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Evaluation of phytochemicals and the role of oxidative stress pathways during fruit development in strawberries (*Fragaria×ananassa*)

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Abstract: Strawberries (*Fragaria* × *ananassa*) are one of the Rosaceae family, considered economically and nutritionally important berry fruits. Phytochemicals are crucial ingredients that contribute to characteristics such as the fruit's aroma and flavor. The current study investigated the phytochemical characteristics of strawberry fruit at three developmental stages (green, white, and red). HPLC measurements determined the glucose, fructose, and sucrose concentrations significantly increased in the red fruit, whereas glucose was identified as the major sugar in ripe fruit (2.8 $g.g^{-1}FW$). Evaluation of the phenylpropanoid pathway implied that the amount of total phenol and flavonoid following phenylalanine ammonia-lyase enzyme activity decreased during fruit development. Unlike phenolic compounds, vitamin C was considerably increased in the red stage, while antioxidant capacity was almost constant in all stages of fruit development. The activities of all antioxidant enzymes at the ripe fruit stage were higher than in the green fruit. At the final stage, the superoxide dismutase enzyme had the highest activity. Increasing carbohydrates in the red fruit is due to the role of these compounds in regulating metabolic pathways during fruit ripening. The reduction of phenolic contents and the stability of antioxidant capacity indicates the role of nonphenolic antioxidant compounds such as vitamin C in the ripening and preservation of antioxidant properties. Identifying the phytochemicals and antioxidant capacity of strawberries during ripening reveals the role of various compounds and oxidative stress pathway signaling in fruit ripening; it can also contribute to the widespread application of strawberries in the edible and nonedible industries at different stages of fruit development.

Key words: Carbohydrates, catalase enzyme, glucose, phenolic compounds, strawberries, vitamin C

1. Introduction

Strawberry (*Fragaria* × *ananassa*) is a hybrid species belonging to the Rosaceae family (Gonzalez et al., 2009). So far, more than 23 species and approximately 2000 different varieties of strawberries have been identified worldwide, with an average of over 9 million t produced worldwide in a year (Koyama et al., 2022). Moreover, strawberries are one of the most consumed berries in the world. Strawberries contain significant amounts of micronutrients, sugars, metabolites and phytochemical compounds, organic acids, and iron salts, as well as vitamins E, K, B, and A, which play a significant role in the human diet and are widely used in various food industries (Gunduz et al., 2016). In addition to their nutritional value, strawberries have medicinal properties due to their abundant mineral elements, such as calcium, phosphorus, and antioxidant compounds. It has been reported that after the consumption of strawberries, plasma antioxidant capacity, and blood vitamin C concentration increase (Samykanno et al., 2013). Other significant applications of strawberries in the industry include their use as a skin cleanser and in making aromatic candles, perfumes, and detergents in cosmetic products. Also, the compounds in strawberry seed oil are used to maintain skin elasticity (Gonzalez et al., 2009). The medicinal and cosmetic properties of strawberries include antiinflammatory applications for skin care in the cosmetics industry, use in supplements and drugs due to anticancer and antiobesity effects, use in cardiovascular drugs, diabetes, and protective features against neurological disorders (Samykanno et al., 2013).

Reactive oxygen species (ROS) are reactive chemicals that are produced as inevitable byproducts in normal metabolic processes during development and stress conditions and have many positive and negative roles in the survival of plants (Miller et al., 2019). ROS causes the oxidation of molecules such as proteins and nucleic acids,

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causing extensive cell damage and death. Moreover, the positive roles of ROS can be noted in their effect on triggering cell signaling pathways, gene expression, and regulation of cyclase guanylate activity in cells (Bektas et al., 2005). Furthermore, ROS is a factor in reducing fruit firmness during development. The increase in H_2O_2 is associated with the oxidation of membrane lipids by lipoxygenases, often in the early stages of fruit softening (Dumville and Fry, 2003). To maintain a stable cellular component, plants use specific mechanisms to deactivate ROS, including a nonenzymatic and enzymatic antioxidant system (Vogt, 2010). In the nonenzymatic system, phenolic compounds, as a large group of secondary metabolites, can neutralize ROS due to their redox reaction (Karaman et al., 2010). As a result, the role of these compounds is prominent during the phenylpropanoid pathway, in which the biosynthesis of the secondary metabolite initiates with the help of the essential enzyme phenylalanine ammonia-lyase (PAL) (Vogt, 2010). Strawberries are known as a source of vitamin C, and studies indicate that phenolic compounds can prevent the degradation of L-ascorbic acid against ROS in this fruit (Miller et al., 2019). In the enzymatic mechanism against ROS, the activity of various antioxidant enzymes is known to enhance defense responses and interfere with the oxidative process of fruit ripening. For instance, superoxide dismutase (SOD) and glutathione reductase (GR) are the main enzymes for removing the ROS produced in plant cells. In addition to the significant role of peroxidase (PRX) and catalase (CAT) enzymes in response to environmental stress, PRXs also play a role in lignin biosynthesis, auxin metabolism, and plant cell development. CAT plays a role in scavenging H_2O_2 produced by processes such as β-oxidation of fatty acids and fruit ripening in plants (Miller et al., 2019).

The easy propagation of the strawberry fruit and its small size makes it a valuable model species for studying physiological and phytochemical changes during fruit ripening (Hartl et al., 2017). This study aims to evaluate the phytochemical and antioxidant changes of strawberry fruit during development. In the current research, vital metabolites in the ripening of strawberry fruit, the evaluation of primary and secondary metabolites, and the role of the enzymatic and nonenzymatic antioxidant systems during fruit development have been investigated. The identification of phytochemicals and antioxidant capacity helps to comprehend the role of antioxidant signals during strawberry fruit development. Understanding the phytochemical properties of strawberry fruit in different developmental stages leads to the optimal application of fruits in edible and nonedible industries such as cosmetics and pharmaceutical products.

2. Materials and methods

2.1. Sample collection

Albion cultivars of strawberry plants were cultivated in a greenhouse (hydroponically in cocopeat substrate) under light and temperature conditions (16 h of light and 8 h of darkness at 25–27 °C, 60%–75% humidity). Water and nutrient solutions were provided directly to the plant and growth was carried out under controlled conditions (without stress). Fruits were selected in three different developmental stages (Figure 1): green fruit (15 days after flowering, 11.5 mm), white fruit (25 days after flowering, 24.6 mm), and red fruit (35 days after flowering, 31.5 mm). Then, strawberry fruits free from physical or biological damage were visually selected. Finally, the fruits were transferred to the laboratory at 4 °C.

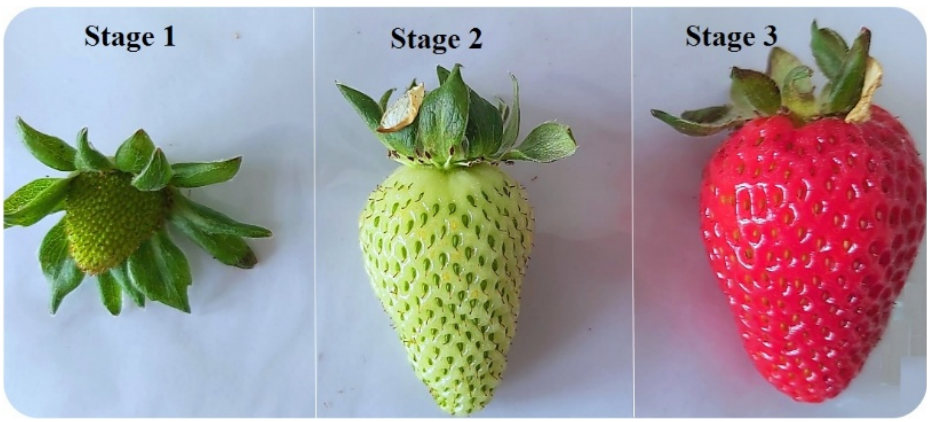

Figure 1. Different developmental stages of strawberry fruit. Stages 1 to 3 are green, white, and red fruits, respectively.

2.2. Measuring the texture firmness and total soluble solids (TSS)

A SANTAM-1 model texture device was used via a 20 kg cell load to check the firmness of the fruit texture. A few drops of the prepared extract (5 g of tissue with 20 mL of distilled water) were dropped on the sensitive screen of the Refractometer device. The amount of total soluble solids was recorded (Sogvar et al., 2016).

2.3. Detection of total carbohydrates and sugar content

For total carbohydrates, the phenol-sulfuric acid method was used. The absorbance of the samples was detected with a spectrophotometer at a wavelength of 490 nm, and the concentration of carbohydrates was expressed with the help of a glucose calibration curve (Dokhani et al. 1998). The measurement of sugars was done via HPLC (SCR-101N separating column with Ion Exclusion mechanism, the flow rate was 0.7 mL per min, the temperature of 60 °C, attenuation of 4, a chart speed of 5 mL per min). After the samples were filtered (0.45 microns), 1 µL of the sample was injected into the device, and the data was recorded. Then, the type and concentration of the main sugars were calculated according to each sugar's standard curves and the retention time (Dokhani et al., 1998).

2.4. Measurement of the metabolic pathway of phenylpropanoids

The methanolic extract was prepared from the fruit tissue at each stage. The amount of total phenol was measured using the Folin-Ciocalteu method. Then, the absorbance of the samples was read at a wavelength of 760 nm using a spectrophotometer, the standard curve was constructed based on gallic acid, and the amount of total phenol compounds was determined (Velioglu et al., 1998). To measure the concentration of total flavonoids, first, an acidic methanol extract (methanol: hydrochloric acid 1:99 v/v) was prepared. The measurement was carried out by the method of Velioglu et al. (1998). The absorption of the samples was determined at a wavelength of 433 nm. The standard curve was drawn based on quercetin, and the total flavonoid compounds were detected. PAL enzyme activity was measured using cinnamic acid, reported by the method of Wang et al. (2006). The concentration of cinnamic acid was calculated by measuring the absorbance at a wavelength of 290 nm (Wang et al., 2006).

2.5. Detection of the antioxidant capacity and vitamin C

To measure the antioxidant capacity, the ABTS method was used. To measure vitamin C, 2,6-Dichlorophenolindophenol (DCIP) was used based on the titration method of Hernandez et al. (2006).

2.6. Investigating the activity of antioxidant enzymes

The CAT enzyme extract was extracted in potassium phosphate buffer with a concentration of 50 mM, pH = 7, and the enzyme activity was calculated at a wavelength of 240 nm (Chance and Maehly, 1955). The activity of the PRX enzyme was determined according to the method of Arora et al. (2002) through the guaiacol test. Then, the absorbance of the samples was determined at a wavelength of 470 nm by a spectrophotometer. SOD enzyme activity was measured according to the method of Arora et al. (2002). Changes in the absorbance of the reaction solution at a wavelength of 560 nm were determined (Arora et al. 2002). Glutathione transferase (GT) activity was measured by the method of Carmagnol et al. (1981). GR activity was performed according to the method of Foyer et al. (1976). Absorption changes of both enzymes were recorded at 340 nm wavelength (Foyer et al., 1976).

2.7. Statistical analysis

Statistical analysis of the data was performed via SPSS V20 software. The data were analyzed by the ANOVA test, and the averages were compared based on Duncan's test at the 0.05 level (3 replicates). Then the charts were constructed with Excel software.

3. Results

3.1. Changes in quality features during the developmental stages of strawberry fruit

Data showed that the firmness of strawberry fruit texture decreased gradually from the green to the red stage and reached 2.1 N at the red fruit stage. Moreover, fruit weight increased significantly in each stage; the weight was measured at 1.8 g in green, 7.5 g in white, and 8.5 g when it is red. During the development of strawberry fruit, there was a significant difference in the amount of total soluble solids (8.5%) in the red fruit stage ($p < 0.05$) (Table 1).

Table 1 Fruit characteristics of the developmental stages of strawberry.

Fruit developmental stages	Weight (g)	Firmness (N)	Total SS (%)
Green	1.8 a	69.1 a	5.7 a
White	7.5 b	10.1 b	7.3 b
Red	15.8 c	2.1 c	8.5 c

Different letters in each column indicate significant differences ($p < 0.05$).

3.2. Changes in carbohydrate content during the developmental stages of strawberry fruit

The results indicated that fructose, glucose, and sucrose concentrations significantly changed during fruit developmental stages. Fructose, glucose, and sucrose levels in the green stage were determined as 0.8, 0.7, and 0.6 $g.g^{-1}FW$, and in the final stage were increased to 2.5, 2.8, and 2.1 $g.g^{-1}FW$, respectively. Fructose had the highest amount in white fruit, while glucose was the dominant sugar in the red stage (Figure 2). Evaluation of total carbohydrates in the developmental stages of strawberry fruit also showed significant changes, so total carbohydrate levels increased during fruit ripening. The total carbohydrate level in the green stage was 4.3 $g.g^{-1}FW$, and in the white and red stages increased to 7.8 and 9.8 $g.g^{-1}FW$, respectively ($p < 0.05$) (Figure 2).

3.3. Changes in the metabolic pathway of phenylpropanoids during the developmental stages of strawberry fruit

The results indicated that the amount of phenol in the green stage was 298 $mgGAEg^{-1}DW$, and in the white and red stages, it was 152.7 and 127 $mgGAEg^{-1}DW$, respectively. Evaluation of fruit developmental stages showed that total phenol content decreased significantly in the red stage compared to the green (Figure 3a). The results indicated a significant difference in flavonoid content during fruit development so in the green fruit stage, the highest amount of total flavonoid was observed, while flavonoid content decreased significantly in other stages (Figure 3b). Flavonoid content in the green stage was 14.68 $mgQEg^{-1}DW$, and in the white and red stages, it decreased to 6.09 and 5.57 $mgQEg^{-1}DW$, respectively. The flavonoid content showed no significant difference in the white and red fruit stages. Furthermore, PAL enzyme activity was 60 $U.mg^{-1}$ at the green stage and 33 $U.mg^{-1}$ at the white stage, while increased to 42 $U.mg^{-1}$ in the red fruit stage ($p < 0.05$) (Figure 3c).

3.4. Evaluation of antioxidant capacity and vitamin C content during the developmental stages of strawberry fruit

The results showed that there was no significant difference in antioxidant capacity in the developmental stages of strawberry fruit. Antioxidant capacity at the green, white, and red fruit stages was 99%, 98.9%, and 98.8%, respectively (Figure 4a). Moreover, the data in the three developmental stages of strawberry fruit implied that vitamin C levels increased significantly during fruit ripening. Vitamin C concentration at the green, white, and red stages was 0.05, 0.07, and 0.51 $mgAG^{-1FW}$, respectively ($p < 0.05$) (Figure 4b).

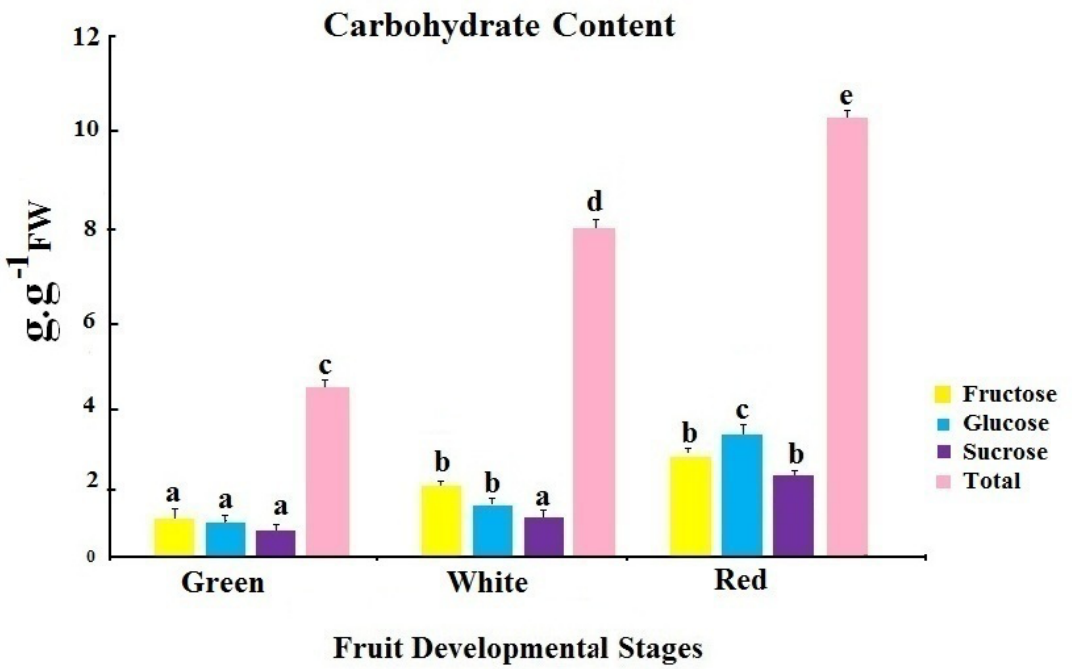

Figure 2. Sugar content during the developmental stages of strawberry fruit. Different letters in each column indicate significant change ($p < 0.05$). Error bars represent standard deviation (SD). Data are the mean ± SD of three replicates.

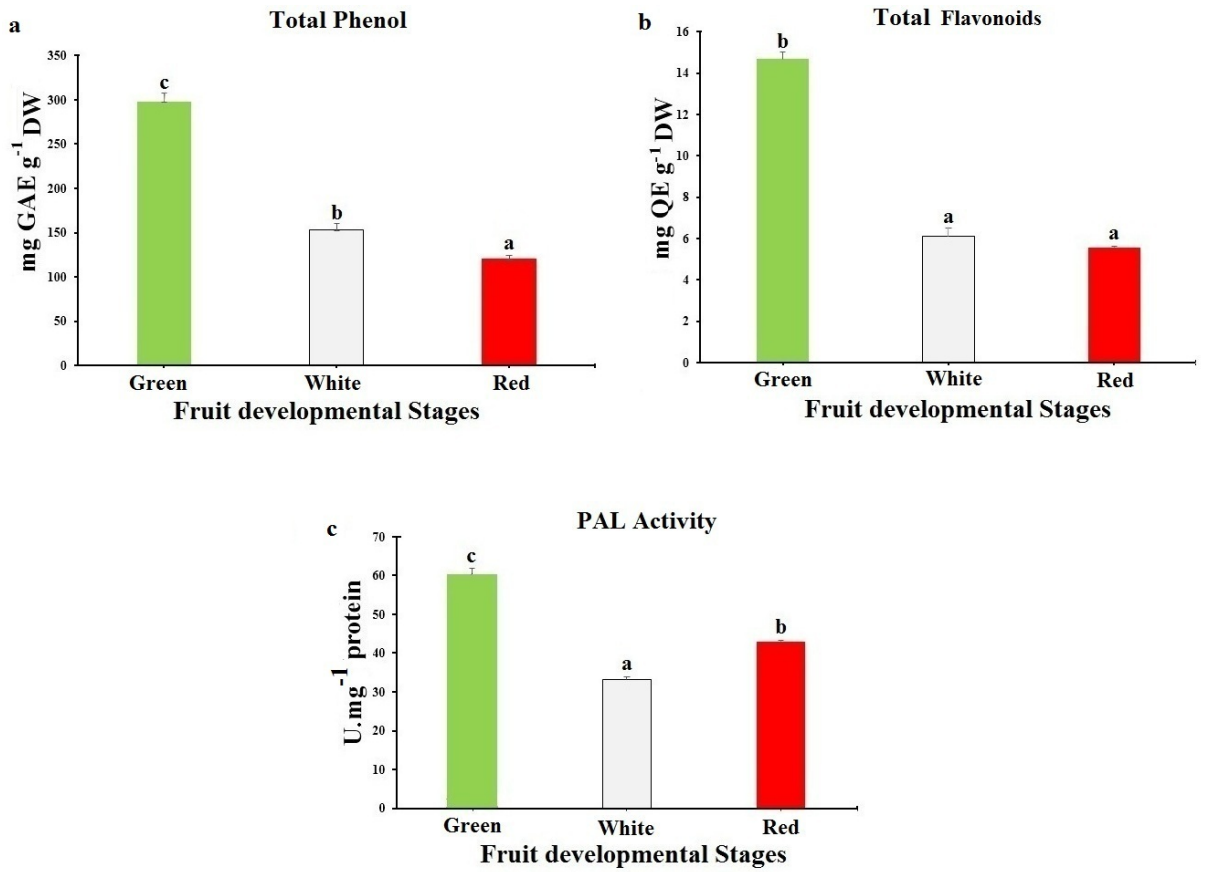

Figure 3. Changes in the metabolic pathway of phenylpropanoids during the developmental stages of strawberry fruit. a Total phenol, b Total flavonoid, c PAL enzyme activity. Different letters in each column indicate significant differences ($p < 0.05$). Error bars represent standard deviation (SD). Data are the mean ± SD of three replicates.

3.5. The activity of antioxidant enzymes during the developmental stages of strawberry fruit

The results of CAT enzyme activity during fruit developmental stages showed that the activity of CAT enzyme has no significant changes in the white stage to the green stage, while in the red stage increased significantly from 9.8 U.mg^{-1} (green fruit) to 16.8 U.mg^{-1} (red fruit) (Figure 5a). Changes in the activity of the SOD enzyme at the green, white, and red fruit stages were 44, 67.4, and 69 U.mg^{-1}, respectively. The comparison of SOD activity changes showed a significant increase in the white and red fruit stages in comparison to the green stage with no significant difference revealed between the red and white stages (Figure 5b). Furthermore, the results indicated no significant changes in PRX activity during the developmental stages of strawberry fruit (Figure 5c). According to the results, the activity of GR and GT enzymes increased during fruit developmental stages. Although the activity of both enzymes in the white and red stages revealed no significant difference, a significant difference was observed in the green fruit stage ($p < 0.05$) (Figure 5d, 5e).

4. Discussion

Different qualitative and phytochemical traits, including texture firmness, soluble solids, carbohydrate, total phenol, flavonoids, vitamin C contents, and antioxidant enzyme activity, were investigated to study the developmental changes in strawberry fruit during three stages. According to the present research, the firmness of strawberry fruit texture decreased gradually from the green to the red stage. The fruit ripening stage includes genetic, biochemical, and physiological changes such as pigment aggregation, sugar change, aromatic volatile compounds production, and fruit texture softening (Paniagua et al., 2014). Softening of fruit texture during its development is one of the last stages of fruit ripening (Paniagua et al., 2014). Softness and juiciness are the most essential characteristics of fruit texture. Both parameters are determined mainly by the characteristics of parenchymal cells, such as shape,

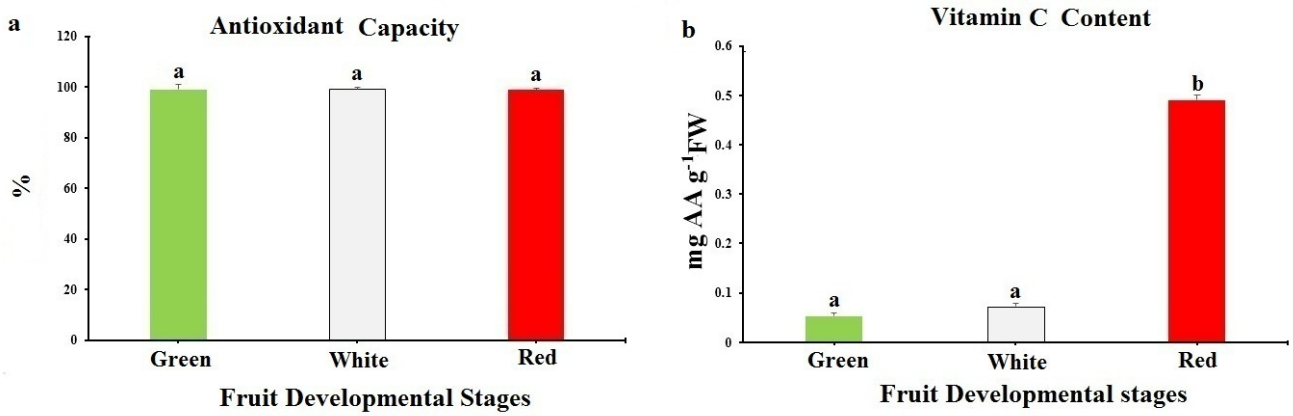

Figure 4. Evaluation of antioxidant capacity (a) and vitamin C (b) during the developmental stages of strawberry fruit. Different letters in each column indicate significant differences ($p < 0.05$). Error bars represent standard deviation (SD). Data are the mean ± SD of three replicates.

size, turgor pressure of cells, thickness, strength of cell walls, and adhesion between adjacent cells (Toivonen and Brummell, 2008). Softening of fruit tissue results from changes in cell wall structure such as reduction of hemicellulose and galactose, the activity of cell wall hydrolyzing enzymes, polymerization of glycan, and depolymerization of pectin. Pectin methyl esterase, pectate lyase, beta-galactosidase, and cellulase enzymes degrade the cell wall and consequently reduce the firmness of fruit tissue (Mercado et al., 2011). Moreover, the results of the present study showed that the concentration of soluble solids increased gradually during fruit development and reached the highest amount in the red stage. Three factors of changes in fruit weight, fruit juice, and dissolving of cell wall pectin and cell wall constituents in ripe strawberry fruit help to increase soluble solids (Bouzari et al., 2015). Since there is no significant storage starch in strawberry fruit cells, the increase in soluble solids can be associated with cell wall degradation (Cordenunsi et al., 2003).

Measurement of sugar content in strawberry fruit showed that fructose and glucose concentrations at the white and red stages were higher than sucrose concentrations at these stages. Glucose, fructose, and sucrose as soluble sugars, in addition to their role in glycolysis processes, have been identified as signaling molecules in plant metabolic processes associated with fruit ripening, such as pigment aggregation as well as association with hormone transfer pathways (Duran-Soria et al., 2020). On the other hand, these sugars are controlled by regulating gene expression and affecting mRNA or protein translation stability (SamKumar et al., 2022). Sucrose is the main sugar for long-term transportation to nonphotosynthetic organs such as fruits during development. When sucrose reaches the sink organ, it is converted into glucose and fructose by invertases (Duran-Soria et al., 2020). Glucose and fructose play a role in fruit development through intervention in specific biological pathways. Furthermore, sucrose can activate several genes and induce fruit-ripening processes (SamKumar et al., 2022). Changes in total carbohydrate content in the present study indicate that the concentration of sugars is higher in the final stages of fruit development than in the previous stages. During plant development, carbohydrates are transferred from photosynthetic organs (source) such as leaves to sink, including fruits. The amount and type of sugars accumulated during fruit ripening also affect fruit quality (Solfenalli et al., 2006). The increase in total carbohydrates in the white stage compared to the green stage of strawberry fruit indicates that sugar compounds, in addition to influencing taste, may also be involved in anthocyanin pathway biosynthesis (Rizwan et al., 2018). All substances synthesized in the leaf become available to the vegetative organs during the vegetative stage. However, later, with the formation of fruits (the reproductive stage of the plant), the produced material moves to the fruits (Solfenalli et al., 2006). On the other hand, there is a direct correlation between the total sugar content of fruits and leaves. Depending on the fruit's demands, the leaves also increase photosynthetic activity to meet the needs of the fruit. Therefore, source activity depends on sink requests (Solfenalli et al., 2006). A study of three strawberry cultivars also implied that the increase in sugar content might be due to the biosynthesis of carbohydrates from possible carbon sources such as organic acids and cell wall degradation (Cordenunsi et al., 2003). Organic acids and cell wall compounds are a more likely explanation because strawberry fruits do not contain enough starch to support this biosynthesis (Rizwan et al., 2018).

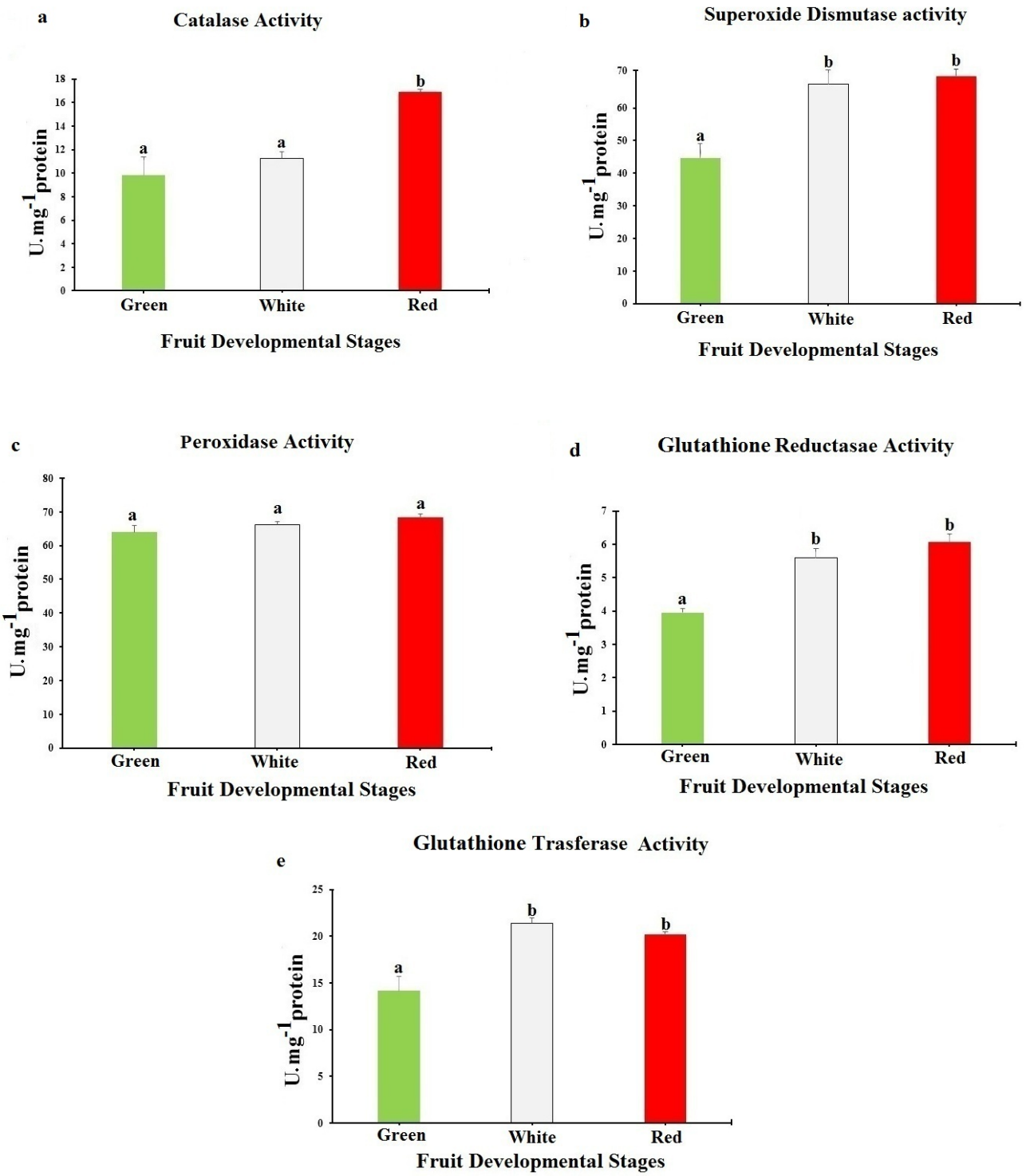

Figure 5. The activity of antioxidant enzymes during the developmental stages of strawberry fruit. a CAT, b SOD, c PRX, d GR, e GT. Different letters in each column indicate significant differences ($p < 0.05$). Error bars represent standard deviation (SD). Data are the mean ± SD of three replicates.

Evaluation of the metabolic pathway of phenylpropanoid showed that phenol, flavonoid content, and PAL activity during fruit development (red fruit) decreased significantly compared to the green fruit stage. Carbon enters the secondary metabolites pathway from primary metabolic compounds for synthesizing phenyl propionic (including flavonoids) by amino acid phenylalanine and PAL enzyme activity. PAL is the fundamental enzyme in the primary pathway of the synthesis of many natural compounds, such as flavonoids (Samykanno et al., 2013). In other words, a positive correlation exists between PAL activity and phenol and flavonoid content. The reduction of total phenol can be due to the release of polyphenol oxidase enzyme and the degradation of mono-phenolic and di-phenolic compounds in fruit (Nguyen et al., 2021). Decreased amounts of flavonoids may also be due to their use for the biosynthesis of other metabolites or covalent associations with other cellular components (Zhang et al., 2011). Differences in results may be due to growing conditions, maturity at harvest, cultivar, harvesting time, and using different solvents during sample extraction (Sun et al., 2018). Meanwhile, the transcription of genes encoding enzymes in the phenolic compound biosynthesis pathway also affects the amount of these compounds in different *Fragaria* species (Baldi et al., 2018).

This study also implied that vitamin C increases gradually during fruit development and is highest in the red stage. In contrast, the amount of antioxidant capacity was almost consistent at all three developmental stages. Strawberries, due to their vitamin C content, have a high antioxidant capacity against free radicals such as hydrogen peroxide, hydroxyl radicals, and singlet oxygen (Aharoni and Connell, 2002). Antioxidants affect fruit quality and can protect the fruit from environmental stress during the developmental stages by detoxifying ROS (Andreotti et al., 2022). Under optimal conditions, ROS such as superoxide, hydrogen peroxide, and hydroxyl radicals are produced as products of normal metabolism in different cular compartments (Li et al., 2013). Increased hydrogen peroxide levels during fruit ripening may also be associated with the onset of lipid peroxidation (Apel and Hirt, 2004). Membrane damage provides the basis for increased ROS leakage in ripening fruits, confirmed by K^+ redistribution, increased permeability, and oligosaccharide accumulation in the apoplast (Dumville and Fry, 2003). Transcription and gene expression during fruit ripening is also a stress-induced oxidative process (Andreotti et al., 2022). Although phenol content decreases, anthocyanin and vitamin C content increase in the ripening stage, thereby preserving the fruit's antioxidant capacity. Consequently, due to the stability of antioxidant capacity during fruit ripening, this function plays a vital role in many biochemical pathways during plant ripening (Aharoni et al., 2002). The pattern of changes in ascorbic acid content (the bioactive form of vitamin C) during fruit development is variable, and ascorbic acid content may increase, decrease or remain unchanged (Gomez and Lajolo, 2008). Changes in vitamin C content are due to changes in gene expression and the activity of enzymes involved in vitamin C metabolism during fruit maturation (Kim et al. 2017). Several chemical reactions during fruit ripening alter the vitamin C content of plant tissues, including chemical processes between plant tissue components, causing changes in color, taste, and odor (Moori et al., 2017). Indeed, vitamin C has limited stability, and environmental stimuli influence its degradation after fruit ripening. Mellidou et al. (2021) reported that vitamin C degradation is initially slow but gradually increases due to endogenous metabolism. The presence of ascorbate oxidase inhibitors in plant tissues may protect vitamin C from oxidation (Mellidou et al., 2021).

A study of the antioxidant enzyme activity (CAT, SOD, PRX, GR, and GT) at different developmental stages of strawberry fruit showed that the activity of these enzymes significantly increased during fruit development compared to green fruits. Oxidative stress during fruit development is the result of changes in osmotic pressure due to the accumulation and storage of compounds such as hexose, electron flow in mitochondria, and abiotic and biotic factors (Gill et al., 2010). It has also been reported that H_2O_2 increases during the ripening stage due to membrane lipid oxidation during softening and loosening of the fruit cell wall (Schweikert et al., 2002). Consequently, increased activity of antioxidant enzymes may be a means for plant resistance to ROS in ripe fruits. The activity of the enzymes PRX, CAT, GT, and SOD increases during fruit development, which may be related to the protective response of fruit to remove ROS. CAT is involved in cellular signaling processes by regulating hydrogen peroxide (Moori et al., 2017). When CAT activity decreases, the activity of other antioxidant enzymes increases through compensatory pathways. PRXs also commonly use different phenolic substrates to remove H_2O_2, which is why they are considered useful identifiers of oxidative processes (Moori et al., 2017). The GR enzyme influences the function of other enzymes and is involved in NADPH consumption during plant development. Indeed, GR causes the glutathione cycle to continue and is thus indirectly involved in the breakdown of hydrogen peroxide. It is strongly correlated with PRX and glutathione peroxidase, suggesting an important role for these enzymes in removing hydrogen peroxide generated (Gill et al., 2010). The activity of the ROS inhibitor system is also directly related to the CAT, GT, and GR enzymes, while GR acts as a cofactor in the activity of several enzymes (Sharma et al., 2018). In general, the antioxidant

content is not determined just by the variability of specific substances; the activities of different enzymes and chemical compounds are also involved in this phenomenon due to a complex process during fruit development (Sun et al., 2018). In other words, the activity of antioxidant enzymes in fruits is mainly influenced by variety, environmental conditions, fruit maturity, developmental stages, and harvest time (Chen et al., 2016).

5. Conclusion

Many physiological and biochemical changes are observed during strawberry fruit ripening. The present study evaluated changes in carbohydrate, phenolic compound content, and antioxidant enzyme activity of strawberry fruits at three developmental stages: green, white, and red fruit. The results showed that soluble solids increased significantly during fruit development. The results of carbohydrate studies on strawberry fruits revealed the important role of monosaccharides and disaccharides in strawberry ripening. Further research indicated glucose plays a more important role in later fruit development stages, while fructose levels are more involved in the middle stages of fruit development. It was also shown that phenolic and flavonoid content and PAL enzyme activity decreased during fruit development, while antioxidant capacity remained consistent at all three stages. It seems phenolic compounds and enzyme related play a major role in the early stages of strawberry development. The high antioxidant capacity of strawberries at all developmental stages, although phenol and flavonoid content decreased in the ripping stages, suggests that other antioxidant compounds, such as vitamin C and anthocyanin, play an important role in the maintenance of antioxidant capacity and the ripening of fruit. The decrease in phenolic compounds and the increase in carbohydrates during fruit ripening may also indicate the switching of primary and secondary metabolic pathways towards each other during fruit development; in other words, the coordination, alteration, and transformation of primary and secondary metabolites is part of the fruit ripening process. The results of antioxidant enzymes showed the CAT enzyme plays a vital role in the final stage of fruit maturation compared to other antioxidant enzymes. In contrast, the SOD, GR, and GT enzymes play a role in the intermediate stages of fruit maturation. The PRX enzyme is also highly active at all stages of fruit development. Increasing the activity of antioxidant enzymes is one of the ways to increase plant resistance to produce ROS during fruit development and ripening. The high activity of antioxidant enzymes during the developmental stages of strawberry fruits may also indicate the activation of oxidative stress signaling pathways during fruit ripening. The identification of phytochemicals and antioxidant capacity of strawberries during ripening reveals the role of various compounds and oxidative stress pathway signaling in fruit ripening; it can also contribute to the widespread application of strawberries in the edible and nonedible industries at various stages of fruit development.

Statements and declarations

The authors report no declarations of interest and that they are responsible for the content and writing of the article.

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