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Dynamic changes of anthocyanins during 'Ziyan' tea wine processing

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ABSTRACT

In this study, the dynamic changes of different anthocyanins in the processing of 'Ziyan' tea wine were investigated quantitatively. Results showed that six types of anthocyanins, namely petunidin, malvidin, pelargonidin, delphinidin, cyanidin and peonidin, as well as two co-pigmented substances, procyanidins and flavonoids, were detected in 'Ziyan' tea wine. As fermentation proceeded, the contents of petunidin, pelargonidin, delphinidin, cyanidin and peonidin decreased. Among them, petunidin, peonidin and pelargonidin showed a tendency of decreasing first, then increasing and finally decreasing, whereas delphinidin and cyanidin continued to decrease during fermentation. Variation trend of procyanidins and flavonoids was consistent with those of petunidin. Furthermore, metabolism of delphinidin, cyanidin and pelargonidin were main pathways responsible for the anthocyanin changes during 'Ziyan' tea wine processing. These findings suggested that the color of 'Ziyan' tea wine was achieved by the combination of various anthocyanins in different ratios and the co-pigmentation of procyanidins and flavonoids.

1. Introduction

Tea wine is a beverage using tea leaves as the main raw material, fermented or prepared, which combines the flavor of both tea and wine. According to the processes, it can be subdivided into fermented tea wine, prepared tea wine and gas tea wine (Chen et al., 2023). Tea wine is popular for its unique flavor and antioxidant effects (Liang et al., 2024). As a kind of wine, the criteria for evaluating the quality of tea wine and the factors affecting its consumption are consistent with those of other wines. In addition to the aroma and taste, color is also a critical factor affecting the consumption of wines, and it is the most intuitive sensory index for consumers (Feng et al., 2024). In general, the color of wine consists of chromogenic components and co-pigmentation substances, mainly including anthocyanins, tannins and phenols (Heras-Roger et al., 2016). Of which, anthocyanin is the basis of color, its structure and content affect the depth and characteristics of tea wine color (He et al., 2012). The higher the content of anthocyanins, the deeper the color. Co-pigmentation substances mainly include flavanols (flavan-3-ols and procyanidins) and flavonols (Klisurova et al., 2019).

Anthocyanin is the glycosylated product of anthocyanidin, its glycosides are mostly monosaccharides and disaccharides, such as glucose, fructose and galactose. Anthocyanidin is a natural pigment belongs to flavonoids. It is enriched in grapes, purple sweet potatoes, blueberries and other plants, with dibenzopyran as the basic structural unit (Cai et al., 2022). Currently, 650 natural anthocyanins have been identified (Yin, 2023). Of which, petunidin, malvidin, pelargonidin, delphinidin, cyanidin and peonidin are common, which presents strong antioxidant, free radical scavenging and anti-aging abilities (Garcia & Blesso, 2021; Wang et al., 2024). Anthocyanidins in wine exist in the form of anthocyanins, mainly derived from the grape peel. It has been found that both the type and content of anthocyanins undergo drastic changes during wine processing (Ai et al., 2021). During the aging of Cabernet Sauvignon wine, anthocyanins were transformed to anthocyanin-vinyl derivatives and anthocyanin-flavanol adducts, such as malvidin 3-O-glucoside-pyruvate, malvidin 3-*O*-acetylglucoside-pyruvate, malvidin 3-O-glucoside-4-vinylphenol, malvidin 3-*O*-acetylglucoside-4-vinylphenol, etc. (Wang et al., 2003). It was found that the types of anthocyanin in wine brewed with strawberries are less than those in the initial

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matrix, which was the main reason for the color change from red to orange in strawberry wine (Hornedo-Ortega et al., 2017). Similar phenomenon was observed during the blueberry wine processing, where the content of flavonoids, anthocyanins and total phenolics was significantly reduced (Zhang et al., 2022). This shows that anthocyanin alteration is an important part of the processing of pigment-rich wine. However, no systematic research on the change of anthocyanin during the processing of tea wine.

Camellia sinensis (L.) O. Kuntze (*C. sinensis*) 'Ziyan' is an anthocyanin-rich tea cultivar bred in recent years, and the anthocyanin content of fresh leaves (one bud and two leaves) in spring is 0.88 mg·g^{-1} (Lai et al., 2016). Previously, we made 'Ziyan' tea wine by mixing and fermenting 'Ziyan' green tea with yeast, sugar and other food additives like potassium metabisulfite. The obtained product combined both tea and wine aroma, with red color, and was widely popular in the market. In addition, a significant difference between the color of 'Ziyan' green tea infusion and 'Ziyan' tea wine was observed, as well as a markedly decrease in the total amount of anthocyanins (Xu, Wang, Jia, Wen, Liao, Zhao et al., 2023). Color change from tea infusion to tea wine is not only the result of the reduction of pH values, but the variation in anthocyanin content and species may also be important. However, no systematic studies have been conducted yet.

The liquid chromatography-tandem mass spectrometry (LC-MS/MS) technology is one of the most utilized bio-analytical tools owing to its advantage of selectivity, sensitivity and multitasking (Gautam & Singh, 2023). In recent years, high-resolution LC-MS/MS approaches coupled with multivariate statistics have been widely used to realize the so-called "metabolomic profiling" of plant foods for human nutrition. For example, Borowiec et al. (2024) revealed that anthocyanins, flavonols, and tannins are the pro-health components of fruit smoothie via LC-MS/MS. Spinelli et al. (2023) analyzed the major components in the Guabiju fruit and found that anthocyanins were the major phenolic compounds using LC-MS/MS. This indicates LC-MS/MS is efficient in analyzing the anthocyanins in different foods.

Therefore, in the present study, we quantitative analyzed the dynamic changes of anthocyanins during the processing of 'Ziyan' tea wine by using LC-MS/MS. We found that the content of anthocyanidin reduced in the processing of 'Ziyan' tea wine, and different types of anthocyanin present distinct variation trends. This finding will help to improve the color of 'Ziyan' tea wine, enrich the research on the quality characteristics of fermented tea wine, and provide guidance for the color control of anthocyanin-rich fermented wine in the subsequent industrial production.

2. Materials & methods

2.1. Materials and reagents

Fresh leaves (one bud and two leaves) of 'Ziyan' (*Camellia sinensis* (L.) O. Kuntze) were harvested in Muchuan County, Sichuan Province, China, and then were processed into green tea. All the 'Ziyan' green tea were stored at 4 °C until use. Yeast (*EnartisFerm SC*) was purchased from Enartis (Beijing, China). The grade and source of food additives such as fructose glucose syrup (Shandong Scents Jianyuan Biotech, Shandong, China), potassium metabisulfite (Microbio Co., Ltd., Shanghai, China) and ammonium sulfate (Wuhan Kangcan Biotech, Wuhan, China) were the same as those used in the previous study (Xu et al., 2023). HPLC-grade methanol (MeOH) was purchased from Merck (Darmstadt, Germany). All the standards with purity over 99 % were purchased from isoReag (Shanghai, China). Formic acid was purchased from Sigma-Aldrich (St Louis, MO, USA). Hydrochloric acid was bought from Xinyang Chemical Reagent (Changsha, China). The stock solutions of standards were prepared at the concentration of 1 mg·mL^{-1} in 50 % MeOH. All stock solutions were stored at −20 °C. The stock solutions were diluted with 50 % MeOH to working solutions before analysis.

2.2. Preparation of 'Ziyan' tea wine

Preparation of 'Ziyan' tea wine was performed in accordance with our previous study (Xu et al., 2023) and was repeated for three times. For each fermentation, three replications were conducted. The fermentation broth was collected before the fermentation (day 0), on the 2nd (the end of aerobic fermentation), 4th, 6th, 8th, 10th and 12th day of fermentation, respectively. The specifical procedure was presented in Fig. S1. All the samples were stored at −80 °C until use.

2.3. Measurement of major biochemical components

Content of sugar, total acid, total anthocyanin, alcohol and pH values were determined as the methods described by Xu et al. (2023).

2.4. Qualitative and quantitative analysis of anthocyanins

2.4.1. Sample preparation and extraction

About 2 mL of tea wine samples were lyophilized and then diluted with 1000 μL extract (50 % aqueous methanol solution containing 0.1 % hydrochloric acid). The sample was vortexed for 1 min and then centrifuged at 12,000*g* under 4 °C for 2 min. The supernatants were collected and transferred for the LC-MS/MS analysis.

2.4.2. UPLC conditions

The sample extracts were analyzed using an UPLC-ESI-MS/MS system (UPLC, ExionLC™ AD , https://sciex.com.cn/; MS, Applied Biosystems 6500 Triple Quadrupole, https://sciex.com.cn/). The analytical conditions were as follows, UPLC: column, WatersACQUITY BEH C18 (1.7 μm, 2.1 mm × 100 mm); solvent system, water (0.1 % formic acid): methanol (0.1 % formic acid); gradient program, 95:5 *V*/V at 0 min, 50:50 V/V at 6 min, 5:95 V/V at 12 min, hold for 2 min, 95:5 V/V at 14 min; hold for 2 min; flow rate, 0.35 mL·min^{-1}; temperature, 40 °C; injection volume, 2 μL.

2.4.3. ESI-MS/MS conditions

Linear ion trap (LIT) and triple quadrupole (QQQ) scans were acquired on a triple quadrupole-linear ion trap mass spectrometer (QTRAP), QTRAP® 6500+ LC-MS/MS System, equipped with an ESI Turbo Ion-Spray interface, operating in positive ion mode and controlled by Analyst 1.6.3 software (Sciex, MA, USA). The ESI source operation parameters were as follows: ion source, ESI+; source temperature: 550 °C; ion spray voltage (IS) 5500 V; curtain gas (CUR) was set at 35 psi, respectively. Anthocyanins were analyzed using scheduled multiple reaction monitoring (MRM). Data acquisitions were performed using Analyst 1.6.3 software (Sciex, MA, USA). Multiquant 3.0.3 software (Sciex, MA, USA) was used to quantify all metabolites. The standard curve of different metabolites was shown in Table S1. Mass spectrometer parameters including the declustering potentials (DP) and collision energies (CE) for individual MRM transitions were done with further DP and CE optimization. A specific set of MRM transitions were monitored for each period according to the metabolites eluted within this period.

Significantly regulated metabolites between groups were determined by $|Log_2FC$ (fold change) $| \geq 1$. Identified metabolites were annotated using KEGG compound database (http://www.kegg.jp/kegg/compound/), annotated metabolites were then mapped to KEGG Pathway database (http://www.kegg.jp/kegg/pathway.html). Pathways with significantly regulated metabolites mapped to were then fed into MSEA (metabolite sets enrichment analysis), their significance was determined by hypergeometric test's *p*-values.

2.5. Statistical analysis

All the experiment was repeated for three times. Data are expressed as means ± SEM. Statistical analyses were performed using SPSS (IBM Corp, v23, Armonk, NY, USA). For samples that pass the homogeneity of variance test, one-way ANOVA with Duncan multiple comparisons tests was used. For samples that did not pass the homogeneity of variance test, significance was calculated using the Tamhane T2 test. A value of $p < 0.05$ indicated statistical significance.

3. Results

3.1. Dynamic changes of the major biochemical components during 'Ziyan' tea wine fermentation

To understand the changes of taste and color, we examined the changes of total sugar, acid, anthocyanin content and pH values in the fermentation process of 'Ziyan' tea wine. As shown in Fig. 1A, the pH value decreased gradually as the fermentation progressed, with statistical significance observed both from the original tea broth (day 0) to the end of aerobic fermentation (day 2) and from the end of aerobic fermentation to the 2nd day of anaerobic fermentation (day 4) (Fig. 1A). In the later stage of anaerobic fermentation (day 6-day 12), the pH values almost remained unchanged. This is owing to the reduced yeast activity and slow production of carbon dioxide at this stage. Meanwhile, total acid of the fermentation broth increased as the fermentation progressed, and by the 10th day of fermentation (8th day of anaerobic fermentation), significant differences began to observed when compared to the initial tea infusion (Fig. 1B). However, the total sugar content showed a decreasing trend from the initial 15 % to approximately 8 %, which was attributed to the action of the yeast (Fig. 1C). Similarly, we determined the total amount of anthocyanidin during the fermentation of tea wine. The anthocyanidin content continued to decrease as the fermentation proceeded. From the start of fermentation to the 2nd day, the anthocyanidin content decreased by about one-third, and by the end of fermentation (day 12), the total amount of anthocyanidins was about 3 $\mu g \cdot mL^{-1}$, which was only approximately 1/5 of the initial fermentation broth (Fig. 1D).

3.2. Overall change of anthocyanin before and after fermentation of 'Ziyan' tea wine

Anthocyanin is a natural water-soluble pigment widely distributed in 'Ziyan' tea. As shown in Fig. 2A, the color of the fermentation broth changed from purple-blue to deep-red before and after fermentation, which is not merely a result of pH variation. To explore the reasons for the color change of 'Ziyan' tea wine, we analyzed the content of anthocyanin components in the fermentation broth. The results showed that a total of 125 anthocyanins, 2 flavonoids, and 5 procyanidins were detected in 'Ziyan' tea wine. The 6 types of anthocyanins commonly found in food were all presented in 'Ziyan' tea wine, with cyanidin being the most abundant at 50, followed by delphinid and pelargonidin (Fig. 2B, Table S2). Furthermore, only 49 anthocyanins, 2 flavonoids and 5 procyanidins were detected in all fermentation broth (Fig. 2B, Table S2). Compared with the original fermentation broth, the number of differential anthocyanins gradually increased with fermentation. At the end of fermentation, the number of differential anthocyanins was up to 41(Fig. 2C, Table S2). Among them, the content of 24 type of substances decreased whereas 17 substances increased in comparison with the original fermentation broth (Fig. 2D, Table S2). The heatmap showed that most of the anthocyanins decreased from tea infusion to tea wine, and only the levels of cyanidin-3-O-sophorotriose, malvidin-3-O-(glucosyl)-glucuronide and pelargonidin-3-O-(6-O-malonyl-beta-D-

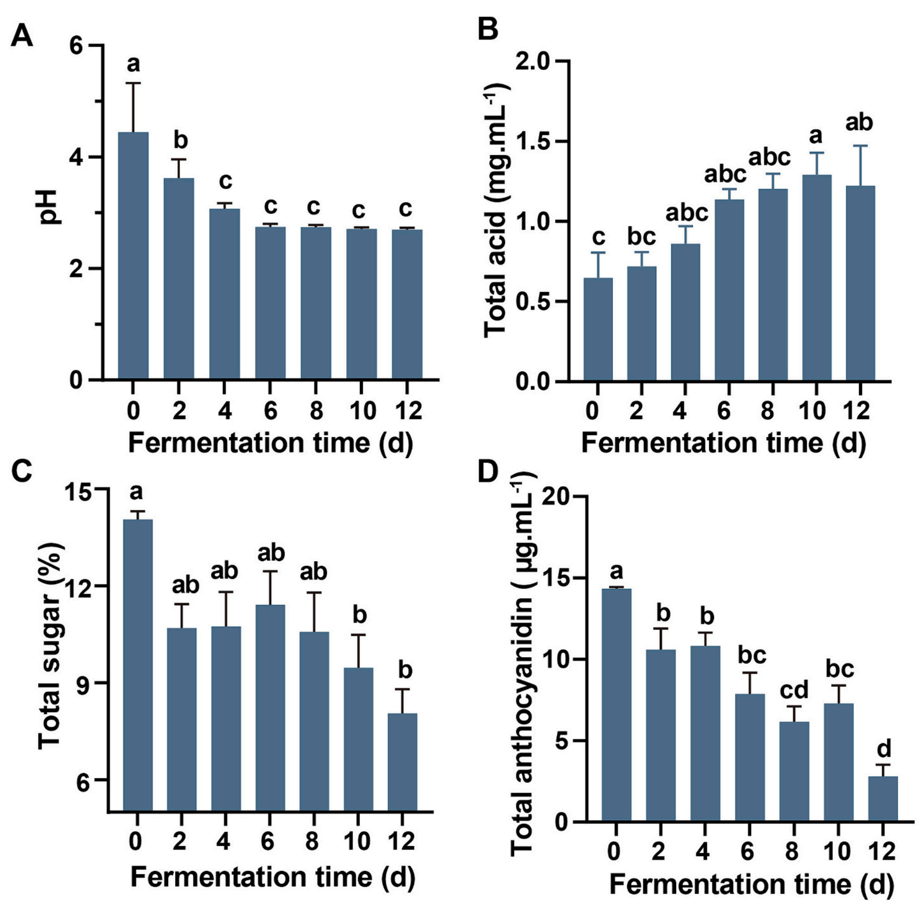

Fig. 1. Dynamic changes of major biochemical components during 'Ziyan' tea wine fermentation. The pH values (**A**), content of total acid (**B**), total sugars (**C**) and total anthocyanidin (**D**) in different stages of 'Ziyan' tea wine fermentation. Data are means ± SEM. Bars with different letters indicate a significant difference ($p < 0.05$).

glucoside) increased (Fig. 2E).

3.3. Dynamic changes of different anthocyanins during the fermentation of 'Ziyan' tea wine

As evident in Fig. 3, the anthocyanins with high levels in 'Ziyan' tea wine were mainly delphinidin, cyanidin and pelargonidin, the content of which were all exceeded 1 μg·mL^{-1}. Besides, flavonoids and procyanidins, especially procyanidins, were abundant in 'Ziyan' tea wine. As fermentation proceeded, the total amount of the six anthocyanins showed two different trends, in which the contents of petunidin, malvidin, peonidin and pelargonidin showed a tendency of decreasing first, then increasing, and decreasing again (Fig. 3A,D,E,G). On the 12th day of fermentation, the concentrations of petunidin, peonidin and pelargonidin were significantly lower than those of the original fermentation broth, whereas no significant difference was observed in the malvidin content during 'Ziyan' tea wine fermentation (Fig. 3A,D,E,G). The content of delphinidin and cyanidin continued to decrease during the fermentation of tea wine. Both decreased significantly from the 2nd to the 4th day of fermentation, and their contents were significantly lower than those of the original fermentation broth by the 12th day (Fig. 3B and F). Interestingly, the total amount of procyanidins and flavonoids showed a consistent trend of decreasing, then increasing and decreasing again during the fermentation process. However, no significant

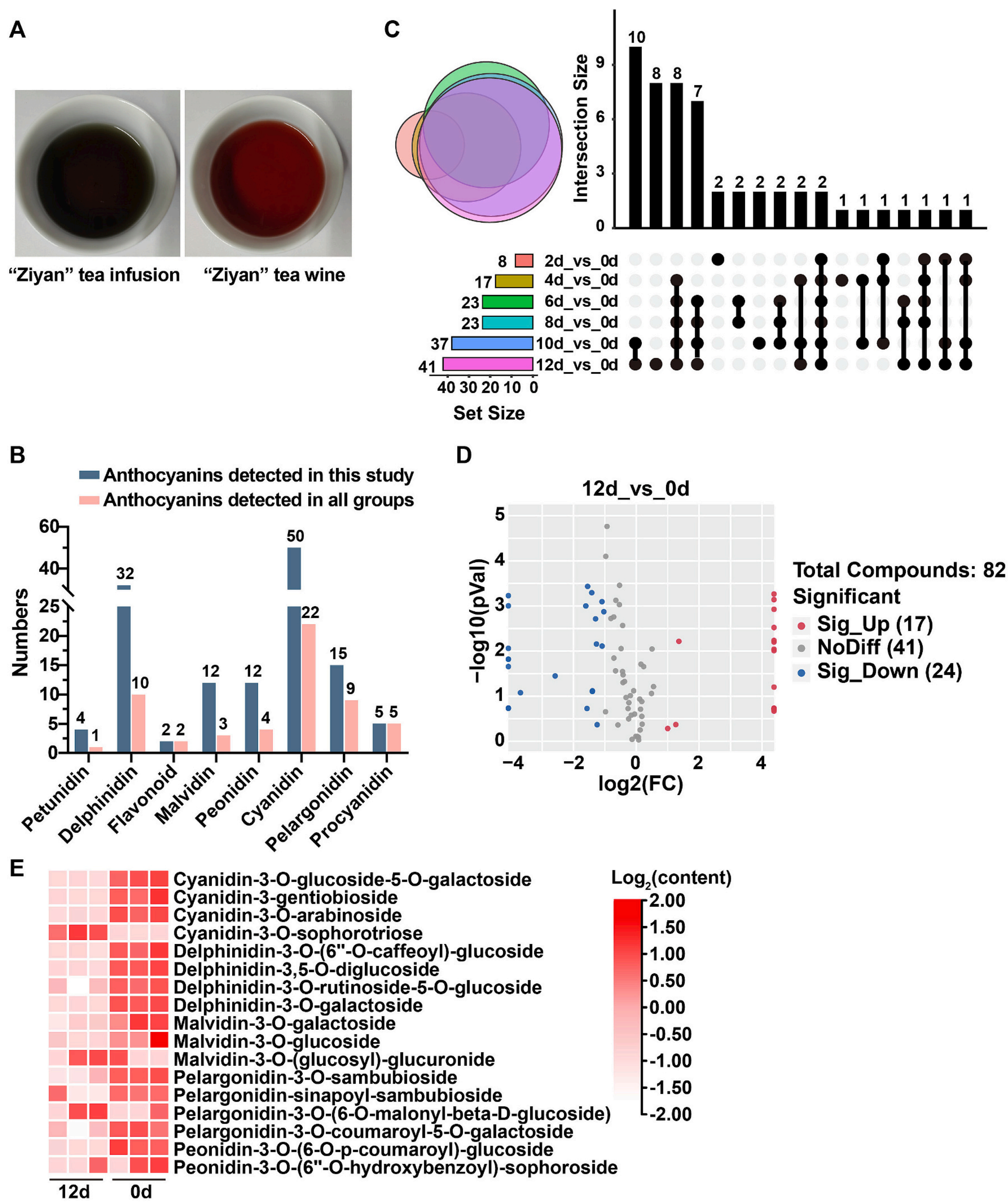

Fig. 2. Overall changes of anthocyanin before and after fermentation of 'Ziyan' tea wine. (**A**) 'Ziyan' tea infusion and 'Ziyan' tea wine. (**B**) Numbers of different types of anthocyanins detected in the present study. (**C**) Upset plot of differential anthocyanins during 'Ziyan' tea wine processing. (**D**) Volcano plot of differential anthocyanins between the 12th day and the original fermentation broth. (**E**) Heatmap of differential anthocyanins screened from 12d_vs._0d.

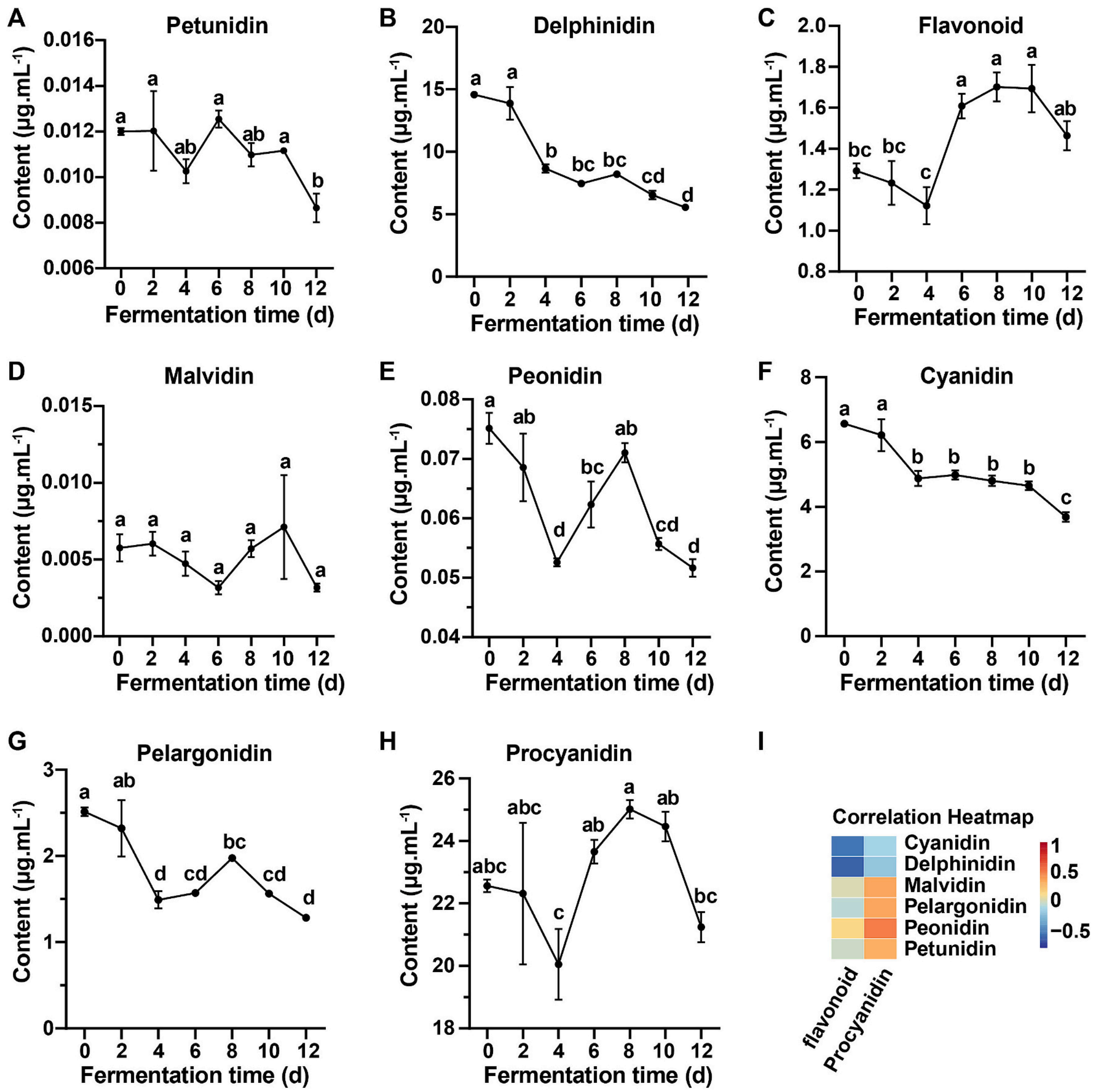

Fig. 3. Line chart of different types of anthocyanins during fermentation of 'Ziyan' tea wine. Changes of Petunidin (**A**), Delphinidin (**B**), Flavonoid (**C**), Malvidin (**D**), Peonidin (**E**), Cyanidin (**F**), Pelargonidin (**G**) and Procyanidin (**H**) in the processing of 'Ziyan' tea wine. (**G**) Correlation analysis of flavonoid and procyanidin with other six types of anthocyanins.

difference in the concentration of procyanidins and flavonoids was observed between the tea wine on the 12th day and the initial fermentation broth (Fig. 3C and H). This suggests that the changes of these two substances during the fermentation of 'Ziyan' tea wine may be related to the degradation of other types of anthocyanins. Correlation analysis showed that the content of flavonoids was negatively correlated with cyanidin, delphinidin, pelargonidin and petunidin, whereas positively related with peonidin. In contrast, except for cyanidin and delphinidin, procyanidin was positively correlated with the other four types of anthocyanins (Fig. 3I).

Then the dynamics changes of anthocyanins with relative higher level during the fermentation of tea wine was analyzed. The changes of quercetin-3-O-glucoside and naringenin (two types of flavonoids) were consistent with those of procyanidin B2, procyanidin B3, procyanidin B4 and procyanidin C1 (four types of procyanidins), which were the same as the total amount of flavonoids and procyanidins (Fig. 4A-4F). Except for naringenin and procyanidin C1, the contents of all other components exhibited a minimum value at the 4th day whereas a maximum at the 8th–10th day of fermentation (Fig. 4A–F). Pelargonidin-3-O-galactoside, the highest constituent of pelargonidin detected in this study, the content of which was the most in the original fermentation infusion. With the progress of fermentation, it decreased first, then increased and further decreased. On the 6th–8th day of fermentation, it increased slightly, and by the end of fermentation, the level was only half that of the initial fermentation broth (Fig. 4G). The content of peonidin-3-O-(6-O-p-coumaroyl)-glucoside, the most abundant peonidin, decreased continuously during the fermentation process, and reduced by about a half at the end of fermentation, which was significantly different from that of the original fermentation broth (Fig. 4H). Meanwhile, the trend of petunidin-3-O-(6-O-p-coumaroyl)-glucoside (the highest component of petunidin in the present study) was consistent with that of pelargonidin-3-O-galactoside during 'Ziyan' tea wine fermentation (Fig. 4I). The content of delphinidin-3-O-galactoside, delphinidin-3-O-(6"-O-caffeoyl)-glucoside and delphinidin-3,5-O-diglucoside decreased during the fermentation of 'Ziyan' tea wine, and the trend was in line with that of cyanidin-3-O-arabinoside and cyanidin-3-O-(6"-O-coumaryl)-galactoside. All these substances were decreased first, then increased, and finally decreased, with the most dramatic reduction occurring from the 2nd day to 4th day of fermentation (the earlier stage of anaerobic fermentation) (Fig. 5A-5C, 5E and 5F). The content of

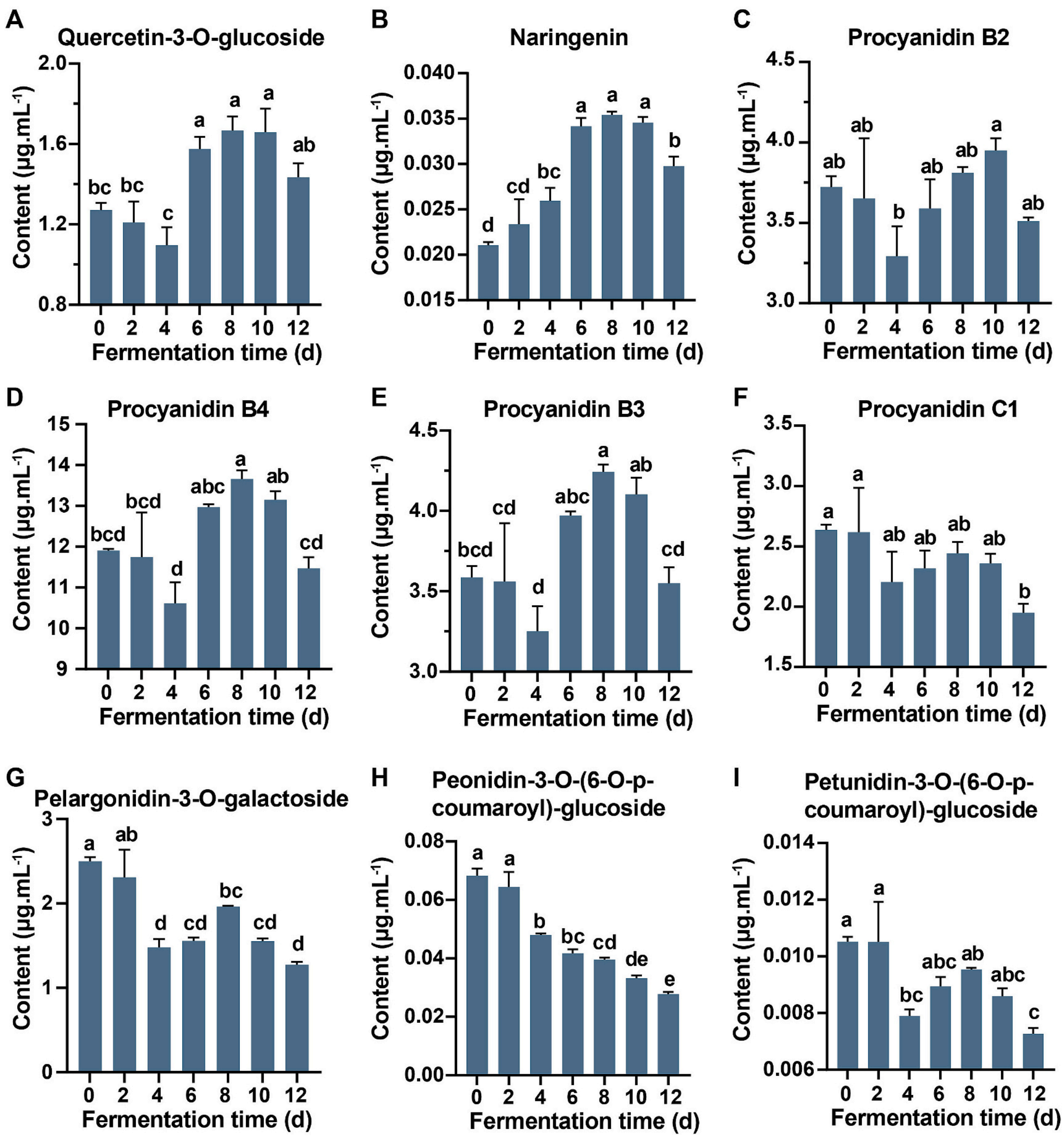

Fig. 4. Variation of higher content components of flavonoids, procyanidins, pelargonidins, peonidins and petunidins over fermentation time. Changes in Quercetin-3-O-glucoside (**A**), Naringenin (**B**), Procyanidin B2 (**C**), Procyanidin B4 (**D**), Procyanidin B3 (**E**), Procyanidin C1 (**F**), Pelargonidin-3-O-galactoside (**G**), Peonidin-3-O-(6-O-p-coumaroyl)-glucoside (**H**), and Petunidin-3-O-(6-O-p-coumaroyl)-glucoside (**I**) during the processing of 'Ziyan' tea wine.

cyanidin-3-O-galactoside continued to decrease during fermentation and a significant difference was observed by day 12 (Fig. 5D).

3.4. Delphinidin, cyanidin and pelargonidin metabolism is the main pathway of anthocyanin changes during the processing of 'Ziyan' tea wine

To elucidate the mechanism of anthocyanins changes during the processing of 'Ziyan' tea wine, we analyzed the KEGG pathways for the differential anthocyanins screened by day 12 vs. day 0. The result showed that these differential anthocyanins were enriched in flavonoid biosynthesis, secondary metabolite biosynthesis and anthocyanin biosynthesis pathways (Fig. 6A). Furthermore, these differential metabolites were mainly enriched in delphinidin, cyanidin and pelargonidin metabolic pathways (Fig. 6B–D), indicating that the metabolism of delphinidin, cyanidin and pelargonidin was the main pathway of anthocyanin changes during the processing of 'Ziyan' tea wine.

4. Discussion

In the present study, we investigated the dynamic changes of anthocyanins during the fermentation of tea wine processed from 'Ziyan' (a tea cultivar rich in anthocyanin) green tea. Consistent with the previous study (Xu et al., 2023), the total amount of anthocyanidin decreased during the fermentation of 'Ziyan' tea wine, and six typical anthocyanidin types were presented in 'Ziyan' tea wine. Besides, two co-pigmented substances, procyanidins and flavonoids, were also detected. However, we found that the content of petunidin, pelargonidin, delphinidin, cyanidin and peonidin decreased during the fermentation process, except for malvidin. Furthermore, delphinidin, cyanidin and pelargonidin metabolism were the main pathways of anthocyanin changes during the processing of 'Ziyan' tea wine. This indicates that the color of 'Ziyan' tea wine is the result of the combination of anthocyanins with different concentrations and proportions, as well as the co-pigmentation of procyanidins and flavonoids.

Anthocyanidin is a natural water-soluble flavonoid pigment that is widely used in the food, pharmaceutical and cosmetic industries due to

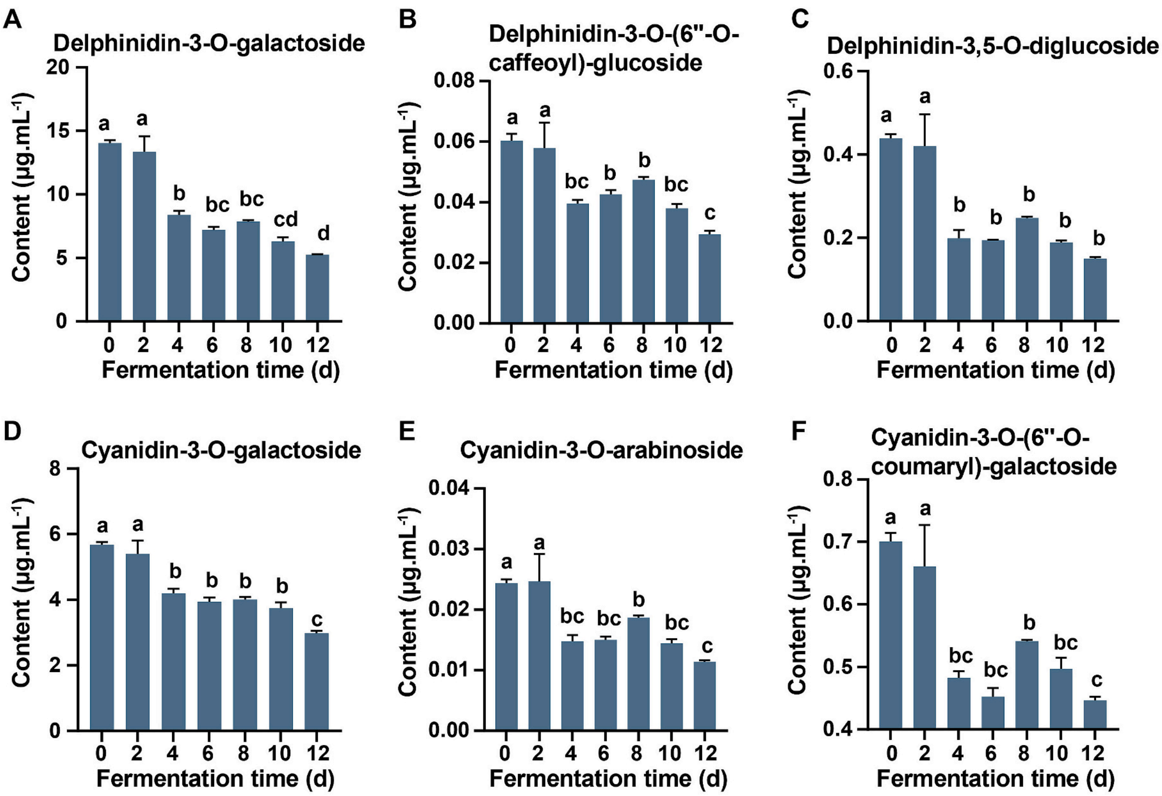

Fig. 5. Dynamic changes of components with higher content in delphinidin and cyanidin over fermentation time. Histograms illustrate changes of Delphinidin-3-O-galactoside (**A**), Delphinidin-3-O-(6"-O-caffeoyl)-glucoside (**B**), Delphinidin-3,5-O-diglucoside (**C**), Cyanidin-3-O-galactoside (**D**), Cyanidin-3-O-arabinoside (**E**), and Cyanidin-3-O-(6"-O-coumaryl)-galactoside (**F**).

its strong antioxidant properties. Anthocyanins are structurally based on flavin cations, including delphinidin, cyanidin, petunidin, peonidin, pelargonidin and malvidin (Xu et al., 2018). In the present study, all these six types of anthocyanidins were detected in 'Ziyan' tea wine, and this result is consistent with that in other wines (Laitila et al., 2019; Nyman & Kumpulainen, 2001). Typically, the higher the anthocyanin content, the deeper the color. During the fermentation and aging of red wines, anthocyanin content decreases (Hornedo-Ortega et al., 2017), and an identical result was observed in 'Ziyan' tea wine. The reduction in anthocyanin content is not only induced by alterations in temperature and pH during fermentation, but also may be attributed to the action of β-glucoside secreted by *Saccharomyces cerevisiae* as well as the direct adsorption of anthocyanins by the yeast cell wall (Echeverrigaray et al., 2020). In this study, anthocyanins with higher content were delphinidin, cyanidin, and pelargonidin. This result is in agreement with that observed by Lai et al. (2016) in 'Ziyan' fresh leaves, where high levels of delphinidin, cyanidin, and pelargonidin, while no other types of anthocyanins were detected (Lai et al., 2016). Nevertheless, in addition to the three anthocyanins mentioned above, petunidin, peonidin, and malvidin were also detected in 'Ziyan' tea wine. This may be caused by the effect of temperature and enzymes during the processing of 'Ziyan' green tea or the fermentation of 'Ziyan' tea wine, whereas the specific details need to be explored further. Delphinidin appears bright blue, pelargonidin presents orange to brick red, and cyanidin shows pink to red, and the color of which depends on their concentration. Low concentrations of cyanidins confer reddish-purple, while high concentrations of cyanidins present blue (Deng et al., 2020). The variation in the content of delphinidin and cyanidin during fermentation also confirms the change in color from tea infusion to wine illustrated in Fig. 2A to some extent. However, the concentration of mallow did not change during the fermentation of 'Ziyan' tea wine, which was inconsistent with the results observed in blueberry wine (Xiong et al., 2022), and this may be due to the diverse parameters used in the fermentation process (Setford et al., 2019; Zhang et al., 2022). Interestingly, the glycosides of anthocyanins with high level (>1 μg·mL^{-1}) in this study were galactosides, such as pelargonidin-3-O-galactoside (Fig. 3G), cyanidin-3-O-galactoside (Fig. 4D) and delphinidin-3-O-galactoside (Fig. 4A). This is because in purple or red-bud tea cultivars like 'Ziyan' and 'Zijuan', the anthocyanidins were mainly delphinidin-3-O-galactoside and cyanidin-3-O-galactoside (He et al., 2021). Furthermore, it was found that peonidin, petunidin and malvidin were formed by further modification of the structure of cyanidin and delphinidin (Tanaka & Ohmiya, 2008). In other words, pelargonidin, cyanidin and delphinidin are the three basic types of anthocyanins. Therefore, in the present study, the metabolism of anthocyanins in 'Ziyan' tea wine mainly focused on the metabolic pathways of these three types of pigments (Fig. 6B–D).

It has been found that the color of wine is also related to the co-pigmentation substances (cofactors) (Ujihara & Hayashi, 2020). These co-pigmentation substances enhance the color of red wine by stabilizing the structure of colored substances through intermolecular interactions (Bimpilas et al., 2016; Figueiredo-Gonzalez et al., 2013). Co-pigmentation substances in red wine mainly include phenolic acids, flavonols and flavonoid derivatives, alkaloids, and procyanidins (Rustioni et al., 2012). In the present study, high levels of procyanidins and flavonoids were detected in 'Ziyan' tea wine, suggesting that procyanidins and flavonoids are most likely to be involved in the formation of the color of 'Ziyan' tea wine through intermolecular co-pigmentation. A previous study showed that the co-pigmentation effect of anthocyanins is affected by pH, which is stronger when the pH is around 3.3 or even lower (Forino et al., 2019). As evident in Fig. 1A, the pH of the fermentation broth was below 4 from the anaerobic fermentation stage to the completion of fermentation (day 2-day 12), indicating that the co-pigmentation of anthocyanins has been involved in the formation of 'Ziyan' tea wine color from the beginning of anaerobic fermentation. In

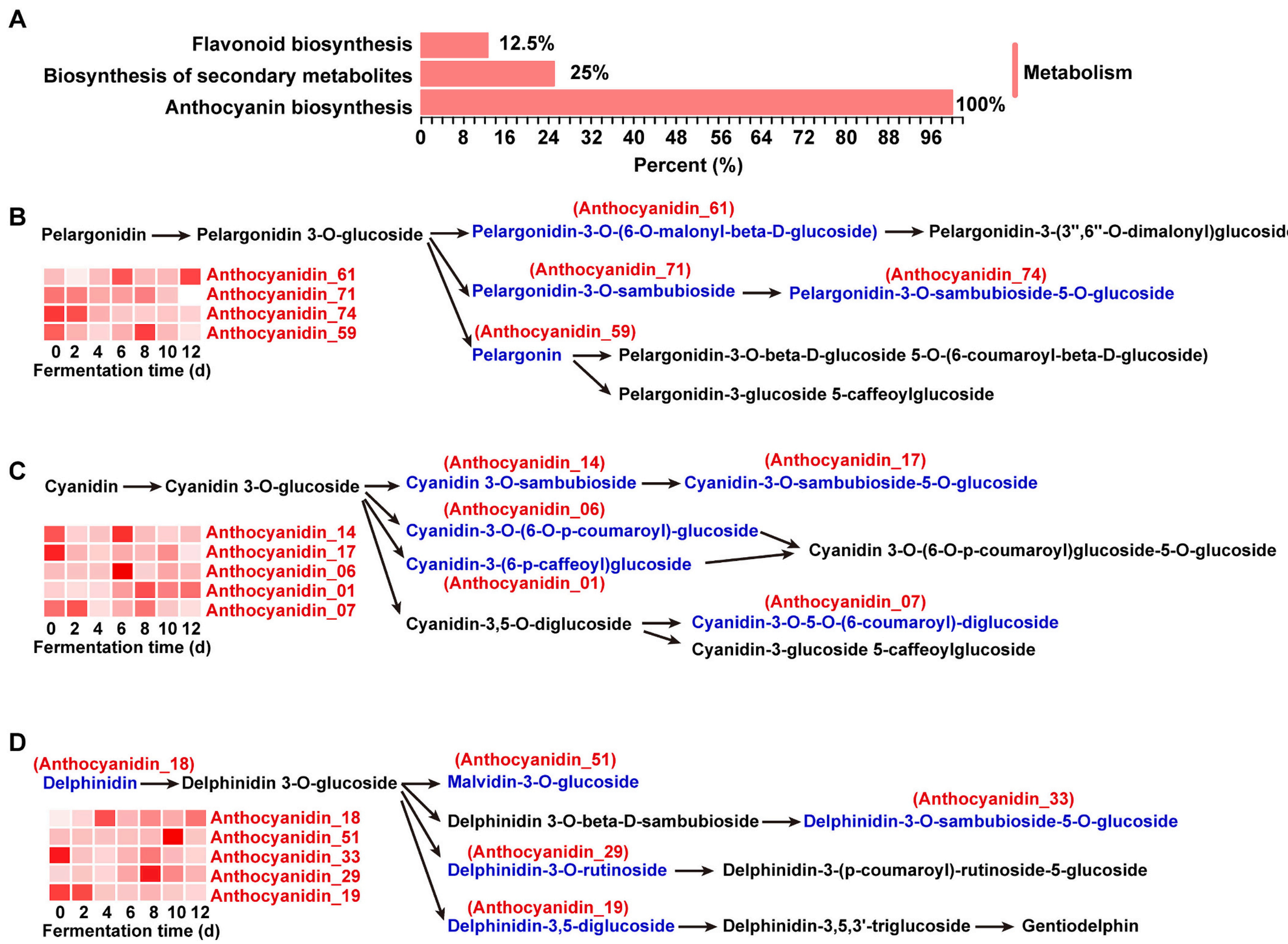

Fig. 6. Metabolic pathways for the anthocyanin changes during 'Ziyan' tea wine fermentation. (A) KEGG pathways of differential metabolites screened from 12d_vs._0d. (**B**—**D**) Pathways responsible for the variation of anthocyanin during 'Ziyan' tea wine processing.

fresh red wines, anthocyanin co-pigmentation accounts for more than 30 % of the red wine color, whereas during aging, the color of red wines was dominated by polymeric pigments of anthocyanins and procyanidins (Bimpilas et al., 2016). In the present study, the content of procyanidins was much higher than that of anthocyanins, and showed a tendency of decreasing first, then increasing, and finally decreasing. This may be explained by the fact that the synthesis pathways of anthocyanins and procyanidins share the public phenylpropane pathway and the core flavonoid-anthocyanin pathway, thus anthocyanins can be served as substrates to synthesize anthocyanins and procyanidins in different tissues and conditions (Zhao et al., 2023). During the fermentation of 'Ziyan' tea wine, the conversion between anthocyanin and procyanidin synthesis may occur in the presence of enzymes, leading to the variation of procyanidins. Additionally, studies have shown that yeast (Mekoue Nguela et al., 2015), insoluble fibres (Bindon & Smith, 2013), and plant-derived proteins (Granato et al., 2010) can adsorb procyanidins. Therefore, the changes in procyanidin content in this study may also be attributed to crude fibre as well as protein in *Saccharomyces cerevisiae* yeast and tea. However, it remains to be explored further.

5. Conclusion

In conclusion, this study systematically investigated the dynamic changes of anthocyanins during the fermentation of 'Ziyan' tea wine. We found that the total amount of anthocyanidins decreased during fermentation, and six typical anthocyanins were detected in tea wine. Except for mallow, the content of the other five anthocyanins decreased during fermentation, but their trend was not consistent. In addition, a large amount of procyanidins and flavonoids were identified in the fermentation broth, and their variation trend was coherent. The result indicates that the color change from 'Ziyan' tea infusion to 'Ziyan' tea wine is not only the result of the mixture of different anthocyanins with different concentrations and ratios, but the co-pigmentation of procyanidins and flavonoids with anthocyanins is also important. The findings provide strong evidence to understand the color changes of fermented wines rich in anthocyanins, and promote the deeper use of tea as well as the diversification of the tea industry.

CRediT authorship contribution statement

Ling Lin: Writing – original draft, Project administration, Methodology, Conceptualization. **Keke Li:** Writing – original draft, Project administration, Investigation. **Yajie Hua:** Validation, Software, Investigation. **Siyu Liao:** Software, Resources, Investigation. **Jiaru Chen:** Software, Investigation. **Liqiang Tan:** Writing – review & editing, Software. **Yang Yang:** Resources. **Bo Sun:** Writing – review & editing, Supervision, Software. **Qian Tang:** Writing – review & editing, Supervision. **Wei Xu:** Writing – review & editing, Funding acquisition, Conceptualization.

Declaration of competing interest

There are no conflicts to declare.

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Data availability

Data will be made available on request.

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