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Article

Drainage Recycling Ratio and Fruit Developmental Stages Affect Metabolite Profiles and Mineral Accumulation in Strawberries Under Hydroponics Cultivation

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Abstract

Drainage recycling management in recycling hydroponic systems affects root-zone conditions and fruit metabolism. However, its interaction with fruit developmental stages on strawberry biochemical quality and mineral content is unclear. Therefore, this study investigated how developmental stages and drainage recycling ratio influence antioxidant properties, pigment contents, carbohydrate accumulation, and mineral composition in hydroponically grown 'Seolhyang' strawberry. The experiment was conducted from 20 September 2024 to 31 March 2025 in a smart greenhouse. Plants were grown in coconut coir in a closed hydroponic system with 30%, 50%, 70% and 100% (open-system) drainage recycling ratios. Fruits were harvested at green, white, turning, and red stages on March 11, 2025. Fruit developmental stages primarily determined biochemical composition, while drainage recycling ratio modified traits in a stages-dependent way. Antioxidant properties including total phenolic content (TPC), total flavonoid content (TFC), ferric reducing antioxidant power (FRAP), and 2,2-diphenyl-1-picrylhydrazy (DPPH) radical-scavenging activity were highest in green fruit, especially under 30% drainage, reaching 45.8 mg GAE g^{-1} FW for TPC and 5.5 mg QE g^{-1} FW for TFC. Conversely, anthocyanin and soluble sugar accumulation increased during ripening, with 50% drainage recycling yielding the highest concentrations in red fruit (30.0 and 17.8 mg g^{-1} DW, respectively) and lower starch accumulation. Chlorophyll a, chlorophyll b, and total chlorophyll were also highest in green fruit and enhanced by 30% drainage. Mineral responses were less pronounced and primarily driven by developmental stages. Correlation analysis showed associations among phenolic compounds, antioxidant capacity, pigments, Ca, and Mg, while soluble sugars were negatively associated with early-development traits. Partial least squares analysis identified DPPH, TPC, TFC, FRAP, and anthocyanins as key variables distinguishing developmental stages and drainage recycling treatments. Drainage recycling management does not have a single optimum for strawberry fruit quality; its effects depend on the developmental target. A 30% drainage recycling regime favored phenolic-antioxidant characteristics in early-stages fruit, while 50% drainage recycling enhanced sugar accumulation and pigmentation at full ripeness. In contrast, a 70% drainage recycling ratio promotes the accumulation of Na^+, Cl^-, and SO_4^{2-}, disrupts nutrient balance, whereas an open system showed lower phytochemical accumulation. This stages-specific response

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helps guide drainage recycling management based on the harvest goals and shows that immature strawberries may be a useful source of phytochemicals.

Keywords: drainage recycling management; fruit maturity; antioxidant compounds; mineral composition; recycling hydroponics

1. Introduction

Strawberry *(Fragaria × ananassa* Duch.) is widely consumed due to its sensory attributes and recognized health benefits. It is rich in bioactive compounds, including phenolic acids, flavonoids, anthocyanins, vitamin C, and antioxidants, which contribute to its functional and nutraceutical value [1]. The accumulation of these compounds changes as the fruit develops and ripens, from the green and white stages to the fully red stages [2]. Therefore, understanding the biochemical changes that occur during strawberry maturation is essential for optimizing fruit quality and expanding its industrial applications.

Ripening involves physiological and biochemical changes that affect fruit quality and nutritional value. The distinct stages of ripening including green, white, turning, and red are marked by changes in color, texture, sugar accumulation, organic acid metabolism, aroma formation, and secondary metabolite synthesis [3]. Hormonal signaling, metabolic pathways, and environmental factors regulate these transitions. While most studies focus on fully ripe red fruits [4,5], immature and intermediate stages also exhibit valuable phytochemical profiles [6]. Green and white fruits often contain higher levels of certain phenolic acids and tannins (Figure 1). At the same time, the turning and red stages are enriched in anthocyanins, soluble sugars, and volatile aroma compounds (Figure 1) [7]. These metabolic profiles suggest that strawberries at different developmental phases may offer distinct functional properties relevant to fresh consumption and industrial applications.

In commercial strawberry production, thinning the fruit is commonly practiced to improve its final size, uniformity, and quality. Consequently, a significant quantity of fruit, especially that in the green stages, is removed and discarded as agricultural waste [8]. This results in the loss of valuable biological resources. However, evaluating the phytochemical composition, mineral content, and functional properties of these early-stages fruits could transform them into valuable raw materials for the pharmaceutical industry, functional foods, nutraceutical formulations, and food processing [6,8]. Therefore, understanding the biochemical potential of early-stages strawberries is significant from scientific, economic, and environmental perspectives.

Cultivation conditions determine the metabolic and phytochemical properties of fruits. Hydroponics cultivation supplies essential inorganic nutrients in a balanced manner through irrigation, unlike soil-based cultivation. However, in closed hydroponic systems, nutrient recirculation can alter the proportions of nutrients in the solution over time. Moreover, the drainage recycling ratio, a critical management factor, influences root-zone salinity, nutrient availability, oxygen status, and water stress, which in turn impacts primary and secondary metabolite synthesis, and mineral accumulation in fruits [4]. While previous research shows that cultivation systems and nutrient management affect fruit quality and metabolite accumulation [5], the combined effects of fruit ripening stages and drainage recycling ratio on the phytochemical composition of strawberries grown in closed hydroponic systems are not fully understood. Investigating these interactions is crucial given that phytochemical composition changes throughout fruit development and in response to cultivation management. Therefore, this study aims to evaluate the phytochemical composition of hydroponically grown strawberries at different ripening stages under varying drainage recycling ratios. The findings are expected to clarify how ripening stages and hydroponic management influence strawberry phytochemical properties, identify the functional value of early-stages fruits, and provide insights for developing sustainable hydroponic management strategies and expanding industrial applications of strawberries.

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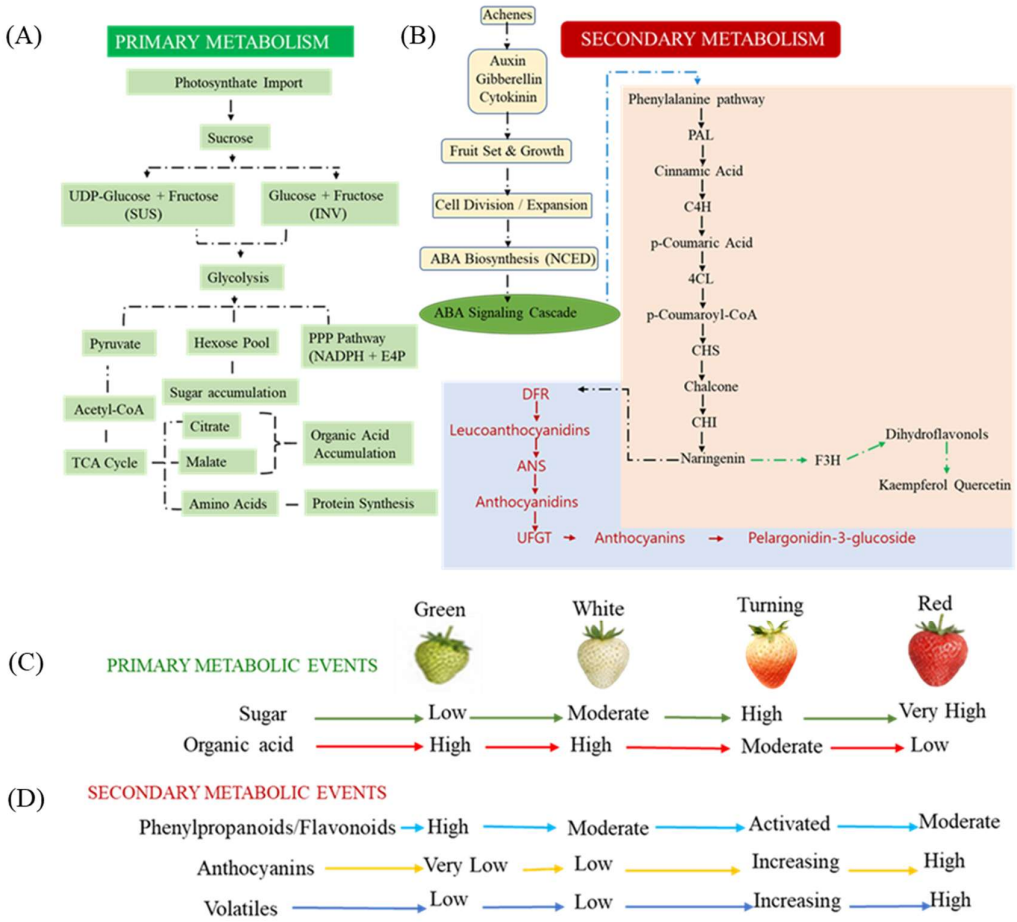

Figure 1. Schematic representation of primary and secondary metabolic pathways involved in strawberry fruit development and ripening. (A) Primary carbohydrate and organic acid metabolism; (B) phenylpropanoid and flavonoid biosynthesis; (C) sugar and organic acid metabolism across fruit developmental stages; and (D) phenylpropanoid, flavonoid, anthocyanin, and volatile compound biosynthesis during fruit development and ripening. The strawberry images illustrate the progression from the green stage to the red-ripe stage, highlighting coordinated changes in primary and secondary metabolism.

2. Materials and Methods

The experiment was conducted using the strawberry cultivar 'Seolhyang' in a smart greenhouse at Kangwon National University (Chuncheon, Gangwon-do, Republic of Korea). Uniform transplants were obtained from a commercial nursery located in PyeongChang, Korea. At the time of transplanting, seedlings had an average of 3.6 fully expanded leaves and a mean crown diameter of 12.1 mm, and were established at a planting density of 7.2 plants m^{-2}. Plants were cultivated in coconut coir substrates (100 × 20 × 12 cm; dust:chip ratio 10:0, v/v; Duck Yang Co., Sri Lanka) arranged on four suspended gutter lines (11 m × 27 cm, length × width) from 20 September 2024 to 31 March 2025. Greenhouse environmental conditions were automatically regulated using a Ridder CX500 climate control system (Ridder HortiMax, Harderwijk, The Netherlands). During the cultivation period, the mean daytime and nighttime air temperatures were maintained at 19.9 °C and 13.0 °C, respectively, while relative humidity averaged 74.8% during the day and 83.4% at night. Integrated solar radiation ranged from 801.5 to 2078.8 J cm^{-2}, with a mean value of 1292.9 J cm^{-2}.

Plants were supplied with the UoS nutrient solution developed by University of Seoul (UoS). The concentrations of nutrient solution are TN 7.0, P 2.1, K 4.0, Ca 3.0, Mg 1.5, and S 1.5; mEq L^{-1}. The electrical conductivity (EC) of the nutrient solution was maintained between 0.8 and 1.3 dS m^{-1}, and

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the pH was controlled within a range of 5.5–6.0 according to plant developmental stages. To mitigate potential nutrient deficiencies and physiological disorders, mono-potassium phosphate was supplemented at 18 g per 200 L (20% stock solution) from 11 January 2025 until the termination of the experiment. The collected drainage solution underwent two filtration steps: sand filtration followed by NUF membrane filtration (Caesarea Industrial Park, Caesarea, Israel). The solution was sterilized simultaneously during membrane filtration before being recycled. For sample analysis, additional filtration was performed for each treatment. Samples were first filtered through filter paper (Whatman No. 6, Kent, United Kingdom) and then through a syringe filter (25 mm, 0.45 μm; ANYLAB, Seoul, Republic of Korea). The concentrations of macro ions were then determined using ion chromatography (Eco IC, Metrohm AG, Herisau, Switzerland). A semi-closed hydroponic system was implemented with four drainage recycling ratio treatments: 30%, 50%, 70%, and 100% (open system as control). In the recycling treatments, irrigation was automatically regulated using a frequency domain reflectometry sensor (Coco Sensor; Mirae Sensor, Seoul, Korea), maintaining substrate volumetric moisture content between 66% and 71%, depending on plant developmental stages. In the control treatment, irrigation was timer-based, with 4–6 irrigation events per day depending on seasonal conditions. Each irrigation event delivered 25 mL of nutrient solution per plant, commencing at 09:00 and terminating three hours prior to sunset. The detail of irrigation and drainage recycling characteristics throughout the treatment period are presented on Table 1. On March 11, 2025, fruits from each drainage recycling ratio were harvested at four developmental stages: green (G), white (W), turning (T), and red (R). The harvested fruits were washed with distilled water, and stored at −20 °C until freeze-drying. Then the samples were chopped and freeze-dried using a freeze dryer (FDUT-6002; Operon, Gimpo-si, South Korea) at −110 °C. Representative fruit developmental stages are presented in Figure 2.

Table 1. Average irrigation and drainage characteristics and inorganic ion concentrations in nutrient solutions across different drainage recycling ratios during the treatment periods.

Treatment	Irrigation			Drainage			Drainage recycling ratio	Retained volume	Nutrient solutions (mEq·L⁻¹)-							
	Volume (mL·plant⁻¹)	EC (dS·m⁻¹)	pH	Volume (mL·plant⁻¹)	EC (dS·m⁻¹)	pH	(%)	(mL·plant⁻¹)	N	P	K	Ca	Mg	Na	Cl	S
Control	152.1	1.3	5.7	58.2	1.3	6.9	35.7	91.9	6.8	3.6	4.2	4.9	1.7	1.6	1.5	1.8
30%	132.6	1.3	6.0	42.5	1.5	6.9	28.2	90.1	6.0	3.0	3.9	4.3	1.6	2.0	1.8	2.2
50%	136.7	1.3	5.8	43.6	1.4	7.0	28.6	93.2	4.2	2.3	3.4	4.3	1.6	2.9	1.8	2.2
70%	129.2	1.3	5.8	48.7	1.4	7.0	34.4	80.7	3.5	2.2	2.8	4.6	1.7	3.9	2.2	2.5

The nutrient solution data represent the average values of measurements collected from January to March (n = 6).

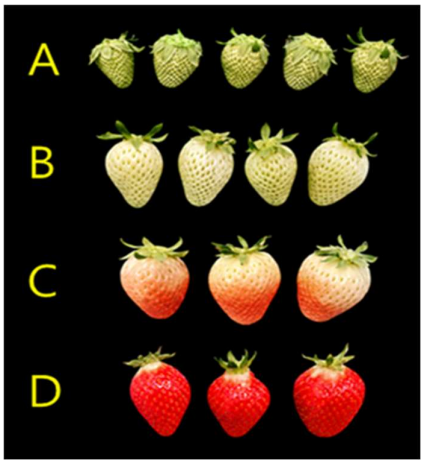

Figure 1. Different stages of 'Seolhyang' strawberry fruits used in this study; (A) green, (B) white, (C) turning and (D) red.

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2.1. Polyphenolic Compound Extraction and Antioxidant Assays

The dried samples were ground into a fine powder using a mortar and pestle. For extraction, 100 mg of powdered sample was placed in a 50 mL Falcon tube and mixed with 7 mL of 100% methanol. The mixture was vortexed and sonicated for 30 min using an ultrasonic cleaner (UCP-10; Seoul, South Korea). The extract was centrifuged at 4500 rpm for 20 min using a refrigerated centrifuge (LZ-1248R, Gyozen Co., Ltd., Gimpo, Korea). The supernatant was collected, and the remaining pellet was re-extracted with 3 mL of 80% methanol under the same conditions. Supernatants from both extractions were combined and stored at −20 °C until analysis. Total phenolic content (TPC) was determined using the Folin–Ciocalteu method. Briefly, 50 µL of extract was mixed with 2.95 mL of Folin–Ciocalteu reagent, followed by 0.75 mL of 20% sodium bicarbonate and 1.25 mL of dis-tilled water. After incubation in the dark for 30 min at room temperature, absorbance was measured at 760 nm using a UV–Vis spectrophotometer (UV-1800; Shimadzu Corporation). Results were expressed as mg gallic acid equivalents per gram of dry weight (mg GAE g^{-1} DW). Total flavonoid content (TFC) was determined by mixing 50 µL of extract with 1.5 mL methanol, 0.1 mL of 1 M sodium acetate, 0.1 mL of 10% aluminum chloride, and 2.95 mL distilled water. The mixture was incubated in the dark for 30 min, and absorbance was measured at 415 nm. Results were expressed as mg quercetin equivalents per gram of dry weight (mg QE g^{-1} DW). 2,2-diphenyl-1-picrylhydrazy (DPPH) radical scavenging activity was evaluated by adding 0.05 mL of extract to 2.95 mL of 0.1 mM DPPH solution in methanol. After incubation in the dark for 30 min at room temperature, absorbance was measured at 517 nm. Scavenging activity (%) was calculated as:

DPPH (%) = (Control absorbance-Sample absorbance)/ (Control absorbance) (1)

Ferric reducing antioxidant power (FRAP) was measured using freshly prepared FRAP reagent containing 10 mM TPTZ (in 40 mM HCl), 300 mM acetate buffer, and 20 mM $FeCl_3$ (2.5:2.5:5, v/v/v). A 0.05 mL aliquot of extract was mixed with 2.95 mL of FRAP reagent and incubated at 37 °C for 4 min. Absorbance was recorded at 593 nm, and results were expressed as mM Trolox equivalents per gram of dry weight (mM TE g^{-1} DW).

2.2. Chlorophyll Analysis

Chlorophyll content was determined using an acetone extraction method. Fresh fruit tis-sue (0.5 g) was homogenized in 10 mL of 80% (v/v) acetone. The homogenate was vortexed and sonicated for 20 min to ensure complete pigment extraction, then centrifuged at 5,000 rpm for 20 min at 4°C using a refrigerated centrifuge. The supernatant was collected and stored at −20°C in the dark until analysis. Absorbance was measured at 663, 645, and 470 nm using spectrophotometer to determine chlorophyll a, chlorophyll b, and total carotenoids, respectively. Pigment concentrations were calculated using the equations described by Zebro et al. [5].

2.3. Determination of Total Anthocyanin Content

Anthocyanins were extracted from strawberry fruit following a methanol-based extraction method. Fresh samples were freeze-dried at −110 °C for 3 days and subsequently ground into a fine powder using a mortar and pestle. A 1 g fruit powder was mixed with 10 mL of 50% (v/v) methanol solution adjusted to pH 2.0. The mixture was subjected to ultrasonic extraction for 10 min to enhance anthocyanin release, followed by centrifugation at 10,000 rpm for 10 min at 4 °C. The supernatant containing anthocyanins was collected and stored at −20 °C until further analysis. Total anthocyanin content was determined using a UV–Vis spectrophotometric method. The absorbance of the extracts was measured at 530, 620, and 650 nm. Anthocyanin concentration was calculated using the following equation:

Total anthocyanin content (µg·g−1) = 46,200×[(A530−A620) − 0.1×(A650−A620)] (2) where A530, A620, and A650 represent the absorbance values at 530, 620, and 650 nm, respectively.

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2.4. Determination of Soluble Sugars and Starch Contents

Soluble sugars were extracted from 0.2 g of freeze-dried strawberry powder using 80% (v/v) ethanol. The samples were incubated at 80 °C for 60 min, followed by centrifugation, and the supernatant was collected for soluble sugar analysis. Total soluble sugars were deter-mined using the anthrone method. Briefly, 0.05 mL of the extract was mixed with 3 mL of freshly prepared anthrone reagent (150 mg anthrone in 100 mL of 72% H_2SO_4), incubated at 100 °C for 10 min, and rapidly cooled in an ice bath. Absorbance was measured at 620 nm using a spectrophotometer. Soluble sugar content (mg g^{-1} dry weight) was calculated as 2.34 × (A + 0.07)/W, where A is the absorbance and W is the sample dry weight (g) [9].

For starch analysis, the residue remaining after ethanol extraction was treated with 30% (v/v) perchloric acid and incubated at 60 °C for 60 min. After centrifugation, the supernatant (acidic extract) was collected. Starch content was determined using the anthrone method by mixing 0.05 mL of extract with 3 mL of anthrone reagent, followed by incubation at 90 °C for 10 min and cooling to room temperature. Absorbance was recorded at 620 nm. Starch content (mg g^{-1} dry weight) was calculated as 0.578 × (A + 0.0295)/W, where A is the absorbance and W is the sample dry weight (g) [9].

2.5. Mineral Analysis

To determine macro- and micronutrient concentrations, fruits grown under different drainage recycling ratios were harvested at various developmental stages. The harvested fruits were freeze-dried and finely ground using a mortar and pestle. A 0.5 g portion of the powdered sample was transferred into a glass digestion flask, followed by the addition of 10 mL of 50% perchloric acid ($HClO_4$) and 1 mL of sulfuric acid (H_2SO_4). The mixture was thoroughly homogenized and subjected to acid digestion. Digestion was performed on a hot plate using a stepwise temperature program of 88 °C for 120 min, 180 °C for 30 min, and 350 °C for 30 min, until a clear solution was obtained. After cooling, the digested solutions were diluted with distilled water at a ratio of 1:1000 for macronutrient analysis and 1:100 for micronutrient analysis. The diluted samples were filtered sequentially through Whatman No. 6 filter paper (3 μm pore size) and a 0.45 μm syringe filter. Mineral concentrations were then determined using inductively coupled plasma optical emission spectrometry (Avio 220 Max, PerkinElmer, New York, USA). Standard solutions for both macro- and micronutrient analyses were purchased from PerkinElmer (New York, USA).

2.6. Statistical Analysis

All measured parameters were evaluated using three biological replicates. Statistical analyses were performed using SPSS software (version 26.0; IBM Corp., Armonk, NY, USA). Differences among treatments were determined by one-way analysis of variance (ANOVA), and mean comparisons were conducted using Duncan's Multiple Range Test (DMRT) at $p < 0.05$. Correlation analyses among variables were performed using SRplot and partial least squares (PLS) analysis was analyzed using MetaboAnalyst 6.0.

3. Results

3.1. Soluble Sugar and Starch Accumulation at Drainage Recycling Ratio and Different Stagess of Fruits

Figure 3 shows soluble sugar and starch contents of strawberry fruits at different maturity stages and drainage recycling ratios. Soluble sugar content increased throughout fruit development, peaking at the red stages (Figure 3A). During the green stages, soluble sugar was highest under 50% drainage recycling (9.1 mg g^{-1} DW) and lowest under 30% (6.6 mg g^{-1} DW). Starch was highest under 70% drainage recycling (6.1 mg g^{-1} DW) and lowest under 50% (4.2 mg g^{-1} DW) (Figure 3B). In the white stages, soluble sugar did not significantly differ among control, 30%, and 50% drainage recycling (10.6–11.1 mg g^{-1} DW), but decreased under 70% (9.8 mg g^{-1} DW). Starch was highest under control (4.7 mg g^{-1} DW), while 30%, 50%, and 70% drainage recycling showed significantly lower and

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similar contents (3.9–4.0 mg g^{-1} DW). At the turning stages, soluble sugar was highest under 50% drainage recycling (12.6 mg g^{-1} DW), not significantly different from control (12.1 mg g^{-1} DW), and lowest under 70% (10.5 mg g^{-1} DW). Starch was significantly highest in control (4.3 mg g^{-1} DW) and lowest under 30% (3.4 mg g^{-1} DW). In the red stages, soluble sugar was highest under 50% drainage recycling (17.8 mg g^{-1} DW), while control, 30%, and 70% drainage recycling had significantly lower values (15.1–16.2 mg g^{-1} DW). Starch peaked under control (3.8 mg g^{-1} DW) and decreased significantly with increasing drainage recycling intensity, reaching its lowest value (2.7 mg g^{-1} DW) under 50% drainage recycling.

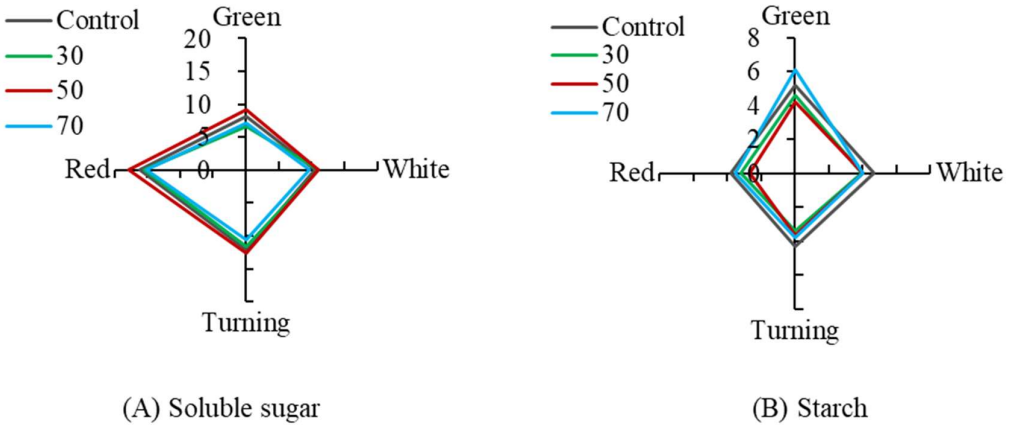

Figure 3. Effects of drainage recycling ratio and fruit developmental stages on soluble sugar and starch accumulation.

3.2. Chlorophyll Content at Different Drainage Recycling Ratio and Stagess of Fruits

Table 3 indicates that chlorophyll a, chlorophyll b, and total chlorophyll concentrations were significantly affected by fruit maturity stages, drainage recycling ratio, and their interaction. Carotenoid concentration was only significantly affected by the drainage recycling ratio. Highest chlorophyll concentrations occurred at the green stages, particularly with 30% drainage recycling. At this stages, 30% drainage recycling yielded significantly higher chlorophyll a (76.5 μg g^{-1} FW), chlorophyll b (107.3 μg g^{-1} FW), and total chlorophyll (183.8 μg g^{-1} FW) compared to control, 50%, and 70% treatments ($P < 0.05$). Carotenoid concentration was also highest at 30% drainage recycling (21.97 μg g^{-1} FW) during the green stages, with no significant differences among treatments. Lowest green stages chlorophyll concentrations were observed at 70% drainage recycling: chlorophyll a (30.0), chlorophyll b (42.6), and total chlorophyll (72.6 μg g^{-1} FW). At the white and red stages, no significant differences were found in chlorophyll or carotenoid concentrations among drainage recycling treatments. At the turning stages, 30% drainage recycling resulted in the highest chlorophyll a (24.5 μg g^{-1} FW), chlorophyll b (42.8 μg g^{-1} FW), total chlorophyll (67.4 μg g^{-1} FW), and carotenoid concentration (13.1 μg g^{-1} FW). Chlorophyll a, chlorophyll b, and total chlorophyll were significantly lower at 50% drainage recycling (21.8, 37.23, and 59.0 μg g^{-1} FW, respectively). Carotenoid concentration decreased at 50% and 70% drainage recycling, with the lowest value (10.9 μg g^{-1} FW) at 70%.

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Table 3. Effects of drainage recycling ratio and fruit developmental stages on chlorophyll and carotenoid concentrations.

Maturity stages (A)	Treatment (B)	Chlorophyll a	Chlorophyll b	Total chlorophyll	Carotenoid
		----------μg·g^{-1} FW----------			
Green	Control	32.6b	42.7b	75.4c	7.2a
	30%	76.5a	107.3a	183.8a	22.0a
	50%	37.4b	50.5b	87.9b	9.3a
	70%	30.0b	42.6b	72.6c	9.0a
	Average	44.2	60.8	104.9	11.9
White	Control	22.9a	36.1a	59.0a	9.4a
	30%	24.9a	43.0a	68.0a	12.9a
	50%	23.4a	38.9a	62.2a	11.1a
	70%	23.3a	36.3a	59.6a	9.3a
	Average	23.6	38.6	62.2	10.7
Turning	Control	23.4ab	40.3ab	63.7ab	12.1ab
	30%	24.5a	42.8a	67.4a	13.1a
	50%	21.8b	37.3b	59.0b	11.1bc
	70%	22.5ab	37.7b	60.2b	10.8c
	Average	23.1	39.5	62.6	11.8
Red	Control	23.0a	40.8a	63.8a	12.8a
	30%	22.5a	39.2a	61.7a	11.9a
	50%	19.8a	35.1a	54.9a	10.9a
	70%	21.3a	37.4a	58.7a	11.9a
	Average	21.6	38.1	59.8	11.8
A		***	***	***	ns
B		***	***	***	**
A × B		***	****	***	ns

Different lowercase letters within the same column indicate significant differences among treatments based on the multiple-comparison test applied ($p < 0.05$).

3.3. Antioxidant Properties at Different Drainage Recycling Ratio and Stagess of Fruits

Table 2 shows fruit maturity stages, drainage recycling ratio, and their interaction significantly affected TPC, TFC, FRAP, and DPPH. Green stages fruits had higher antioxidant properties than later stages. A 30% drainage recycling ratio resulted in the highest antioxidant concentrations (TPC: 45.8 mg GAE g^{-1} FW, TFC: 5.5 mg QE g^{-1} FW, FRAP: 7.9 mg TE g^{-1} FW, DPPH: 79.1%), significantly greater than the control, 50%, and 70% treatments ($P < 0.05$). Conversely, 70% drainage recycling yielded the lowest values (TPC: 16.3 mg GAE g^{-1} FW, TFC: 0.9 mg QE g^{-1} FW, FRAP: 3.7 mg TE g^{-1} FW, DPPH: 43.4%). At the white stages, TPC did not significantly differ among treatments (8.9–9.1 mg GAE g^{-1} FW). However, TFC, FRAP, and DPPH were significantly affected. The 30% ratio resulted in the highest TFC (1.8 mg QE g^{-1} FW), while 70% showed the highest FRAP (2.3 mg TE g^{-1} FW) and DPPH (20.2%). The control had the lowest DPPH (10.1%). At the turning stages, the 30% drainage recycling ratio showed the highest TFC (1.5 mg QE g^{-1} FW) and DPPH (18.9%). TPC was reduced under 50% and 70% treatments (7.4 and 7.2 mg GAE g^{-1} FW), while the control and 30% treatments showed comparable, higher TPC values (9.8 and 9.2 mg GAE g^{-1} FW). FRAP values ranged from 1.8 to 2.3 mg TE g^{-1} FW, with the control recording the highest. At the red stages, antioxidant concentrations were generally lower than at the green stages. The 30% drainage recycling treatment produced the highest

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TPC (8.5 mg GAE g^{-1} FW), FRAP (2.0 mg TE g^{-1} FW), and DPPH (21.6%), while the control showed the lowest DPPH (7.9%). The highest FRAP (2.1 mg TE g^{-1} FW) was recorded under the 70% treatment, statistically comparable to the 30% treatment. TFC was highest in the control (1.4 mg QE g^{-1} FW) and decreased significantly under 50% and 70% treatments.

Table 2. The effects of drainage recycling ratio and fruit developmental stages on antioxidant concentration.

Maturity stagess (A)	Treatment (B)	TPC (mg GAE·g^{-1} DW)	TFC (mg QE·g^{-1} DW)	FRAP (mg TE·g^{-1} DW)	DPPH (%)
Green	Control	27.2b	2.0b	5.2b	57.4b
	30%	45.8a	5.5a	7.9a	79.1a
	50%	27.3b	1.6b	5.4b	54.9b
	70%	16.3c	0.9c	3.7c	43.4c
	Average	29.1	2.5	5.6	58.7
White	Control	8.2a	1.1ab	1.7b	10.1c
	30%	9.1a	1.8a	2.1a	15.1b
	50%	9.0a	0.5b	2.0a	17.2ab
	70%	8.5a	0.5b	2.3a	20.2a
	Average	8.7	1.0	2.0	15.7
Turning	Control	9.8a	0.8b	2.3a	15.9b
	30%	9.2a	1.5a	2.2a	18.9a
	50%	7.4b	0.8b	1.8b	13.7c
	70%	7.2b	0.6b	2.0ab	15.8b
	Average	8.4	0.9	2.1	16.1
Red	Control	7.3ab	1.4a	1.7b	7.9c
	30%	8.5a	1.1ab	2.0a	21.6a
	50%	6.8b	0.7b	1.7b	13.7b
	70%	7.2ab	0.7b	2.1a	17.3ab
	Average	7.4	1.0	1.9	15.1
A		***	***	***	***
B		***	***	***	***
A× B		***	***	***	***

Different lowercase letters within the same column indicate significant differences among treatments based on the multiple-comparison test applied ($p < 0.05$). Abbreviations: TPC, total phenolic content; TFC, total flavonoid content; FRAP, ferric reducing antioxidant power; and DPPH, 2,2-diphenyl-1-picrylhydrazyl.

3.4. Anthocyanin Content at Different Stagess of Fruits And Drainage Recycling Ratio

Anthocyanin content increased as fruit matured (Figure 4). During the green stages, the 30% and 70% drainage recycling treatments had the highest anthocyanin contents (2.3 and 2.2 mg g^{-1} DW, respectively), significantly exceeding the control and 50% drainage recycling treatments ($p < 0.05$). In the white stages, the 30% drainage recycling treatment displayed the highest anthocyanin content (1.5 mg g^{-1} DW), followed by the 70% drainage recycling treatment (1.4 mg g^{-1} DW), while the control had the lowest value (0.8 mg g^{-1} DW). At the turning stages, the control treatment showed the highest anthocyanin content (10.5 mg g^{-1} DW), followed by the 50% drainage recycling treatment (9.2 mg g^{-1} DW), with the lowest value under the 30% drainage recycling treatment (3.7 mg g^{-1} DW). In the red stages, the 50% drainage recycling treatment showed the highest anthocyanin content (30.0 mg g^{-1}

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DW), significantly greater than the other drainage recycling treatments ($P < 0.05$). The lowest anthocyanin content (10.0 mg g^{-1} DW) was observed under the 70% drainage recycling treatment.

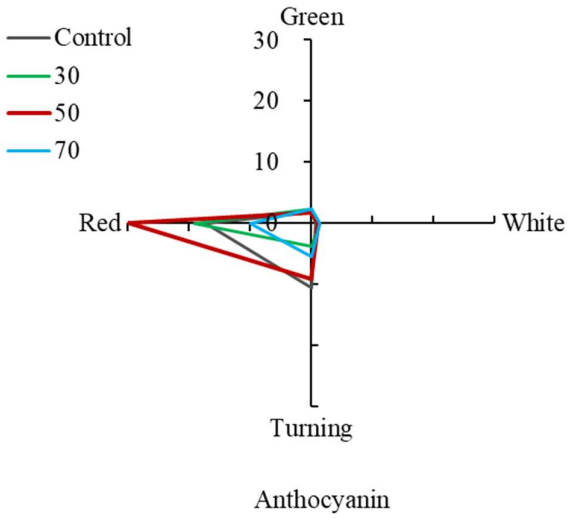

Figure 4. Effects of drainage recycling ratio and fruit developmental stages on anthocyanin accumulation.

3.5. Fruits Mineral Content at Different Stages of Fruits and Drainage Recycling Ratio

Table 4 details the mineral content of strawberries at different developmental stages and drainage recycling ratios. Fruit developmental stages significantly affected Ca, Mg, and P, while drainage recycling ratio significantly affected only P. No significant interaction was observed, and K and Na were unaffected by either factor. At the green stages, K, Na, and P were similar across drainage recycling treatments. However, 30% drainage recycling significantly increased Ca (1,864 mg kg^{-1}) and Mg (1,170 mg kg^{-1}) compared to control, 50%, and 70% drainage recycling ($P < 0.05$). The lowest Ca (1,329 mg kg^{-1}) and Mg (928 mg kg^{-1}) were in the control. At the white stages, no significant differences were found among drainage recycling treatments for any macronutrient. Numerically, 50% drainage recycling yielded the highest K (12,625 mg kg^{-1}), Na (344 mg kg^{-1}), P (3,786 mg kg^{-1}), and Ca (1,199 mg kg^{-1}), while 70% drainage recycling showed the lowest values for all measured nutrients. At the turning stages, no significant differences were observed among drainage recycling treatments for any macronutrient. The 70% drainage recycling resulted in the highest K (11,554 mg kg^{-1}), Ca (937 mg kg^{-1}), and Mg (758 mg kg^{-1}), while 30% drainage recycling produced the highest Na (361 mg kg^{-1}) and P (3,083 mg kg^{-1}). The lowest K (9,491 mg kg^{-1}), Ca (782 mg kg^{-1}), and Mg (613 mg kg^{-1}) were under 50% drainage. At the red stages, K, Ca, Mg, and P were not significantly different among drainage recycling treatments. However, Na was significantly affected by drainage recycling ratio; 30% drainage recycling had the highest Na (377 mg kg^{-1}), significantly greater than 50% drainage recycling (282 mg kg^{-1}) ($P < 0.05$). Numerically, 30% drainage recycling also showed the highest K (9,954 mg kg^{-1}), Ca (785 mg kg^{-1}), and Mg (632 mg kg^{-1}), while 70% drainage recycling recorded the lowest values for K (8,966 mg kg^{-1}), Ca (563 mg kg^{-1}), Mg (534 mg kg^{-1}), and P (2,447 mg kg^{-1}).

Furthermore, fruit developmental stages significantly affected Mn concentration, while drainage recycling ratio had no significant effect on any micronutrient (Table 4). A significant interaction was observed only for Mn. Fe, Cu, Zn, and B concentrations were not significantly influenced by either factor or their interaction. At the green stages, Fe, Cu, Mn, and B concentrations did not significantly differ across drainage recycling treatments. However, Zn concentration was significantly influenced by the drainage recycling ratio ($P < 0.05$). The 30% drainage recycling treatment yielded the highest Zn concentration (19.9 mg kg^{-1}), significantly greater than both the control (11.7 mg kg^{-1}) and 70% drainage recycling (13.0 mg kg^{-1}) treatments. Numerically, 30% drainage recycling also resulted in

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the highest Fe (51.9 mg kg^{-1}), Cu (9.0 mg kg^{-1}), and B (46.9 mg kg^{-1}) concentrations, while 70% drainage recycling recorded the highest Mn (14.4 mg kg^{-1}). At the white stages, no significant differences were observed among drainage recycling treatments for Fe, Cu, Zn, or B. However, Mn concentration was significantly affected by the interaction between fruit developmental stages and drainage recycling ratio. The control treatment produced the highest Mn concentration (11.2 mg kg^{-1}), significantly higher than that under 70% drainage recycling (8.5 mg kg^{-1}) ($P < 0.05$), with 30% and 50% drainage recycling showing intermediate values. Numerically, 50% drainage recycling resulted in the highest Cu (13.8 mg kg^{-1}), Zn (12.9 mg kg^{-1}), and B (67.3 mg kg^{-1}) concentrations, whereas 70% drainage recycling had the lowest Fe (45.3 mg kg^{-1}), Mn (8.5 mg kg^{-1}), and B (27.7 mg kg^{-1}) concentrations. At the turning stages, no significant differences were detected among drainage recycling treatments for any micronutrient. Numerically, 70% drainage recycling produced the highest Fe (52.4 mg kg^{-1}) and Zn (18.2 mg kg^{-1}) concentrations, while the control treatment recorded the highest Cu (14.9 mg kg^{-1}) and B (42.8 mg kg^{-1}) concentrations. The highest Mn (9.7 mg kg^{-1}) was observed under 30% drainage, whereas the lowest Fe (45.9 mg kg^{-1}), Cu (7.0 mg kg^{-1}), Mn (6.2 mg kg^{-1}), and B (31.8 mg kg^{-1}) concentrations occurred under 50% drainage. At the red stages, no significant differences were observed among drainage recycling treatments for Fe, Cu, Mn, Zn, or B. Numerically, the control treatment recorded the highest Fe (50.0 mg kg^{-1}) and Zn (11.8 mg kg^{-1}) concentrations. The highest Cu (16.3 mg kg^{-1}) was produced by 70% drainage, while the highest Mn (6.5 mg kg^{-1}) and B (48.1 mg kg^{-1}) were observed under 30% and 50% drainage, respectively. The lowest Fe (45.5 mg kg^{-1}), Mn (5.7 mg kg^{-1}), and B (39.7 mg kg^{-1}) concentrations were recorded under 70% drainage, while 50% drainage recycling resulted in the lowest Zn (8.7 mg kg^{-1}).

Table 4. Effects of drainage recycling ratio and fruit developmental stages on macro and micronutrient contents.

Maturation stage (A)	Treatment (B)	K	Ca	Mg	Na	P	Fe	Cu	Mn	Zn	B
		mg·kg^{-1}									
Green	Control	10,345a	1,329b	928b	252a	3,608a	46.1a	4.2a	12.5a	11.7b	31.5a
	30%	10,372a	1,864a	1170a	307a	4,244a	51.9a	9.0a	12.3a	19.9a	46.9a
	50%	10,653a	1,498ab	963b	234a	4,074a	50.9a	7.9a	12.7a	14.0ab	42.7a
	70%	10,355a	1,481ab	970b	289a	3,574a	47.9a	8.6a	14.4a	13.0b	41.0a
	Average	10,355	1,481	970	289	3,574	49.2	7.4	13.0	14.7	40.5
White	Control	11,859a	1,081a	883a	271a	3,600a	50.3a	4.0a	11.2a	11.4a	29.6a
	30%	10,757a	1,124a	884a	291a	3,617a	45.8a	2.0a	9.4ab	10.0a	27.1a
	50%	12,625a	1,199a	884a	344a	3,786a	50.3a	13.8a	9.6ab	12.9a	67.3a
	70%	8,626a	779a	625a	251a	2,879a	45.3a	10.5a	8.5b	10.5a	27.7a
	Average	10,967	1,046	819	289	3,471	47.9	7.6	9.7	11.2	37.9
Turning	Control	10,054a	867a	675a	287a	3,117a	50.0a	14.9a	6.2a	11.8a	42.8a
	30%	9,886a	831a	685a	361a	3,083a	47.2a	8.5a	9.7a	11.3a	32.8a
	50%	9,491a	782a	613a	335a	2,871a	45.9a	7.0a	7.6a	13.3a	31.8a
	70%	11,554a	937a	758a	353a	2,863a	52.4a	12.0a	8.7a	18.2a	38.6a
	Average	10,246	854	683	334	2,984	48.9	10.6	8.1	13.7	36.5
Red	Control	9,846	721	616	327ab	2,769	50.0a	14.9a	6.2a	11.8a	42.8a
	30%	9,954	785	632	377a	2,778	47.1a	8.4a	6.5a	10.9a	43.8a
	50%	9,794	696	601	282b	2,964	45.8a	13.9a	6.3a	8.7a	48.1a
	70%	8,966	563	534	304ab	2,447	45.5a	16.3a	5.7a	11.7a	39.7a
	Average	9,640	691	596	323	2,740	47.1	13.4	6.2	10.8	43.6
A		ns	***	***	ns	***	ns	ns	***	ns	ns
B		ns	ns	ns	ns	**	ns	ns	ns	ns	ns
A× B		ns	ns	ns	ns	ns	ns	ns	*	ns	ns

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3.6. Correlation Analysis

Correlation analysis revealed several strong associations among the measured parameters across strawberry fruit developmental stages under different drainage recycling ratios (Figure 5).

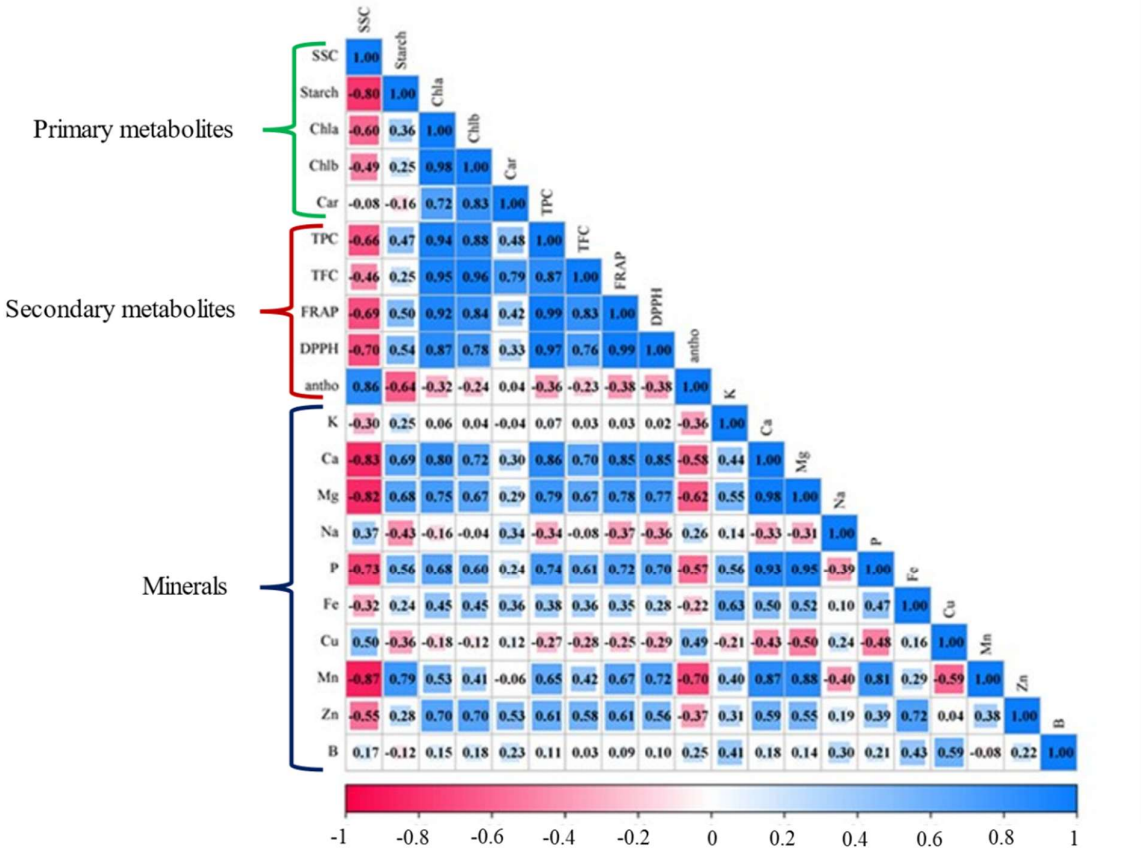

Figure 5. Correlation heatmap illustrating the pairwise relationships among biochemical, physiological, and mineral nutrient parameters measured in fruits at different developmental stages under varying drainage recycling ratios. Pearson's correlation coefficients (r) are displayed within each cell, with values ranging from −1 to +1. Blue shades indicate positive correlations, red shades indicate negative correlations, and the color intensity reflects the strength of the association. Where: Chla, chlorophyll a; Chlb, chlorophyll b; Car, carotenoids; SSC, soluble sugar content; TPC, total phenolic content; TFC, total flavonoid content; FRAP, ferric reducing antioxidant power; Antho, anthocyanin.; DPPH, 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity; K, potassium; Ca, calcium; Mg, magnesium; Na, sodium; P, phosphorus; Fe, iron; Cu, copper; Mn, manganese; Zn, zinc; B, boron.

The antioxidant-related parameters showed very strong positive correlations with each other. TPC was highly correlated with FRAP (r = 0.99), DPPH (r = 0.97), chlorophyll a (r = 0.94), chlorophyll b (r = 0.88), Ca (r = 0.86), and Mg (r = 0.79). Similarly, TFC exhibited strong positive correlations with DPPH (r = 0.76), FRAP (r = 0.83), chlorophyll a (r = 0.95), chlorophyll b (r = 0.96), and carotenoids (r = 0.79). FRAP was strongly associated with DPPH (r = 0.99), chlorophyll a (r = 0.92), Ca (r = 0.85), and Mg (r = 0.78), while DPPH showed strong positive correlations with chlorophyll a (r = 0.87), chlorophyll b (r = 0.78), Ca (r = 0.85), Mg (r = 0.77), and Mn (r = 0.72). The photosynthetic pigments were closely related. Chlorophyll a was strongly correlated with chlorophyll b (r = 0.98), carotenoids (r = 0.72), Ca (r = 0.80), Mg (r = 0.75), and Zn (r = 0.70). Chlorophyll b also showed strong positive correlations with carotenoids (r = 0.83) and Ca (r = 0.72). Among mineral nutrients, the strongest

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positive relationship was observed between Ca and Mg (r = 0.98). P was also strongly correlated with Ca (r = 0.93) and Mg (r = 0.95). In addition, Mn showed strong positive correlations with Ca (r = 0.87), Mg (r = 0.88), P (r = 0.81), and starch (r = 0.79). Fe and Zn were also strongly associated (r = 0.72). Strong negative correlations were mainly associated with fruit ripening traits. SSC was negatively correlated with DPPH (r = −0.70), starch (r = −0.80), Ca (r = −0.83), Mg (r = −0.82), P (r = −0.73), and Mn (r = −0.87). Anthocyanin showed a strong positive correlation with SSC (r = 0.86) but strong negative correlations with Mn (r = −0.70).

3.7. Partial Least Squares (PLS) Analysis

The Partial Least Squares (PLS) analysis was performed to identify the variables that contributed most to the discrimination of strawberry fruit developmental stages under different drainage recycling ratios (Figure 6). The Variable Importance in Projection (VIP) scores showed that DPPH radical scavenging activity was the most influential variable, with the highest VIP score. This was followed by TPC, TFC, FRAP, and anthocyanin content. These variables all had VIP values greater than 1, suggesting that antioxidant capacity and phenolic metabolites were the principal factors explaining the variation among fruit developmental stages and drainage recycling treatments. Chlorophyll a and chlorophyll b, together with copper (Cu), exhibited intermediate importance, with VIP values close to 1, indicating a moderate contribution to the PLS model. In contrast, mineral nutrients including Ca, B, Zn, Mn, Mg, Na, K, P, and Fe, as well as starch, carotenoids, and SSC, had VIP scores below 1.

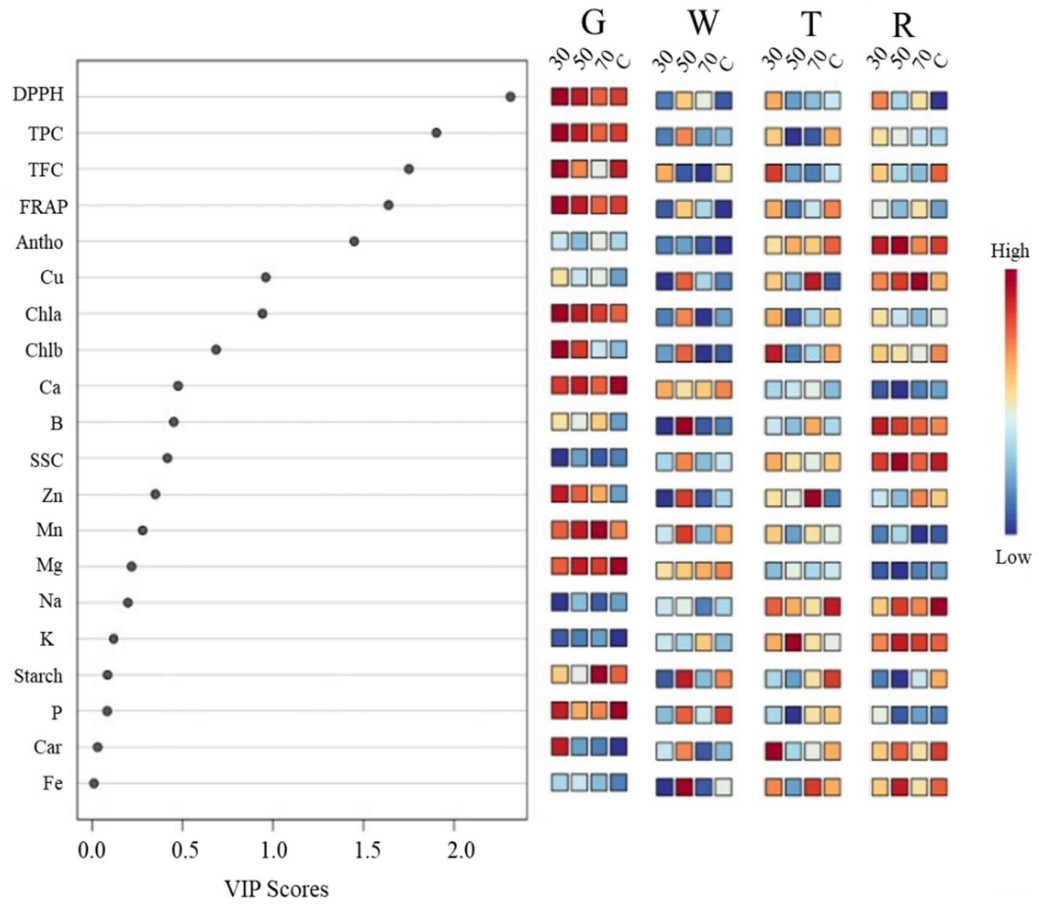

Figure 6. Variable Importance in Projection (VIP) scores and standardized heatmap of physicochemical, biochemical, and mineral attributes contributing to the discrimination of strawberry fruit developmental stages under different drainage recycling ratios. The VIP plot (left) ranks variables according to their importance in the partial least squares (PLS) model, where variables with VIP > 1 are considered the major contributors to sample

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dis-crimination. The heatmap (right) illustrates the relative abundance of each variable across the four fruit developmental stages, green (G), white (W), turning (T), and red (R) under 30%, 50%, 70% drainage recycling ratios and the control (C). Color intensity represents standardized values, with red indicating higher relative levels and blue indicating lower relative levels. Where: Chla, chlorophyll a; Chlb, chlorophyll b; Car, carotenoids; SSC, soluble sugar content; TPC, total phenolic content; TFC, total flavonoid content; FRAP, ferric reducing antioxidant power; Antho, anthocyanin.; DPPH, 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity; K, potassium; Ca, calcium; Mg, magnesium; Na, sodium; P, phosphorus; Fe, iron; Cu, copper; Mn, manganese; Zn, zinc; B, boron.

4. Discussion

Soluble sugar and starch contents showed a clear developmental transition: soluble sugars increased continuously from green to red fruit, whereas starch declined (Figure 3). This pattern aligns with the increased demand for soluble carbohydrates during strawberry ripening. Glucose, fructose, and sucrose are the principal soluble sugars in strawberry fruit, and their accumulation increases during maturation [10]. Sugars can also function as signaling molecules involved in strawberry fruit development and ripening [11]. The results therefore support that starch reserves are progressively mobilized and/or replaced by soluble carbohydrate accumulation as the fruit approaches full ripeness. The correlation analysis also reflected the inverse relationship between starch and soluble sugar (Figure 5). SSC was negatively correlated with starch ($r = -0.80$), while anthocyanin was strongly positively correlated with SSC ($r = 0.86$) (Figure 5). These associations are biologically coherent with the transition from growth-related carbon storage toward ripening-associated sugar accumulation and pigmentation. The correlation therefore indicates coordinated ripening rather than demonstrating that soluble sugars alone caused anthocyanin accumulation. Drainage recycling ratio modified carbohydrate accumulation in a stage-specific manner. At the red stage, 50% drainage recycling produced both the highest soluble sugar and the highest anthocyanin concentration, while starch was comparatively low (Figure 3). The higher retained nutrient solution volume under the 50% drainage recycling treatment likely maintained adequate root-zone water, supporting photosynthesis and the transport of photoassimilates to developing strawberry fruits (table 1). This improved carbon supply may have contributed to greater soluble sugar accumulation through enhanced sugar translocation and metabolism [12]. Sugars serve as metabolic substrates for flavonoid biosynthesis and can act as signaling molecules that regulate fruit ripening and anthocyanin accumulation through interactions with abscisic acid (ABA) signaling [13]. In contrast, the 70% treatment had lower soluble sugar and anthocyanin concentrations, consistent with the possibility that stronger ionic imbalance constrained carbon assimilation, transport, or allocation (Table 1, Figures 3 and 4). Recent work on semi-closed hydroponic strawberry systems similarly indicates that excessive recycling can progressively alter nutrient balance and reduce fruit growth [4].

Fruit developmental stage was the dominant factor determining the antioxidant profile of 'Seolhyang' strawberry, although drainage recycling ratio significantly modified these responses. TPC, TFC, FRAP, and DPPH were highest at the green stage and generally declined as fruit progressed toward the red stage (Table 2). This pattern is consistent with the developmental reorganization of strawberry secondary metabolism. During early fruit development, the flavonoid pathway is strongly active, favoring proanthocyanidins and other phenolic compounds, whereas later ripening redirects metabolism toward anthocyanin production and other compounds associated with fruit coloration and sensory quality [2].

Independent profiling of strawberries at several ripening stages has likewise shown that flavanols and hydroxybenzoic acid derivatives predominate in unripe fruit, whereas anthocyanins and hydroxycinnamic acid derivatives become more important in ripe fruit [14]. The particularly high antioxidant values in green fruit indicate a change in the composition of the antioxidant pool during development. The present results show that green fruit contained much higher TPC and TFC than white, turning, and red fruit (Table 2), while anthocyanin concentration increased strongly toward the red stage (Figure 4). Baldi et al. [2] demonstrated that flavonoid-related genes are activated during

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both early development and ripening, but the products associated with these phases differ: early activation is associated with proanthocyanidins and ellagic-acid-derived tannins, whereas ripening favors anthocyanins. Thus, the decrease in total phenolics and flavonoids observed here may reflect metabolic redistribution rather than a simple loss of antioxidant metabolites. The strong positive relationships among antioxidant properties further support the contribution of phenolic compounds to the measured antioxidant capacity (Figure 4). In particular, TPC was strongly correlated with FRAP and DPPH, while TFC was also strongly associated with FRAP and DPPH (Figure 5). Because Folin–Ciocalteu and colorimetric flavonoid assays are not compound-specific, these correlations should be interpreted as evidence of coordinated variation among broad antioxidant-related pools rather than proof that a particular phenolic compound caused the radical-scavenging response. Nevertheless, the consistency among the four assays indicates that developmental changes in phenolic metabolism were a major determinant of the antioxidant phenotype in this experiment.

Drainage recycling ratio significantly impacted antioxidant properties, with the 30% recycling treatment yielding the highest green-stage values and maintaining relatively high levels through the turning and red stages (Table 2). Conversely, the 70% recycling treatment generally resulted in the lowest green fruit antioxidant concentrations (Table 2). This suggests that drainage recycling effects on antioxidant properties are fruit developmental stage-dependent, possibly reflecting plant response changes during biochemical reprogramming. The 30% recycling treatment's high antioxidant values may rise from a favorable root-zone environment balancing nutrient availability and water conditions with moderate environmental stimulation. This treatment maintained intermediate concentrations of N, P, K, Ca, and Mg, unlike the control and 70% recycling treatments which showed the highest and lowest concentrations, respectively (Table 1). Therefore, the antioxidant response under 30% recycling may reflect a balance between sufficient nutrient supply and moderate root-zone stress, rather than solely higher nutrient availability. This is consistent with previous 'Seolhyang' strawberry research where moderate drainage recycling maintained stable ionic relationships, while higher ratios promoted Na^+, Cl^-, and SO_4^{2-} accumulation, potentially disrupting nutrient balance [4]. Mild abiotic stress can stimulate strawberry secondary metabolism; thus, increased antioxidant activity doesn't necessarily indicate improved plant performance. At the white stage, individual antioxidant assays showed varied responses. FRAP and DPPH values were highest with 70% recycling, TFC was highest with 30%, and TPC showed no significant difference (Table 2). These differences likely reflect the distinct chemical properties and sensitivities of each assay. DPPH assesses free-radical-scavenging, while FRAP measures ferric-reducing power. The contrasting white-stage responses suggest drainage recycling may influence antioxidant metabolite composition or activity without altering total phenolic content. These findings underscore the importance of using complementary antioxidant assays to fully capture drainage recycling's multifaceted effects on fruit antioxidant properties.

Pigment data explain the visible transition from green to red fruit. Chlorophyll a, chlorophyll b, and total chlorophyll were highest in green fruit, declining sharply before the white stage (Table 3). Anthocyanin concentration increased progressively, peaking in red fruit (Figure 4). These changes characterize strawberry ripening and reflect coordinated pigment metabolism remodeling. Transcriptomic and metabolomic studies show extensive reprogramming of pathways associated with flavonoid biosynthesis, starch and sucrose metabolism, and color development between green, white, turning, and red stages [15]. High chlorophyll concentrations in green fruit under 30% drainage recycling correlated with high TPC, TFC, FRAP, and DPPH values (Figure 5). Chlorophyll a and b also strongly correlated with TPC and TFC across the dataset (Figure 5), consistent with photosynthetic pigments and phenolic metabolism being prominent in actively developing green fruit. However, these correlations do not prove chlorophyll directly stimulates phenolic synthesis; both variables are influenced by developmental stage. In contrast, anthocyanin accumulation increasing significantly from green to red fruit (Figure 4). Red-stage fruit under 50% drainage recycling had the highest anthocyanin concentration, while 70% drainage recycling produced the lowest (Figure 4). The highest retained volume was observed under the 50% drainage recycling

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treatment, which may have enhanced the transport of photoassimilates to developing strawberry fruits and thereby contributed to increased soluble sugar accumulation (Figure 3). Given the positive relationship between soluble sugar content and anthocyanin accumulation (Figure 5), the observed response under the 50% drainage recycling treatment may be associated with the combined effects of greater retained volume and increased soluble sugar availability. In contrast, the 70% drainage recycling treatment showed the lowest retained volume (Table 1), which may have limited photoassimilate transport to the developing fruits and consequently reduced anthocyanin accumulation. Furthermore, anthocyanin biosynthesis is strongly associated with ripening and regulated by transcriptional, hormonal, and environmental signals [16]. Sucrose acts as both a carbon source and signaling molecule in strawberry ripening, while abscisic acid is linked to ripening transition and anthocyanin accumulation [8,17]. Environmental factors like light and temperature further modify secondary metabolism during ripening [18]. Thus, the high anthocyanin level under 50% drainage recycling may reflect an interaction between carbon availability, nutrient status, and the ripening-associated regulatory network.

Fruit mineral composition responded less to drainage recycling than antioxidant and pigment traits. Fruit developmental stage significantly affected Ca, Mg, and P, while drainage recycling significantly affected P overall and caused several stage-specific differences (Table 4). Potassium was stable across treatments and stages. Conversely, Ca and Mg declined significantly as fruit matured, particularly from the green to red stage (Table 4). The green-stage increase in Ca and Mg under 30% drainage recycling is notable because these nutrients correlated positively with TPC, FRAP, DPPH, and chlorophyll (Figure 5), suggesting adequate mineral nutrition supported physiological activity and secondary metabolism during early fruit development. Ca and Mg concentrations, antioxidant traits, and chlorophyll were all strongly affected by developmental stage and may therefore covary due to common developmental regulation. The strong Ca–Mg relationship and correlations of P with Ca and Mg further indicate coordinated mineral accumulation (Figure 5). Micronutrient concentrations were comparatively stable (Table 4). Only Mn showed a significant overall developmental effect, decreasing progressively from green to red fruit, with a significant stage × drainage recycling interaction (Table 4). This decline aligns with the transition from metabolically active green fruit to mature tissue, reflecting the reduced demand for Mn in photosynthetic processes and enzyme activation. Fe, Cu, Zn, and B were relatively stable, suggesting limited influence from fruit developmental stage and drainage recycling on their accumulation (Table 4). Correlation analysis indicates strawberry fruit quality was organized into two broads, partially contrasting metabolic patterns (Figure 5). The first consisted of antioxidant and photosynthetic traits, including TPC, TFC, FRAP, DPPH, chlorophyll a, and chlorophyll b. The second was associated with ripening, particularly soluble sugars and anthocyanins. Strong negative associations between SSC and DPPH, starch, Ca, Mg, P, and Mn reflect the shift from immature, phenolic-rich tissue toward mature, sugar- and anthocyanin-rich fruit (Figure 5). Similar developmental separation between phenolic profiles in unripe fruit and anthocyanin-rich profiles in ripe fruit has been documented in strawberry [2,14]. The PLS analysis provides a useful multivariate summary of these changes. DPPH, TPC, TFC, FRAP, and anthocyanin had VIP values above 1 and were therefore the variables contributing most strongly to discrimination among developmental stages and drainage recycling treatments (Figure 6). This result is consistent with the univariate findings, in which antioxidant traits showed large differences among stages and drainage recycling treatments, while many mineral traits showed relatively modest variation. The prominence of anthocyanin together with the antioxidant variables also confirms that the transition from green to red fruit is primarily characterized by a shift in secondary-metabolite composition.

5. Conclusions

This study demonstrated that fruit developmental stage was the primary factor influencing the biochemical composition, antioxidant properties, pigment accumulation, and mineral composition of ‘Seolhyang’ strawberries, while drainage recycling ratio modified these responses in a trait- and

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stage-dependent manner. During fruit development, soluble sugar and anthocyanin concentrations increased toward the red stage, whereas starch, chlorophyll, TPC, TFC, and antioxidant activities measured by FRAP and DPPH generally decreased. Among the drainage recycling treatments, 30% recycling generally promoted higher antioxidant properties, particularly in green fruit, whereas 50% recycling resulted in the highest soluble sugar and anthocyanin concentrations in red fruit. In contrast, 70% recycling generally reduced antioxidant properties and the accumulation of soluble sugars and anthocyanins in mature fruit. Mineral composition was comparatively less affected by drainage recycling, although several minerals, including calcium, magnesium, phosphorus, and manganese, exhibited developmental changes. Correlation and partial least squares analyse further highlighted the distinct metabolic patterns associated with antioxidant properties in immature fruit and sugar and anthocyanin accumulation during ripening. Further research across cultivars, seasons, and production environments is needed to validate these findings and establish optimal recycling strategies that improve strawberry fruit quality while promoting efficient water and nutrient management in hydroponic production systems.

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Abbreviations

ABA	**Abscisic acid**
ANOVA	Analysis of variance
DMRT	Duncan's multiple range test
DPPH	2,2-Diphenyl-1-picrylhydrazyl
DW	Dry weight
EC	Electrical conductivity
FRAP	Ferric reducing antioxidant power
FW	Fresh weight
GAE	Gallic acid equivalents
ICP-OES	Inductively coupled plasma optical emission spectrometry
PLS	Partial least squares
QE	Quercetin equivalents
SSC	Soluble sugar content
TFC	Total flavonoid content
TPC	Total phenolic content
TPTZ	2,4,6-Tris(2-pyridyl)-s-triazine

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