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Quality formation of living bamboo wine from *Dendrocalamus brandisii* culms

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ABSTRACT

To verify the beneficial ingredients contained in bamboo wine, this study simulated the production of living bamboo wine by injecting alcohol solution into the bamboo cavity of *Dendrocalamus brandisii*, and further analyzed the nutrient components of the alcohol solution and their relations to the physiological changes of the culms based on multi-omics analysis. The results demonstrated that the living bamboo wine derived from *D. brandisii* was rich in four types of nutrients, among which glucose (450.0–1230.0 μg/ml) and fructose (600.0–1240.0 μg/ml) were the primary sugars. Among the amino acids, the sweet-tasting Ala (3.1–10.5 μg/ml) and Ser (1.1–8.3 μg/ml), as well as the umami-tasting Glu (4.5–12.0 μg/ml) and Asp (2.0–5.6 μg/ml), were prominent. Furthermore, the polyphenols were primarily composed of vitexin (0.09–0.18 μg/ml), isovitexin (0.14–0.16 μg/ml), 4-hydroxybenzoic acid (426.8–520.7 μg/ml), vanillic acid (220.3–355.2 μg/ml), and syringic acid (193.0–556.0 μg/ml). Overall, the nutritional value was significantly greater in the alcohol solution obtained from 1- and 2-year-old culms compared to that obtained from 3-year-old culms. The quality of the living bamboo wine was closely linked to the physiological changes and gene expression levels of the bamboo culms. This could provide theoretical guidance for the commercial production of living bamboo wine.

1. Introduction

The bamboo beverage was a kind of functional drink developed with bamboo resources as its main raw material. It was categorized into two types, i.e., alcoholic and non-alcoholic beverage (Chen et al., 2020). These products contained bamboo juice, flavonoids and other bamboo-derived ingredients (Chen et al., 2018).Bamboo wine, specifically, was an alcoholic beverage primarily made from bamboo materials (Sangija and Wu, 2020). As a kind of traditional alcoholic beverage in China, it had been extensively produced and consumed for a long time in various areas, such as Yunnan, Fujian, Hunan and Guizhou. It played a pivotal role in the traditional wine culture and had recently emerged as a significant market in recent years (Chen et al., 2020).

In the past, the production of bamboo wine was categorized into two types: soaking and fermentation type. Sun et al. (2008)employed fermentation technology to create low-alcohol wine using the water extract of *D. hamiltonii*. Tan (2009) developed a novel beer by fermenting a mixture of bamboo juice and wort. Chen et al. (2018) soaked bamboo leaves into a high-concentration sugar solution to make bamboo leaf sugar extract, which was then fermented into bamboo leaf wine. Sangija and Wu (2020) also reported a type of bamboo wine, which was a fermented product of shoot juice of *Oxytenanthera abyssinica*, rich in water-soluble vitamins, essential and non-essential amino acids, and various antioxidants, including free radical scavengers and anti-aging agents

Living bamboo wine, also known as the fresh bamboo wine, was mainly produced by injecting the basic liquors of Baijiu into alive bamboo culms of *Phyllostachys edulis*, and was then pumped out of the culm cavity after several months (Wang, 2017). During the production process of most living bamboo wine, the basic liquors of Baijiu were injected into the bamboo cavity at the middle part of the living bamboos using microporous technique, and the cavity was then sealed with

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paraffin (Zhang, 2011). After being injected, the basic liquors of Baijiu gradually absorbed the rich nutrients from the living culms, resulting in a healthier and better flavor (Guo et al., 2018).

Based on the degree classification of Baijiu, high-proof liquor typically ranged from 50 %vol to 68 %vol, medium-proof liquor falled between 40 %vol and 50 %vol, and low-proof liquor was less than 40 % vol (Yan, 2016). During the production process of living bamboo wine, high-proof wine could cause irreversible damage or even death to the living culms. However, if the alcohol concentration was too low, the alcohol would be metabolized along with the growth of the living bamboo culms, resulting in low alcohol content, poor taste and even microorganism breeding in the wine. Consequently, the medium-proof liquor was usually chosen for the production of living bamboo wine. Additionally, due to the complex composition of the wine sold on the market, it was not suitable for the composition analysis and quantitative detection of the living bamboo wine. Hence, an alcoholic solution prepared from the high purity alcohol was used to simulate the living bamboo wine process, which was more suitable for the components analysis of living bamboo wine during production.

Dendrocalamus brandisii, a sympodial bamboo species, was primarily distributed in the Yunnan province of China and sporadically found in Burma, Laos, Vietnam, and Thailand (Viswanath et al., 2013). Locals in Southwest China preferred consuming their new shoots, which were sweet and had nutritional value. Compared with *P. edulis*, the culms and leaves of *D. brandisii* were rich in organic acids, polysaccharides, mineral elements, flavonoids and other chemical components (Yue et al., 2007). Their culms also possessed a unique sweet and milky taste. Therefore, the culms of *D. brandisii* might be more suitable for the production of living bamboo wine, which needed to be further confirmed. Although bamboo wine had a long history in China, few studies were focused on what components of culms were infiltrated into the living bamboo wine and how the bamboo culms reacted after the wine was injected.

In this study, a comprehensive approach was employed to investigate the composition of living bamboo wine, so as to evaluate the quality of the living bamboo wine and reveal the formation process of living bamboo wine. Additionally, it could also elucidate the impact of physiological changes in culms on the quality of the living bamboo wine. This study provided fundamental information pertaining to the commercial production of bamboo wine, thereby laying a theoretical foundation for subsequent standardized production.

2. Materials and methods

2.1. Material and sampling

The 45 % alcohol solution (chromatographically pure) was injected into the 6th internodes aboveground of *D. brandisii* culms of 1, 2 and 3 years old, respectively, in the bamboo garden of Southwest Forestry University located in Kunming, Yunnan Province, China (25° 03 N , 102° 45E).

A total of 9 samples of the living bamboo wine were collected from the culms of the three age classes for subsequent nutrient components analysis. A total of 18 culm samples were collected from the alcohol-injected bamboo culms and the culms without alcohol injection at all age classes for subsequent anatomical and physiological analysis. For physiological analysis, the samples were stored at −80 ℃. For anatomical observations, the samples were fixed in FAA solution (1.85 % formaldehyde + 45 % alcohol + 0.25 % acetic acid) and then stored in a mixture of 50 % alcohol and 50 % glycerin before slicing. A total of 5 ml living bamboo wine samples were taken for subsequent untargeted and targeted metabolomics determination, and meanwhile, a total of 5 g bamboo culm samples of each age class were also taken for the analysis of transcriptomics, untargeted and targeted metabolomics.

2.2. Color and alcohol content of the living bamboo wine

The color differences of the living bamboo wine obtained from the culms of different ages were distinguished according to the observation. The alcohol content by volume of the wine was determined at 20 ℃ according to the method in GB/T 10345–2007.

2.3. Localization of starch grain in culms after alcohol injection

The softened culms were embedded in polyethylene glycol 6000 (PEG 6000) for one week, and then cut into slices with 20 μm thickness using a sliding microtome (Leica SM2010R, Germany). To localize the starch grains, the method of periodic acid-Schiff (PAS) reaction was employed. The dewaxed sections were firstly soaked in 0.5 % KIO_4 for 10 minutes, followed by the Schiff reagent for 20 minutes, and then stained in Fast green FCF (Ameresco 0689, Solarbio, Beijing, China), and photographed with the microscope (Nikon E400, Tokyo, Japan).

2.4. Soluble sugar and starch contents determination

The contents of soluble sugar and starch were determined according to the method of Glassop et al. (2007). About 0.5 g samples were ground to powder in a mortar and pestle in liquid nitrogen, and then were extracted with deionized water. The supernatants collected by centrifugation at 1800 g for 10 min were reacted with 5 % phenol and 98 % sulfuric acid for half an hour to determine the soluble sugar content. The absorbance at 485 nm was determined by spectrophotometer (UV-8000, MWTASH, shanghai, China). An additional 9.2 mol/L perchloric acid was added during the reaction to determine the starch content.

2.5. Activities of sucrose- and starch-metabolizing enzymes

The activities of carbohydrate metabolizing enzymes were determined in the culms before and after the alcohol injection according to the methods of Lingle and Dunlap (1987) and Sergeeva et al. (2012). For soluble acid invertase (SAI) assay and insoluble extracellular invertase (CWI) assay, the methods referenced to Lingle and Dunlap (1987). The SAI and CWI activities were expressed in μmol NADH per min per g fresh tissue. The activity of SuSy was determined according to Hoffmann et al. (1996). The activities were expressed in μmol NADH per min per g fresh tissue. For the activity determination of starch-metabolizing enzymes, such as soluble starch synthase (SSS), granule-bound starch synthase (GBSS), ADP-glucose pyrophosphorylase (ADPase) and starch phosphorylase (STP)and the crude enzyme was extracted according to Sergeeva et al. (2012). The activity was calculated in terms of μmol NADH per min per g fresh tissue. The activities were also expressed in μmol NADH per min per g fresh tissue.

2.6. Transcriptome sequencing

The total RNA was extracted and the cDNA library was constructed for RNA isolation and sequencing. The library was sequenced by using Illumina novaseq 6000 in Suzhou PANOMIX Biomedical Tech Co., LTD. The raw sequence reads had been submitted into the NCBI Sequence Read Archive (SRA) with the BioProject accession ID PRJNA995377. The transcriptome was then subjected to sequence processing, assembly, and unigene annotation. To functionally annotate the assembled unigenes, the sequences were compared against Kyoto Encyclopedia of Genes and Genomes (KEGG) and GeneOntology (GO) databases. The unigenes with adjusted $P < 0.01$ and $|\log2 \text{ (fold change)}| > 1$ were identified as significantly regulated DEGs (differentially expressed genes), while those with $P < 0.05$ and $|\log2 \text{ (fold change)}| > 0.5$ were identified as slightly regulated DEGs.

2.7. Quantitative real-time PCR (qRT-PCR) verification

For estimating the validity of the transcriptome sequencing, a total of 12 candidate genes were selected for RNA extraction, reverse transcription, primer design, synthesis, and debugging, followed by analysing with qRT-PCR. The protein phosphatase 2 A (PP2A) was chosen as an internal control. The test was commissioned in Suzhou PANOMIX Biomedical Tech Co., LTD.

2.8. Untargeted and targeted metabolomics

Firstly, the untargeted metabolomics were chosen to detect the components of culms and the living bamboo wine from the bamboo culms of different ages. The chromatographic detection was performed using the Thermo Vanquish ultra-high-performance liquid chromatography system (Thermo Fisher Scientific, USA), and the Thermo Q Exactive Focus mass spectrometer (Thermo Fisher Scientific, USA) to conduct the non-targeted metabolomic. The differential metabolites were focused and analyzed and the secondary classification of differential metabolites focused on four major categories of nutrients, including soluble sugars, amino acids, flavonoids and phenolic acids. Combined with the differential metabolic pathways according to the KEGG pathway (Pvalue < 0.01), the targeted metabolomics of four categories nutrients in culms and the living bamboo wine were determined. After processing samples of the living bamboo wine and bamboo culms, the targeted metabolomic measurements were conducted using ultra-high-performance liquid chromatography (Vanquish, UPLC, Thermo, USA) coupled with high-resolution mass spectrometry (Q Exactive, Thermo, USA). These tests were commissioned in Suzhou PANOMIX Biomedical Tech Co., LTD.

2.9. Transcriptomics and targeted metabolomics correlation analysis

The correlation analysis between the transcriptome of culms and the targeted metabolomics results of the living bamboo wine, could reveal the quality formation basis of the living bamboo wine. Therefore, the main differential metabolites in the living bamboo wine were chosen to analyse the correlations with their contents in the culms and the expression levels of the key genes in their synthesis pathways in the culms. The results were presented by using the correlation heat maps and the correlation network diagrams.

2.10. Statistical analysis

All of the datas presented in this experiment were derived from the average of three independent biological replicates. The datas were processed and mapped by Excel 2010 software. The least significant difference (LSD) analysis and the correlation analysis were performed by using SPSS 22.0 software (SPSS, Inc., Chicago, IL, USA). The results were plotted using GraphPad Prism (Prism 8.0.2, San Diego, USA). The correlation heat maps as well as the correlation network diagrams were generated using Hiplot (https://hiplot-academic.com). All the data were presented as means ± standard deviation.

3. Results and discussion

3.1. Color and alcohol content of the living bamboo wine

Compared with the alcohol solution (Fig. 1A), the color of the living bamboo wine from 2-year-old culms was the darkest, showing yellow (Fig. 1C), followed by the wine from the 1-year-old culms, showing light yellow (Fig. 1B), and the lightest color was the living bamboo wine from the 3-year-old culms (Fig. 1C). After one month, the highest alcohol content was shown in the living bamboo wine from 1-year-old culms, which was 11.75 ± 0.5 %, followed by those from the culms of 2 years, which contained 6.75 ± 0.5 % alcohol. However, only 1.75 ± 0.5 % alcohol could be detected in the wine from the 3-year-old culms.

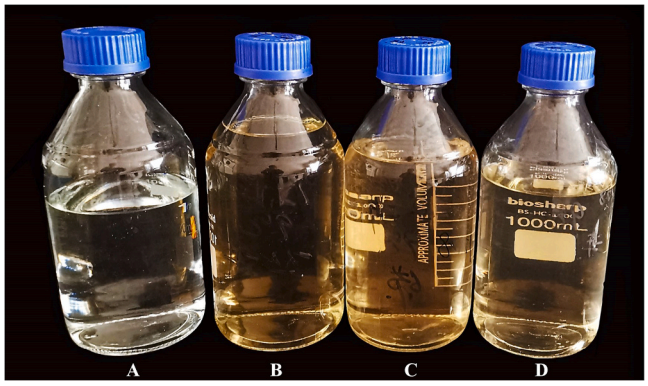

Fig. 1. Color of living bamboo wine. (A) 45 % Alcohol. (B) Living bamboo wine obtained from 1-year-old culms. (C) Living bamboo wine obtained from 2-year-old culms. (D) l iving bamboo wine obtained from 3-year-old culms.

3.2. Untargeted and targeted metabolomics analysis of the living bamboo wine

3.2.1. Untargeted metabolomics analysis of the living bamboo wine

Based on the untargeted metabolomics analysis, a total of 17808 metabolites were detected in the living bamboo wine. By comparing the living bamboo wine produced from the culms of different ages, a total of 5954 differential metabolites were identified (Fig. S1A and B). The cluster analysis revealed that these differential metabolites were mainly classified into 17 categories, including alcohols and polyols, amino acids and analogues, amines, alpha-keto acids and derivatives, benzoic acids and derivatives, carbohydrates and carbohydrate conjugates, benzoyl derivatives, dicarboxylic acids and derivatives, carboxylic acid and derivatives, carbonyl compounds, flavones, fatty alcohols, hydroxycinnamic acids and derivatives, monosaccharides, sesquiterpenoids, 1-hydroxy-2-unsubstituted benzenoids, short-chain keto acids and derivatives and medium-chain hydroxy acids and derivatives (Fig. S1C).

The KEGG pathway enrichment analysis indicated that the differential metabolites were mainly involved in 11 metabolic pathways, including Biosynthesis of other secondary metabolites, Carbohydrate metabolism, Amino acid metabolism, Taste transduction, ABC transporters, Biosynthesis of plant hormones, Lipid metabolism, Mineral absorption, Biosynthesis of alkaloids, Energy metabolism, and others (Fig. S1D and E). Based on the findings from the untargeted metabolomics, it was evident that the nutrients present in the living bamboo wine were predominantly clustered into three main categories, i.e., soluble sugars, amino acids, and phenolic compounds (flavonoids and phenolic acids). To further examine these specific metabolites, we employed targeted metabolomics to assess their changes in content (Figs. S2–4).

3.2.2. Soluble sugars of the living bamboo wine

According to the determining results of soluble sugars, the glucose and fructose contents were found to be higher than the levels of other soluble sugars in the living bamboo wine from the culms of all age classes, among which the living bamboo wine obtained from the 2-year-old culms exhibited the highest contents of glucose and fructose, whereas the wine obtained from the 3-year-old culms displayed the lowest values (Fig. S2A, C and D). The sucrose content was significantly higher in the living bamboo wine obtained from 1-year-old culms compared to that from 2-year-old culms, but it was undetectable in the wine from 3-year-old culms (Fig. S2B). Similarly, the maltose and galactose levels also peaked in the living bamboo wine from 2-year-old culms but the lowest in the wine from 1-year-old culms (Fig. S2E and F). Raffinose and arabinose were also detected in the living bamboo wine

(Fig. S2A, G and H). The raffinose content in the living bamboo wine gradually decreased with the increase of bamboo age classes (Fig. S2G). The arabinose content was the highest in the wine from 2-year-old culms and lowest in the wine from 3-year-old culms (Fig. S2H).

Previous studies have demonstrated that the content of total sugar in the living bamboo wine made by injecting into the *Ph. edulis* culms was about 1.1 % (Zhao et al., 2018). However, the total sugar content of the living bamboo wine produced from the culms of *D. brandisii* was 4.00–6.00 %, which was about 4 times higher than that of *Ph. edulis*. This difference might be attributed to the fact that the *D. brandisii* bamboos produced the shoots with a distinctly sweet flavor, and their culms could also accumulate more contents of soluble sugars as compared to those of *Ph. edulis* (Huang et al., 2019). Therefore, the living bamboo wine produced from the *D. brandisii* culms displayed a more prominent sweet taste than those produced from other bamboo species. Furthermore, fructose was the sweetest naturally occurring carbohydrate and was about 1.7 times sweeter than sucrose (Zhang et al., 2021). Therefore, higher fructose content could further enhance the sweetness of the living bamboo wine. Overall, the living bamboo wine produced from the 2-year-old culms displayed a superior sweet flavor compared to those produced from the 1- and 3-year-old culms.

3.2.3. Free amino acids of the living bamboo wine

Free amino acids were a crucial class of flavor-active ingredients that exhibited unique flavor characteristics (Tan et al., 2018). Their content and type were often used as important indicators for evaluating the nutritional value and taste of food (Egydio et al., 2013). Based on the results of amino acid determination, a total of 21 kinds of amino acids were identified in the living bamboo wine (Fig. S3), seven amino acids exhibited higher concentrations than the others in the living bamboo wine from the culms of all age classes, i.e., Glu, Ala, Ser, Asp, Gln, Asn, GABA (gamma-aminobutyric acid) (Fig. S3B-H). Additionally, 8 essential amino acids (EAA) were also detected in the living bamboo wine from the culms of all age classes, i.e., Lys, Trp, Phe, Met, Thr, Ile, Leu and Val. Generally, the living bamboo wine obtained from the 1-year-old culms exhibited the highest amino acid content, followed by those obtained from 2-and 3-year-old culms (Fig. S3).

Previous studies had shown that the amino acids in wine made an important contribution to the flavor of wine (Zhu et al., 2010). The flavor amino acids in wine could enrich the taste level and made the wine more soft, coordinated and tasteful. Based on their flavor characteristics, free amino acids could be broadly categorized into four groups: umami taste amino acids (Glu, Asp, Gln, etc.), sweet taste amino acids (Ala, Gly, Ser, etc.), bitter taste amino acids (Arg, Val, Ile, etc.) and sour taste amino acids (Asn, His, Glu, etc.) (Yang et al., 2023). In the brewed bamboo wine, higher contents of Glu, Arg, Asp, Leu, Lys and Ala were observed (Yang et al., 2023). A total of 17 kinds of amino acids were identified in apple wine, and the amino acid of which were mainly characterized by sweet and bitter taste (Liu and Guo, 2015). Another study also showed that a total of 14 kinds of amino acids were detected in the fresh bamboo wine from the *Ph. edulis* culms (Guo et al., 2018).

The present results showed that the amino acid composition of the living bamboo wine produced from *D. brandisii* primarily consisted of sweet taste amino acids (Ala and Ser) and umami taste amino acids (Glu and Asp), accounting for more than 75 % of the total amino acids and surpassing the contents of those in the fresh bamboo wine from *Ph. edulis* culms (Guo et al., 2018). The sweet taste amino acids made the wine moist and mellow, and the umami taste amino acids made the wine entrance delicious, lasting and long aftertaste time (Liu et al., 2023). Therefore, the bamboo wine from *D. brandisii* could show better mouthfeel as compared to those obtained from *Ph. edulis* (Guo et al., 2018). Additionally, the living bamboo wine obtained from the 1- and 2-year-old culms exhibited higher amino acid contents compared to that obtained from 3-year-old culms. Consequently, the living bamboo wine produced from the younger culms demonstrated superior taste profiles and enhanced nutritional values in comparison to those originating from the older culms. No significant disparity was observed in the composition of bamboo wine between 1- and 2-year-old culms.

3.2.4. Flavonoids of the living bamboo wine

There were a total of 5 different of flavonoids detected in the living bamboo wine, including vitexin, isovitexin, cynaroside, apigenin and naringenin. Among these, vitexin and isovitexin exhibited the highest contents (Fig. S4A). The highest vitexin content was observed in the living bamboo wine obtained from 1-year-old culms, followed by those from 2- and 3-year-old culms (Fig. S4C). Regarding isovitexin content, it peaked in the living bamboo wine from 2-year-old culms but the lowest in those from 3-year-old culms (Fig. S4D). Trace amounts of cynaroside and apigenin were detected in the living bamboo wine from 1-year-old culms, but were undetectable in those from 2- and 3-year-old culms. Minimal amounts of apigenin and naringenin were separately detected in the living bamboo wine from the culms of 2 and 3 years old, respectively (Fig. S4E-G).

Flavonoids are plant compounds with a variety of health benefits. Excellent nutritional qualities of flavonoids in bamboo leaves, including food safety, flavor enhancement, stable pigmentation, and unique nutritional functions, have been highlighted in previous studies (Cheng et al., 2023). However, few studies had been focused on the specific flavonoids presented in the living bamboo wine. A total of 5 kinds of flavonoids were identified in the living bamboo wine produced from the culms of *D. brandisii*, with isovitexin and vitexin exhibiting the highest concentrations. Vitexin and isovitexin were active components of many traditional Chinese medicines, and were found in various medicinal plants, which recently received increased attention due to its multiple pharmacological properties, including relaxing blood vessels, promoting the blood circulation, antioxidant, anti-inflammatory and anti-cancer activities (Babaei et al., 2020). Vitexin and isovitexin have been identified as active constituents in various plant species (Kalinova et al., 2021), but their presence had not been reported in bamboo wine and bamboo culms. Isovitexin was also reported to have strong hepatoprotective effects (Qiao et al., 2020). This study showed the living bamboo wine obtained from the 1- and 2-year-old culms contained higher levels of vitexin and isovitexin compared to those produced from the 3-year-old culms. This significantly increased the nutritional value of living bamboo wine from *D. brandisii*. High content of isovitexin detected in the living bamboo wine might reduce the harm of alcohol intake to the human body.

3.2.5. Phenolic acids of the living bamboo wine

Based on the results of the phenolic acids assay, a total of 17 kinds of phenolic acids were identified in the living bamboo wine (Fig. S4B and H-X). Among these, p-hydroxybenzoate, vanillic acid, syringic acid, trans-ferulic acid and ρ-coumaric acid were the primary components, exhibiting higher contents compared to others in the living bamboo wine (Fig. S4H-L). With culms aging, the contents of 4-hydroxybenzoic acid, ρ-hydroxycinnamic acid, vanillin, salicylic acid and phenylalanine gradually decreased in the living bamboo wine (Fig. S4I, L, N-O and U). Conversely, the contents of syringic acid, vanillic acid, 3,4-dihydroxybenzoic acid, syringaldehyde, sinapic acid, gallic acid, trans-cinnamic acid, caffeic acid, hydrocinnamic acid and trans-cinnamic acid increased gradually (Fig. S4H, J, M, P-Q, S-T and V-X). However, the contents of trans-ferulic acid and benzoic acid initially increased and then decreased with age (Fig. S4K and R).

Phenolic acids could better anti-hypertensive, stop diarrhea, anti-inflammatory analgesia and protect blood vessels, and also had important effects such as anti-oxidation, antibacterial and anti-cancer (Agunloye et al., 2019). There were two types of phenolic acids, i.e., hydroxybenzoic and hydroxycinnamic acid (Lourenço et al., 2018). Hydroxycinnamic acid had higher antioxidant activity than hydroxybenzoic acid (Lourenço et al., 2018). In the bamboo juice of the *Ph. edulis*, ρ-hydroxycinnamic acid was reported to be the main phenolic acid component (Zhu et al., 2020). In the living bamboo wine from

culms of *D. brandisii*, the content of ρ-hydroxycinnamic acid was about 4–8 times that of the *Ph. edulis* bamboo juice. Among the detected phenolic acids, the contents of 4-hydroxybenzoic acid, vanillic acid, syringic acid, as well as trans-ferulic acid and ρ-hydroxycinnamic acid were higher than those of salicylic acid, sinapic acid and caffeic acid. The present studies showed that the living bamboo wine from 3-year-old culms contained higher levels of phenolic acids, followed by those from 2-year-old culms, and the less content was those from 1-year-old culms.

3.3. Untargeted metabolomics analysis of culms before and after alcohol injection

The untargeted metabolomics results revealed that a total of 30143 metabolites were detected, among which a total of 3086 differential metabolites were identified in the 1-year-old culms (Fig. S6A). Additionally, a total of 3729 and 1625 differential metabolites were separately identified in the 2- and 3-year-old culms (Fig. S6B and C). By classifying the detected differential metabolites, the main focuswas on 22 classes, including amino acids, peptides and analogues, carbohydrates and carbohydrate conjugates, amines, alcohols and polyols, benzoic acids and derivatives, carbonyl compounds, fatty acids and conjugates, eicosanoids, furanocoumarins, flavonoid glycosides, flavans, hydroxycinnamic acids and derivatives, monoterpenoids, phenylpyruvic acid derivatives, phenols and derivatives, pyrimidines and pyrimidine derivatives, purines and purine derivatives, sesquiterpenoids, tryptamines and derivatives, 1-hydroxy-2-unsubstituted benzenoids and tricarboxylic acids and derivatives (Fig. S6D-F).

According to the relative contents of differential metabolites, a large number of metabolites of culms changed their contents after the alcohol injection, especially in carbohydrate and amino acids and their derivatives. The KEGG pathway enrichment analysis showed that the differential metabolites primarily converged on 6 classes of metabolic pathways, i.e., amino acid metabolism, ABC transporters, Carbohydrate metabolism, Signal transduction, Lipid metabolism and Energy metabolism (Fig. S6G-I). The changes of differential metabolites identified in culms after the alcohol injection were highly similar to those determined in the living bamboo wine, which mainly involved amino acids, soluble sugars and phenolic compounds. Consequently, a targeted metabolomics analysis was conducted on these three types of metabolites in culms, both before and after the injection of alcohol.

3.4. Sugar metabolism in culms after alcohol injection

It was observed that the starch grains were mainly distributed in the parenchyma cells, and the quantity of starch grains were the highest in 2-year-old culms but the least in 1-year-old culms (Fig. S5). After the alcohol injection, the quantity of starch grains decreased significantly in the 1- and 2-year-old culms, especially in the 2-year-old culms. However, few changes were observed in the 3-year-old culms.

The starch and soluble sugar contents were measured in the culms both before and after the injection of alcohol solution. Under natural conditions, the highest levels of both starch and soluble sugar were observed in the 2-year-old culms, followed by the 3-year-old culms and the lowest were found in the 1-year-old culms. After the alcohol injection, the contents of both starch and soluble sugar decreased in the culms of all age classes, with the most significant decrease occurring in the 2-year-old culms ($P < 0.01$) (Fig. S7A and B). Based on the targeted metabolomics analysis, a total of 5 kinds of soluble sugars were detected in the culms (Fig. S8A). The injected alcohol led to a decrease in the contents of all soluble sugars in the 1-year-old culms, with sucrose experiencing a particularly significant decrease. In 2-year-old culms, the contents of sucrose and maltose decreased significantly, while the contents of glucose, fructose and galactose increased significantly after the alcohol injection. The increase in glucose and fructose contents in 2-year-old culms might be attributed to their increased production and decreased consumption in the culms after alcohol injection. However, the trends observed in the 3-year-old culms after alcohol injection were opposite to those in the 2-year-old culms. Specifically, the contents of sucrose and maltose increased, while the contents of other soluble sugars decreased in the 3-year-old culms after the alcohol injection (Fig. S8A).

For sucrose metabolism, it was noticed that the activities of SuSy and SAI increased significantly, while CWI activities increased slightly in the culms of all ages after the alcohol injection (Fig. S7C-E). The most significant change in the activities of the three enzymes was observed in the 2-year-old culms. This implied that the alcohol injection stimulated sucrose catabolism more prominently in the 2-year-old culms, in comparison to 1- and 3-year-old culms.

In sucrose metabolism, SuSy and INV catalyzed the degradation of sucrose into glucose and fructose during plant growth (Moscatelloa et al., 2011). In 2-year-old culms, the increase in activities of SAI, CWI and SuSy demonstrated that sucrose was decomposed and a large amount of glucose and fructose were accumulated in the culms after alcohol injection. The glucose and fructose in culms constantly infiltrated into the bamboo wine, which not only accelerated the sucrose degradation in culms, but also increased the contents of glucose and fructose in the living bamboo wine. This result was consistent with the results of Liu et al. (2015) who reported that the contents of sucrose, glucose and fructose were increased in plants under stressful conditions. Therefore, the alcohol accelerated the sugar catabolism in bamboo culms, which further accelerated the permeation of glucose and fructose into the alcohol solution.

The activities of STP, AGPase, SSS and GBSS were also assayed to analyze the starch metabolism in culms after the alcohol injection (Fig. S7F-I). Notably, the activities of AGPase, GBSS and SSS decreased significantly, while the STP activity increased significantly in 1-year-old culms after the alcohol injection. In 2-year-old culms, the activities of AGPase, GBSS and SSS also decreased after the alcohol injection, while the STP activity increased slightly. However, in 3-year-old culms, all the activities of GBSS, SSS, AGPase and STP increased after the alcohol injection. Overall, the alcohol injection appeared to inhibit starch synthesis while enhancing starch degradation and sucrose catabolism in the culms, thereby increasing the soluble sugar contents within them.

In starch metabolism, AGPase, SSS and GBSS were closely related to the starch accumulation (Wang et al., 2020). STP was one of the important enzymes involved in starch degradation (Wang et al., 2020). After the alcohol injection, the increased activities of STP and the decreased activities of AGPase, SSS and GBSS indicated the increase in starch degradation but the decrease in starch synthesis in 1- and 2-year-old culms, resulting in a decline in starch content in the culms after alcohol injection. Therefore, the alcohol injection increased the starch degradation in culms.

3.5. Changes in the contents of amino acids and phenolic compounds after the alcohol injection

The targeted metabolomics of culms revealed that a total of 21 kinds of amino acids were identified in the culms, among which GABA, Gln, Asp, Glu, Ser, Ala, Arg, Lys, Trp and Tyr exhibited higher contents than the other amino acids (Fig. S8B). For the 1-year-old culms, the injected alcohol significantly decreased the levels of GABA, Asp, Gly, Val, Asn, Gln, Trp and Ser significantly but significantly increased the contents of Glu, Ala, Phe, His, Ile and Arg. The concentrations of the remaining amino acids remained unchanged. In the 2-year-old culms, the contents of GABA, Glu, Val, Pro, Ile, Gln, Phe and Ala increased significantly after the alcohol injection, whereas the contents of Asp, Ser, Tyr, Trp and Arg decreased significantly. In the 3-year-old culms, the contents of most amino acids increased with the exception of Met and Gly (Fig. S8B). The changes of amino acids in plants encompassed a multitude of physiological regulatory activities, intricately linked to growth, development, and stress resilience (Abd El-Samad et al., 2011). The elevation of amino acid contents in the culms results in more amino acids entering the living bamboo wine, thereby enhancing its nutritional value and flavor.

A total of 12 kinds of flavonoids were detected in the culms of all ages according to the results of the targeted metabolomics, among which isovitexin and vitexin exhibited the highest contents, followed by apigenin, naringenin, cynaroside, luteolin, naringin and rutin. The contents of astragalin, genistin, diosmin and chrysin were relatively low (Fig. S8C). The alcohol injection significantly increased the contents of isovitexin, vitexin and rutin in the 1-year-old culms, but significantly decreased the contents of other flavonoids. In the 2-year-old culms, the contents of isovitexin, naringenin, astragalin and apigenin decreased significantly, whereas the contents of the other flavonoids significantly increased after the alcohol injection. As for the 3-year-old culms, the contents of isovitexin, vitexin, cynaroside, genistin, astragalin and rutin decreased, while the others showed an upward trend after the alcohol injection (Fig. S8C).

Furthermore, a total of 16 kinds of phenolic acids were identified in the culms (Fig. S8D). The highest contents of phenolic acids were shown in ρ-hydroxycinnamic acid and trans-ferulic acid, followed by vanillin, syringic acid, vanillic acid, 4-hydroxybenzoic acid, syringaldehyde and sinapic acid. In 1-year-old culms, the levels of most phenolic acids were significantly decreased after the alcohol solution injection, with the exception of 4-hydroxybenzoic acid, phenylalanine, gallic acid and trans-cinnamic acid in the culms. In 2-year-old culms, the contents of phenolic acids increased after the alcohol injection, except vanillin, syringaldehyde, salicylic acid, hydrocinnamic acid and benzoic acid. In contrast, in 3-year-old culms, the levels of phenolic acids fluctuated slightly, and almost no gallic acid and hydrocinnamic acid were detected (Fig. S8D). Overall, the influence of alcohol injection on amino acid and phenolic compound content in the culms diminished with age.

Phenolic compounds not only served as natural pigments but also played a critical role in plant growth and development, stress defense, signal transduction and antioxidant mechanism (Shen et al.,2022). Flavonoids had physiological functions such as antioxidant, antitumor and bactericidal anti-inflammatory (Zhu et al., 2016). The alcohol injection caused significant variations in the contents of phenolic compounds in culms, especially for those flavonoids and phenolic acids with strong antioxidant capacity, such as vitexin, isovitexin, 4-hydroxybenzoic acid, vanillic acid, syringic acid, trans-ferulic acid and ρ-hydroxycinnamic acid, which increased significantly after the alcohol injection. Generally, the variations in nutritional components in bamboo culms were basically the same to those in the alcohol solution injected in the culms.

3.6. Transcriptomics analysis of culms after the alcohol injection

3.6.1. RNA-sequencing and functional annotation

After being filtered using fastp (version 0.18.0), the percentage of high quality clean reads for each sample reached 90.49–91.32 %. The Q20 and Q30 base in the samples were respectively greater than 97.47 % and 93.43 % (Table S1). The results showed that the data was suitable for further analysis.

The functional annotation was performed by comparing with six databases. A total of 16879 unigenes were successfully annotated, including 122478 (52.90 %) in NR, 48729 (21.05 %) in KEGG, 109003 (47.08 %) in NOG, 58213 (25.14 %) in GO, 58038 (25.07 %) in Pfam and 83681 (36.14 %) in SwissProt. FPKM values were employed to represent the abundance of unigenes. A total of 10504, 328 and 4163 unigenes were identified to show significantly differential expression in the culms of three age classes after the alcohol injection, among which 3756, 54 and 3966 DEGs were down-regulated and 6748, 274 and 197 DEGs were up-regulated.

3.6.2. Pathway analysis of DEGs enriched in KEGG

Through KEGG enrichment analysis, the DEGs were mainly enriched in four primary categories: Metabolism, Genetic information processing, and Environmental information processing and organism (Fig. S9). Based on the KEGG annotation, a total of 123, 44 and 117 pathways were enriched, and 27, 14 and 44 of these pathways were found to be significantly enriched at $P<0.05$ levels (Table S2–4). The top 20 pathways with the highest abundance DEGs were primarily concentrated on the pathways of Carbohydrate metabolism, Amino acid metabolism, Plant hormone signal transduction, Biosynthesis of other secondary metabolites, and Metabolism of other amino acids (Table S5). The results suggested that the endogenous carbohydrate, hormone signal transduction, secondary metabolites and amino acids metabolism played crucial roles in the alcohol stress resistance of bamboos. These findings were also consistent with the results of metabolome assay obtained from the living bamboo wine.Therefore, the quality of the living bamboo wine is likely to be closely associated with the physiological activities of the bamboo culms.

3.6.3. qRT-PCR validation

A total of 12 DEGs were used for qRT-PCR analysis in order to verify the reliability of the RNA-Seq DEGs data (Fig. S10). The expression of the DEGs measured by qRT-PCR exhibited a completely consistent trend with the transcriptome sequencing results, indicating the reliability of the RNA-Seq data.

3.6.4. Analysis of DEGs in the metabolic pathways of sugar, amino acids, flavonoid and phenolic acid

In starch metabolism, the expression levels of most DEGs related to starch synthesis were down-regulated whereas those of most DEGs related to the starch degradation were up-regulated after the alcohol injection. *INV* and *SUS* genes played important role in the sugar metabolism and normal development of plants. *SUS* gene also could promote the cellulose synthesis. In sucrose metabolism, alcohol injection led to an increase in the expression levels of *INV* and *SUS* genes (Fig. S11), which enhancing the activities of INV and SuSy enzymes. This finding aligned with the dynamical changes in starch and soluble sugar contents, as well as the activities of starch catabolizing enzymes, further indicating hyperactive starch metabolism in the culms after alcohol injection. Regarding cellulose synthesis, the expression level of *CESA* gene, which was the key gene for cellulose synthesis, was upregulated in the 1-year-old culms but downregulated in 2- and 3-year-old culms after alcohol injection (Fig S11). This was consistent with what Wei et al. (2015) found that overexpression of poplar xylem SUS2 in tobacco increased the cellulose content. In the pathways of glycolysis, TCA cycle and fermentative metabolism, it was observed that the expression levels of most DEGs were up-regulated. In addition, the pentose phosphate pathway (PPP) was also upregulated due to higher expression levels of *PGD*, *TKT* and *TAL* genes. This result suggested an increase in glucose catabolism in the culms after alcohol injection.

In the amino acid synthesis pathway, a total of 20 DEGs were identified and enriched (Fig. S12). Upon alcohol injection, the expressions of the majority of genes involved in this pathway were upregulated across all bamboo culms, with a particular emphasis on key genes such as *ALT*, *SHMT*, and *ASNS*. Notably, the alcohol injection significantly elevated the expression levels of these genes in 2- and 3-year-old culms compared to the 1-year-old ones. This finding provides a plausible explanation for the alcohol injection's ability to enhance amino acid accumulation, especially in the 2- and 3-year-old bamboo culms.

The biosynthesis of flavonoids and phenolic acids in plants originated from the phenylpropane pathway, and shared the first three enzymatic reactions, with *PAL*, *C4H* and *4CL* genes serving as the key genes for these reactions. In the flavonoid synthesis pathway, a total of 6 DEGs were identified, among which the expression levels of *CHS* and *F3H* genes were significantly up-regulated, whereas the expression of *PAL* and *CHI* genes was significantly down-regulated in the culms of 1 year after alcohol injection (Fig. S10). An opposite trend was observed in the 2- and 3-year-old culms. The expression pattern of *CYP73 A* followed a similar trend, which were up-regulated in the culms of 1 and 2 years after alcohol injection, but down-regulated in the culms of 3 years. The expression levels of the *CYP75A* gene in the culms exhibited an opposite

pattern to that of the *CYP73A* gene.

In the phenolic acid synthesis pathway, a total of 6 DEGs were enriched, including *PAL*, *C4H*, *4CL*, *AroG*, *CYP98A3* and *ICS* genes (Fig. S13). *AroG*, *CYP98A3* and *ICS* genes were essential regulators of phenolic acid synthesis (Xue, et al., 2024). The expressions of *AroG* and *CYP98A3* genes were significantly downregulated in the 1-year-old culms, whereas they were upregulated in the 2-year-old culms, with minimal changes observed in the 3-year-old culms. Conversely, the expression patterns of *ICS gene* in the culms were the opposite of those of *AroG* and *CYP98A3* genes. Specifically, the ICS gene was upregulated in the 1-year-old culms but downregulated in the 2-year-old culms after alcohol injection.

3.7. Correlations analysis between the targeted metabolites in culms and in the living bamboo wine, and the related gene expressions

Correlation coefficient (r) represented the correlation between two variables, which was usually divided into four levels: strong correlation ($|r|\geq0.75$), moderate correlation ($0.5\leq|r|<0.75$), weak correlation ($0.25\leq|r|<0.5$), no correlation ($|r|<0.25$) (Statology Systems, 2021). The correlation analysis could effectively reveal the intricate relationships between the quality of bamboo wine and the physiological changes observed in the culms after alcohol injection.

3.7.1. Formation mechanism of sugars in the living bamboo wine

Sugar metabolites not only served as the key compounds in bamboo's response to alcohol stress but were also critical for the formation of the living bamboo wine quality. The soluble sugars contents in the living

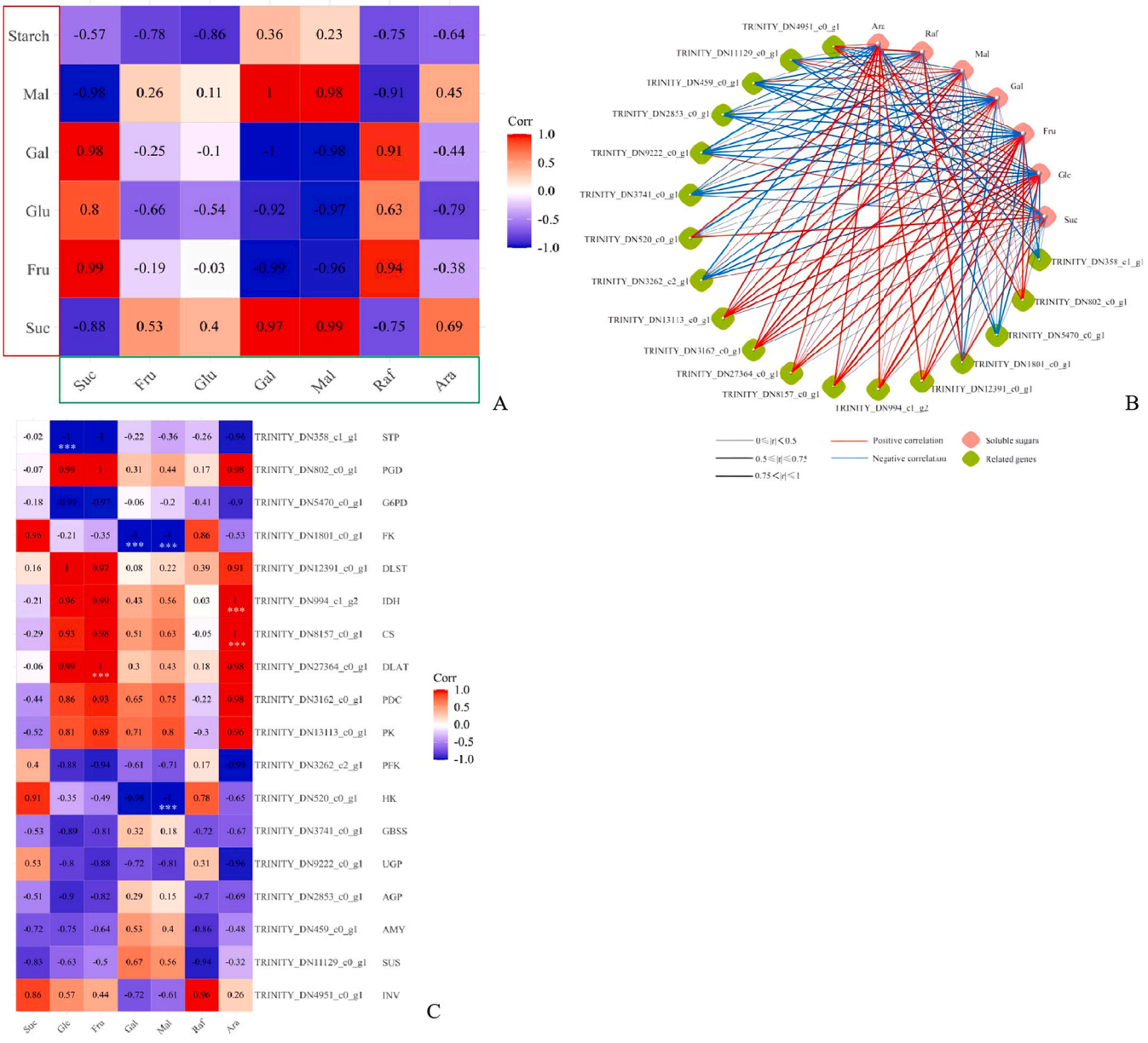

Fig. 2. Correlation of soluble sugars contents in culms and living bamboo wine and their related gene expressions. (A) Correlation heat map of soluble sugars contents in culms and living bamboo wine. The red box represented the changes of soluble sugars and starch contents in culms. The green box represented those in the living bamboo wine. Color scale from blue to red represented the correlation in heat map. (B) The igraph of soluble sugars between the transcriptomics of culms and the targeted metabolomics of living bamboo wine. (C) Correlation analysis of soluble sugars between the targeted metabolomics of living bamboo wine and the transcriptomics of culms. Color scale from blue to red represented the changes of correlation values in heat map.

bamboo wine exhibited close correlations with those in the culms after alcohol injection (Fig. 2A). There were strong and positive correlations between the sucrose content in the living bamboo wine and the fructose, glucose and galactose contents in culms, which respectively reached 0.99, 0.80 and 0.98. However, the sucrose content in the living bamboo wine was strongly and negatively correlated with the contents of sucrose in culms, reaching −0.88. This suggested that the sucrose in culms degraded and penetrated into the living bamboo wine. Moreover, there were strongly negative correlations between the glucose and fructose contents in the living bamboo wine and the starch content in the culms, which reached about −0.8. The fructose and glucose contents in the living bamboo wine were weakly and negatively correlated with the glucose content, but weakly and positively correlated with the sucrose content in the culms. The results suggested that the glucose and fructose in the living bamboo wine were closely related to the decomposition of sucrose and starch in the culms after the alcohol injection. The close correlations between the soluble sugars contents in the injected alcohol solution and those in the culms indicated that the sugars in the living bamboo wine originated from the culms.

For the living bamboo wine, the contents of sucrose and raffinose in which were significantly and positively correlated with the expression levels of *INV*, *HK*, and *FK* genes in the culms, with correlation coefficients reaching 0.96. However, they were greatly and negatively correlated with *SUS*, *AGP*, *GBSS* and *AMY* genes,with correlation coefficients exceeding 0.51 (Fig. 2B and C). The contents of glucose, fructose and arabinose in the living bamboo wine were strongly and positively correlated with the expression levels of *INV* gene and most genes in the TCA pathway. Conversely, they exhibited a negative correlation with the expression levels of most genes involved in the starch metabolism and glycolytic pathway (Fig. 2B and C). The correlation patterns between the contents of galactose and maltose in the living bamboo wine and the expression levels of sugar-metabolizing genes in the bamboo culms showed an opposite trend to those observed for glucose and fructose. Overall, the contents of soluble sugars in the living bamboo wine were positively correlated with the expression levels of *INV* in the bamboo culms. Sugar metabolism played a crucial role in the

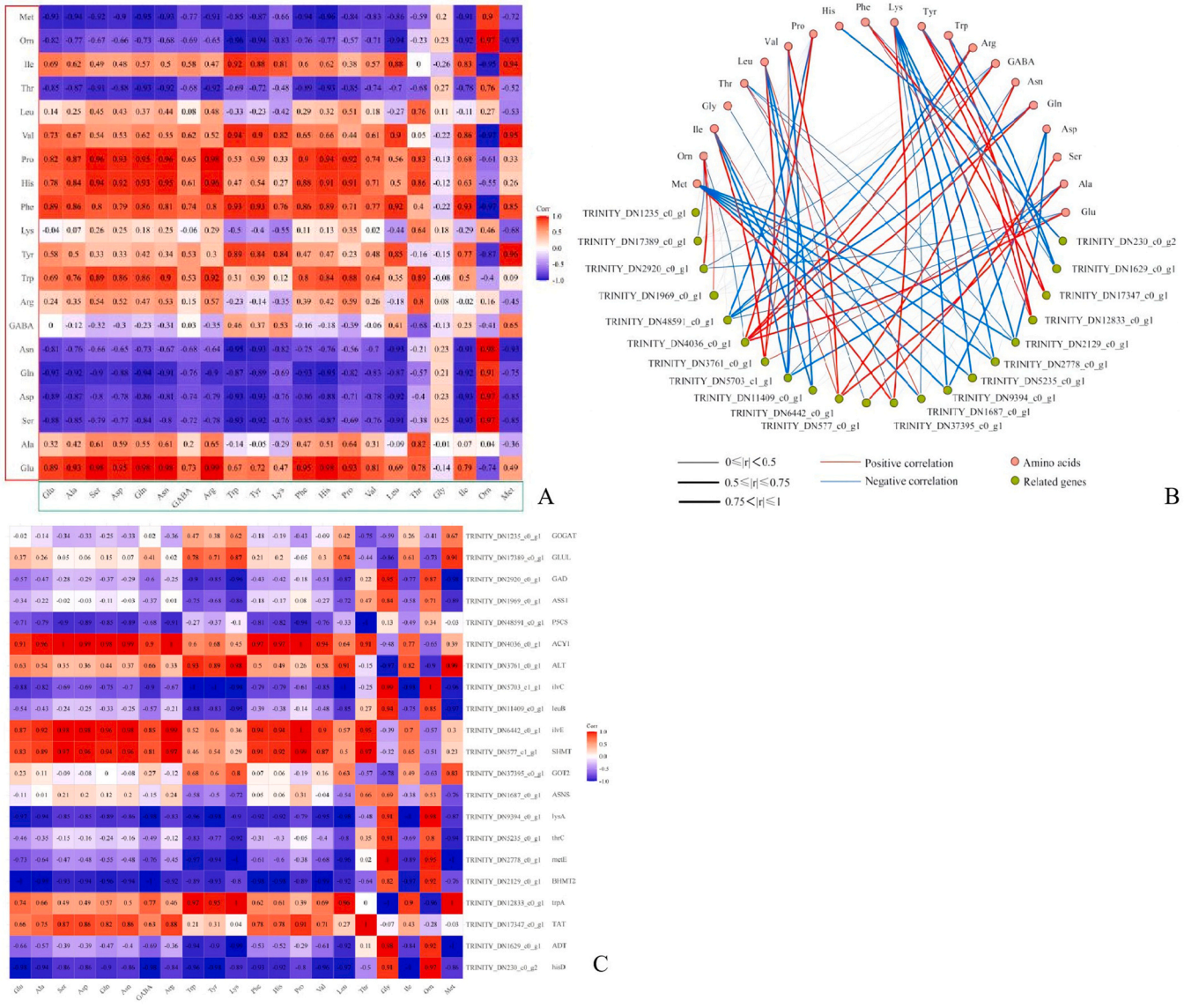

Fig. 3. Correlation of amino acids contents in culms and living bamboo wine and their related gene expressions. (A) Correlation heat map of amino acids contents in culms and living bamboo wine. The red box represented the changes of the soluble sugars and starch contents in culms. The green box represented those in living bamboo wine. Color scale from blue to red represented the correlation in heat map. (B) The igraph of amino acids between the transcriptomics of culms and the targeted metabolomics of living bamboo wine. (C) Correlation analysis of amino acids between the targeted metabolomics of living bamboo wine and the transcriptomics of culms. Color scale from blue to red represented the changes of correlation values in heat map.

quality formation of the living bamboo wine. Soluble sugars were formed in the culms and subsequently entered the living bamboo wine through infiltration. The active gene expressions in glycolysis and TCA pathways might be attributed to the fact that alcohol stress accelerated carbohydrate metabolism in the bamboo culms. The close correlations between the contents of soluble sugars in the injected alcohol solution and the gene expression levels in sugar metabolism pathways in the culms further indicated that the alcohol injection accelerated the starch and sucrose metabolism in culms, producing a large amount of soluble sugars such as glucose and fructose,and then penetrated into the injected alcohol solution and enriched the sweetness of the living bamboo wine.

3.7.2. *Formation mechanism of amino acids in the living bamboo wine*

Significant correlations were also observed between the concentrations of most amino acids in the living bamboo wine and their corresponding contents in the culms. (Fig. 3A). Among these, the contents of Glu, Phe, Pro, Val, Orn, His, Tyr and Ile exhibited significant and strong positive correlations in both the culms and the living bamboo wine, all reaching values higher than 0.8. Conversely, the contents of Ser, Asp, Gln, Asn, Thr and Met in the living bamboo wine displayed moderate negative correlations with their contents in the culms, all exceeding 0.6. Additionally, the contents of Ala, Arg, Trp, Lys, Leu and Val in the living bamboo wine exhibited weak correlations ($|r|<0.5$) with their corresponding contents in the culms. In the synthesis pathway, a strong positive correlation was observed between the Ser content in the living bamboo wine and the gene expression levels of *SHMT* (Fig. 3B and C). Furthermore, the contents of Ala and Ile in the living bamboo wine were closely and positively correlated with the expression levels of *ALT* and *ilvE* genes, respectively. Conversely, the contents of Val and Leu exhibited negatively correlations with the expression levels of *ilvC* and *leuB* genges. Lastly, the content of Trp also demonstrated a strong positive correlation with the expression level of the key gene (*trpA*) in the bamboo culms, reaching a value of 0.97 (Fig. 3B and C).

The amino acid metabolism usually increased significantly in plants

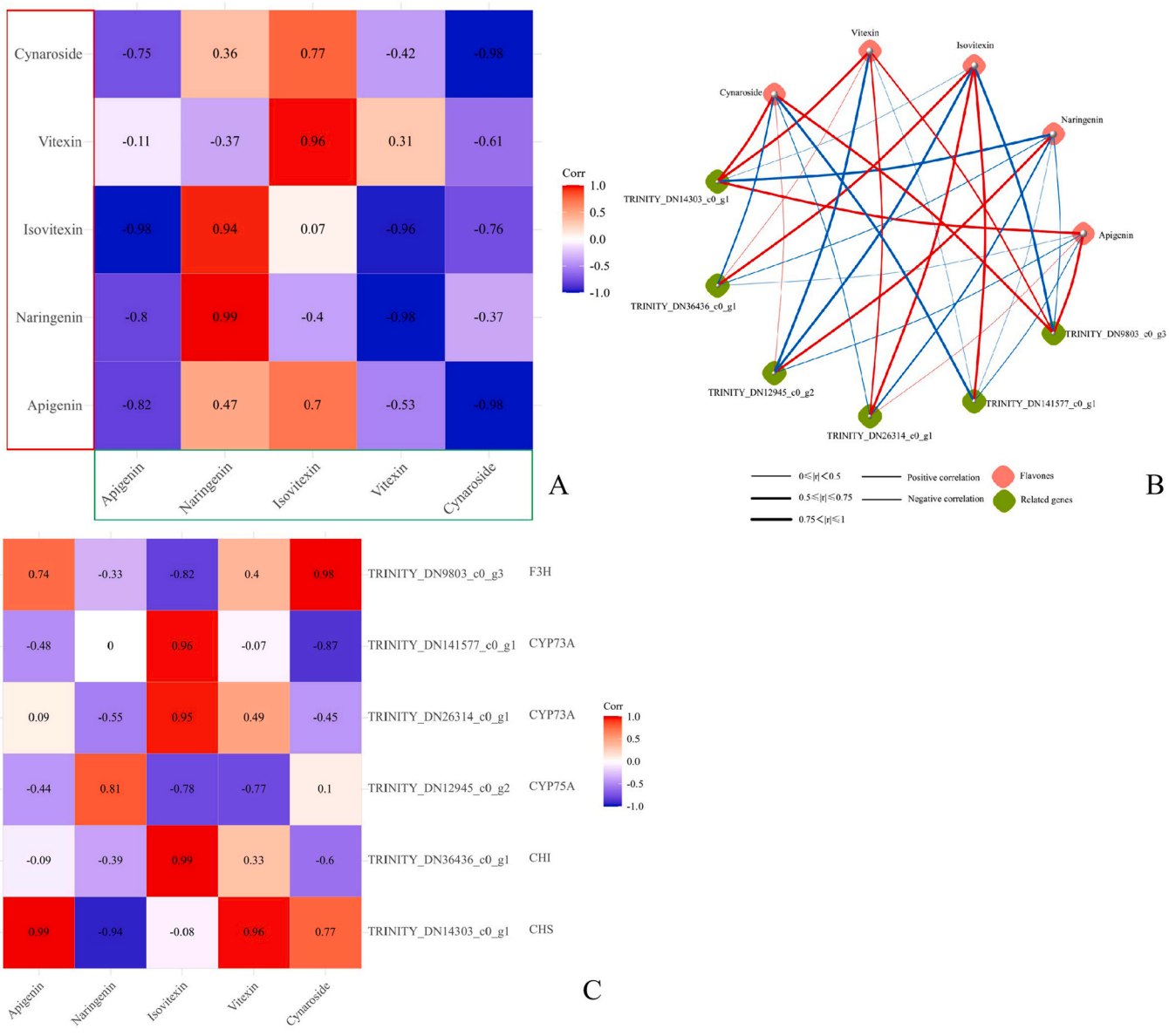

Fig. 4. Correlation of flavonoids contents in culms and living bamboo wine and their related gene expressions. (A) Correlation heat map of flavonoids contents in culms and living bamboo wine. The red box represented the changes of soluble sugars and starch contents in culms. The green box represented those in living bamboo wine. Color scale from blue to red represented the correlation in heat map. (B) The igraph of flavonoids between the transcriptomics of culms and the targeted metabolomics of living bamboo wine. (C) Correlation analysis of flavonoids between the targeted metabolomics of living bamboo wine and the transcriptomics of culms. Color scale from blue to red represented the changes of correlation values in heat map.

under stressful environments (Panda et al., 2021). In this study, the correlation analysis showed that the contents of amino acids in the living bamboo wine had a strong correlation with their contents in the culms after alcohol injection, and with the expressions of their related genes in the culms after alcohol injection. This indicated that the alcohol injection promoted the synthesis of amino acids in bamboo culms in order to increase the resistance to the alcohol stress, and further promoted the amino acids accumulation. Hence, the amino acids constantly penetrated from culms into the living bamboo wine, and enriched the sweetness and umami of the living bamboo wine, and also improved its antioxidant capacity.

3.7.3. *Formation mechanism of polyphenols in the living bamboo wine*

For the flavonoids, it was observed that their contents in the living bamboo wine exhibited strong correlations with their respective contents in the culms (Fig. 4A). Specifically, the contents of apigenin and cynaroside in the wine displayed strong negative correlations with their contents in the culms, which reached −0.82 and −0.98 respectively (Fig. 4B and C). There was also a strong positive correlation (r=0.99) between the naringenin content in the wine and that in the culms, as well as a weak positive correlation for vitexin contents. Furthermore, the contents of apigenin, vitexin and cynaroside were significantly and positively correlated with the expression levels of *CHS* gene, which reached higher than 0.77. Additionally, the isovitexin content exhibited a strong positive correlation with the expression levels of *CYP73A* and *CHI* genes, reaching correlation coefficients higher than 0.95 (Fig. 4B and C). Therefore, it could be concluded that the contents of flavonoids in the living bamboo wine were significantly correlated with their syntheses and the expression levels of related genes in the bamboo culms after alcohol injection.

The phenolic acids contents in the living bamboo wine were closely related to their contents in the culms after the alcohol injection

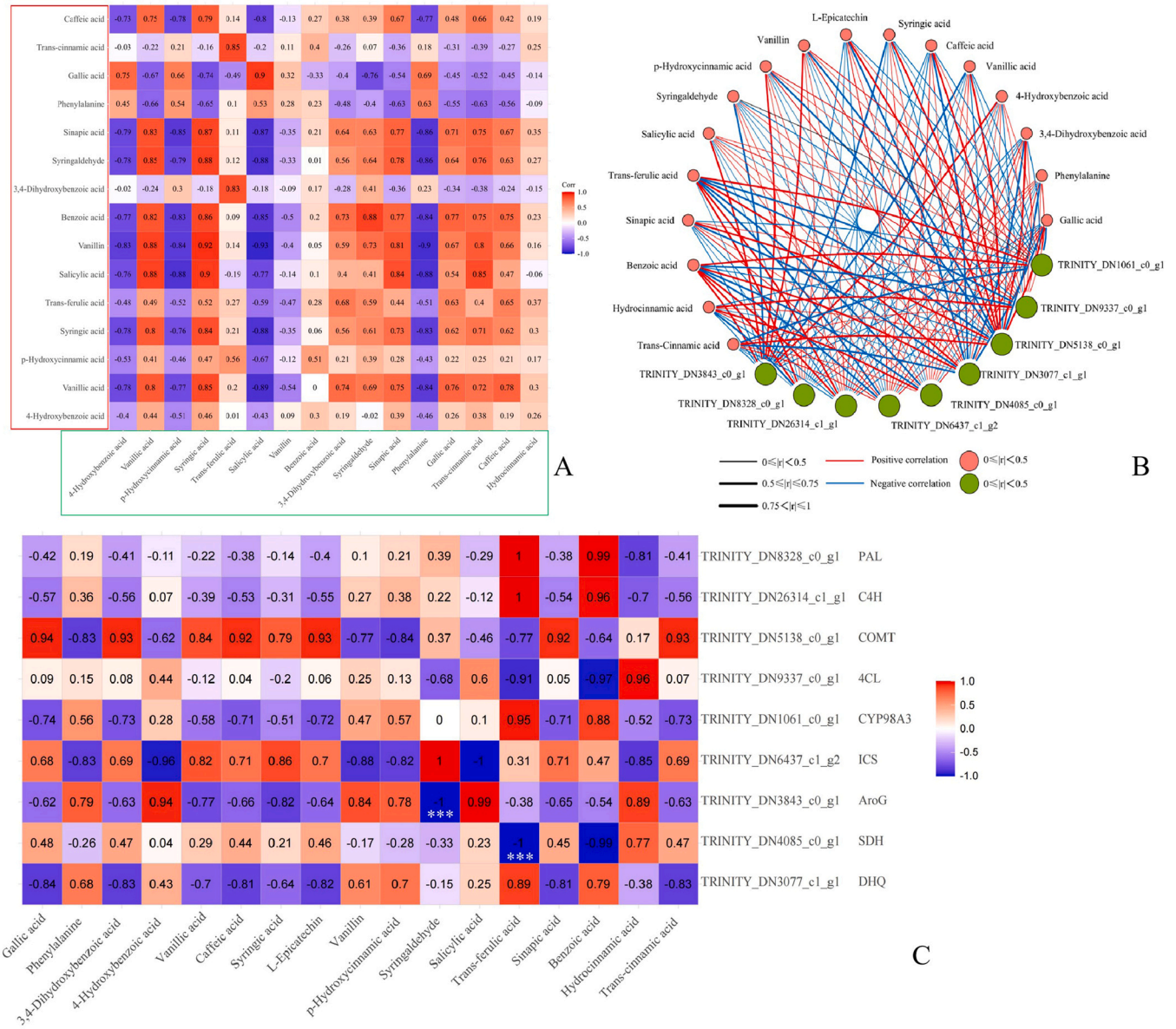

Fig. 5. Correlation of phenolic acids contents in culms and living bamboo wine and their related gene expressions. (A) Correlation heat map of phenolic acids in culms and living bamboo wine. The red box represented the changes of soluble sugar and starch contents in culms. The green box represented those in living bamboo wine. Color scale from blue to red represented the correlation in heat map. (B) The igraph of phenolic acids between the transcriptomics of culms and the targeted metabolomics of living bamboo wine. (C) Correlation analysis of phenolic acids between the targeted metabolomics of living bamboo wine and the transcriptomics of culms. Color scale from blue to red represented the changes of correlation values in heat map.

(Fig. 5A). There was a strong positive correlation between the levels of vanillic acid, syringic acid and sinapic acid in the living bamboo wine and their respective contents in the culms, exceeding 0.77. Conversely, phenylalanine and syringaldehyde displayed moderate positive correlations ($0.5 \leq |r| < 0.75$) when comparing their concentrations in the living bamboo wine to those in the culms (Fig. 5A). The levels of gallic acid, 3,4-dihydroxybenzoic acid, vanillic acid, caffeic acid, syringic acid, L-epicatechin, syringaldehyde, sinapic acid, and trans-cinnamic acid in the living bamboo wine exhibited strong positive correlations with the expression levels of *COMT* gene in the culm, as well as moderate correlations with the expression levels of *ISC* gene, reaching approximately 0.9 and 0.5 respectively. However, they were closely and negatively correlated with the expression levels of the *POD, AroG*, *DHQ, CYP98A3* and *C4H* genes (Fig. 5B and C).

The contents of trans-ferulic acid and benzoic acid were strongly and positively correlated with the expression levels of *PAL*, *C4H*, *DHQ* and *CYP98A3* genes, but greatly and negatively correlated with the expression levels of *COMT*, *4CL*, *ICS and SDH* genes. Additionally, the levels of phenylalanine, 4-hydroxybenzoic acid, vanillin, ρ-hydroxycinnamic acid, and salicylic acid were closely and positively correlated with the expression levels of *POD, DHQ* and *AroG* genes, but greatly and negatively correlated with the expression levels of *COMT* gene, all of which reached higher than 0.6 (Fig. 5B and C). These findings indicated that the *COMT*, *POD*, *DHQ* and *SDH* genes in the culms played a key role in the accumulation of phenolic acids in the bamboo wine after alcohol injection.

Rangani et al. (2020) considered that polyphenols was a kind of major functional class of metabolites in plants under stressful conditions. Zhou et al. (2020) also reported that the UV-C irradiation could promote the synthesis of phenols, flavonoids and anthocyanins. Low nitrogen supply treatment could increase the phenolic accumulation in lettuce (*Lactuca sativa*) by effectively redirecting more carbon and nitrogen resources to the phenolic biosynthesis pathway (Zhou et al., 2021). In this study, the contents of flavonoids in the living bamboo wine showed strong correlations with their contents and the expression levels of the related genes in culms after the alcohol injection. The correlation analysis of phenolic acids also showed the same trend. This indicated that the alcohol injection promoted the synthesis of flavonoids and phenolic acids in the bamboo culms, and the accumulation of flavonoids and phenolic acids in the living bamboo wine. Generally, the quality of living bamboo wine was closely related to the physiological activities in the culms after the alcohol injection. Bamboos increased their synthesis and degradation of sugars, amino acids, flavonoids and phenolic compounds in the culms to resist the alcohol stress, which further enriched the nutrient values of bamboo wine.

4. Conclusion

The nutritional values of the living bamboo wine obtained from the 1- and 2-year-old culms of *D. brandisii* were higher than those from the 3-year-old culms. The quality of the living bamboo wine was closely related to the physiological metabolism and gene expression within the alcohol-injected culms. The alcohol solution injection triggered an increase in gene expression levels related to sugar metabolism, amino acid metabolism and phenolic metabolism of bamboo culms, which led to the synthesis of a significant quantity of metabolites such as soluble sugars (sugars and glucose, ect.), amino acids (Glu, Asp, ect.), flavonoids (vitexin and isvitexin, ect.) and phenolic acids (4-hydroxybenzoic acid, ρ-hydroxycinnamic acid), which were then incorporated into the living bamboo wine.

CRediT authorship contribution statement

Todd Shupe: Methodology. **Jiaxin Liu:** Supervision, Methodology. **Ying'dan Yan:** Writing – review & editing, Writing – original draft, Software, Methodology, Investigation, Formal analysis. **Chongsheng Zhao:** Resources, Investigation. **Fangwei Zhu:** Supervision, Resources. **Shuguang Wang:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization. **Yufang Wu:** Supervision, Methodology, Data curation.

Declaration of Competing Interest

The authors declared that there were no conflicts of interest or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jfca.2024.106406.

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