



Article

The Main Physicochemical Characteristics and Nutrient Composition during Fruit Ripening of *Stauntonia Obovatifoliola* Subsp. *Urophylla* (Lardizabalaceae)

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Abstract: *Stauntonia obovatifoliola* Hayata subsp. *urophylla* is a novel edible and healthy fruit in China, commonly known as “Jiuyuehuang” (September yellow). The fully ripe fruit of *S. obovatifoliola* subsp. *urophylla* has a soft fruit pulp texture, golden flesh, and sweet flavor which is very popular with the locals. In this paper, we have investigated the fruit appearance quality, physicochemical quality, and nutritional quality of *S. obovatifoliola* subsp. *urophylla* that was harvested at six stages (S1: 60 DAFB, S2: 90 DAFB, S3: 130 DAFB, S4: 160 DAFB, S5: 190 DAFB, S6: 205 DAFB). An increase in fruit size (including single fruit weight, fruit length, and fruit diameter) was related to the ripeness stage of fruit development. The total soluble solids, firmness, dry matter, sugar and starch showed remarkable changes as the fruit approached ripening (S5–S6 stage). The main sugar components in the fruit were fructose, glucose, and maltose. The contents of fructose, glucose, and total sugars in *S. obovatifoliola* subsp. *urophylla* fruit progressively increased from the S1 to the S6 stage while increasing sharply from the S4 to the S5 stage. As for the content of maltose and starch, they both showed an increasing trend from the S1 to the S4 stage but decreased sharply at the S5 stage. The vitamin B, vitamin C, total phenolics, total flavonoids, and amino acid levels showed an overall downward trend during fruit development. To our knowledge, this is the first study to compare the phytochemical characteristics, nutrient composition, and antioxidant content during the different fruit development stages. The results of this study may provide a scientific basis for clarifying the growth and development characteristics of *S. obovatifoliola* subsp. *urophylla* fruit and the further utilization of these excellent medicinal and edible germplasm resources.

Keywords: *Stauntonia obovatifoliola* subsp. *urophylla*; physicochemical; nutritional; ripening; maturity stage

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1. Introduction

Stauntonia obovatifoliola Hayata subsp. *urophylla* (Hand.-Mazz.) H. N. Qin is a perennial woody liana. It belongs to the *Stauntonia* genus, has large and edible fruits, and is endemic to China [1]. The species is widely distributed in the Yangtze River basin provinces of China (Guangdong, Guangxi, Hunan, Jiangxi, Fujian, Zhejiang, etc.) (Figure 1), normally occurring at an altitude of 500–1500 m in forest edges, roadsides, or along streams in valleys [2]. Its fruits are usually called September yellow (Jiuyuehuang in Chinese) by local villagers, which is due to the fruit appearing yellow in color when ripe in Chinese lunar September [1]. The fully ripe fruit of *S. obovatifoliola* subsp. *urophylla* has a soft fruit pulp texture, golden flesh, and a sweet flavor, tasting like a mixture of persimmon and litchi. *S. obovatifoliola* subsp. *urophylla* fruits are rich in sugars, crude proteins, vitamins, amino acids, mineral elements, fat, total dietary fiber, and reducing sugars [3,4]. In addition to its edible value, *S. obovatifoliola* subsp. *urophylla* is also a traditional Chinese

medicinal material. Its stems and fruits are rich in phenolics, flavonoids, triterpenes, sterides, and polysaccharides, and is usually used as an important medicinal material to treat rheumatic arthralgia, neuralgia, headache, heat strangury, trigeminal neuralgia, sciatica, and trauma pain [5–9]. These excellent nutritional and chemical compositions indicate that *S. obovatifoliola* subsp. *urophylla* is an alternative functional fruit that is worthy of development and utilization.

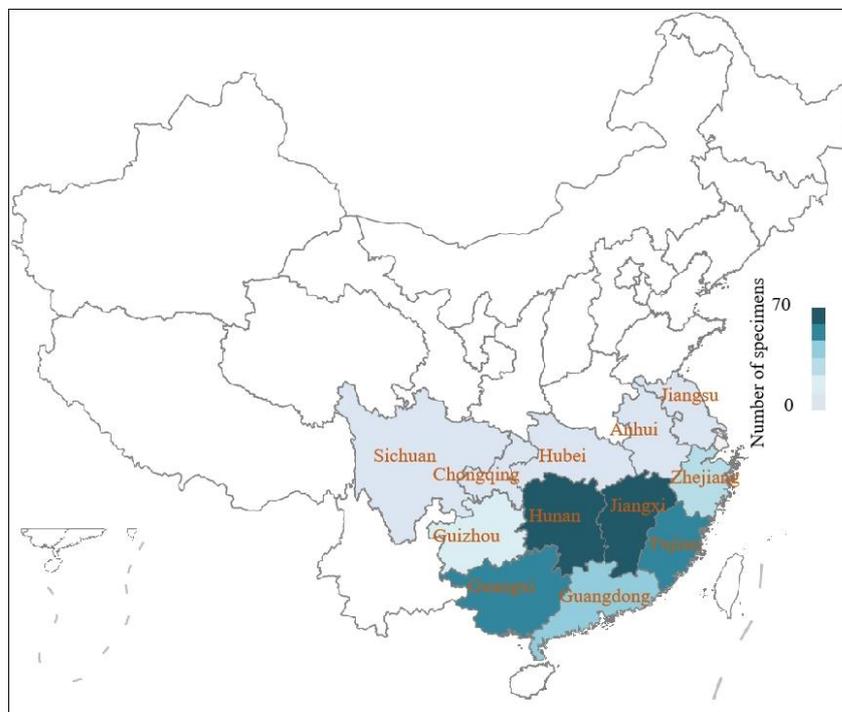


Figure 1. The geographic distribution of *Stauntonia obovatifoliola* subsp. *urophylla*. The distribution heat map was made based on the specimen data of *S. obovatifoliola* subsp. *urophylla* in a Chinese virtual herbarium.

In recent years, *S. obovatifoliola* subsp. *urophylla*, as a characteristic health fruit, has been widely cultivated in China. Even so, *S. obovatifoliola* subsp. *urophylla* is still an under-exploited wild fruit in the infancy stage of domestication. Only a few studies on *S. obovatifoliola* subsp. *urophylla* breeding, germplasm evaluation and cultivation have been attempted, with even most efforts on phytochemical analyses [1,3,5,10,11]. Moreover, most of the reports about *S. obovatifoliola* subsp. *urophylla* were found on the local flora with only a brief description [12–16]. The lack of information on the change patterns of the fruit's physiological quality and physicochemical characteristics during fruit ripening impedes the development and utilization of *S. obovatifoliola* subsp. *urophylla*.

Fruit ripening is a very important physiological process in the later stage of fruit development which is usually accompanied by tremendous changes in the physical and chemical characteristics, such as sugar composition, fruit texture and color change, aromatic substances release, cell wall degradation, and so on, which ultimately affects the quality of fruit [17–20]. The analysis of these physical and chemical changes occurring during the development and ripening of fruit gives an insight into the underlying biochemical and physiological processes taking place [21] and is especially useful for determining fruit maturity. Although ripening is the process by which fruits attain their desirable color, flavor, nutritional quality, and textural properties, appropriate ripeness can effectively guarantee the commercial value of the fruit and prolong the storage time of the fruit. For instance, yellow peach will have a poor flavor if harvested too early, while the fruit can present a strong characteristic flavor when harvested late; however, if the olive fruit is harvested too late, the sugar content in the pulp will decrease and be prone to

fibrosis [22,23]. In another example, in order to prolong the shelf life, winter jujube is usually harvested at the white maturity stage [24]. However, few studies are relevant to the change patterns in physicochemical indicators and nutrient composition of *S. obovatifoliola* subsp. *urophylla* at different fruit maturity stages. Therefore, it is necessary to explore the change patterns of *S. obovatifoliola* subsp. *urophylla* fruit's physiological parameters and physicochemical characteristics during fruit ripening, which provide basic physiological information for a better understanding of the ripening process of *S. obovatifoliola* subsp. *urophylla* fruit.

Since the optimum harvesting period is a considerable evaluation criterion for both food industries and consumers, the aim of the present study was to investigate the changes in the physicochemical characteristics, antioxidant content, and nutritional composition of *S. obovatifoliola* subsp. *urophylla* fruit at different maturity stages. The results of this work provide the basic dynamic change patterns of the fruit's physicochemical characteristics and explore a proper maturity stage at the harvest of *S. obovatifoliola* subsp. *urophylla* fruit with better quality, longer shelf life, and better market acceptability simultaneously.

2. Materials and Methods

2.1. Plant Materials

The plant materials were grown from seeds which were collected from Jiangxi Province, China, with relatively good comprehensive traits. A total of 56 plant materials were planted in 3×1.5 m in a fruit garden in Jiujiang City ($29^{\circ}38' N$, $115^{\circ}59' E$), Jiangxi Province, China. The fruits of *S. obovatifoliola* subsp. *urophylla* free from insects, pests, and diseases were randomly harvested at six developmental stages (S1: 60 days after full bloom (DAFB), S2: 90 DAFB, S3: 130 DAFB, S4: 160 DAFB, S5: 190 DAFB, S6: 205 DAFB) until the fruits became fully ripe (S6 stage) in November (Figure 2). The *S. obovatifoliola* subsp. *urophylla* trees were four years old. Open pollination and a sprinkling irrigation system were used in the experimental farm. Due to a single tree of *S. obovatifoliola* subsp. *urophylla* being unable to provide enough fruit samples for all six stages, different trees were used at each stage in this experiment. In total, twenty fruit trees were used in this study, and ten fruits were harvested from each tree; thirty fruits were harvested at each stage. The fruits harvested from the same tree were considered as a sample at each stage. Three fruits' pulp was mixed into one biological replicate for sugars, vitamin B, vitamin C, total phenolics, total flavonoids, starch, and amino acid content analysis. We cut the fruit into slices with a knife and removed the peel and seeds to separate the pulp, and then the pulp was quickly treated with liquid nitrogen and stored at $-80^{\circ}C$ until further analysis. In total, three biological replicates were conducted for each stage. The first sampling time was in June 2021, and the last sampling time was in November 2021. In this sampling period, the average daily temperature in Jiujiang was 20 – $28^{\circ}C$, and the total precipitation was 471.80 mm.



Figure 2. The fruits of *Stauntonia obovatifoliola* subsp. *urophylla* at different maturity stages.

2.2. Determination of Fruit Physical Parameters

The single fruit weight, fruit length, fruit diameter, and fruit shape index were evaluated immediately after the fruit samples were picked. The single fruit weight was recorded using an electronic balance. The fruit length and fruit diameter were measured using a digital caliper, and the ratio of fruit length to fruit diameter was the fruit shape index. The firmness of *S. obovatifoliola* subsp. *urophylla* fruit was determined by using a digital fruit firmness tester (GY-4, Zhejiang, China) equipped with a 3.5 mm cylinder probe, and the results were expressed as kg/cm². The dry matter content and moisture content in *S. obovatifoliola* subsp. *urophylla* fruit were measured by the drying–weighing method. Thin slices of fresh fruit were weighed using an electronic balance and then dried at 70 °C in an oven until their weights became constant. The dry matter content is calculated as the percentage of dry weight to fresh weight. Conversely, the moisture content is the percentage of lost weight to fresh weight.

2.3. Determination of Biochemical Parameters

The total soluble solids content (TSS) of *S. obovatifoliola* subsp. *urophylla* fruit was measured by an ATAGO (PAL-1) handheld digital refractometer, and the results were expressed in °Brix. The content of titratable acid (TA) was measured by the acid–base neutralization titration method, and the values were calculated as % of citric acid. The protein content in *S. obovatifoliola* subsp. *urophylla* fruit pulp was measured by the Kjeldahl method, and the results of protein content were expressed as N × 6.25 [25].

2.4. Determination of Carbohydrates

The sugar components (glucose, fructose, maltose, and sucrose) were measured by high-performance liquid chromatography (HPLC-E2695, Waters, Milford, MA, US) equipped with a differential refraction detector, and the sample preparation and chromatographic conditions were conducted as described in the previous study [26].

The starch content of *S. obovatifoliola* subsp. *urophylla* fruit pulp was also determined by the method described in our previous study [26]. Briefly, approximately 1 g of fruit pulp samples and 20 mL of 80% ethanol were added together and vortex mixed to promote particle dispersion. Subsequently, the sample was incubated in the water bath at 80 °C for 30 min and stirred continuously until cooling. Then, the sample was filtered with 80% ethanol, and the filter residue was washed in a centrifuge tube by using hot distilled water and placed in a boiling water bath for gelatinization. Then, we added 2 mL of cold 9.2 mol/L perchloric acid and incubated for 15 min in a boiling water bath, and then filtered the mixture. The filtrate was poured into a 100 mL volumetric flask, and the filtrate residue was washed with distilled water. The absorbance was read at 490 nm by a differential refraction detector.

2.5. Determination of Vitamin B, Vitamin C, Total Phenolics, and Total Flavonoids

The Vitamin B₁, Vitamin B₂, Vitamin B₃, Vitamin B₆, and Vitamin C contents of *S. obovatifoliola* subsp. *urophylla* fruit pulp were measured by HPLC (HPLC-E2695, Waters, Milford, MA, US) equipped with a diode array detector. Briefly, approximately 0.5 g of fruit pulp samples were weighed into a 15 mL centrifuge tube, and 5 mL of 0.1% hydrochloric acid aqueous solution was added. After 2 min of shock, the sample was extracted by ultrasonic wave (20 kHz, 30 °C) for 20 min and then centrifuged at a low temperature (4 °C) at a rate of 8000 r/min for 10 min. After the supernatant was filtered by a 0.45 µm filter membrane, then the sample was detected by HPLC. The detection conditions were as follows: a C18 chromatographic column (4.6 mm × 250 mm, 5 µm) was used for analysis; the mobile phase consisted of monopotassium phosphate and acetonitrile (80% and 20%, respectively) at a flow rate of 1.00 mL/min. The column temperature was 30 °C, and the injection volume was 10 µL.

The total phenolics content in the *S. obovatifoliola* subsp. *urophylla* fruit pulp was determined with Folin–Ciocalteu reagent following the method of Razzaq et al. [27]. Additionally, the total flavonoids content was measured following the method of Zhao et al. [28]. The detailed detection methods could be found in our previous study [26].

2.6. Determination of Amino Acids

The amino acid content in the *S. obovatifoliola* subsp. *urophylla* fruit pulp was measured by HPLC. Briefly, approximately 0.5 g of ground sample was put into the hydrolysis tube, 6 mL of 6 mol/L hydrochloric acid was added into the hydrolysis tube, and 3–4 drops of phenol were added, and then filled with high-purity nitrogen and sealed. The hydrolysis tube was placed in a constant temperature drying oven and hydrolyzed at 115 °C for 23 h. Then, we took out the hydrolysis tube from the oven and cooled it, adjusted the PH = 7 with sodium hydroxide solution, and added water to 7 mL for further analysis. Subsequently, 10 µL of extract solution was absorbed into the derivative tube, and 70 µL AccQ-Fluor borate buffer was added for vortex mixing. Then, another 20 µL AccQ-Fluor derivator was added to the tube, and vortex mixing was maintained for 10 s. We placed the tube at room temperature for one minute and then heated it in the oven at 55 °C for 10 min. Finally, we took out the hydrolysis tube from the oven and cooled it to ambient temperature, then transferred it to the fully recovered sample bottle for machine determination. AccQ-Tag column (3.9 × 150 mm) for amino acid analysis was used. The detection conditions were as follows, Mobile phase A: sodium acetate buffer solution; Mobile phase B: acetonitrile; Mobile phase C: pure water. Gradient elution was performed according to the Table 1.

Table 1. The elution gradient of mobile phase.

Time (min)	Flow Rate (mL/min)	Sodium Acetate Buffer (%)	Acetonitrile (%)	Pure Water (%)
start	1.0	100	0	0
0.5	1.0	98	2	0
13	1.0	95	5	0
19	1.0	91	9	0
29.5	1.0	83	17	0
33	1.0	0	60	40
36	1.0	100	0	0
45	1.0	100	0	0

The column temperature was 37 °C, and the injection volume was 10 µL. The excitation wavelength of the fluorescence detector was 250 nm, and the emission wavelength was 395 nm.

2.7. Statistical Analysis

SPSS v20.0 software was used to analyze the difference significance of the data (one-way ANOVA, LSD test, $p < 0.05$), and the results were expressed as means ± standard errors.

3. Results

3.1. Dynamic Changes of Appearance Quality during Fruit Development

The measurement results of the appearance quality of *S. obovatifoliola* subsp. *urophylla* fruit at different development stages were shown in Table 2. The single fruit weight began to increase rapidly at 60 DAFB, and the growth trend slowed down after 160 DAFB, entering the slow growth stage, and the single fruit weight increased to 192.72 ± 10.51 g during the period of the S1 to the S4 stages. As the fruit was close to ripening, the single

fruit weight increased more slowly and had the highest weight value at the S6 stage. The growth patterns of the fruit length and fruit diameter were basically the same, and the rapid growth period of them was from 60 DAFB to 130 DAFB, and then they entered the slow growth period. The fruit shape index did not change much throughout the development period; the fruit length was about twice as long as the fruit diameter, indicating that the fruit length and fruit diameter developed simultaneously during the fruit growth.

Table 2. Dynamic changes in appearance quality during *S. obovatifoliola* subsp. *urophylla* fruit development.

Stages	Single Fruit Weight/g	Fruit Length/mm	Fruit Diameter/mm	Fruit Shape Index
S1	14.08 ± 1.28 e	47.79 ± 1.98 d	24.12 ± 0.58 d	1.98 ± 0.07 ab
S2	83.86 ± 4.45 d	84.24 ± 3.90 c	39.54 ± 0.87 c	2.14 ± 0.15 a
S3	133.75 ± 3.02 c	96.40 ± 3.95 b	52.54 ± 1.54 b	1.84 ± 0.08 b
S4	192.72 ± 10.51 b	111.62 ± 5.03 a	53.94 ± 1.65 b	2.08 ± 0.12 ab
S5	230.57 ± 8.15 a	114.21 ± 3.94 a	59.57 ± 0.24 a	1.92 ± 0.07 ab
S6	240.40 ± 2.22 a	118.77 ± 3.27 a	62.86 ± 1.93 a	1.89 ± 0.03 ab

Means with different letters within the same column indicate statistical differences at the $p < 0.05$ level. S1: 60 DAFB (days after full bloom), S2: 90 DAFB, S3: 130 DAFB, S4: 160 DAFB, S5: 190 DAFB, S6: 205 DAFB.

3.2. Dynamic Changes of Total Soluble Solids, Titratable Acidity, Dry Matter, and Fruit Firmness during Fruit Development

The total soluble solids content fluctuated below 2° Brix in the early stages (S1–S4). Whereas there were no significant differences between those stages, thereafter, the total soluble solids content of the fruit pulp increased sharply during the S5 and S6 stages and reached the maximum value of 13.52° Brix in the ripening stage S6 (Figure 3A). Although the titratable acidity content maintained an increasing trend during the whole fruit development period, the titratable acidity content of the *S. obovatifoliola* subsp. *urophylla* fruit maintained at a low level, and the values fluctuated in the range of 0.62–0.84% during the period of the S1 to the S6 maturity stage (Figure 3B).

The content of dry matter content increased continuously in the early stage of fruit development (S1–S4) and reached the maximum value of 27.90% at the S4 stage but decreased slightly during the period from S5 to S6 (Figure 3C).

The fruit firmness of *S. obovatifoliola* subsp. *urophylla* was significantly ($p < 0.05$) affected by the fruit development stage. The fruit firmness also maintained an increasing trend during the early fruit development period and reached the maximum value of 127.53 kg/cm² at the S4 stage but decreased sharply during the period from S5 to S6, and had a minimum value of 15.75 kg/cm² at the S6 stage (Figure 3D).

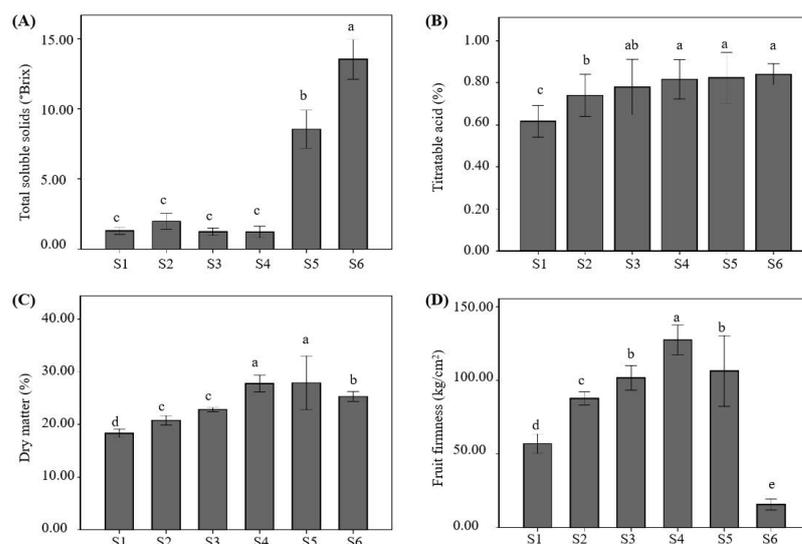


Figure 3. Total soluble solids (A), titratable acidity (B), dry matter (C), and fruit firmness (D) in *S. obovatifoliola* subsp. *urophylla* fruit at different ripening stages. Means with different letters within the same fruit character indicate statistical differences at the $p < 0.05$ level using the LSD test. S1: 60 DAFB (days after full bloom), S2: 90 DAFB, S3: 130 DAFB, S4: 160 DAFB, S5: 190 DAFB, S6: 205 DAFB.

3.3. Dynamic Changes of Carbohydrate Contents during Fruit Development

Carbohydrate contents in *S. obovatifoliola* subsp. *urophylla* fruit showed significant changes during fruit development and ripening (Figure 4). Only three (fructose, glucose, and maltose) of the four tested soluble sugars were detected in the fruit pulp of *S. obovatifoliola* subsp. *urophylla*, of which sucrose was not detected by HPLC. The fructose contents were very low before the S5 stage and fluctuated from 0.10 g/100 g FW to 0.33 g/100 g FW. As Figure 4A showed, the fructose content accumulated rapidly during the S5 and S6 stages and reached the maximum value of 5.35 g/100 g FW at the mature stage (S6). Similarly, the glucose showed the same accumulation pattern as the fructose, mainly accumulated during the S5 and S6 stages and reaching the maximum value of 7.02 g/100 g FW at the S6 stage (Figure 4B). The maltose content of *S. obovatifoliola* subsp. *urophylla* fruit continuously accumulated from the S1 to the S3 stage (0.72–1.22 g/100 g FW) and reached the maximum value at the S3 stage, although it had a slight decrease at the S4 stage, whereafter, a sharp and significant decline during the S5 and S6 stages were detected (0.16 g/100 g FW and 0.08 g/100 g FW, respectively) (Figure 4C). The contents of the total sugars of the *S. obovatifoliola* subsp. *urophylla* fruit were relatively low and changed little in the early stages of fruit development (S1–S4) and began to increase significantly from the S5 stage and reached the maximum value at the S6 stage (12.45 g/100 g FW) (Figure 4D). The starch content of *S. obovatifoliola* subsp. *urophylla* fruit continuously accumulated from the S1 to the S4 stage (0.21–1.09 g/100 g FW) and reached the maximum value at the S4 stage, but had a sharp decrease at the S5 stage, and finally reached the minimum value at the mature stage (S6) (0.07 g/100 g FW) (Figure 4E).

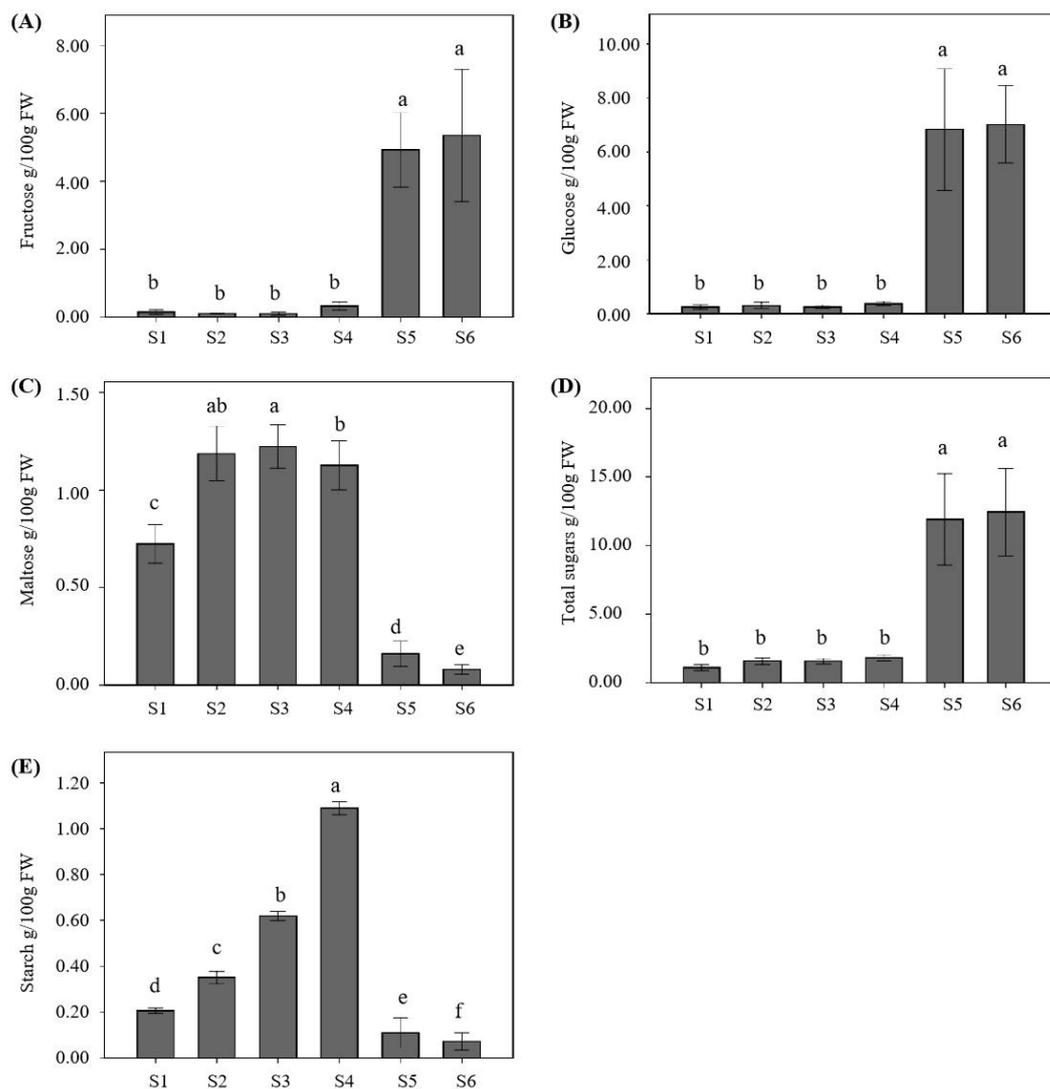


Figure 4. Dynamic changes of concentrations of fructose (A), glucose (B), maltose (C), total sugars (the sum of glucose, fructose, and maltose) (D), and starch (E) during the fruit development of *S. obovatifoliola* subsp. *urophylla*. The different letters in each figure indicate significant differences at the $p < 0.05$ level using the LSD test. S1: 60 DAFB (days after full bloom), S2: 90 DAFB, S3: 130 DAFB, S4: 160 DAFB, S5: 190 DAFB, S6: 205 DAFB.

3.4. Dynamic Changes of Vitamin B, Vitamin C, Total Phenolics, Total Flavonoids, and Protein Contents during Fruit Development

The changes in antioxidant component and protein contents during *S. obovatifoliola* subsp. *urophylla* fruit development were shown in Table 3. The vitamin B1 content increased from the S1 to the S2 stage and reached the maximum value at the S2 stage (20.24 ± 1.68 mg/100 g FW); thereafter, the content of vitamin B1 continued to decline from the S2 to the S6 stage; particularly, between the S5 and S6 stages, a sharp and significant decline has been observed. The content of vitamin B2 has the maximum value at the S1 stage (191.21 ± 2.99 mg/100 g FW), then fluctuated between 31.08 ± 1.27 mg/100 g FW and 38.65 ± 1.57 mg/100 g FW; subsequently, the vitamin B2 content declined slightly and tended to be stable during the S5 and S6 stages. The vitamin B3 content also has the maximum value at the S1 stage (10.53 ± 0.71 mg/100 g FW), then fluctuated between 3.08 ± 0.16 mg/100 g FW and 4.68 ± 0.28 mg/100 g FW, and finally reached the minimum value at the S6 stage (2.03 ± 0.30 mg/100 g FW). As displayed in Table 3, the content of vitamin B6 at the six maturity stages declined continuously with the delaying of the fruit's development, and finally reached the minimum value at the mature stage (S6) (0.13 ± 0.01 mg/100 g FW). The

content of vitamin C indicated the same accumulation pattern as vitamin B1. That is, the content of vitamin C increased from the S1 to the S2 stage and reached the maximum value at the S2 stage (608.58 ± 7.28 mg/100 g FW). Thereafter, the content of vitamin C continued to decline from the S2 to the S6 stage; particularly, between the S4 and S5 stages, a sharp and significant decline was observed, and it finally tended to be stable during the S5 and S6 stages.

As shown in Table 3, the protein content continuously declined during the whole development period and reached the minimum value at the S6 stage (0.48 ± 0.00 g/100 g FW). The content of total phenolics declined from the S1 to the S2 stage, then increased at the S3 stage, and declined again at the S4 stage, whereas a significant rise was found from the S4 to the S5 stage; thereafter, there was no significant difference between the S5 and S6 stages. Similarly, the total flavonoids indicated the same accumulation pattern as the total phenolics in the early stage of fruit development (S1–S3) and then showed a continuing downward trend until reaching the minimum value at the mature stage (13.85 ± 0.80 mg/100 g FW).

Table 3. Dynamic changes of antioxidant component and protein content during *S. obovatifoliola* subsp. *urophylla* fruit development.

Stages	Vitamin B ₁ mg/100 g FW	Vitamin B ₂ mg/100 g FW	Vitamin B ₃ mg/100 g FW	Vitamin B ₆ mg/100 g FW	Vitamin C mg/100 g FW	Protein Content g/100 g	Total Phenolics mg/100 g FW	Total Flavonoids mg/100 g FW
S1	13.88 ± 0.66 b	191.21 ± 2.99 a	10.53 ± 0.71 a	1.39 ± 0.08 a	553.88 ± 18.12 b	2.08 ± 0.04 a	1491.92 ± 10.48 a	622.92 ± 0.47 a
S2	20.24 ± 1.68 a	31.08 ± 1.27 c	4.12 ± 0.32 bc	0.62 ± 0.03 b	608.58 ± 7.28 a	1.54 ± 0.01 b	295.40 ± 3.65 d	25.20 ± 0.46 d
S3	18.62 ± 0.47 a	33.77 ± 1.07 bc	3.08 ± 0.16 cd	0.51 ± 0.03 b	464.12 ± 13.49 c	1.39 ± 0.00 c	376.91 ± 1.80 c	73.09 ± 0.28 b
S4	14.87 ± 1.21 b	38.65 ± 1.57 b	4.68 ± 0.28 b	0.58 ± 0.03 b	332.62 ± 6.52 d	1.35 ± 0.04 c	323.39 ± 5.19 d	39.08 ± 0.55 c
S5	14.53 ± 1.17 b	18.17 ± 0.97 d	4.66 ± 0.75 b	0.18 ± 0.03 c	1.02 ± 0.04 e	0.93 ± 0.08 d	531.10 ± 20.23 b	15.09 ± 0.93 e
S6	2.58 ± 0.21 c	22.38 ± 2.47 d	2.03 ± 0.30 d	0.13 ± 0.01 c	1.22 ± 0.19 e	0.48 ± 0.00 e	512.38 ± 9.62 b	13.85 ± 0.80 e

Means with different letters within the same column indicate statistical differences at the $p < 0.05$ level. S1: 60 DAFB (days after full bloom), S2: 90 DAFB, S3: 130 DAFB, S4: 160 DAFB, S5: 190 DAFB, S6: 205 DAFB.

3.5. Dynamic Changes of Amino Acid Composition during Fruit Development

We detected the content of 17 common free amino acids in the fruit pulp of *S. obovatifoliola* subsp. *urophylla* at different development stages, and the results were shown in Table 4. In general, the content of amino acids at the six development stages declined continuously with the fruit development and has the minimum value at the mature stage, including glutamic acid (Glu), glycine (Gly), alanine (Ala), tyrosine (Tyr), valine (Val), methionine (Met), lysine (Lys), isoleucine (Ile), leucine (Leu), proline (Pro), and phenylalanine (Phe). However, some amino acids showed a spike and then declined in an overall decline trend, such as aspartic acid (Asp) showing a spike at the S2 stage, threonine (Thr) showing a spike at the S4 stage, and cysteine (Cys) showing a spike at the S5 stage, whereas the content of serine (Ser), histidine (His), and arginine (Arg) have maximum values in the S1 stage and showed a spike at the S5 stage. The content of total amino acids (TAAs) declined continuously with the fruit development and has the minimum value at the mature stage (336.89 ± 11.98 mg/100 g). Similarly, essential amino acids (EAAs) indicated the same accumulation pattern as the TAAs. In particular, the proportion of EAAs, including Thr, Val, Met, Lys, Ile, Leu, and Phe, has a maximum value at the mature stage (38.94%).

Table 4. Dynamic changes of amino acid composition during *S. obovatifoliola* subsp. *urophylla* fruit development.

Amino Acids (mg/100 g)	Fruit Development Stages					
	S1	S2	S3	S4	S5	S6
Aspartic acid (Asp)	324.90 ± 4.88 b	433.71 ± 4.40 a	393.44 ± 5.56 a	332.24 ± 8.34 b	162.33 ± 32.59 c	33.45 ± 1.15 d
Serine (Ser)	148.56 ± 1.63 a	75.05 ± 2.08 c	75.91 ± 0.36 c	78.36 ± 0.82 c	86.12 ± 2.35 b	31.30 ± 1.21 d
Glutamic acid (Glu)	103.97 ± 3.37 a	50.13 ± 0.77 b	44.46 ± 0.50 b	44.58 ± 0.45 b	32.20 ± 4.29 c	23.89 ± 1.57 d
Glycine (Gly)	67.18 ± 0.71 a	33.29 ± 0.34 b	28.81 ± 1.15 c	31.92 ± 0.63 bc	23.91 ± 2.51 d	17.53 ± 0.9 e
Threonine (Thr) *	71.02 ± 1.25 a	34.38 ± 0.70 b	30.31 ± 0.92 c	34.50 ± 0.48 b	15.01 ± 0.72 d	17.01 ± 0.78 d
Histidine (His)	38.18 ± 0.76 a	21.05 ± 0.95 c	17.18 ± 0.43 cd	20.25 ± 0.71 c	27.77 ± 3.71 b	12.72 ± 0.29 d
Arginine (Arg)	141.98 ± 2.05 a	69.54 ± 0.90 c	65.17 ± 0.60 c	66.58 ± 1.25 c	85.75 ± 2.66 b	41.31 ± 1.86 d
Alanine (Ala)	40.21 ± 0.78 a	19.47 ± 0.45 b	18.48 ± 0.42 b	18.83 ± 0.20 b	17.92 ± 1.10 b	9.38 ± 0.31 c
Proline (Pro)	47.07 ± 14.05 a	33.62 ± 1.49 ab	30.17 ± 0.94 ab	29.49 ± 0.83 ab	34.73 ± 1.49 ab	15.35 ± 0.57 b
Cysteine (Cys)	6.35 ± 0.27 b	4.31 ± 0.18 bcd	4.58 ± 0.25 bc	2.81 ± 0.16 cd	27.43 ± 1.80 a	1.86 ± 0.18 d
Tyrosine (Tyr)	55.03 ± 1.35 a	26.55 ± 1.60 b	25.94 ± 0.68 b	26.80 ± 0.63 b	24.72 ± 0.95 b	18.90 ± 0.72 c
Valine (Val) *	87.50 ± 1.45 a	42.78 ± 1.16 b	36.90 ± 1.37 c	39.51 ± 1.12 bc	27.37 ± 2.36 d	20.30 ± 0.71 e
Methionine (Met) *	13.41 ± 0.49 a	5.88 ± 0.36 bc	6.83 ± 0.17 b	4.82 ± 0.11 cd	5.44 ± 0.54 cd	4.31 ± 0.27 d
Lysine (Lys) *	85.47 ± 1.70 a	40.02 ± 2.07 b	37.60 ± 0.93 b	38.10 ± 0.97 b	27.42 ± 2.60 c	20.82 ± 0.61 d
Isoleucine (Ile) *	112.80 ± 3.08 a	55.06 ± 1.54 b	52.58 ± 1.15 b	51.39 ± 1.21 b	37.46 ± 3.67 c	27.60 ± 0.91 d
Leucine (Leu) *	98.02 ± 4.13 a	49.33 ± 1.65 b	44.96 ± 2.14 b	47.07 ± 1.80 b	32.64 ± 3.49 c	24.97 ± 0.69 c
Phenylalanine (Phe) *	64.15 ± 1.25 a	31.12 ± 0.94 b	27.75 ± 0.56 b	28.40 ± 0.82 b	20.89 ± 2.22 c	16.17 ± 0.49 d
Total amino acids (TAAs)	1505.81 ± 25.32	1025.28 ± 14.88	941.07 ± 13.59	895.65 ± 17.42	689.08 ± 59.57	336.89 ± 11.98
	a	b	bc	c	d	e
Essential amino acids (EAAs)	532.37 ± 10.03 a	258.58 ± 7.74 b	236.93 ± 4.58 b	243.78 ± 5.54 b	166.21 ± 15.54 c	131.20 ± 4.26 d
	35.35%	25.22%	25.18%	27.22%	24.12%	38.94%

Means with different letters within the same line indicate statistical differences at the $p < 0.05$ level. * indicates essential amino acids, and the percentage number (%) represents the proportion of essential amino acids among the total amino acids. S1: 60 DAFB (days after full bloom), S2: 90 DAFB, S3: 130 DAFB, S4: 160 DAFB, S5: 190 DAFB, S6: 205 DAFB.

4. Discussion

Stauntonia obovatifoliola subsp. *urophylla* is a novel edible and healthy fruit and has tremendous potential for exploitation and utilization. In this study, the dynamic changes in the fruit quality, sugar composition and content, antioxidant component and content, and amino acids content of *S. obovatifoliola* subsp. *urophylla* during fruit development were detected and analyzed.

The detection results of the fruit's appearance quality showed that the fruit increased rapidly in the early stages of development. The single fruit weight of *S. obovatifoliola* subsp. *urophylla* at the S2, S3, S4, S5, and S6 stages increased by 495.60%, 59.49%, 44.09%, 19.64%, and 4.26%, respectively. For the fruit length, the growth rates at each stage were 76.27%, 14.43%, 15.79%, 2.32%, and 3.99%, respectively. The highest growth rate of fruit diameter also occurred at the S2 stage (63.93%), and the lowest growth rate was found at the S4 stage (2.66%), whereafter, the growth rate showed a spike at the S5 stage (S5–S6, 10.44%, and 5.52%, respectively). The results of the fruit shape index showed that the fruit shape index of *S. obovatifoliola* subsp. *urophylla* was basically stable between 1.8–2.2 during the fruit development period, and the difference between each stage was not obvious. In particular, the values of fruit weight, fruit length, and fruit diameter at the mature stage were much higher than those that grew in the wild [3]. Moreover, most of the wild *S. obovatifoliola* subsp. *urophylla* fruits are small and have a low edible ratio, low stress resistance, and poor appearance quality. Fortunately, the wide range of geographical distribution of *S. obovatifoliola* subsp. *urophylla* provides substantial genetic diversity and rich wild

germplasm resources for breeders to select superior genotypes with excellent comprehensive characteristics through resource exploration and evaluation.

Total soluble solids and titratable acid are the main components of fruit flavor quality and nutritional composition which were also considered as crucial parameters of fruit ripening [29]. Additionally, the importance of detecting the change of TSS, TA, and TSS/TA has also been demonstrated by many studies in different fruits, such as strawberry, sweet cherry, orange, mulberry, etc. [30–32]. In this experiment, the TSS content of the *S. obovatifoliola* subsp. *urophylla* fruit showed an increasing trend during the whole fruit development period. Exactly, the TSS content kept at low levels during the early fruit development period (S1–S4), while the TSS content accumulated sharply during the S5–S6 stages, which was mainly due to the breakdown of starch and the accumulation of sugars. Meanwhile, the TA content of the *S. obovatifoliola* subsp. *urophylla* fruit remained at low levels during the fruit ripening process, which is consistent with previous reports on *S. obovatifoliola* subsp. *urophylla* fruit [3]. Thus, the high ratio of TSS to TA resulted in the sweet flavor of *S. obovatifoliola* subsp. *urophylla* fruit. Moreover, the TSS content changed sharply when the fruit neared ripening, which could be considered as an alternative maturity indicator of *S. obovatifoliola* subsp. *urophylla* fruit.

The dry matter content not only is a significant parameter to evaluate the carbon incorporation at different development stages of fruits but is also an important indicator of fruit flavor quality and texture [33,34]. The dry matter content of the *S. obovatifoliola* subsp. *urophylla* fruit continuously increased in the early stages and then declined significantly at the mature stage, which was mainly due to the direct relation between an increase in fruit size and the degradation of starch. Fruit firmness usually has a significant impact on fruits' market value, consumer acceptance, and shelf life [26,35]. The firmness declined sharply when the fruit of *S. obovatifoliola* subsp. *urophylla* neared maturity and softening. Unlike *Akebia* fruits (a relative genus of *Stauntonia*), the *S. obovatifoliola* subsp. *urophylla* fruit did not crack when fully ripe; thus, it could be picked after full maturity (S6 stage), and it tasted better as a fresh fruit. However, considering the long-distance transportation, we suggest that the best time point to harvest the fruit may be at the S5 stage as the fruit is maintained at a suitable hardness that could bear long-distance transportation.

Sugars are not only important components of fruit flavor and nutritional composition but also play important signaling factor roles during plant growth and are also involved in regulating gene expression during plant development [36,37]. The major sugars identified in *S. obovatifoliola* subsp. *urophylla* fruit pulp are fructose, glucose, and maltose. The contents of fructose, glucose, and total sugars showed the same accumulation trend, which firstly increased slightly in the early fruit development periods (S1–S4), thereafter increased sharply at the S5 stage, and there were no significant dynamic changes at the S5 and S6 stages. The changing trends of maltose and starch during the fruit's development and ripening were basically similar; both continuously accumulated during the early fruit development periods but declined sharply as the fruit approached ripening. This opposite change trend of monosaccharides and polysaccharides was mainly due to the hydrolysis of carbohydrates as the fruit ripened, similar to strawberry, sweet cherry, bananas, etc. [30,38,39].

Vitamins, apart from being an important nutrient in daily diet, are also potent antioxidant components [40–42]. The results of this study showed that the fruit of *S. obovatifoliola* subsp. *urophylla* was rich in vitamin B (B₁, B₂, B₃, B₆), vitamin C, phenolics, and flavonoids. In particular, the content of vitamin B₂ was the most abundant vitamin B at the mature stage, while the vitamin C content at the mature stage was in accordance with a previous report [6]. The vitamin content of *S. obovatifoliola* subsp. *urophylla* fruit showed an overall downward trend during fruit development, but there was a large fluctuation in this process, especially the vitamin C content, which decreased sharply when the fruit was near ripening (S5–S6). Phenolics and flavonoids compounds are important secondary metabolites of plants, which can help plants' resistance to bacteria and protect cells from oxidative damage [43,44]. In this study, the content of phenolics declined from the S1 to

the S2 stage, followed by a subsequent increase in fluctuation. The flavonoids content declined firstly from the S1 to the S2 stage and increased at the S3 stage, followed by a subsequent sustained decrease in further fruit development in *S. obovatifoliola* subsp. *urophylla* fruit. The dynamic change of antioxidant content was closely related to fruit development and ripening; the content of antioxidants could also be influenced by structural genes, temperature, light intensity, etc. [45,46]. The general decrease in the content of vitamins, phenolics, and flavonoids is mainly due to the oxidation of oxidase during fruit maturity or the effect of dilution as fruit increases in size [47,48].

Amino acids are important nutrient elements for most of life and also play some key roles in various biological reactions, such as signaling pathways, ATP generation, redox balance, nucleotide synthesis, cellular immunity, etc. [49–53]. In this study, 17 common free amino acids and 7 essential amino acids were detected by HPLC. The content of amino acids showed an overall downward trend during fruit development, which might be related to the conversion and expenditure of amino acids. Another reason may be the dilution effect of increasing fruit size. The abundance of amino acids in the fruit of *S. obovatifoliola* subsp. *urophylla* documented its excellent medicinal and edible value.

Compared to some common fruits, *S. obovatifoliola* subsp. *urophylla* has more advantages in certain nutrients (Table 5). For example, the total soluble solids, total sugars, fructose, and glucose are higher than apple, peach, kiwifruit, and strawberry, indicating that the *S. obovatifoliola* subsp. *urophylla* fruit has a sweeter flavor than some common fruits. *S. obovatifoliola* subsp. *urophylla* fruit has more protein than apple and cherry but less than banana, grape, peach, kiwifruit, and strawberry. As for the content of total amino acids, *S. obovatifoliola* subsp. *urophylla* is on par with grape, kiwifruit, strawberry, and cherry whereas less than apple, banana, and peach. Surprisingly, the contents of vitamin B₁, vitamin B₂, and vitamin B₃ of *S. obovatifoliola* subsp. *urophylla* are much higher than those common fruits, whereas it has less vitamin C than those common fruits. It should be pointed out that the determination of the nutritional composition of *S. obovatifoliola* subsp. *urophylla* in this paper is not comprehensive, but it can still prove that *S. obovatifoliola* subsp. *urophylla* is worthy of being exploited and utilized as a medicinal and edible fruit crop.

Table 5. The nutrient composition of *S. obovatifoliola* subsp. *urophylla* fruit at the ripening stage [54,55].

Parameter	<i>S. Obovatifoliola</i> Subsp. <i>Urophylla</i>	Apple	Banana	Grape	Peach	Kiwifruit	Strawberry	Cherry
Total soluble solids (%)	13.52	12.00	22.63	17.51	7.82	7.58	7.08	14.30
total sugars (g/100 g)	12.45	11.07	19.55	15.30	6.17	6.99	5.93	11.90
fructose (g/100 g)	5.35	4.66	7.96	6.02	0.89	1.96	1.74	7.63
glucose (g/100 g)	7.02	1.39	2.41	7.56	0.75	2.38	2.42	1.44
protein (g/100 g)	0.48	0.41	1.26	0.55	0.78	1.15	0.90	0.32
Total amino acids (mg/100 g)	689.08	2706.92	7623.91	368.33	4580.00	629.27	667.00	573.00
vitamin B ₁ (mg/100 g)	2.58	0.02	0.02	0.03	0.01	0.05	0.02	0.02
vitamin B ₂ (mg/100 g)	22.38	0.02	0.04	0.02	0.02	0.02	0.02	0.02
vitamin B ₃ (mg/100 g)	2.03	0.20	0.70	0.25	0.30	0.30	0.30	0.60
vitamin C (mg/100 g)	1.22	2.84	8.00	4.79	5.17	89.89	74.80	11.12

5. Conclusions

In conclusion, the dynamic changes in the fruit's appearance, physiochemical quality, and nutritional quality during the development of *S. obovatifoliola* subsp. *urophylla* were detected and analyzed. This study suggested that the maturity stage had a significant effect on nutritional properties and physiochemical parameters during *S. obovatifoliola* subsp. *urophylla* fruit ripening and softening. Particularly, the values of TSS, firmness, dry matter, fructose, glucose, maltose, starch, vitamin B, vitamin C, total phenolics, and total flavonoids of *S. obovatifoliola* subsp. *urophylla* fruit showed significant changes during the transition to physiological maturity. In view of the nutrient content and pulp texture, the fruit of *S. obovatifoliola* subsp. *urophylla* picked at the S5 maturity stage was more suitable for long-distance transportation. The results of this study laid a foundation for clarifying the growth and development characteristics of *S. obovatifoliola* subsp. *urophylla* fruit and the further utilization of these excellent medicinal and edible germplasm resources.

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