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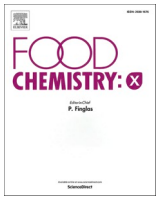

Comparative study of polysaccharide metabolites in purple, orange, and white *Ipomoea batatas* tubers

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ABSTRACT

We employed LC-MS/MS to investigate the metabolic profiles of polysaccharide compounds in white, orange, and purple sweet potato flesh. Comparisons between Orange vs White, Purple vs Orange, and Purple vs White identified 69 polysaccharide metabolites, including 23, 36, and 44 differential metabolites, respectively, with distinct differentiation. Among the three sample groups, 14 polysaccharide compounds and 2 anthocyanins exhibited significant differences. Our further analysis indicated that anthocyanins occupy a central position in the related network diagram and are interconnected with polysaccharides. In metabolic pathways, sucrose and the anthocyanin precursor UDP-glucose were upregulated in purple sweet potatoes, along with elevated levels of pelargonidin 3-O-β-D-sambubioside and delphinidin 3,5-diglucoside. Conversely, sucrose was downregulated in purple sweet potatoes while increasing in white and orange varieties. Therefore, we hypothesize that the competition between sugars and anthocyanins for shared biosynthesis precursors is attributed to differential polysaccharide metabolites among sweet potato tubers of three colors.

List of compounds

Pelargonidin 3-O-beta-D-sambubioside (PubChem CID: 71627264)
Delphinidin 3,5-diglucoside (PubChem CID: 10100906)
Cyanidin 3-sambubioside-5-glucoside (Pub-Chem CID: 74976933)
Sucrose (PubChem CID: 5988)
Gentianose (PubChem CID: 117678)
Paromomycin (PubChem CID: 165580).

1. Introduction

The sweet potato (*Ipomoea batatas*), known as kumara, batata, or by various regional names, belongs to the Convolvulaceae family and holds substantial economic importance (Jiang & Wei, 1979). This perennial plant is widely revered for its highly nourishing tuberous roots, which hold the impressive distinction of being the seventh most important staple food crop globally. Sweet potatoes are rich in carbohydrates, polysaccharides, and dietary fiber. They are significant sources of multiple vitamins, including vitamins A and C, and minerals, rendering them a highly nutritious dietary staple (Chen et al., 2012; Zhang, 2018; Remya & Subha, 2014). Sweet potatoes originated in Central America and have undergone extensive cultivation and refinement throughout the millennia. Currently, they are widely spread around the globe and play a vital role as a significant source of sustenance and animal feed in numerous areas. Beyond their substantial caloric contribution, sweet potatoes contain various health-promoting compounds, including antioxidants, anti-inflammatory agents, and elements that may regulate blood sugar and lipid levels (Luo et al., 2015). Empirical research has demonstrated that sweet potatoes' nutritional and bioactive components substantially impact human health (Lu et al., 2016; Luo et al., 2021). These benefits support essential physiological functions and may contribute to the prevention of certain diseases (Wang & Nie, 2016). As a result, sweet potatoes are vital as a food resource and a natural food with potential health advantages.

Studies have shown that polysaccharides, intricate carbohydrates composed of lengthy chains of monosaccharide units, display a broad spectrum of biological and pharmacological effects (Li et al., 2022;). Their anti-inflammatory, antineoplastic, antioxidant, hypoglycemic, and hepatoprotective activities have been intensively studied in recent studies (Wu et al., 2015). This research emphasizes their potential as significant resources for developing health supplements and pharmaceuticals (Zhang, 2016; Hong et al., 2019). Studies on the various soluble fractions of polysaccharides derived from purple sweet potatoes, namely water-soluble polysaccharides (WSP), dilute alkali-soluble

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polysaccharides (DASP), and concentrated alkali-soluble polysaccharides (CASP), have demonstrated their ability to restore standard macrophage structure, suppress excessive production of inflammatory mediators, and decrease abnormal macrophage cell death. As a result, these compounds provide immunomodulatory and protective advantages (Tang et al., 2018). Further studies have isolated an alkali-soluble polysaccharide from purple sweet potatoes that can modulate gut microbiota and display anti-inflammatory activity (Sun et al., 2020). Additionally, research has indicated that polysaccharides derived from several traditional Chinese medicinal herbs possess extraordinary abilities to eliminate harmful free radicals, suggesting their potential efficacy in treating conditions such as cancer, diabetes, and Alzheimer's disease (Aun et al., 2016).

Although there has been extensive research on the impact of color on some molecules, including flavonoids (Cai, 2020; Li, 2016), anthocyanins (Zhao et al., 2023), and polyphenols (Liu, 2020), there remains a notable dearth of comprehensive studies that examine the influence of color on the metabolism of polysaccharides. To fill this void, the present study examines explicitly sweet potato tubers with purple, orange, and white colors. This work employs sophisticated metabolomic techniques and quantitative analysis to investigate the variations in polysaccharide metabolites across sweet potato tubers of three distinct colors. Furthermore, through the enrichment analysis of several metabolic pathways, the study provides a first examination of the role of color in the production of polysaccharide metabolites and the interconnected pathways associated with them. The findings contribute novel insights and scientific evidence to understanding the relationship between tuber coloration and polysaccharide metabolism.

2. Materials and methods

2.1. Materials

Sweet Potato Tuber Sampling: For this study, sweet potato tubers were harvested from the experimental plots at the School of Forestry and Horticulture, Hubei Minzu University, at the peak of their maturity in late October 2023. The mass of each plant was meticulously documented. We selected tubers based on three distinct pigmentation types: purple for purple sweet potatoes, orange for watermelon sweet potatoes, and white for chestnut sweet potatoes. Only specimens exhibiting average growth and devoid of disease or pest damage were included in the sample (Fig. 1A). We promptly processed a subset of these tubers by dicing them, placing them in centrifuge tubes, snap-freezing them in liquid nitrogen, and securing them in dry ice for transport. We subsequently preserved these samples at −80 °C for later analysis. We thoroughly washed, peeled, sliced, and dried another subset in an oven at 50 °C. We then pulverized the dried samples and passed them through an 80-mesh screen to prepare for the polysaccharide content analysis.

2.2. Methods

2.2.1. Determination of polysaccharide content in sweet potato tubers

We meticulously cleaned, removed the outer layer, cut it into thin pieces, and dehydrated another portion in an oven set at 50 °C. Next, we ground the dry samples into a fine powder and sifted them through an 80-mesh screen to facilitate the detection of polysaccharide content.

The method for extracting polysaccharides was adapted from previous studies (Bai et al., 2023): Initially, we mixed a suitable amount of dried sweet potato powder with petroleum ether in a solid-to-liquid ratio of 1:50 g/mL in a beaker. The mixture was refluxed at 50 °C for three hours to remove pigments and lipids. The mixture was subjected to ultrasonic-assisted hot water extraction for one hour. Subsequently, the extract was centrifuged at 5000 rpm for 15 min to isolate the supernatant. Subsequently, we reduced the volume of this supernatant to one-sixth of its initial amount at a temperature of 60 °C. Following the concentration process, we introduced four liters of 95 % ethanol to the polysaccharide solution and allowed the combination to precipitate overnight at a temperature of 4 °C. The polysaccharide precipitate was centrifuged at 8000 rpm for 10 min. Subsequently, it was dissolved in distilled water and subjected to continuous dialysis against running water for 48 h. We subjected the dialysate to freeze-drying under vacuum conditions, forming a raw brown polysaccharide. Subsequently, we kept it in a desiccator for future utilization.

The polysaccharide content was quantified using the sulfuric acid-anthrone colorimetric method (Shanghai Institute of Plant Physiology, 1999).

2.2.2. Metabolite extraction and untargeted metabolomics analysis conditions

Dry sample extraction: Using vacuum freeze-drying technology, place the biological samples in a lyophilizer (Scientz-100F), then grind (30 Hz, 1.5 min) the samples to powder form by using a grinder (MM 400, Retsch). Next, weigh 50 41 mg of sample powder using an electronic balance (MS105DM) and add 1200 μL of −20 °C pre-cooled 70 % methanolic aqueous internal standard extract (less than 50 mg added at the rate of 1200 μL extractant per 50 mg sample). Vortex once every 30 min for 30 s, for six times. After centrifugation (rotation speed 12,000 rpm, 3 min), the supernatant was aspirated, and the sample was filtered through a microporous membrane (0.22 μm pore size) and stored in the injection vial for UPLC-MS/MS analysis.

HPLC conditions: All samples were acquired by the LC-MS system following machine orders. The analytical conditions were as follows: UPLC: column, Waters ACQUITYUPLCHSST31.8 μm 2.1 mm*100 mm; column temperature, 40 °C; flow rate, 0.40 mL/min; injection volume, four μL; solvent system, water (0.1 % formic acid): acetonitrile (0.1 % formic acid); Sample measurements were performed with a gradient program that employed the starting conditions of 95 % A, 5 % B. Within 5 min, a linear gradient to 35 % A, 65 % B was programmed, within 1 min, a linear gradient to 1 % A, 99 % B was programmed, kept for 1.5 min. Subsequently, a composition of 95 % A and 5.0 % B was adjusted within 0.1 min and kept for 2.4 min.

MS conditions (AB): The data acquisition was operated using the information-dependent acquisition(IDA) mode using Analyst TF 1.7.1 Software (Sciex, Concord, ON, Canada). The source parameters were set as follows: ion source gas 1 (GAS1), 50 psi; ion source gas 2 (GAS2), 60 psi; curtain gas (CUR), 35 psi; temperature(TEM), 550 °C, or 550 °C; declustering potential (DP), 80 V, or-80 V in positive or negative modes, respectively; and ion spray voltagefloating (ISVF), 5500 V–4500 V in positive or negative modes, respectively. The TOF MS scan parameters were set as follows: mass range, 50–1250 Da; accumulation time, 200 ms; and dynamic background subtract, on. The product ion scan parameters were set as follows: mass range, 50-1250 Da; accumulation time, 40 ms; collision energy, 30 or 30 V in positive or negative modes, respectively; collision energy spread, 15; resolution, UNIT; charge state, 1 to 1; intensity, 100 cps; exclude isotopes within 4 Da; mass tolerance, 50 mDa; maximum number of candidate ions to monitor per cycle, 12.

2.2.3. Multivariate statistical analysis

The original data file acquisited by LC-MS was converted into mzXML format by ProteoWizard software. The XCMS program respectively performed peak extraction, peak alignment, and retention time correction. The "SVR" method was used to correct the peak area. The peaks with a detection rate lower than 50 % in each group of samples were discarded. Afterward, metabolic identification information was obtained by searching the laboratory's self-built, integrated public, AI, and mtDNA databases.

We utilized the MetaboAnalyst platform (https://www.metaboanalyst.ca/) to perform principal component analysis (PCA) on the identified polysaccharide metabolites, revealing the intrinsic relationships between different samples. Through the Bioinformatics Cloud Platform website (https://www.bioinformatics.com.cn/), we applied the Ward. D2 clustering method for systematic classification of the samples and

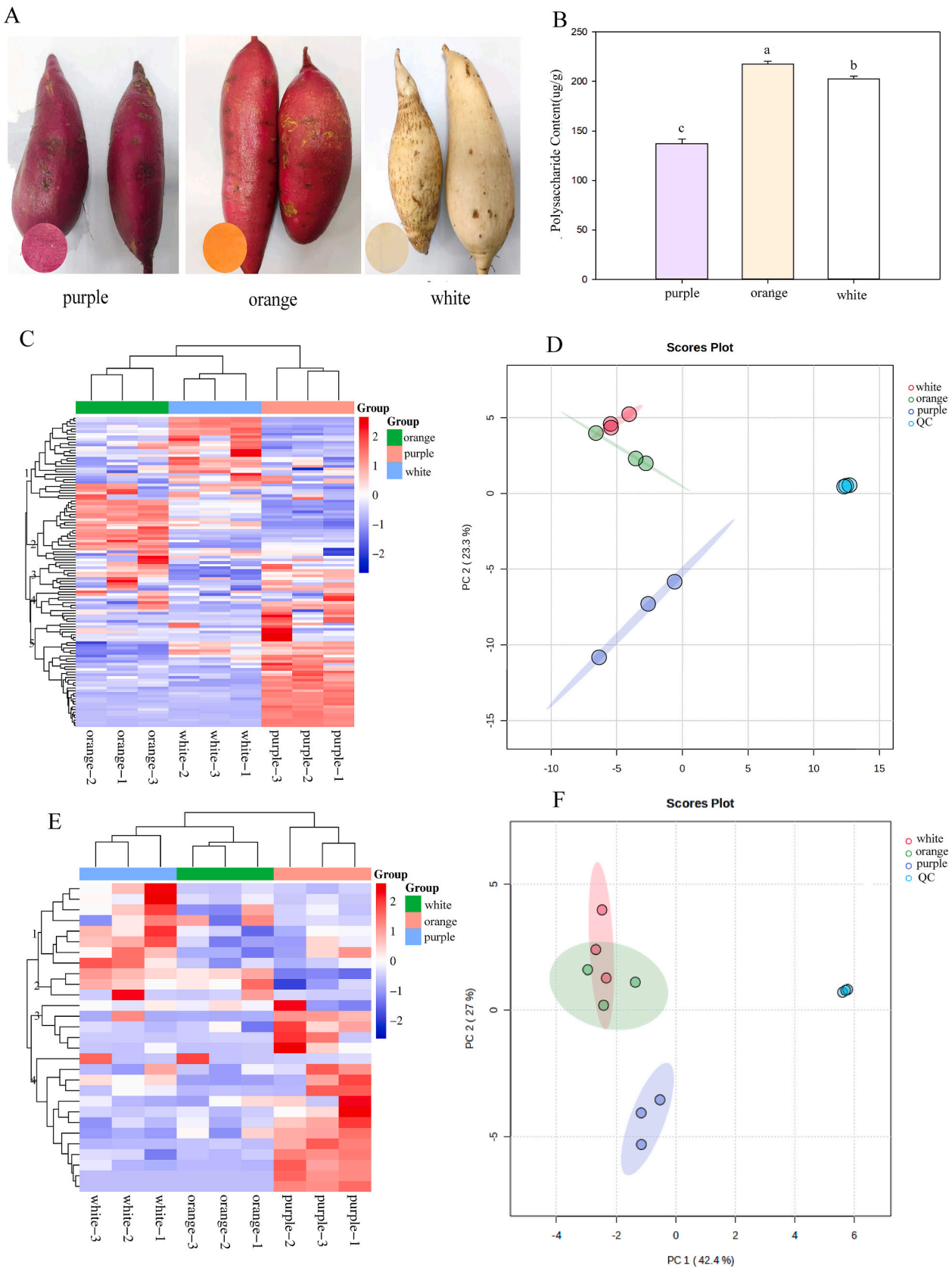

Fig. 1. A: Exterior characteristics of *Ipomoea batatas* and their tubers of different colors; B: The polysaccharide content in the *Ipomoea batatas* tubers of various colors. The various letters in the figure represent statistically significant differences ($P < 0.05$); C & E: Clustering heat map; Red represents high-level expression, and blue represents low-level expression; D & F: Principal Component Analysis (PCA) score chart. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

visually represented the clustering relationships among the samples using a heatmap. Combining the results of PCA and clustering analysis, we conducted orthogonal partial least squares discriminant analysis (OPLS-DA) on paired samples and validated the reliability of the model through 200 permutation tests. We employed the *t*-test to analyze the significance of differences in each compound and, in conjunction with the fold change (FC), *P*-value, and variable importance in projection (VIP) values from the OPLS-DA model, selected polysaccharide metabolites with significant differences to identify critical variables. The criteria for selection were set as a fold change greater than 1, a P-value less than 0.05, and a VIP value greater than 1. Finally, we utilized the Metware Cloud Platform (https://cloud.metware.cn/) to create volcano plots and to perform enrichment analysis of metabolic pathways.

3. Results

3.1. Comparison of polysaccharide content in sweet potatoes of different colors

Using a glucose standard solution, we created a standard curve for polysaccharides, resulting in the regression equation $y = 0.006\times + 0.0004$. The determination coefficient (R^2) was 0.9992, indicating a strong correlation. Hence, this regression equation is suitable for calculating sweet potato samples' polysaccharide content.

The polysaccharide content was analyzed in sweet potato tubers of different hues. It was found that the purple sweet potato variety had 137.31 μg/g of polysaccharides, the orange sweet potato variety had 217.46 μg/g, and the white sweet potato type had 202.57 μg/g. The polysaccharide content of the orange, white, and purple types differed significantly, as indicated by a *p*-value of less than 0.05 (Fig. 1B).

3.2. Analysis of polysaccharide metabolite profiles in colored sweet potato tubers

This study analyzed the polysaccharide metabolite profiles in sweet potato tubers exhibiting three distinct colors, utilizing multivariate statistical techniques. We identified a total of 69 polysaccharide compounds across the three sweet potato varieties. The relative abundance of these sugars is clearly depicted in the Total Ion Chromatogram (TIC) (Fig. S1). A clustering heatmap identified five distinct clusters. Cluster 1 showed the highest abundance of these metabolites in white sweet potatoes, followed by orange, and the least in purple sweet potatoes. Clusters 2 and 3 exhibited the highest metabolite levels in orange, with white and purple following in clusters 2 and 3, respectively. In contrast, cluster 4 and cluster 5 displayed the highest metabolite abundance in purple, with descending levels in orange and white. The consistency of biological replicates within the same color group underscores the reliability of the data. The variation in polysaccharide content among the tubers suggests a correlation with the color of the sweet potato (see Fig. 1C).

Principal component analysis (PCA) further elucidated the differences in polysaccharide metabolites among the colored sweet potato tubers. The analysis revealed distinct separation among the metabolites from differently colored tubers, aligning with the sample classifications and highlighting the unique metabolite profiles of each color variant. The metabolites in purple sweet potatoes diverged markedly from those in the orange and white varieties, as evidenced by the PCA score plot. In contrast, the metabolites in sweet potatoes in white and orange exhibited subtle differences. The quality control (QC) samples clustered in the same region on the PCA plot, confirming the consistency and reproducibility of the metabolic profiles (refer to Fig. 1D).

In conclusion, the clustering heatmap and PCA analysis consistently demonstrate significant differences in the polysaccharide metabolite profiles of sweet potato tubers according to their color.

3.3. OPLS-DA analysis and permutation test

We performed pairwise comparisons between the three sweet potato tubers of different colors using Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) and Permutation Test. We used OPLS-DA to further investigate their unique polysaccharide metabolism. Our research shows that panels A to C in all three data models exhibit apparent clustering of samples with different colors and a noticeable tendency towards separation (Fig. 2).

In the Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) model, R^2X represents the model's suitability for fitting the X matrix, while R^2Y represents the same for the Y matrix. Conversely, Q^2 represents the predictive capacity of the model. The R^2X values for the comparative groups fluctuate between 0.744 and 0.8, the R^2Y values range from 0.995 to 0.998, each surpassing 0.5, and the Q^2 values lie between 0.985 and 0.992, each exceeding 0.9. These outcomes suggest that the developed models are proficient in elucidating the data and demonstrate reliable predictive performance (Table 1).

3.4. Identification of differential polysaccharide metabolites in sweet potato tubers of varied colors

The OPLS-DA analysis results facilitated the screening of differential polysaccharide metabolites in the three control groups, employing fold change (FC), variable importance in projection (VIP) values, and *P*-values derived from univariate analysis. Metabolites satisfying the criteria of FC exceeding 1, VIP surpassing 1, and a *P*-value under 0.05 were deemed differential metabolites. Subsequently, we created Venn and volcano plots to visually display the screening process results. The results illustrate the identification of 14 distinct polysaccharide metabolites among the three comparison groups, with color being the varying factor. A total of 23 distinct polysaccharide metabolites were identified by comparing white and orange. Among them, 14 metabolites were shown to be upregulated, whereas 9 metabolites were downregulated. In the comparison between purple and white, we identified 44 distinct polysaccharide metabolites, with 26 showing upregulation and 18 showing downregulation. 36 distinct polysaccharide metabolites were identified by comparing purple and orange. Among these, 24 metabolites were shown to be elevated, while 12 were downregulated (Fig. 3).

3.5. The differences in polysaccharides and anthocyanin metabolites among sweet potatoes of three colors

In this study, we identified 14 types of polysaccharides as common differential metabolites among the orange, white, and purple sweet potato varieties, as well as 2 common anthocyanin compounds. The anthocyanin content in the flesh of purple sweet potatoes is significantly higher than that in the orange and white varieties. Specifically, the content of Peonidin 3-rhamnoside in the purple sweet potatoes is 13.31 times that of the orange and 74.77 times that of the white sweet potatoes; the content of Theaflavin monogallates is 8.74 times that of the orange and 124.96 times that of the white sweet potatoes. Among the polysaccharide compounds 3 to 6, the content in the white variety is markedly higher than in the purple and orange varieties, being approximately 5.23 to 20.85 times and 1.52 to 2.70 times higher, respectively. In compounds 7 to 10, the content in the orange variety has a significant advantage over the white and purple varieties, being about 1.37 to 38.33 times and 1.44 to 30.68 times higher, respectively. In compounds 11 to 16, the content in the purple variety is significantly higher than in the white and orange varieties, being approximately 5.76 to 74.44 times and 2.70 to 70.33 times higher, respectively (see Fig. 4).

3.6. Differential metabolite KEGG enrichment analysis

We analyzed the distinct polysaccharide metabolites found in purple, white, and orange sweet potato tubers. This analysis aimed to identify

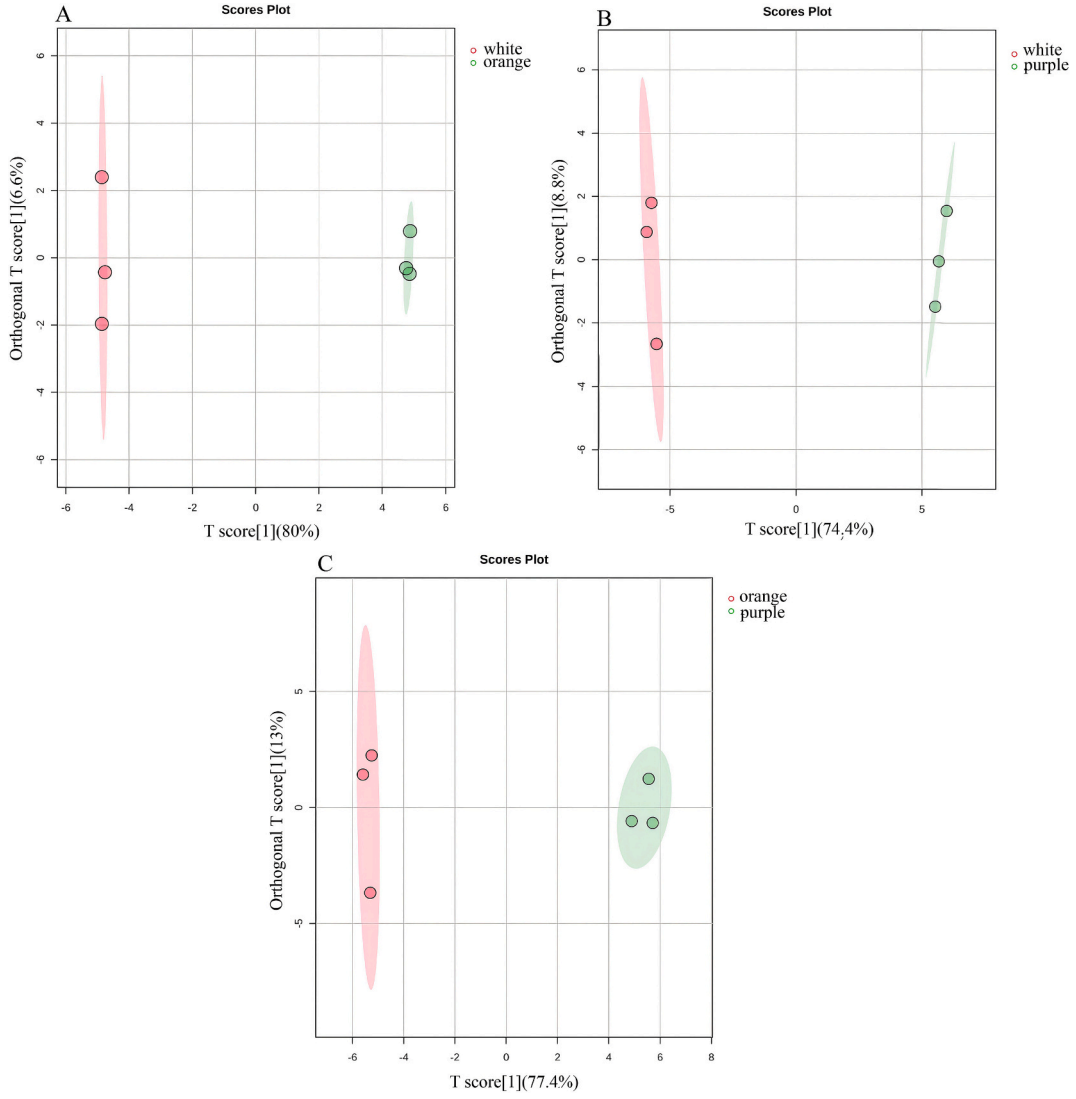

Fig. 2. Score chart of metabolite Orthogonal Partial Least Squares-Discriminant Analysis (OPLS-DA). A: illustrates the comparison between orange and white; B: denotes the comparison between purple and white; C: portrays the comparison between purple and orange. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Prediction indexes of the OPLS-DA model for different groups.

Group	R^2X	R^2Y	Q^2
white vs orange	0.800	0.998	0.992
purple vs white	0.744	0.996	0.985
purple vs orange	0.776	0.995	0.989

enriched pathways using the KEGG database. The investigation showed that metabolites from the three colored sweet potato tubers were mainly concentrated in 11 metabolic pathways. These pathways include nucleotide sugar biosynthesis, amino and nucleotide sugar metabolism, fructose and mannose metabolism, glycolysis/gluconeogenesis, pentose and glucuronate interconversion, the pentose phosphate pathway, and galactose metabolism, among others. In the white versus orange comparison, the primary enriched pathways were similar, with the addition of polyketide biosynthesis. Enriched pathways also included the biosynthesis of mannosyl-O-glycan, starch, and sucrose metabolism for the purple versus white comparison. In the purple versus orange comparison, the pathways further included the biosynthesis of thioglucosides. The findings suggest notable disparities in the chemical makeup and biosynthetic pathways across sweet potato tubers of varying colors (Fig. 5A ~ C).

3.7. Analysis of anthocyanin metabolite profiles

We comprehensively investigated the profiles of anthocyanin metabolites, generating a heatmap containing five distinct clusters. We identified a total of 26 anthocyanin compounds across the three sweet potato varieties. The relative abundance of these anthocyanins is illustrated in the Total Ion Chromatogram (TIC) (Fig. S2). Within clusters 1 and 2, anthocyanin metabolites appear most abundant in the white sweet potato. Conversely, in clusters 3 and 4, anthocyanin metabolites are most abundant in purple. Signifying good homogeneity among replicates and implying high data reliability (Fig. 1E).

We performed a Principal Component Analysis (PCA) on the anthocyanin metabolites in differently colored sweet potato tubers. The metabolites were separated based on color, with their clustering corresponding to the sample classification, indicating distinct differences. Specifically, polysaccharide metabolites in purple tubers diverge more significantly from those in orange and white, as evidenced by a clear separation in the score plot. The differences between white and orange tubers are less pronounced. Quality Control (QC) samples in the

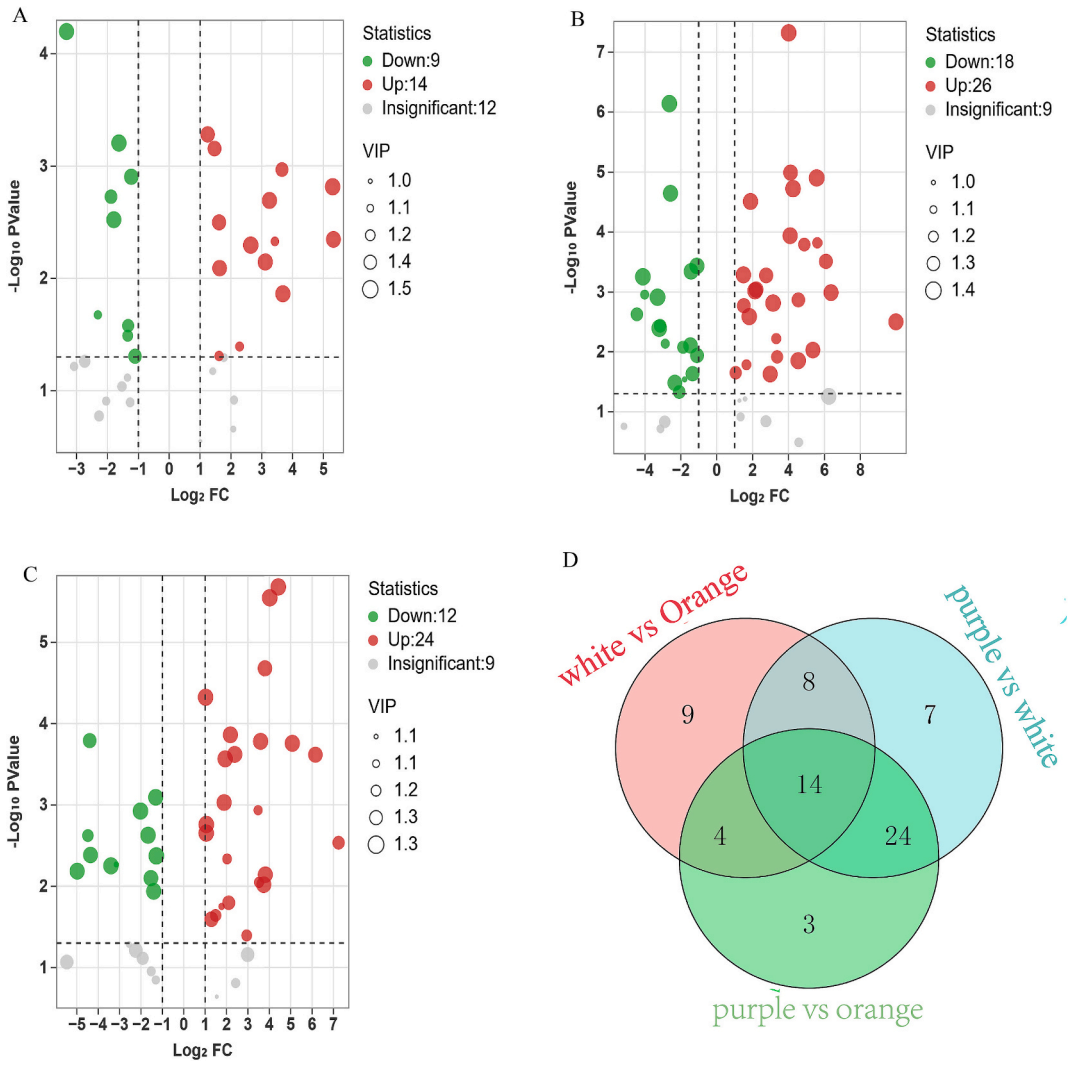

Fig. 3. Volcanic map and Vein of differential metabolites. A-C: Respectively represent orange vs white, purple vs white, purple vs orange; red indicates upregulation, and green indicates downregulation; D: represents the Venn diagram. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

PCA plot are projected into the same area, indicating similar metabolic characteristics and a stable, reproducible analysis (Fig. 1F).

Our differential analysis of anthocyanins in variously colored sweet potato tubers revealed 13 differential anthocyanin metabolites in orange compared to white, with four upregulated and nine downregulated. In purple, compared to white, there are 15 differential anthocyanin metabolites, with 11 upregulated and four downregulated. Comparing purple to orange, 16 differential anthocyanin metabolites were identified, with 14 upregulated and two downregulated (Fig. S3).

KEGG analysis identified differential pathways related to anthocyanin synthesis. Within the Galactose Metabolism pathway, the levels of UDP-glucose and galactose were found to be upregulated in purple. In contrast, raffinose and sucrose were downregulated Within the Anthocyanin Biosynthesis pathway, pelargonidin 3-O-β-D-sambubioside and delphinidin 3,5-diglucoside are upregulated in purple, while cyanidin-5-glucoside-3-sambubioside is upregulated in white. This suggests that purple possesses unique metabolic characteristics and regulatory mechanisms in the anthocyanin synthesis pathway, with increased pathway activity potentially leading to a greater allocation of carbon flux towards anthocyanin synthesis. Conversely, in orange and white, the carbon flux may be more directed towards other metabolic pathways (Fig. 6).

3.8. Polysaccharide and anthocyanin correlation network analysis

Analysis of the correlation network between polysaccharides and anthocyanins revealed that anthocyanins have a strong level of connectivity, indicating significant interactions with different polysaccharides and playing a crucial role in the network. Flavonoid 25 was discovered to have a connection with group 36 of saccharides, and there was a notably high positive link with Saccharide 38; Furthermore, flavonoids 3, 5, 7, and 18 connected with group 34 of saccharides. Flavonoid 17 showed associations with saccharide group 31, while Flavonoid 16 showed connections with saccharide group 30. In contrast, polysaccharide molecules exhibit reduced connectedness, implying that in biological systems, these molecules may engage with anthocyanins through fewer paths. We established a correlation between Saccharides 38 and 45 and a group of 11 anthocyanins. Remarkably, saccharide 38 displayed substantial inverse associations with ten anthocyanins.

On the other hand, there was a notable and favorable relationship between saccharide 45 and flavonoid 3. Furthermore, saccharides 32, 36, 47, 51, and 52 were found to be linked to the identical group of 10 anthocyanins. Saccharide 32 had inverse solid associations with nine anthocyanins. Saccharides 36 and 52 both exhibited strong positive associations with Flavonoid 7. In addition, Saccharide 47 had a notable positive association with Flavonoid 16, while Saccharide 51 showed a

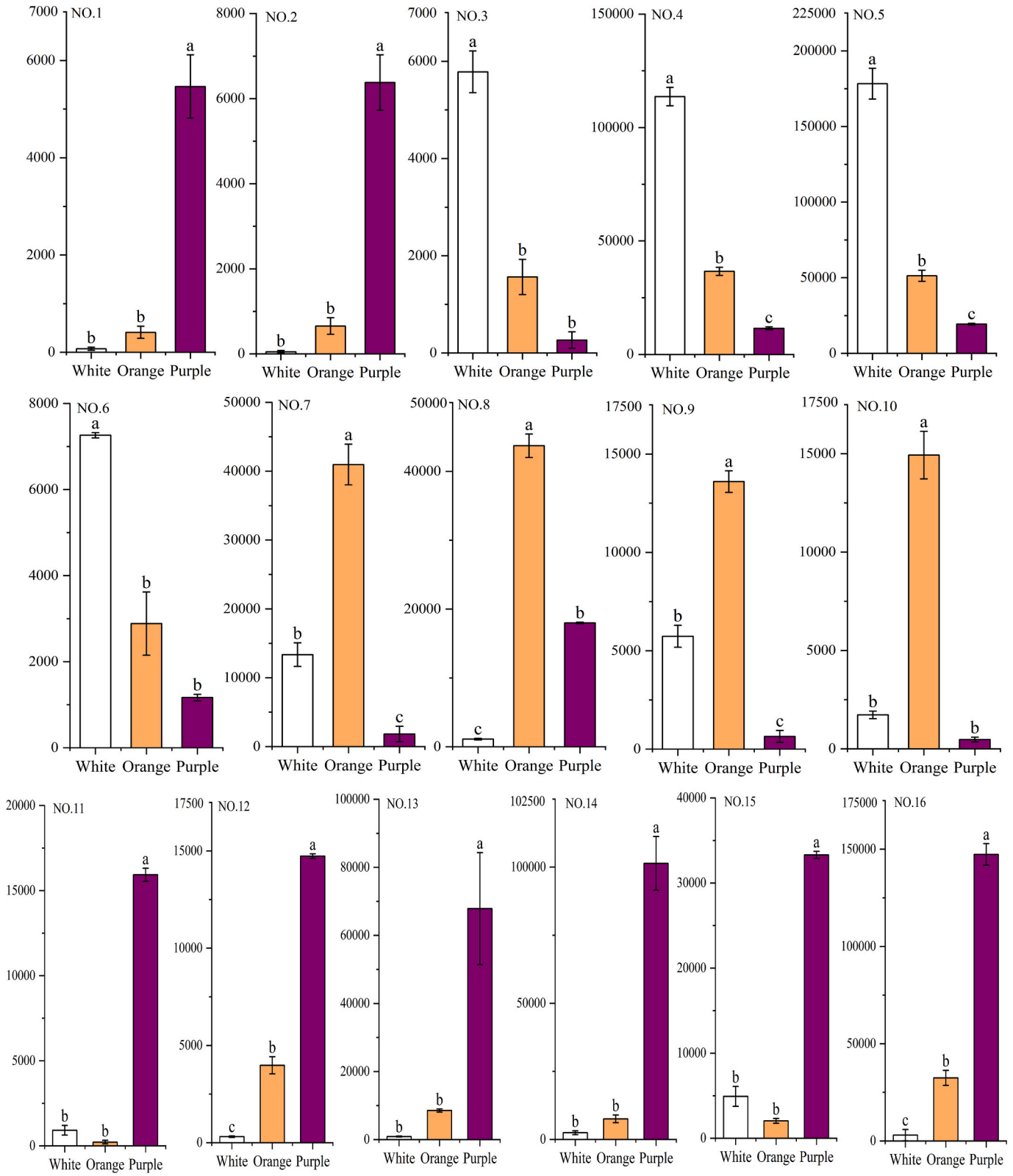

Fig. 4. Peak areas of 16 polysaccharides and anthocyanin metabolites were identified. Significance was evaluated using Duncan's test. The compound number corresponds to the same in Table S1. Different letters indicate significant differences ($P < 0.05$).

substantial link with flavonoid 18. These findings highlight the complex connections between certain sugars and anthocyanins throughout the metabolic system. Furthermore, the study demonstrated the specificity of these interactions, with specific polysaccharides selectively binding to anthocyanins, while others may have the ability to bind to many anthocyanins (Fig. 7 and Table S2).

4. Discussion

After being identified in sweet potatoes, polysaccharides, a crucial

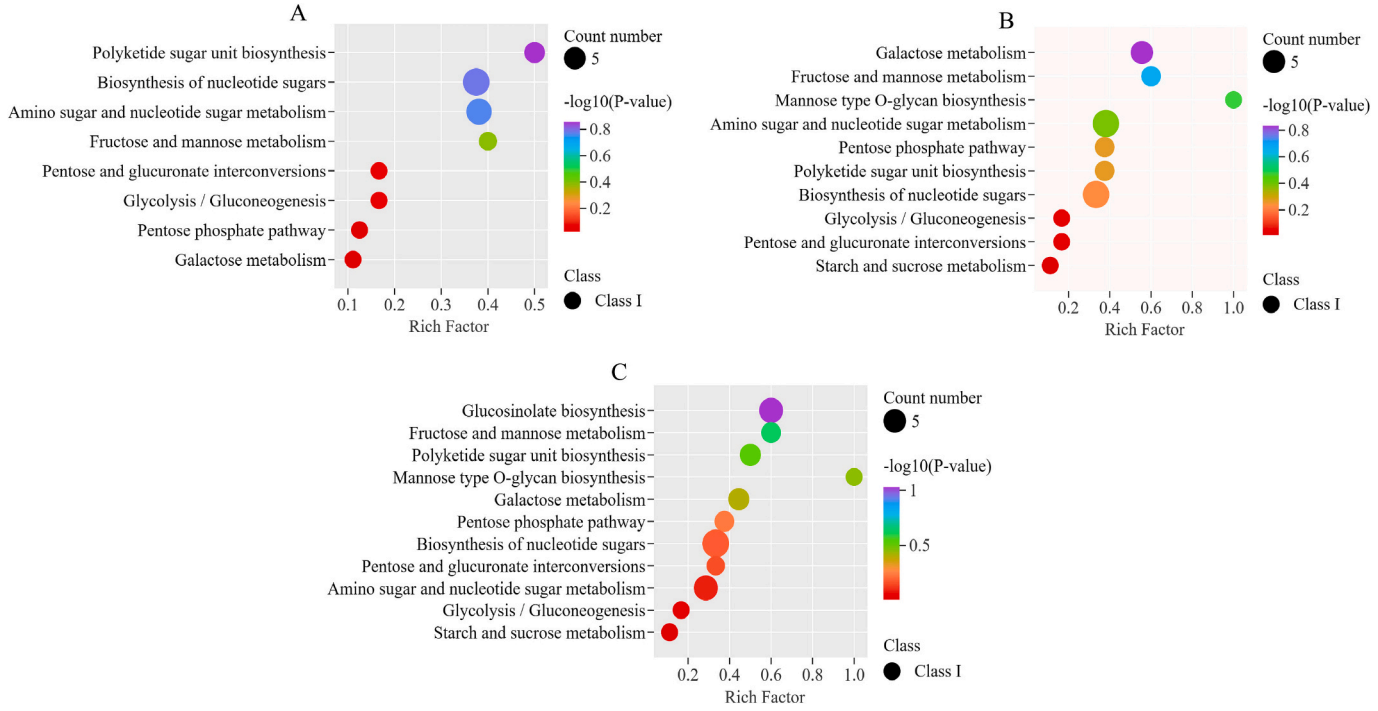

Fig. 5. Bubble diagram of enrichment analysis of various metabolic pathways across different groups. A: represents orange vs white; B: represents purple vs white; C: represents purple vs orange; The size of the bubbles represents the significance of the metabolites, and the color of the bubbles indicates the concentration of the metabolites. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

category of plant bioactive compounds, have garnered considerable interest among scientists. Macromolecules have several functions in plant growth, such as providing structural support, storing energy, and contributing to defensive systems. Sweet potatoes are sometimes referred to in news reports as 'red sweet potatoes', 'purple sweet potatoes', and 'white sweet potatoes', depending on the color of their flesh. This variation in color may be linked to the presence of anthocyanins. Research indicates that the diversity of tuber flesh color is primarily determined by pigment components such as carotenoids and anthocyanins (Tanaka et al., 2008). The yellow and purple hues of root tubers are attributed to the accumulation of lipid-soluble β-carotene and water-soluble anthocyanin, respectively (Odake et al., 1992). The yellow hue of root tubers can gradually transition to orange as the β-carotene content increases (Tanaka et al., 2008). In contrast, white root tubers contain negligible amounts of anthocyanins.

Anthocyanins are crucial compounds responsible for color variations, with studies indicating that anthocyanidins, in their aglycone state, exhibit instability within plants. To attain stability, anthocyanins undergo glycosylation, wherein sugars substitute for part or all of the hydroxyl groups (Guo et al., 2022). The stability of this process is influenced by the type and quantity of glycosyl groups involved (Vidana Gamage et al., 2021). This glycosylation results in the formation of shared metabolic intermediates between anthocyanins and polysaccharides, leading to competitive biosynthesis (Jones & Dang, 2020; Zhang et al., 2018). It is generally accepted that triglycoside anthocyanidins show superior stability to monoglycoside anthocyanidins (Chen et al., 2022). Furthermore, the precursors necessary for anthocyanin synthesis are derived from the shikimic acid pathway, which is dependent on robust pentose metabolism (Zhuang et al., 2018). In our study, the purple sweet potato variety demonstrated the lowest polysaccharide content; however, anthocyanin levels were significantly upregulated. This observation aligns with previous research, suggesting that anthocyanins may preferentially bind with polysaccharides to form triglycoside anthocyanidins.

Besides, it is generally accepted that white sweet potato tubers predominantly accumulate starch, supporting growth and development (Li et al., 2019; Zhao et al., 2018). In contrast, orange sweet potato tubers possess elevated carotenoids, sucrose, and fructose levels (Tan & Xie, 2016). Purple sweet potato tubers, meanwhile, are abundant in anthocyanins and functional anthocyanin polysaccharides (He et al., 2022; Zhang et al., 2020). Our study found that orange sweet potatoes had the highest polysaccharide content, followed by white sweet potatoes and purple sweet potatoes. These findings are consistent with prior research. Some studies have highlighted glucose as the most ancient and conserved regulatory signaling molecule in plants, playing a pivotal role in the allocation of carbon sources within the plant (Sheen & Jen, 2014). In the current research, the compounds pelargonidin 3-O-β-D-sambubioside and delphinidin 3,5-diglucoside were notably increased in purple; this upregulation suggests that these varieties may preferentially direct carbon flux towards anthocyanin biosynthesis, in contrast to other varieties, such as orange and white sweet potatoes, which may channel carbon into polysaccharide metabolic pathways. Consequently, the polysaccharide content was higher in the white and orange varieties compared to the purple sweet potato. A metabolic network regulation diagram illustrates the interrelationships among metabolites and their regulatory networks. Research indicates that highly connected metabolites are more likely to be deemed essential compared to those with fewer connections (Jeong et al., 2001). In the correlation network analysis, anthocyanins were central to the interactive network, with each anthocyanin linked to corresponding polysaccharides. These results suggest that the polysaccharide profiles of sweet potato tubers differ by color and are associated with anthocyanin content, corroborating previous findings. Notably, specific anthocyanins such as cyanidin-5-glucoside-3-sambubioside were detected in the white variety.

Research on the antioxidant mechanisms of *Lyceum ruthenium* Murr anthocyanin extracts has revealed that the biosynthetic pathways for anthocyanins and polysaccharides have common precursors and enzymes (Deng et al., 2023). UDP-glucose is the sugar donor in anthocyanin glycosylation, escalating anthocyanin content (Gu et al., 2023; Xie, Sun et al., 2013). Research on the UDP-glucose flavonoid 3-O-glucosyltransferase (UFGT) gene in grapes has demonstrated its critical role in

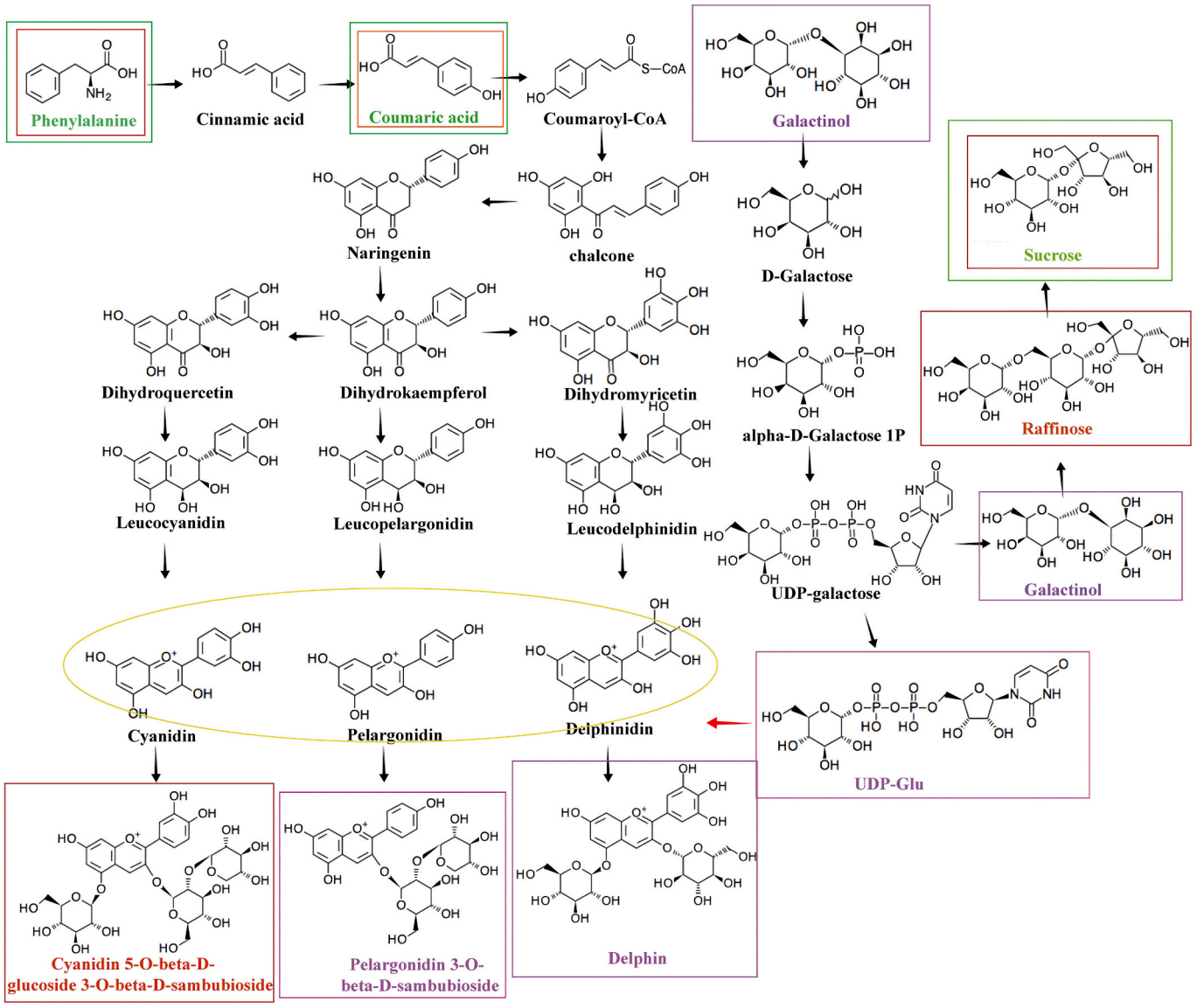

Fig. 6. Galactose metabolism and anthocyanin biosynthesis pathway diagram. Red boxes signify the upregulated expression of critical metabolites in white potato, green box denotes the upregulated expression of crucial metabolites in orange potato. In contrast, purple box denotes the upregulated expression of critical metabolites in purple potato. Yellow ovals denote the compounds bound to the UDP-Glu. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the biosynthesis of anthocyanins within grape berries (Kobayashi et al., 2001). In higher plants, UDP-glucose is synthesized from sucrose through the catalytic action of sucrose-phosphate synthase (SPS; EC2.4.1.14) and sucrose synthase (SuSy; EC2.4.1.13) (Huber & Huber, 1996). Sucrose is the primary form of carbon and energy transport within the plant, and its synthesis and accumulation are contingent upon source-sink communication (Verma et al., 2020). Moreover, studies have revealed that sucrose regulates structural genes within the anthocyanin biosynthetic pathway, precisely modulating specific regulatory genes (Solfanelli et al., 2006). In this investigation, UDP-glucose upregulation within purple was noted; however, a concomitant downregulation of sucrose was observed, indicating a reallocation of carbon resources towards the anthocyanin synthetic pathway. This finding implicates that anthocyanins may influence polysaccharide synthesis and compete with polysaccharides for carbon sources.

5. Conclusion

This study employed metabolomics techniques to perform a comprehensive comparative analysis of polysaccharide metabolites in sweet potato tubers of three distinct colors. We identified 23 differential polysaccharide metabolites between white and orange tubers, 36 between purple and orange, and 44 between purple and white tubers. Our research found that anthocyanins occupy a central position in the correlation network graph and are interconnected with polysaccharides. The competition between sugars and anthocyanins for shared biosynthesis precursors is attributed to differential polysaccharide metabolites among sweet potato tubers of three colors. This study provides a novel perspective on the metabolic pathways of sugars and anthocyanins in sweet potatoes, thereby establishing a scientific foundation for enhancing the development of functional foods derived from this crop.

Institutional review board statement

Ethical review and approval were waived for this study, because sweet potatoes samples used in the study are consumed in daily life.

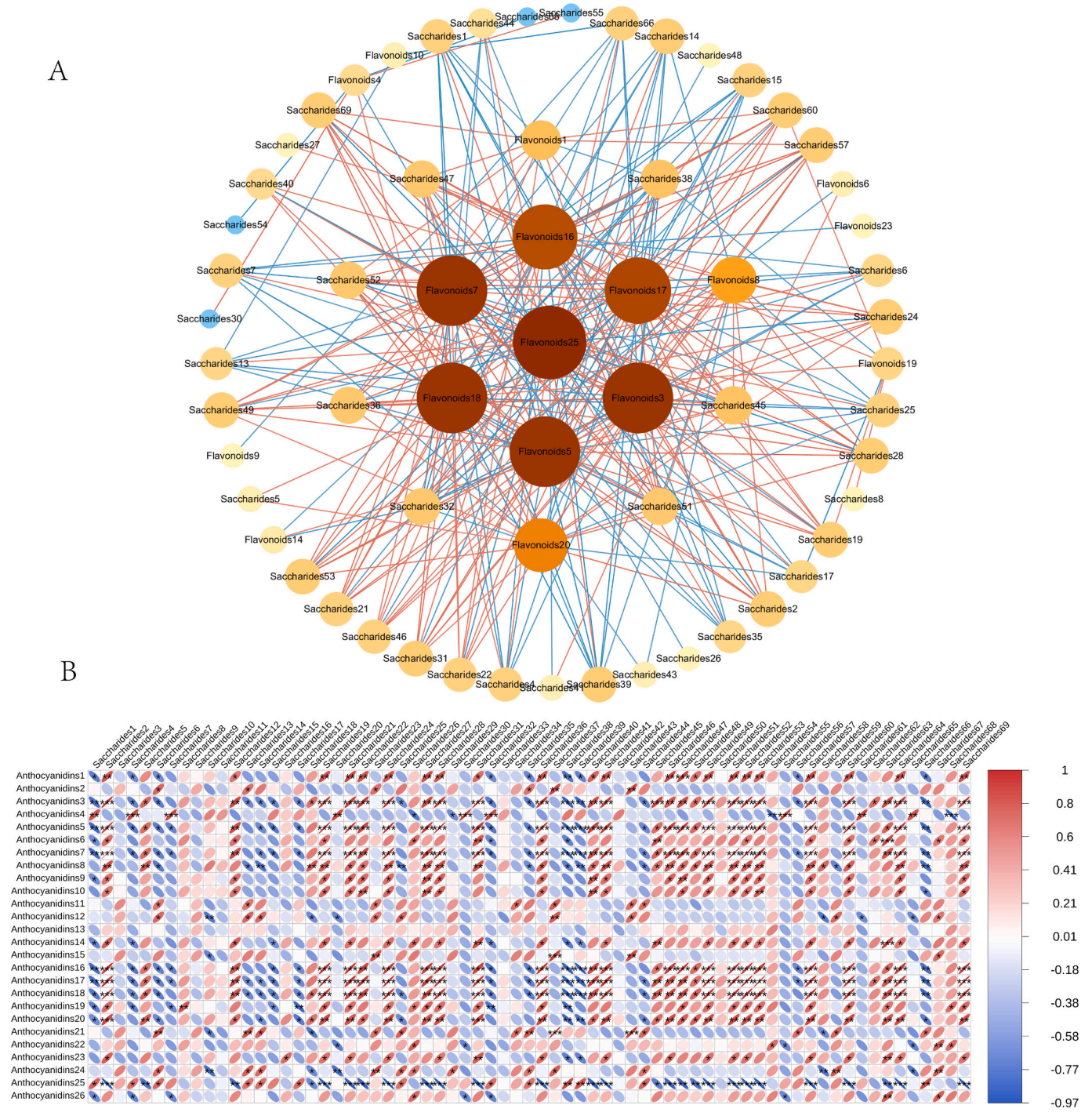

Fig. 7. A: Association network. B: Correlation Analysis. The color gradient from deep red to light yellow represents a decrease in connectivity, with the deeper red indicating higher connectivity and the lighter yellow indicating less connectivity. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Informed consent statement

Informed consent was obtained from all subjects and panelists involved in the study.

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CRediT authorship contribution statement

Xiuzhi Wang: Writing – original draft, Validation, Methodology, Investigation, Conceptualization. **Jiaqi Wu:** Validation, Investigation, Formal analysis. **Lingjun Cui:** Supervision, Funding acquisition. **Qiang Xiao:** Validation, Methodology, Investigation, Formal analysis.

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.fochx.2024.101855.

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