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Nutrients in 'Opal' apples and key metabolites in delayed browning of their pulps were analyzed based on comparative omics

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Abstract

The 'Opal' apple, a crossbreed between the 'Topaz' and 'Golden Delicious' varieties, has a unique crunchy, a tangy flavor, slow browning rate that can keep long-lasting freshness after being sliced up. With 'Fuji' apples as reference controls, the content of mineral elements, and their key metabolites in the pulps of two kinds of apples were analyzed to clarify nutritional properties and slow browning features in 'Opal' apples. In this study, the differential metabolites of the two cultivars of apple were statistically analyzed with the differential principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) using UHPLC-LTQ-Orbitrap MS, combined with non-targeted metabolomics technology, to identify the key differential metabolites in the two varieties of apple. The results showed that there was a total of 147 significantly differential metabolites in 10 categories, of which phenylpropanoids and polyketides account for the highest proportion, Through KEGG pathway analysis, the differential compounds in the two apple cultivars were annotated into 52 pathways, and they were significantly enriched in the two pathways ABC transporters and Flavonoid biosynthesis. The results can lay a theoretical basis for researches on apple quality and provide data support for the indepth development of different apple products.

Keywords: apple; UHPLC-LTQ-Orbitrap MS; non-targeted metabolome; quality.

Practical Application: The findings in this study can provide reference for researches on function, processing, development and utilization of apple fruits, and also lay a theoretical basis for the breeding of good apple varieties and the development of functional health food.

1 Introduction

Apple (*Malus domestica*) is a stone fruit of the family Rosaceae (Karaaslan & Ekinci, 2022). It is the most important fruit produced and eaten around the world (Musacchi & Serra, 2018; Akšić et al., 2022) and constitutes an important part of the human diet (Ján & Ivana, 2018). The industrial structure of apples in China is unreasonable, with a lack of excellent early- and mid-maturing varieties and the allocation of yellow, green, and functional varieties, resulting in insufficient diversification, differentiation, and specialization of fresh apples, and making it difficult to meet people's demand for high-quality, safe, and diverse fruits. Currently, late-maturing Fuji apples continue to account for the majority of the market in China, with a more prevalent phenomenon of variety simplification and homogenization. In terms of variety structure, the disparity between fresh and processing apples is also a noticeable issue. More than 90% of apples planted in China are used for fresh food, with only 10% for deep processing, which leads to the uneven development of China's apple processing industry. Additionally, problems like browning and weak aroma make it difficult for most apples to meet the processing requirements. Therefore, current research ought to attach more importance to finding apple varieties with slow browning and optimizing varieties suitable for processing through grafting, selective breeding, and other methods.

The apple industry in Ningxia of China is experiencing a period of structure adjusting of the local fruit variety. The main apple varieties planted in Ningxia are 'Fuji' and 'Red Delicious'. Their small area cultivars also diversity as 'Gala', 'Jonathan', 'Granny Smith', etc. (Xia & Shi, 2020). The current pattern of cultivation in Ningxia is changing from mainly planting single and high-yield varieties ('Fuji' series) for meet market demands, to seeking cultivars with different maturity periods, different regional characteristics, different colors and different tastes. The overall apple planting pattern here steers to the direction of focusing on diversification, dislocation and quality. Especially, fresh apple fruit with low browning rate is a kind of ideal raw material for fruit processing and juice processing enterprises to develop new products.

The 'Opal' apple provide excellent quality for cultivation in Ningxia. The trial planting results demonstrate that 'Opal' apples have the correct shape, rich aroma, close texture, and full juice, which taste sweet and delicious. Moreover, experiments

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show that the browning rate of 'Opal' apples is relatively slow. The 'Opal' apple was the first U.S. apple cultivar verified by the Non-GMO project, the only independent verification in North America for non-GMO food. The 'Opal' apple is a cross between the 'Topaz' and 'Golden Delicious' cultivars, The 'Opal' apple shows specific quality as: (1) unique crunchy; (2) tangy flavors; (3) slow browning rate that can keep long-lasting freshness after being sliced up (Site built by RedFin Group, 2022).

In this study, to explore the 'Opal' apple quality characteristic in Ningxia, the 'Opal' and 'Fuji' apple were taken as the research objects, and comprehensive assessment the nutrients and the metabolites, and analysis.

Researches on the quality of apple stored postharvest and on the factors affecting the quality of stored apples (Zhao & Yin, 2018), which mainly analyzed known nutrients and bioactive components, while few studies have been conducted on many unknown nutrients and active components. The research on the quality of fruit by metabolomics technology started late, it had developed rapidly and has been widely used. UHPLC-LTQ-Orbitrap MS was used to carry out non-targeted metabolomic analysis to screen bioactive substances with significant differences, aiming to provide a theoretical basis for the development of functional apple products and lay a foundation for the researches on the mechanism of apple browning.

2 Materials and methods

2.1 Analytical objects

The samples of 'Opal' and 'Fuji' apple were collected from the apple germplasm resource bank of the farms in Ningxia, China. 'Opal' and 'Fuji' apples with similar maturity, size, and color were collected, peeled, and sampled by quartering, with about 5 g of pulp selected as a replicate, a total of 6 biological replicates for each variety. 6 fruits were included in each biological experiment. All the samples were frozen in liquid nitrogen and stored at -80°C. The details of apple fruits are shown in Table S1. This study analyzed the physicochemical indicators such as sugar and acid and determined the contents of trace elements, fresh sample was used; for mineral elements, dried sample was used.

2.2 Chemicals

Acetonitrile and Methanol were purchased from Millipore (USA); Ammonium acetate, a mobile phase additive, was obtained from Sigma.

2.3 Elemental composition of minerals

The contents of mineral elements (Mg, Ca, K, P, Zn, Mn, Cu, Fe, Se) were determined using dried apple sample. About 1.0 g of the sample was accurately weighed and put into a glass digestion tube. Then, a mixture of nitric acid and perchloric acid (10:2) was used for digesting the sample on an automatic digestion apparatus (purchased from Beijing Polytech Instrument Co., Ltd., China). The digested sample was made into final volume of 25 mL, and it was shaken well before use. K element was determined with a flame photometer (purchased from Sherwood Technology Company, USA), P element was deter-mined with the Olsen method, and other elements were determined with atomic absorption spectrophotometer (220FS/220Z, purchased from Varian Corporation, USA).

2.4 Metabolites extraction

Take out the test samples stored at -80 °C, weigh 100 mg per sample, grind them with liquid nitrogen, add 200 μL of pre-cooled water and 800 μL of pre-cooled methanol/acetonitrile (1:1, v/v), mix the solution well, sonicate it in an ice bath for 60 min, keep it at -20 °C for 2 h, centrifuge it at 16,000 g at 4 °C for 30 min, and take the supernatant. The supernatant is evaporated for dryness using a high-speed vacuum centrifuge. During mass spectrometry detection, add 100 μL of acetonitrile-water solution (1:1, v/v) to reconstitute it, centrifuge it at 16,000 g at 4 °C for 20 min, and take the supernatant for analysis. Meanwhile, QC (Quality control) sample is prepared: suck up 10 μL of each sample to be tested and mix them into a quality control sample, which is used to correct the deviation of the analytical results and the error caused by the analytical instrument itself.

2.5 UHPLC-LTQ-Orbitrap MS analysis

Metabolomics profiling was analyzed using a UPLC-ESI-Q-Orbitrap-MS system (UHPLC, Shimadzu Nexera X2 LC-30AD, Shimadzu,Japan) coupled with Q-Exactive Plus (Thermo Scientific, San Jose, USA). For hydrophilic interaction liquid chromatography (HILIC) separation, samples were analyzed using a 2.1 mm*100 mm ACQUIY UPLC BEH Amide 1.7 μm column (Waters, Ireland). The flow rate was 0.3 mL/min and the mobile phase contained: A: 25 mM ammonium acetate and 25 mM ammonium hydroxide in water, and B: 100% acetonitrile (ACN). The metabolites were eluted by gradient as follows: 0~1 min, 95%B; 1~7 min, 95%B→65%B; 7~9 min, 65%B→35%B; 9~10.5 min, B35%; 10.5~11 min, 35%B→95%B; 11~15 min, 95%B. Each sample was detected by electrospray ionization (ESI) in positive (+) and negative (-) modes, respectively. After the samples were separated by UPLC, they were analyzed by QE Plus mass spectrometer and were ionized using a HESI source with the following ionization conditions: Spray Voltage: 3.8 kv (+) and 3.2 kv (-); Capillary Temperature: 320 (±); Sheath Gas: 30 (±); Aux Gas: 5 (±); Probe Heater Temp: 350 (±); S-Lens RF Level: 50. The MS acquisition settings were as follows: MS acquisition time: 12 min. Precursor ion scanning range: 80-1200 m/z, MS1 resolution: 70,000 @m/z 200, AGC target: 3e6, primary maximum IT: 100 ms. MS2 analysis was acquired as follows: MS2 scan was triggered after each full scan to acquire the 10 precursor ions with the highest-intensity, MS2 resolution: 17,500 @ m/z 200, AGC target: 1e5, Level 2 maximum IT: 50 ms, MS2 activation type: HCD; Isolation window: 2 m/z, Normalized collision energy (Setpped): 27, 29, 32.

2.6 Quality control analysis

The Non-targeted metabolomic analysis of the apple fruit extracts was conducted to evaluate the differences in metabolite components of the two apple cultivars. The specific experimental procedures are as follows: sample collection, extraction of metabolites, mass spectrometry analysis, data processing, and

screening and identification of differential metabolites. In the study of metabolomics, quality control analysis (QC) is an important method used to evaluate the reliability and high quality of mass spectrometry technology. Before testing the samples, QC test was conducted for the samples to balance and stabilize the "chromatography-mass spectrometry" system. The spectrum obtained through continuous scanning was the total ion chromatogram (TIC). TIC analysis of different quality control samples showed that the response intensity and retention time of each chromatographic peak basically overlapped with each other. Therefore, it could be judged that the mass spectrometry instrument in this study was in good and stable condition (Lu et al., 2022), its systematic error was within the controllable range, and the experimental data were deemed stable and reliable (Figure S1).

The principal component analysis (PCA) was performed on the peaks extracted from all experimental samples and QC samples after UV treatment. After 7 cyclic interactive verifications, the PCA model was obtained. that in the positive and negative ion modes, the QC samples were closely clustered, indicating that the data obtained by using UHPLC-LTQ-Orbitrap MS in the test had good repeatability and stability. The differences in the metabolic profiles obtained in the test can show the biological differences between the samples themselves (Figure S2).

2.7 Data processing and statistical analysis

Statistical significance analysis (ANOVA, Tukey Pairwise Comparisons) and graphing were conducted in Excel 2016.

The raw data were analyzed using MSDIAL for peak alignment, retention time correction and extraction of peak areas. The identification of metabolite structures was performed through accurate mass number matching and MS2 matching, and public databases such as HMDB, MassBank, the standard library of metabolites built by Shanghai Bioprofile Co., Ltd., were searched. The total peak areas of the positive and negative ion data were processed for normalization, respectively. The positive and negative ion peaks were integrated and the models were recognized by using R, and the data were normalized using SIMCA14.0. After that, chemometric and multivariate statistical analyses such as PCA (Principal component analysis) and OPLS-DA (orthogonal partial least squares discriminant analysis) were conducted.

3 Results

3.1 'Opal' and 'Fuji' apples were significant different at flesh browning rate

The 'Opal' and 'Fuji' apples were significant different at appearance of peel color and flesh browning condition. They were different at the peel color, as 'Opal' is yellow while 'Fuji' is red. 'Opal' is a fresh yellow gold apple often with an attractive delicate blush and a russeted stem end 'halo' It combines a crisp and juicy texture with an intense sweet and slightly tangy, aromatic flavour.

It's worth noting that the 'Opal' apple showed slow flesh browning rate than 'Fuji' apple (Figure 1). showed that freshly sliced 'Opal' apple and 'Fuji' apple had no browning. With time passing by, the browning areas of the two cultivars of apple tended to be significantly different. After being placed for 3 hours, browning occurred in both cultivars of apple. However, compared with their state of being freshly-sliced, 'Opal' apple slices had slight browning without obvious change in color, while 'Fuji' apple slices showed obvious browning. After being placed for 6 hours, 'Opal' apple slices showed obvious traces of browning compared to their state 3 hours ago. At the same time, it can be seen that the browning degree of 'Fuji' apple slices was significantly higher than that of 'Opal' apple slices after being placed for 6 hours. After being placed for 9 hours it can be clearly seen that the browning degree of 'Fuji' apple slices was much higher than that of 'Opal' apple slices. The results showed that the browning rate of 'Fuji' apple was much faster.

3.2 'Opal' and 'Fuji' apples were significant different at mineral elements

This study determined the nutritive elements in 'Opal' and 'Fuji' apple. According to the results shown in Figure 2, the two apple cultivars are rich in mineral elements but their contents differ greatly. Among the 9 mineral elements, the content of Fe in 'Fuji' apple was higher than that in 'Opal' apple. However, the contents of other 8 mineral elements in 'Opal' apple were

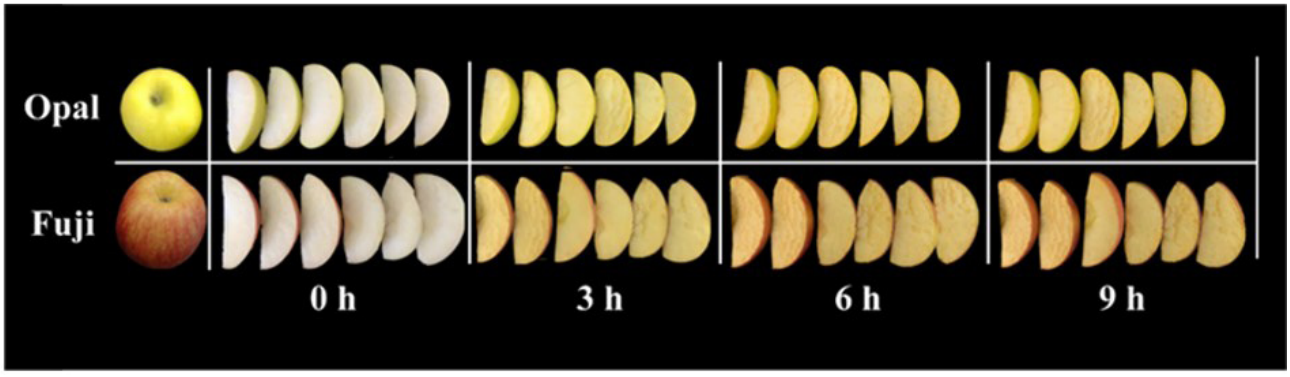

Figure 1. Characteristics of apple browning at different times. A 9-hour experiment was designed for this study, by which fresly silced samples were placed for different time periods (time interval: 3 hours) to observe their browning degrees.

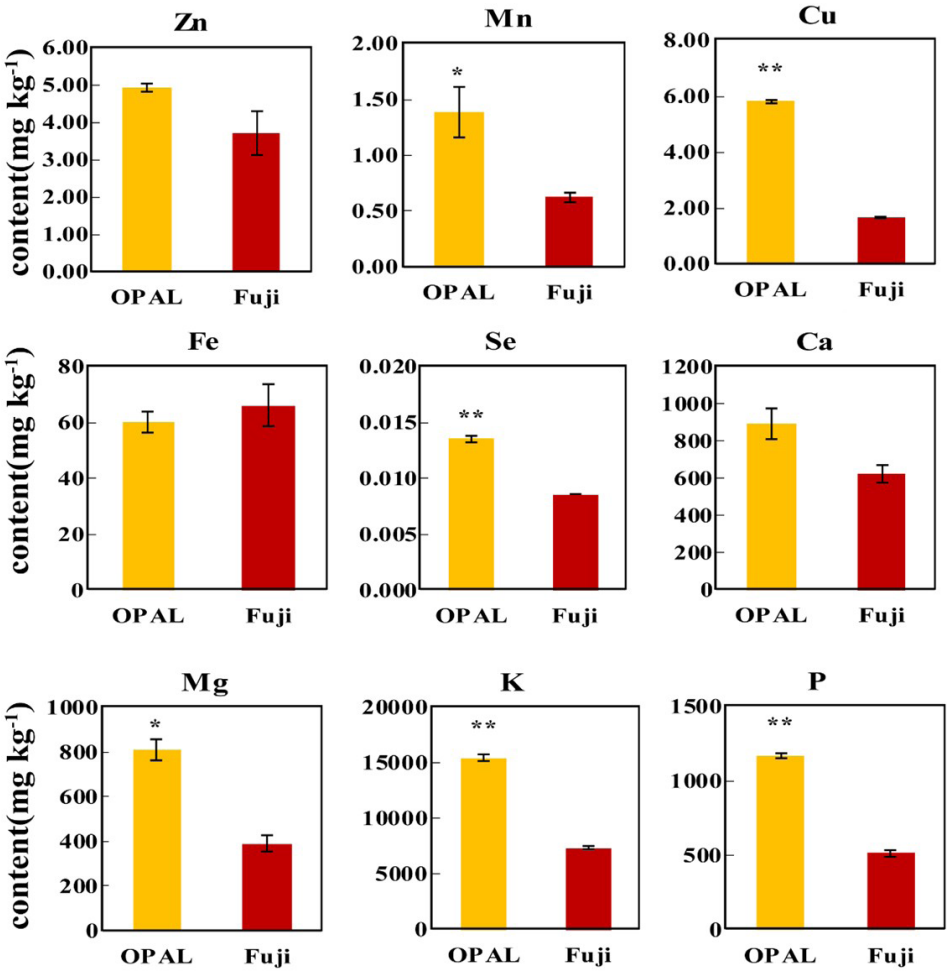

Figure 2. Concentrations of Zn, Mn, Cu, Fe, Se, Ca, Mg, K and P (mg $kg^{-1}$of dry weight), in the different apple cultivars. Data (n = 3) were analyzed by one-way ANOVA. Tukey's post-hoc test at 0.05 probability level was applied and * indicated significant difference among means.

higher than those in 'Fuji' apple, with the amounts of Zn, Se, Ca in 'Opal' apple more than twice as much as those in 'Fuji' apple. The biggest difference by 3.53 times was observed in the content of Cu in the two apple cultivars. This shows that the two apple cultivars are quite different, resulting in a significant difference in their quality. This finding provides a theoretical basis for the selection and breeding of apple cultivars.

3.3 Analysis of non-targeted metabolomics data profile of apple cultivars

The Non-targeted metabolomic analysis of the apple fruit extracts was conducted to evaluate the differences in metabolite components of the two apple cultivars. The differences in the metabolic profiles were obtained.

We identified 9918 positive ion features and 5245 negative ion features by using UHPLC-LTQ-Orbitrap MS. Furthermore, we got 265 annotated chemicals from 9918 positive ion features and mapped these 265 chemicals on 34 KEGG pathways. In parallel, we got 121 annotated chemicals from 5245 negative ion features and mapped these 121 chemicals on 23 KEGG pathways.

To analyze the differences between metabolites in different apple varieties. The PCA showed a total of 2 principal components were obtained, with cumulative $R^2X = 0.563$ and $Q^2X = 0.549$, and the contribution of the first principal component was 31.96% while that of the second principal component was 14.50%. Both 'Opal' and 'Fuji' samples fell within the confidence interval. The samples of the two apple varieties were separated from each other completely and could be clearly distinguished in space, showing a significant clustering trend. This means that the metabolites of the two groups of samples differed in terms of category and quantity (Figure 3A).

To obtain more accurate results and analyze the differences between the two groups quickly and accurately, a supervised OPLS-DA model was used to perform the analysis. As shown in Figure 3B, 'Fuji' apple samples were mainly distributed on the left side, while 'Opal' apple samples were mainly distributed on the right side, suggesting that the two groups of samples were of good discriminability, that the stability of the model was good, and that the predictive power of the cross-validation was high ($R^2 = 0.927$, $Q^2 = 0.929$) (Figure 3C). At the same time, the OPLS-DA model was verified, and all Q^2 points from left

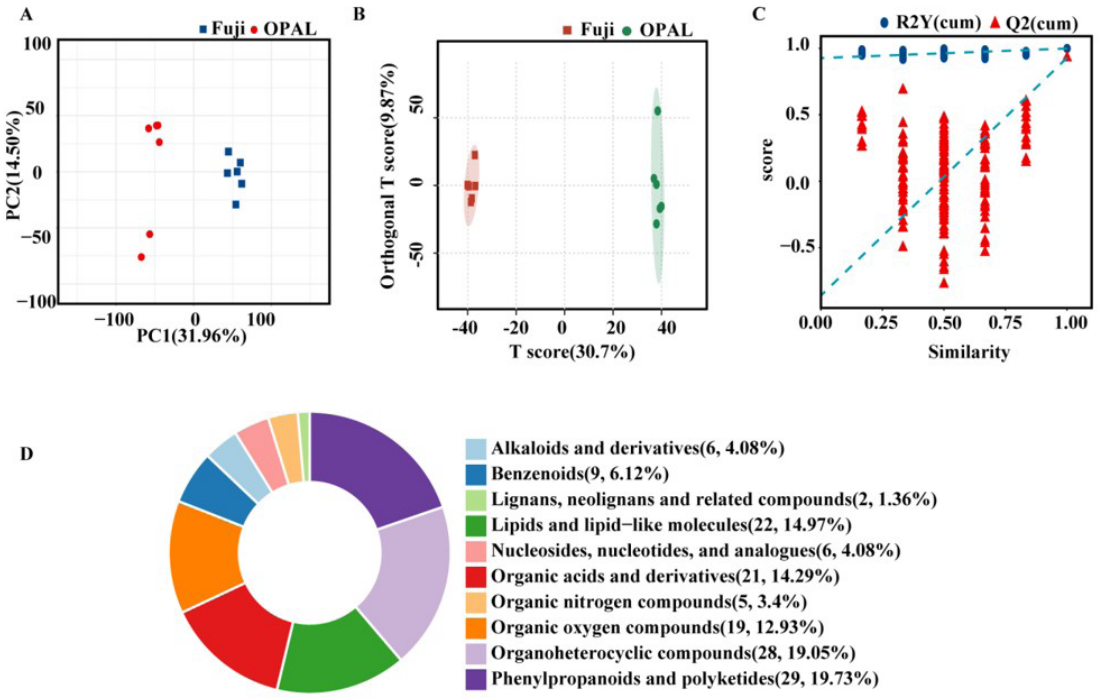

Figure 3. PCA scores of different apple samples (A), OPLS-DA scores (B) and permutation test (C) of 'Opal' vs. 'Fuji', Pie chart of differential metabolites by category (D). The PCA and PLS-DA were used to screen out several representative principal components from the massive original data, and a plot is made based on the principal components to visually describe the classification trend of the samples and the clustering between different groups as a whole.

to right were lower than the original Q^2 points on the far right, indicating that the OPLS-DA model was reliable and it can be used to explain the sample differences between the groups very well. In addition, the original model did not have over-fitting, showing that the model had good robustness.

3.4 Screening the importance of differential metabolites between apple cultivars

Differential markers were screened according to VIP value combined with t test, with VIP > 1, P < 0.05 taken as the screening standard. By comparing with the HMDB database, a total of 147 significantly differential metabolites (in 10 categories) were screened out from the pulp of 'Opal' apple and 'Fuji' apple. It can be seen in Figure 3D that the main differential metabolites identified in the two apple varieties are as follows: Alkaloids and derivatives, benzenoids, liganans, neolignans and related compounds, lipids and lipid-like molecules, nucleosides,nucleotides, and analogues, organic acids and derivatives, organic nitrogen compounds, organic oxygen compounds, organoheterocyclic compounds, phenylpropanoids and polyketides, etc. The differential metabolites with the biggest share in terms of quantity was phenylpropanoids and polyketides with 19.73%, followed by organoheterocyclic compounds with 19.05%, lipids and lipid-like molecules with 14.97%, organic acids and derivatives with 14.29%, organic oxygen compounds with 12.93%, benzenoids with 6.12%, alkaloids and derivatives with 4.08%, nucleosides, nucleotides, and analogues also with 4.08%, organic nitrogen compounds with 3.4%, and liganans, neolignans and related compounds with 1.36%. It can be seen that the most different metabolites were phenylpropanoids and polyketides.

In order to explore the nutritional and active components of these two cultivars, this study provided a cluster heatmap of phenylpropanoids and polyketides, Organic oxygen compounds, organic acids and derivatives using 'Fuji' apples as the control, so as to clarify the flavor characteristics of 'Opal' apples.There were clear areas with high or low expression in different samples (Figure 4). the cluster heatmap analysis results of phenylpropanoids and polyketides are as follows (Figure 4A): The relatively important metabolites in 'Opal' apple in the control group include trans-p-hydroxycinnamate, rutamarin, hesperidin, daunorubicin, epicatechin, phlorhizin, naringin, pimecrolimus, 5,7-dihydroxyflavanone,procyanidin B1, sinensetin, isosinensetin, mulberroside F, the relatively important metabolites in 'Fuji' apple are glabrone, 5-O-demethylnobiletin, diosmin, catechin, fisetin, chlorogenic acid, trifolirhizin, medicagol, methyl rosmarinate, myricitrin, isosakuranin, esculin, naringenin, rosmarinic acid, decursinol angelate, oxytetracycline, isoferulic acid.

The soluble sugars and organic acids are important components of fruit taste, and together with the aroma, they have a strong impact on the overall organoleptic quality of fruits. The cluster heatmap analysis results of organic oxygen compounds are as follows: Figure 4B shows that the metabolites with relatively higher content in 'Opal' apple are sucrose, mannose, acarbose, lusitanicoside, panthenol, stachyose, maltotriose, 3,7-dimethyl-1-propargylxanthine, tobramycin, acetaminophen glucuronide. while the metabolites with relatively higher content in 'Fuji'

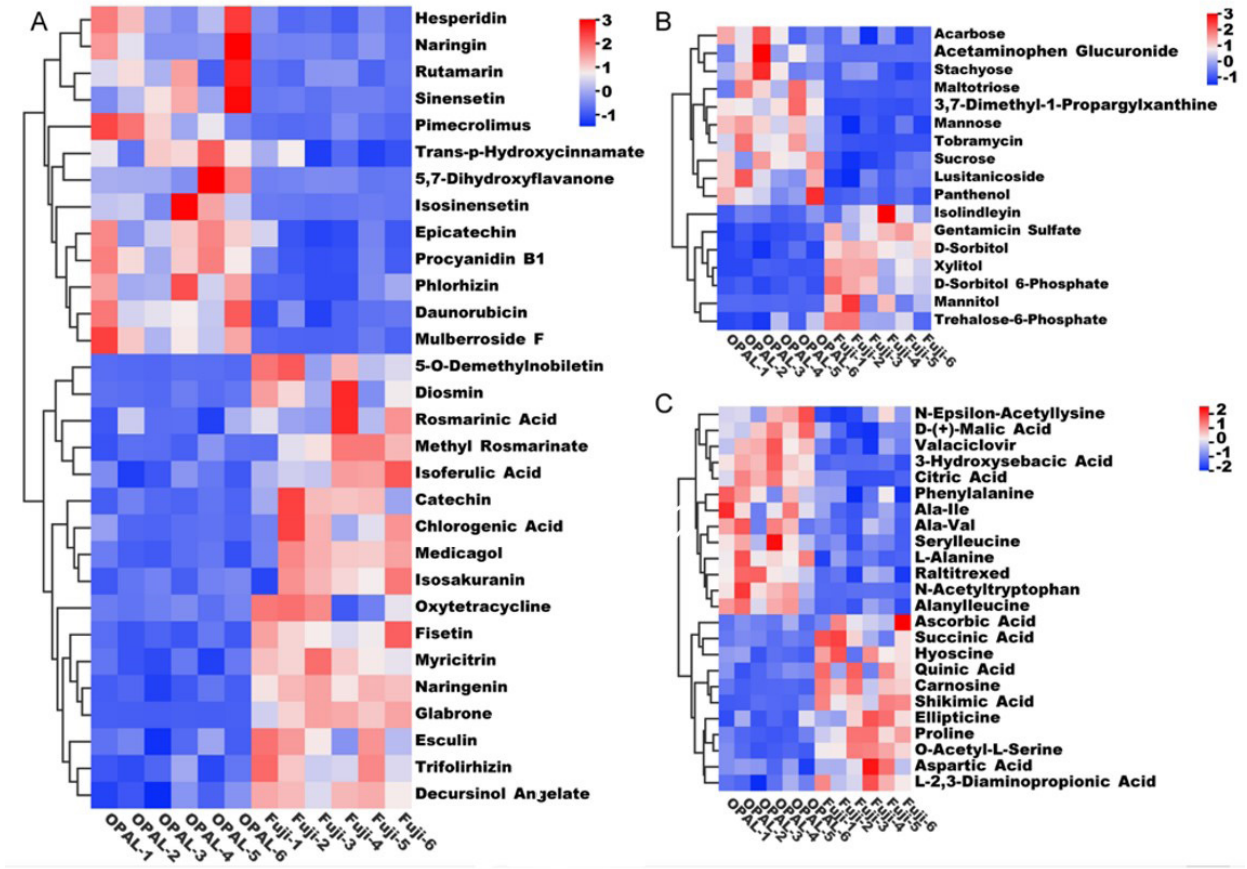

Figure 4. Phenylpropanoids and polyketides (A), organic oxygen compounds (B), organic acids and derivatives (C). The above figure depicts the hierarchical clustering results of differential metabolites in the comparison group. The redder the color, the higher the relative expression; the bluer the color, the lower the relative expression. The bands in the figure indicate that there are clear areas of high or low expression in both varieties of apples after clustering analysis of the identified metabolites.

apple are mannitol, D-sorbitol 6-phosphate, gentamicin sulfate, isolindleyin, xylitol, D-sorbitol, trehalose-6-phosphate.

The cluster heatmap analysis results of organic acids and derivatives are as follows: Figure 4C shows that the metabolites with relatively higher content in 'Opal' apple are D-(+)-malic acid, phenylalanine, N-epsilon-acetyllysine, alaval, valaciclovir, raltitrexed, alaile, L-alanine, alanylleucine, serylleucine, 3-hydroxysebacic acid, citric acid, N-acetyltryptophan, while the metabolites with relatively higher content in 'Fuji' apple are succinic acid, hyoscine, O-acetyl-L-serine, shikimic acid, proline, carnosine, ascorbic acid, aspartic acid, quinic acid, l-2,3-diaminopropionic acid, ellipticine.

3.5 Analysis of key differential metabolites

Figure 5A shows the top 25 differential metabolites by importance, with the log transformation of FC on the abscissa and the metabolites on the ordinate. The blue and red dots on the left and right sides represent the down- and up-regulated differential metabolites, respectively. The size of the point represents the VIP value, and the larger the point, the larger the VIP value, or the higher the importance of the variable. The relatively important metabolites in 'Fuji' apple in the control group include trigonelline, D-sorbitol 6-phosphate, chlorogenic acid, succinic acid, O-acetyl-L-serine, xylitol, D-sorbitol, naringenin, beta-nicotinamide mononucleotide, choline, pinostrobin, sinapoyl malate, L-2,3-diaminopropionic acid, and adenosine. The relatively important metabolites in 'Opal' apple are sucrose, mannose, cytosine, cytidine, trans-p-hydroxycinnamate, deoxyuridine, stachyose, phlorhizin, naringin, eriodictyol, and maltotriose.

Annotation and KEGG pathway enrichment analysis of the differential metabolites in 'Opal' apple and 'Fuji' apple. Pathway enrichment map of 'Opal' apple and 'Fuji' apple, it was found that 57 differential metabolites were annotated in KEGG, mainly distributing in 52 KEGG pathways. The Figure 4B shows the 10 most important pathways relevant to the distribution of differential metabolites: (1) ABC transporters; (2) Flavonoid biosynthesis; (3) Phenylpropanoid biosynthesis; (4) Pyrimidine metabolism; (5) Nicotinate and nicotinamide metabolism; (6) Galactose metabolism; (7) Fructose and mannose metabolism; (8) Citrate cycle (TCA cycle); (9) Biosynthesis of various secondary metabolites - part 3; (10) Alanine, aspartate and glutamate metabolism. A total of 42 metabolites were observed in the 10 pathways. Differential metabolites were significantly enriched

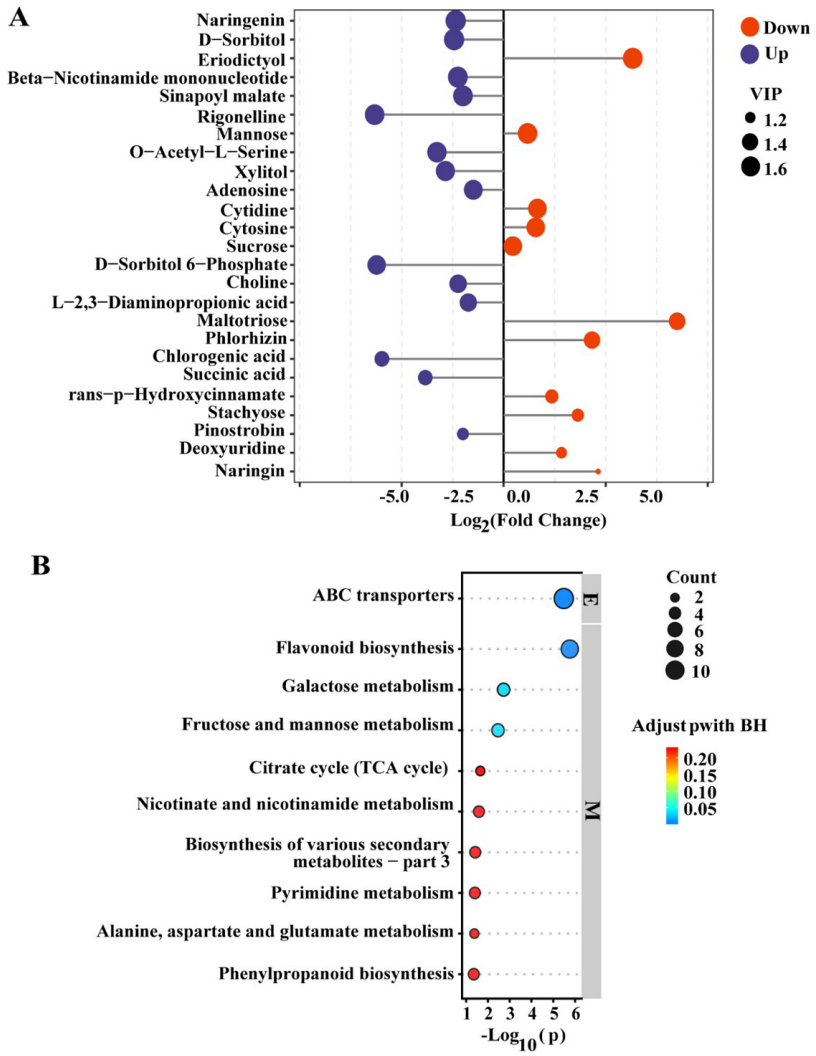

Figure 5. Histogram of differential metabolites (A), pathway enrichment map of 'Opal' apple and 'Fuji' apple, where the xaxis represents the negative logarithmic transformation of p-value, and the yaxis represents the name of pathway. The size of the circle represents the count of differential metabolites annotated into the pathway; the color of the circle corresponds to the calibrated p-value, and its significance rises from red to blue (B).

in two pathways, indicating that the differential metabolites of the two apple varieties mainly were Flavonoids and Carbohydrates and carbohydrate conjugates.

Apple fruits are widely used to make juice, processed products, etc. The metabolites in apple not only affect its growth and development, but also are important factors affecting the quality of related products. The primary metabolites of plant include sugars, amino acids, nucleotides, organic acids and other substances, which are essential substances and energy sources for the growth, development and reproduction and other life activities of plant. These metabolites mainly participate in the energy metabolism of cells and are important indicators for evaluating fruit quality (Liu et al., 2016). Figure 6 shows that the metabolites with relatively higher content in 'Fuji' apple are D-sorbitol 6-phosphate, chlorogenic acid, while the metabolites with relatively higher content in 'Opal' apple are phlorhizin, sucrose, mannose, and maltotriose. These metabolites may contribute a lot to the development of taste and quality of the two apple varieties.

4 Discussion

The 'Opal' and 'Fuji' apples were significant different at qualitative characteristics: their specific aroma, taste and appearance (as the peel of 'Opal' is a yellow while 'Fuji' is a red) and flesh browning rate. This study comprehensive assessment the nutrients and bioactive components of two cultivars in Metabolomics level for evaluating the metabolic basic of the 'Opal' apple special flavors.

There are pertinent publications on the nutritional assessment of apples and their metabolome research (Wang et al., 2021; Xu et al., 2021; Ding et al., 2021) However, few relevant systematic studies and just a few pieces of literature address the research on 'Opal' apples (Táborský et al., 2021). In this study, Fuji apples were used as the control group, and the mineral element content of

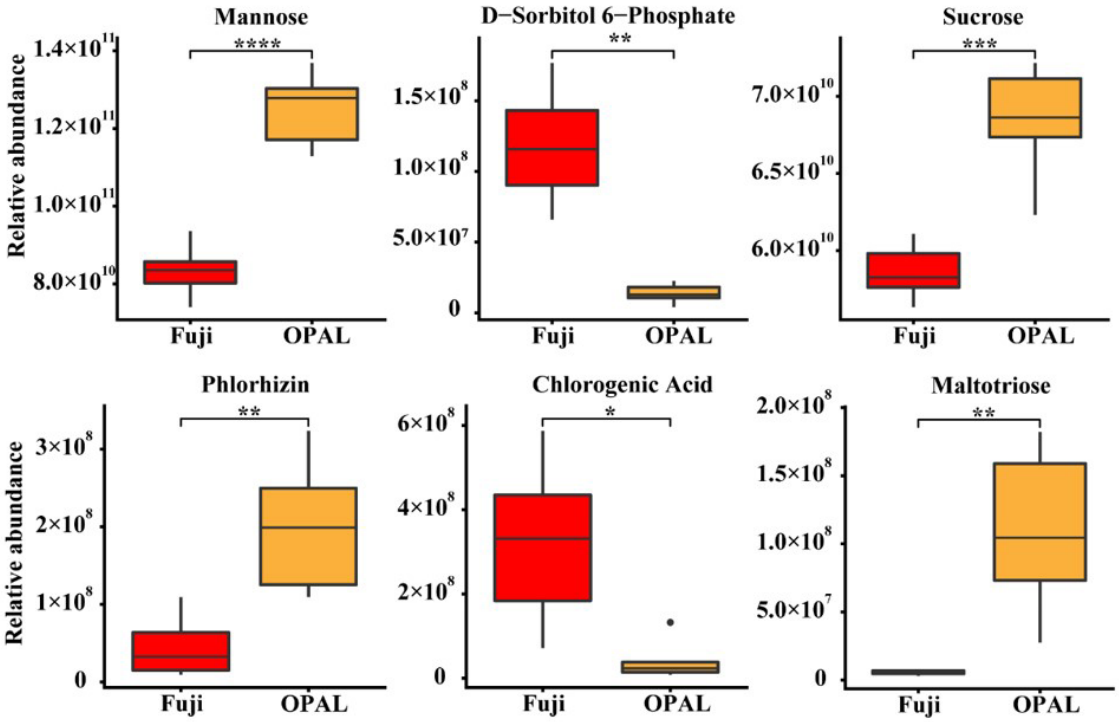

Figure 6. Box plots of the contents of differential metabolites in the two apple fruits. * represents an obvious difference ($P < 0.05$), ** represents a significant difference ($P < 0.01$), and *** represents a very significant difference ($P < 0.001$).

'Opal' apples was systematically analyzed. However, the analysis of microelements was still inadequate. Additionally, this study employed untargeted metabolomics to analyze the metabolites of 'Opal' apples, providing relevant data for the adjustment and optimization of apple variety selection in Ningxia, China.

Apples contain a great deal of elements such as magnesium (Mg), calcium (Ca), iron (Fe) and others that are beneficial to human body (Liu et al., 2021). It is scientifically recognized that mineral elements are essential components indispensable for keeping human body healthy (Medveckienė et al., 2022). It is well known that phosphorus (P), potassium (K), calcium (Ca) and trace element iron (Fe) are essential for fruit quality. Ca was established to play a key role in horticultural fruit storage (Ban et al., 2021). In recent years, many studies have explored the effects of mineral nutrients on plant growth, fruit development, quality and preservation of fruit and their molecular mechanisms, especially the effects of P and K on fruit quality (Bai et al., 2021). Experiments conducted in this study have proven that apples contain a wealth of mineral elements. The comparison results illustrate that 'Opal' apples have a higher content of mineral elements, which can serve as the basic data for the follow-up study on variety differences.

Metabolomics has emerged as a fascinating scientific field in recent years (Raza, 2022), and non-targeted metabolomics involves trying to detect as many metabolites as possible in the sample, and has been widely used in different life science fields (Sumner et al., 2015). Metabolomics has been used for testing food adulteration (Jandrić et al., 2014; Zhang et al., 2018; Uttl et al., 2019), identifying planting pattern (Bueno et al., 2018), and exploring the effect of cultivar genotype on plant phenotype (Zhang et al., 2016), the effect of environment on plant phenotype and the effect of stress on plant phenotype (Jorge et al., 2016). Also, it has been used to study the differences in metabolism of different parts of plants (Guodong et al., 2017), distinguish the medicinal values of plant organs, etc. (Cui et al., 2018), study nutrient substances in apple (Xie et al., 2021; Ceci et al., 2021), and explore the mechanism of apple browing (Shi et al., 2022; Tang et al., 2020). In this study, the differential metabolites of the two varieties of apple were statistically analyzed with the differential principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) using UHPLC-LTQ-Orbitrap MS, combined with non-targeted metabolomics technology, to identify the key differential metabolites in the two varieties of apple. Fruit sweetness is determined by the content and composition of various sugars and sugar alcohols. During fruit development, the fruit tissue firstly accumulates starch with the cell expansion and then the starches begin to breakdown at about 100 days after blooming to form palatable sugars (Gong et al., 2021). It is popularly believed that the content and proportion of carbohydrates and organic acid are the critical factors to determine the flavor and quality of fruit (Zheng et al., 2022). The nutritional value of bioactive substances such as anthocyanins, flavonoids and vitamins has become increasingly important with the improvement of public health awareness, which should be considered in the analysis of apple quality (Liu et al., 2015; Smanalieva et al., 2021). Phenolics compounds are the characteristic bioactive compounds in plants,

which play a critical role in the defense mechanism to fungal infections and pests as well as in the antioxidant expression of the plants (Xu et al., 2021). It is reported that chlorogenic acid and epicatechin are the main phenolic compound in apples, their contents in apple juice excel than others (Xu et al., 2020; Pollini et al., 2022; Menbari et al., 2021).

Enzymatic browning was the main reason for fruit browning, as polyphenols such as caffeic acid, epicatechin and chlorogenic acid can be oxidized to quinones by polyphenol oxidase. These quinones react and compound with each other, and then they are encapsulated by proteins to form melanin, thus leading to fruit browning (Bajwa et al., 2015; Deutch, 2018). Chlorogenic acid quinone is the necessary substance to generate browning in apples (Liao et al., 2021). 'Opal' apple slices go brown slowly and can be kept fresh for a long, while Fuji apple slice goes brown faster. As a result, it can be inferred that chlorogenic acid is an important metabolite that can be used to distinguish the two apple fruits. We are also aware of the limitations of this study. Firstly, more information regarding the delayed browning mechanism of 'Opal' apples is required. Secondly, further identification and validation of the important metabolites discovered by screening are required. Finally, it is necessary to investigate the aroma substances of 'Opal' apples to provide more theoretical support for breeding varieties.

5 Conclusion

This study comprehensive assessment the nutrients and bioactive components of two cultivars in Metabolomics level for evaluating the metabolic basic of the 'Opal' apple special flavors. The findings in this study can provide reference for researches on function, processing, development and utilization of apple fruits, and also lay a theoretical basis for the breeding of good apple varieties and the development of functional health food. As a result, the results have broad prospects for further development and utilization.

To sum up, the results in this study are conducive to tapping and explaining the mechanism of slow browning in 'Opal' apple, and also provide a theoretical basis for the researches and development of browning regulation technology in the future.

Conflict of 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.

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Supplementary Material

Supplementary material accompanies this paper.

Table S1. Relevant physicochemical.

Figure S1. TIC of QC samples.

Figure S2. PCA scores of QC samples.

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