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Study on the browning mechanism of apple juice based on untargeted metabolomics

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Abstract: Browning of apple juice is a major quality defect that occurs during storage, yet the molecular basis of the browning process remains unclear. This study utilised an untargeted metabolomics approach to investigate the untargeted metabolomic differences in Xinjiang Aksu sugarheart apple juice before and after 100 days of storage. Employing high-resolution liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF/MS), we identified 6 264 metabolites, with 1 588 significantly upregulated and 1 158 downregulated. Multivariate statistical analysis, including principal component analysis (PCA) and orthogonal partial least squares-discriminant analysis (OPLS-DA), revealed that storage time was the primary factor affecting metabolic differences (PCA1 : 55.54%; OPLS-DA $Q^2Y = 0.971$). Key findings suggest that browning is triggered by enzyme activation through tyrosine metabolism activation (substrate supply) and dopaquinone accumulation. Changes in transmembrane transport by ABC transporters also contribute to this process. Non-enzymatic browning is exacerbated by Maillard intermediate products and lipid peroxidation products. Simultaneously, disrupted glutathione metabolism and antioxidant system failure lead to redox imbalance. KEGG enrichment analysis indicated coordinated changes in phenylpropanoid biosynthesis (secondary metabolic polymerisation), alkaloid metabolism, and the pentose phosphate pathways. These results suggest that oxidative stress, cell membrane damage, and polyphenol metabolism disturbances are key drivers of apple juice browning, offering a molecular foundation for quality control in apple juice production.

Keywords: apple juice browning; oxidative stress; tyrosine metabolism; transmembrane transport; untargeted metabolomics

In recent years, consumers have shown growing acceptance of the health benefits associated with unprocessed or minimally processed fruit products (Rico et al. 2007). Not-from-concentrate (NFC) apple juice,

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in particular, has gained popularity for its superior nutrient content and taste compared to juices made from concentrate. As demand for minimally processed foods rises, NFC apple juice has become a premium beverage option, better preserving apple nutrients such as vitamins, polyphenols, and fibre (Moon et al. 2020). However, there are still many problems in the juice industry during processing and storage, primarily due to browning reactions that adversely affect product quality, shelf life, and consumer acceptance. Browning leads to the loss of original colour and nutrients, causing substantial economic losses for producers. Browning is a biochemical reaction system that includes enzymatic browning, mediated by polyphenol oxidase (PPO), which oxidises polyphenols to quinones that subsequently polymerise into melanin (Kanteev et al. 2015; Persic et al. 2017a).

Enzymatic browning primarily stems from polyphenol oxidase catalysis, which causes the oxidation of polyphenols to quinones, and then the quinones polymerise to form melanin (Muñoz-Pina et al. 2022a; Lee et al. 2022a). The rate of this reaction is influenced by the polyphenol content, enzyme activity, and oxygen levels in the juice. Non-enzymatic browning, on the other hand, encompasses the Maillard reaction and ascorbic acid oxidation pathways, with the accumulation of reactive carbonyl species (RCS), such as glyoxal and methylglyoxal, playing a pivotal intermediary role (Paravisini and Peterson 2018a; Xu et al. 2023a). Recent research endeavours have focused on developing natural inhibitors and novel technologies to mitigate browning. These include plant polyphenols (Yu et al. 2021a), synthetic compounds (Peng et al. 2024a), and metal-organic frameworks (Marrufo-Hernández et al. 2024a), which aim to inhibit enzymes or trap carbonyl intermediates, offering innovative solutions to browning challenges in the juice industry. Advancements in molecular-level analytical techniques have provided fresh perspectives on fruit browning research through analytical equipment. Metabolomics technology, capable of accurately characterising changes in small-molecule metabolites during storage-related quality deterioration, offers a novel approach. Metabolites, as the basis of biological phenotypes, enable people to have a more direct and effective understanding of biological processes and mechanisms. By conducting qualitative and quantitative analyses, metabolomics can elucidate metabolic pathways and networks, examine metabolic patterns in biospecimens from diverse individuals, or investigate metabolic responses to physical, chemical, or pathogenic stimuli. It also contributes to food and drug safety assessments. For example, metabolomics has revealed a connection between cutin biosynthesis and browning in oriental melons (Park et al. 2023) and, through a combination of metabolomics, enzyme analysis, and *in vitro* simulation systems, has unveiled specific anti-browning mechanisms in goji berries (Gao et al. 2024). The primary objective of metabolome analysis is to detect metabolites that are biologically relevant and statistically distinct between samples, thereby revealing metabolic pathways and underlying processes. This involves experimental and data analysis phases, including differential metabolite screening and metabolic pathway analysis. Based on data from experimental design, sample processing, and metabolite extraction and detection, researchers can perform metabolite identification, quality control of metabolomic samples (QC) and functional predictive analysis of differential metabolites.

Modern high-resolution mass spectrometry platforms, in combination with multivariate statistical analysis, can systematically identify key metabolic markers and pathways associated with browning. The precision of these techniques has been validated in dietary metabolomics research (Playdon et al. 2024), and metabolomics has also been applied to study the metabolic pathways of bamboo shoots during storage (Wang et al. 2025). These technical advancements are crucial for meeting market demands for NFC apple juice as consumers expect extended shelf life, preservation of fresh quality, and clean-label products without artificial preservatives. Research on relevant mechanisms lays the foundation for preservation techniques that do not rely on chemical means.

This study used advanced untargeted metabolomics technology to explore the metabolic network underlying browning in NFC apple juice. LC-QTOF-MS (liquid chromatography quadrupole time-of-flight mass spectrometry) technology was used to investigate metabolite changes in apple juice under controlled storage conditions. Advanced chemometric methods, such as PCA (principal component analysis) and OPLS-DA (orthogonal partial least square discriminant analysis), were employed to identify metabolites with significant changes during the browning process. In addition, pathway enrichment analysis was conducted to interpret metabolic changes and determine the most critical biochemical pathways related to the quality deterioration. This study not only enhances our understanding of the browning mechanism at the molecular level but also provides potential targets for quality preservation strategies in juice processing and storage.

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MATERIAL AND METHODS

Material

Freshly squeezed juice from Xinjiang Aksu Red Fuji apples (*Malus domestica*) was used in this study. The apples were stored for 100 days under simulated showroom conditions of 25 ± 2 °C, 85–90% humidity. The juice was packaged in commercial NFC apple juice cans made of polypropylene, with temperature and humidity fluctuations set to mimic real-world storage conditions.

The initial-time-point samples (Group A) were set as the baseline control group, and a parallel control group was established at each sampling time point. All operations were performed in a laminar-flow cabinet to maintain sterility. Samples were taken both before storage (Group A) and after the storage period (Group B) for a metabolomics study.

Reagents and instruments

The chemicals used in the experiment included methanol (CAS No. 67-56-1, LC-MS grade, Merck KGaA, Germany), acetonitrile (CAS No. 75-05-8, LC-MS grade, Merck KGaA, Germany), L-2-chloro-L-phenylalanine (CAS No. 103616-89-3, purity ≥ s98%, Shanghai Aladdin Biochemical Technology Co., Ltd., China), and formic acid (CAS No. 64-18-6, LC-MS grade, Tokyo Chemical Industry Co., Ltd., Japan). For the purpose of instrumental analysis, a Waters UPLC Acquity I-Class PLUS ultra-high-performance liquid chromatography system, a Waters UPLC Xevo G2-XS QTOF high-resolution mass spectrometer, and an Acquity UPLC HSS T3 column (1.8 μm, 2.1 × 100mm), all from Waters Corporation (USA), were employed.

Metabolite extraction

Metabolites were extracted by adding 100 μL of apple juice and 500 μL of extraction solvents (methanol : acetonitrile 1 : 1, *v/v*) containing 20 mg·L^{-1} L-2-chlorophenylalanine as an internal standard. The mixture was vortexed for 30 s, placed in an ice-water bath for 10 min, and then stored at –20 °C for 1 h to precipitate proteins. After centrifugation at 12 000 × g for 15 min at 4 °C, 500 μL of the supernatant was collected and vacuum-concentrated to dryness. The residue was reconstituted in 160 μL of acetonitrile : water (1 : 1, *v/v*), vortexed, and sonicated under the same conditions. Following another centrifugation (12 000 × g, 15 min, 4 °C), 120 μL of the supernatant was transferred to an injection vial. For QC, 10 μL from each sample was pooled to create QC samples to monitor system performance during the analysis process (Want et al. 2010; Dunn et al. 2011).

LC-MS/MS analysis

Analysis was performed using a Waters Corporation Xevo G2-XS QTOF high-resolution mass spectrometer controlled by MassLynx v. 4.2 software in MSe mode. Each acquisition cycle involved parallel dual-channel data acquisition at low and high collision energies. Specifically, the low collision energy was turned off, while the high collision energy was ramped from 10 to 40 V, with a mass spectrometric scan rate of 0.2 s per spectrum. Electrospray ionisation source parameters were set as follows: capillary voltage of 2 500 V (positive mode)/–2 000 V (negative mode), cone voltage of 30 V, source temperature of 100 °C, desolvation gas temperature of 500 °C, cone gas flow of 50 L·h^{-1}, and desolvation gas flow of 800 L·h^{-1} (Wang et al. 2016).

Qualitative and quantitative identification of metabolites

Metabolite identification was carried out using Progenesis QI (Waters Corporation) following a specific procedure: raw data obtained from MassLynx v. 4.2 software was imported into Progenesis QI software. Peak-picking, retention time correction, and peak-alignment were performed using the software's internal algorithms. Then, metabolite identification was primarily conducted based on the accurate mass values from high-resolution mass spectrometry and tandem mass spectrometry fragmentation ion information. Meanwhile, cross-matching was conducted with the in-house library included in Progenesis QI software, the online METLIN database, and other public databases. Theoretical fragmentation pattern recognition was employed to enhance the accuracy of identification results.

Statistical analysis

Multivariate analysis. PCA and OPLS-DA were conducted using the ropls package in the R programming environment (version 3.6.1, R Core Team, Austria) and the validity of the models was verified through 200 permutation tests to ensure robustness and reliability.

Differential metabolite screening. Metabolites were deemed significantly different if all three conditions were satisfied: FC > 1, VIP score > 1 from OPLS-DA and a P-value < 0.05 from Student's t-test.

Pathway enrichment analysis. KEGG pathway enrichment analysis was performed using a hypergeo-

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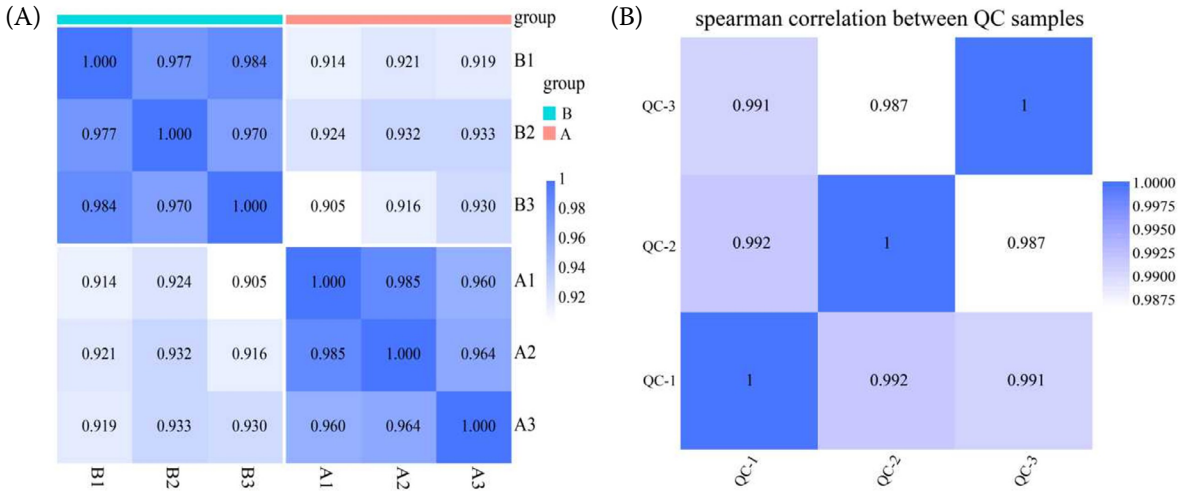

Figure 1. Samples (A) and quality control of metabolomic samples (QC) samples (B) correlation diagram
The horizontal and vertical coordinates are sample names, the intensity of the colour reflects the size of the correlation coefficient (*r*), and group represents the classification

metric distribution test to identify metabolic pathways associated with browning.

RESULTS AND DISCUSSION

Data quality assessment. Correlation analysis between samples was conducted to determine the biological reproducibility of apple juice samples before and after storage. As illustrated in Figure 1A, the Spearman rank correlation coefficient (*r*) among the three replicate samples in the pre-storage group (A1–A3) ranged from 0.960 to 1.000, indicating a high degree of consistency. In comparison, the post-storage group (B1–B3) showed slightly lower correlation coefficients (r = 0.905–0.984). The QC samples demonstrated excellent reproducibility ($r > 0.92$), suggesting that the analytical platform had good stability (Figure 1B). The scatter plot of QC samples further validated the method's performance (Figure 2). After log transformation, most metabolites clustered closely around the diagonal, indicating minimal technical variation and reliable detection of metabolic changes. The high correlation ($r > 0.96$) among QC replicates underscored the precision of sample preparation and LC-MS/MS analysis. Additionally, correlation analysis revealed that all QC samples had correlation coefficients exceeding 0.8 (Figure 2, upper-right panels), meeting the conventional reliability threshold (> 0.8) and further confirming the trustworthiness of our experimental data.

All in all, under controlled conditions, the biological variance of apple juice could be decreased. The high reproducibility of QC samples supports the suitability of untargeted metabolomics for investigating the browning mechanisms in apples during storage.

Metabolite annotation before and after browning. All detected metabolites were annotated against the KEGG database (Kanehisa and Goto 2000), and the top 20 most abundant KEGG Orthology (KO) pathway terms (level 3) were selected for presentation (Figure 3). Through untargeted metabolomics analysis,

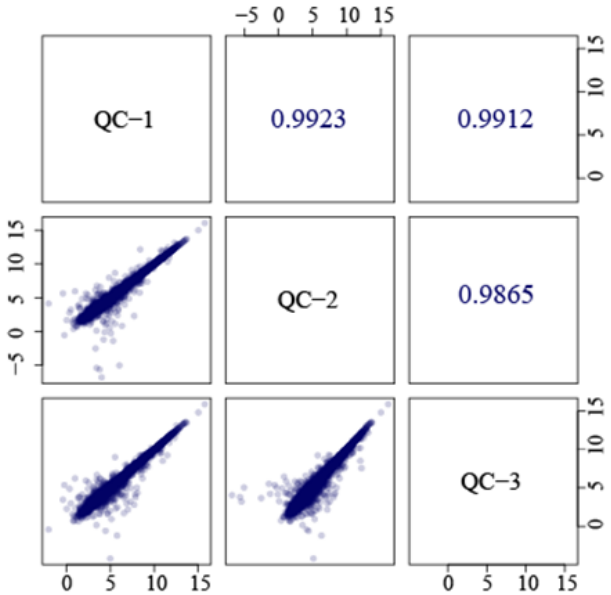

Figure 2. quality control of metabolomic samples (QC) sample correlation scatter plot
The diagonal square is the QC sample names; the lower-left squares below the diagonal are the corresponding QC sample correlation scatter plot; the x-axis and y-axis coordinates of the data are the log2-transformed levels of the metabolites, and each point is a metabolite; above the diagonal in the upper-right squares are the Spearman rank correlation coefficients (*r*) of the corresponding QC samples

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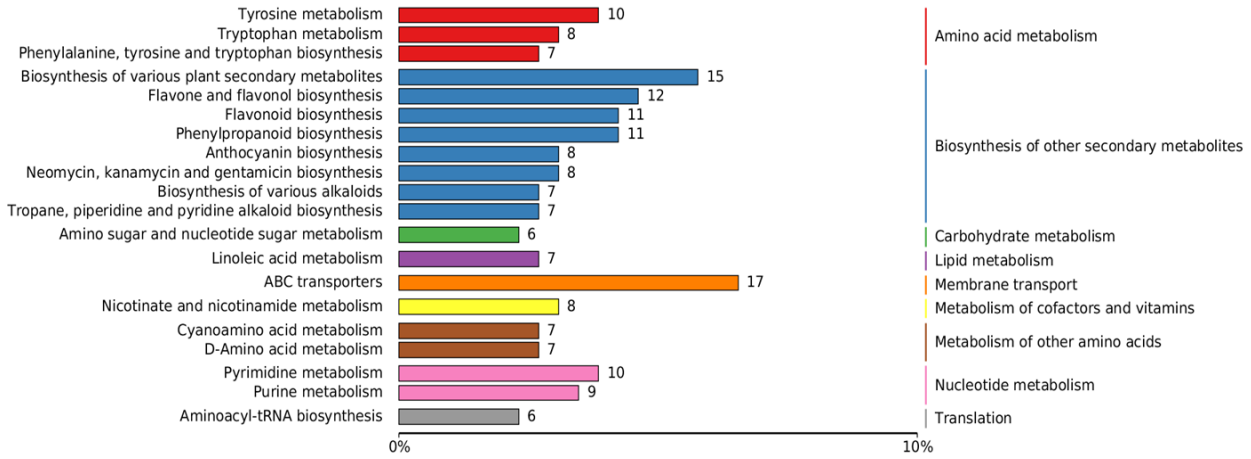

Figure 3. Summary of KEGG database classification

a total of 6 211 metabolites were identified in the apple juice samples from groups A (pre-browning) and B (post-browning). After classification and annotation using the KEGG database, the top 20 pathways were determined. These pathways encompass a diverse range of metabolic processes, including tyrosine metabolism, biosynthesis of phenylalanine, tyrosine, and tryptophan, production of various plant secondary metabolites, flavone and flavonol biosynthesis, phenylpropanoid biosynthesis, anthocyanin biosynthesis, biosynthesis of neomycin, kanamycin, and gentamicin, synthesis of various alkaloids, tropane, piperidine, and pyridine alkaloid biosynthesis, amino-sugar and nucleotide-sugar metabolism, fatty acid biosynthesis, ABC transporter function, nicotinic acid and nicotinamide metabolism, cyanoamino acid metabolism, glycerolipid metabolism, pyrimidine and purine metabolism, aminoacyl-tRNA biosynthesis, and RNA degradation. These annotation results provide a comprehensive overview of the metabolite category distribution and core characteristics of Aksu sugarheart apple juice before and after browning, offering valuable insights into the underlying metabolic changes associated with the browning process.

Grouped principal component analysis. According to the results of group-different PCA in different types of samples (Figure 4) and in the same samples from different groups, all kinds of multi-angle validation methods were employed to assess the stability and repeatability of the sample results. The PCA outcomes revealed that PC1 accounted for 55.54% of the total variance, indicating that storage time was the primary driver of metabolic changes. Moreover, it was confirmed that PC2, explaining 17.97% of the variance, and PC3, contributing 11.67%, were identified as secondary factors influencing group differences. In order to test the statistical significance of the model, we carried out 200 permutation tests, confirming that the observed group separation was not due to non-random factors. The tight clustering of post-storage samples (Group B) on the PC1–PC2 plot, along with intra-group correlation coefficients exceeding 0.9, aligned with expectations for biological replicates. Additionally, the aggregation of QC samples in PCA space and a cumulative variance contribution rate of exceeding 70% (PC1 + PC2) collectively demonstrated instrumental stability and model interpretability. At the same time, methods, such as log transformation and Pareto, were used to make the data better meet the requirements of multivariate statistics. This systematic verification approach not only elucidated the metabolic reprogramming in Group B, which segregated along the positive direction of PC1, but also uncovered inherent metabolic heterogeneity within Group A. These findings provide a more reliable, multi-dimensional data foundation for the screening of subsequent differential metabolites.

Orthogonal partial least squares-discriminant analysis (OPLS-DA). As depicted in Figure 5, OPLS-DA was utilised to screen for differential metabolites based on variable importance in the projection (VIP) values, enabling the identification of potential characteristic biomarkers and providing critical data for product QC (Thévenot et al. 2015). The OPLS-DA model constructed for Aksu sugarheart apple juice revealed that (Figure 4A) the metabolic profile changed significantly after three months of storage, and it was closely related to the browning process. Model validation parameters, including R^2Y = 1 and Q^2Y = 0.971, indicated high model quality without overfitting, achiev-

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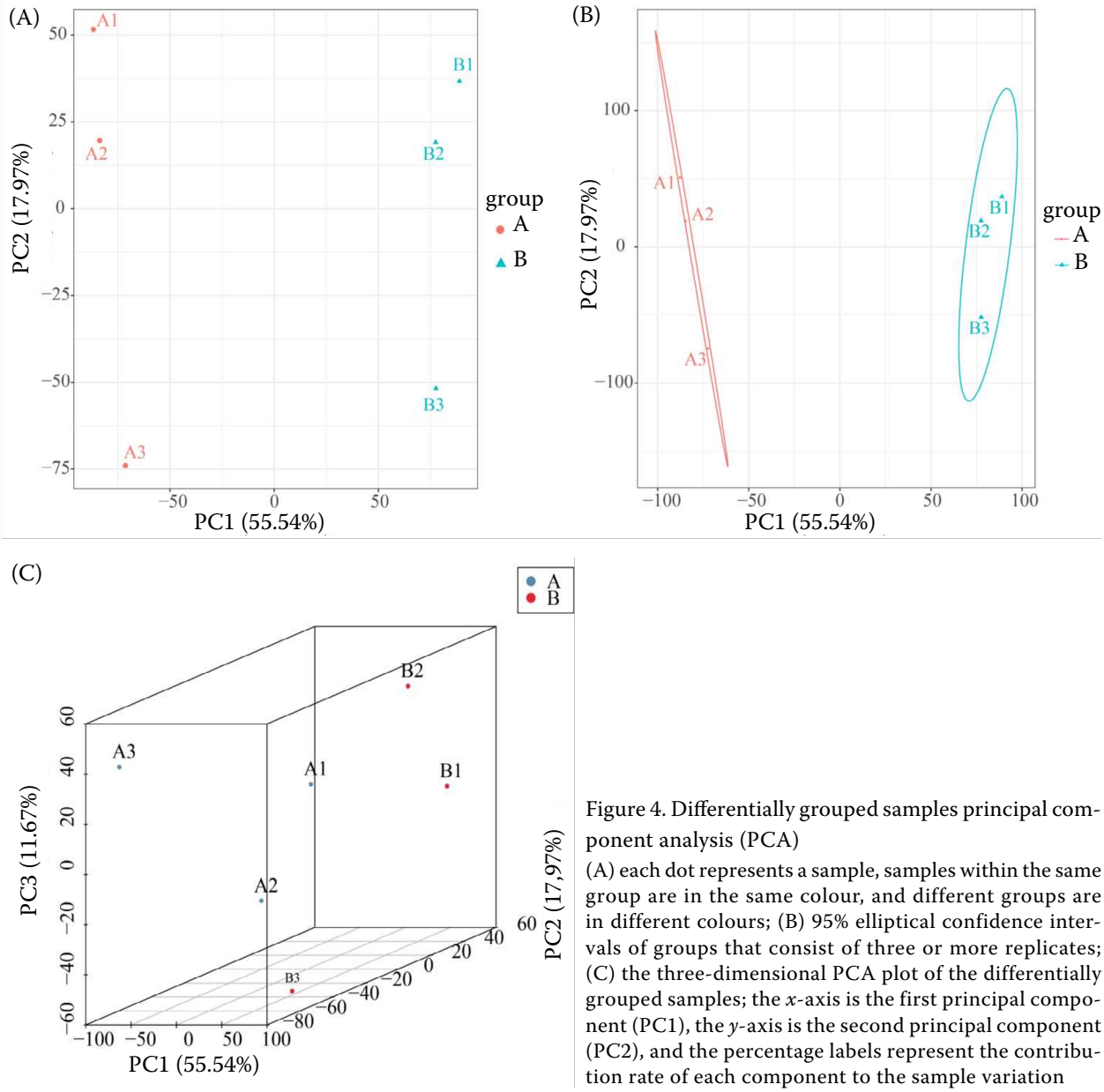

Figure 4. Differentially grouped samples principal component analysis (PCA)

(A) each dot represents a sample, samples within the same group are in the same colour, and different groups are in different colours; (B) 95% elliptical confidence intervals of groups that consist of three or more replicates; (C) the three-dimensional PCA plot of the differentially grouped samples; the *x*-axis is the first principal component (PC1), the *y*-axis is the second principal component (PC2), and the percentage labels represent the contribution rate of each component to the sample variation

ing a balanced trade-off between interpretability and predictive accuracy. To further validate the model and prevent overfitting, seven-fold cross-validation with response permutation (200 permutations) was performed. The R^2Y and Q^2 values of the original model were significantly higher than those of the permuted model ($P < 0.001$), confirming the model's statistical significance. Furthermore, all differential metabolites identified with a VIP cutoff of 1.0 were validated using univariate statistics with an FDR-adjusted *P*-value < 0.05, enhancing the reliability of the screening results. The first principal component (t1) accounted for 61% of the variance, with storage duration identified as the most influential factor driving metabolic changes. The root mean square error of estimation (RMSEE) value of 0.006 further confirmed the model's excellent fitting performance. The model's correlation validation plot (B) supported the validity of both the model and the experimental data. In combination with Figure 5A, these findings demonstrate that the OPLS-DA model effectively characterises the metabolic changes in apple juice during storage, providing a robust foundation for further analysis.

Differential metabolite analysis. A radar chart, also known as a Deborah chart, spider web chart, or spider chart, offers a comprehensive visualisation of financial or, in this case, metabolic ratios. For each comparison group, we calculated the quan-

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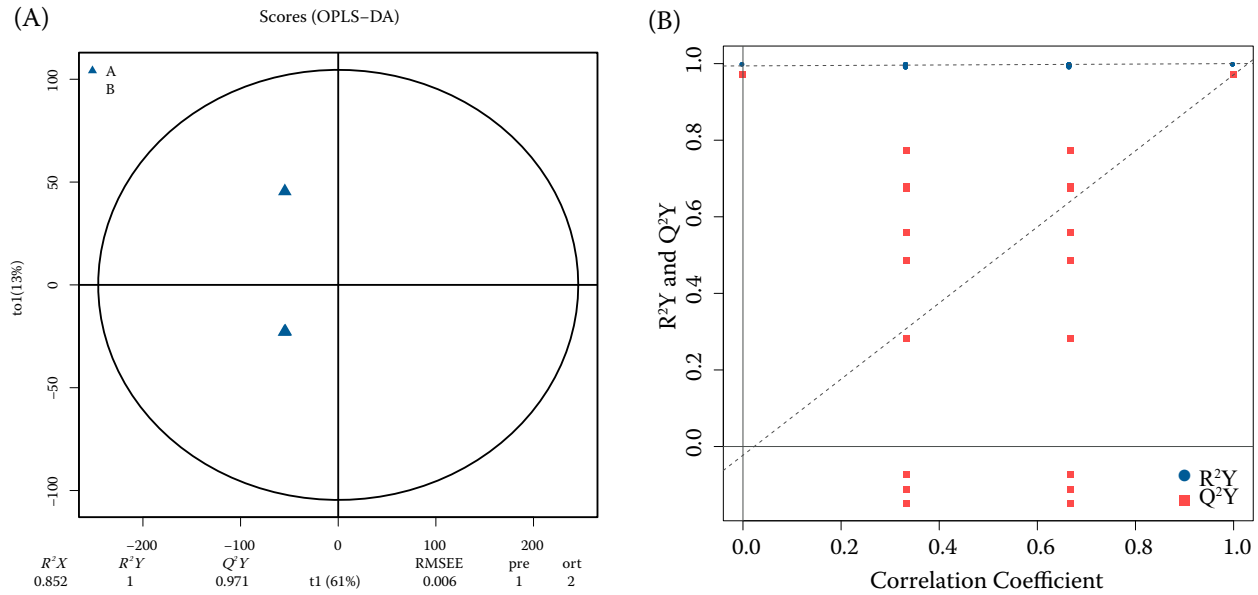

Figure 5. Orthogonal partial least squares-discriminant analysis (OPLS-DA)

titative ratios of differential metabolites and selected the top 10 with the highest absolute log2*FC* values for radar chart visualisation (Figure 6). The most significantly upregulated metabolites, with absolute log2*FC* values around 35.49, included 2H-pyran-2,4(3H)-dione, 3-(aminomethylene)dihydro-; 2-hydroxy-3-methyl-H-pyran-4-one; 2"-O-monosuccinylguanosine 3',5'-cyclic monophosphate; *cis*-(2S)-5-oxooxolane-2-carboxylic acid; 5-(2-fluoro-4-methylphenyl)isoxazole; PSI-6130; and tetrahydro-2-oxo-2H-1,3-thiazine-4-carboxylic acid.

Conversely, the most significantly downregulated metabolites, with absolute log2*FC* values around 38.87, included 2"-O-monosuccinylguanosine 3":5"-cyclic monophosphate (appearing twice in the list, likely indicating a significant reduction); *cis*-(2S)-5-oxooxolane-2-carboxylic acid; (1R)-6t-hepta-1,3t-dien-t-yl-3c,4c-dihydroxy-2-oxo-cyclohexan-r-carbaldehyde; cis-4-carboxymethylenebut-2-en-4-olide; and another instance of (2S)-5-oxooxolane-2-carboxylic acid.

The browning mechanism in apple juice before and after storage likely involves three key pathways: first,

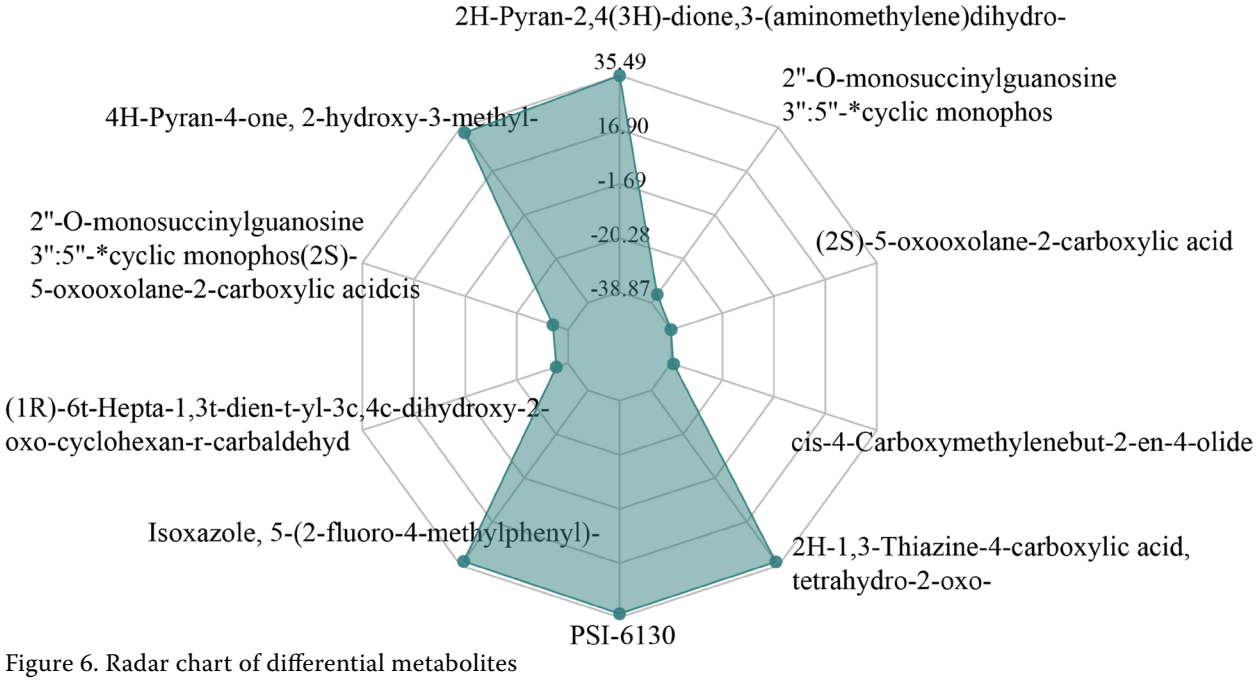

Figure 6. Radar chart of differential metabolites

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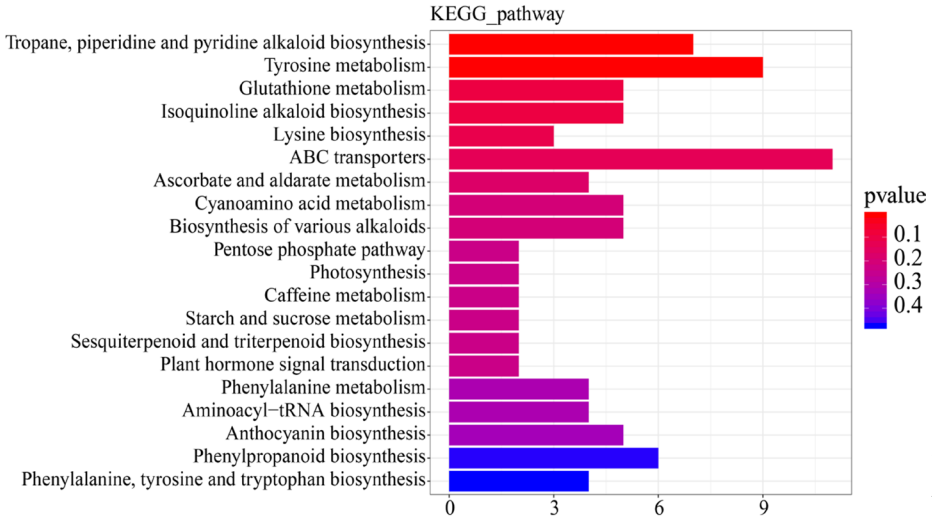

Figure 7. Bar plot of differential metabolite enrichment

it is oxidised via a non-enzymatic pathway to form a reactive pyran and thiazine ring; second, the consumption of nucleotide analogues, such as PSI-6130, and antioxidant metabolites; third, structural modification of the terpenoid skeleton. These metabolic changes collectively suggest a time-dependent progression from early Maillard reaction intermediates to an eventual increase in advanced glycation end products. The depletion of 3-(aminomethylene) dihydro-2H-pyran-2,4(3H)-dione may serve as an indicator of oxidative stress. Furthermore, the positive correlation between the accumulation of certain heterocyclic compounds and the degree of browning provides a theoretical basis for understanding the quality deterioration mechanism in stored apple juice.

KEGG functional annotation and enrichment analysis of differential metabolites. Differential metabolites are biologically linked to various metabolic pathways. By annotating these metabolites using the KEGG database, we identified the top 20 pathways based on the abundance of the metabolites. These pathways are visually represented through enrichment bar plots, abundance score plots, and a KEGG enrichment factor bubble chart (Yu et al. 2012).

Utilising the KEGG pathway analysis system, we elucidated the metabolic regulatory network responsible for the browning of NFC apple juice. As depicted in the bar chart (Figure 7), it can be seen that tyrosine metabolism and ABC transporters were notably induced. Tyrosine metabolism directly contributes to the browning process by generating melanin precursors such as dopaquinone (Kanteev et al. 2015; Sui et al. 2023; Liu et al. 2024). Meanwhile, functional changes in the ABC transporter pathway affect the transmembrane transport efficiency of phenolic compounds (Persic et al. 2017b). Glutathione metabolism exhibited fluctuations, indicating an imbalance in the antioxidant system. Additionally, the decline in the PPP heightened oxidative stress (Cruz-Rus et al. 2011; Ahmad et al. 2023). A decrease in glucose-6-phosphate dehydrogenase activity

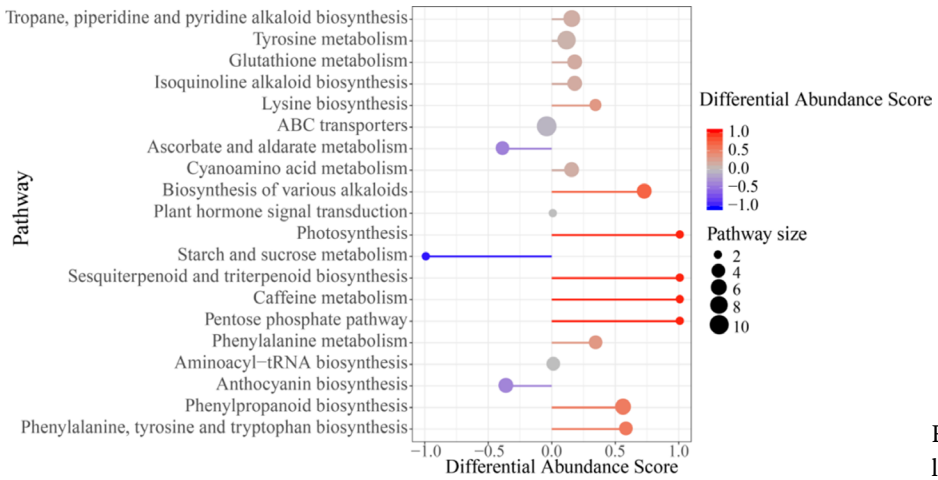

Figure 8. Differential metabolite abundance score plot

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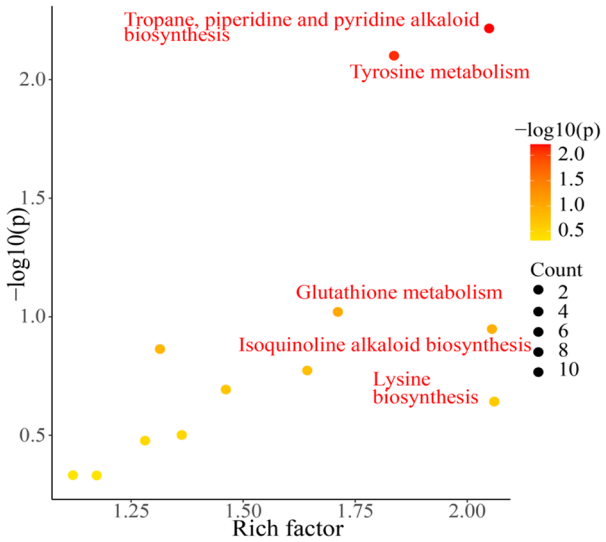

Figure 9. Bubble plot of KEGG enrichment factors for differential metabolites

impaired regeneration of NADPH, compromising cellular reduction capacity and thereby reducing antioxidant effectiveness (Peng et al. 2024b).

Abundance score analysis (Figure 8) underscores the pivotal role of secondary metabolic pathways in the browning of apple juice. The upregulation of the photosynthesis pathway heightens photooxidative stress (Paravisini and Peterson 2018b), while the inhibition of anthocyanin and ascorbic acid metabolism diminishes the presence of natural antioxidants (Lee et al. 2022b). Concurrently, the activation of phenylpropanoid biosynthesis fosters the accumulation of lignin precursors, which, in concert with alkaloid metabolism, expedites colour deterioration (Marrufo-Hernández et al. 2024b). These interconnected pathways form a sophisticated regulatory network that collectively propels the browning process.

The bubble plot in Figure 9 clearly illustrates the correlations among core metabolic pathways. The co-enrichment of tyrosine metabolism with several alkaloid pathways suggests a potential link between dopaquinone biosynthesis and the oxidative polymerisation of secondary metabolites such as alkaloids (Xu et al. 2023b). Additionally, the observed changes in glutathione metabolism underscore its role in maintaining redox balance (Yu et al. 2021b). In addition, the enrichment of lysine biosynthesis implies that proline metabolism may indirectly influence browning by modulating cellular osmotic pressure.

A comprehensive analysis reveals that the browning of NFC apple juice results from a synergistic four-stage process: substrate supply, transmembrane transport, antioxidant system failure, and secondary metabolic polymerisation. Tyrosine metabolism and the ABC transporter pathway contribute to browning by supplying oxidation substrates. Meanwhile, imbalances in glutathione metabolism and the pentose phosphate pathway lead to the collapse of the antioxidant defence system. The metabolism of phenylpropanoids and alkaloids accelerates pigment polymerisation (Moon et al. 2020). Inhibition of ABC transporter activity causes phenolic compounds to accumulate intracellularly, increasing their likelihood of contact with polyphenol oxidase (Muñoz-Pina et al. 2022b). These insights offer valuable references for further investigation into the browning mechanism.

The KEGG enrichment network of differential metabolites (Figure 10), visually outlines the interaction patterns among the top 20 pathways with the most annotated differential metabolites, along with the distribution characteristics of core node metabolites. Combining multivariate statistical analysis with pathway functional validation, it is found that heat-stress-responsive genes involved in carbon storage and metabolism, oxidant defence, and hormone metabolism and signalling promote or interact in the browning effect through physiological regulations. It is worth noting that these metabolic changes are closely linked to the sensory quality deterioration of apple juice. Tyrosine metabolism, as the central hub of this network, may be activated, leading to the accumulation of dopaquinone, which directly promotes enzymatic browning and darkens the juice colour. At the same time, this pathway is also directly connected to the isoquinoline alkaloid biosynthesis pathway and the tropane, piperidine and pyridine alkaloid biosynthesis pathways, thereby further confirming its role in promoting enzymatic browning by providing melanin precursors. Based on these findings, appropriate measures can be applied in practical juice processing, such as regulating the key enzyme activity in the tyrosine metabolism pathway or employing inhibitors to control the browning process (Meng et al. 2025).

The glutathione metabolism pathway involves multiple types of metabolites, including dehydroascorbic acid and glutathionylaminopropylcadaverine, whose contents inversely correlate with the ABC transporter pathway responsible for transporting phenolic substances across the membrane. This metabolic disruption not only triggers a ROS-induced stress response to produce and accumulate a large amount of phenolic substances but also creates a favourable microenvironment for catalysis by polyphenol oxidase.

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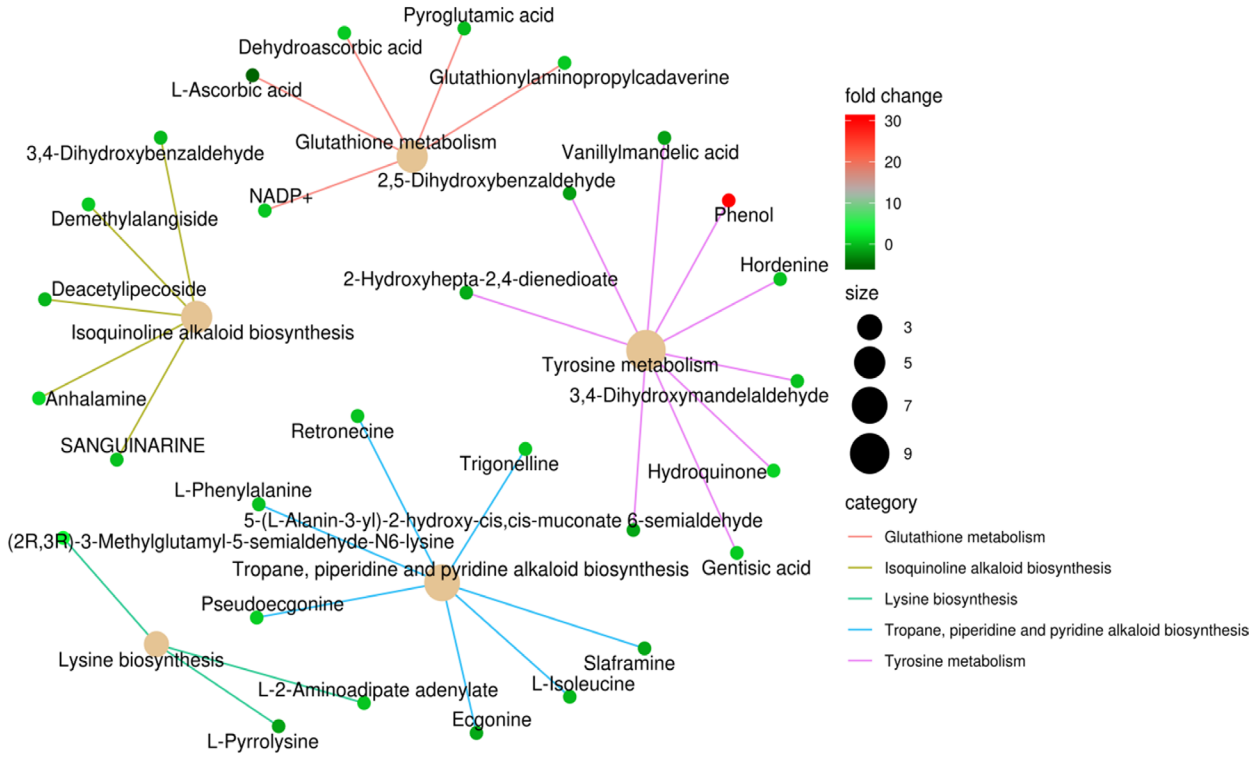

Figure 10. KEGG pathway enrichment correlation network diagram

More importantly, functional changes in ABC transporters can regulate the metabolism and distribution of phenolic compounds, thus affecting the release of flavour precursors. In addition, glutathione metabolism disruption and antioxidant system collapse may promote the formation of off-flavour substances such as aldehydes and ketones.

During juice processing, storage conditions can be controlled to promote natural glutathione synthesis or regulate its metabolic key nodes to maintain redox balance. Processing technology can be optimised to mitigate the adverse effects of transmembrane transport on the distribution of phenolic compounds. The phenylpropanoid biosynthesis and alkaloid biosynthesis secondary metabolic pathways are densely clustered in the network. The synergistic effects of metabolites such as 3,4-dihydroxybenzaldehyde and sanguinarine, as well as the up-regulation of substances like vanillylmandelic acid and pseudopelletierine, not only promote the polymerisation of secondary metabolites and enhance the pigmentation effect but also may further influence the colour clarity of the juice by aggregating pigments (Guan et al. 2024).

All these metabolic changes collectively validate the four-stage browning mechanism of 'substrate supply – transmembrane transport – antioxidant collapse – secondary metabolic polymerisation'. At the same time, they provide a molecular explanation for the sensory quality changes in apple juice during storage, such as colour darkening, taste and smell deterioration, and clarity reduction (Paravisini and Peterson 2018c). The key metabolic nodes screened out in this experiment offer a theoretical basis for the future development of new browning prevention methods. It is recommended to further develop or modify specific inhibitors or process parameters based on these key issues, thereby providing clear pathways and related metabolites for targeted quality management programs for NFC apple juice.

CONCLUSION

This study initially revealed the complex metabolic network involved in apple juice browning through untargeted metabolomics. LC-QTOF/MS, combined with multivariate statistical methods, revealed that browning might be a bimolecular process involving both enzymatic and non-enzymatic pathways. This mechanism may be related to the joint action of substrate supply (tyrosine metabolism), transmembrane transport (ABC transporters), weakening of the antioxidant system (reduced glutathione levels), and

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polymerisation of secondary metabolites (phenylpropanoid/alkaloid pathways). The main conclusions are as follows: the activation of tyrosine metabolism, ABC transporters may be involved in phenolic compound transport, and some Maillard reaction intermediate products accumulate. Alterations in glutathione metabolism and reduced antioxidant capacity may lead to more severe oxidative stress, while the phenylpropanoid and alkaloid pathways are probably involved in pigment synthesis. These results imply that oxidative stress, membrane system modification, and metabolic reprogramming might occur jointly during browning.

Validation of metabolic pathway interactions through enrichment network analysis offers new insights for developing targeted preservation strategies and provides references for maintaining the quality of Aksu apple juice from Xinjiang. Future work will focus on validating and elucidating the regulatory mechanisms of key enzymes in key metabolic pathways and the regulation mechanism in browning. In addition, intermediate storage time points will be observed, and metabolic changes will be dynamically tracked to further improve the time-oriented understanding of browning mechanisms.

For industrial applications, juice makers are advised to establish an exact control system based on a metabolic perspective. This includes breeding apple varieties with lower tyrosine content, promoting dynamic oxygen-controlled filling technology, optimising low-temperature instantaneous sterilisation processes to precisely control the glutathione metabolic network, and establishing monitoring indexes for the transmembrane transport of phenolic compounds to build a complete quality assurance system. This approach aims to achieve additive-free quality improvement and extend shelf life.

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