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Study on the accumulation pattern of anthocyanins, sugars and organic acids in medicinal *Vitis vinifera* 'SuoSuo' during ripening

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ABSTRACT

In this study, targeted metabolomics technology was used to accurately and quantitatively analyze the metabolic pathways of anthocyanin, sugars and organic acid metabolites during the ripening of 'SuoSuo' grape berries. Results, 33, 10 and 36 metabolites of anthocyanins, sugars and organic acids, respectively, were detected. The anthocyanin with the highest content was cyanidin-3-O-glucoside (136.343 ng/g), which reached a maximum at 135 days after full bloom. The highest fructose content in sugar was 167.69 ng/g (135 days after full bloom). Among the organic acids, tartaric acid exhibited the highest content (37,196.67 mg/kg, 105 days after full bloom). The content of oleanolic acid (230.064 mg/kg, 135 days after full bloom) was higher in organic acids. These results clarify how anthocyanin, sugar and organic acid metabolites accumulate and change as 'SuoSuo' grapes ripen and provide a reference for the development and utilization of 'SuoSuo'.

1. Introduction

Grapes are amount the oldest and most economically valuable fruits worldwide, and grape plants are traditionally cultivated globally. In addition, grapes are consumed fresh, dried into table grapes or fermented to produce wines and spirits (Dong et al., 2023). Living a healthy lifestyle is very important to people; thus, the demand for nutritional and healthy food is increasing, and studies on functional plants have become a hot spot in research on medicine food homology plants (Lu et al., 2022). 'SuoSuo' grapes (*Vitis vinifera* L.) is a perennial woody vine red grape family with small pepper-like grains. 'SuoSuo' grapes are only distributed in Turpan, Hetian, Shanshan and other places in Xinjiang, China. 'SuoSuo' is a traditional Chinese medicine used by local ethnic minorities and exhibits many pharmacological effects, such as antioxidant, antibacterial, anti-inflammatory and antiviral effects (Askari-Khorasgani & Pessarakli, 2019). 'SuoSuo' is widely used in Uygur medicine (The four major ethnic medicines in China are Tibetan medicine, Mongolian medicine, Uyghur medicine and Dai medicine. Uyghur medicine is one of the treasures of outstanding national culture.) to treat hepatitis, low energy, blood deficiency, cough caused by lung deficiency, spleen and stomach maladies, dizziness, soreness in the waist, restlessness, measles in children and other diseases (Liu et al., 2010). Liu et al. (2012) identified total flavonoids, total polysaccharides, and total triterpenes from 'SuoSuo' grapes, which are the main medicinal components. At present, only the main therapeutic effects of medicinal 'SuoSuo' grapes are known, but their medicinal value has not been fully exploited. Therefore, a comprehensive and in-depth study on the quality of 'SuoSuo' grape berries is needed to provide a theoretical basis for the development and utilization of 'SuoSuo' grapes in medical applications.

In this decade, consumers have increasingly focused on the quality of grapes, including the quality of their taste and appearance. The appearance traits mainly include the size, shape and colour of the grape, while the taste is mainly influenced by the sugar and organic acid content and their composition ratio (Poni et al., 2018). The colour of grape skin is mainly determined by the anthocyanin content. Anthocyanin is a flavonoid compound that is a secondary metabolite of plants and is synthesized during maturation (Kőrösi et al., 2022). Cyanidin, delphinidin, peonidin, petunidin and malvidin are the main anthocyanin

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inducers in grape berries (Hwang et al, 2017; Liang et al, 2008). Anthocyanin is synthesized in the peel of red varieties and accumulates, while no anthocyanin is produced in the berries of white varieties (Massonnet et al., 2017). The types and contents of sugar and organic acids in grape berries are important indicators that affect the taste and flavour of berries (Coelho et al., 2018). Different grape varieties have different types and contents of sugar and organic acids, and their differences determine the unique style of different grape varieties (Liang et al., 2011). At present, domestic and foreign scholars have carried out many related studies on the glucose and organic acid components and achieved remarkable results. Silva et al. (2015) showed that organic acids, such as malic acid, tartaric acid and citric acid, were the main components of grapes, and fructose and glucose were the main sugars in grapes. Liu et al. (2006) showed that the content and composition of sugar and organic acids in grape berries depend largely on the genotype of grapes, and wine grape varieties are characterized by high sugar and high tartaric acid contents. Niu et al. (2022) found that among different grape populations, the total acid content of the East Asian population was the highest, followed by the North American population, and the Eurasian population was the lowest. Jang et al. (2017) reported that Chinese wild grapes have low sugar and high acid contents, and the composition of organic acids in different wild grapes is quite different, while the composition of sugar is basically the same. However, studies on the quality of 'SuoSuo' grapes and their characteristics remain relatively limited compared with other grape varieties, with only a few reports on their pharmacological activity, clinical application and extraction process.

Targeted metabolomics is an important part of metabolomics research and achieves strong specificity, high detection sensitivity and accurate quantification (Ribbenstedt et al., 2018). With this method, targeted detection and analysis are usually performed with several specific or several types of metabolite groups and reveal changing metabolic mechanisms; this is achieved by collecting accurate quantitative data for the metabolic pathways corresponding to the candidate metabolites and all components upstream and downstream (Ren et al., 2018). As a raw material of medicine and health care products with wide application value, 'SuoSuo' grapes have very broad development prospects in the treatment and prevention of many diseases. However, the composition and content of its berry metabolites have not been fully revealed and utilized. The metabolic accumulation patterns for anthocyanins, sugars, and organic acids that occur as trivial grapes ripen have not been reported. Therefore, in this study, anthocyanin, sugar and organic acid fractions were measured in 'SuoSuo' grapes during three critical ripening periods; this was achieved by using targeted metabolomics techniques to clarify the accumulation and changes in berry quality. The aim was to improve the evaluation system for the quality of medicinal grape resources characteristic to Xinjiang. Xinjiang's rich grape resources have an economic advantage. Simultaneously, the study provides new ideas for the development of safe, effective and inexpensive immunomodulatory agents or antiviral national new drugs.

2. Materials and methods

2.1. Plant materials

The experimental site was the 'SuoSuo' grapes plantation (42°56′ N, 89°05′ E) in Shanghu Village, Gaochang District, Turpan City, Xinjiang. The site has a warm temperate continental arid desert climate. The average annual precipitation is 16.4 mm, the average annual evaporation is 2837.8 mm, the annual sunshine is 3200 h, the annual average temperature is 14.5 °C, the maximum temperature is above 35 °C on more than 100 d, the maximum temperature is above 40 °C on 35 ~ 40 d and the frost-free period is 268 d.

The test sample was a 12-year-old 'SuoSuo' grapes. The plant-row spacing was 1.5 × 5.5 m, the shape was dragon-trunk, the branch direction was from north to south, and the orchard was managed with conventional water and fertilizer. Healthy plants with consistent growth were selected in the orchard, with 3 plants per plot, and the procedure was repeated 3 times. Fruit samples were collected at three growth stages between August and September 2021, including when the berries turned green and began to soften (S1, 95 days after full bloom); when the berries gained colour and expanded and the sugar content reached the medium equivalent (S2, 105 days after full bloom); and when the maturity of berries reached the harvest requirements (S3, 135 days after full bloom). Three biological replicates were obtained at each stage. Fifty berries were randomly picked from the east, south, west, and north directions of the middle and upper parts of the crown of the trees at 10 a.m. on sunny days. The samples from each stage were divided into two parts. One part was used to determine appearance indexes (vertical and transverse diameter of berries, single fruit weight.0, etc.), and the other was immediately frozen in liquid nitrogen and stored at −80 °C to measure total phenols, soluble sugars content and other physiological indexes as well as anthocyanin, sugars and organic acid metabolites.

2.2. Determining the appearance quality of the berries

Thirty berries were randomly taken at each of the three critical stages of ripening. The vertical diameter and transverse diameter of a single grain were measured by a Vernier calliper, and the mass of a single grain was measured by an electronic balance. A berry hardness tester was used to measure the hardness of berries (Eideburg, GY-3). Two symmetrical parts near the equator line of each berry were measured. The instrument was pressed vertically and slowly until the berry was deformed, and the peak mean value was used as the berry hardness.

2.3. Determining the internal quality of the berries

2.3.1. Soluble solids content

To measure the content of soluble solids, an electronic display sugar metre was used (PAL-1; Atago Co., Japan). Before use, the sugar metre was placed in a bright place, and the zero point was corrected with distilled water. The juice of grape berries was squeezed, and the juice was dripped in the centre of the mirror of the sugar metre for determination. After the measurement was performed for each berry, the mirror was washed with distilled water, and the Soluble solids content value was recorded.

2.3.2. Total phenols

The total phenols were determined by the Folin-Ciocalteu method (Matić et al., 2017). 10.0 g of berries were weighed and ground into a homogenate, which was diluted to 100.0 mL in a quantitative bottle. 1.0 mL of the homogenate was drawn into a 10.0 mL test tube, and 0.5 mL of Folin-Ciocalteu reagent was added. The reaction was carried out in the dark for 5 min at room temperature, and 0.4 mL of 7.5% sodium carbonate solution was added. The solution was diluted to 10.0 mL with distilled water, and the reaction was carried out in the dark for 60 min at room temperature. The absorbance values for the samples and different mass concentrations of gallic acid were measured at 765 nm. The mass concentration of gallic acid (g/kg) was used as the abscissa. The standard curve was created with the absorbance as the ordinate. The total phenol content of each sample was calculated according to the standard curve.

2.3.3. Soluble sugars

The soluble sugars were measured by the anthrone colorimetric method (Wang et al., 2015). The grape berries were weighed and ground in liquid nitrogen to 0.2 g and transferred into a 10.0 mL centrifuge tube. Then, 5.0 mL ethanol–water (80:20 M, v/v) was added to dissolve the grape berries. After the solution was cooled in a water bath at 80 °C for 30 min, 0.5 mL of the extract was taken and placed in a 10.0 mL test tube. Then, 1.5 mL of distilled water, 0.5 mL of anthrone ethyl acetate reagent and 5 mL of concentrated sulfuric acid were added. The blank (without extract) was set, and the colour was measured at 620 nm. The blank was used as the standard curve to further obtain the soluble sugar content.

2.3.4. Titratable acid

The titratable acid was determined by NaOH titration (Darias-Martín et al., 2003). A total of 10 mL juice stock solution was added to a 100.0 mL volumetric flask, distilled water was added to a constant volume and the solution was shaken well. The diluent was transferred into a conical flask, 1% phenolphthalein indicator was added (1 drop), and the solution was titrated with the calibrated sodium hydroxide solution until the solution turned red for 30 s. Parallel tests were performed three times, and the average value was taken. The volume of sodium hydroxide solution consumed by titration was recorded, and the titratable acid content in the sample juice was calculated according to the volume consumed.

2.3.5. Tannin

The tannin content was determined by the Folin-Dennis method (Feitosa et al., 2018). 10 g berries were homogenized and placed in a 100.0 mL volumetric flask. A total of 1.0 mL sample was put into 10.0 mL test tube and 0.5 mL FoLin-Denis reagent was added. Add saturated sodium carbonate solution 1.0 mL; distilled water was diluted to 10.0 mL and placed indoors for 30 min. The absorbance values of the samples and tannic acid at different concentrations were measured at 510 nm. The standard curve was created with the mass concentration of tannic acid (g/kg) as the horizontal coordinate and the absorbance as the vertical coordinate. The tannin content of each sample was calculated according to the standard curve.

2.3.6. Vitamin C

Vitamin C determination by the 2,6-dichloroindophenol method (Borba et al., 2021). Weigh 10.0 g berries into homogenate and transferred into a 100.0 mL volumetric flask. The 10.0 mL filtrate was placed in a 50.0 mL conical flask and titrated with the calibrated 2,6-dichloroindophenol solution until the solution remained pink for 15 s. The volume of 2,6-dichloroindophenol solution consumed by titration was recorded, and the vitamin C content in the sample juice was calculated according to the volume consumed.

2.4. Determination of the anthocyanin, sugar and organic acid metabolites in berries

2.4.1. Determination of anthocyanin content

Berries ground to powder through liquid nitrogen freezing. Fifty milligrams of powder weighed and dissolved in 500 μL of extract (50% methanol aqueous solution containing 0.1% hydrochloric acid). The cells were vortexed for 5 min, sonicated for 5 min, and centrifuged for 3 min (12,000 r/min, 4 °C), and the supernatant was obtained and the procedure was repeated once. The supernatant was combined twice, and the sample was filtered with a microporous membrane (0.22 μm pore size) and stored in an injection bottle for LC-MS/MS (SCIEX, QTRAP 6500 +) analysis.

2.4.2. Determination of sugar content

Berries were frozen and ground in liquid nitrogen to powder. A total of 20 mg of powder was weighed, and 500 μL of methanol: isopropanol: water (3:3:2 *v*/*v*/*v*) extract was added, vortexed for 3 min, and sonicated in ice water for 30 min. After centrifugation at 14,000 r/min for 3 min at 4 °C, 50 μL of supernatant was collected, and 20 μL of 100 μg/mL ribitol internal standard solution was added. Then, 100 μL of methoxyammonium pyridine (15 mg/mL) was added and incubated at 37 °C for 2 h. Then, 100 μL of BSTFA (N, O-Bis (trimethylsilyl) trifluoroacetamide) was added and incubated at 37 °C for 30 min to obtain the derivatization solution. The 50 μL derivatization solution was diluted with *n*-hexane to 1 mL and stored in a brown injection bottle for GC–MS (Agilent, 8890-5977B) analysis.

2.4.3. Determination of organic acid content

Berries were separated and frozen in liquid nitrogen to powder. Fifty milligram (±2.5 mg) samples were weighed into a 2 mL centrifuge tube, and the weight of each sample was recorded. The weighed samples were immediately added to 500 μL of 70% methanol water extract precooled at −20 °C for 3 min. At 4 °C, the samples were centrifuged at 12,000 r/min for 10 min, and 300 μL of the supernatant was placed into a 1.5 mL centrifuge tube. The sample was refrigerated at −20 °C for 30 min and centrifuged at 4 °C and 12,000 r/min for 10 min. Two hundred microlitres of the supernatant was transferred to a vial for LC-MS/MS (SCIEX, QTRAP 6500 +) analysis.

2.5. Statistical analyses

All three biological replicates were collated using Microsoft Excel 2020, and the data were analysed by analysis of variance and plotted using GraphPad Prism 8 (Graphpad, California); correlation heatmaps were produced using Origin 2017 (OriginLab, USA). Principal component analysis (PCA), hierarchical cluster analysis (HCA) and KEGG bubble plots were performed with R software.

3. Results

3.1. Comparison of the growth changes in 'SuoSuo' grape berries at three ripening stages

The phenotypes of berry development at different stages during the maturation of 'SuoSuo' grapes are shown in (Fig. 1). S1: 95 days after full bloom, the berries were green and began to soften. S2: 105 days after full bloom, the berries turned and expanded, and the sugar content reached the medium equivalent. S3: 135 days after full bloom, the maturity of berries reached the harvest requirements. The vertical diameter, transverse diameter, single fruit weight and soluble solids content of berries showed an obvious upwards trend as time continued. The vertical diameters of berries from S1 to S3 were 5.67 mm, 6.31 mm and 6.60 mm, respectively, and S3 increased by 0.93 mm compared with S1, and compared with S1, S2 increased 0.64 mm. The transverse diameters of berries were 6.56 mm, 7.10 mm and 7.68 mm in the three periods, respectively, and S3 increased by 1.12 mm compared with S1, and S2 was larger than S1, 0.54 mm. The single fruit weight from S1 to S3 was 0.19–0.30 g, and S3 was 0.11 g heavier than S1, and S2 was 0.06 g heavier than S1. The soluble solids content reached 22.96%–31.96% from S1 to S3, and S3 was 9.00% higher than S1, and S2 was 3.88% higher than S1. In contrast, the hardness of berries decreased gradually with the growth of berries. The hardness of the berries was 0.65 kg/cm^2, 0.58 kg/cm^2 and 0.54 kg/cm^2 in the three periods. The hardness of S1 was 0.11 kg/cm^2 higher than that of S3, and S1 was 0.07 kg/cm^2 higher than S2. The above shows that the phenotype of 'SuoSuo' grapes changed greatly at the mature stage.

3.2. The physiological changes in 'SuoSuo' grape berries during three maturation processes were compared

There were significant differences in the intrinsic quality of grape berries at different growth stages (Fig. 2). Among the six main quality indicators, total phenol, tannin, soluble sugar and sugar acid ratio increased overall, and the vitamin C content and titratable acid content gradually decreased as the berries ripened. The largest difference between S1 and S3 was the ratio of sugar to acid. The ratio of sugar to acid was 63.67 in S3, 50.02 in S2, 29.67 in S1, and 2.15 times in S3. The contents of soluble sugar from S1 to S3 were 18.94 g/kg, 29.36 g/kg and 38.93 g/kg, respectively, and S3 was 51.33% higher than S1. The difference in titratable acid content was the smallest; the contents of S1 to S3 were 0.64%, 0.61% and 0.59%, respectively, and the content of S1 to

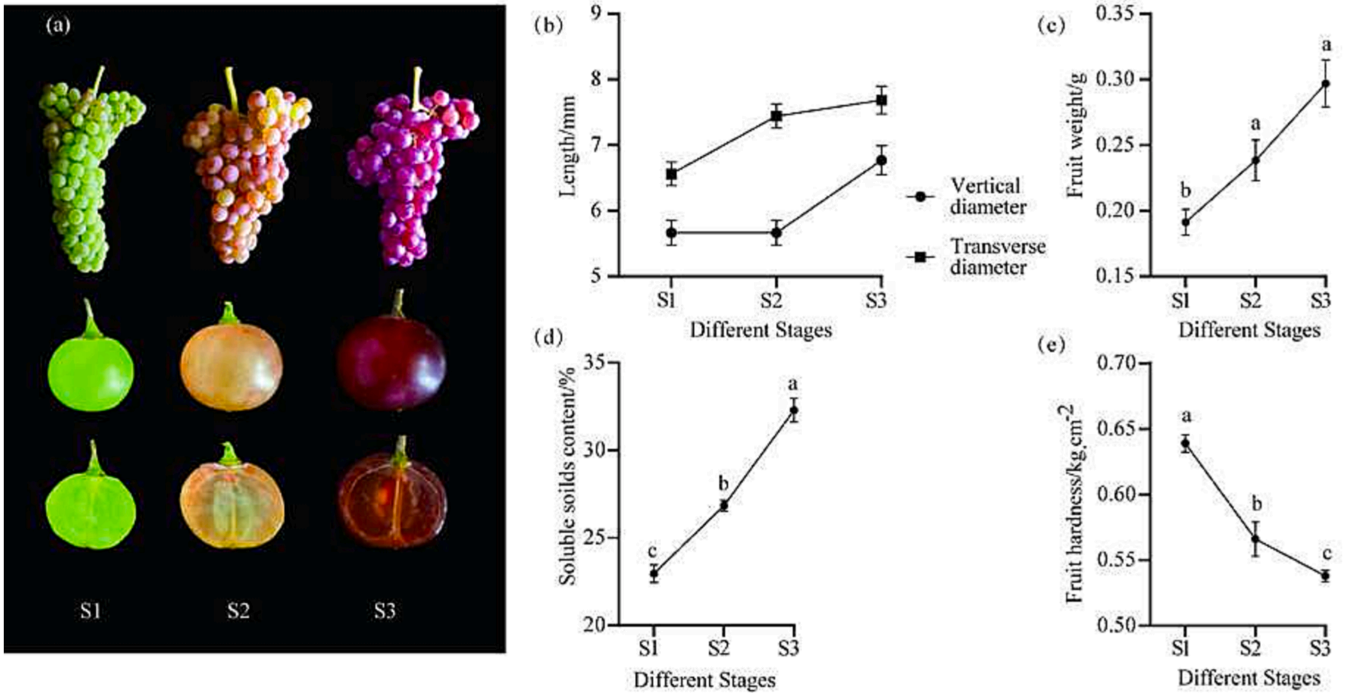

Fig. 1. Determination of morphological indexes that occur during the ripening of 'SuoSuo' grapes. (a) Three stages of fruit ripening, (b) Changes in vertical and transverse diameters of berries during the three stages, (c) Changes in the single fruit weight during the three stages, (d) Changes in soluble solids content during the three stages, (e) Changes in fruit hardness during the three stages. Different lowercase letters represent significant differences ($P < 0.05$).

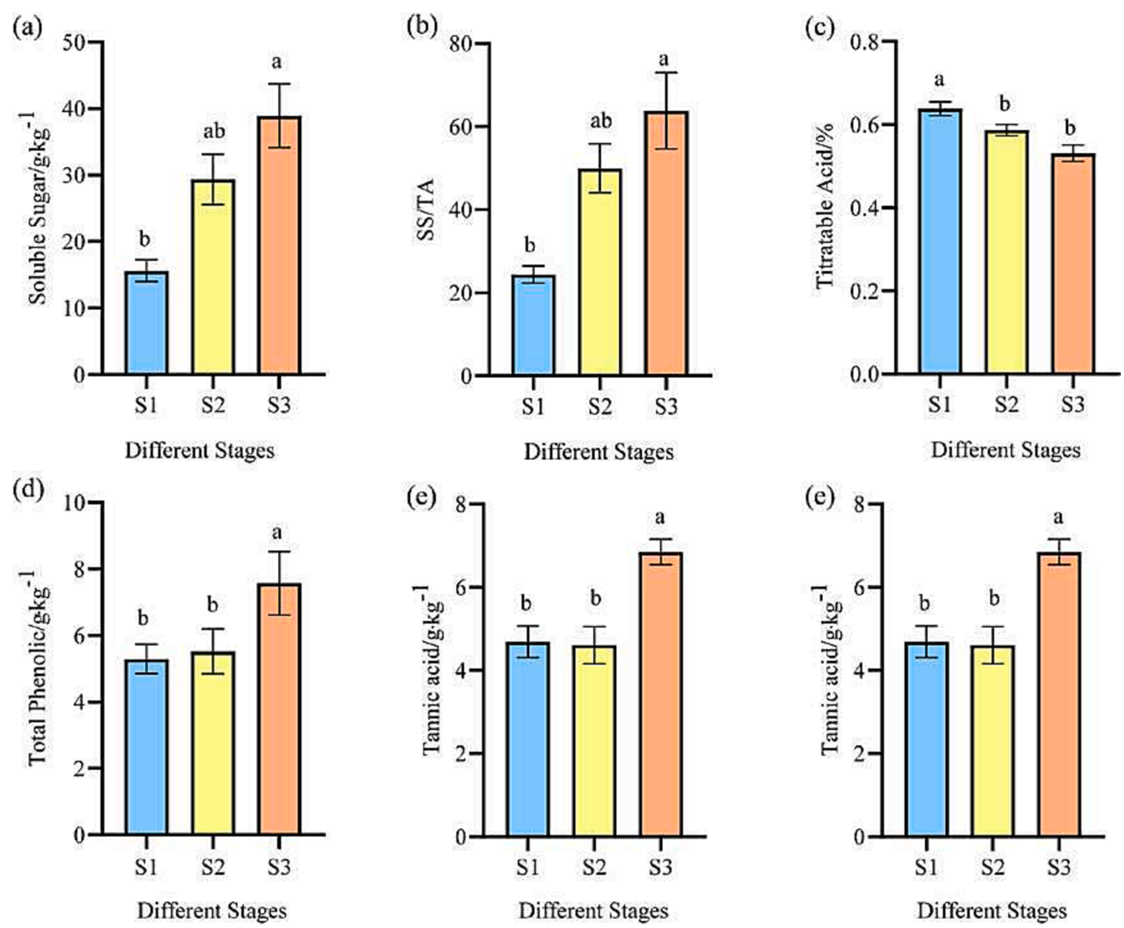

Fig. 2. Difference analysis of physiological indexes in different growth stages of 'SuoSuo' grapes. (a): Soluble sugar, (b): Sugar acid ratio, (c): Titratable acid, (d): Total phenol, (e): Tannin, (f): Vitamin C. Different lowercase letters represent significant differences ($P < 0.05$).

S3 decreased by only 0.05%. As berry maturity increased, acidity decreased and sugar accumulated in large quantities. Differences were observed in total phenol, tannin and vitamin C between S3 and S2 and S1, and the content was higher than that of S2 and S1. The contents of total phenol S1 to S3 were 5.29 g/kg, 5.52 g/kg and 7.57 g/kg, respectively. The content of S3 was 30.04% higher than that of S1. The tannin content in the three periods was 4.36 g/kg, 4.62 g/kg and 6.18 g/kg, respectively, and the content of S3 was 29.54% higher than that of S1. There were differences between S1, S2 and S3 in vitamin C, and the content of S3 was the lowest, which was 6.74 g/kg, 5.87 g/kg and 4.07 g/kg in the three periods, respectively. Compared to S3, S1 was 39.57% higher. It shows that as the 'SuoSuo' grapes tends to mature, phenols and sugars are continuously synthesized and accumulated, and acids are continuously consumed.

3.3. Morphological changes between the appearance and physiological indexes of 'SuoSuo' grape berries during ripening based on correlation analysis

The growth and development of berries are determined by sensory size and are influenced by intrinsic quality. To clarify the berry ripening process, we correlated the phenotype with the physiological changes in grapes. As shown in (Fig. 3), berry vertical diameter was positively correlated with soluble sugars and the sugar-acid ratio; berry transverse diameter was significantly and positively correlated with single fruit weight. Soluble solids content and soluble sugars; single fruit weight was significantly and positively correlated with total phenols. Soluble sugars and the sugar-acid ratio were positively correlated with soluble solids and tannins; berry hardness was positively correlated with vitamin C content and titratable acids. Total phenols were significantly and positively correlated with tannins and positively correlated with soluble sugars and the sugar-acid ratio. The berry vertical diameter was negatively correlated with vitamin C content and titratable acidity and negatively correlated with berry hardness; single fruit mass was negatively correlated with titratable acidity and negatively correlated with berry hardness and vitamin C content; berry hardness was negatively correlated with sugar-acid ratio; and total phenols were negatively correlated with vitamin C and titratable acidity. Thus, berry size and single fruit weight were closely related to total phenols, soluble sugars and titratable acids during the growth of 'SuoSuo' grapes.

3.4. Analysis of the difference in anthocyanin content of 'SuoSuo' grape berries during ripening

To compare the composition of metabolites related to anthocyanin accumulation during the maturation of 'SuoSuo' grapes, we used LC-MS/MS to determine the anthocyanin content. A total of 33 anthocyanins (Table S1) were detected, and 21, 29 and 33 were detected in S1, S2 and S3, respectively. PCA of the detected anthocyanin derivatives (Fig. 4a) showed that PC1 accounted for 73.28% of the total variables, PC2 accounted for 11.58% of the total variables, and there was a clear separation between S1, S2 and S3, and there were differences. In addition, the anthocyanin metabolites were analysed by a cluster heatmap (Fig. 4b). Most of the anthocyanin metabolites were concentrated in the S3 period, and a small amount of anthocyanin metabolites were concentrated in the S1 period. In addition, 33 anthocyanin metabolites were divided into 8 categories (Fig. 4c); the largest category was peonidin (n = 7), and S1, S2 and S3 were detected at 1, 2 and 7, respectively.

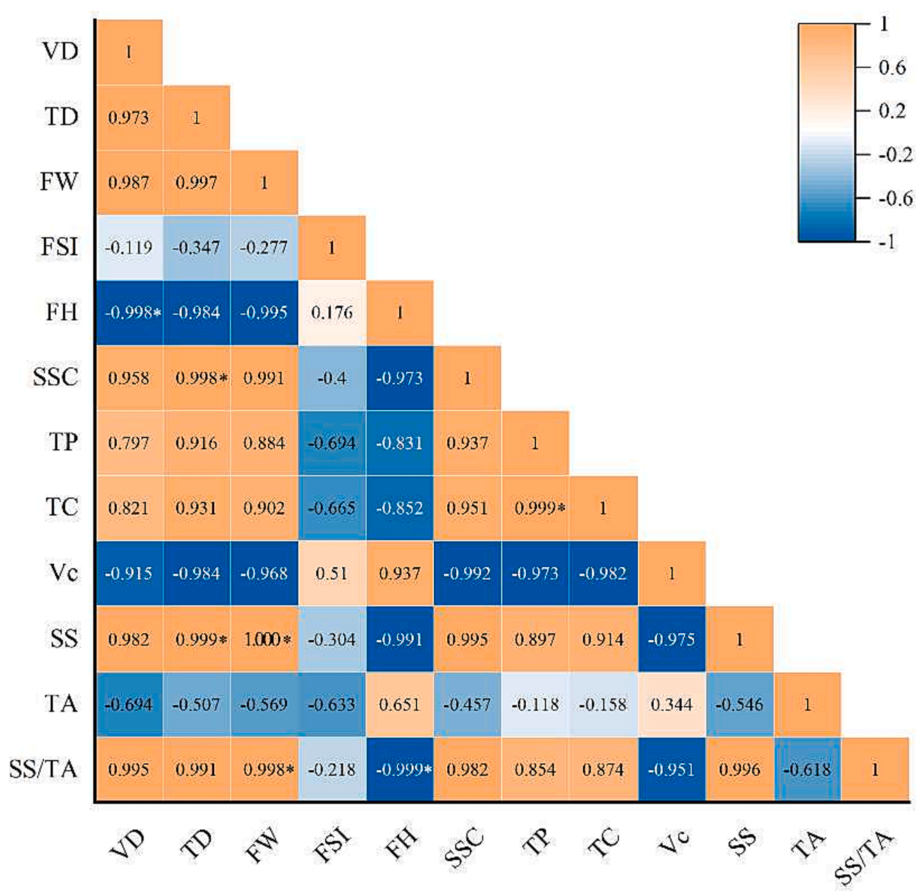

Fig. 3. Correlation analysis of grape berry quality. * was significantly correlated at the 0.05 level (bilateral). VD: Vertical Diameter; TD: Transverse Diameter; FW: Fruit Weight; FH: Fruit Hardness; SSC: Soluble Solids Content; TP: Total Phenolic; TC: Tannic Acid; SS: Soluble Sugar; TA: Titratable Acid; SS/TA: Soluble Sugar/Titratable Acid.

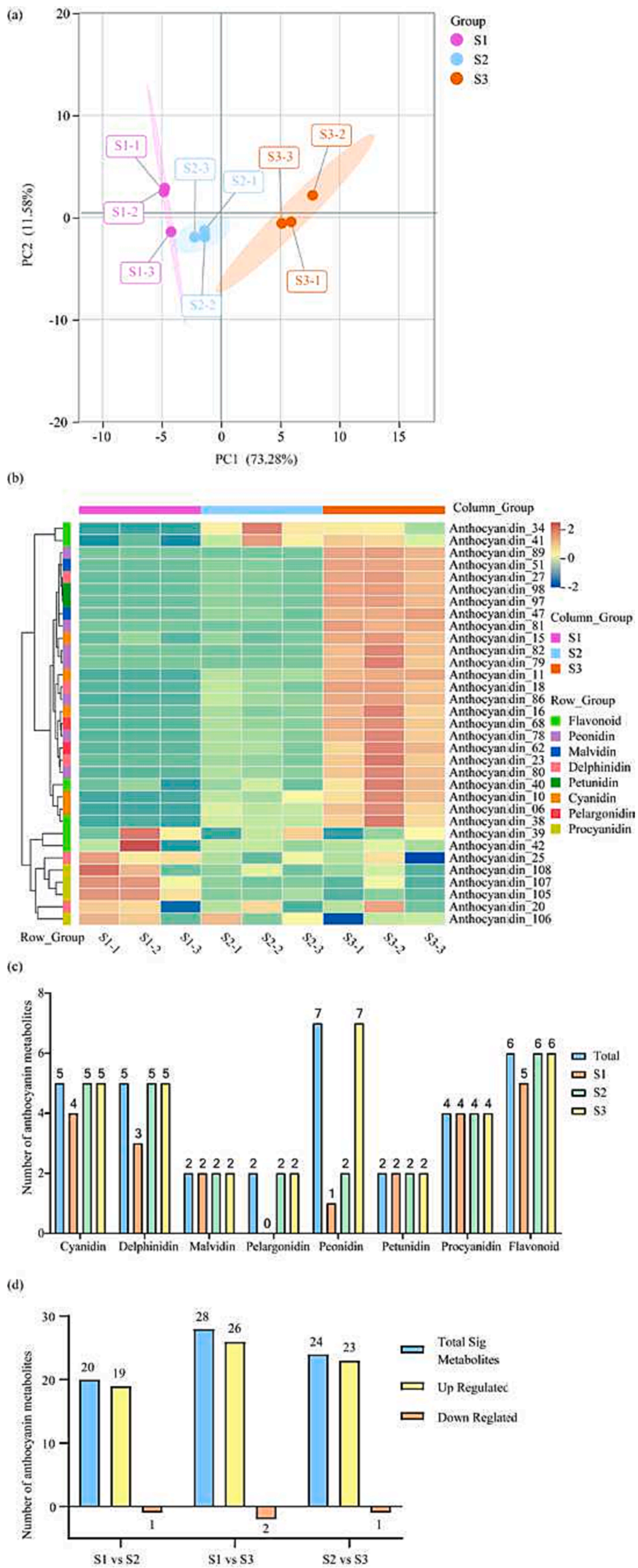

Fig. 4. Anthocyanin metabolites in different stages of grape ripening. (a) Cluster heatmap of anthocyanin metabolite content at different stages, (b) Anthocyanin classification maps at different stages, (c) Differential metabolites of anthocyanins at different stages.

The content of peonidin-3-O-glucoside in S3 was the highest, 94.611 ng/g. The second was flavonoids (n = 6), S1 to S3 were detected in 5,6,6, quercetin-3-O-glucoside in S2 is the highest content, 117.750 ng/g. Cyanidin (n = 5), S1, S2 and S3 were detected in 4, 5 and 5 species, respectively. The content of cyanidin-3-O-glucoside was the highest in S3, which was 136.343 ng/g. Three, five and five delphinidins were detected in S1, S2 and S3, respectively, and delphinidin-3-O-glucoside was 11.375 ng/g in S3. The least were malvidin (n = 2), pelargonidin (n = 2) and petunidin (n = 2), and the least was pelargonidin-3-(6-O-p-coumaroyl)-glucoside, 0.055 ng/g.

Differential metabolites were screened by combining the fold change value (fold change ≥2 and fold change ≤0.5) and *P* value (*P* value < 1) (Fig. 4d). There were 20 differential metabolites in S1 vs. S2, including 19 up-regulated metabolites and 1 down-regulated metabolite. There were 28 differential metabolites in S1 vs. S3, including 26 up-regulated metabolites and 2 down-regulated metabolites. There were 24 differential metabolites in S2 vs. S3, including 23 up-regulated metabolites and 1 down-regulated metabolite. The results of the three groups were transformed into log_2FC by difference multiple treatment, and the metabolites with the largest difference multiple and down-regulated metabolites were screened out (Fig. S1a–c). The common up-regulated differential metabolites of S1, S2 and S3 were as follows: cyanidin-3-O-glucoside, petunidin-3-O-glucoside, malvidin-3-O-glucoside, delphinidin-3-O-glucoside, malvidin-3-O-(6-O-p-coumaroyl)-glucoside and peonidin-3-O-(6-O-p-coumaroyl)-glucoside. The down-regulated differential metabolites were cyanidin-3-O-(6-O-malonyl-β-D-glucoside) and naringenin. The KEGG database was used to enrich the metabolic pathways involved in different metabolites of berries at different maturities (Fig. S1d–f). The differential metabolites of S1 vs. S2, S1 vs. S3, and S2 vs. S3 were enriched in 5–6 metabolic pathways and were coenriched in metabolic pathways for anthocyanin biosynthesis, flavone and flavonol biosynthesis and flavonoid biosynthesis. The detection results of anthocyanin metabolites were basically consistent with the trend of total phenol determination. In the metabolic pathway, anthocyanin was enriched to the most differential metabolism, and the highest content metabolite was cyanidin-3-O-glucoside. It shows that cyanidin-3-O-glucoside is the main substance of purple peel of 'SuoSuo' grapes.

3.5. Analysis of the difference in sugar content of 'SuoSuo' grapes berries during ripening

Ten sugar components were detected in berries at different growth stages (Fig. 5), and there were significant or extremely significant differences in sugar content at different ripening stages. It was found that lactose, D-sorbitol, trehalose, sucrose, L-fructose and D-arabinose 'SuoSuo' grapes increased as the berries ripened. Inositol, rhamnose, fucose and arabinose decreased from S1 to S3. Fructose, glucose and sucrose were the main components in berries, and the content of fructose was the highest. The contents of S1, S2 and S3 were 144.977 ng/g, 158.920 ng/g and 167.696 ng/g, respectively. The glucose contents were 129.493 ng/g, 140.290 ng/g and 152.211 ng/g, respectively. The sucrose content was relatively small, and the contents in the three periods were 32.731 ng/g, 34.745 ng/g and 42.061 ng/g. The content of D-arabinose in other sugars in berries was the lowest and gradually decreased as the berries ripened. The contents of S1, S2 and S3 were only 0.040 ng/g, 0.037 ng/g and 0.025 ng/g, respectively. Followed by fucose, S1 is 0.043 ng/g, S2 is 0.034 ng/g and S3 is 0.030 ng/g.

A combination of differential ploidy fold change values (fold change greater than 2 and fold change < 0.5) and *P* value values (*P* value < 1) were utilized, and one differential metabolite each was screened for sorbitol (S2) for S1 vs. S2, S1 vs. S3 and S2 vs. S3. The metabolic pathways involved in the differential metabolites of berries at different maturity levels were enriched using the KEGG database (Fig. S2). The differential metabolites of S1 vs. S2, S1 vs. S3, and S2 vs. S3 were consistently enriched in metabolic pathways and were jointly enriched in the ABC transporter protein, fructose and mannose

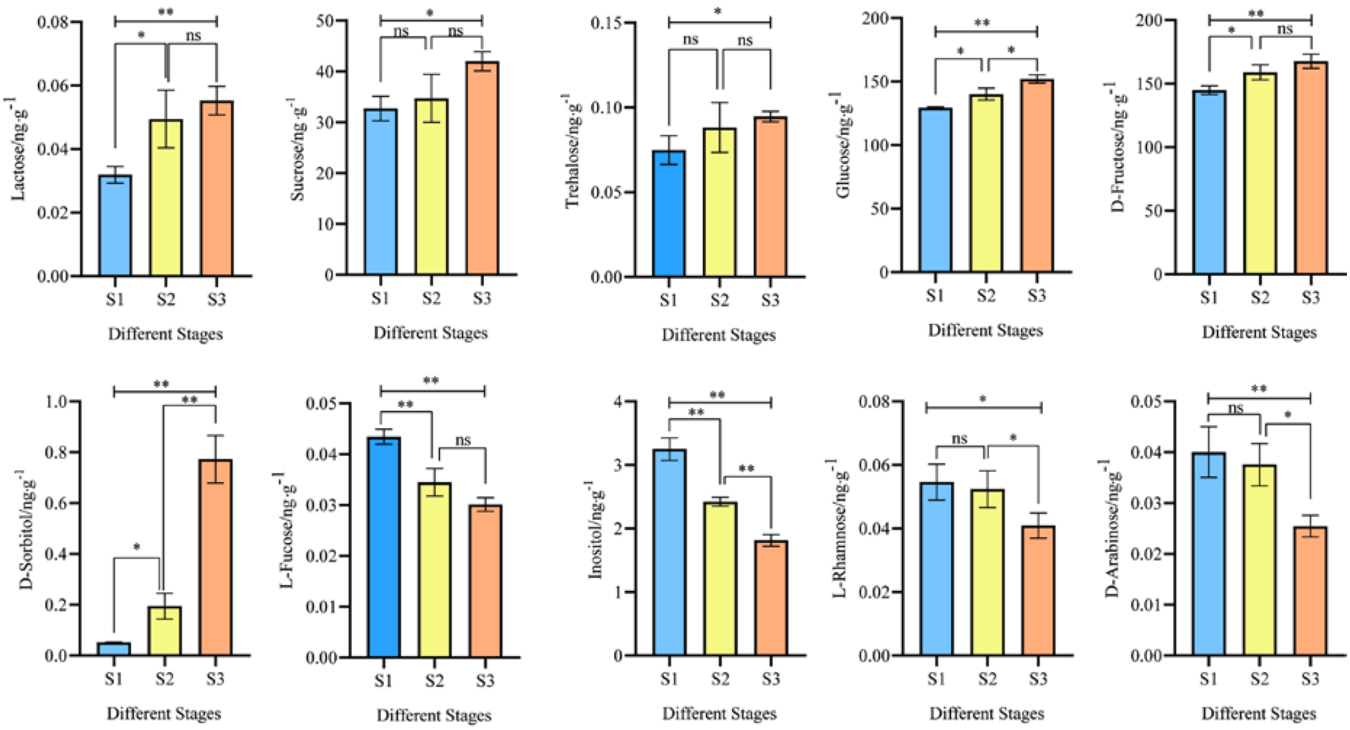

Fig. 5. Analysis of the differences between sugar content in different developmental stages of 'SuoSuo' grapes. (a) Changes in the content of sugar metabolism derivatives at different stages of berry ripening, '*' indicates a significant difference ($P < 0.05$), '**' indicates an extremely significant difference ($P < 0.01$), 'ns' indicates not significant.

metabolism and galactose metabolic pathways. The results of sugar metabolism in grape berries were basically consistent with the results of soluble sugar determination. These results indicated that the sugar metabolism and accumulation in berries were strictly regulated by the development process, and sorbitol played an important role in the transport and accumulation of berries.

3.6. Analysis of the differences in organic acid content of 'SuoSuo' grape berries during ripening

To compare the composition of metabolites related to the accumulation of organic acids during the ripening of 'SuoSuo' grapes, we used LC3000 high-performance liquid chromatography to determine the content of organic acids. In this study, 36 organic acids (Table S2) were detected. Based on the results obtained for organic acid metabolism, principal component analysis (PCA) was performed. The contribution rate of the first principal component (PC1) to the sample was 55.12%, and the contribution rate of the second principal component (PC2) to the sample was 18.85%. The distribution of each sample was concentrated, indicating that the samples in the group exhibited good repeatability and that the organic acid metabolites were highly consistent. The distribution of sample points at different stages of maturation was significantly separated, indicating that there were significant differences between organic acid metabolites during maturation (Fig. 6a). In addition, cluster analysis of metabolites detected by organic acids (Fig. 6b) showed that approximately 1/2 of the organic acid metabolites, such as salicylic acid, pantothenic acid and gallic acid, gradually decreased with the maturation of berries, mainly concentrated in the S1 period. Approximately 1/4 of the organic acid metabolites, such as tartaric acid, fumaric acid and benzoic acid, were the most abundant in the S2 period. More than 1/4 of the organic acid metabolites, such as shikimic acid, L-malic acid and oleanolic acid, gradually increased as the berries ripened, mainly in the S3 period. The content of tartaric acid was the highest in 'SuoSuo' grape berries, and the highest content was 37,196.67 mg/kg at S2, which was 13.76% higher than that at S3. Fumaric acid was the second most abundant, with a content of 43,203.33 mg/kg in S2, 53.41% more than that in S3. The lowest content was anthranilic acid, which was 0.535 mg/kg in S3. The contents of oleanolic acid in S1, S2 and S3 were 194.33 mg/kg, 224.424 mg/kg and 230.064 mg/kg, respectively.

A combination of fold change (fold change greater than 2 and fold change < 0.5) and P value (P value < 1) was used to screen 24 differential metabolites (Fig. 6c). Among them, there were 6 up-regulated and 2 down-regulated differential metabolites in S1 vs. S2 and S1 vs. S3; S2 vs. S3 contained 3 up-regulated and 5 down-regulated differential metabolites. The results of the three groups were transformed into $\log_2$FC by difference multiple treatment, and the metabolites with the largest difference multiple and down-regulated metabolites were screened out (Fig. S3a-c). In S1 vs S2, the largest up-regulated metabolite was lactic acid, and the smallest down-regulated metabolite was shikimic acid. In S1 vs S3, the largest up-regulated metabolite was cinnamic acid, and the smallest down-regulated metabolite was anthranilic acid. The largest up-regulated metabolite in S2 vs S3 was cinnamic acid, and the smallest down-regulated metabolite was levulinic-acid. Succinic acid was an up-regulated differential metabolite in S1 vs S2, and a down-regulated differential metabolite in S2 vs S3. It indicated that when SuoSuo grapes reached maturity at S3, the organic acid was decomposed and succinic acid played an important role. The KEGG database was used to enrich the metabolic pathways involved in the differential metabolites of berries with different maturities (Fig. S3d). The differential metabolites of S1 vs. S2, S1 vs. S3, and S2 vs. S3 were enriched in the same metabolic pathways and were coenriched in metabolic pathways, such as metabolic pathways, biosynthesis of secondary metabolites, TCA cycle, glyoxylic acid and dicarboxylic acid metabolism, butyric acid metabolism, amino acid biosynthesis, and alanine, aspartic acid and glutamic acid biosynthesis. The results of organic acid metabolism and titratable acid determination were basically consistent. It indicated that organic acids were consumed as substrates for glycolysis, tricarboxylic acid cycle and gluconeogenesis when organic acids decreased in 'SuoSuo' grapes.

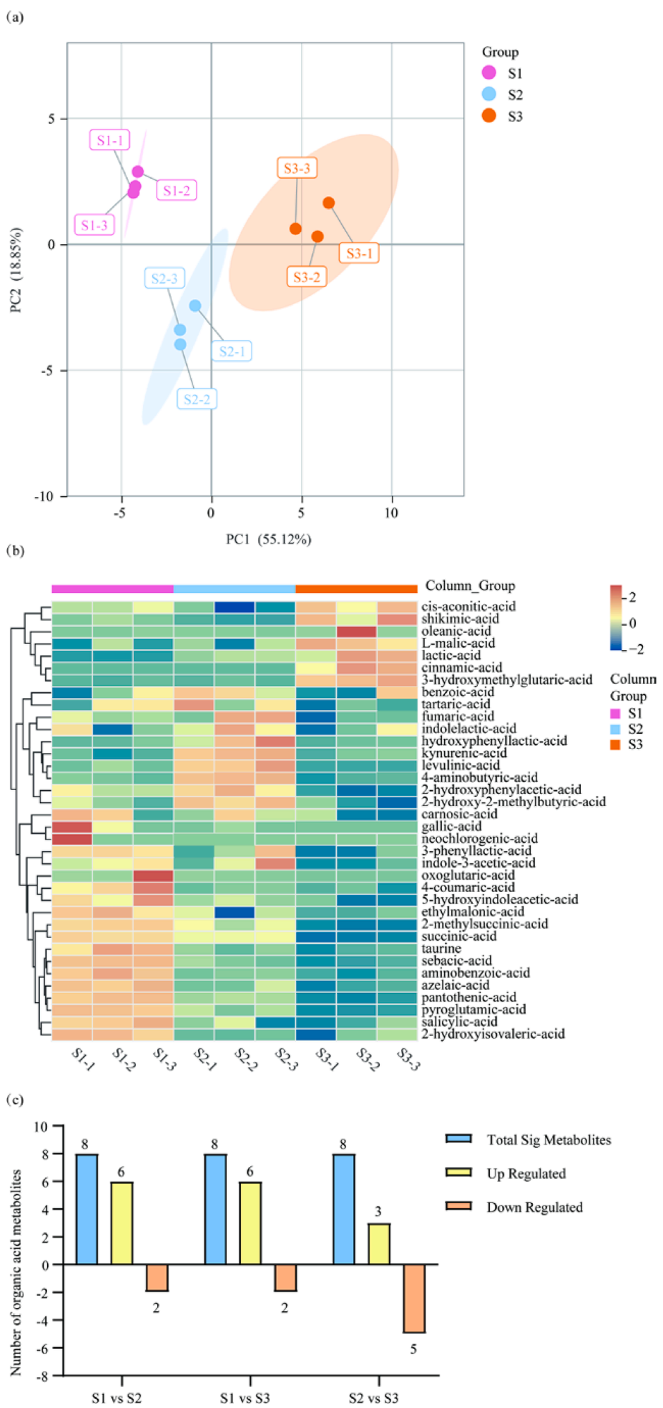

Fig. 6. (a) PCA plot, (b) Clustering heatmap, (c) The number of up-regulated and down-regulated differential metabolites of organic acids.

4. Discussion

Natural active substances in plants are a hot research topic and key source for biopharmaceutical development (Uwineza and Waśkiewicz, 2020). As a medicinal and edible material, 'SuoSuo' grapes exhibit rich pharmacological activities. The types, contents and proportions of anthocyanins, sugars and organic acids in 'SuoSuo' grapes berries change greatly during the ripening process, which also plays a decisive role in the formation of grape berries with medicinal value. In this study, we used targeted metabolomics analysis to investigate the changes in anthocyanins, sugars, organic acids and related metabolite pathways that occur as 'SuoSuo' grapes berries ripen to explore the accumulation patterns that influence the quality of 'SuoSuo' grapes as they ripen.

Anthocyanins, one of the most important traits that influences the quality of grape berry colour, undergo significant changes during berry ripening. In this study, 33 different anthocyanin metabolites were identified in 'SuoSuo' grapes, and 21, 29 and 33 were detected in S1, S2 and S3, respectively. When studying the phenolic substances of BRS Vitoria seedless grapes, Colombo et al. (2021) found that the content and type of anthocyanins in the mature stage were higher than those in other stages, which was consistent with the results of this study. In this study, the highest content of cyanidin-3-O-glucoside was detected in S3, which was the main purple red substance in the peel of 'SuoSuo' grapes. At the same time, it plays a very important role in anthocyanin metabolism. By analysing the anthocyanin components in the peel of more than 90 red grapes in Greece, Kyraleou et al., (2020) found that malvidin-3-O-glucoside is the main component of anthocyanins in the peel of most grapes. At the same time, paeoniflorin glucoside and delphinium pigment were the most abundant in the extract of Mavrotragano peel. The study on Cabernet Sauvignon grapes showed that the anthocyanin in peel was expressed by *F3′H* and *F3′5′H* simultaneously, and their proportion controlled the composition of anthocyanin in grape peel. The varieties with high expression levels of *F3′H* accumulated more cyanidin anthocyanins in peel, and the varieties with high expression levels of *F3′5′H* accumulated more delphinidin anthocyanins (Jeong et al., 2006). The results showed that the main components of anthocyanins in grapes were different due to different varieties or gene regulation. In addition, centaureidin-3-O-glucoside, a natural product, exhibits antioxidant, anti-inflammatory, anticancer, antiobesity, cardioprotective, diabetes prevention and blood circulation enhancing effects. For example, Yang et al. (2019) found that centaureidin-3-O-glucoside interferes with cardiomyocyte apoptosis and proliferation and changes in glycolytic enzymes by inhibiting the expression of CD38, thus playing a role in reducing senescence damage in cardiomyocytes. This indicates that cornetin-3-O-glucoside plays an important role when 'SuoSuo' grapes are used as medicinal herbs.

The content and composition of sugar have an important influence on the flavour, colour and other nutrients of grape berries. Sugar is an important nutrient and alcohol fermentation substrate in grape berries and among the important signs of grape maturity (Bigard et al., 2018). Glucose and fructose are present in almost equal amounts in grape berries and are the main sugar fractions. however, trace amounts of other sugars, such as sucrose, galactose and maltose, are also present in European grape berries (Wang et al., 2022). In this study, fructose and glucose were the main components in the ripening process of 'SuoSuo' grapes. The differential metabolites in the three growth cycles were sorbitol, and the metabolic pathways involved were mainly ABC transporters. Studies have found that ABC transporters are involved in the transport of metabolites and growth hormones, plant organ formation and cell development (Cakir and Kilickaya, 2013). The results indicated that sorbitol played an important role in sugar accumulation and berry growth in 'Suosuo' grape berries. Previous studies have found that polysaccharides composed of monosaccharides such as sucrose, rhamnose, galactose, and arabinose extracted from 'SuoSuo' grapes exhibit strong antiviral, immune-enhancing and neuroprotective effects (Zhang et al., 2022). Ma et al. (2018) studied the effect of polysaccharides in 'SuoSuo' grapes on Alzheimer's disease model rats and found that polysaccharides in 'SuoSuo' grapes can reduce the damage and apoptosis of neurons in the hippocampal CAI area, improve the ability of rats with Alzheimer's disease to learn and protect hippocampal neurons.

The composition and content of organic acids in grape berries are important indicators that determine the flavour and quality of berries (Lima et al., 2022). Organic acids in grape berries mainly include tartaric acid and malic acid, as well as a small amount of citric acid and succinic acid. Their content and composition are the main basic substances for the formation of grape berry flavour and quality (Kısaca and Gazioglu Sensoy, 2023). In a unique grape germplasm resource in Xinjiang, we detected 36 organic acids, mainly tartaric acid, fumaric acid and L-pyroglutamic acid. In addition, we detected more oleanolic acid, which is the main component of the medicinal value of 'SuoSuo' grapes. This may be due to the differences in acid types of different growth climate conditions or different grape varieties. Chahine and Tong (2019) identified organic acids in 41 grapes and found that the concentration of titratable acid was higher in grapes from colder climates, and the concentration of lactic acid was higher in grapes from warmer climates. There are differences in lactic acid, pyruvic acid, volatile acidity and acetic acid among different varieties. Oleanolic acid exhibits good pharmacological activity and antibacterial, anticancer, antioxidant and hepatoprotective effects. Liu et al. (2012) found that oleanolic acid in 'SuoSuo' grape berries can resist the enlargement of the liver and spleen in mice caused by immune injury and effectively restore the activity of ALT and AST in liver tissue.

5. Conclusions

In this study, targeted metabolomics was used to accurately and quantitatively analyse anthocyanins, sugars and organic acid metabolites in the three critical periods of berry ripening. The results showed that 33 kinds of metabolic derivatives were detected in anthocyanins, among which peonidin (n = 7) had the most types, and cyanidin-3-O-glucoside had the highest content in S3 (136.343 ng/g). Ten kinds of metabolic derivatives were detected, among which fructose and glucose were the highest, which were 167.69 ng/g and 152.21 ng/g at S3, respectively. For organic acids, 36 metabolic derivatives were detected. Among them, tartaric acid was the main organic acid, with the highest content of 37196.67 mg/kg at S2. A large amount of oleanolic acid was also found in the berries and gradually increased as the berries ripened, with a content of 230.064 mg/kg at S3. KEGG enrichment analysis of differential metabolites of anthocyanin, sugar and organic acid showed that anthocyanin differential metabolites were mainly concentrated in anthocyanin biosynthesis, flavone and flavonol biosynthesis and flavonoid biosynthesis pathways. Sugars differential metabolites were mainly enriched in ABC transporter, fructose and mannose metabolism and galactose metabolism pathways. The differential metabolites of organic acids were mainly concentrated in the TCA cycle, glyoxylic acid and dicarboxylic acid metabolism, butyric acid metabolism, amino acid biosynthesis, and alanine, aspartic acid and glutamic acid biosynthesis. In this study, trends for the accumulation of anthocyanins, sugars and organic acids during the ripening of 'SuoSuo' grape berries were preliminarily explored, and a theoretical basis was established for the accurate extraction of effective components in 'SuoSuo' grapes.

CRediT authorship contribution statement

Lingzhe Wang: Conceptualization, Methodology, Software, Formal analysis, Investigation, Writing – review & editing. **Weiquan Zhou:** Conceptualization, Methodology, Data curation. **Chunyan Liu:** Investigation, Data curation. **Pengfei Chen:** Data curation, Software. **Long Zhou:** Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2023.137294.

References

Askari-Khorasgani, O., & Pessarakli, M. (2019). Fruit quality and nutrient composition of grapevines: A review. *Journal of Plant Nutrition, 42*(17), 2133–2150. https://doi.org/10.1080/01904167.2019.1643369

Bigard, A., Berhe, D. T., Maoddi, E., Sire, Y., Boursiquot, J. M., Ojeda, H., … Torregrosa, L. (2018). *Vitis vinifera* L. Fruit diversity to breed varieties anticipating climate changes. *Frontiers Plant Science, 9*, 455. https://doi.org/10.3389/fpls.2018.00455

Borba, K. R., Spricigo, P. C., Aykas, D. P., Mitsuyuki, M. C., Colnago, L. A., & Ferreira, M. D. (2021). Non-invasive quantification of vitamin C, citric acid, and sugar in 'Valência' oranges using infrared spectroscopies. *Journal of Food Science and Technology, 58*(2), 731–738. https://doi.org/10.1007/s13197-020-04589-x

Cakir, B., & Kilickaya, O. (2013). Whole-Genome survey of the putative ATP-binding cassette transporter family genes in Vitis vinifera. *PLoS One, 8*(11), e78860.

Chahine, S. Z., & Tong, A. (2019). Effect of climatic conditions on organic acid composition of some wines obtained from different sources. *AIMS Agriculture and Food, 4*(1), 27–40. https://doi.org/10.3934/agrfood.2019.1.27

Coelho, E. M., Da Silva Padilha, C. V., Miskinis, G. A., De Sá, A. G. B., Pereira, G. E., De Azevêdo, L. C., & Dos Santos Lima, M. (2018). Simultaneous analysis of sugars and organic acids in wine and grape juices by HPLC: Method validation and characterization of products from northeast Brazil. *Journal of Food Composition and Analysis, 66*, 160–167. https://doi.org/10.1016/j.jfca.2017.12.017

Colombo, R. C., Roberto, S. R., Cruz, M. A., Carvalho, D. U., Yamamoto, L. Y., Nixdorf, S. L., … Hermosín-Gutiérrez, I. (2021). Characterization of the phenolic ripening development of 'BRS Vitoria' seedless table grapes using HPLC-DAD-ESI-MS/MS. *Journal of Food Composition and Analysis, 95*, Article 103693. https://doi.org/10.1016/j.jfca.2020.103693

Darias-Martín, J., Socas-Hernández, A., Díaz-Romero, C., & Díaz-Díaz, E. (2003). Comparative study of methods for determination of titrable acidity in wine. *Journal of Food Comppositing and Analysis, 16*, 555–562. https://doi.org/10.1016/S0889-1575(03)00032-2

Dong, Y., Duan, S., Xia, Q., Liang, Z., Dong, X., Margaryan, K., … Chen, W. (2023). Dual domestications and origin of traits in grapevine evolution. *Science, 379*(6635), 892–901. https://doi.org/10.1126/science.add8655

Feitosa, S., Greiner, R., Meinhardt, A. K., Müller, A., Almeida, D., & Posten, C. (2018). Effect of Traditional Household Processes on Iron, Zinc and Copper Bioaccessibility in Black Bean (Phaseolus vulgaris L.). *Foods, 7*(8), 123. https://doi.org/10.3390/foods7080123

Hwang, J. W., Natarajan, S. B., Kim, Y. S., Kim, E. K., Lee, J. W., Moon, S. H., … Park, P. J. (2017). Biosynthesis of Oligomeric Anthocyanins from grape skin extracts. *Molecules, 22*(3), 497. https://doi.org/10.3390/molecules22030497

Jeong, S. T., Goto-Yamamoto, N., Hashizume, K., & Esaka, M. (2006). Expression of the flavonoid 3′-hydroxylase and flavonoid 3′,5′-hydroxylase genes and flavonoid composition in grape (Vitis vinifera). *Plant Science, 170*(1), 61–69. https://doi.org/10.1016/j.plantsci.2005.07.025

Jang, Y., Meng, J. F., Liu, C. H., Jiang, J. F., Fan, X. C., Yan, J., & Zhang, Z. W. (2017). Quality characteristics, phenolics content and antioxidant activity of chinese wild grapes. *Food Science, 38*(7), 142–148. https://doi.org/10.7506/spkx1002-6630-201707023

Kısaca, G., & Gazioglu Sensoy, R. I. (2023). Phenolic contents, organicacids and antioxidant capacities of twenty grape (Vitis vinifera L.) cultivars having different berry colors. *Journal of Food Measurement and Characterization, 17*(2), 1354–1370. https://doi.org/10.1007/s11694-022-01698-3

Kőrösi, L., Molnár, S., Teszlák, P., Dörnyei, Á., Maul, E., Töpfer, R., … Röckel, F. (2022). Comparative study on grape berry anthocyanins of various teinturier varieties. *Foods, 11*(22), 3668. https://doi.org/10.3390/foods11223668

Kyraleou, M., Kallithraka, S., Gkanidi, E., Koundouras, S., Mannion, D. T., & Kilcawley, K. N. (2020). Discrimination of five greek red grape varieties according to the anthocyanin and proanthocyanidin profiles of their skins and seeds. *Journal of Food Composition and Analysis, 92*, Article 103547. https://doi.org/10.1016/j.jfca.2020.103547

Silva, L. N. F., Schmidt, E. M., Messias, C. L., Eberlin, M. N., & Sawaya, H. F. A. C. (2015). Quantitation of organic acids in wine and grapes by direct infusion electrospray ionization mass spectrometry. *Analytical Methods, 7*(1), 53–62. https://doi.org/10.1039/C4AY00114A

Liang, Z., Sang, M., Fan, P., Wu, B., Wang, L., Duan, W., & Li, S. (2011). Changes of polyphenols, sugars, and organic acid in 5 vitis genotypes during berry ripening. *Journal of Food Science, 76*(9), C1231–C1238. https://doi.org/10.1111/j.1750-3841.2011.02408.x

Liang, Z., Wu, B., Fan, P., Yang, C., Duan, W., Zheng, X., … Li, S. (2008). Anthocyanin composition and content in grape berry skin in *Vitis* germplasm. *Food Chemistry, 111* (4), 837–844. https://doi.org/10.1016/j.foodchem.2008.04.069

Lima, M. M. M., Choy, Y. Y., Tran, J., Lydon, M., & Runnebaum, R. C. (2022). Organic acids characterization: Wines of Pinot noir and juices of 'Bordeaux grape varieties'. *Journal of Food Composition and Analysis, 114*, Article 104745. https://doi.org/10.1016/j.jfca.2022.104745

Liu, H.-F., Wu, B.-H., Fan, P.-G., Li, S.-H., & Li, L.-S. (2006). Sugar and acid concentrations in 98 grape cultivars analyzed by principal component analysis. *Journal of the Science of Food and Agriculture, 86*(10), 1526–1536. https://doi.org/10.1002/jsfa.2541

Liu, T., Zhao, J., Li, H., & Ma, L. (2010). Evaluation on anti-hepatitis viral activity of *Vitis vinifer* L. *Molecules, 15*(10), 7415–7422. https://doi.org/10.3390/molecules15107415

Liu, T., Zhao, J., Ma, L., Ding, Y., & Su, D. (2012). Hepatoprotective effects of total triterpenoids and total flavonoids from *Vitis vinifera* L against immunological liver injury in mice. *Evidence-based complementary and alternative medicine, 2012*, 1–8. https://doi.org/10.1155/2012/969386

Lu, Q., Li, R., Yang, Y., Zhang, Y., Zhao, Q., & Li, J. (2022). Ingredients with anti-inflammatory effect from medicine food homology plants. *Food Chemistry, 368*, Article 130610. https://doi.org/10.1016/j.foodchem.2021.130610

Ma, L., Wang, X., Li, Y., Xiao, H., & Yuan, F. (2018). Effect of polysaccharides from *Vitis vinifera* L. on NF-κB/IκB-α signal pathway and inflammatory factors in Alzheimer's model rats. *Biotechnology & Biotechnological Equipment, 32*(4), 1012–1020. https://doi.org/10.1080/13102818.2018.1464948

Massonnet, M., Fasoli, M., Tornielli, G. B., Altieri, M., Sandri, M., Zuccolotto, P., … Pezzotti, M. (2017). Transcriptomic program in red and white grapevine varieties correlates with berry skin anthocyanin accumulation. *Plant Physiology, 174*(4), 2376–2396. https://doi.org/10.1104/pp.17.00311

Matić, P., Sabljić, M., & Jakobek, L. (2017). Validation of spectrophotometric methods for the determination of total polyphenol and total flavonoid content. *Journal of Aoac international, 100*(6), 1795–1803. https://doi.org/10.5740/jaoacint.17-0066

Niu, S. Y., Liu, C. B., Liu, Q., Fan, X. C., Zhang, Y., Sun, L., Zhang, X. L., & Jiang, J. F. (2022). Composition and contents of organic acids in different grape germplasms. *Food Science, 43*(12), 228–234. https://doi.org/10.7506/spkx1002-6630-20210704-030

Poni, S., Gatti, M., Palliotti, A., Dai, Z., Duchêne, E., Truong, T. T., … Tombesi, S. (2018). Grapevine quality: A multiple choice issue. *Scientia Horticulturae, 234*, 445–462. https://doi.org/10.1016/j.scienta.2017.12.035

Ren, J. L., Zhang, A. H., Kong, L., & Wang, X. J. (2018). Advances in mass spectrometry-based metabolomics for investigation of metabolites. *RSC Advances, 8*(40), 22335–22350. https://doi.org/10.1039/C8RA01574K

Ribbenstedt, A., Ziarrusta, H., & Benskin, J. P. (2018). Development, characterization and comparisons of targeted and non-targeted metabolomics methods. *PLoS One1, 13*(11), e0207082.

Uwineza, P. A., & Waśkiewicz, A. (2020). Recent advances in supercritical fluid extraction of natural bioactive compounds from natural plant materials. *Molecules, 25*(17), 3847. https://doi.org/10.3390/molecules25173847

Wang, C., Wang, L., Ye, J., & Xu, F. (2022). Fruit quality of Vitis vinifera: How plant metabolites are affected by genetic, environmental, and agronomic factors. *Scientia Horticulturae, 305*, Article 111404. https://doi.org/10.1016/j.scienta.2022.111404

Wang, H., Cui, X., Zhao, X., Gao, S., Zhao, J., & Yuan, Z. (2015). Differences of biochemical constituents and contents of eight cultivars flowers of camellia sinensis. *Journal of Essential Oil Bearing Plants, 18*(2), 320–328. https://doi.org/10.1080/0972060X.2014.961036

Yang, D., Zhang, L., Zhang, Z., Hu, S., Fu, Y., Laukkanen, J. A., & Li, G. (2019). Silencing of C3G increases cardiomyocyte survival inhibition and apoptosis via regulation of p-ERK1/2 and Bax. *Clinical and Experimental Pharmacology and Physiology, 46*(3), 237–245. https://doi.org/10.1111/1440-1681.13027

Zhang, P., Maimaiti, Z., Aili, G., Yuan, F., & Xiao, H. (2022). *Vitis vinifera* L. flavones regulate hippocampal neurons via autophagy in APP/PS1 alzheimer model mice. *Evidence-Based Complementary and Alternative Medicine, 2022*, 1–7. https://doi.org/10.1155/2022/8554184