DOI: 10.29050/harranziraat. 1747031
Harran Tarım ve Gıda Bilimleri Dergisi, 2025, 29(4): 638-648

Research Article/Araştırma Makalesi

Pomological and biochemical changes in Angeleno plum (*Prunus salicina* Lindl.) during fruit development

Angeleno eriğinin (Prunus salicina Lindl.) meyve gelişimi sırasında pomolojik ve biyokimyasal değişimler

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To cite this article:
Adilova, F., Tonguç, M., Önder, S & Mutlucan, M. (2025). Pomological and biochemical changes in Angeleno plum (*Prunus salicina* Lindl.) during fruit development. Harran Tarım ve Gıda Bilimleri Dergisi, 9 (4): 638-648.

DOI: 10.29050/harranziraat. 1747031

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Received Date:
21.07.2025
Accepted Date:
06.10.2025

ABSTRACT

Fruits of Japanese plum (*Prunus salicina Lindl.*) cultivar Angeleno were harvested from Egirdir, Isparta, between June and September 2023 at four intervals (H1-H4) to investigate pomological and biochemical changes during fruit development and ripening. Fruit width increased by approximately 67%, fruit length by 89% and fruit flesh diameter by 128% and the average fruit weight increased over 500% from June to September. Colorimetric variables showed that fruit color changed from green to red during fruit maturation. Total chlorophyll, carotenoid and protein content of fruits decreased at the commercial harvest stage (H4) compared to the pit hardening stage (H1). Total phenolic content increased, but flavonoid content did not change significantly. Though the antioxidant capacity of fruits changed between the ripening stages, both CUPRAC and DPPH values were similar at the H1 and H4 stages. However, total soluble and reducing sugar contents increased steadily throughout the fruit growth stages. The results showed that fruit development stages have different biochemical contents, and plum fruit could be a good source of polyphenols and antioxidants for the human diet.

Key Words: Fruit weight, Fruit color, Chlorophyll, Phenolic substances, Antioxidant capacity

ÖZ

Japon eriği (*Prunus salicina Lindl*) çeşidi olan Angeleno'nun meyvelerinin gelişimi ve olgunlaşması sırasındaki pomolojik ve biyokimyasal değişiklikleri incelemek amacıyla Haziran-Eylül 2023 tarihleri arasında Isparta'nın Eğirdir ilçesinden dört farklı dönemde (H1-H4) hasat edilmiştir. Meyve genişliği yaklaşık %67, uzunluğu %89 ve meyve eti çapı %128 artmıştır ve ortalama meyve ağırlığı ise Haziran-Eylül ayları arasında %500'ün üzerinde artmıştır. Kolorimetrik parametreler, meyve olgunlaşması sırasında meyve renginin yeşilden kırmızıya döndüğünü göstermiştir. Meyvelerin toplam klorofil, karotenoid ve protein içerikleri çekirdek sertleşme aşamasına (H1) kıyasla ticari hasat aşamasında (H4) azalmıştır. Toplam fenolik madde içeriği artmıştır, ancak flavonoid içeriği önemli bir değişim gözlenmemiştir. Meyvelerin antioksidan kapasitesi olgunlaşma aşamaları arasında değişim gösterse de, hem CUPRAC hem de DPPH değerleri H1 ve H4 aşamalarında benzer eğilim göstermiştir. Bununla birlikte, toplam çözünebilir ve indirgenebilir şeker içerikleri meyve gelişim aşamaları boyunca istikrarlı bir şekilde artmıştır. Sonuçlar, meyve gelişim aşamalarının farklı biyokimyasal içeriklere sahip olduğunu ve erik meyvesinin insan beslenmesi için iyi bir polifenol ve antioksidan kaynağı olabileceğini göstermiştir.

Anahtar Kelimeler: Meyve ağırlığı, Meyve rengi, Klorofil, Fenolik maddeler, Antioksidan kapasitesi

Introduction

Many different fruits and vegetables can be cultivated commercially in Türkiye due to its diverse environmental conditions and agricultural lands, which support cultivation of approximately 75 out of 138 fruits and 60 out of 80 vegetable species commonly grown throughout the world (Ağaoğlu et al., 2001). Fruits can be classified in different ways according to their characteristics and uses. Stone fruits belong to the *Rosaceae* family, *Prunus* genus contains more than 35 species and these species are very rich in sugar (Butac and Budan, 2009; Sezer and Çetin, 2021). Stone fruit production reaches 25% of the total fruit production in the country, including sour cherry, sweet cherry, apricot, peach, nectarine and plums (Duru et al., 2022). Plums, one of the most consumed stone fruits in Türkiye, were domesticated approximately 2000 years ago around the Caucasus and Anatolia. Plum species identified in Türkiye include *Prunus cerasifera* Ehrh., *P. domestica* L., *P. salicina* Lindl., *P. insititia* L., *P. spinosa* L. and *P. simonii* Carr. (Davis, 1965). Commercially grown plum varieties belong to *P. cerasifera* Ehrh. (green plums), *P. domestica* L. (European plums) and *P. salicina* L. (Japanese plums) species in Türkiye (Özçağıran et al., 2003).

Due to the cultivation of different plum species and the large number of varieties, the expansion of cultivation area and production of plums has been increasing (Bolat et al., 2017). The number of trees and production area in Türkiye is also increasing and annual plum production increased from 175 thousand tons in 1988 to 355 thousand tons in 2023. The total planted plum tree area in Türkiye is 216.903 da and 2.7% of the planted trees are located in Isparta (5.826 da) (TUİK, 2024). Plum species and varieties have different ripening times; therefore, they are available in markets for 6-7 months as fresh produce (Sezer and Çetin, 2021).

Plums are widely used in food as they can be consumed as fresh, dry, jam, juice and paste, pharmaceutical, cosmetic and other industries, and the effects of plum consumption on disease prevention, such as cardiovascular diseases and diabetes have been reported (Stacewicz-Sapuntzakis et al., 2001; Önal and Cinsoy, 2003). Fruit consumption is recommended due to their high nutritious value, antioxidant capacity and low glycemic index (Bulantekin et al., 2020). Plums can have an important place in the human diet due to their rich flavonoid, anthocyanin, carotene, polyphenol and fiber content (Kim et al., 2003; Sahamishirazi et al., 2017).

Fruit development in plums shows a double sigmoid change and consists of four stages. In the first (S1) and the second stages (S2), cell division and elongation, and seed hardening occur, but fruit development does not occur. In the third stage (S3), cell division starts again and the fruit reaches its final size and in the fourth stage (S4), fruit ripening occurs (Khan, 2016; Farcuh et al., 2017). As stone fruits develop and reach the commercial harvest stage, many pomological and biochemical changes occur. They have different biochemical contents and pomological properties at different periods and the changes in these properties vary depending on variety, cultivar and environmental conditions (Duru et al., 2022). With these changes, the acidity level of the fruits decreases and the soluble solids content increases with ripening (Özcan, 2020).

Fruit is the most important part of a fruit tree, which determines its acceptance and commercial success. During the ripening period, fruits undergo pomological, biochemical and physiological alterations that turn immature fruits into mature fruits with edible quality. Therefore, it is necessary to investigate those changes to better understand the underlying properties. In this study, Angeleno plum fruits picked at four fruit development stages were used to determine the pomological and biochemical changes that occur during fruit development.

Material and Method

Plant Material

Fruits of the late-maturing Japanese plum (*P. salicina* Lindl.) cultivar Angeleno were used as

plant material. The orchard (commercial plum orchard) was located in Bedre, Eğirdir, Isparta (37° 54′ 56" (N) latitude, 30° 47′ 24" (E) longitude; 927 m altitude) and the trees were planted 4 m x 4 m spacing. The trees were grafted on *P. ceracifera* rootstock and were 9 years old. Standard cultural practices including pruning, fertilization, pesticide sprays and soil management had been used with the trees for several years. The orchard was irrigated with drip irrigation system during the study period. Meteorological data was obtained from the Eğirdir station of State Meteorological Services (MGM) and presented in Table 1. Sampling was carried out at four different periods starting from two months after blooming corresponding to pit hardening stage (H1, 26/06/2023) (Khan, 2016). The second sampling was done when fruits started to expand and become larger (H2, 24/07/2023). The third (H3, 28/08/2023) and the fourth (H4, 25/09/2023) samplings were carried out at one-month intervals when coloration of fruits started and at the commercial harvest stage, respectively (Figure 1). Fruit samples were taken from 9 representative trees in the orchard with 3 replicates and 3 trees in each replicate. In each replicate, 25 fruit samples were taken. The harvested fruits were transferred to the laboratory on the same day by refrigerated vehicle (approximately 30 min) and pomological measurements were completed. Fruits were stored at -20°C until biochemical analyses.

Pomological Measurements

Morphological measurements were taken using 15 randomly chosen fruits with three replicates (15 × 3= 45). Fruit width and length and fruit flesh diameter were measured with an electronic caliper (Stainless Hardened) and the fresh weights of the fruits (g) were measured with an electronic scale (Radwag AS 220.R2, Poland).

The skin color of fruits was measured using a portable colorimeter (PCE-CSM 1, PCE Instruments, Germany). Colorimetric parameters based on the PCE system for skin color measurements were recorded: *a**: green (−) to red (+), *b**: blue (−) to yellow (+), *C**: chroma (color brightness), *L**: black (−) with white (+), *h°*: hue angle values. The color scale was: *a** value [(-60) green – (+60) red], *b** value [(-60) blue – (+60) yellow] and *L** value [(0) black – (+100) white]. The *h°* value is expressed in degrees from 0 to 360, where 0° = red, 90° = yellow, 180° = green and 270° = blue.

Biochemical Analysis

One g of fresh fruit sample was crushed in liquid nitrogen then 10 mL of 100% (v/v) dimethyl formamide solution was added and the samples were homogenized. The homogenates were

Table 1. Long term (1950-2023) and monthly mean temperature (°C), precipitation (L m^2) and humidity (%) for Isparta province (State Meteorological Service).

Months	Temperature (°C)		Precipitation (L m^2)		Humidity, %	
	2023	1950 – 2023	2023	1950 – 2023	2023	1950 – 2023
January	5.4	1.8	125.3	81.4	73.3	75.2
February	3.6	3.0	5.8	67.5	59.3	71.5
March	9.1	6.0	73.8	59.0	67.1	65.8
April	10.8	10.8	69.9	51.4	65.0	61.1
May	15.5	15.5	111.0	56.5	71.7	59.9
June	20.1	19.9	47.3	35.7	65.8	52.9
July	25.8	23.5	8.0	15.5	40.2	45.4
August	27.3	23.4	3.1	14.0	43.1	46.2
September	21.7	18.9	29.1	18.6	47.0	52.0
October	16.7	13.4	9.7	37.5	56.5	62.1
November	11.9	7.9	53.3	44.4	73.8	69.8
December	7.7	3.7	46.3	86.0	79.0	76.0
Total	–	–	582.6	567.5	–	–
Mean	14.6	12.3	–	–	61.8	61.4

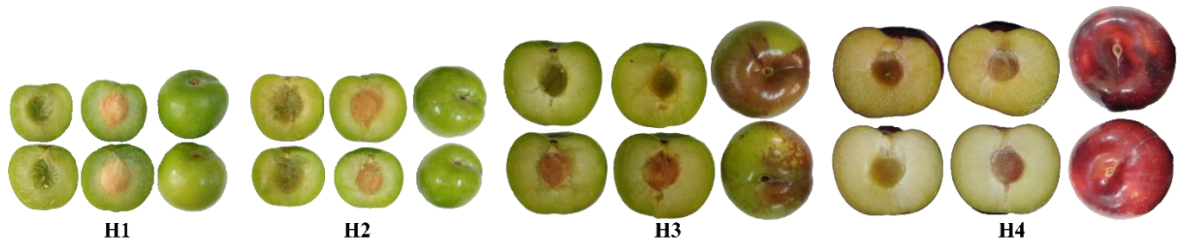

Figure 1. Angeleno fruits harvested for study between June to September.

centrifuged at 10,000 × g for 10 min at 4 °C and liquid phase were used to determine total chlorophyll and carotenoid content at 480, 664 and 647 nm wavelengths (Shimadzu UV-1280, Japan). Chlorophyll a, chlorophyll b and total carotenoid contents were calculated according to the formulas below (Torres et al., 2014):

Chlorophyll a (µg mL^{-1}) = 12.7 x A_{664} — 2.79 x A_{647}
Chlorophyll b (µg mL^{-1}) = 20.7 x A_{647} — 4.62 x A_{664}
Total chlorophyll = 17.9 x A_{647} + 8.08 x A_{664}
Total carotenoid (µg mL^{-1}) = (1000 x A_{480} — 0.89 x Chlorophyll a) / 245

To extract phenolic and flavonoids from the plum fruits, 0.2 g dry samples were weighed and pulverized in liquid nitrogen. Then 10 mL of 80% (v/v) cold methanol was added and the homogenate mixed at 120 rpm for 15 min, later it was centrifuged at 6,000 x g for 10 min at 4 ° C. The same process was repeated twice and the supernatants were combined. The collected supernatant was filtered with Whatman No 1 filter paper and the final volume was brought to 25 mL with 80% (v/v) cold methanol.

To determine the total phenolic content of the samples, 100 µL of methanolic extract, 2.5 mL deionized water and 100 µL Folin-Ciocalteu solution (2N) were mixed in a test tube and the reaction was incubated in the dark for 6 min. Then, 500 µL of 20% (v/v) sodium carbonate solution added and the solution was kept in the dark for 30 min to allow color development to occur (Folin and Ciocalteu, 1927). The color development was determined at 760 nm wavelength. The standard curve was obtained using gallic acid.

Total flavonoid content was determined using the aluminum chloride method (Sakanaka et al., 2005). The methanolic extract (250 µL), 1.25 mL deionized water, 75 µL of 5% (w/v) sodium nitrate solution were mixed and incubated in the dark for 6 min, then 150 µL of 10% (w/v) aluminum chloride solution was added. The solution was incubated in the dark for 5 min, then 500 µL of 1 M sodium hydroxide was added and incubated in the dark for additional 15 min for the reaction to occur and color development. The absorbance values of the samples were read at 510 nm. Total flavonoid content of the samples was calculated using a catechin standard curve.

For total soluble and reducing sugars extraction, dry fruits (1 g) were homogenized with 10 mL of 80% (v/v) cold-ethanol and incubated overnight at -20 °C. The homogenate was centrifuged at 4 °C for 5 min at 4,000 × g and both total soluble and reducing sugars content were determined from the obtained supernatant. For total soluble sugars content, 1 mL of ethanolic extract, 0.5 mL of 5% (v/v) phenol and 2.5 mL of concentrated sulfuric acid were added, and the mixture was vortexed. The reaction was left at room temperature for 10 min for color development and the absorbance values were determined at 490 nm (DuBois et al., 1956).

Total reducing sugars content, 1 mL of ethanolic extract, 2.5 mL of 100 mM sodium borate (pH 9.0) and 0.5 mL of 1% (w/v) 2-cyanoacetamide were mixed and vortexed. The reaction was incubated in a boiling water bath for 10 min, after incubation the samples were quickly cooled, and the absorbance values were determined at 280 nm (Somogyi, 1952). The standard curves for soluble and reducing sugars

determination were prepared using glucose.

For total protein extraction, 0.2 g of dry fruit tissue was pulverized in liquid nitrogen. One mL of 0.05 M cold-tris buffer (pH 8.0) solution (0.1% (w/v) ascorbic acid, 0.1% (w/v) cysteine hydrochloride, 1% (w/v) polyethylene glycol 4000, 0.15% (w/v) citric acid, 0.008% (v/v) 2-mercaptoethanol and 0.05 g (w/v) PVPP) was added and the homogenate was centrifuged at 14,000 rpm for 20 min at 4 °C. To determine the total protein content of fruits, 3 mL of Bradford reagent (0.01% (w/v) Coomassie Brilliant blue G-250, 95% (v/v) ethanol and 85% (v/v) phosphoric acid) was added to 100 µL of supernatant and vortexed, and after incubation for 5 min at room temperature, absorbance values were determined at 595 nm (Bradford, 1976). The standard curve was prepared with Bovine Serum Albumin (BSA).

For antioxidant capacity and radical scavenging activity analyses, 2 g of dry fruit tissue was pulverized in liquid nitrogen, 20 mL of methanol: water (v/v, 80:20) solution was added and the mixture was treated in an ultrasonic water bath at 45 °C for 30 min. Then, the samples filtered through a 0.45 µm membrane filter and stored in a refrigerator at 4 °C. Total antioxidant capacity was determined according to the CUPRAC method (Apak et al., 2006). Free radical scavenging capacity was determined using the DPPH method (Bener et al., 2022). The methods of CUPRAC and DPPH methods were performed as described in Önder et al. (2024). Antioxidant capacity and radical scavenging capacity in the samples are given as trolox equivalent (µmol Trolox g^{-1}).

Statistical Analysis

All analyses were performed with three replications. The results were subjected to one-way analysis of variance (ANOVA) using IBM SPSS Statistics 27.0 software (SPSS Inc., Chicago, IL, USA). The results were given as mean ± standard deviation (SD). The significance levels between the means were determined according to the least significant difference (LSD) method.

Results and Discussion

Angeleno cultivar belongs to Japanese plums, which shows climacteric growth pattern, and it is a late maturing variety. To study pomological changes associated with fruit development, fruits were harvested at four intervals starting from pit hardening and ended at the commercial harvest stage (Figure 1). The average fruit weight, fruit width, fruit length and fruit flesh diameter were determined at different development and harvest times. Significant differences were determined between the development stages for measured traits and the results and their significance levels were given in Table 2.

Plum fruits exhibit a double sigmoid growth pattern, characterized by two rapid growth phases. The first growth continues approximately 70 days after full blooming; between 72 and 86 days after blooming stone hardening takes place. During the pit hardening period growth of fruits slows down and later they resume the growth process (Khan, 2016). Though pomological variables generally increase during fruit development, other physiological variables, such as acidity, carotenoid and phenolic accumulation, show significant differences and fluctuations at the same development period (Khan, 2016; Vlaic et al., 2018; Li et al., 2019). Therefore, it is necessary to examine fruit development and associated changes to understand their roles and effects in fruit development and commercial harvest periods.

Table 2. Pomological measurements of Angeleno fruits harvested at different periods.

Pomological variables	H1	H2	H3	H4
Fruit width (mm)	28.65±3.56^{c}	29.52±4.27^{c}	44.75±7.22^{b}	48.01±5.29^{a}
Fruit length (mm)	22.83±4.61^{d}	26.63±3.35^{c}	39.03±7.03^{b}	43.23±5.15^{a}
Fruit flesh diameter (mm)	8.22±1.74^{d}	12.44±2.15^{c}	16.42±2.47^{b}	18.76±1.83^{a}
Fruit weight (g)	14.13±3.89^{d}	42.20±6.57^{c}	65.90±11.24^{b}	85.40±13.01^{a}

Means followed by the same letters within the same row are not significantly different from each other at $p \leq 0.05$

Fruit width and length increased with fruit development and differences between the harvest times were significant, except between H1 and H2 for fruit width. Fruit length increased significantly throughout fruit development from 22.83 mm at H1 to 43.23 mm at H4. Similarly, fruit width was 28.65 mm at H1 and 48.01 mm at H4. Mesocarp thickness, measured as fruit flesh diameter, also increased significantly between the harvest times as fruit development progressed. While fruit flesh diameter was 8.22 mm at the beginning, it was 18.76 mm at the commercial harvest stage. Fruit width increased by approximately 67%, fruit length by 89% and fruit flesh diameter by 128% from H1 to H4 of the study. Different researchers reported significant increases in fruit dimensions during the development of plum fruits (Khan, 2016; Sarıdaş et al., 2016; Çatak et al., 2022).

Continuous fruit weight increases for different Japanese plum cultivars were reported until harvest (Khan, 2016). Depending on harvest times, the average weights of the fruits also showed significant differences. The average fruit weight was 14.1 g at the first collection time; the average fruit weight increased by 198% to 42.2 g at H2 and the increase continued in the subsequent harvest periods. In the third harvest period, the fruit weight increased by 56% compared to H2 and was 65.9 g. At the commercial harvest stage, the fruit weight increased by 23% compared to H3 and reached 85.4 g. The increase in average fruit weight from H1 to H4 was over 500%. Fruit weight is an important quality parameter and Japanese, European and green plum fruit weights were reported to be between 10 and 130 g (Önal and Cinsoy, 2003; Bilgü and Seferoğlu, 2005; Açar, 2016; Çatak et al., 2022). Significant increases in fruit weights of Japanese plum cultivars during fruit development periods have also been reported (Khan, 2016; Zhang et al., 2022).

The color measurements of the fruit skins were made with a colorimeter according to the Hunter color system (*L* a* b**) after harvest (Table 3). The *L** value is used to measure the black (0) to white (100) values of the colors. Plum fruits collected at the H1 period were darker than the fruits at the H2 and H3 periods; an increase in *L** values showed that the whiteness of the fruits increased at those harvest times. However, the fruits darkened as they became mature, as was assessed by their *L** values, which decreased significantly at H4. The *L** value of two different colored plum cultivars showed that the colored variety's *L** value increased at mid-season compared to the beginning, but later it decreased as the fruits ripened. However, green fruits *L** values increased as fruit development and ripening progressed, which was similar to the results of the dark-colored Angeleno cultivar used in this study (Zhang et al., 2022). Fruit appearance is an important quality parameter, which can be assessed by measuring color parameters, and significantly impacts consumers' demand (Sarıdaş et al., 2016). The *a** value in the color scale is used to determine the change between green (-60) and red (60). The green-red values of the fruits did not show a significant difference at the H1 and H2 stages, and they were in green tones. As fruits developed, a significant decrease occurred in value with the start of coloration of the fruits (H3), and the *a** value became positive, indicating that fruits turned red at maturity (Figure 1). The *b** value, which determines the change in blue (-60) and yellow (60) colors, did not change in the first two harvests and started decreasing, indicating that the color of the fruits started changing from yellow to blue from the H3 to H4 periods (Table 3). Similar increases in *a**

Table 3. Color measurements of Angeleno fruits sampled at different maturity periods.

Color parameters	H1	H2	H3	H4
*a**	-10.91±1.37^{c}	-10.630±0.86^{c}	-3.59±6.55^{b}	16.25±4.14^{a}
*b**	32.67±1.91^{a}	32.02±1.58^{a}	26.44±7.61^{b}	9.16±4.01^{c}
*C**	34.46±2.12^{a}	33.90±1.76^{a}	26.60±3.75^{b}	18.68±5.07^{c}
*L**	52.64±2.33^{b}	54.10±1.96^{a}	54.98±4.68^{a}	31.41±4.33^{c}
h°	108.43°±1.72^{a}	108.36°±0.89^{a}	93.95°±16.55^{b}	28.15°±8.06^{c}

Means followed by the same letters within the same row are not significantly different from each other at $p \leq 0.05$

and decreases in *b** values during plum fruit development were reported for the red colored Cuihongli cultivar, which had the same changes as the purple skinned Angeleno cultivar (Zhang et al., 2022). Chroma (*C**) values are a value used to determine the liveliness or brightness of the fruit or tissues and the chroma values did not differ significantly between the H1 and H2 periods. However, as fruits started to develop further, significant decreases were recorded at H3 and H4 periods. The *h°* values of the color also varied with fruit development. While the fruits were in the green region at H1 and H2 periods, the corresponding *h°* values changed to the red region with fruit development (Table 3). Reductions in *C** and *h°* values during maturation of green plums have also been reported (Sarıdaş et al., 2016).

Some physiological and biochemical changes that occur in Angeleno fruits during development were determined by measuring total protein, soluble and reducing sugars, chlorophyll, carotenoid, phenolic and flavonoid content. In addition, the antioxidant capacity of fruits at each harvest time was measured with CUPRAC and DPPH assays. It was found that each measured parameter, except the total flavonoid content, showed significant differences throughout the development periods of Angeleno fruits (Table 4).

Chlorophyll is an essential molecule where photosynthesis takes place, which produces sugars from carbon dioxide and water under sunlight. Chlorophyll degradation is a natural process during senescence and fruit maturation (Solovchenko et al., 2005), and chlorophyll absorbance could be used to determine the ripeness of plums (Infante et al., 2011); therefore, chlorophyll and carotenoid changes during fruit development were monitored. Both compounds followed a similar pattern between the sampling dates (Table 4). Total chlorophyll and carotenoid content changed significantly between the sampling dates. The highest chlorophyll and carotenoid content was observed at H1, and later reduction occurred at H2. However, both chlorophyll and carotenoid content increased significantly at H3 compared to H2, and the lowest amounts were observed at H4. Similar chlorophyll and carotenoid decreases were reported during fruit development in different fruit species (Solovchenko et al., 2005; Vlaic et al., 2018).

Plums are rich in health beneficial phytochemicals and many reports indicated that plum fruits are especially rich for flavonoids and phenolic substances (Kim et al., 2003; Sarıdaş et al., 2016; Li et al., 2019; Vlaic et al., 2018). Though total flavonoid content did not exhibit significant changes, total phenolic content was significantly different between the harvest dates. Total phenolic content decreased in H2 and H4 compared to H1 and H3 harvest dates, but the reduction between H3 and H4 was not significant. Angeleno fruits had higher phenolic, but lower flavonoid content at H3 and H4 than at H1 and H2 harvest dates (Table 4). Total phenolic content of different plum varieties changed between 38 and 1245 mg CAE 100 g^{-1} FW (Kim et al., 2003; Cevellos-Cavals et al., 2006; Sahamishirazi et al., 2017; Vlaic et al., 2018). Total flavonoid content of plum varieties was reported to be between 65 and 258 mg QE 100 g^{-1} (Kim et al., 2003; Vlaic et

Table 4. Biochemical parameters measured at different development stages of Angeleno fruits.

Biochemical Variables	H1	H2	H3	H4
Total chlorophyll (µg mL^{-1})	8.56±0.03^{a}	5.21±0.03^{c}	6.17±0.55^{b}	2.72±0.12^{d}
Total carotenoid (µg mL^{-1})	1.85±0.01^{a}	1.35±0.01^{b}	1.76±0.08^{a}	0.96±0.02^{c}
Total phenolics (mg g^{-1} DW)	233.24±7.94^{b}	199.11±11.66^{c}	315.73±7.63^{a}	298.67±7.63^{a}
Total flavanoid (mg g^{-1} DW)	66.85±5.00^{a}	69.07±3.49^{a}	62.41±2.07^{a}	62.04±2.30^{a}
Soluble sugars (mg g^{-1} DW)	135.72±2.32^{d}	254.22±21.07^{c}	366.91±22.28^{b}	449.18±14.19^{a}
Reducing sugars (mg g^{-1} DW)	107.26±1.51^{d}	176.11±7.79^{c}	189.46±5.30^{b}	254.83±2.36^{a}
Protein (mg g^{-1} DW)	20.65±0.97^{b}	23.75±0.50^{a}	13.96±1.71^{d}	17.74±1.23^{c}
CUPRAC (µmol Trolox g^{-1} DW)	245.35±11.54^{b}	247.47±5.32^{b}	276.61±1.83^{a}	255.17±3.79^{b}
DPPH (µmol Trolox g^{-1} DW)	42.75±1.15^{b}	50.83±6.14^{a}	44.58±0.68^{ab}	39.08±0.35^{b}

Means followed by the same letters within the same row are not significantly different from each other at $p \leq 0.05$

al., 2018). The average phenolic content of over 100 plum cultivars was reported to be 271 mg 100 g^{-1} FW, and dark-skinned fruits had higher phenolic content than yellow fruits (Sahamishirazi et al., 2017). Vlaic et al. (2018) stated that during fruit development, phenolic compound concentration usually decreases, but flavonoid content increases and a decrease-increase trend has been reported for phenolic and flavonoid content during fruit development of Sanhua plum (Li et al., 2019). Çatak et al. (2022) reported that unripe (30.9 mg 100 g^{-1}) Angeleno fruits had higher phenolic content than the ripe (27.4 mg 100 g^{-1}) fruits, which was not the case in the present study, where flavonoid content did not change significantly, but phenolic content exhibited a decrease-increase trend during fruit growth. Besides differences in phenolic and flavonoid content due to fruit development and genotypes, fluctuations due to harvest times, development stage, different years, location, temperature fluctuations and fruit position within trees were reported (Miletic et al., 2012; Sarıdaş et al., 2016; Kırbağ and Göztok, 2017; Vlaic et al., 2018; Li et al., 2019; Zhang et al., 2022). Therefore, to understand phenolic compound accumulation in fruits, it is necessary to examine more than one genotype over the years.

Sugars are produced by chloroplasts and transported to different parts of plants, including fruits where they are used to make and expand new cells, provide necessary energy and sugar composition and content along with other substances determine fruits' distinct tastes, sweetness and palatability (Kim et al., 2015). Fructose, glucose, sucrose and sorbitol are major sugars along with some minor sugars found in plum fruits (Kim et al., 2015; Farcuh et al., 2017). Soluble and reducing sugars content shows a similar pattern in which their content increased significantly with fruit development throughout the study period. While the soluble sugar content was 135.7 mg g^{-1} DW at H1, corresponding to the dark green period, the soluble sugar content of the fruits increased with fruit development and reached 449.2 mg g^{-1} DW at H4, which was the commercial harvest period. The reducing sugars content was 107.3 mg g^{-1} DW at the first sampling period; its amount increased by 65% to 254.8 mg g^{-1} DW at the last harvest period. Though sugar levels increase with ripening, levels of individual sugars show differences during fruit development and ripening (Kim et al., 2015; Sarıdaş et al., 2016).

The total protein amount did not show a regular increase or decrease during the ripening periods of plum fruits. In the second and fourth harvest periods, the protein amount increased compared to the first and third harvest periods. The highest protein amount (23.7 mg g^{-1} DW) was obtained from the H2 and the lowest protein amount (13.9 mg g^{-1} DW) was obtained from the fruits at H4 periods (Table 4). Protein content of European plum fruits was reported to be 38 mg g^{-1} DW (Mehta et al., 2014), and green plum fruits were 1.32 mg g FW^{-1} (Kırbağ and Göztok, 2017). On a dry weight basis, Angeleno fruits had lower protein content than reported values.

The antioxidant capacity of plum fruits depends on phenolic substances, vitamins and carotenoids (Arion et al., 2014). Antioxidant activity of fruits at different development stages was measured by CUPRAC and DPPH methods since these assays are different for selectivity, sensitivity and reaction mechanisms (Apak et al., 2006). CUPRAC activity of plum fruits did not differ between the H1, H2 and H4 periods, but a significant increase was observed at the H3 period (Table 4). The DPPH method measures radical scavenging activity that also exhibited significant differences between the harvest periods. The DPPH value increased in the second period and had the highest value, then it decreased in the third and fourth harvest times. In addition, antioxidant activity as measured by DPPH and CUPRAC assays differed for their antioxidant values (Table 4). Antioxidant activity is influenced by cultivars, environmental conditions of the growing years and seasons, cultural practices, development stages of fruits and storage (Kim et al., 2003; Miletic et al., 2012; Arion et al., 2014; Vlaic et al., 2018; Li et al., 2019). Even though

antioxidant activity measured by two methods differed between harvest dates in the present study, antioxidant capacity of different cultivars did not show any significant differences at different harvest stages and positions on trees (Sarıdaş et al., 2016; Vlaic et al., 2018). However, significant changes in *in vitro* antioxidant activity were reported at different maturity stages of plum varieties, similar to the results of this paper (Li et al., 2019; Zhang et al., 2022). In addition, a significant correlation was detected between total phenolic and flavonoid content and antioxidant activity (Sarıdaş et al., 2016; Li et al., 2019; Zhang et al., 2022).

We have studied pomological and biochemical changes associated with fruit development and ripening in Angeleno plum. Fruit weight and dimensions increased throughout the study period. Coloration of fruits started at H3 and fruits were fully red/purple at H4. Biochemical analysis of fruits revealed that total chlorophyll, carotenoid, protein, phenolic and antioxidant capacity did not show regular decrease or increase trends, while sugar content showed regular increase during fruit development. Results also showed that Angeleno plum fruits are a good source of phenolic compounds with a good level of antioxidant capacity.

Acknowledgments

This research was partly supported by the Scientific and Technological Research Council of Türkiye (TÜBİTAK), under 2209-A research project for undergraduate students.

Conflict of interest

The authors declare that they have no conflict of interest

Author contributions

FA: investigation. MT: conceptualization, supervision, writing–original draft. SÖ: investigation, visualization, writing–review & editing. MM: investigation, writing–review & editing.

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