was the highest in the immature stage (8.83 ± 2.06 fruits per bunch) and decreased continuously till the over-ripe stage (1.92 ± 0.17 fruits per bunch). The ripe fruit had a higher fruit weight (6.57 ± 0.04 g), fruit length (24.90 ± 0.16 mm), fruit diameter (21.31 ± 0.90 mm), seed weight (3.72 ± 0.08 g), and pulp content (43.33 ± 1.34%), which were similar to the corresponding values measured at the mature stage.

Table 1 Changes in the physical characteristics of the fruits of *Prunus jenkinsii* at different stages of maturity

The values given are the mean (n = 25), with ±SE followed by different letters on each column indicating a significant difference from each other according to Tukey's test ($p < 0.05$)

	Stages			
	Over ripe	Ripe	Mature	Immature
Number of fruits/bunches	1.92 ± 0.17^{b}	2.36 ± 0.16^{b}	4.54 ± 0.65^{b}	8.83 ± 2.06^{a}
Fruit weight (g)	6.37 ± 0.29^{a}	6.57 ± 0.04^{a}	6.24 ± 0.04^{a}	5.55 ± 0.17^{b}
Fruit length (mm)	24.71 ± 0.3^{a}	24.90 ± 0.16^{a}	24.53 ± 0.49^{a}	22.75 ± 0.82^{b}
Fruit diameter (mm)	21.27 ± 0.89^{a}	21.31 ± 0.91^{a}	20.52 ± 0.33^{ab}	18.98 ± 0.33^{b}
Seed weight(g)	3.70 ± 0.08^{a}	3.73 ± 0.09^{a}	3.63 ± 0.07^{ab}	3.46 ± 0.03^{b}
Pulp (%)	41.93 ± 2.2^{a}	43.33 ± 1.34^{a}	41.90 ± 0.74^{a}	37.55 ± 1.83^{b}
Fruit firmness (N)	0.66 ± 0.11^{d}	1.78 ± 0.13^{c}	3.05 ± 0.23^{b}	19.32 ± 2.02^{a}
Yield (kg/tree)	13.20 ± 1.68^{c}	16.69 ± 1.46^{bc}	30.35 ± 3.17^{b}	52.63 ± 11.32^{a}

These results indicated a progressive reduction in the number of fruits with an increase in the stages of fruit growth and development. The number of fruits dropped increased as the fruits ripened, i.e., by 48.65%, 73.30%, and 78.26%, at the mature, ripe, and over-ripe stages, respectively, compared to that recorded in the immature stage. However, the fruit weight in the ripe stage was higher than that recorded in the mature stage (5.30%), but it decreased in the over-ripe stage (−2.98%). Similarly, fruit dimension, pulp content, and seed characteristics increased till the ripe stage, and then, their values decreased. The growth of fruits led to an increase in their weight and volume till a certain phase (Fleancu 2007).

This might be due to a genetic trait of the species and growth hormones. Auxin, gibberellic acid (GA), and other hormones strongly affect the growth of fruits. Auxin and GA work together to regulate the fruit size through cell division, elongation, and expansion (Hai and Chizuko 2022), which is a quantitative process that increases fruit weight and volume till a certain stage of development (Wongmetha et al. 2015). During this process, the cells in different parts of the fruit often enlarge at different rates and in different directions, resulting in noticeable gradients in cell size from the surface to the center of mature fruits. The transition from growth to maturation is characterized by noticeable changes in the phytohormone levels, which lead to the cessation of fruit expansion and promotion of fruit senescence and ripening, depending on the type of fruit (Forlani et al. 2019).

Seeds are rich sources of hormones, especially auxins, GA, and cytokinin, which are involved in stimulating the growth of surrounding tissues and even determining fruit size (Ozga et al. 2003). We found that the firmness of the fruits was the highest at the immature stage (19.32 ± 2.02 N) and the lowest at the over-ripe stage (0.66 ± 0.11 N).

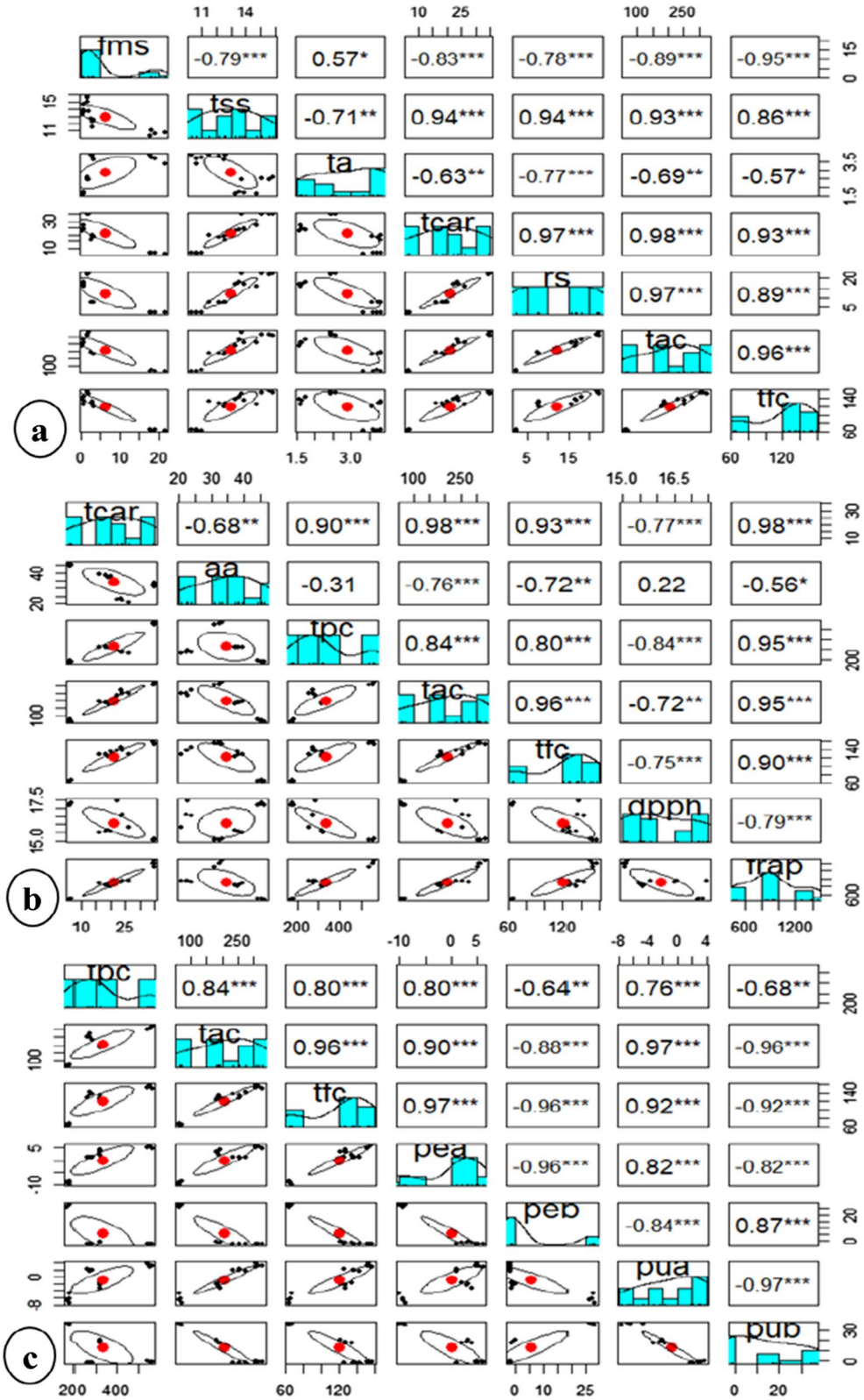
Fruit firmness and total soluble solids (TSS) are useful indices for determining fruit quality and the optimum stage of harvesting in the fruit industry. Our results showed that fruit firmness decreased from the immature stage till the over-ripe stage. Flesh firmness decreased by 84.23% at the mature stage relative to that recorded in the immature stage; however, the decrease in firmness was 41.80% in the ripe stage (vs. the mature stage) and 63.21% (in the over-ripe vs. the ripe stage). This might be due to a higher starch content in the cell wall in the early stage of fruit growth, which was subsequently converted into soluble solid content (sugars) in the latter stage, leading to a decrease in firmness.

Our results also indicated that fruit firmness was negatively correlated with TSS (−0.785**), total carbohydrates (−0.828**), and reducing sugar (−0.783**) (Fig. 2). The reduction in flesh firmness might be associated with changes in the cell wall composition caused by hydrolytic enzymes (Shattir and Abu-Goukh 2010). The softening of various fruits is primarily due to the degradation of the cell wall. This process involves the partial or complete solubilization of cell wall polysaccharides, such as pectins and/or cellulose (Paliyath and Murr 2008).

The hydrolysis of starch and other storage polysaccharides also contributes to the textural changes associated with fruit softening (Selvaraj et al. 1989). These enzymatic processes collectively contribute to the overall softening of fruits.

Biochemical changes

The biochemical content was significantly different among the different stages of maturity of the fruits (Table 2). The content of total soluble solids was the highest in the ripe stage (15.04 ± 1.14 °Brix), followed by the over-ripe stage (13.86 ± 0.76 °Brix), and it was the lowest in the immature stage (10.67 ± 0.36 °Brix). The titratable acidity of the fruits was the highest in the immature stage (3.63 ± 0.27%) and the lowest in the over-ripe stage (1.64 ± 0.09%). The total carbohydrate content was the highest in the ripe stage (34.74 ± 0.08 mg glucose/g fw), followed by the over-ripe stage. Similarly, the reducing sugar content was the highest in the ripe stage (22.33 ± 0.43 mg glucose/g fw) and the lowest in the immature fruit (2.27 ± 0.06 mg glucose/g fw). Our results showed that TSS increased during the ripening process, which was higher by 14.41% in the mature stage (relative to the immature stage); it increased considerably in the ripe stage (by 23.22% relative to the mature stage), and then, decreased in the over-ripe stage (by 7.87% compared to the ripe stage). Similarly, the total carbohydrate content increased by 175.09% (from the immature to the mature stage) and 87.47% (from the mature to the ripe stage) but decreased by 30.39% in the over-ripe stage compared to that in the ripe stage.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-0.84*** 0.87***
-0.97***

◂**Fig. 2** A pairs panel for different biochemicals, antioxidants, and pigmentations of *Prunus jenkinsii* fruits is shown. The pairs panel represents a scatter plot of matrices, with bivariate scatter plots below the diagonal, histograms on the diagonal, and Pearson's correlation above the diagonal. Abbreviations: fms—fruit firmness (N); tss—total soluble solids (°Brix); ta—titratable acidity (%); tcar—total carbohydrates (mg glucose/g pulp fw); rs—reducing sugar (mg glucose/g pulp fw); aa-ascorbic acid (mg/100 g fw); tpc—total phenolic content (mg GAE/100 g fw); tac—total anthocyanin content (mg/100 g fw); tfc—total flavonoid content (mg QE/g); dpph—DPPH antioxidants capacity (IC50 value mg/mL); frap—FRAP antioxidants capacity (mg AAE/g); pea—peel a* value; peb—peel b* value; pua—pulp a* value; pub—pulp b* value

Similar changes were found for the content of reducing sugars, as indicated by a continuous increase in the reducing sugar content from the immature stage to the ripe stage, which then decreased in the over-ripe stage. In contrast, the titratable acidity increased from the immature stage to the mature stage by 3.53%, but decreased considerably in the ripe (34.09%) and over-ripe (56.32%) stages compared to its value recorded in mature fruits. These results indicated that titratable acidity probably started increasing in young fruitlets and reached its peak value in mature fruits, and then decreased during the ripening process. Soares et al. (2007) found that the titratable acidity of guava increased in the immature and intermediary stages of maturation and decreased from the mature stage to the over-ripe stage. We found that TSS was negatively correlated with titratable acidity (−0.711**) but positively correlated with the content of total carbohydrates (0.937**) and reducing sugars (0.938**) (Fig. 2a). Thus, the sourness of the fruits of *P. jenkinsii* decreased as they ripened, and correspondingly, the content of TSS increased, which might be due to a higher content of total carbohydrates and reducing sugar. In most fleshy fruits, the sugar content increases, and organic acid content decreases while ripening (Giovannoni Giovannoni 2001). The content of sugars and organic acids increases with fruit growth but at different stages of development. Therefore, the content of organic acids in fruits was high till the maturation stage, and then, it decreased as the fruits ripened. Organic acids and starch are the major respiratory substrates for most fruits. However, due to the high respiration rate during fruit ripening, organic acids are consumed and converted into sugars (Jiménez et al. 2011).

Amylase and phosphorylase can convert starch during the ripening process into soluble sugars (Mitra and Baldwin 1997; Moneruzzaman et al. 2008), which in turn may lead to an increase in the content of TSS, total carbohydrates, and reducing sugars. An increase in the total carbohydrates and reducing sugar with the ripening of fruits was also due to the accumulation of carbohydrates in the fruits from the plant till the ripe stage (Mitra and Baldwin 1997; Moneruzzaman et al. 2008). The depletion of these substrates in the over-ripe stage probably led to a lower content of TSS, total carbohydrates, and reducing sugar. In a study, the results of a global transcriptome analysis showed that during the middle and later stages of citrus fruit development, carbohydrate synthesis and catabolism decreased considerably, whereas sugar transport increased (Wang et al. 2017). The content of organic acids and sugars in fruits increases after cell division, reaching higher levels in the middle of stage II and decreasing, mostly due to catabolism, during ripening (Hussain et al. 2017). These processes lead to an overall increase in the concentrations of sugars, thus enhancing the flavor and sweetness of the ripened fruits. TSS and acidity are the two main indicators of the quality traits in a fruit. They also influence the taste and sweetness and serve as an indicator of the maturity of the fruit and its suitability for processing (Rymbai et al. 2023). Therefore, TSS and acidity together enhance fresh consumption, processing, and value addition, which can be endorsed for different processed foods.

Changes in the functional and antioxidant properties

The antioxidant and functional properties of *P. jenkinsii* also differed significantly among different fruit maturity stages (Table 2). Specifically, the ripe stage had the highest TPC (552.40 ± 11.63 mg GAE/100 g fw), TFC (153.81 ± 3.43 mg QE/100 g fw), TAC (313.03 ± 11.79 mg cyd-3-gluE/100 g fw), and FRAP (1374.54 ± 92.47 mM FeSO4E/100 g fw). The lowest values were recorded in the immature stage for all parameters, except for the ascorbic acid content and DPPH. Ascorbic acid content was the highest in the immature stage (45.32 ± 0.95 mg/100 g fw) and the lowest in the over-ripe stage. Similarly, the DPPH value was the highest in the immature fruit stage (IC_{50} value 17.23 ± 0.08 µg/mL) and the lowest in the ripe stage (IC_{50} value 15.08 ± 0.06 µg/mL); the IC_{50}

Table 2 Changes in the biochemical and functional characteristics of the fruits of *Prunus jenkinsii* at different stages of maturity

	Stages			
	Over ripe	Ripe	Mature	Immature
TSS (°Brix)	13.86 ± 0.76^{ab}	15.04 ± 1.14^{a}	15.04 ± 1.14^{a}	10.67 ± 0.36^{c}
Titratable acidity (%)	1.64 ± 0.09^{c}	2.48 ± 0.08^{b}	3.76 ± 0.15^{a}	3.63 ± 0.27^{a}
Total carbohydrates (mg glucose/g fw)	24.18 ± 2.02^{b}	34.74 ± 0.08^{a}	18.53 ± 1.93^{c}	6.74 ± 0.38^{d}
Reducing sugar (mg glucose/g fw)	16.87 ± 1.22^{b}	22.33 ± 0.43^{a}	7.81 ± 0.06^{c}	2.27 ± 0.06^{d}
Ascorbic acid (mg/100 g fw)	2.32 ± 1.56^{d}	32.35 ± 1.24^{c}	38.23 ± 1.09^{b}	45.32 ± 0.95^{a}
Total phenols (mg GAE/100 g fw)	273.05 ± 14^{c}	552.41 ± 11.63^{a}	318.76 ± 0.94^{b}	179.22 ± 6.28^{d}
Total anthocyanin (mg cyd-3-glu eq./100 g fw)	254.15 ± 20.81^{b}	313.03 ± 11.79^{a}	186.66 ± 6.17^{c}	66.47 ± 5.24^{d}
Total flavonoid (mg QE/100 g fw)	135.77 ± 8.72^{b}	153.81 ± 3.44^{a}	127.62 ± 6.34^{b}	64.77 ± 1.35^{c}
DPPH (IC_{50}, µg/mL)	16.61 ± 0.83^{ab}	15.08 ± 0.07^{c}	15.56 ± 0.08^{bc}	17.24 ± 0.08^{a}
FRAP (mM $FeSO_4$ eq./100 g fw)	936.78 ± 13.14^{b}	1374.55 ± 92.47^{a}	841.21 ± 41.26^{b}	491.63 ± 15.62^{c}

The values given are the mean (n = 25), with ±SE followed by different letters on each column indicating a significant difference from each other according to Tukey's test ($p < 0.05$)

for standard ascorbic acid was 13.05 µg/mL. Ascorbic acid is a potent antioxidant that can scavenge and neutralize free radicals. It acts as an electron donor, effectively quenching reactive oxygen species (ROS) and preventing oxidative damage to cells and tissues (Fischer-Nielsen et al. 1992). In this study, we found that ascorbic acid decreased from the immature stage to the mature stage (15.64%), the ripe stage (15.38% relative to the mature stage), and the over-ripe stage (31.03% relative to the ripe stage). Similar changes were found in other fruits, such as wild *Rosa laevigata* Michx and peaches, where the ascorbic acid content decreased during the ripening process (Imai et al. 2009; Xie et al. 2016). This decrease might be attributed to the activity of an ascorbate oxidase enzyme, which is more prominent in fully ripe fruits than in unripe fruits (Butt 1980). The higher activity of the enzyme probably contributed to the greater degradation of ascorbic acid in the ripe stage.

The total phenol content also increased as the fruit ripened, as determined by its increase from the immature stage to the mature stage (77.87%) and from the mature stage to the ripe stage (73.30%). However, an abrupt decrease in TPC (− 75%) occurred in the over-ripe stage relative to that in the ripe stage. Similar changes were reported in other fruits, such as bitter gourd, red pepper, and *Berberis buxifolia*, where the ripening stages exhibited higher total phenolic content than the unripe and over-ripe stages (Aminah and Anna 2011; Bhandari et al. 2013). Similarly, the total anthocyanin content increased considerably from the immature stage to the ripe stage (370.91%), but it decreased slightly (by 18.81%) from the ripe stage to the over-ripe stage. The anthocyanin content in fruits increases with maturity and ripening (Miletic et al. 2012). An increase in the total flavonoid content (by 137.47%) was recorded from the immature stage to the ripe stage and a decrease in the over-ripe stage (by 11.73%) relative to that in the ripe stage. Similarly, the total flavonoid content increased by 256.41% in the ripe fruits relative to that in the immature fruits of *Eucalyptus marginata* (Ghazghazi et al. 2021). The TPC showed a significant positive correlation with the total flavonoid content (0.801**) and TAC (0.835**) (Fig. 2b), which indicated that phenols, anthocyanins, and flavonoids function synergistically during various growth stages of fruits. The higher antioxidant levels in ripe fruits might also be attributed to the ripening process, which increases oxidative stress in plants. During the ripening stage, plants produce antioxidants as a defensive mechanism against biological and environmental stressors (Rabiu et al. 2018). This oxidative stress during ripening may stimulate the synthesis and accumulation of antioxidants, leading to higher levels of these compounds in ripe fruits compared to that in unripe fruits. In tomatoes, the lowest total flavonoid content was reported in the breaker stage across all cultivars; however, it subsequently increased and reached its peak level during the pink or light red stage (Bhandari and Lee 2016). This finding suggested that the ripe stage plays a crucial role in increasing the flavonoid

and anthocyanin content in fruits. This information is of interest due to the potential health benefits associated with flavonoid consumption. We found that the total flavonoid content had a significant positive correlation with total soluble solids (0.883**), reducing sugars (0.883**), and total carbohydrates (0.883**) (Fig. 2a). Sugars like sorbitol, fructose, glucose, and minor sugars, along with their biosynthesis-related genes, are positively associated with the production of flavonols and flavan-3-ols (Farcuh et al. 2022). Therefore, the increase in sugar content via the ripening process might be associated with higher levels of flavonoids during fruit ripening.

We also found differences in the DPPH free radical scavenging activity and FRAP antioxidant activity among the different stages of maturity. The IC_{50} value decreased by 9.76% in the mature stage relative to the immature stage and by 3.04% in the ripe stage relative to the mature stage; however, the IC_{50} value increased by 10.12% in the over-ripe stage relative to the ripe stage. A lower IC_{50} value indicates higher antioxidant capacity (Matuszewska et al. 2018). Therefore, the fruits in the ripe stage had the highest antioxidant properties, as shown by the lower IC_{50} of the DPPH value. The FRAP antioxidant activity in the fruits increased by 71.11% from the immature stage to the mature stage and by 63.40% from the mature stage to the ripe stage, and then, decreased in the over-ripe stage. We found that FRAP values were positively correlated with TPC (0.953**) and the flavonoid content (0.801**), but they were negatively correlated with DPPH (−0.791**) (Fig. 2b). Some research found similar relationships, i.e., ripened fruits had the lowest DPPH values and the highest FRAP values, and vice versa in fruits, indicating stronger antioxidant activity (Aminah and Anna 2011; Mokhtar et al. 2021). The higher antioxidant activity during fruit ripening might be associated with the relative increase in the content of various polyphenol compounds (Fischer et al. 2011). We found that phenolic, anthocyanin, and flavonoid compounds strongly influence the antioxidant activity of *P. jenkinsii*, as indicated by a lower IC_{50} of the DPPH value and a higher FRAP value. This finding matched the results reported by Chandra et al. (2014) and Rymbai et al. (2023), who found that most of the antioxidant activity in fruits occurred due to phenolic compounds, anthocyanins, and flavonoids. Some researchers have suggested that as the fruit matures and ripens, its antioxidant capacity increases; however, in this study, we found that till the ripe stage, the antioxidant capacity decreased. The results of this study suggested that the ripe fruit of *P. jenkinsii* can scavenge DPPH radicals and form a stable reduced DPPH molecule. Thus, the ripe fruits of *P. jenkinsii* are natural antioxidants (Rymbai et al. 2023; Matuszewska et al. 2018).

Changes in pigmentation

The color of the fruits changed as they transitioned from the immature stage to the ripe stage (Table 3). The a* value of the peel color increased from the immature stage (−9.44 ± 0.59) to the ripe stage (5.36 ± 0.76). The red color turned dark purple in the over-ripe stage, as indicated by a decrease in the a* value (1.10 ± 0.05) and b* value (−2.01 ± 0.15). The a* value of pulp showed that it was green till the fruits matured (a*, −2.34 ± 0.79), and then, abruptly turned red in the ripe fruit stage (a*, 3.22 ± 0.51). In contrast, the b* value of pulp decreased as the stage of maturity progressed. Our findings indicated that the reddish-purple color of the peel progressively enhanced till maturity, after which it turned dark purplish. We also found that color development first occurred in the peel and then in the pulp. The enhanced color development in the peel also

Table 3 Changes in the peel and pulp color at different maturity stages of the fruits of *Prunus jenkinsii*

Stages	Peel color			Pulp color		
	L*	a*	b*	L*	a*	b*
Over ripe	22.17 ± 1.74^{c}	1.10 ± 0.05^{c}	-2.01 ± 0.15^{b}	22.69 ± 1.25^{c}	1.92 ± 0.23^{a}	-1.57 ± 0.11^{c}
Ripe	25.53 ± 0.59^{b}	5.36 ± 0.76^{a}	-1.82 ± 0.03^{b}	20.77 ± 0.68^{c}	3.22 ± 0.51^{a}	-1.00 ± 0.57^{c}
Mature	26.71 ± 0.34^{b}	3.13 ± 1.03^{b}	-1.00 ± 0.79^{b}	42.87 ± 1.80^{b}	-2.34 ± 0.79^{b}	18.31 ± 1.95^{b}
Immature	49.07 ± 1.68^{a}	-9.44 ± 0.59^{d}	27.35 ± 0.91^{a}	68.24 ± 1.89^{a}	-6.38 ± 1.50^{c}	35.47 ± 1.09^{a}

The values given are the mean (n = 25), with ±SE followed by different letters on each column indicating a significant difference from each other according to Tukey's test ($p < 0.05$)

Table 4 Correlation among different biochemicals, antioxidants, and pigmentations of the fruits of *Prunus jenkinsii*

Characters	fms	tss	ta	tcar	rs	aa	tpc	tac	tfc	dpph	frap	pel	pea	peb	pul	pua	pub
fms	1.00	− 0.785**	0.575*	− .0.828**	− .0.783**	.801**	− 0.621*	− 0.890**	− 0.954**	.663**	− 0.772**	.991**	− 0.938**	.989**	.924**	− 0.866**	0.897**
tss		1.00	− 0.711**	0.937**	0.938**	− 0.747**	0.788**	0.927**	0.857**	− 0.668**	0.893**	− 0.776**	0.807**	− 0.765**	− 0.920**	0.919**	− 0.910**
ta			1.00	− 0.628**	− 0.773**	0.918**	− 0.30	− 0.692**	− 0.568*	0.05	− 0.537*	0.614*	− 0.40	0.523*	0.785**	− 0.778**	0.835**
tcar				1.00	0.966**	− 0.680**	0.902**	0.979**	0.931**	− 0.766**	0.978**	− 0.806**	0.878**	− 0.823**	− 0.937**	0.955**	− 0.916**
rs					1.00	− 0.763**	0.824**	0.971**	0.888**	− 0.595*	0.939**	− 0.771**	0.784**	− 0.761**	− 0.948**	0.969**	− 0.952**
aa						1.00	− 0.31	− 0.764**	− 0.722**	0.22	− 0.564*	0.829**	− 0.596*	0.749**	0.876**	− 0.837**	0.903**
tpc							1.00	0.835**	0.801**	− 0.839**	0.953**	− 0.578*	0.803**	− 0.640**	− 0.717**	0.756**	− 0.679**
tac								1.00	0.962**	− 0.717**	0.953**	− 0.873**	0.898**	− 0.880**	− 0.976**	0.966**	− 0.963**
tfc									1.00	− 0.753**	0.901**	− 0.939**	0.965**	− 0.958**	− 0.948**	0.920**	− 0.919**
dpph										1.00	− 0.791**	0.613*	− 0.850**	0.710**	0.608*	− 0.596*	.535*
frap											1.00	− 0.740**	0.869**	− 0.772**	− 0.881**	0.896**	− 0.857**
pel												1.00	− 0.918**	0.987**	0.924**	− 0.852**	0.898**
pea													1.00	− 0.958**	− 0.869**	0.820**	− 0.819**
peb														1.00	0.903**	− 0.842**	0.867**
pul															1.00	− 0.972**	0.995**
pua																1.00	− 0.972**
pub																	1.00

fms fruit firmness (N), *tss* total soluble solids (°Brix), *ta* titrable acidity (%), *tcar* total carbohydrates (mg Glucose/g pulp fw), *rs* reducing sugar (mg Glucose/g pulp fw), *aa* ascorbic acid (mg/100 g fw), *tpc* total phenolic content (mg GAE/100 g fw), *tac* total anthocyanin content (mg/100 g fw), *tfc* total flavonoids content (mg QE/g), *dpph* DPPH antioxidants capacity (IC_{50} value mg/mL), *frap* FRAP antioxidants capacity (mg AAE/g), *pel* peel L*, *pea* peel a* value, *peb* peel b* value, *pul* pulp L*, *pua* pulp a* value, *pub* pulp b* value

**Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed)

stimulated color development in the pulp, as shown by the positive correlation between the a* values of the peel and the pulp (0.820**) (Fig. 2c). The change in color during fruit maturation and ripening occurred due to the enzymatic degradation of chlorophyll and the breakdown of the photosynthetic machinery. This process released the primordial pigments and led to the synthesis of various carotenoids and anthocyanin pigments, resulting in their accumulation in vacuoles (Majeed and Jawandha 2016). The reddish-purple color appeared due to the presence of anthocyanins that masked the content of carotenoids and chlorophyll (Infante et al. 2011a, b). We also found that the b* value was positively associated with the total anthocyanin content and the flavonoid content, which might be responsible for the dark purplish peel color of *P. jenkinsii*. Another study reported that anthocyanins were most probably responsible for the dark purple and blue color of the fruits of *Haematocarpus validus* and *Prunus nepalensis* (Rymbai et al. 2023). The lowest L* value, which indicated the dark purple color of the fruits, was recorded in the over-ripe stage in the peel and pulp of the fruits. Similarly, another study found that the darkest blue table grape cultivars had the lowest L* values; these values were associated with the blue-black and violet-black peel in different cultivars of table grapes (Muzolf-Panek and Waskiewicz 2022). Another study showed that anthocyanins are the major pigments responsible for the development of the dark blue color of fruit berries; darker fruits were found to have a greater content of anthocyanins (Ponder et al. 2021). The total anthocyanin content was positively correlated with the total carbohydrate content (0.673**) and reducing sugar (0.673**) (Fig. 2a), which indicated that the effect of sugar on pigment accumulation was specific to anthocyanins. Dai et al. (2014) cultivated Cabernet Sauvignon grape berries in vitro and found similar results (Table 4).

Conclusion

Prunus jenkinsii is an important tree species found in hilly regions and bears edible fruits. The trees are hardy and thus contribute significantly to the food security, healthcare systems, and livelihood security of the community. This species may also be cultivated in other parts of the world with suitable agro-climatic conditions. Our findings provided valuable insights into the changes in the physical, biochemical, and functional properties at different stages of maturity. The fruits in the ripe stage had optimum quality, including physical, biochemical, and antioxidant properties, which indicated an optimum harvesting stage. Total phenols, flavonoids, and total anthocyanins were found to have a synergistic effect on the fruits at various growth stages, and they were the major contributors to antioxidants. The increase in sugar content during the ripening process might be associated with higher levels of flavonoids during fruit ripening. The a* value of the peel color increased from the immature stage to the ripe stage of the fruits. The peel turned dark purple in the over-ripe stage. The pulp a* value showed that the pulp was green until the mature stage and abruptly turned red in the ripe fruit stage. Color development first occurred in the peel of the fruits and subsequently in the pulp. Our findings highlighted the rich biochemical and antioxidant properties of this fruit and its potential application in natural color extraction in the food processing industry. Our results also indicated the importance of precisely determining the harvest period and providing adequate information to the farmers about the ideal time for harvesting the fruit to obtain products of the highest quality.

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Declarations

Conflict of interest The authors hereby declare no conflict of interest that may have influenced either the conduct or the presentation of the research.

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