Influence and metabolomic basis of an indigenous yeast CECA, from Ningxia wine region of China, on the aroma and flavor of Cabernet Sauvignon wines

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A R T I C L E   I N F O

A B S T R A C T

In this study, three fermentation treatments of spontaneous fermentation (SF), direct inoculation of CECA (YF), and inoculation with CECA after addition of dimethyl dicarbonate (YDF) were carried out. Multivariate statistical analysis approved that CECA inoculation significantly influenced the composition of 141 metabolites (15 volatile organic compounds (VOCs) and 126 non-VOCs), mainly consisting of 36 acids and derivatives and 25 lipids and lipid-like molecules. YF and YDF wines exhibited similar correlations with aroma types, while there were differences in the kinds and number of VOCs. Moreover, CECA-inoculated fermentation was more favorable to the formation of aftertaste-A, umami, sourness, and richness. The KEGG metabolic pathway analysis indicated that the inoculation strategy significantly affected the amino acid metabolism. The antimicrobial treatment effectively enhanced bitterness, astringency, umami and saltiness while reducing acidity. Further studies are needed to assess the effects of antimicrobial treatment on lipid metabolism.

1. Introduction

Wine has commercial economic, and sociocultural values, and its chemical composition and quality are influenced by several factors. Among these, the yeast plays a decisive role in manipulating the wine's flavor, aroma, and style during fermentation, providing far more reliability for vinification (Pinu et al., 2023; Van Wyk, Grossmann, Wendland, Von Wallbrunn, & Pretorius, 2019). Ningxia is a premium winemaking region in China. According to the statistics of People's Daily (2023), as of May 2023, the wine grape planting area in Ningxia has reached 583,000 mu, with 228 existing wineries and an annual production of 138 million bottles. Currently, the industrialized wine production in Ningxia, China, mainly relies on imported active dry yeasts, which leads to serious homogenization of wines and is not beneficial to the shaping of regional wine styles. Moreover, the stability and repeatability of spontaneously fermented wines were unsatisfactory (D. Liu, Legras, Zhang, Chen, & Howell, 2021; Mas & Portillo, 2022). Using indigenous *Saccharomyces cerevisae* as inocula is an ideal alternative to

traditional commercial starter and spontaneous fermentation. This controlled "spontaneous" fermentation ensures the quality of the wines and enhances the regional typicality (Topić Božič et al., 2022; Tufariello et al., 2023; Wang, Liang, Yang, Wu, & Qiu, 2022). Studies have been widely reported on applying indigenous *S. cerevisiae* with excellent fermentative and oenological properties as a starter to enhance wine aroma and quality.

CECA (formerly named N11424) is an excellent indigenous *S. cerevisiae* strain isolated from the spontaneous fermentation process of Cabernet Sauvignon wines from Ningxia, China, and has been commercially used in recent years (N. Liu et al., 2016). It has been reported that CECA produced wines with relatively high levels of alcohols, aldehydes, ketones, esters, and proanthocyanins, with honey, pepper, more fruit flavors, and a more stable color than other commercial *S. cerevisiae* strains (Lin et al., 2018; N. Liu, Song, Qin, Gong, & Liu, 2015, Liu et al., 2016; Wang et al., 2022). Moreover, the excellent fermentation characteristics and high implantation percentages of CECA during Cabernet Sauvignon wine fermentations in Ningxia, and floral

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and fruity aromas were confirmed to be the characteristics of CECA (Zhang, Wang, Zhang, & Sun, 2023). Whereas these studies have described the characteristics of metabolites, how does indigenous starters affect the wine flavor compounds and the interaction between the microorganisms need further investigation.

The kinds and content of wine matrix compounds affect wine qualities. According to the volatility of these compounds, these compounds are classified as volatile organic compounds (VOCs) and non-VOCs. Among them, VOCs contribute to the postnasal aromas of wine, while non-VOCs mainly contribute more to the wine's flavor, mouthfeel, and color (Sáenz-Navajas, Fernández-Zurbano, & Ferreira, 2012; Villamor & Ross, 2013). Synergistic and masking effects between different kinds of VOCs and non-VOCs can alter their partition coefficients, thus affecting the aroma and flavor of wine (Nicolli et al., 2018; Styger, Prior, & Bauer, 2011). Traditional flavor chemistry studies were based on targeted approaches to detect and quantify specific compounds, which can only detect a limited number of compounds. In comparison, flavoromics relies on the non-targeted approach, which allows for the simultaneous detection of unspecified metabolites and establishes a link between metabolites and organoleptic properties of wines (Cuadros-Inostroza, Verdugo-Alegría, Willmitzer, Moreno-Simunovic, & Vallarino, 2020; Z. Wang et al., 2023). For instance, Bettenhausen et al. (2020) investigated the effects of barley varieties on beer flavor by integrating sensory and non-targeted metabolomics approaches. Moreover, non-targeted metabolomics could effectively characterize the metabolite profiles of wine flavors. Further studies on flavor (taste and aroma) and metabolite correlations using chemometrics techniques will provide new ideas for studying the metabolic pathways of flavor compounds.

Therefore, the aims of this study were to investigate the effects of CECA on the overall metabolite profile of Cabernet Sauvignon wines, and to assess its contribution to the flavor quality of the wines. Cabernet Sauvignon wines were fermented using three different fermentation strategies: spontaneous fermentation without inoculation (SF), inoculated fermentation with CECA yeast (YF), and inoculated fermentation with CECA yeast after grape must antibacterial by dimethyl dicarbonate (YDF). Metabolite profiles of Cabernet Sauvignon wines under the three fermentation strategies were analyzed by headspace solid phase microextraction in combination with a gas chromatography coupled to a mass spectrometric detection (HS-SPME-GC–MS), ultra-high performance liquid chromatography-Q Exactive HF-X mass spectrometer (UHPLC-Q Exactive HF-X). Then, the correlation between the metabolic profiles and sensory properties were discussed. Finally, the differential metabolites were screened by multivariate statistical analysis and their metabolic pathways were analyzed. The results of this study are expected to enrich our understanding of the effects of indigenous *S. cerevisiae* strains on wine aroma and flavor composition, and on shaping the wines quality in Ningxia region.

2. Materials and methods

2.1. Materials

Ripe and physically undamaged Cabernet Sauvignon grapes (50 kg per fermentation treatment, total 150 kg grape samples) were aseptically collected in 2020 in Ningxia, China. Grape samples were transported to the laboratory in refrigerated condition at 4 °C. The *S. cerevisiae* CECA was purchased by AngelYeast Co., Ltd.

2.2. Wine fermentation

The wine fermentation process was consistent with the previous study (Zhang et al., 2023). The basic oenological parameters of the grapes must were as follows: pH was 3.64, total sugar was 268.82 g/L, and total acidity was 3.45 g/L. Briefly, Cabernet Sauvignon grapes were manually destemmed and crushed, followed by the addition of 60 mg/L potassium metabisulfite and 30 mg/L pectinase. Three different

fermentation strategies: spontaneous fermentation (SF), direct inoculation with 20 g/hL CECA (YF), and addition of 240 mg/L dimethyl dicarbonate followed by inoculation of 20 g/hL CECA were conducted (YDF). Three biological replicates were set up for each fermentation treatment, and the volume of the fermenter was 10 L containing 8 L of grape must, and the alcoholic fermentation was carried out at 24–26 °C, with punching for 15 min every 8 h. Each flask was sealed with a sterile rubber cork with fermentation airlocks. The density and temperature of the fermentation broth were monitored daily until the completion of alcoholic fermentation. Malolactic fermentation (MLF) was not performed at the end of alcoholic fermentation during winemaking. The physicochemical properties of the Cabernet Sauvignon wine samples under the three fermentation modes were as follows: residual sugar content was 12.94 to 13.34 g/L, total acidity was 5.75 to 6.37 g/L, pH was 3.82 to 4.03, and alcohol content was 14.33 to 14.62% in the final wines (Zhang et al., 2023). The results showed no significant difference in the above-mentioned physicochemical properties in SF, YF, and YDF wines.

2.3. Electronic senses analysis

Taste properties of wine samples were collected by the Alpha ASTREE *E*-tongue (Alpha M.O.S. Toulouse, France), which consists of an LS48 autosampler unit, a set of crossselective chemosensors (consisting of 7 working electrodes and 1 reference electrode (Ag/AgCl electrode)) and a chemometric software package. The samples were diluted onefold in distilled water before E-tongue analysis to achieve optimum sensitivity of the E-tongue sensor. Wines samples from all three fermentation strategies were taken in triplicate, and the E-tongue was repeated 6 times from each sample, with the data from the last 3 times being selected as raw data for subsequent analysis (Fliszár-Nyúl, Zinia Zaukuu, Szente, Kovacs, & Poór, 2023). The time for each acquisition was set to 120 s, and measurements were obtained once per second. The stirring rate was 60 rpm, and the cleaning time was 10 s. The average of the last 20 s (100–120 s) measurements was used as the output value, representing the stable and optimal sensitivity of the different sensors.

2.4. Volatile metabolites determination

HS-SPME was used to extract VOCs from wine samples. 2 mL of wine sample and 1 μL of 50 μg/mL N-pentacosane-d32 (internal standard, CAS# 36340–20-2) were added to the headspace vial for quantifying VOCs, and the detection was carried out twice as a technical repetition. The vials were equilibrated for 10 min in a heating box at 60 °C, injected SPME fiber (with DVB/CAR/PDMS 50/30 μm) into the headspace, and extracted under the same conditions for 28 min. Subsequently, using VF-WAXms capillary column (25 m × 0.25 mm × 0.2 μm, Agilent CP9204) separated VOCs and absorbed by GC–MS (PAL RTC 120–8890-5977B, Agilent Technologies, USA) for their identification and quantification. Helium (1.5 mL/min) was used as the carrier gas, and the temperature of the column chamber at 40 °C for 2 min, then increased to 100 °C (5 °C/min), then to 230 °C (15 °C/min) and maintained for 5 min. The MS temperatures of the quadrupole and ion source were 150 °C and 230 °C, respectively. The ionization mode was electron impact mode (70 eV), and the mass scan range of 50–500 *m/z* (3.2 scans/s). Identification was performed using the public databases NIST (2017), Fiehn (2013), and MS-DIAL (2021) to screen for metabolites with ≥80% match, and VOCs were annotated in combination with commercially available standard data reported in the relevant literature. The relative content of each volatile compound was determined using the internal standard method, and the concentration of VOCs was calculated semiquantitative based on N-pentacosane-d32 (IS): the relative content of VOCs was the ratio of the VOC to the peak area of the IS, multiplied by the IS concentration.

2.5. Non-volatile metabolites determination

A centrifuge tube was filled with 300 μL of wine sample and 600 μL of methanol-acetonitrile ($v/v = 1/1$, IS: 0.02 mg/mL 2-Chloro-*L*-phenylalanine) and sonicated for 30 min (5 °C, 40 kHz). The samples were placed in a refrigerator at −20 °C for 30 min to precipitate proteins and then centrifuged for 15 min (4 °C, 13000 g). The collected supernatant was blown dry under nitrogen, 100 μL of acetonitrile-water ($v/v = 1/1$) was added, sonicated for 5 min (5 °C, 40 kHz), and then centrifuged for 10 min (4 °C, 13000 g), collected the supernatant for subsequent LC-MS/MS analysis, and the detection was carried out twice as a technical replicate. Chromatographic conditions: the injection volume was 3 μL, and the column was ACQUITY UPLC HSS T3 column (100 × 2.1 mm, 1.8 μm; Waters Corporation, Milford, MA, USA) at 40 °C; the mobile phase A was acetonitrile-water (v/v = 19/1), and B was acetonitrile-isopropanol-water (v/v/v = 47.5/47/5), both containing 0.1% formic acid, and the mobile phase elution gradient is shown in Table S1.

The samples were subjected to electrospray ionization, followed by applying ESI+ and ESI-scanning modes to acquire the mass spectral signals; detailed parameter information is shown in Table S2. Metabolomics processing software Progenesis QI (Waters Corporation, Milford, USA) was used to identify metabolites. The databases were KEGG and HMDB, and the qualitative comparisons and annotations were performed on the I-Sanger Cloud platform (Majorbio, Shanghai, China).

2.6. Statistical evaluations

Metabolome data were analyzed by orthogonal normal partial least squares-discriminant (OPLS-DA) model analysis and calculation of variable importance in the projection (VIP) by SIMCA Software package (version 13, Umetrics AB; Umeå, Sweden), combined with IBM SPSS Statistics 27.0 software for one-way analysis of variance (ANOVA) with Duncan's multiple comparisons test to calculate *P*-value for further screening of differential metabolites. Different criteria were selected to screen for differential metabolites based on the test results (significantly different VOCs: VIP > 1 and *P* < 0.01; significantly different non-VOCs: VIP > 1.5 and *P* < 0.01). Principal component analysis (PCA) and volcano plots used R package ropls (version 1.6.2), with downscaling to look at within-group reproducibility and between-group variability between wine samples. Heatmaps were plotted using TBtools (Toolbox for Biologists; version 1.120, China) to demonstrate differences in the expression of differential metabolites in wines from the three fermentation strategies. Rose diagram, PCA biplot, and correlation plots of differential VOCs with aroma properties (Correlation Plot APP, version 1.31) were generated using Origin Pro 2021 (OriginLab Corporation, Northampton, MA, USA). PLSR models were constructed with The Unscrambler® X version 10.4 (64-bit) (Camo Software AS, Oslo, Norway) to analyse the relationship between predictors (independent variables: VOCs/differential non-VOCs) and responses (dependent variables: aroma properties/taste properties). The "pheatmap" package in the R software package (version 3.5.3) to calculated Spearman correlation coefficients (r), where |r| > 0.8 and *P* < 0.05 were considered significant correlations (Huang et al., 2019), and the correlation network was visualized by Cytoscape 3.7.2 (The Cytoscape Consortium, San Diego, CA, USA). Finally, differential metabolites were analyzed for metabolic pathway enrichment using MBRole 2.0 (MBRole 2.0 - Home (csic.es)), *P* < 0.05 was selected to construct the metabolic network, and metabolites in wine were mapped to metabolic pathways using the KEGG database (C.-F. Li et al., 2018).

3. Results and discussion

3.1. Impacts of CECA inoculation on VOCs

3.1.1. Differential VOCs screening
The tested compounds were screened in public databases for matches

≥80% metabolites, and 32 VOCs were obtained by combining the relevant literature and databases. As shown in Table S3, 32 VOCs, including 13 acids, 9 alcohols, 5 esters, and 5 other compounds were obtained in this study. The PCA and OPLS-DA models were used to compare the similarities and differences of VOCs among SF, YF, and YDF. PCA is an unsupervised method commonly used in metabolomics studies to demonstrate inter-group variability and intra-group reproducibility. In this study, the PCA score plots showed significant separation between the inoculated fermentation (YF and YDF) and SF (Fig. S1A). Table 1 shows that the R^2X was 0.842 (close to 1), indicating high retention in the X-axis direction; The Q^2 of PCA model was 0.669 (>0.5), indicating the model's good predictive ability according to previous study (Lever, Krzywinski, & Altman, 2017). In addition, OPLS-DA was also used to explore the differences in the metabolites of the SF, YF and YDF wines (Fig. S1B). OPLS-DA is a supervised discriminant analysis statistical method that emulates the relationship between metabolite expression and sample category. The parameters R^2Y and Q^2 contribute to judging the accuracy and reliability of the OPLS-DA model. The model parameters R^2Y (0.986) and Q^2 (0.966) were both >0.90 (Table 1), indicating that these samples had good discriminatory and predictable properties according to previous studies (Da Silva et al., 2017; Yang, Dai, Ayed, & Liu, 2019). The OPLS-DA score plot showed inter-group differences and intra-group consistency, which identified 15 VOCs with significant differences (VIP > 1, *P* < 0.001; Table S3). The permutation test ($N = 200$) results indicated that the OPLS-DA model was well predictable and reproducible without excessive randomness or over-fitting, and could be used to screen for differential metabolites as reported by Li et al. (2021).

To visualize the differences in VOC abundance among the three different fermentation treatments, the 15 significantly different VOCs screened in Table S3 were subjected to HCA according to their abundance (Fig. 1). The results showed that different VOCs were clustered into 5 classes, namely I to V. The SF samples were distinguishable from the inoculated fermentation, with YF and YDF having greater chemical similarity.

Higher alcohols are secondary products of the alcoholic fermentation of yeasts, and their varying concentrations can positively or negatively affect the aroma of wine (Swiegers & Pretorius, 2005). The content of pentan-1-ol (fruity, balsamic), 1-octanol (intense citrus, rose), and benzyl alcohol (almond) in YDF was significantly higher than that in SF (Fig. 1). Notably, 1-octanol was only present in YDF, suggesting that YDF increased the concentration and variety of higher alcohols, thus contributing to the desired complexity of the wine's aroma. Red fruit aromas in red wine are often associated with acetate esters, which are considered as pleasant aroma descriptors (Welke, Nicolli, Hernandes, Biasoto, & Zini, 2022). Studies have shown that ethyl decanoate has low threshold, larger proportion, and high concentration, significantly impacting the overall aroma of wine (Hu, Jin, Mei, Li, & Tao, 2018). In this study, ethyl decanoate has significantly higher relative content in YF than that in YDF and SF, providing YF wines with a more pronounced fruit aroma profile (Fig. 1). Volatile organic acids are produced during fatty acid metabolism in yeast and bacteria, and a certain amount of

Table 1
Detailed parameters of the PCA and OPLS-DA models.

Model classes	Metabolites	Mode	R^2X (cum) (%)	R^2Y (cum) (%)	Q^2 (cum) (%)	Differential metabolites
PCA	VOCs	–	0.842	–	0.669	–
	non-VOCs	ESI+	0.761	–	0.478	–
		ESI-	0.805	–	0.620	–
OPLS-DA	VOCs	–	0.876	0.986	0.966	15
	non-VOCs	ESI-	0.749	0.984	0.955	55
		ESI+	0.757	0.994	0.953	71
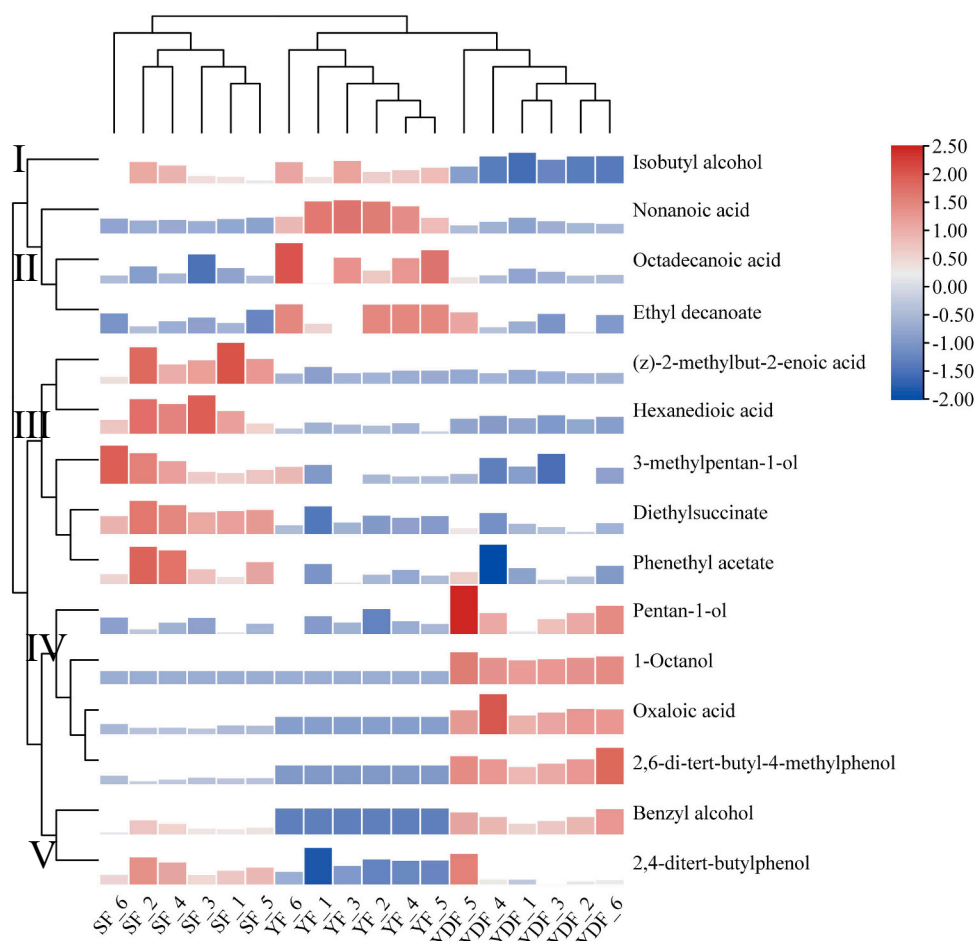
Fig. 1. Hierarchical cluster analysis (HCA) heatmap with 15 significantly different VOCs (VIP > 1, P < 0.01). SF: Spontaneous fermentation without inoculation; YF: Inoculated fermentation with CECA yeast; YDF: Inoculated fermentation with CECA yeast after addition of dimethyl dicarbonate. SF_1 and SF_2 are two technical replicates in the first biological replicate of SF sample, SF_3 and SF_4 are two technical replicates in the second biological replicate of SF sample, and so on.

volatile organic acids, increase the complexity of wine aroma and taste. In this study, 5 volatile organic acids (oxaloic acid, (z)-2-methylbut-2-enoic acid, nonanoic acid, octadecanoic acid, and hexanedioic acid) showed significant differences among treatments, suggesting that the inoculated fermentation and the inoculation method influenced their accumulation in wines, which could lead to the differences in the aroma and taste. 2,4-ditert-butylphenol and 2,6-di-tert-butyl-4-methylphenol also showed significant differences among SF, YF, and YDF. They showed the lowest expression in YF, suggesting that direct inoculation with CECA decreased the content of volatile phenolics, and the antimicrobial treatment increased the content of these compounds. In summary, inoculation with CECA altered the kind and content of VOCs and favored the formation of complex aroma properties in Cabernet Sauvignon wines.

3.1.2. Correlation analysis between VOCs and aroma profiles

The data on sensory analyse were derived from the results of the previous study (Zhang et al., 2023). Fig. S2 analyzed significant differences for 10 aroma profiles (citrus, berry, tropical fruit, temperate fruit, dried fruit, floral, herbal, spice, toasted, and undesirable flavors). The results showed that significant differences existed for all 9 aroma profiles except dried fruit, with berry aroma being the most prominent in terms of sensory intensity.

PLSR modeling was constructed to investigate the relationship between 32 VOCs and 10 aroma profiles (Fig. 2A). The PLSR model's principal components were VOCs and aroma profiles, with a 62% (40%

+ 22%) and 26% (18% + 8%) explanation, respectively. Among them, herbal, undesirable flavors, and 20 VOCs were located between the two ovals, indicating that they are strongly correlated in the model. In Fig. 2A, the herbal and undesirable flavors as well as (z)-2-methylbut-2-enoic acid and hexanedioic acid were close to each other and located in the same quadrant, indicating a positive correlation between them. According to our knowledge, no studies have yet showed the correlation of these two compounds with aroma, which needs further investigation. However, six kinds of VOCs (hexadecanoic acid, valeric acid, decanoic acid, hexanoic acid, ethyl isobutyrate, and ethyl decanoate) were in diagonal quadrant showing a negative correlation with herbal and undesirable flavors. Based on the loading weights and the regression coefficients, 3-methylbutan-1-ol (exhibiting ripe fruit aromas in red wines) could be considered the best predictor of wine aroma profiles in this study (Petretto et al., 2021).

Subsequently, heatmaps analyzed the differences in the correlation between the 32 VOCs and 10 aroma profiles in the SF, YF and YDF wines (Fig. 2B). A total of 4, 7, and 28 VOCs were detected in SF, YF, and YDF, respectively, that positively or negatively correlated with aroma profiles. As for aroma types, YF and YDF exhibited the similar correlations with them, while there were differences in the kinds and number of VOCs affecting them. In addition, the antimicrobial treatments affected the microbial composition and metabolic activities, thus presenting different correlation profiles. Specifically, undesirable flavors were negatively correlated with 4 VOCs of octanoic acid, hexadecenoic acid, hexanedioic acid and phenethyl acetate in YF, while 11 VOCs including

Fig. 2. (A) PLSR analysis and (B) Correlation analysis between the aroma profiles and the VOCs of three fermentation strategies wines. X. 32 VOCs; Y. 10 aroma profiles; Significant mark: "*" *P*-value <0.05. SF: Spontaneous fermentation without inoculation; YF: Inoculated fermentation with CECA yeast; YDF: Inoculated fermentation with CECA yeast after addition of dimethyl dicarbonate.

octanoic acid and hexanedioic acid in YDF showed negatively correlated with them. There were 16 VOCs had negative correlation with tropical fruit aroma in YDF, while tropical fruit was negatively correlated with 1 VOC (ethyl decanoate) in SF and 2 VOCs (octanoic acid and hexanedioic acid) in YF, respectively. It is worth noting that herbal aroma was positively correlated with 3-methylpentan-1-ol, hexanol and nonanoic acid in YDF. In contrast, it exhibited a negative correlation with 1-decanol in YF. These findings are consistent with the previous conclusion that VOCs and aroma profiles have varying degrees of positive and negative correlations (C. Liu et al., 2022), suggesting that aroma perception in wine was generated by the interaction of various aromatic compounds (Tchabo et al., 2017).

3.2. Impacts of CECA inoculation on non-VOCs

3.2.1. Differential non-VOCs screening

The composition of non-VOCs was characterized by UHPLC-Q Exactive HF-X. 1152 and 952 non-VOCs were identified in ESI+ and ESI- modes, respectively. The PCA and OPLS-DA models were used to compare the similarities and differences of non-VOCs among SF, YF, and YDF. The PCA score plots showed significant separation between the inoculated fermentation (YF and YDF) and SF (Fig. S3A—B). There was a partial overlap between the confidence intervals of the YF and YDF samples. Table 1 shows that the R^2X of PCA in ESI- and ESI+ models were 0.805 and 0.761, and Q^2 were 0.620 and 0.478, respectively. These results implied that inoculation of CECA significantly altered the metabolic profiles of non-VOCs in SF, while antimicrobial treatment had some effects but to a limited extent.

Moreover, the OPLS-DA models (ESI+ and ESI–) showed significant separation between samples with different fermentation treatments (Fig. S3C-D). The model parameters R^2Y and Q^2 were both >0.90 (Table 1). To verify that the OPLS-DA models were not overly random or over-fitted, we performed a random permutation test with 200 iterations. The R^2 and Q^2 parameters of the experimental data were higher than the data generated by the 200 permutation test, indicating that the OPLS-DA model was stable and reproducible without over-fitting. Therefore, multivariate statistical analyses of PCA and OPLS-DA confirmed statistically significant differences in non-VOCs among SF, YF, and YDF samples.

OPLS-DA analysis was used to explore the effect of inoculated fermentation (YF and YDF) on wine flavor. Table S4 shows the 126 significantly differential non-VOCs (VIP > 1.5, $P < 0.01$) with 71 in ESI + mode and 55 in ESI - mode. These 126 differential non-VOCs contained 31 organic acids and derivatives, 25 lipids and lipid-like molecules, 20 organic oxygen compounds, 18 organoheterocyclic compounds, 13 benzenoids, 9 phenylpropanoids and polyketides, 4 nucleosides, nucleotides, and analogues, and 6 others. The composition and content of differential non-VOCs in wines from the three fermentation strategies are shown in Fig. 3. Organic acids and derivatives were the most abundant differential non-VOCs (e.g., malic acid and argininic acid, etc.), which could play an essential role in the overall sensory quality of the wines. Malic acid and arginine, as precursors and metabolites of MLF, significantly impacted the quality of the wine (Mira De Orduña, Patchett, Liu, & Pilone, 2001; Styger et al., 2011). Malic acid in SF is higher than the inoculated fermentation, probably because more malic acid was converted to lactic acid through MLF, making the wine softer and palatable. MLF is usually carried out by lactic acid bacteria (LAB), and we detected the presence of LAB by high-throughput sequencing in the same batch of wine fermentation (unpublished data). The content of argininic acid in YDF was significantly lower than that in SF and YF, suggesting that adding dimethyl dicarbonate was a key factor influencing the difference in argininic acid. Argininic acid, as a primary amino acid in both must and wine, significantly impacts wine quality (Tonon & Lonvaud-Funel, 2002).

3.2.2. Correlation analysis between taste properties and differential non-VOCs

Electronic sensors in the *E*-tongue mimic human taste by plotting a rose diagram with the mean response values of 8 taste properties (sourness, bitterness, astringency, aftertaste-B, aftertaste-A, umami, richness, and saltiness (Fig. 4A). It can be observed that the taste of Cabernet Sauvignon wines fermented by SF, YF and YDF is notably distinguishable, because most of taste indexes are not overlapping each other. For SF wine, the intensity of bitterness, astringency, aftertaste-B, aftertaste-A, umami and saltiness was higher than the other, while the intensity of sourness and richness taste was lower. The inoculation of CECA decreased the taste of bitterness, astringency, aftertaste-B, umami and saltiness, while increased the taste of sourness. Moreover, the results of one-way ANOVA showed significant differences in bitterness, astringency, aftertaste-B, and saltiness taste properties (Table S5). Their response values were ranked in order of magnitude as SF > YDF > YF. However, the richness of SF, YF and YDF wines showed no significant differences. It is worth noting that the E-tongue data combined with the sensory analysis showed the higher acidity and lower umami of YDF wine.

To gain an intuitive and comprehensive understanding of the changes in wine taste, we performed PCA biplot analyses of wine samples under different treatments. The PCA scores and loading biplot showed the contribution of taste properties to wine differences, with PC1 and PC2 providing 55.0% and 28.3% of the explanation, respectively (Fig. 4B). The results showed that none of the three wine samples of the fermentation treatments overlapped, demonstrating significant differences in taste quality. CECA inoculation and antimicrobial treatments significantly altered the taste of Cabernet Sauvignon wines. Specifically, the SF wine was more strongly associated with bitterness, astringency, aftertaste-B, aftertaste-A, umami, and saltiness. In contrast, the YDF wine clustered in the upper left was characterized mainly by sourness and richness. It is worth noting that YF wine was weakly correlated with all 8 taste properties. The result may be due to YF treatment resulting in the most harmonious and balanced wine in case of taste quality in this study, which is consistent with wine-tasting results. The harmony and balance of the wines favored the perception of wine complexity (Schlich, Medel Maraboli, Urbano, & Parr, 2015).

To investigate the relationships between differential non-VOCs and taste properties, 126 differential non-VOCs (X variables) and 8 taste properties (Y variables) were used to generate the PLSR model (Fig. 4C). Consistent with the results of the one-way ANOVA, 7 taste properties except richness were significantly different. These 7 taste properties were located between the two circumferences of the correlation circle plot, showing significant correlations with different non-VOCs. The variance explained 86% (63% + 23%) and 78% (32% + 46%) for the X and Y variables, respectively, which was between 50% and 100%, having good explanatory power according to Yin et al. (2020). To further investigate the correlation differences between differential non-VOCs and taste properties in the SF, YF and YDF wines, this study constructed a correlation network based on Spearman's correlation ($|r| > 0.8$, $P < 0.05$) analysis (Fig. S4). The network parameters showed 61 (SF), 60 (YF), and 53 (YDF) pairs of significant and robust relationships (edges), with 38, 17 and 21 pairs showing positive correlation, and 23, 43 and 32 pairs having negative associations (Table 2). In addition, we found that the network complexity (showing as average degree) of the inoculated fermentation was higher than that of SF. Notably, the value of average spearman's correlation coefficients for bitterness, saltiness, astringency and aftertaste-B in SF wine was higher than in YF and YDF wines. On the contrary, the correlation coefficients of SF for aftertaste-A, umami, sourness, and richness were lower, in agreement with the PCA biplot results of Fig. 4B, which indicated that inoculated fermentation was more favorable for the formation of these four taste properties in Cabernet Sauvignon wine.
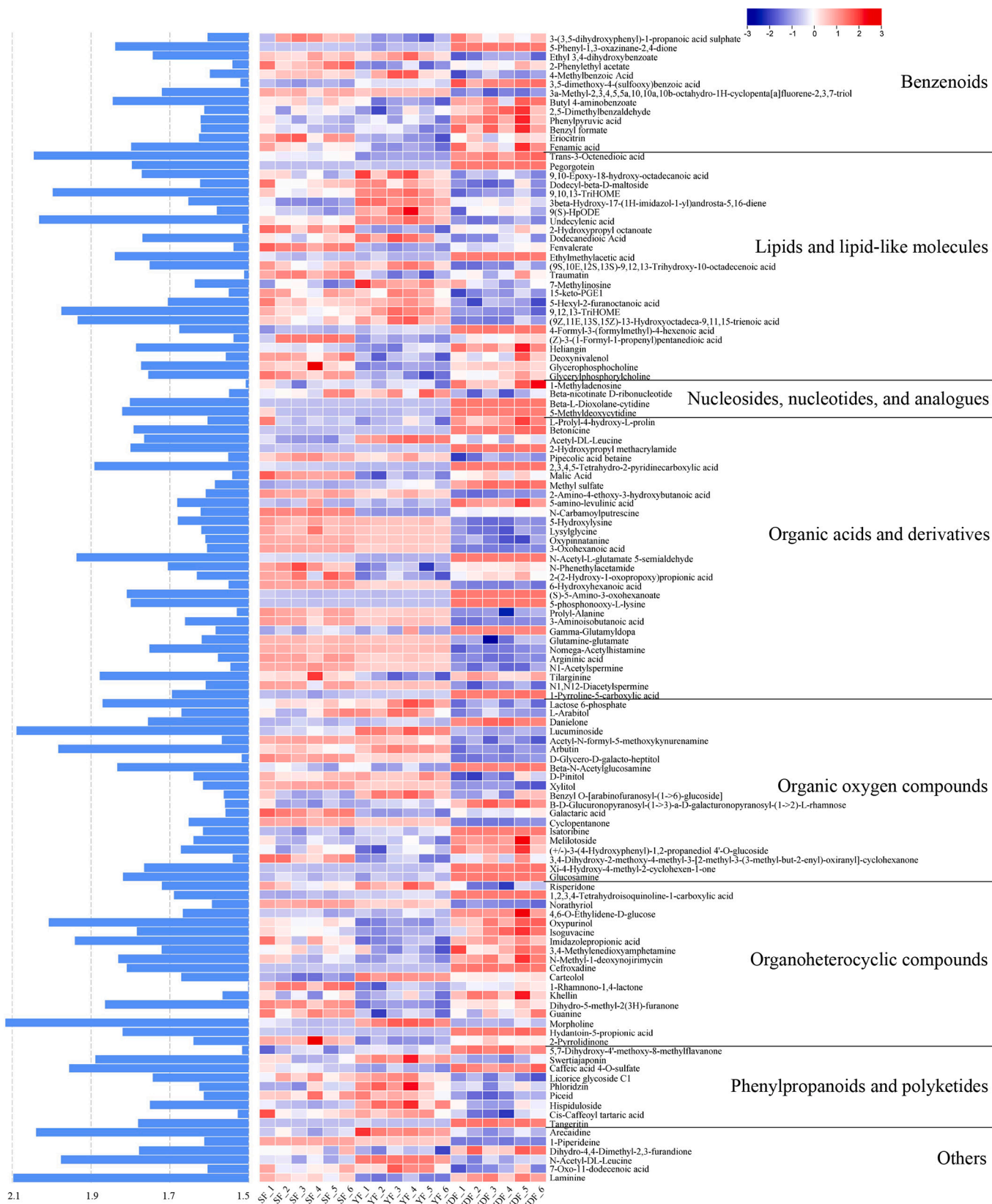
Fig. 3. Heatmap analysis and VIP scores with 126 significant differences non-VOCs (VIP > 1.5, $P < 0.01$) from the three fermentation strategies wines. SF: Spontaneous fermentation without inoculation; YF: Inoculated fermentation with CECA yeast; YDF: Inoculated fermentation with CECA yeast after addition of dimethyl dicarbonate. SF_1 and SF_2 are two technical replicates in the first biological replicate of SF sample, SF_3 and SF_4 are two technical replicates in the second biological replicate of SF sample, and so on.
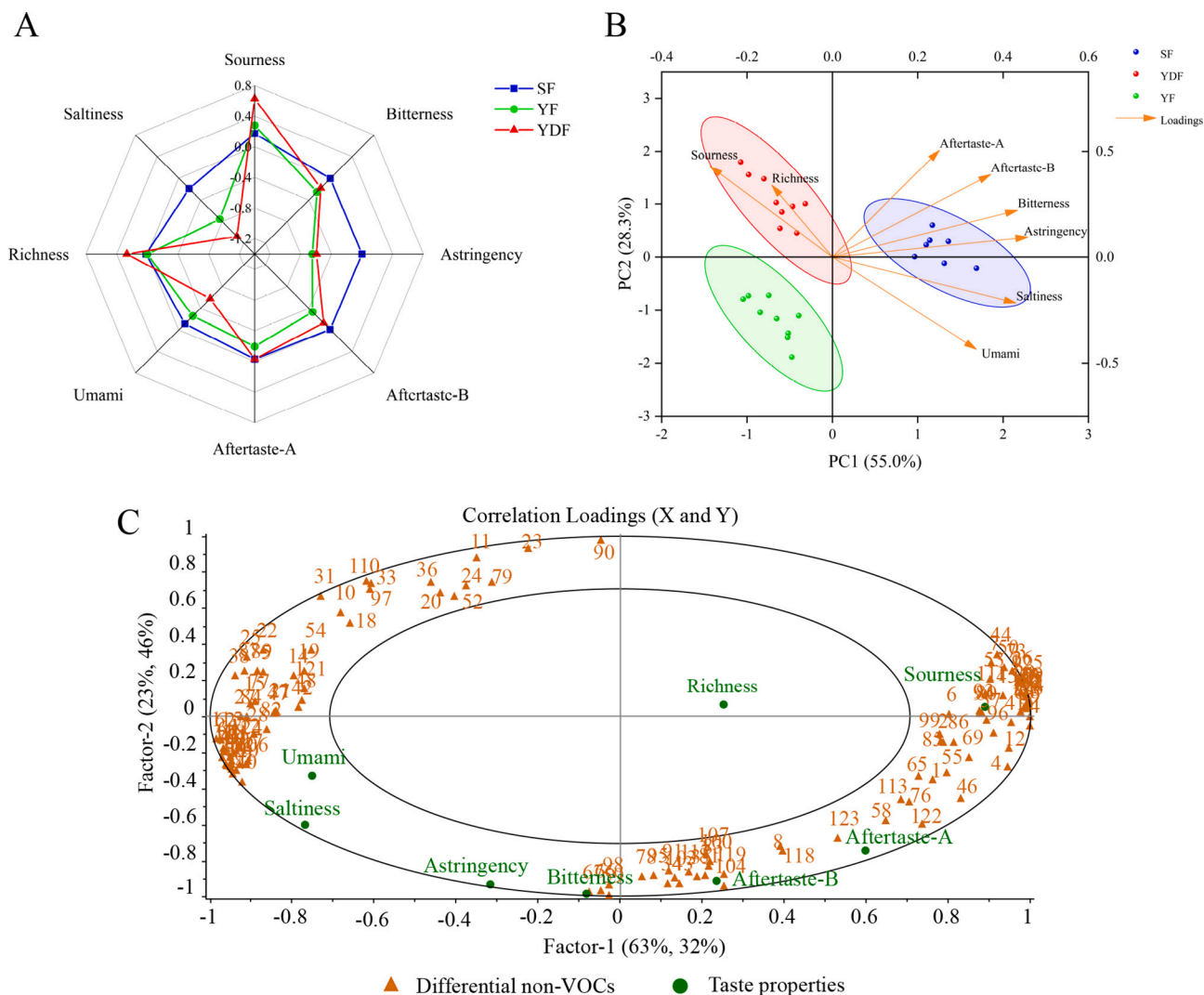
Fig. 4. Sensory analysis. (A) Rose diagram and (B) PCA biplot for *E*-tongue data of taste quality among the three fermentation strategies wines; (C) Correlation loadings plot of the PLSR model between 126 significant differences non-VOCs and 8 taste profiles. Orange triangles represent the 126 significant differences non-VOCs in Table S4. SF: Spontaneous fermentation without inoculation; YF: Inoculated fermentation with CECA yeast; YDF: Inoculated fermentation with CECA yeast after addition of dimethyl dicarbonate. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
The correlation network parameters between taste profiles and differential non-VOCs of three fermentation strategies wines.

Network parameters		SF	YF	YDF
Nodes		61	54	47
links		61	60	53
Positive links		38	17	21
Negative links		23	43	32
Average degree		2.000	2.222	2.255
	Sourness	0.857	0.905	0.886
	Bitterness	0.910	0.883	0.897
	Astringency	0.888	0.860	0.829
	Aftertaste-B	0.914	0.886	0.812
Average Spearman's correlation coefficient	Aftertaste-A	0.866	0.880	0.820
	Umami	0.872	0.857	0.892
	Richness	0.853	0.886	0.886
	Saltiness	0.922	0.860	0.900

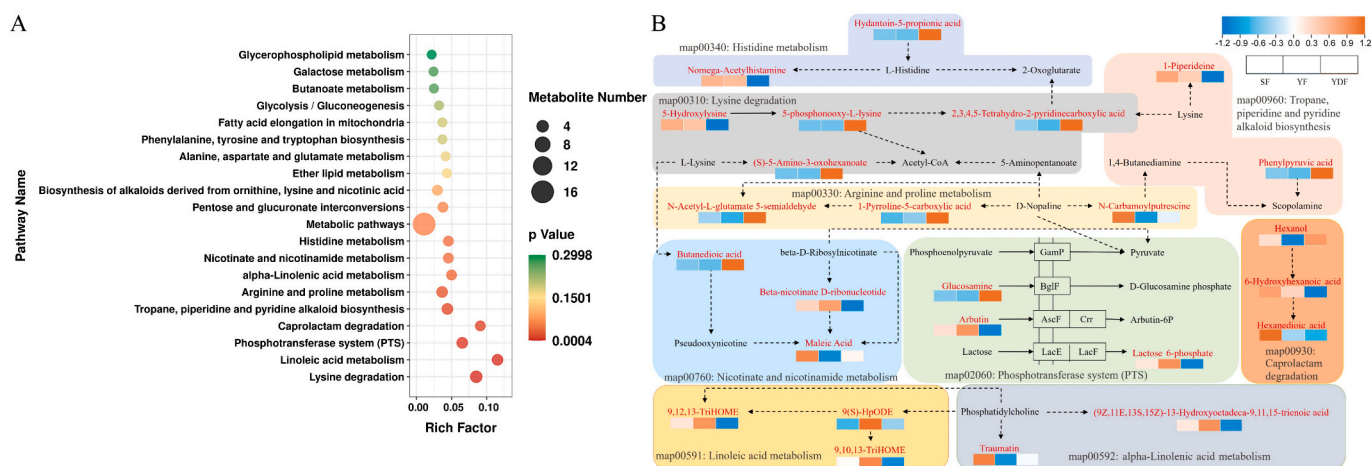### 3.3. Metabolic pathway analysis

Metabolic pathways could show metabolic differences in wines with different fermentation strategies and the preferential energy consumption of microorganisms during wine fermentation. This study aimed to investigate the effects of CECA inoculation on Cabernet Sauvignon wine aromas and flavors as well as the involved metabolic pathways. We analyzed the possible metabolic pathways of 141 differential metabolites (15 VOCs and 126 non-VOCs) by KEGG enrichment. As shown in Fig. 5A, the enrichment analysis by MBRole 2.0 showed that 141 differential metabolites were significantly correlated ($P < 0.05$) with 9 pathways (red circles): lysine degradation, linoleic acid metabolism, phosphotransferase system (PTS), caprolactam degradation, tropane, piperidine and pyridine alkaloid biosynthesis, arginine and proline metabolism, alpha-linolenic acid metabolism, nicotinate and nicotinamide metabolism, histidine metabolism.

Metabolic networks were constructed to visualize the 9 significant metabolic pathways ($P < 0.05$) mapped by differential metabolites, where red and black metabolites represent differential metabolites and intermediates (Fig. 5B). Three pathways (lysine degradation, arginine and proline metabolism, and histidine metabolism), accounting for one-third of the total number of significantly different metabolic pathways, were associated with amino acids, suggesting that inoculation strategy significantly affects amino acid metabolism. There were nine significant differences non-VOCs among these three metabolic pathways, among which the relative content of six non-VOCs (hydantoin-5-propionic acid,

Fig. 5. Pathway analysis of differential metabolites from three fermentation strategies wines. (A) KEGG enrichment scatterplot with 141 differential metabolites; (B) Significantly correlated pathways ($P < 0.05$) and differential metabolite heatmap analysis. SF: Spontaneous fermentation without inoculation; YF: Inoculated fermentation with CECA yeast; YDF: Inoculated fermentation with CECA yeast after addition of dimethyl dicarbonate.

5-phosphonooxy-L-lysine, (*S*)-5-amino-3-oxohexanoate, 1-pyrroline-5-carboxylic acid, 2,3,4,5-tetrahydro-2-pyridinecarboxylic acid, and *N*-acetyl-L-glutamate 5-semialdehyde) in YDF was significantly higher than that in SF and YF. In addition, PLSR analysis showed that the first four non-VOCs were positively correlated with bitterness, astringency, umami and saltiness, while showed negative correlation with sourness taste profiles. However, the last two non-VOCs exhibited a positive correlation with aftertaste-B and aftertaste-A (Fig. 4C). It indicates that antimicrobial treatment increased bitter (bitterness and aftertaste-B), astringent (astringency and aftertaste-A), umami and saltiness tastes while reducing the wine acidity through amino acid-related metabolic pathways. It is worth noting that the significant correlations identified through the metabolic pathway analysis were "statistical" and not due to the high intensity of the sensory descriptors (Tempère et al., 2018). For the aforementioned reasons, it is necessary to carry out further sensory analysis to fully understand the actual impact of metabolite changes on sensory differences.

Especially, the lysine degradation was most affected by the fermentation strategies ($P = 0.015$), supported by the significant differences in the abundance of 4 compounds (5-hydroxylysine, 5-phosphonooxy-L-lysine, 2,3,4,5-tetrahydro-2-pyridinecarboxylic acid, and (*S*)-5-amino-3-oxohexanoate) among different fermentation strategies. 5-phospho-nooxy-L-lysine and (*S*)-5-amino-3-oxohexanoate in YDF wine was higher than in SF and YF wines, which could contribute to the accumulation of acetyl-CoA. Study has shown that an increased supply of acetyl-CoA from amino acid metabolism can further increase lipid biosynthesis flux in oleaginous yeast and fungi (Vorapreeda, Thammarongtham, Cheevadhanarak, & Laoteng, 2012). Therefore, we hypothesized that YDF had a robust lipid metabolism. However, the content of five compounds associated with lipid metabolic pathways (alpha-linolenic acid metabolism and linoleic acid metabolism) was lower in YDF wine than in SF and YF wines (Fig. 5B). Future work is needed to understand how the antimicrobial treatment affects the metabolism of lipids.

4. Conclusions

CECA, a young indigenous commercial yeast, was characterized by floral and fruity aromas. To our knowledge, very few studies have published on CECA and mostly written in Chinese; more research is needed to introduce CECA to international researchers and winemakers. This study used flavoromics to establish a link between wine composition and sensory properties, which can help comprehensively understand the role of indigenous *S. cerevisiae* CECA on the aroma and flavor of Cabernet Sauvignon wines, three different fermentation treatments

(SF, YF and YDF) were carried out.

Our results demonstrate that inoculation with CECA altered the kinds and content of VOCs and non-VOCs, enhanced the citrus, temperate fruit, floral and berry aroma properties, and improved the undesirable flavors of the wine. Meanwhile, CECA inoculation was more favorable for the formation of aftertaste-A, umami, sourness and richness taste properties, and YF treatment resulted in the most balanced wine in case of taste quality. The metabolite enrichment analysis showed that 141 differential metabolites were significantly correlated ($P < 0.05$) with 9 pathways, with the amino acid metabolism pathway (one-third) being most affected by the fermentation strategy. In the three pathways, the antimicrobial treatment increased bitter (bitterness and aftertaste-B), astringent (astringency and aftertaste-A), umami and saltiness tastes while reducing the wine acidity. These differential metabolite analyses provided an outline for the interpretation of the CECA contribution to the aroma and flavor of Cabernet Sauvignon wines. In addition, wine fermentation is a complex biochemical process, and microbial interactions directly or indirectly affect wine perception. Therefore, the relationship between metabolites and microorganisms and their effects on wine style and quality needs further investigation.

In summary, CECA provide a theoretical basis for the industrial production of regional wines by enhancing the "terroir" of local wines. These results suggest that inoculated fermentation has an essential impact on the microbial community, central metabolic pathways and metabolite content. Future studies will identify critical enzymes and functional microorganisms during wine fermentation and refine the microbial impact on wine aroma and flavor.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101525.

CRediT authorship contribution statement

Fang Zhang: Data curation, Conceptualization, Formal analysis, Writing – original draft, Writing – review & editing. **Jing Zhang:** Writing – review & editing, Conceptualization. **Yue Sun:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data availability.

Data will be made available on request.

Data availability

Data will be made available on request.

Acknowledgement

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