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Physicochemical Characteristics and Nutritional Composition during Fruit Ripening of *Akebia trifoliata* (Lardizabalaceae)

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Abstract: *Akebia trifoliata* is a high-value medicinal and edible fruit crop in China, and it has begun to be widely cultivated as a new fruit crop in many areas of China. Its fruits crack longitudinally when fully ripe and should be harvested before fruit cracking. Physicochemical characteristics and nutritional composition of the ripening process are prerequisites to establishing proper harvest maturity windows. In the current study, we have investigated the fruit quality characteristics of two *A. trifoliata* clonal lines ('Luqing' and 'Luyu') that were harvested at four time points (S1: 120 days after full bloom (DAFB), S2: 134 DAFB, S3: 148 DAFB, S4: 155 DAFB). An increase in fruit size (fruit weight, fruit length, and fruit diameter) was associated with delayed harvest maturity. The firmness of *A. trifoliata* fruit exhibited a decreasing trend with delaying the harvest stage. In particular, the firmness decreased sharply from S2 to S3 stage. The TSS, fructose, and glucose content in *A. trifoliata* fruit continuously increased from the S1 to S4 stage and accumulated sharply from S2 to S3 stage. However, the sucrose and starch content showed an increasing trend from the S1 to S2 stage but declined sharply in the S3 or S4 stage. Ascorbic acid progressively increased with the advancement of *A. trifoliata* maturity stages, while total phenolics and total flavonoids levels declined with fruit ripening. Considering the results of all quality parameters mentioned above, the *A. trifoliata* fruit harvested at the S3 maturity stage was the ideal harvest maturity for long-distance transportation and higher consumer acceptability before fruit cracking. Our research reveals the dynamic changes in physicochemical characteristics and nutritional composition during fruit ripening of *A. trifoliata*. Results in this study reflect the importance of maturity stages for fruit quality and provide basic information for optimal harvest management of *A. trifoliata*.

Keywords: *Akebia trifoliata*; maturity stage; physicochemical; nutritional; ripening

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1. Introduction

Akebia trifoliata is a perennial woody vine that belongs to the family Lardizabalaceae, has large and edible fruits, and is mainly distributed in East Asia [1]. Its fruits are known as "Bayuezha" in China; ripe fruit could crack longitudinally in the Chinese lunar August. The fully ripe fruit of *A. trifoliata* has a soft pulp texture and sweet flavor, tasting like a mixture of banana and litchi. The fruits of *A. trifoliata* are rich in sugars, crude proteins, amino acids, vitamins, minerals, ash, and fiber [2,3]. As an edible fruit plant, the fruit of *A. trifoliata* can also be processed into commodities such as desserts, wine, juices, vinegar, tea, edible oil, etc. [4,5]. The value of *A. trifoliata* fruit lies not only in its nutrition or commercialization but also in its bioactive ingredients with anti-inflammatory, diuretic, antimicrobial, anticancer, anti-obesity, and antioxidative properties [6–11]. In recent years, *A. trifoliata*, as a high-value medicinal and edible fruit crop, has been widely cultivated in China [12,13]. Moreover, the genomic data of *A. trifoliata* subsp. *australis* were published last year, which will greatly accelerate the extensive and intensive research on this new fruit crop [14].

The fruit of *A. trifoliata* cracks open longitudinally when completely ripe on the vine. However, pericarp cracking causes a series of problems in that pulp is easily contaminated

by impurities, infected by pathogens, or eaten by birds, resulting in short shelf life, low visual quality, and decreasing acceptance by consumers and the commercial value. Namely, the fruit quality decreases rapidly when it is harvested after full ripening and cracking. The maturity stage at harvest is a fundamental preharvest factor that could determine the final fruit quality and storage potential. For example, in order to prolong the shelf life, winter jujube was harvested at the white maturity stage and stored at a low temperature for a period of time and then moved to market [15]; strawberries were frequently harvested at the turning stage (75% red), or even at the green stage (50% red) for long-distance transport or export markets [16]; mulberries harvested at the fully ripe stage are suitable for fresh consumption while those harvested at semi ripe stage are appropriate for the processing industry [17]. Therefore, it is of great practical significance for the fruit of *A. trifoliata* to explore a proper maturity stage at harvest which can meet the demand for good fruit quality, shelf life, and market value simultaneously.

The fruit maturity stage is usually judged by external attributes such as size, appearance, texture, and color, or internal attributes such as total soluble solids, acidity, and starch. The colors of *A. trifoliata* fruit are varied in different cultivars at maturity, from green to cyan, from yellow to brown, from purple to pink, etc. Thus, the maturity stage of *A. trifoliata* fruit is hardly assessed just by appearance or color in some cultivars before fruit cracking. Thus, we should investigate the changes in physicochemical characteristics and nutritional composition during *A. trifoliata* fruit development to better understand the fruit ripening process of *A. trifoliata*.

Contents of nutrients, phytochemical compounds, and volatiles in fruits are usually influenced by numerous preharvest or postharvest factors, but the maturity stage at harvest is probably the most important factor in determining the final fruit quality [18–20]. Studies showed that the physical properties, biochemical properties, and proximate composition of tomatoes were more affected by maturity stages than genotype [21]. For winter jujube, the physicochemical properties during fruit ripening were influenced by the maturity stage at harvest [22,23]. However, there is little information on the changes in physicochemical characteristics and nutritional composition of *A. trifoliata* at different fruit maturity stages. Thus, the objective of this study was to investigate the changes in physicochemical characteristics, nutritional composition, and antioxidant content of *A. trifoliata* fruit at different stages of on-the-vine ripening. The results of this study can help us to understand the basic dynamic change patterns of fruit quality and to identify the optimum harvest maturity stage of *A. trifoliata* with better quality and longer marketability before fruit cracking.

2. Materials and Methods

2.1. Plant Material

Fruits of two *A. trifoliata* clonal lines ('Luqing' and 'Luyu' selected from our genetic improvement programs of *Akebia* [12,13]) free from pests, insects, and diseases were randomly harvested at four time points (S1: 120 days after full bloom (DAFB), S2: 134 DAFB, S3: 148 DAFB, S4: 155 DAFB) until the fruits begin cracking in the experimental farm located at Zhangjiajie county (29°41′ N, 111°09′ E), China (Figure 1). At the S3 time point, the fruits of two clonal lines begin to soften to some extent with no fruit cracking. At the S4 time point, the fruits begin to crack (about 50%), and the S4 time point was set as the fruit cracking time (Figure 1D). The *A. trifoliata* trees were five years old, and these plants were freely pollinated, and the sprinkling irrigation system was used for field water balance; the vines were pruned in the December or January and applied 4–5 kg organic fertilizer per plant in the winter. Because the fruit from one tree of *A. trifoliata* did not provide enough samples for four stages, fruits were harvested from different stages of ten trees in each clonal line at the same time and considered as a sample. Three fruits pulp were mixed into one biological replicate for sugars, starch, ascorbic acid, total phenolics, total flavonoids, and proximate analysis, and the fruit pulp was immediately treated with liquid nitrogen and stored at −80 °C until further analysis. Sampled fruits from each stage were

immediately evaluated for fruit weight, length, diameter, and moisture on 5 fruits. In total, three biological replicates were collected for each maturity stage.

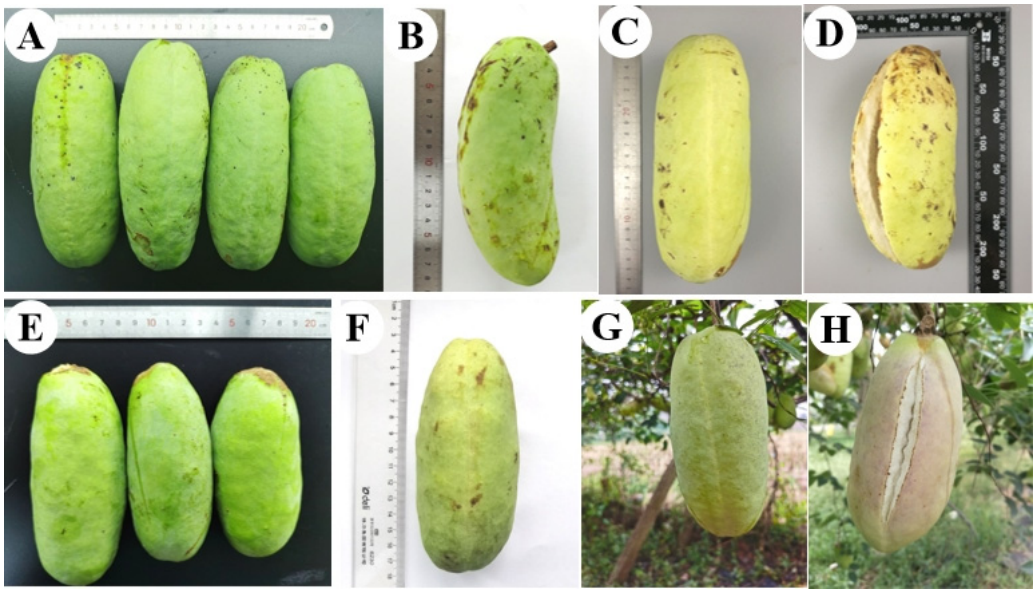

Figure 1. The fruits of *A. trifoliata* at different maturity stages. (**A**–**D**) the fruit of Luqing at S1, S2, S3, and S4 stages, respectively. The pericarp is pale green when it cracks. (**E**–**H**) the fruit of Luyu at S1, S2, S3, and S4 stages, respectively. The pericarp is light pink when it cracks. S1: 120 days after full bloom (DAFB), S2: 134 DAFB, S3: 148 DAFB, S4: 155 DAFB.

2.2. Physical Parameters

The average fruit weight of each sample (5 fruits) was recorded by using an electronic balance and expressed in grams. Fruit length (FL) and fruit diameter (FD) were determined using a digital caliper with 0.01 mm accuracy, and their values were expressed in millimeters. The firmness of *A. trifoliata* was determined independently for each of 5 fruit per replicate using a digital fruit firmness tester (GY-4, Zhejiang, China) outfitted with a 3.5-mm cylinder probe. A knife was to peel off the cuticle of the fruit peel, then the cylinder probe was aligned vertically to the test position of the fruit peel, and pressure was slowly applied to make the probe insert the fruit peel. The results of the firmness values were expressed as the kg/cm^2.

2.3. Biochemical Parameters

Total soluble solids content (TSS) of *A. trifoliata* fruit pulp was determined by a digital refractometer (PAL-1, Atago, Tokyo, Japan), and the results were expressed in °Brix. Titratable acidity (TA) was determined by titrating with 0.1 N NaOH until reaching pH 8.1, and the values were expressed as % of citric acid. The pulp pH was calculated using a pH meter (Jenway, Staffordshire, UK).

2.4. Determination of Sugars

The levels of individual sugars (glucose, fructose, and sucrose) were simultaneously determined by high-performance liquid chromatography (HPLC). Firstly, the fruit pulp sample (accurate to 0.001 g) was weighed and placed in a 100 mL triangle flask. Then 10 mL of extraction solution (0.02 mol/L NaOH) was added accurately. The extraction solution was fully oscillated at 180 r/min for 40 min, and the qualitative filter paper was used for filtration. Finally, 2 mL filtrate was filtered through a 0.45 μm aqueous phase filter membrane and then used for HPLC analysis with an evaporative light scattering detector. The detection conditions were as follows: the carrier gas was nitrogen, the gain was 500, the spray mode was cooling, the drift tube temperature was 50 °C, the gas pressure

was 40 psi, and the analysis time was 30 min. Shodex's AsahiPak NH2P-50 4E column (150 mm × 4.6 mm) was used for analysis. The mobile phase consisted of water and acetonitrile at the flow rate of 0.8 mL/min. The column temperature was 35 °C, and the injection volume was 10 µL.

2.5. Determination of Starch

About 1 g of sample of fruit pulp was extracted with 20 mL of 80% ethanol using a high-speed dispersion machine for 30 min. Then the sample was transferred to a water bath at 80 °C for 30 min, stirred for 30 min until cool and filtered. The above step was repeated once, and then the sample was extracted with 80% ethanol and a filter. The filter residue was washed into a centrifuge tube with 20 mL of hot distilled water and placed in a boiling water bath for gelatinization for 15 min. Then, 2 mL of cold 9.2 mol/L perchloric acid was added into the centrifuge tube and stirred continuously, extracted for 15 min, and filtered. The filtrate was poured into a 100 mL volumetric flask and 10 mL distilled water was added to the filter residue. Then, 1 mL 9.2 mol/L perchloric acid was added, and the filtrate residue was placed in a boiling water bath and heated for 15 min, and filtered. The filter residue was washed twice with distilled water, then poured into the voluminous flask. Absorbance was recorded at 490 nm. The calibration curve was derived by the phonel-sulfate method.

2.6. Determination of Ascorbic Acid, Total Phenolics, and Total Flavonoids

The ascorbic acid (AsA) content of *A. trifoliata* fruit pulp was measured by the indophenol's titration method [24]. Briefly, 2 g of fruit pulp was mixed with distilled water (10 mL), and a solution of 0.4% oxalic acid (90 mL) was taken in a 100 mL volumetric flask. Then the mixture was filtered with filter paper. After that, 5 mL filtrated aliquot was taken and titrated against 2,6-dichlorophenolindophenol to a light pink color endpoint. The amount of AsA was expressed in milligrams per 100 g of fresh weight (FW).

Total phenolics content in the *A. trifoliata* fruit pulp was determined with Folin-Ciocalteu reagent by the method of Razzaq et al. [25]. A total of 1.0 g of fruit pulp was extracted with 97% (*v*/*v*) methanol. Then, 1.0 mL of extract solution was mixed with 1.0 mL Folin–Ciocalteu solvent, let stand for 5 min, and 8.0 mL of Na_2CO_3 (75 mg/mL) was added to the mixture. After being kept in the dark for 30 min at room temperature, the absorbance was measured at the wavelength of 765 nm. A standard curve of gallic acid was used for calculating the total phenolics in *A. trifoliata* fruit pulp and the results were expressed as mg gallic acid equivalents (GAE) per 100 g of FW.

Total flavonoids content was measured as described by Zhao et al. [26]. Briefly, fruit pulp (2.0 g) was extracted with 80% (*v*/*v*) ethanol and centrifuged at 10,000 g for 30 min at 4 °C. Then, 0.5 mL of supernatant was mixed with 0.1 mL of 5% $NaNO_2$. After 5 min, 0.1 mL of 10% $AlCl_3$ solution was added, followed by incubating for 6 min, then 1 mL 1 mM NaOH was added. The reaction solution was kept for 30 min at room temperature, and the absorbance was assayed at 510 nm. The total flavonoids content in *A. trifoliata* fruit pulp was calculated using a standard curve of rutin and was expressed as mg rutin equivalents per 100 g of FW.

2.7. Proximate Analysis

Dry matter content and moisture content in *A. trifoliata* fruit were measured by oven drying at 70 °C till its weight became constant. The crude fiber was measured by extraction of fruit sample in the presence of sulphuric acid and potassium hydroxide reagents, based on the method outlined by Abdullahi et al. [27]. Fat content was measured by the Soxhlet method based on the solubility of the free lipid content in organic solvents such as ethyl ether and petroleum ether. The Kjeldahl method was used for the determination of the protein content in *A. trifoliata* fruit pulp and protein content were calculated as N × 6.25 based on the method of Imran et al. [28].

2.8. Statistical Analysis

Statistical analyses were performed using one-way analysis of variance (ANOVA), and the significant differences between means were determined by the least significant difference (LSD) test at $p < 0.05$ by using IBM SPSS Statistics 20.0 software. The results were expressed as means ± standard errors.

3. Results

3.1. Changes in the Physical Parameters of A. trifoliata Fruit during Maturity Stages

The changes in the physical parameters of *A. trifoliata* (clonal lines 'Luqing' and 'Luyu') at four different maturity stages (S1–S4) are shown in Table 1. In both clonal lines of 'Luqing' and 'Luyu', fruit weight, fruit length, and fruit diameter increased significantly with increasing maturity. For the 'Luqing', the values of fruit weight increased from 203.19 ± 11.59 g at the S1 stage to 536.33 ± 18.93 g at the S4 stage. The fruit length increased significantly from 128.17 ± 2.62 mm at the S1 stage to 172.35 ± 4.29 mm at the S4 stage. The fruit diameter increased significantly from 52.44 ± 1.23 mm at the S1 stage to 72.04 ± 2.02 mm at the S4 stage. For the 'Luyu', the values of fruit weight, fruit length, and fruit diameter all increased at various extents from the S1 stage to the S4 stage. For example, fruit weight increased from 152.93 ± 5.81 g to 302.82 ± 8.22 g; the fruit length increased significantly from 117.86 ± 1.67 mm to 139.90 ± 2.37 mm. Meanwhile, the fruit diameter increased significantly from 48.19 ± 0.95 mm to 62.12 ± 1.34 mm.

Table 1. Effect of harvest maturity on physical parameters of two *Akebia trifoliata* clonal lines 'Luqing' and 'Luyu' fruit.

Clonal Lines	Maturity Stages	Fruit Weight/g	Fruit Length/mm	Fruit Diameter/mm
Luqing	S1	203.19 ± 11.59 d	128.17 ± 2.62 c	52.44 ± 1.23 c
	S2	289.54 ± 13.38 c	148.10 ± 3.03 b	59.45 ± 1.43 b
	S3	463.10 ± 14.66 a	168.27 ± 3.32 a	69.69 ± 1.56 a
	S4	536.33 ± 18.93 a	172.35 ± 4.29 a	72.04 ± 2.02 a
Luyu	S1	152.93 ± 5.81 d	117.86 ± 1.67 d	48.19 ± 0.95 c
	S2	191.37 ± 6.22 c	123.77 ± 1.79 c	51.76 ± 1.01 b
	S3	259.50 ± 6.71 b	129.41 ± 1.93 b	61.49 ± 1.09 a
	S4	302.82 ± 8.22 a	139.90 ± 2.37 a	62.12 ± 1.34 a

Values are the means ± standard errors. Means with different letters within the same column indicate statistical differences at the $p < 0.05$ level. S1: 120 days after full bloom (DAFB), S2: 134 DAFB, S3: 148 DAFB, S4: 155 DAFB.

3.2. Changes in Fruit Firmness, Total Soluble Solids, and Titratable Acidity during Maturity Stages

The fruit firmness of *A. trifoliata* was significantly ($p < 0.05$) affected by the harvest maturity stages during fruit development. The firmness of *A. trifoliata* fruit at four maturity stages decreased continuously with the delaying of the harvest stage (Figure 2A). The firmness of 'Luqing' fruit in S1, S2, S3 and S4 stages were 29.32 kg/cm^2, 26.29 kg/cm^2, 8.58 kg/cm^2 and 5.00 kg/cm^2, respectively. The firmness decreased significantly from S2 to S3 stage, while there were no significant differences between the S1 and S2 stage or S3 and S4 stage. The firmness of 'Luyu' fruit at S1, S2, S3, and S4 stages was 33.13 kg/cm^2, 28.02 kg/cm^2, 4.74 kg/cm^2, and 3.41 kg/cm^2, respectively, and the firmness decreased significantly from S1 to S3 stage, while there was no significant difference between S3 and S4 stage. On the whole, the firmness of 'Luqing' fruit at S1 and S2 stages was lower than 'Luyu', while higher than 'Luyu' at S3 and S4 stages, which meant that the firmness of 'Luqing' fell faster than 'Luyu' in the early mature stage, whereas in late maturity it declined more slowly than 'Luyu'.

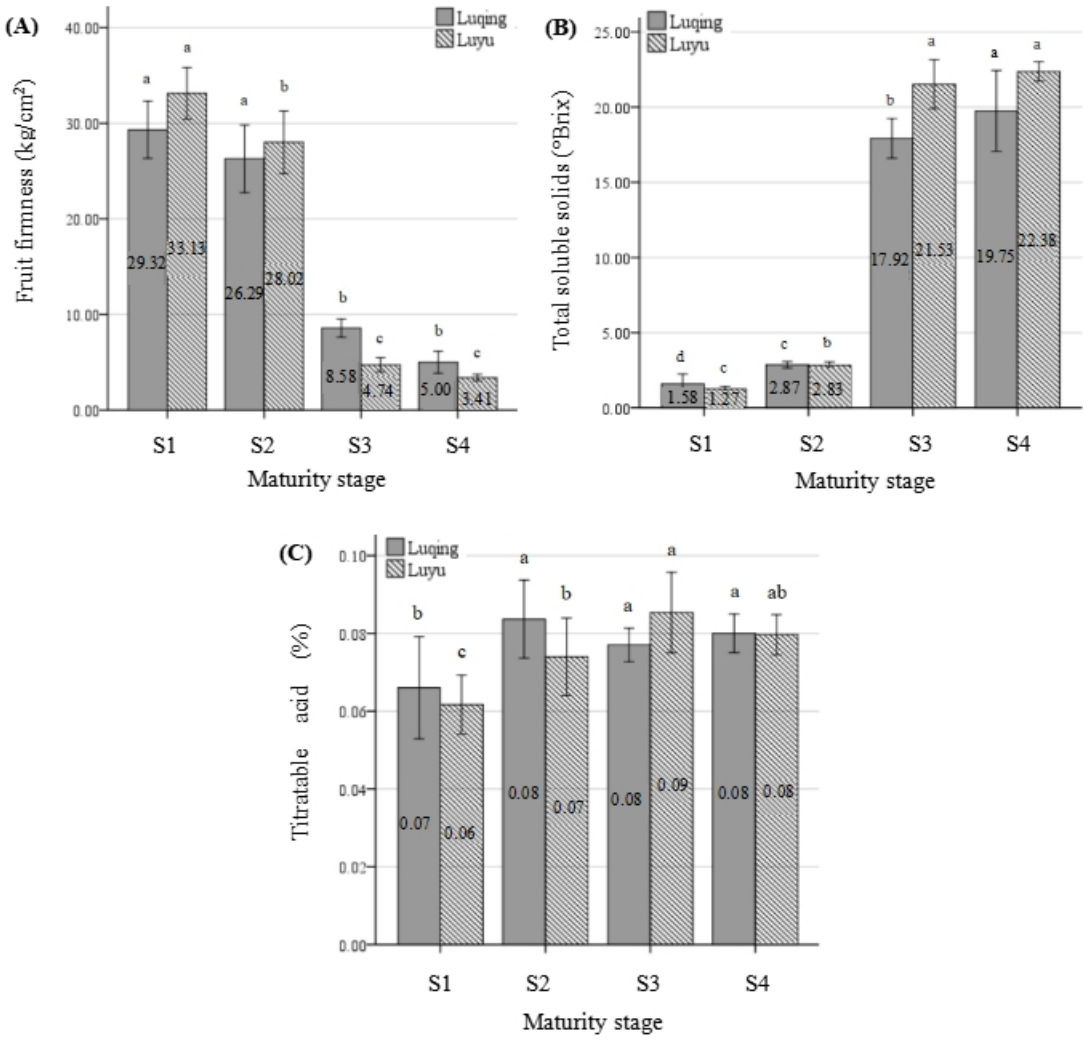

Figure 2. Fruit firmness (**A**), total soluble solids (**B**), and titratable acidity (**C**) in *Akebia trifoliata* fruit at four stages of ripening. Error bars indicate standard error from 10 replicates. Means with different letters within each clonal line indicate statistical differences at the $p < 0.05$ level using the LSD test. S1: 120 days after full bloom (DAFB), S2: 134 DAFB, S3: 148 DAFB, S4: 155 DAFB.

During the fruit maturation and ripening period, the changes in TSS content of 'Luqing' and 'Luyu' in four mature stages displayed a similar pattern, namely continuously increased and reached maximum values at the fourth harvest stage (Figure 2B). The TSS content of 'Luqing' fruit at S1, S2, S3, and S4 stages was 1.58 °Brix, 2.87 °Brix, 17.92 °Brix, and 19.75 °Brix, respectively. For 'Luyu' fruit, the TSS content at S1, S2, S3, and S4 stages was 1.27 °Brix, 2.83 °Brix, 21.52 °Brix, and 22.38 °Brix, respectively. For both clonal lines, TSS initially increased slightly as the fruit developed from the S1 to S2 stage; thereafter, TSS rose sharply from S2 to S3 stage, and after that, it increased slightly from S3 to S4 stage.

The TA content of *A. trifoliata* fruit was kept at low levels, and the values were in the range of 0.06–0.08% from the S1 to S4 maturity stage (Figure 2C).

3.3. Changes in Carbohydrates Contents during Maturity Stages

Carbohydrate contents in *A. trifoliata* fruit displayed significant changes during fruit growth and development (Figure 3). The fructose contents of 'Luqing' fruit at S1, S2, S3, and S4 stages were 0.31 g/100 g FW, 0.68 g/100 g FW, 7.39 g/100 g FW, and 7.80 g/100 g FW, respectively. From the graph of the fructose content of 'Luqing', it was obvious that fructose accumulated rapidly, mainly from S2 to S3 stage (Figure 3A). For 'Luyu' fruit, fructose content at S1, S2, S3, and S4 stages were 0.16 g/100 g FW, 0.21 g/100 g FW, 5.33 g/100 g FW,

and 8.83 g/100 g FW, respectively, which showed 'Luyu' fruit accumulated a large amount of fructose from S2 to S4 stage. Similarly, glucose indicated the same accumulation pattern as the fructose (Figure 3B). That is, the glucose of 'Luqing' mainly accumulated from the S2 to S3 stage, and 'Luyu' from the S2 to S4 stage.

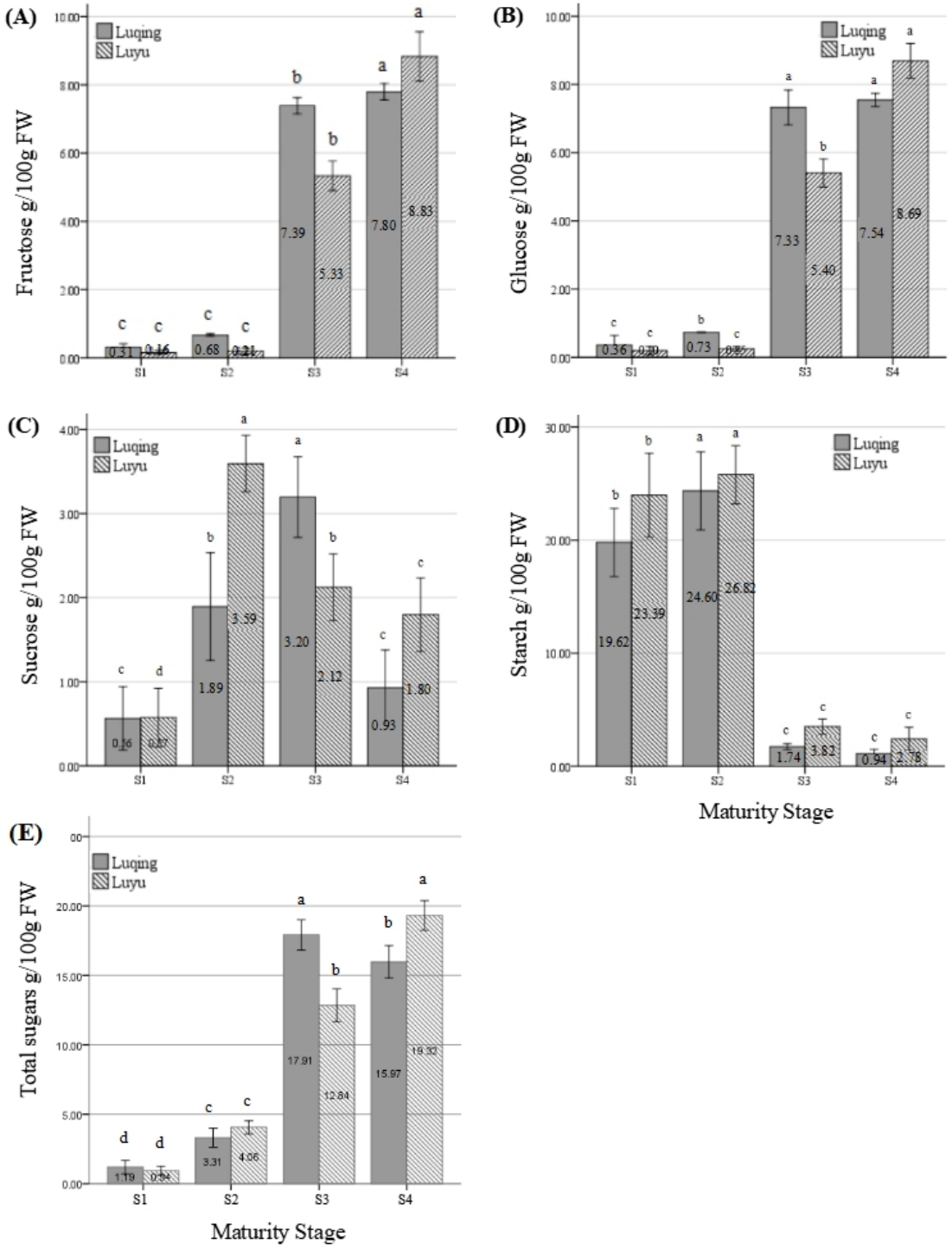

Figure 3. Fructose (**A**), glucose (**B**), sucrose (**C**), starch (**D**), and total sugars (**E**) (the sum of fructose, glucose, and sucrose) concentrations in *Akebia trifoliata* fruit at four stages of ripening. Error bars indicate standard error from 5 replicates. Means with different letters within each clonal line indicate statistical differences at the $p < 0.05$ level using the LSD test. S1: 120 days after full bloom (DAFB), S2: 134 DAFB, S3: 148 DAFB, S4: 155 DAFB.

The sucrose content of 'Luqing' fruit continuously accumulated from the S1 to S3 stage (0.56–3.20 g/100 g FW) and reached maximum values at the S3 stage but had a sharp, significant decline from S3 to S4 stage (3.20–0.93 g/100 g FW) (Figure 3C). While sucrose content of 'Luyu' fruit increased from S1 to S2 stage (0.57–3.59 g/100 g FW), thereafter sucrose declined from S2 to S4 stage (3.59–1.80 g/100 g FW). Starch concentrations of 'Luqing' and 'Luyu' fruit both increased significantly from the S1 to S2 stage (19.62–24.60 g/100 g FW and 23.30–26.82 g/100 g FW, respectively) (Figure 3D), while starch declined sharply from S2 to S3 stage (24.60–1.74 g/100 g FW and 26.82–3.82 g/100 g FW, respectively) and reached minimum values at the S4 stage (0.94 g/100 g FW and 2.78 g/100 g FW, respectively). The total sugars of 'Luqing' increased significantly from the S1 to S3 stage (1.19–17.92 g/100 g FW) while showing some decline at the S4 stage (15.97 g/100 g FW) (Figure 3E). For the 'Luyu', the total sugars continuously increased from the S1 to S4 stage and reached maximum values at the S4 stage (0.94–19.32 g/100 g FW).

3.4. Changes in AsA, Total Phenolics, and Total Flavonoids during Maturity Stages

As displayed in Figure 4A, on the whole, the AsA content of both clonal lines fruit continuously increased during the whole ripening period, except for a slight decline at the S2 stage. The AsA content of 'Luqing' fruit at S1, S2, S3, and S4 stages were 0.87 mg/100 g FW, 0.83 mg/100 g FW, 1.10 mg/100 g FW, and 1.26 mg/100 g FW, respectively. The AsA contents of 'Luyu' fruit at S1, S2, S3, and S4 stages were 0.87 mg/100 g FW, 0.86 mg/100 g FW, 1.07 mg/100 g FW, and 1.16 mg/100 g FW, respectively.

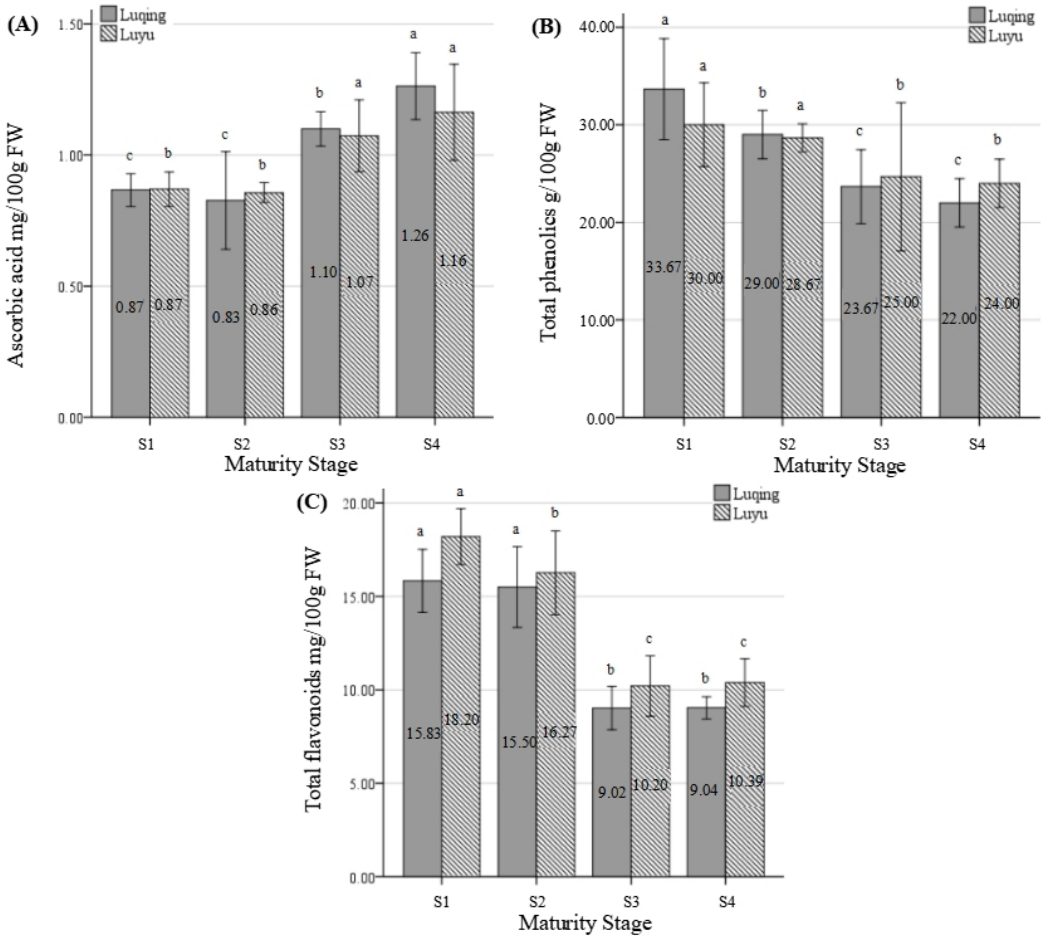

Figure 4. Ascorbic acid (**A**), total phenolics (**B**), and total flavonoids (**C**) contents in *Akebia trifoliata* fruit at four stages of ripening. Error bars indicate standard error from 3 replicates. Means with different letters within each clonal line indicate statistical differences at the $p < 0.05$ level using the LSD test. S1: 120 days after full bloom (DAFB), S2: 134 DAFB, S3: 148 DAFB, S4: 155 DAFB.

The contents of the total phenolics of *A. trifoliata* fruit at four maturity stages are presented in Figure 4B. Overall, the total phenolics declined from the S1 to S4 stage. In comparison with 'Luyu', the content of total phenolics in 'Luqing' was slightly higher than 'Luyu' at S1 (33.67 mg/100 g FW and 30.00 mg/100 g FW, respectively) and S2 (29.00 mg/100 g FW and 28.67 mg/100 g FW, respectively) stages, but slightly lower than 'Luyu' ($p < 0.05$) at the S3 and S4 stages.

As shown in Figure 4C, the maturity stage had a significant influence on the total flavonoids in *A. trifoliata* fruit. During the ripening process, 'Luqing' and 'Luyu' fruit at four maturity stages exhibited similar total flavonoids change patterns. Specifically, 'Luqing' and 'Luyu' showed a slight decrease from the S1 to S2 stage (15.83 mg/100 g FW to 15.50 mg/100 g FW, 18.20 mg/100 g FW to 16.27 mg/100 g FW, respectively), whereas a sharp decrease was found from S2 to S3 stage (15.50–9.02 mg/100 g FW, 16.27–10.20 mg/100 g FW, respectively), thereafter, there was no significant difference between S3 and S4 stage.

3.5. Proximate Composition

The proximate composition of two *Akebia trifoliata* clonal lines is presented in Table 2. The dry matter content of 'Luqing' continuously decreased from the S1 to S4 stage (25.78 ± 1.21% to 17.19 ± 1.10%). For 'Luyu', the dry matter content of 'Luqing' continuously decreased from the S1 to S4 stage (29.68 ± 0.63% to 18.71 ± 1.28%). The moisture content of 'Luqing' continuously increased from the S1 to S4 stage (74.22 ± 1.21% to 82.81 ± 1.10%). Similarly, the change pattern of moisture content in 'Luyu' fruit, like 'Luqing's', increased from the S1 to S4 stage (70.32 ± 0.63% to 81.29 ± 1.28%). The crude fiber content of 'Luqing' fruit at S1, S2, S3, and S4 stages were 0.67 ± 0.03%, 0.60 ± 0.06%, 0.20 ± 0.00%, and 0.17 ± 0.03%, respectively, indicating a continuously decreasing pattern. However, the decrease of crude fiber content in 'Luyu' mainly occurred during the S2 and S3 stages. 'Luqing' and 'Luyu' fruits had a low fat content, and the fat content decreased as the fruits matured. The protein content of 'Luqing' and 'Luyu' fruits fell in half from the S1 stage to the S4 stage. At each stage of maturity, the protein content of 'Luqing' and 'Luyu' was basically the same. In summary, moisture content showed an increasing trend as ripening progressed, while crude fiber, fat, and protein content showed a decreasing pattern along with the maturity of the fruit.

Table 2. Proximate composition of *Akebia trifoliata* fruit at four maturity stages (mean ± standard error).

Clonal Lines	Maturity Stage	Dry Matter Content (%)	Moisture (%)	Crude Fiber(%)	Fat Content(%)	Protein Content(%)
Luqing	S1	25.78 ± 1.21 b	74.22 ± 1.21 b	0.67 ± 0.03 a	0.50 ± 0.00 a	1.14 ± 0.05 a
	S2	23.46 ± 1.23 b	76.54 ± 1.23 b	0.60 ± 0.06 a	0.40 ± 0.06 a	0.99 ± 0.01 b
	S3	19.63 ± 0.80 a	80.37 ± 0.80 a	0.20 ± 0.00 b	0.20 ± 0.00 b	0.56 ± 0.02 c
	S4	17.19 ± 1.10 a	82.81 ± 1.10 a	0.17 ± 0.03 b	0.17 ± 0.03 b	0.54 ± 0.01 c
Luyu	S1	29.68 ± 0.63 b	70.32 ± 0.63 b	0.70 ± 0.06 a	0.47 ± 0.03 a	1.05 ± 0.01 a
	S2	26.98 ± 1.89 b	72.90 ± 1.89 b	0.70 ± 0.06 a	0.43 ± 0.03 a	0.98 ± 0.03 b
	S3	21.26 ± 0.84 a	78.74 ± 0.84 a	0.20 ± 0.00 b	0.23 ± 0.03 b	0.52 ± 0.02 c
	S4	18.71 ± 1.28 a	81.29 ± 1.28 a	0.20 ± 0.00 b	0.20 ± 0.00 b	0.52 ± 0.01 c

Means in the same column for the same clonal line with different letters are significantly different at $p < 0.05$. S1: 120 days after full bloom (DAFB), S2: 134 DAFB, S3: 148 DAFB, S4: 155 DAFB.

4. Discussion

This study demonstrated that the maturity stage had significant effects on the physicochemical and nutritional properties of *A. trifoliata* fruit. An increase in fruit size (fruit weight, fruit length, and fruit diameter) was associated with delaying harvest maturity. The fruit weight of 'Luqing' at S2, S3, and S4 stages increased by 42.50%, 59.94%, and 15.81%, respectively. While in the 'Luyu' clonal line, the fruit weight increased by 25.14%, 35.60%, and 16.69%, respectively, and the fruit weight in both clonal lines increased rapidly from S2

to S3 stage. The fruit weight increased in 'Luqing' more quickly than 'Luyu' at all stages, except for the S4 stage, but the fruit weight of 'Luqing' was still higher than 'Luyu' at each stage. The highest growth rate of fruit length in 'Luqing' occurred at the S2 stage (15.55%), then the growth rate gradually declined at S3 and S4 stages (13.62% and 2.42%, respectively). While the increasing trends of fruit length in 'Luyu' were different from 'Luqing,' whose highest growth rate was found at the S4 stage (8.11%), the lowest growth rate arose at the S3 stage (4.56%) and increased by 5.01% at S2 stage. For the fruit diameter, the highest growth rate in both clonal lines occurred at the S3 stage (17.22% for 'Luqing' and 18.80% for 'Luyu'), and the lowest growth rates were found at the S4 stage (3.37% for 'Luqing' and 1.02% for 'Luyu'). In summary, the fruit weight and fruit diameter increased rapidly from the S2 to S3 stage, while the fruit length had different growth patterns between 'Luqing' and 'Luyu'. Fruit growth showed an S-shaped curve in both clonal lines, consistent with previous reports in *A. trifoliata* fruit [3]. In particular, the values of fruit weight, fruit length, and fruit diameter at the S3 and S4 stages were close to fully ripened fruit [12]. Previous studies have shown that the quality of ripe fruit and storage life are greatly influenced by the maturity stage at harvest [22,29,30]. Fruit firmness, as one of the foremost quality parameters, has a direct impact on the fruit shelf life and consumer acceptance [31]. The fruit of *A. trifoliata* starts softening and cracking with fruit ripening, associated with easy rotting mediated by bacterial infection and mechanical damage. Therefore, although the fruit of *A. trifoliata* could be picked after cracking, the fruit quality decreases rapidly within 3–5 days at room temperature after harvest. Therefore, harvesting fruits before softening and cracking may be an effective method to prolong the shelf life of *A. trifoliata*. In the current study, the firmness of both clonal lines of *A. trifoliata* exhibited a decreasing trend with the delaying of the harvest stage. The fruit firmness of 'Luqing' and 'Luyu' from S1 to S4 declined by 82.95% and 89.71%, respectively. In particular, the firmness decreased sharply from the S2 to S3 stage (67.36% for 'Luqing' and 83.08% for 'Luyu'), which indicated that fruit textural property has tremendous changes during the period. Niu et al. [32] found that the cell wall became thinner, looser, and showed a substantial breakdown in the pericarp of cracking fruit compared with that in noncracking fruit in *A. trifoliata*. In this study, the firmness of *A. trifoliata* decreased sharply before cracking, consistent with the pericarp structure observation results described by Niu et al. [32]. Fruit ripening and cracking are complex physiological, genetically programed processes that are accompanied by the transcription of many genes and the synthesis of large amounts of proteins [33]. Studies have indicated that pectin metabolism-related genes showed a strong link with cracking phenotypes in sweet cherry fruit [34]. Studies on atemoya pericarp cracking have suggested that starch decomposition into soluble sugars and cell wall polysaccharides metabolism are closely related to the ripening and cracking of African Pride atemoya [35]. Those results in this study may provide useful physiological data for advanced research about the fruit cracking of *Akebia*.

TSS and TA are not only the main compounds responsible for the fruit flavor but are also considered crucial indicators of fruit maturity and postharvest fruit quality evaluation during storage [36]. In this study, the TSS content in both clonal lines of *A. trifoliata* fruit showed an upward trend from the S1 stage to the S4 stage. Exactly, the TSS content of 'Luqing' at S2, S3, and S4 stages increased by 102.78%, 904.11%, and 2.86%, respectively. Furthermore, the TSS content of 'Luyu' at S2, S3, and S4 stages increased by 25.00%, 2068.00%, and 60.33%, respectively. Obviously, the TSS content accumulated sharply from the S2 stage to the S3 stage mainly because of the accumulation of sugars and the breakdown of starch and reached physiological maturity at the S3 stage. Niu et al. [37] found that the activity of beta-amylase (BAM) increased 10-fold when fruit begins to crack longitudinally along with its ventral suture, and BAM may play a significant role in starch degradation during fruit ripening. Meanwhile, the TA content of *A. trifoliata* fruit remained at a very low level during the fruit ripening process, which could be almost ignored and is consistent with previous reports in *A. trifoliata* fruit [38,39]. The continuous accumulation of TSS and low level of TA resulted in the very sweet taste of *A. trifoliata* fruit. Therefore,

the balance of sugars and acids in the fruit could strongly affect the taste of the fruit. For this reason, TSS and TA, along with firmness, are always considered the main parameters for determining fruit quality. At different maturity stages, the changes in TSS, TA, and TSS/TA have also been documented by many studies in different fruits [40–42]. For *A. trifoliata*, the firmness and TSS changed sharply between S2 and S3 stages and then tended to level off; we can consider firmness and TSS as the maturity indicators of *A. trifoliata* fruit.

Carbohydrates play important roles not only in the human diet but also in plants' stress responses, metabolic processes, and biological processes [43]. Sugars are important signaling molecules during plants development and are involved in modulating gene expression in plants [43,44]. Results in this study indicated dynamics of carbohydrates with fruit ripening in *A. trifoliata*. Fructose and glucose contents in 'Luqing' and 'Luyu' showed the same accumulation pattern, which initially increased slightly as the fruit developed from S1 to S2 stage, thereafter rose sharply from S2 to S3 stage (the fructose and glucose contents in 'Luqing' and 'Luyu' increased by 10-fold, 9-fold and 27-fold, 20-fold, respectively), after that increased slightly again from S3 to S4 stage. On the other hand, rapid accumulation of fructose and glucose at the S3 stage could be a signal response to reach physiological maturity in *A. trifoliata*. Cao et al. [45] reported that *A. trifoliata* fruit is a kind of climacteric fruit and is easy to rot and deteriorate after harvest. Furthermore, the increasing content of fructose and glucose at advanced stages of fruit ripening has been found in many climacteric fruit crops [46–49]. The sucrose content in 'Luqing' showed an increasing trend from the S1 to S3 stage but declined sharply at the S4 stage, while the turning point of descent in 'Luyu' occurred at the S3 stage, and the strain differences are mainly responsible for this difference. The contents of fructose, glucose, and sucrose at the S4 stage in 'Luyu' are higher than in 'Luqing'; thus, the TSS at the S4 stage was higher in 'Luyu' and tasted sweeter. The starch content in both clonal lines showed an increasing trend from the S1 to S2 stage but declined sharply at the S3 stage, indicating that the starch degraded rapidly as the fruit approached ripening. The increasing trend in fructose and glucose levels was due to the breakdown of carbohydrates (sucrose and starch) with the advancement of fruit maturity [40,50]. The fructose and glucose contents in *A. trifoliata* fruit increased sharply from the S3 stage, while starch content stayed at a low level from the S3 stage, which resulted in the sweet, soft, and glutinous flavor of *A. trifoliata* fruit pulp. The total sugars content of 'Luqing' increased significantly from the S1 to S3 stage, especially at the S3 stage the content of total sugars increased by 6-fold. It is worth noting that the total sugars content of 'Luqing' had a significant decline after the S3 stage, which may be due to hydrolysis or translocation of sugars. While the total sugars content of 'Luyu' continuously increased from the S1 to S4 stage, which showed a different accumulate dynamics of total sugars. In terms of total sugar content, 'Luqing' is more suitable for harvesting at the S3 stage.

Ascorbic acid is not only an essential nutrient for humans but is also considered a powerful antioxidant component [19]. In the present study, the AsA content of *A. trifoliata* fruit had no difference between the S1 and S2 stages while it significantly increased from S2 to S3 stage, indicating that AsA is rapidly synthesized before fruit ripening. In this study, the AsA content in both clonal lines is higher than that reported in wild *A. trifoliata* subsp. *australis* and *A. trifoliata* [38,39,51,52]. Phenolics and flavonoid compounds are important secondary metabolites of plants, which can protect cells from oxidative damage, increase plant resistance to pathogens, and provide antioxidative properties for the human diet as well [53,54]. Our work showed that total phenolics and total flavonoids levels declined with the advancement of *A. trifoliata* maturity stages. Such variations in phenolics and flavonoids have been found in many fruit crops [55,56], while structural genes, light intensity, and temperature have been reported to influence phenolics and flavonoid biosynthesis [57,58].

The dry matter content is a significant attribute that could indicate the carbon incorporation at different ripening stages of fruits. The decreasing trends of dry matter percentage as maturity progressed in the two clonal lines of *Akebia* were mainly due to the indirect relation with moisture content that increased with the advancement of maturity in fruits.

The moisture content of fruits is an essential parameter that determines their flavor character, storage ability, and suitability for consumption. It is worth noting that moisture content in both clonal lines increased significantly at the S3 stage and had maximum values at the S4 stage. The increase in moisture content with maturity could be due to the hydrolysis of starch to sugars as the fruit ripeness. A similar increasing trend of moisture content with maturity has been observed in mulberry fruit and cherry fruit [17,59]. Conversely, the crude fiber, fat, and protein content in fresh fruit decreased significantly at the S3 stage, which may be due to a significant increase in the moisture content of fruit at the S3 stage.

5. Conclusions

A. trifoliata is a delicate and perishable fruit having a short market and shelf life. For the two clonal lines of *A. trifoliata,* 'Luqing' has bigger fruit than 'Luyu', while the fruits' biochemical and nutritional attributes have no significant difference between them. This study suggested that the stage of maturity had a significant effect on physiochemical parameters and nutritional properties during *A. trifoliata* fruit on-vine ripening, with dramatic changes occurring at the S3 stage of ripening. Obviously, the values of firmness, TSS, fructose, glucose, sucrose, starch, ascorbic acid, total phenolics, and total flavonoids showed remarkable changes during the transition to physiological maturity. The earlier the harvest before fruit cracking, the harder the fruit is, more suitable for long-distance transportation and the longer shelf life. However, considering the nutrients content, sugars content, pulp texture, and other physiological parameters, the S3 maturity stage was considered a proper harvesting stage for long-distance transport of *A. trifoliata* fruit to have desirable fruit quality and consumer acceptability.

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