



Change in fruit properties, bioactive contents and individual phenolics of strawberry tree (*Arbutus Unedo* L.) fruits at different developmental stage

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Abstract

The strawberry tree (*Arbutus unedo* L.) is a Mediterranean plant that produces fruits containing important components for health. In this study, fruit quality traits and bioactive contents of *A. unedo* fruits at 4 different fruit developmental stages [Green Stage (GS), Orange Stage (OS), Pink Stage (PS) and Red Stage (RS)] were investigated. Depending on the developmental stages, fruit properties and bioactive contents varied significantly ($p < 0.05$). Fruit weight, length and width were found to be lower at the GS stage and higher at the other stages. In color parameters, a decrease in L^* and b^* values was observed as the red color of the fruits increased. The a^* value increased with the progress of fruit development. In the taste tests, fruits that turned red were found to be sweeter, and the highest taste score was obtained at the RS. While the SSC content increased with the progress of fruit development, the TA amount decreased. Fruits at the OS stage exhibited the highest vitamin C ($312 \text{ mg } 100 \text{ g}^{-1}$), whereas those at the GS stage showed the highest titratable acidity (TA). Total phenolics was highest at the RS stage ($964.9 \text{ mg GAE kg}^{-1}$), while total flavonoids peaked at the GS stage ($244.9 \text{ mg QE kg}^{-1}$). The highest antioxidant activity was also observed at the GS stage ($52.9 \text{ mmol TE kg}^{-1}$), showing stage-dependent increases or decreases depending on the developmental stages. Catechin was identified as the predominant individual phenolic compound. In general, the highest individual phenolic compounds were recorded at the GS, while the lowest were found at the OS and PS. The PCA analysis results also support these findings. Indeed, RS was associated with total phenolics, soluble solids, protocatechuic acid, trans-ferulic acid, and catechin (major phenolic). GS was related to other phenolic compounds, total flavonoid and antioxidant activity. Physical properties were associated with PS. As a result, different developmental stages markedly alter the physical, chemical and bioactive properties of strawberry tree fruits. The results provide new insights into which fruits at different developmental stages have the highest content of specific bioactive compounds associated with health benefits or functional properties.

Keywords Antioxidant · *Arbutus unedo* · Bioactive compounds · Catechin · Development stages · Functional foods

Introduction

In recent years, people's need for affordable and accessible food and nutrients has brought fruits with high bioactive content to an important position. One of these is strawberry

tree fruits. Strawberry tree (*Arbutus unedo* L.), which belongs to the *Arbutus* genus of the Ericales family, can easily grow in regions where the typical Mediterranean climate prevails [1]. It, which has spherical fruit that turn from green to bright red with maturity, contains very rich bioactive compounds. In fact, strawberry tree fruit are very rich in vitamin C, organic acids, carbohydrates, phenols, flavonoids, flavan-3-ols and galloyl derivatives, antioxidants and anthocyanin contents [2–4]. These bioactive compounds are known to have significant effects on human health. *A. unedo* has been reported to be effective in preventing many diseases such as kidney problems, cardiovascular, platelet

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anti-aggregant, diuretic, anti-inflammatory, diarrhea, hypertension and diabetes [5–8].

Strawberry tree fruit are usually consumed fresh when they are completely red. They are also used in making alcoholic beverages, jelly, jam and marmalade [9]. Strawberry tree stands out not only with its fruits but also with other plant parts. In fact, its leaves [10], roots [11] and flowers [10, 12] contain important components for health.

The physical and biochemical properties of fruit are affected by cultivar, ecological conditions, cultivation techniques, maturity stage and biotic and abiotic stress factors [13–15]. Fruits grow, develop and ripen after the pollination and fertilization stages. During the development process until ripening, there are some physical changes in the fruits. The most obvious ones are the fruit getting bigger and changing color. Concurrently, substantial modifications may also occur in the biochemical composition of the fruit, depending on the developmental stage. Determining this change that occurs at different development stages is important in terms of determining the harvest time and fruits suitability for different areas of use. In recent years, the increasing interest in fruits as sources of bioactive compounds has highlighted the importance of identifying the developmental stage at which these constituents reach peak levels. Such information is particularly valuable for the evaluation of horticultural crops in terms of their nutritional and potential medicinal properties [16, 17].

Although there are several studies in the literature on the general characteristics and biochemical contents of strawberry tree fruit, it has been determined that there is limited study to determine the physical and biochemical properties and changes of fruit at different developmental stages [18, 19]. The study question is based on the question of whether the quality characteristics of fruit change during developmental stages? If there is a change, in which developmental stage will the density of bioactive compounds that have

important effects on health be higher and does it change depending on the developmental stages? The aim of this study is to determine the changes in the quality characteristics and bioactive contents of strawberry tree fruit at different developmental stages and to reveal the fruit developmental stage in which bioactive compounds are at the highest level.

Materials and methods

Plant materials

The plant material of the study consists of a strawberry tree (*Arbutus unedo* L.) plant (fruits belonging to a single region and a single genotype) that grows naturally in the Gebze district (Kocaeli, Türkiye). Fruit samples were hand-harvested from the determined genotype at 4 different developmental stages. Different fruit development stages in strawberry tree were classified as Green Stage (GS), Orange Stage (OS), Pink Stage (PS) and Red Stage (RS) (Fig. 1). The ripening stage is evaluated visually.

During harvest, care was taken to ensure that the fruits were not damaged and were free from diseases and pests. 500 g of fruit was harvested for each fruit developmental stage. All of the fruit was harvested on the same day. Harvested fruit samples were directly transported to the laboratory for analyzed. The fruits were kept at -20 °C until the biochemical analysis was performed.

Fruit weight, width, and length

To investigate the fruit weight, width and length of strawberry tree fruit, 30 fruit were measured for each fruit development stage, 120 fruit in total. The average fruit weight (g) was determined using a digital scale (Radwag, Radom, Poland) with a sensitivity of 0.01 g. Fruit width and length

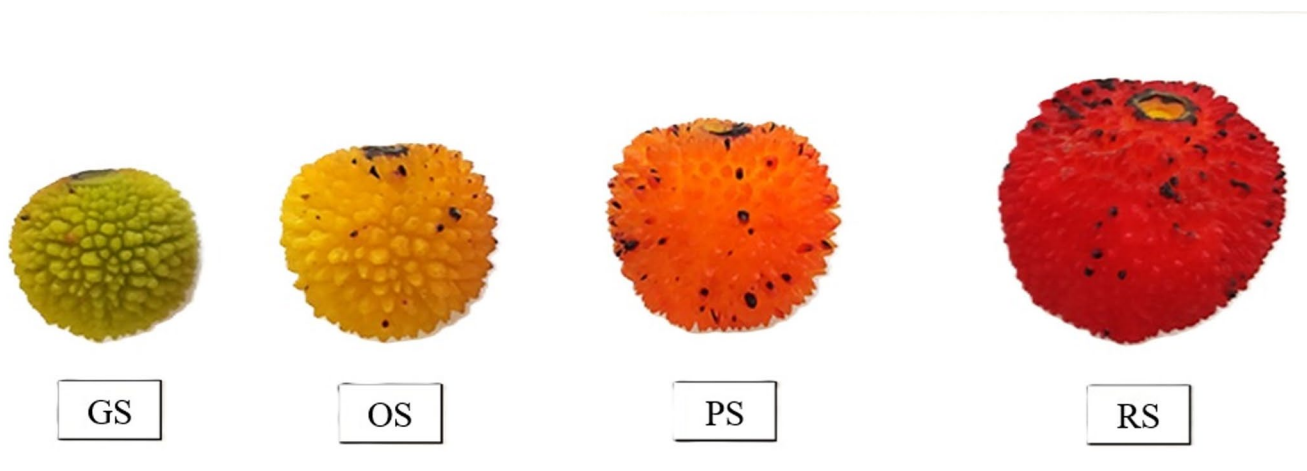


Fig. 1 Different fruit development stages in strawberry tree

(mm) were measured using a digital caliper (sensitivity of 0.1 mm) (Mitutoyo, Japan) [20].

Taste

A team of five was formed to analyze the taste of strawberry tree fruits at different developmental stages. All conditions were created simultaneously and equally to prevent panelists from being influenced by the environment or each other. For taste analysis, the fruits were divided into three replicates and presented to the panelists. Panelists were asked to rate the sweetness level on a scale of 1–5 (1: very tasteless, 2: tasteless, 3: medium, 4: sweet, 5: very sweet). The results were presented as average values.

Color characteristics (L^* , a^* and b^*)

Fruit color measurements were designed to have 15 fruit in each replicate. Minolta, model CR-400, Tokyo, Japan colorimeter was used for measurements. Color measurements were made on two opposite sides of strawberry tree fruit. Color values are presented as L^* , a^* and b^* CIE (Commission Internationale de l'Eclairage) as seen separately on the colorimeter screen McGuire [21].

Soluble solids contents (SSC), titratable acidity (TA) and vitamin C

To evaluate the SSC, TA and vitamin C content of strawberry tree fruit, 30 fruit from each replicate were cleaned with purified water and kept on blotting paper for 20 min. The fruits completely freed from purified water were crushed with a blender (Philips, Türkiye) and turned into puree. Fine-mesh strainer cloth was used to separate the pulp from the pureed fruits and obtain fruit juice. The homogenate that became fruit juice was now completely ready for the determination of SSC, TA and vitamin C content.

The resulting fruit juice was measured using an electronic refractometer (PAL-1, USA). The SSC content were presented as a percentage (%). In order to determine the amount of TA, 15 mL of the obtained fruit juice was taken and combined with 15 mL of purified water. The mixture was stirred on a magnetic stirrer until it became homogeneous. Then, this mixture was titrated by adding 0.1 mol L^{-1} (N) sodium hydroxide (NaOH) solution. When the pH value of the solution reached 8.1, the amount of NaOH consumed (the number seen on the digital screen) was recorded. The obtained results were presented as %. Reflectometers (Merck RQflex plus 10, Germany) were used to determine the vitamin C content. The vitamin C test kit was immersed into the obtained fruit juice. The test kit, which was kept in the fruit juice for approximately 2 s, was removed at

the end of the period and kept outside until the 8 s. During this period, the kit, which was cleaned of excess liquid, was placed in the test adapter and a reading time of 5 s was waited. After 5 s, the value seen on the digital screen was recorded. The results obtained were presented as mg 100 g^{-1} fresh weight (fw) [22].

Total phenolics, total flavonoids and antioxidant activities

For the analysis of bioactive compounds, fruits were first cleaned with distilled water and kept on blotting papers for 20 min. Then, fruits were crushed with a blender (Philips, Turkey) and homogenate was obtained. The homogenates obtained were taken into 50 mL falcon conical tubes and stored until analysis ($-20\text{ }^{\circ}\text{C}$). For analysis, samples taken from the freezer were thawed at room temperature ($22\text{ }^{\circ}\text{C}$) and 1 g of the thawed sample was taken and 10 mL of methanol was added. The prepared extract was kept at $+4\text{ }^{\circ}\text{C}$ for two days. Then, the extract was centrifuged at 12,000 rpm for 30 min at $+4\text{ }^{\circ}\text{C}$, and the upper liquid portion was filtered [22].

Total phenolics were determined as described by Singleton and Rossi [23]. Briefly, 1000 μL of the stock solution was mixed with 3600 μL of distilled water, 100 μL of Folin–Ciocalteu reagent, and 300 μL of sodium carbonate. The samples were then incubated in the dark for 2 h. Total flavonoids were measured following the method of Ozturk et al. [22]. For this assay, 1000 μL of the stock solution was combined with 3300 μL of methanol, 100 μL of aluminum nitrate, and 100 μL of ammonium acetate, and the mixture was incubated for 30 min. FRAP (Ferric Reducing Antioxidant Power) antioxidant activity was assessed according to Benzie and Strain [24]. In this procedure, 20 μL of the stock solution was mixed with 1230 μL of phosphate buffer and 1250 μL of potassium ferricyanide, followed by incubation at $50\text{ }^{\circ}\text{C}$ for 25 min. Subsequently, 1250 μL of trichloroacetic acid (TCA) and 250 μL of ferric chloride were added to the samples. Measurements were made at 760 nm for total phenolics, 415 nm for total flavonoids and 700 nm for FRAP using a spectrophotometer (Shimadzu, Japan). The measured absorbance values were calculated and presented as quercetin equivalents (QE) in milligrams (mg) per kilogram (kg) fresh weight (fw) for total flavonoids, as gallic acid equivalents (GAE) in milligrams (mg) per kilogram (kg) fresh weight (fw) for total phenolics, and as Trolox equivalents (TE) in millimoles (mmol) per kilogram (kg) fresh weight (fw) for FRAP antioxidant activity.

Table 1 Changes in fruit weight, length, width, L*, a*, b* and taste of strawberry fruits in different development stage

Development stage	Fruit weight (g)	Fruit length (mm)	Fruit width (mm)	L*	a*	b*	Taste
GS	19.13 b	14.45 b	15.18 b	64.92 a	-2.22 c	72.09 a	1.0 c
OS	26.47 a	17.03 a	18.11 a	58.23 b	12.85 b	63.18 b	2.3 b
PS	27.27 a	17.50 a	18.41 a	49.04 c	37.05 a	58.33 c	3.0 ab
RS	27.15 a	17.47 a	18.42 a	40.15 d	37.56 a	47.68 d	3.7 a

The difference between the means shown with the same letter in the same column is insignificant (Tukey, $p < 0.05$). GS: Green Stage. OS: Orange Stage. PS: Pink Stage. RS: Red Stage

Individual phenolic compounds

Individual phenolic compounds were quantified according to the method reported by Ozturk et al. [22]. First, the untreated samples (fruit stored at -20°C) were homogenised. Then, 1 g of each sample was extracted with methanol and kept for 6 h. The obtained extracts were centrifuged at 12,000 rpm for 30 min at 4°C , and the supernatant formed on the top was drawn into a syringe and passed through a $0.45\text{ }\mu\text{m}$ millipore filter [22]. The obtained supernatant was transferred to 2 mL vials and injected into the Ultimate 3000 HPLC system manufactured by Thermo Dionex Company. Stock solutions were prepared by dissolving 4-aminobenzoic acid, epicatechin, caffeic acid, trans-ferulic acid, catechin, chlorogenic acid, protocatechuic acid, 4-hydroxybenzoic acid, rutin and *p*-coumaric acid each in methanol solution. Analyses were performed using a high-performance liquid chromatography (HPLC) device (Thermo-Scientific, USA), using a Hypersil Gold (GD) phenyl column, for 40 min at a wavelength of 225 nm. An injection volume of $20\text{ }\mu\text{L}$ and a mobile phase flow rate of 1 mL per minute were used during the analysis. Mobile phase A contained 2.5% formic acid, and mobile phase B contained methanol ($\geq 99.9\%$). The results were reported in $\text{mg kg}^{-1}\text{ fw}^{-1}$.

Statistical analysis

The study was designed to include three replicates, which were to be conducted to provide reliable and repeatable results. For all physical and biochemical analyses, the mean of three biological replicates was calculated. The data were analyzed using the JMP 16.0 statistical package. The normality of the data distribution analysis was confirmed using the Kolmogorov-Smirnov test, and homogeneity was checked using Levene's test. Differences in means were determined using the Tukey multiple comparison test at a significance level of 5%. A principal component analysis was performed based on the physical properties, bioactive compounds and individual phenolics in strawberry tree fruit at different developmental stages.

Table 2 Changes in SSC, TA and vitamin C of strawberry fruits in different development stage

Development stage	SSC (%)	TA (%)	Vitamin C ($\text{mg } 100\text{ g}^{-1}$)
GS	9.30 b	5.97 a	248 c
OS	9.60 a	5.73 a	312 a
PS	9.75 a	5.44 a	278 b
RS	10.05 a	4.76 b	199 d

The difference between the means shown with the same letter in the same column is insignificant (Tukey, $p < 0.05$). GS: Green Stage. OS: Orange Stage. PS: Pink Stage. RS: Red Stage. SSC: Soluble Solids Content. TA: Titratable Acidity

Results

The effect of development stages (the coloring stage of fruits from green to red.) on fruit quality traits of strawberry tree was significant ($p < 0.05$) (Table 1). The lowest fruit weight, length and width were in GS (19.13 g, 14.45 mm, and 15.18 mm, respectively). The highest values for fruit weight and length were recorded in PS (27.27 g and 17.50 mm, respectively). RS had the highest fruit width (18.42 mm). However, the difference between PS, OS, and RS is insignificant. L* and b* values from color parameters showed significant differences in all development stages. In particular, a decrease was observed in L* and b* values with the increase in red color of the fruits. The lowest L* (40.15) and b* (47.68) values were measured in RS, while the highest were found in GS (64.92 and 72.09, respectively). Negative values for a* value from color parameters were observed only in fruits at GS (-2.22). In general, a* value increased with the development of fruits. However, this increase was found to be insignificant in terms of differences between PS (37.05) and RS (37.56), and was significantly higher compared to other development stages. Tasting tests also resulted in parallel with the color change. A significant increase in taste was observed with the red color of the fruits. The highest tasting score was at RS (3.07) and the lowest was at GS (1.0) (Table 1).

The effects of different fruit development stages on SSC, TA and vitamin C contents of strawberry tree fruits were significant ($p < 0.05$) (Table 2). As the development stage progressed towards ripening, an increase in SSC content was observed. SSC content was lowest in GS (9.30%)

and highest in RS, PS and OS (10.05%, 9.75% and 9.60, respectively). Contrary to the increase in SSC content, TA amount decreased parallel to fruit development, especially with the color turning red. The highest TA was measured in GS, OS and PS (5.97%, 5.73% and 5.44, respectively), while the lowest was detected in RS (4.76%). The highest vitamin C was found in OS (312 mg 100 g⁻¹), the lowest in RS (199 mg 100 g⁻¹) (Table 2).

Total phenolics and total flavonoids contents of strawberry tree fruits changed significantly ($p < 0.05$) depending on the fruit development stage (Table 3). Total phenolics remained the same during the fruit development stage, showing an increase in the last stage (RS). The highest total phenolics was found in the RS stage (964.9 mg GAE kg⁻¹), while the lowest was found in the PS stage (736.1 mg GAE kg⁻¹). Total flavonoids content also decreased as the fruit development stage progressed and increased in the last development stage (RS). The highest total flavonoids content was recorded in the first fruit development stage (GS) (244.9 mg QE kg⁻¹), while the lowest was found in PS (69.2 mg QE kg⁻¹). In general, the total flavonoids content was the highest in green (GS) and orange (OS) fruits. Antioxidant activity of strawberry tree fruits changed significantly ($p < 0.05$) depending on the fruit development stage. Antioxidant activity, showed fluctuations depending on the development stage. The highest FRAP antioxidant activity was determined in GS (52.9 mmol TE kg⁻¹), and the lowest in PS (42.1 mmol TE kg⁻¹). In general, the antioxidant changes from largest to smallest were as follows: GS, RS, OS and PS (Table 3).

Depending on the fruit development stage, individual phenolics contents of strawberry tree fruits changed significantly ($p < 0.05$) (Table 4). Catechin was identified as the major phenolic compound and the highest catechin was found in RS and PS (38.48 mg kg⁻¹ and 37.79 mg kg⁻¹, respectively). The lowest catechin was found in OS (23.45 mg kg⁻¹). Except for protocatechuic acid, catechin and trans-ferulic acid, the highest individual phenolic compounds were found in GS. The highest protocatechuic acid and trans-ferulic acid were found in RS. Except for 4-aminobenzoic acid, 4-hydroxybenzoic and caffeic acid, in general, the lowest individual phenolic contents were recorded in OS and PS. The lowest values for these compounds (caffeic acid) were detected in RS (Table 4).

According to PCA analysis, the first two components explained 93.5% of the data. PC1 accounted for 65.4% of the data, while PC2 accounted for 28.1%. Specifically, the GS fruit developmental stage, located in the first region in the PCA plane, were related to total flavonoids, antioxidant activity, chlorogenic acid, epicatechin, rutin, p-coumaric acid, 4-aminobenzoic acid and caffeic acid. The RS fruit developmental stage was associated with SSC, total

Table 3 Changes in total phenolics, total flavonoids, antioxidant activity of strawberry fruits in different development stage

Development stage	Total phenolics (mg GAE kg ⁻¹)	Total flavonoids (mg QE kg ⁻¹)	Antioxidant activity (mmol TE kg ⁻¹)
GS	763.2 b	244.9 a	52.9 a
OS	736.9 b	217.1 b	46.1 c
PS	736.1 b	69.2 d	42.1 d
RS	964.9 a	113.9 c	50.5 b

The difference between the means shown with the same letter in the same column is insignificant (Tukey, $p < 0.05$). GS: Green Stage. OS: Orange Stage. PS: Pink Stage. RS: Red Stage

Table 4 Changes in individual phenolic compounds of strawberry fruits in different development stage

Phenolic compounds (mg kg ⁻¹)	Development stage			
	GS	OS	PS	RS
4-Aminobenzoic acid	2.27 a	1.24 b	1.28 b	0.72 c
Protocatechuic acid	1.56 c	1.27 d	2.17 b	2.56 a
4-Hydroxybenzoic acid	0.40 a	0.39 a	0.34 b	0.20 c
Catechin	29.60 b	23.45 c	37.79 a	38.48 a
Chlorogenic acid	0.49 a	0.42 c	0.37 d	0.45 b
Caffeic acid	0.56 a	0.45 b	0.32 c	0.26 d
Epicatechin	2.76 a	2.39 c	2.43 bc	2.51 b
p-Coumaric acid	1.48 a	0.74 b	0.38 d	0.43 c
Trans-ferulic acid	0.61 b	0.53 c	0.53 c	0.75 a
Rutin	1.23 a	0.97 b	0.88 c	0.91 c

The difference between the means shown with the same letter in the same row is insignificant (Tukey, $p < 0.05$). GS: Green Stage. OS: Orange Stage. PS: Pink Stage. RS: Red Stage

phenolics, protocatechuic acid, catechin and trans-ferulic acid, and located in the second region. Additionally, the OS fruit developmental stage, located in the third region, were related to TA, L*, b*, vitamin C and 4-hydroxybenzoic acid. Furthermore, the PS fruit developmental stage was associated with fruit weight, fruit length, fruit width, taste and a*, and located in the fourth region (Fig. 2).

Discussion

The fruit development stages and the resulting ripening are a genetically programmed and irreversible process in which physical, biochemical and organoleptic changes occur in a sequential and harmonious manner. This process ultimately leads to the formation of a soft and edible fruit with the desired quality characteristics [25, 26]. It has been emphasized that the maturity stage has significant effects on quality characteristics and these effects are directly related to processing systems and consumer satisfaction [27]. Similar to our findings, Tran et al. [28] reported that physical properties such as weight, length, and diameter of Gac (*Momordica cochinchinensis* Spreng.) fruits increased in parallel with

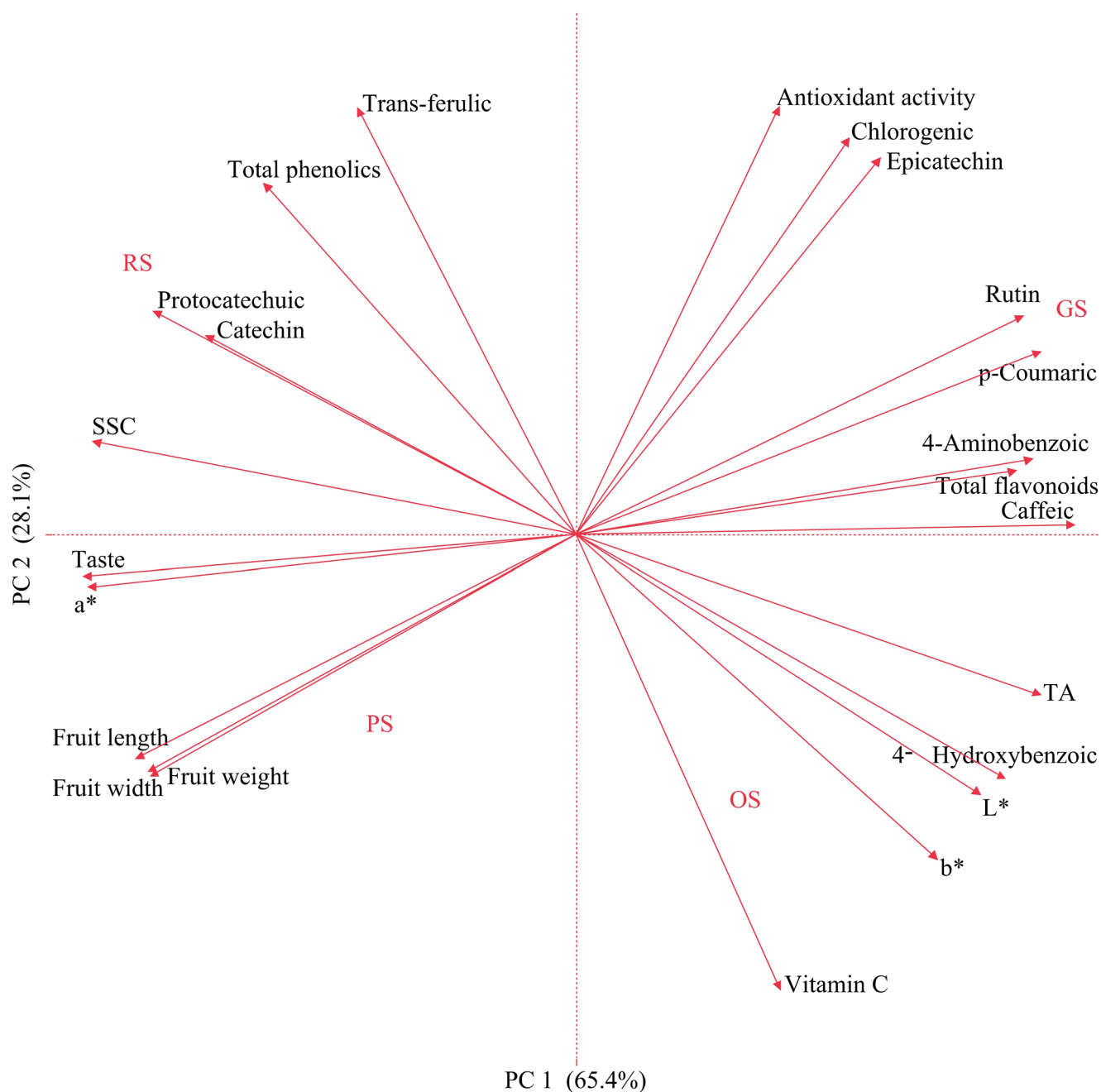


Fig. 2 Principal component analysis based on the physical properties, bioactive compounds and individual phenolics in strawberry fruit at different developmental stages. GS: Green Stage, OS: Orange Stage, PS: Pink Stage, RS: Red Stage, SSC: Soluble Solids Content, TA: Titratable Acidity

fruit development at different stage of ripening. Similarly, Park et al. [29] reported that fruit weight increased significantly in fruits with SSC content of 8.1% and 9.1% in kiwi-fruits harvested at different maturity stages (different SSC amounts). Sbrussi et al. [30] also found that fruit length and diameter in *Physalis peruviana* fruits were higher in yellow and bright yellow colors depending on the coloration stage, while fruit weight increased until the last stage of coloration, but the difference was insignificant (except for green). There are findings that different developmental and ripeness

stages in fruits also have significant effects on SSC and TA contents [31]. Yamankaradeniz [32] stated that SSC and vitamin C content in rosehip varieties increased with ripening, but the amount of TA increased only until half ripe and decreased during the ripening process. However, Bron and Jacomino [33] did not find a significant difference in SSC, TA and ascorbic acid contents of papaya fruits depending on different developmental stages. Sun et al. [34] stated that there may be differences in SSC, TA and vitamin C contents of lemon fruits at different developmental stages (green,

yellow, green-yellow). Similar findings were obtained in other studies examining the effects of fruit developmental stages on SSC and TA content. Santamaría Basulto et al. [35] reported that SSC content in papaya fruits increased with ripening, Tran et al. [28] stated that the SSC value of Gac fruits increased as the color turned orange-red, but decreased after turning dark red. Similarly, Zhu et al. [36] reported that sugar content increased as ripening progressed in banana varieties. Park et al. [29] determined that SSC content in kiwifruits reached the highest TA value when it reached 8.1%.

Color changes occurring in fruits at different developmental stages are an effective parameter on taste and aroma as well as consumer preferences. Iglesias et al. [37] reported that fruit color is related to ripening time and Crisosto et al. [31] reported that this may also affect taste and aroma. Eken and Mavi [38] stated that in *Capsicum baccatum* var. Pendulum, the L^* value decreases with the progression of ripening and this change is observed due to the increase in red color. Çoklar and Akbulut [39] stated that in hawthorn fruits, the L^* values decrease as they turn orange-yellow, while a^* and b^* values increase. Tran et al. [28] reported that the color a^* values of Gac fruits harvested at different color stages were negative in the first 3 harvests, but gradually increased by becoming positive in the last 2 harvests when the fruits turned red, and the color b^* value increased until the fruits reached a dark red color from green. Santamaría Basulto et al. [35] reported that L^* and b^* values increase until the rind color of papaya fruits reaches dark red, while the a^* value changes positively as the orange color becomes more pronounced. Bron and Jacomino [33] also reported that the color H^0 values of 'Golden' papaya fruits at different coloration stages decrease significantly with increasing yellow ripeness color. In the present study, similar to the findings of the researchers, the a^* value increased with ripening, while the L^* and b^* values decreased.

The bioactive contents of fruit are affected by genetic structure, ecological conditions, cultivation techniques, ripening level, fruit color and biotic and abiotic stress factors [14, 15, 40–43]. The bioactive content of fruit changes depending on its developmental stage. Different ripening stages have an effect on bioactive contents both during and after harvest [44]. Sun et al. [34] stated that antioxidant capacity increases with increasing ripening. Çoklar and Akbulut [39] stated that total phenol and antioxidant contents of green and yellow hawthorn fruits were similar, but these values were significantly higher in orange-yellow fruits. Similarly, Hegedüs et al. [45] found that total phenolic content and antioxidant activity in apricot varieties increased with ripening. In addition, Mahmood et al. [46] reported that total phenol and flavonoid contents in unripe, semi-ripe and fully ripe mulberry and strawberry varieties increased

significantly with increasing ripeness stage. Siriamornpun and Kaewseejan [47] stated that total phenolic and antioxidant capacity were higher in green fruits and decreased with ripening, similar to our study findings. Siriamornpun and Kaewseejan [47] found a significant relationship between developmental stages (ripeness) and individual contents (rutin, *p*-hydroxybenzoic acid, chlorogenic acid and quercetin) in their study using different species. The quantitative distributions of individual phenolics were determined to change in fruits harvested at different developmental stages. The highest levels of protocatechuic acid were found in yellow mature stage, while the lowest levels were found in green mature stage. Catechin and epicatechin gradually increased with ripening. Chlorogenic acid had the lowest levels in green mature stage and similar levels in yellow and orange-yellow mature stage. Caffeic acid had the lowest levels in green mature stage and the highest levels in yellow mature stage. Rutin had lower levels in green and yellow mature stage and similar levels in orange-yellow mature stage [38]. Mahmood et al. [46] reported that the amount of *p*-hydroxy-benzoic acid, *p*-coumaric acid, chlorogenic acid, ferulic acid, quercetin and kaempferol in unripe, semi-ripe and fully ripe mulberry and strawberry varieties increased significantly with ripening. In the present study, catechin increased with the advancement of fruit development, while other compounds decreased (4-hydroxybenzoic acid and caffeic acid) or fluctuated (4-aminobenzoic acid, protocatechuic acid, chlorogenic acid, epicatechin, *p*-coumaric acid, trans-ferulic acid, rutin).

According to PCA analysis, the first two components explained 93.5% of the data. A strong positive correlation was determined between fruit weight, fruit length, and fruit width; total flavonoids, antioxidant activity, chlorogenic acid and epicatechin; rutin, *p*-coumaric acid, 4-aminobenzoic acid and caffeic acid; SSC, total phenolics, protocatechuic acid, catechin and trans-ferulic acid. Scarano et al. [19] reported a strong positive correlation between gallic acid and epicatechin in strawberry tree fruits at different maturity stages, and stated that the first two components explained 86.2% of the data. Tas et al. [48] reported that the first two components explained 54.3% of the total variation in service tree genotypes, and noted strong positive correlations between fruit weight, fruit length and fruit width; caffeic acid, catechin and *o*-coumaric acid; *p*-coumaric acid and rutin content of the genotypes. Balta et al. [49] stated strong positive correlations between fruit weight, fruit length and fruit width; total phenolic, total flavonoids and antioxidant activity; caffeic acid, *p*-coumaric acid, catechin, protocatechuic acid, 4-aminobenzoic acid and 4-Hydroxybenzoic acid; epicatechin and rutin in service tree genotypes, and reported that the first two components explained 46.7% of the data. While the obtained variance values were higher

than the researchers' findings, the correlations between the investigated properties showed similarities.

Conclusions

The study showed that the stages of fruit development significantly affect both fruit quality traits and bioactive contents of the strawberry tree, with these changes being one of the main factors determining its quality. As the fruit ripened from the green stage (GS) to the red stage (RS), fruit weight increased by approximately 42%, fruit width by 21%, and sensory taste scores more than tripled; this indicates a strong increase in quality parameters that enhance marketability and consumer appeal. Additionally, total phenolic contents were found to be highest in RS, vitamin C content in OS, and total flavonoids, antioxidant activity, and most individual phenolic compounds were highest in GS. In terms of individual phenolics, catechin was found to be the major compound. Multivariate PCA analysis supported these findings by clearly separating fruit developmental stages according to their dominant quality and bioactive contents. In conclusion, this study provides quantitative evidence that strawberry tree fruits should not be harvested at a single stage of ripening for different uses. Instead, harvesting timing can be optimized according to the intended use: GS for antioxidant-rich extracts; OS stands for vitamin C focused nutritional supplements, while RS stands for fresh consumption functional products. This stage-specific approach offers a practical contribution beyond existing general knowledge and constitutes a valuable resource for producers, processors, and the functional food industry.

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Author contributions OK: Project administration, writing and editing of manuscript, investigation, conceptualization, validation, visualization. UA: Data collection, formal analysis, writing and editing of manuscript, review and editing. BO: Data collection, conducting test, editing of manuscript and analyses. MAÖ: Data collection and analyses. MUH: Data collection and analyses. All authors reviewed the manuscript.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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Consent for publication Not applicable.

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