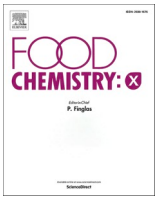
Food Chemistry: X 23 (2024) 101551

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



Accumulation patterns of flavonoids and phenolic acids in different colored sweet potato flesh revealed based on untargeted metabolomics

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ARTICLE INFO

Keywords:
Sweet potato flesh
LC-MS/MS
Metabolic profiles
Flavonoids
Phenolic acids

ABSTRACT

Sweet potatoes are rich in flavonoids and phenolic acids, showing incomparable nutritional and health value. In this investigation, we comprehensively analyzed the secondary metabolite profiles in the flesh of different-colored sweet potato flesh. We determined the metabolomic profiles of white sweet potato flesh (BS), orange sweet potato flesh (CS), and purple sweet potato flesh (ZS) using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The CS vs. BS, ZS vs. BS, and ZS vs. CS comparisons identified a total of 4447 secondary metabolites, including 1540, 1949, and 1931 differentially accumulated metabolites. Among them, there were significant differences in flavonoids and phenolic acids. There were 20 flavonoids and 13 phenolic acids that were common differential metabolites among the three comparison groups. The accumulation of paeoniflorin-like and delphinidin-like compounds may be responsible for the purple coloration of sweet potato flesh. These findings provide new rationale and insights for the development of functional foods for sweet potatoes.
List of compounds: Kaempferol (PubChem CID: 5280863); Peonidin 3-(6"-p-coumarylglucoside) (PubChem CID: 44256849); Swerchirin (PubChem CID: 5281660); Trilobatin (PubChem CID: 6451798); 3-Geranyl-4-hydroxybenzoate (PubChem CID: 54730540); Eupatorin (PubChem CID: 97214); Icaritin (PubChem CID: 5318980); Isorhamnetin (PubChem CID: 5281654); Glucoliquiritin apioside (PubChem CID: 74819335); Brazilin (PubChem CID: 73384).

1. Introduction

Sweet potato (*Ipomoea batatas* (L.) Lam.) is a dicotyledonous vine and the only economically important crop in the family *Cyclophyllaceae* (Ellong, Billard, & Adenet, 2014). Sweet potatoes are not only an important source of energy but also have many valuable by-products (Mussoline & Wilkie, 2017). Its popularity stems from its rich nutritional profile, which includes substantial amounts of dietary fiber, vitamins A and C, protein, and complex carbohydrates. This has led to its widespread cultivation in >100 countries globally (Ayeleso, Ramachela, & Mukwevho, 2017). While the tuber is the primary edible part, the shoots and leaves are also consumed in regions such as Africa and Asia (Su et al., 2019; Zhang et al., 2018).

Shades of white, yellow, orange, and purple are common for sweet potatoes. Research indicates that these sweet potatoes are rich in phenolic compounds (Mohanraj & Sivasankar, 2014; Tanaka, Ishiguro, Oki, & Okuno, 2017). Phenolics are categorized into two primary groups: phenolic acids and flavonoids (Liu, Bruins, de Bruijn, & Vincken, 2020), which constitute significant secondary metabolites in sweet potatoes (Makori, Mu, & Sun, 2020). The phenolic content varies among different colored sweet potatoes (Abalos, Naef, Aviles, & Gomez, 2020; Alam, Rana, & Islam, 2016), and these compounds have demonstrated potent antioxidant properties (Azeem, Mu, & Zhang, 2020). Moreover, fat-soluble polyphenols exhibit antitumor activity (Kato et al., 2021). Anthocyanins from purple sweet potatoes have gotten a lot of attention because they have many health benefits, such as being antioxidants, lowering blood sugar, stopping mutations, fighting tumors, protecting the liver, and lowering blood pressure (Suda et al., 2002; Wang & Chou, 2008; Zhao, Yan, Lu, & Zhang, 2013). Researchers have analyzed the metabolic profile of young sweet potato red leaves up to this point, identifying 193 secondary metabolites, including 82 simple phenols, 85 flavonoids, 18 alkaloids, and 8 terpenoids. This study suggests that the antioxidant capacity of sweet potato leaves is a synergistic effect of anthocyanins and many other colorless compounds (Shi et al., 2022). Furthermore, metabolic profiling of various sweet potato cultivars has identified 163 and 29 differentially expressed metabolites associated with leaf tip color and leaf shape (Tan et al., 2024).

Untargeted metabolomics serves as a powerful tool for investigating

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https://doi.org/10.1016/j.fochx.2024.101551
Received 29 March 2024; Received in revised form 10 June 2024; Accepted 11 June 2024
Available online 12 June 2024

the metabolite composition and dynamics within organisms. Researchers have applied this approach to plants like blueberries (Wu et al., 2022) and pomegranates (Fellah, Rocchetti, Senizza, Giuberti, & Lucini, 2019), resulting in the identification of numerous metabolite patterns. Non-targeted metabolomics has helped us understand the structures of anthocyanins and flavonoids (Bennett, Mahood, Fan, & Moghe, 2021), as well as how the metabolisms of sweet potatoes grown in soil versus hydroponic systems are different (Lin et al., 2021). Integrated transcriptomic and metabolomic analyses have further shed light on various aspects of sweet potato biology. For example, several researchers have employed these techniques to explore the glycation mechanisms in sweet potatoes (Li et al., 2021). Others have utilized transcriptomics and targeted metabolomics to identify the genes and metabolites involved in anthocyanin accumulation within sweet potato tubers (He et al., 2020). However, few studies have analyzed the metabolic differences between different colors of sweet potato flesh via non-targeted metabolomics techniques. This work aims to fill this gap by conducting a non-targeted metabolomics analysis on three distinct sweet potato varieties with significant color variations. The findings will serve as a scientific foundation for the development and utilization of sweet potato-based functional foods, with a focus on comparing the flavonoid and phenolic acid profiles across different sweet potato colors.

2. Materials and methods

2.1. Plant materials

The College of Forestry and Horticulture at Hubei Minzu University (30°17′N latitude, 109°29′E longitude, 495.5 m altitude) selected three distinct sweet potato cultivars: the white-fleshed "Chestnut Potato" (Fig. 1A), the orange-fleshed "Watermelon Red No. 1" (Fig. 1B), and the purple-fleshed "Purple Potato" (Fig. 1C). We rinsed the sweet potatoes with distilled water post-harvest and peeled the flesh (three biological replicates per group). Samples were snap-frozen in liquid nitrogen and subsequently stored at −80 °C until experimentation. We use the abbreviations BS, CS, and ZS in this work to represent the white, orange, and purple sweet potato samples, respectively.

2.2. Sample preparation and metabolomic data acquisition

We freeze-dried the plant samples under vacuum, ground them into a fine powder (30 Hz for 1.5 min), and weighed out 50 mg of the powder. To this, 1200 μL of pre-cooled (−20 °C) 70% methanol aqueous internal standard extraction solution was added. We vortexed the mixture six times, at 30-min intervals, for 30 s each. After being spun at 12000 rpm for 3 min, the supernatant was taken out, filtered through a microporous

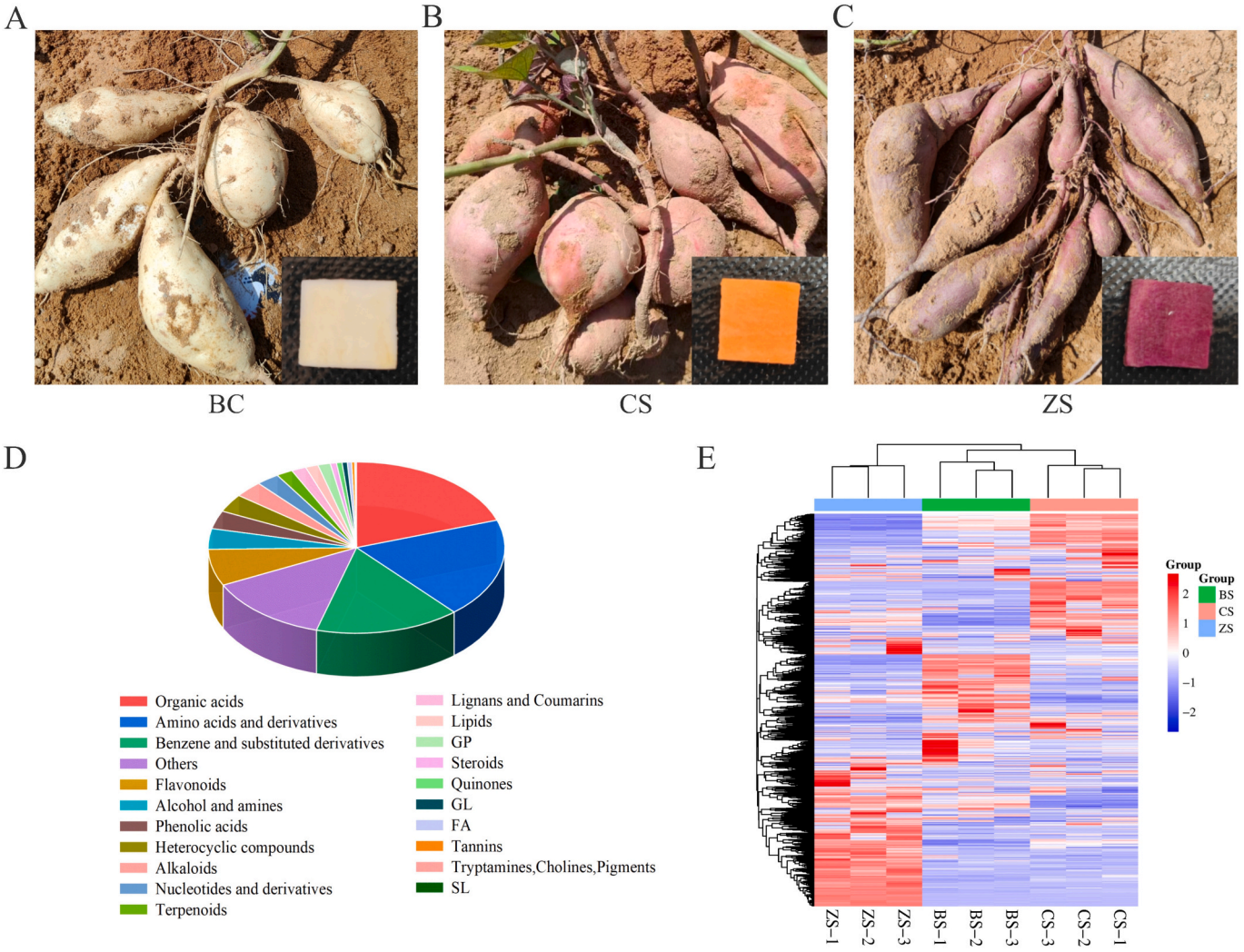

Fig. 1. HCA and identification of metabolites in different colored sweet potato flesh. A: Photographs showing BC color; B: Photographs showing CS color; C: Photographs showing ZS color; D: Metabolite category analysis of the three sweet potato varieties; E: Metabolite clustering HCA of the three sweet potato varieties (red for high content, blue for low content). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

filter membrane with pores that were 0.22 μm in size, and put into an injection bottle so that it could be analyzed later. We further filtered the sample extracts before analyzing them via LC-MS/MS. The MetWare database facilitated the identification of metabolites.

Wuhan MetWare Biotechnology Co. Ltd. (Wuhan, China) (https://www.metware.cn/) carried out the extraction, detection, and quantitative analysis of metabolites in the samples.

2.3. Liquid chromatography and tandem mass spectrometry conditions

We used an LC-MS/MS system. Chromatographic conditions were on a column (1.8 μm, 2.1 mm * 100 mm). The mobile phases consisted of ultrapure water containing 0.1% formic acid (phase A) and acetonitrile containing 0.1% formic acid (phase B). The column temperature was maintained at 40 °C, the flow rate was 0.4 mL/min, and the injection volume was 4 μL. In mass spectrometry, the run time is 10 min, the ion spray voltage is +5.5/−4.5 KV, the temperature is 550 °C, the ion source gas1 is 50 psi, the ion source gas2 is 60 psi, the curtain gas is 35 psi, the de-clustering potential is ±60 V, the MS1 collision energy is ±10 V, the MS2 collision energy is ±30 V, the collision energy spread is 15 V, and the MS1 and MS2 TOF masses are 50–1250 Da.

2.4. Metabolite identification and quantification

Proteo Wizard converted raw data to mzXML format. The XCMS software performed peak extraction, alignment, and retention time correction. We excluded peaks with a missing rate exceeding 50% in any sample set and imputed blank values using the KNN method. We then applied the SVR method to correct the peak areas. We used the processed peaks to identify metabolites by searching against the Meteville Laboratory's proprietary database, integrating with public libraries, predictive libraries, and the Met DNA approach. We selected substances with identification composite scores <0.5 and coefficient of variation (CV) values <0.3 for quality control (QC) samples. We combined positive and negative ion modes to retain compounds with the highest confidence and lowest CV values, resulting in the creation of a comprehensive data file for all samples. Metabolites were characterized using both primary and secondary MS data (Chen et al., 2013). We determined the relative abundance of each metabolite in the various samples based on the chromatographic peak areas.

2.5. Statistical analysis

We uploaded the processed data to the MetWare Cloud Platform (https://cloud.metware.cn/#/user/login) for principal component analysis (PCA), hierarchical cluster analysis (HCA), and orthogonal partial least squares discriminant analysis (OPLS-DA). Metabolites that differentially accumulated among the three sweet potato varieties were identified based on variable importance projection (VIP) ≥1 and fold change (FC) ≥2 or ≤ 0.5. We annotated and classified the functions of different accumulated metabolites in different colored sweet potato flesh using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. Statistical significance was determined using one-way analysis of variance (ANOVA), followed by Tukey's test at a significance level of $P < 0.05$. We derived all data from three biological replicates.

3. Results and discussion

3.1. Metabolic profiling of sweet potato flesh

An untargeted metabolomics approach was used to describe the metabolic makeup of three different types of sweet potatoes, focusing on both shared and divergent metabolites found in their flesh. Total ion current (TIC) analysis of QC samples assessed the reproducibility of the metabolite extraction and detection processes (**Fig. S1A, S1B**). Consistent retention times and peak intensities across multiple identifications of the same sample confirmed the stability of the analytical signals. To evaluate the effectiveness of cross-contamination control, we utilized extracted ion chromatograms (EIC) analysis (**Fig. S2C, S2D**). There were no big peaks in the internal standards of the blank samples that were used throughout the study. This showed that there was very little substance residue, which prevented cross-contamination. Furthermore, we quantified the homogeneity within replicate groups using Pearson's correlation coefficient; the closer the |r| value approached 1, the greater the homogeneity (**Fig. S1E, S1F**).

After quality assessment, we identified 4447 metabolites, most of which were organic acids (863, 19.41%), amino acids and their derivatives (797, 17.92%), benzene derivatives (673, 15.13%), flavonoids (298, 6.70%), alcohols, amines (164, 13.69%), and phenolic acids (157, 3.53%). We identified 2808 differential metabolites among these, primarily comprising organic acids (556 species, 19.80%), amino acids and their derivatives (527 species, 18.77%), benzene derivatives (443 species, 15.78%), flavonoids (194 species, 6.91%), and phenolic acids (96 species, 3.42%) (Fig. 1D). A clustering heat map revealed that the nine sweet potato samples could be distinctly classified into three groups (Fig. 1E). Notably, the metabolite content in ZS was significantly different from that in CS and BS, highlighting substantial compositional variations among the three varieties. These pronounced differences in metabolite profiles are likely influenced by genetic diversity.

There were 194 different types of flavonoids found in this study. These included 10 chalcones, 7 dihydro flavonoids, 1 dihydro flavonol, 84 flavonoids, 22 flavonols, 1 dihydro isoflavone, 2 anthocyanins, 11 flavanols, 2 biflavonoids, 15 isoflavones, and 21 other flavonoids. We discovered that both CS and ZS had elevated contents of kaempferol-3-O-(2″-acetyl) glucoside, sudachitin, and peonidin 3-rhamnoside compared to BS, and these substances were among the top five with the highest fold-change differences in each comparison group. The kaempferol-3-O-(2′-acetyl) glucoside content in BS was 55.98 times lower than in CS and 11.92 times lower than in ZS. The sudachitin content was 6.13 times lower in BS compared to CS and 7.67 times lower in ZS. The peonidin 3-rhamnoside content was 5.62 times lower in BS than in CS, and 74.78 times lower in ZS.

Furthermore, the sweet potato flesh contained 96 phenolic acids at varying levels. Using BS as the reference, we found elevated levels of 1-caffeoylquinic acid, CID 487435, and aspulvinone E in both CS and ZS, consistently ranking in the top five for multiplicity of difference among the controls. Conversely, the levels of 1-caffeoylquinic acid in BS were significantly lower, with a 12.39-fold and 12.53-fold reduction compared to CS and ZS, respectively. CID 487435 contained 6.33 times and 10.85 times less in BS than in CS and ZS, respectively, while aspulvinone E contained 5.69 times and 7.34 times less in BS than in CS and ZS, respectively.

3.2. Multivariate analysis of sweet potato varieties via PCA, CV, and OPLS-DA

PCA simplifies the complex interplay of numerous variables into a few principal components, offering insight into the underlying structure. The combined contribution of the first two principal components (PC1 with 41.08% and PC2 with 23.11%) was 64.19% in the negative ion mode PCA score plot. Similarly, the first two principal components (PC1 with 38.07% and PC2 with 26.05%) accounted for 64.12% of the variance in the positive ion mode (Fig. 2A, B). These plots show a clear clustering of BS, CS, and ZS varieties and highlight significant metabolic differences among them. The tight clustering of replicates for each variety underscores the experimental reproducibility and suitability for further qualitative and quantitative analyses. The CV is a measure of data dispersion. As depicted in Fig. 2C and D, over 75% of QC samples exhibited CV values below 0.3, indicating high stability of the experimental data.

We used OPLS-DA to identify differential metabolites among the sweet potato varieties. When R^2X, R^2Y, and Q^2 values approach 1, we

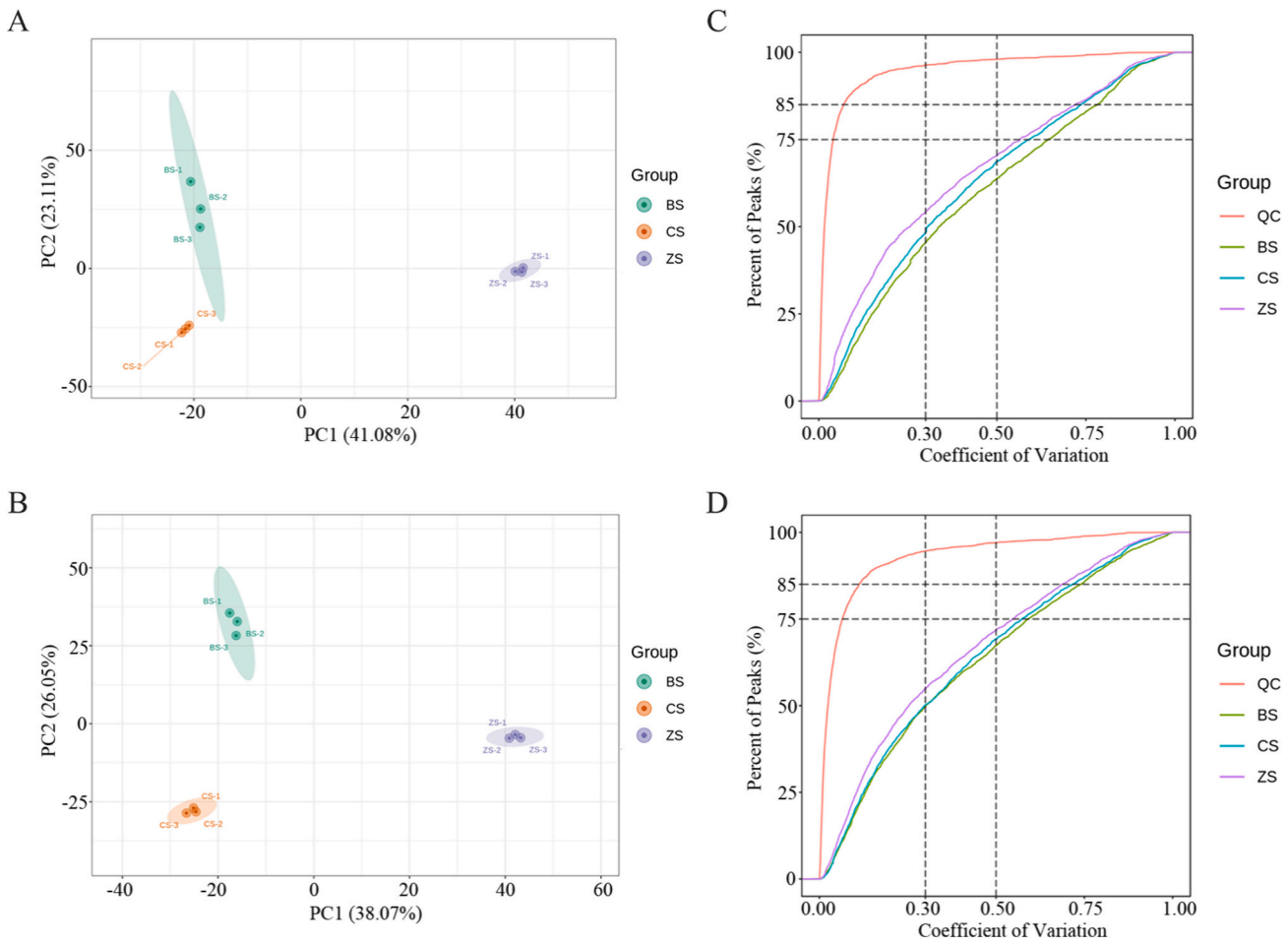

Fig. 2. PCA and CV analyses of the three sweet potato varieties. A and B: PCA scoring plots of metabolite profiles in negative and positive ion modes; green, orange, and purple colors represent the BS, CS, and ZS samples, respectively; and the horizontal and vertical coordinates denote the first and second principal components, PC1 and PC2, respectively. C and D: CV distribution plots of the samples in each group for the negative ion mode and positive ion mode. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

consider a model stable and reliable. This study obtained high R^2X, R^2Y, and Q^2 values, validating the OPLS-DA model for sample comparison. Using the OPLS-DA model, we compared the metabolites in sweet potato flesh two by two. This showed that there were differences between BS and CS ($Q^2 = 0.922$, $R^2X = 0.615$, $R^2Y = 1$; **Fig. S2A**), ZS vs. BS ($Q^2 = 0.958$, $R^2X = 0.687$, $R^2Y = 1$; **Fig. S2B**), and ZS vs. CS ($Q^2 = 0.971$, $R^2X = 0.675$, $R^2Y = 0.1$; **Fig. S2C**). The Q^2 values above 0.9 for all comparison groups indicated that the models were found to be reliable and stable, adequately explaining the metabolic variations among the three varieties for subsequent differential metabolite screening using VIP analysis.

3.3. Differential metabolite profiling in sweet potato flesh

Analyzing multiple samples unveiled distinct metabolite accumulation patterns. To identify the most significant metabolites from the three sweet potato varieties, this study screened 2808 annotated differential metabolites based on FC and VIP. Metabolites with FC ≥ 2 or ≤ 0.5 and VIP ≥ 1 were defined as differential metabolites. We found 1504 differential metabolites (798 up-regulated and 706 down-regulated) between BS and CS (Fig. 3A), 1949 differential metabolites (1107 up-regulated and 842 down-regulated) between BS and ZS (Fig. 3B), and 1931 differential metabolites (1065 up-regulated and 866 down-regulated) between CS and ZS (Fig. 3C). We categorized the differential metabolites of all three comparison groups (CS vs. BS, ZS vs. CS, and ZS vs. CS) into 20 different categories. Differential metabolites analysis showed that flavonoid and phenolic acid metabolites were more common in the ZS group compared to the CS group, making up 6.82% and 4.00% of the total, respectively.

However, the distribution of these compounds varied among the groups. Flavonoid levels (flavonoids, flavonols, and flavanols) were generally lower in CS and ZS compared to BS, while phenolic acid levels were higher. Additionally, the content of alkaloids, lipids, organic acids, amino acids, their derivatives, and terpenoids showed considerable variation among the sweet potato varieties. For instance, the alkaloid content of both CS and ZS flesh was higher than BS, except for phenolamines. Similarly, the terpene content was predominantly higher in CS and ZS than in BS, except for sesquiterpenes and triterpene saponins.

3.4. Phenolic compound variations in sweet potato flesh

Studies have demonstrated the antioxidant properties of flavonoids found in sweet potato leaves, skin, and flesh. The level of activity varies between types of sweet potatoes (Arshad et al., 2021; Krochmal-Marczak et al., 2020; Oloniyo, Omoba, Awolu, & Olagunju, 2021). To understand the variation of phenolic compounds in the flesh of different sweet potato varieties, this study used multivariate analysis such as HCA to determine the differences in the metabolic profiles of the three sweet potato varieties via LC-MS/MS in both positive and negative ionization modes. The HCA indicated that the relative abundance of flavonoids and phenolic acids correlated with the phenotypic color of the varieties. Flavonoid HCA is presented in **Fig. S3A**, with compounds categorized

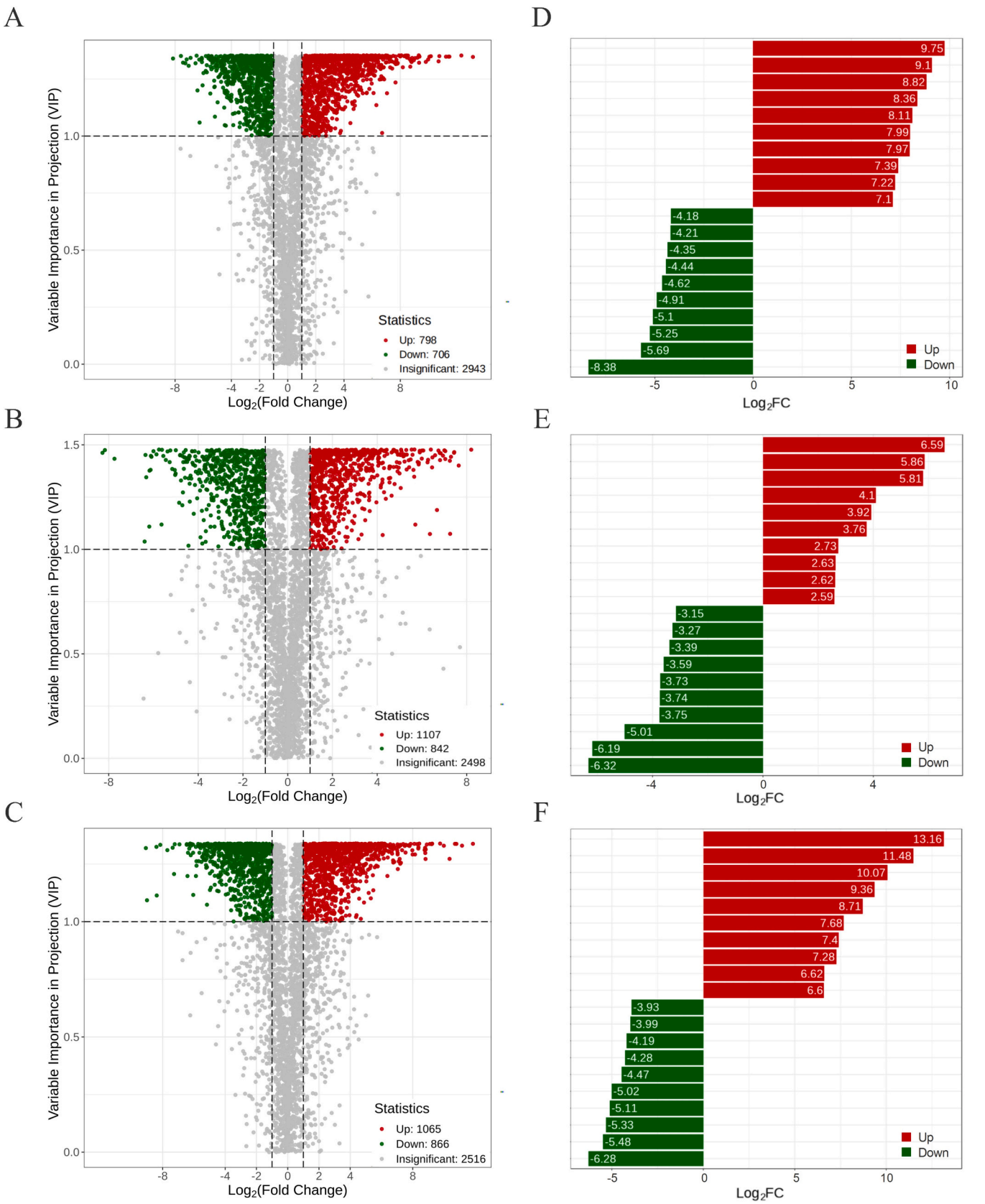

Fig. 3. Chemical differences between three different colors of sweet potato flesh. A-C: volcano plots showing the differential expression levels of metabolites in BS, CS, and ZS samples. Red, green, and gray dots indicate up-regulated, down-regulated, and non-significant differential expression of metabolites, respectively. D-F: top 10 up-regulated and down-regulated compounds in CS vs. BS, ZS vs. BS, and ZS vs. CS, respectively. Red and green colors indicate the up and down-regulation of metabolites, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

into three accumulation groups. The highest flavonoid content was observed in BS, CS and ZS. HCA-based phenolic acids formed four main clusters (**Fig. S3B**). Cluster IV, comprising 48 phenolic acids, showed the highest metabolite levels in ZS. Cluster III exhibited a higher phenolic acid content in BS than in ZS varieties, while most phenolics were down-regulated in CS varieties. Cluster II contained phenolic acids, with higher levels in BS than in the CS and ZS varieties. The maximum phenolic acid content was detected in CS varieties within Cluster I.

This work analyzed ZS vs. CS and found that the multiplicity of difference of 120 flavonoids ranged from 0.003 to 861.61-fold (76 up-regulated and 44 down-regulated). The top 5 up-regulated flavonoids in ZS were swerchirin (861.61-fold), quercetin-3-O-(2''-O-galactosyl) glucoside (549.31 times), kaempferol-3-rhamninoside (451.82 times), kaempferol 3-O-beta-D-galactopyranosyl-7-O-alpha-L- rhamnopyranoside (327.89-fold), and trilobatin (275.57-fold). In previous studies, o-hexoside of quercetin and chrysophanol were the major flavonoids other than anthocyanins, that were different metabolites of different colored sweet potatoes (Wang et al., 2018). Most of the differential metabolites in CS and ZS in this study were quercetin and kaempferol glycosides (Fig. 3D). This finding is similar to previous studies, and the differences may be due to different sweet potato varieties as well as differences in environmental factors. A total of 91 differential flavonoids were found in BC and CS, with the magnitude of the difference ranging from 0.01 to 96.16 times. Among them, the five flavonoid compounds with the highest differences were icaritin (96.16-fold), 5,7-dihydroxy-3′,4′-dimethoxy-8-(3-hydroxy-3-methylbutyl)-isoflavone 7-glucoside (58.04-fold), kaempferol-3-O-(2″-acetyl) glucoside (55.98-fold), glucoliquiritin apioside (17.11-fold), and eupatorin (15.12-fold). These substances were higher in CS varieties (Fig. 3E). Among these, eupatorin has been shown to have antiproliferative activity against a variety of cancer cell lines (Lee, Hyun, Jung, Shin, & Lee, 2016). Glucoliquiritin apioside may have antiviral potency (Tolah et al., 2021). In addition, there were 133 flavonoid compounds with a multiplicity of difference ranging from 0.01 to 9127.56 times in both BC and ZS. The top 3 flavonoid compounds were kaempferol-3-rhamninoside (9127.56-fold), irigenin trimethyl ether (2862.37-fold), and isorhamnetin 3-O-alpha-rhamnopyranosyl-(1–2)-beta-galactopyranoside (1072.33-fold), which were higher in ZS varieties (Fig. 3F). Of interest, kaempferol-3-rhamninoside, a derivative of kaempferol, differed by a factor of 9127.56 in BC and ZS and by a factor of 451.82 in ZS and CS. This substance may be the main one changing the amount of flavonoid compounds present. Prior research has highlighted the correlation between β-carotene, starch, and sucrose levels in storage roots by examining the metabolic profiles of various sweet potato varieties. This serves as a crucial foundation for preserving genetic diversity in favorable ways (Drapal, Rossel, Heider, & Fraser, 2019). This research provides a concise overview of the variations in flavonoid levels among different pigmented sweet potato flesh. This implies that we can choose and reproduce certain sweet potato flesh elements by observing various metabolic alterations.

In prior research, phenolic acids have been identified as predominant phenolic constituents in sweet potatoes (Nakagawa et al., 2021). These compounds are inherently present in plant-derived foods, predominantly manifesting as esters or through glycosidic linkages (Mattila & Hellström, 2007). Among sweet potatoes of varying hues, the predominant phenolic acids identified are acylated forms of quinic and ferulic acids (Wang et al., 2018). In this study, 43 phenolic acids were significantly different between CS and BS (27 up-regulated and 16 down-regulated), 78 were significantly different between BS and ZS (47 up-regulated and 31 down-regulated), and 65 were significantly different between CS and ZS (33 up-regulated and 32 down-regulated). Among them, the ferulic acid content in ZS varieties was 10.21 times higher than that in BS varieties and 6.72 times higher than that in CS varieties, but there was no significant difference between BS and CS varieties. In addition, a total of 14 quinic acids were detected in this study, with only five significant differences. There was no significant difference between chlorogenic acids detected in CS and BS, while the content of 3-O-galloylquinic acid in the ZS variety was 39.76-fold higher than that of the CS variety. The content of syringoylcaffeoylquinic acid-D-glucose in the CS variety was 20.31-fold higher than that of the BS variety but lower than that of the ZS variety by a factor of 10.67 times. This indicated that the phenolic acid content in BS is lower than CS, and CS is lower than ZS. Chlorogenic acid has been implicated as a key active antioxidant component in sweet potato leaves (Chen et al., 2022). Consequently, this work has successfully screened and identified two distinct chlorogenic acid isomers, chlorogenic acid methyl ester, and isochlorogenic acid B. Additionally, a striking 173.93-fold difference in [6]-gingerdione content was observed between CS and BS cultivars, with [6]-gingerdione being recognized as an active antioxidant component (Kumboonma, Senawong, Saenglee, Yenjai, & Phaosiri, 2017). The content of 27 metabolites including pleoside, tenuifoliside A, 3-geranyl-4-hydroxybenzoate, and 1-caffeoylquinic acid was higher in CS varieties than in BS varieties 2.03–173.93 times. Arillatose B in the ZS variety was 148.17 and 94.33 times higher than that in the BS and CS varieties, respectively. In conclusion, our study revealed distinct differences in the profile and concentration of glycosides among sweet potatoes of different colors, a finding that aligns with metabolomic studies conducted on quinoa of diverse hues (Qian et al., 2023).

In summary, our study provides information on the composition of phenolic acids. The majority of the phenolic acids present in sweet potato flesh were discovered to be responsible for its antioxidant effects. Nevertheless, the phenolic acid content exhibited significant variation across sweet potatoes of different colors, potentially indicating disparities in the antioxidant activity of these variously colored sweet potatoes. Prior research has demonstrated that the purple sweet potato contains a greater amount of flavonoids and phenolic acids compared to the white and orange varieties (Park et al., 2016). These findings align with the outcomes of the current investigation. In addition, our investigation revealed a considerable disparity in the levels of kaempferol-3-rhamninoside and arillatose B between ZS varieties and BS and CS varieties. Specifically, the ZS varieties exhibited notably greater levels of kaempferol-3-rhamninoside and arillatose B compared to the BS and CS varieties. These findings indicate that the variations in the levels of kaempferol-3-rhamninoside and arillatose B may significantly influence the overall flavonoid content and phenolic acid concentration.

3.5. Differences in key metabolites in sweet potato of different colors

In this study, a total of 466 compounds were identified as common differential metabolites between CS vs. BS, ZS vs. BS, and ZS vs. CS (Fig. 5A). Among them, 20 flavonoid compounds and 13 phenolic acid compounds were included. As shown in Fig. 4, the contents of compounds 1 to 10 were higher in BS varieties than in CS and ZS varieties. Among them, the flavonoids and phenolic acid contents of BS varieties were about 0.23 to 0.46 and 0.16 to 0.48 times higher than those of CS, respectively. The flavonoid and phenolic acid contents of BS varieties were about 0.02–0.12 and 0.05–0.21 times higher than ZS, respectively. Among compounds 11–24, the CS variety had a higher content than BS and ZS. Among them, the content of isorhamnetin was 2.07 and 0.41 times higher than that of the BS and ZS varieties, respectively. Notably, the content of the derivative of kaempferol (kaempferol-3-O-(2′-acetyl) glucoside) in the CS variety was 11.92 times higher than that in the BS variety. In compounds 25 to 33, the content of ZS varieties was significantly higher than that of BS and CS varieties. Among them, the contents of swerchirin and trilobatin were 861.61 and 275.57 times higher than those of CS varieties, respectively.

In previous studies, extracts of purple and orange sweet potatoes were analyzed, and a large number of acylated paeoniflorin and anthocyanin derivatives were identified (Bennett et al., 2021). This study screened a total of nine significantly different anthocyanins, three of which were paeonidins. The content of peonidin-3-rhamnoside in the ZS variety was 74.78 times higher than that of the BS variety and 13.31 times higher than that of the CS variety, while the content of peonidin-3-

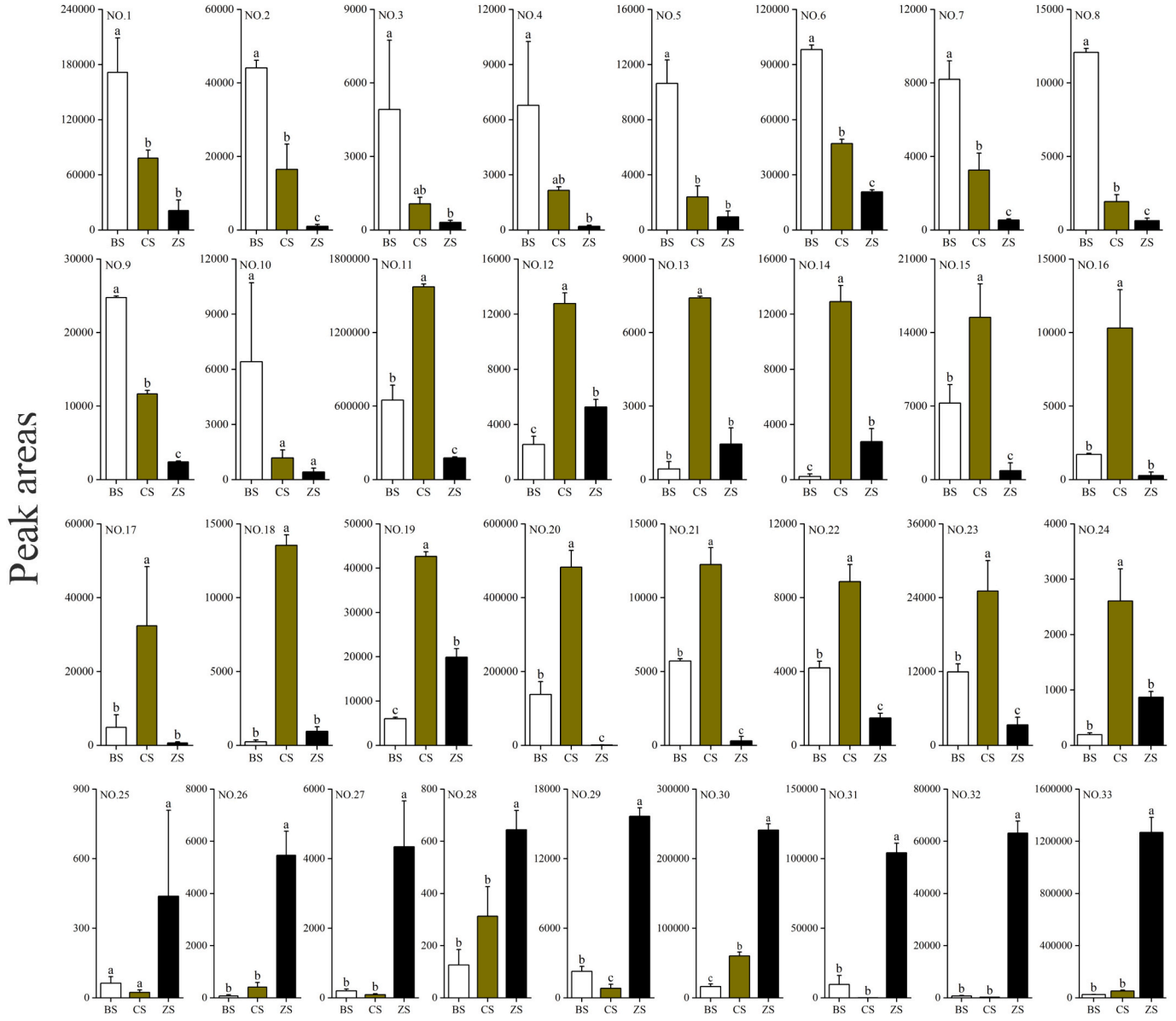

Fig. 4. Peak areas of 33 different metabolites identified in three sweet potato varieties. Significance was evaluated using Duncan's test. The compound number corresponds to the same in Table S1. Different letters indicate significant differences ($P < 0.05$).

rhamnoside in the CS variety was 5.62 times higher than that of the BS variety. The content of Peonidin 3-O-beta-galactopyranoside in ZS varieties was 16.98 times higher than that of BS varieties and 5.76 times higher than that of CS varieties, but the difference in content between BS and CS varieties was not significant. In addition, peonidin 3-(6″-p-coumarylglucoside) differed only between BS and CS varieties and was 2.51 times higher in CS than in BS varieties. The greatest difference in anthocyanin content was found in delphinidin 3,5-diglucoside, a derivative of delphinidin, which was higher in both ZS varieties than in BS and CS. In previous studies, very high levels of delphinidin have been found in the purple leaves of green tea in summer (Zhang et al., 2020) and also in the purple alfalfa petals compared to the yellow and cream-colored petals (Huang et al., 2024). This suggests that the accumulation of paeoniflorin and delphinidin-like compounds may be one of the main reasons for the purple color of sweet potato flesh.

3.6. Metabolic pathway analysis of differential metabolites in the flesh of three sweet potato varieties

The KEGG database is an effective tool for analyzing metabolic pathways and their interrelationships. Therefore, the KEGG database was used to enrich and analyze the differential metabolites in sweet potato flesh samples of different colors to obtain comprehensive functional information on sweet potato flesh. Differential metabolites of CS vs. BS, ZS vs. BS, and ZS vs. CS were involved in 77, 73, and 82 pathways, respectively. The top 20 metabolic pathways identified across these comparisons were notably linked to key biosynthetic processes such as anthocyanin biosynthesis, carotenoid biosynthesis, amino acid biosynthesis, the function of ABC transporters, phenylpropanoid biosynthesis, and flavonoid biosynthesis (Fig. 5B-D). Further investigation into the differential metabolites of flavonoids and phenolic acids through the KEGG database elucidated the complex interactions within these sweet potato varieties. The KEGG enrichment analysis corroborated the well-annotated pathways of anthocyanin biosynthesis, flavonoid biosynthesis, and phenylpropanoid biosynthesis, as depicted in

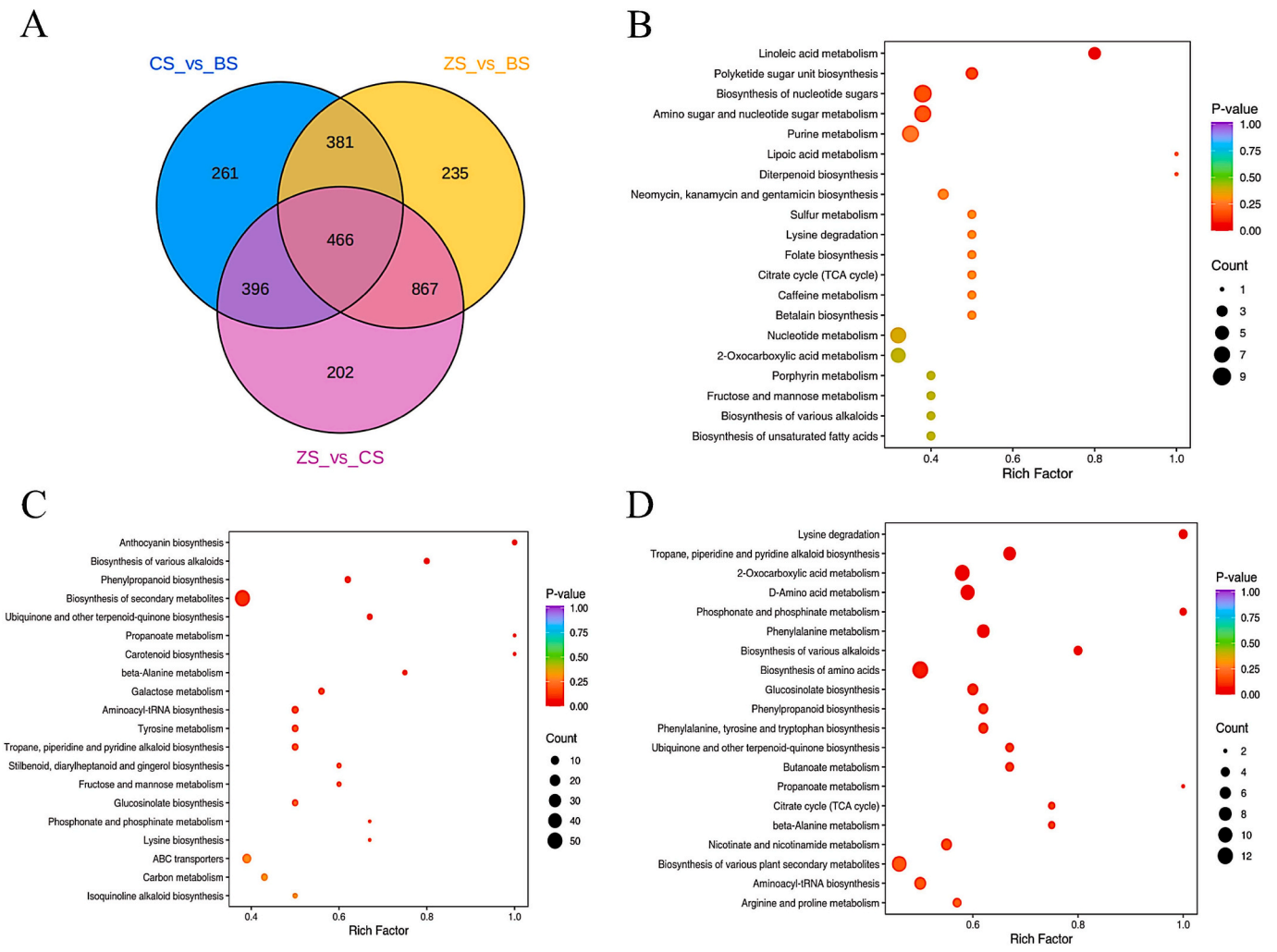

Fig. 5. Venn diagrams and pathway analysis of the differential accumulation of metabolites in the three groups. A: Venn diagrams showing the overlap and culture-specific differential accumulation of metabolites in BS, CS, and ZS samples. B-D: KEGG pathway enrichment of differentially accumulating metabolites among the groups (CS vs. BS, ZS vs. BS, and ZS vs. CS). Each bubble in the graph represents a metabolic pathway, and its horizontal coordinate and bubble size together indicate the magnitude of the pathway's influencing factors. The larger the bubble size, the larger the influence factor. The bubble color represents the *p*-value of the enrichment analysis, with darker colors indicating higher enrichment.

Fig. S3. Precedent literature has established that anthocyanin biosynthesis is modulated by the flavonoid biosynthesis pathway, with increased anthocyanin synthesis observed when there is upregulation of gene expression and heightened activity of the key enzyme, ANS, within the flavonoid biosynthesis pathway (He, Zhu, Sun, Wang, & Zeng, 2021). Additionally, the phenylpropanoid biosynthesis pathway is recognized for its close association with the synthesis of phenolic acids. The present findings regarding the KEGG-enriched metabolic pathways of phenolic acids and flavonoids align with those documented in previous research, further validating the outcomes of this investigation.

4. Conclusion

The present investigation employed an untargeted metabolomics strategy to systematically assess the metabolic profiles across three distinct sweet potato cultivars varying in color. A comprehensive analysis revealed that 4447 detectable metabolites were detected in the flesh, including 863 organic acids, 797 amino acids and their derivatives, 673 benzene derivatives, 298 flavonoids, 164 alcohols and amines, 157 phenolic acids, 150 heterocyclic compounds, 148 alkaloids, and 143 nucleotide derivatives. A detailed examination of the flavonoid and phenolic acid content in sweet potatoes of differing hues uncovered that the presence of paeoniflorin and delphinidin-like entities significantly contributes to the purple pigmentation observed in the flesh. Furthermore, flavonoid biosynthesis stands as a pivotal pathway influencing anthocyanin accumulation in sweet potatoes, while phenylpropanoid metabolism is intrinsically linked to the formation of phenolic acids. Collectively, this research not only enhances our comprehension of the metabolic processes governing phenolic acids and flavonoids in sweet potatoes but also serves as a valuable tool for evaluating the metabolic quality of these tubers. Moreover, it lays a scientific foundation for the future development of superior sweet potato cultivars.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101551.

Funding

This study was jointly funded by the National Natural Science Foundation of China (31260057), Natural Science Foundation of Hubei Province (Joint Fund) (2023AFD077), The Open Fund of Hubei Key Laboratory of Biological Resources Protection and Utilization (Hubei Minzu University) (KYPT012403), and Major Special Project of Technological Innovation of Hubei Provincial Science and Technology Department (2019ACA120).

CRediT authorship contribution statement

Jiaqi Wu: Supervision, Investigation. **Xiuzhi Wang:** Validation, Investigation. **Lingjun Cui:** Supervision, Investigation. **Qiang Xiao:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

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.

The authors declare no conflict of interest.

Data availability

No data was used for the research described in the article.

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