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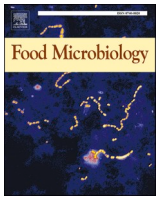

Combined effects of fermentation temperature and *Saccharomyces cerevisiae* strains on free amino acids, flavor substances, and undesirable secondary metabolites in *huangjiu* fermentation

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ABSTRACT

Fermentation temperature (FT) is one of the most critical factors, which can be used to control the growth conditions of *S. cerevisiae* to obtain excellent final products in winemaking. In this study, we analyzed the responses of six *S. cerevisiae* strains with different temperature preferences to FT (20 °C, 30 °C, and 35 °C) in *huangjiu* fermentation. The flavor substances, free amino acids and undesirable secondary metabolites related to *huangjiu* quality were determined. Results indicated that both *S. cerevisiae* strains and FT had significant effects on *huangjiu* fermentation, while the effects were strain-independent and differentiated temperature preferences for different fermentation characteristics. We found that the effects of FT were greater than that of *S. cerevisiae* strains under the premise of satisfying fermentation completion. Low temperature (20 °C) and high temperature (35 °C) fermentation were possible in the production of differentiated industrial *huangjiu* styles, while the effects of FT on undesirable secondary metabolites needed to be considered before industrial application. The results showed that a combination of FT with one or more *S. cerevisiae* strains could be used as a fermentation starter in *huangjiu* production for different types of products.

1. Introduction

Industrial yeast *Saccharomyces cerevisiae* is the main microbial and plays a vital role in modern-day fermented alcoholic beverage (FABs), producing secondary metabolites such as amino acids (AAs), organic acids (OAs), and volatile flavor substances (i.e. alcohols, aldehydes, medium-chained fatty acids, and esters) as well as some hazardous substances (Pires et al., 2014; Robinson et al., 2011; Wang et al., 2014; Zhao et al., 2014). Chinese *huangjiu* is one of the most pleasant FABs and widely popular worldwide due to its unique taste and flavor, local style and high nutritional value (Wang et al., 2014). *S. cerevisiae* strains with superior performance, optimal aromatic profiles, and minimal undesirable metabolic by-products are always desired and selected for industrial production, while a few are used in *huangjiu* brewing (Zhang et al., 2018; Zheng et al., 2021). The quality of *huangjiu* varies highly among different yeast strains and fermentation parameters, which is not only strain-dependent specific, but also affect by fermentation process.

Fermentation parameters affect yeast response during biosynthesis

Abbreviations: FABs, fermented alcoholic beverages; FT, fermentation temperature; LT, low temperature; MT, medium temperature; HT, high temperature; HAs, higher alcohols (HAs); AEs, acetate esters; HS-SPME, headspace solid phase microextraction; DLLME, dispersive liquid-liquid microextraction; GC-MS, gas chromatography–mass spectrometry; HPLC, high performance liquid chromatograph; OAs, organic acids; AAs, amino acids; BAs, biogenic amines; EC, ethyl carbamate.

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of the aromatic or hazardous substances related to the quality of FABs (Pérez et al., 2018; Zhao et al., 2013). Fermentation temperature (FT) is one of the most important fermentation parameters affecting the growth and survival of brewing microbes, which is also the major stresses for *S. cerevisiae* and largely influences the fermentation rate and the final quality of FABs (Deed et al., 2017; Pires et al., 2014; Torija et al., 2003). Since temperature has a significant effect on aroma, the optimum FT selected for different types of FABs to meet the differentiated needs of production is very necessary. The FT of the ale beer is generally controlled at 18–20 °C, while FT of the lager beer is generally controlled at 8–10 °C (Saison et al., 2009). Low FT (10–15 °C) fermentation improved characteristics of taste and aroma are commonly used in wines production. Reducing FT has a potential application prospect for flavor improvement in FABs (Deed et al., 2017; Molina et al., 2007). Compared with other FABs, higher primary FT (20–34 °C) is used for *huangjiu* production due to its unique process and raw materials. The optimal growth temperature for *S. cerevisiae* is 25 °C, lower FT restricts yeast growth and lengthens the fermentations, while higher FT shortens the fermentation period but with the increase of metabolic by-products and risk of contamination by miscellaneous bacteria (Bisson, 1999). Overall, both high FT and low FT can affect the quality and flavor diversity of *huangjiu*, meanwhile, there are also the high risk of stuck or sluggish fermentations in industrial production.

The traditional *huangjiu* brewing process with a history of 9,000 years produces once a year conforming to the nature, low FT and long-term fermentation process (nine months) endows traditional *huangjiu* higher quality. The production of traditional *huangjiu* begins on the winter solstice (Chinese solar terms, around the end of December) and finishes on the spring equinox (Chinese solar terms, around the end of March) considering temperature changes. The production of modern mechanical *huangjiu* is short cycle batch fermentation (20–30 days) and not restricted by the season, achieving year-round production by controlling the temperature, but generally not produced in the hottest season (From July to September) due to too higher FT increasing the chance of rancidity. To obtain higher quality *huangjiu*, differentiated appropriate yeast strains and corresponding optimal FT should take into consideration. Furthermore, it is a shame that there are a few published studies have explored the impact of FT on the composition of *huangjiu*. It is better to control FT under 30 °C, while the FT of modern *huangjiu* usually reaches to 34 °C at the vigorous fermentation period (10–24 h). Strains with a wide range of temperature tolerance and even high temperature adaptation are of great importance in *huangjiu* brewing. In industrial production, reasonable temperature control according to different strains can realize a wider range of *huangjiu* fermentation from low to high FT.

In this study, we firstly analyzed the responses of six *S. cerevisiae* strains with different temperature preferences to FT in *huangjiu* fermentation. Fermentation characteristics related to the quality such as free amino acids, flavor substances (organic acids, volatile flavor), and undesirable secondary metabolites (ethyl carbamate and biogenic amines) related to drinking safety and comfort were determined. The aim of the present study was to determine the changes in different yeast strains performance during low temperature (20 °C), medium temperature (30 °C) and high temperature (35 °C) fermentations in *huangjiu* brewing. Maintaining low or high temperature throughout fermentation to produce the characteristic new world style of *huangjiu* is another possibility of this study.

2. Materials and methods

2.1. Reagents and chemicals

All chemical standards, internal standards (Is) and analytical grade solvents used in this study were purchased from commercial suppliers and used without further purification.

2.2. Yeast strains and media

Five *S. cerevisiae* strains were isolated from fermented *huangjiu* samples, and the fermentation performance in *huangjiu* brewing were evaluated. Six strains of *S. cerevisiae* with different temperature preferences were used to study the combined effects of the FT and *S. cerevisiae* strains on free amino acids, flavor substances and undesirable secondary metabolites in *huangjiu*. The detailed information of strains were shown in Table 1. *S. cerevisiae* TP-555 was chosen due to prior screening of fermentative ability at low temperature (20 °C), while *S. cerevisiae* JH-533, TP-516, and SYH-607 were chosen at high temperature (35 °C). *S. cerevisiae* CYY-661 was chosen due to primary screening of the ability of low-yield high alcohols (HAs) and high-yield acetate esters (AEs) at medium temperature (30 °C). An additional strain 85# was widely used in the industrial production of mechanical *huangjiu* production, included in the experiments as control strain (Zhang et al., 2018) (see Table 2).

Yeast extract peptone dextrose (YPD) medium was prepared with 20 g glucose, 20 g peptone, 10 g yeast extract, and 1000 mL ddH2O, pH 6.0–6.2. YPD agar plates were prepared with 20 g agar powder addition. YPD medium was used for yeast activation. The rice hydrolysate medium (RHM) was used as yeast starter culture (YSC), which was prepared as described below. Firstly, raw rice was soaked in water at 60 °C for 30 min and steamed. Then, the ratio of mixture contained raw glutinous rice (Benchmark), raw wheat Qu (10%), water (400%), then saccharified and liquefied with a saccharifying enzyme (10^6 U/mL) and thermostable α-amylase (2×10^5 U/mL) at 60 °C for 4–6 h until the final sugar concentration of 13 °Brix. The mixture was filtered by eight layers of sterilized gauze. All used media were sterilized at 115 °C for 20 min before using.

2.3. Determination of the growth of the six S. cerevisiae strains at different temperatures

To determine the suitable range of FT of *S. cerevisiae* in *huangjiu* brewing, the growth of the six *S. cerevisiae* strains were determined in the range 5–50 °C on YPD medium by steps of 5 °C. Monoclonal colony yeast cells were firstly activated in 100 mL of YPD medium at 30 °C for 24 h with shaking culture (150 rpm/min), then 100 μL yeast solution was incubation in 100 mL of YPD medium and counting the viable cells, arising after 2 days at different temperatures. Absorbance at 600 nm was measured in 1 mm path length cuvettes (and normalized to 1 cm path length) using SpectraMax 190 Microplate Reader (MD Electronics, USA).

2.4. Huangjiu fermentation experiments

The *huangjiu* simulated fermentation was conducted in the laboratory. Monoclonal colony yeast cells were activated in 15 mL of YPD medium at 30 °C for 24 h with orbital shaking culture (150 rpm/min), and transferred to 135 mL of 13° Brix RHM at 30 °C for 24 h, the harvested cultures were used as YSC. Six kinds of YSCs were prepared from six *S. cerevisiae* strains. *Huangjiu* fermentation was mixed with steamed glutinous rice (750 g), raw wheat Qu and cooked wheat Qu (58.5 g, 9 g), YSC (57 mL), and water (750 mL) in a 3 L flask. The primary fermentations were carried out at 20 °C, 30 °C and 35 °C (2 days)-30 °C (2 days)-20 °C (1 days) (Low, medium, and high temperature, hereafter 'LT', 'MT', and 'HT', respectively) under stable condition for 5 days and post fermentation at 15 °C for 15 days, giving a total of 18 different fermentation treatments. Also, *huangjiu* primary fermentation was carried out at 35 °C (3 days), post fermentation did not proceed due to stuck and sluggish fermentation. All fermentation experimental samples were performed in triplicate. *S. cerevisiae* 85# was conducted (in duplicate) as control.

2.5. Analysis of fermentation parameters

The fermentation parameters (alcohol, pH, total acid, and amino acid

Table 1
Information on strains related in this study.

Strains	Screening strategy (Primary fermentation)			
	High temperature (35°C-72 h)	High temperature step-by-step cooling (35°C-48 h; 30°C-48 h; 20°C-24 h) HT	Medium temperature (30°C-120 h) MT	Low temperature (20°C-120 h) LT
85# (Control)	+	+	+++	+++
TP-555	+	+	+++	+++
CYY-661	+	+	+++	+++
SYH-607	+	++	+++	+++
JH-533	+	++	+++	++
TP-516	+	++	+++	++

"+": The ethanol content of *huangjiu* obtained by the strains at 15 °C for 360 h after the primary fermentation (<14 %vol): "++": (14–15 %vol); "+++": (>15 %vol), respectively.

Table 2
Basic physical and chemical indicators in *huangjiu* fermentation by different *S. cerevisiae* strains at different temperature.

Parameters	Temperature	85#	TP-555	CYY-661	SYH-607	JH-533	TP-516
Alcohol (%vol)	LT	16.10 ± 0.57 B/a	15.36 ± 0.45 B/a	16.45 ± 0.78 B/a	16.14 ± 0.10 B/a	14.76 ± 0.94 AB/a	15.02 ± 0.38 B/a
	MT	14.99 ± 0.72 AB/a	15.63 ± 0.60 B/a	15.83 ± 0.32 B/a	16.38 ± 0.57 B/a	15.40 ± 0.22 B/a	15.11 ± 0.26 B/a
	HT	14.49 ± 0.20 A/b	14.35 ± 0.25 A/b	14.45 ± 0.27 A/b	15.20 ± 0.44 A/c	14.42 ± 0.12 A/b	13.80 ± 0.25 A/a
Residual sugar (g/L)	LT	4.51 ± 0.51 A/b	16.58 ± 1.25 A/c	2.48 ± 0.25 A/a	3.15 ± 0.85 A/ab	15.83 ± 1.02 B/c	14.52 ± 3.12 A/c
	MT	12.32 ± 0.89 B/b	15.23 ± 2.17 A/b	12.14 ± 1.59 B/b	2.24 ± 0.57 A/a	12.51 ± 1.21 A/b	13.21 ± 3.25 A/b
	HT	17.12 ± 1.25C/b	16.58 ± 1.25 A/b	18.56 ± 2.41C/b	12.54 ± 1.25 B/a	17.25 ± 2.14 B/b	17.87 ± 2.12 A/b
pH	LT	4.45 ± 0.11 A/a	4.31 ± 0.16 A/a	4.36 ± 0.05 A/a	4.30 ± 0.02 A/a	4.30 ± 0.11 A/a	4.27 ± 0.09 A/a
	MT	4.47 ± 0.11 A/a	4.43 ± 0.03 A/a	4.50 ± 0.01 B/a	4.48 ± 0.00 B/a	4.51 ± 0.02 B/	4.42 ± 0.05 B/a
	HT	4.42 ± 0.08 A/c	4.34 ± 0.02 A/b	4.37 ± 0.01 A/b	4.27 ± 0.02 A/a	4.35 ± 0.02 A/b	4.25 ± 0.00 A/a
[a]Total acid (g/L)	LT	4.91 ± 0.36 A/a	5.86 ± 0.51 A/bc	5.24 ± 0.11 A/ab	5.37 ± 0.05 A/b	5.75 ± 0.14 A/bc	6.01 ± 0.36 A/c
	MT	6.49 ± 0.32 B/a	6.57 ± 0.29 AB/a	6.23 ± 0.23 B/a	6.42 ± 0.14 B/a	6.20 ± 0.27 B/a	6.12 ± 0.31 A/a
	HT	6.40 ± 0.36 B/a	6.66 ± 0.11 B/a	6.72 ± 0.10C/a	7.20 ± 0.17C/b	6.66 ± 0.39 B/a	7.82 ± 0.66 B/c
Amino nitrogen (g/L)	LT	0.79 ± 0.16 A/a	0.68 ± 0.11 A/a	0.71 ± 0.07 A/a	0.62 ± 0.01 A/a	0.67 ± 0.11 A/a	0.66 ± 0.05 A/a
	MT	1.04 ± 0.03 B/c	0.96 ± 0.04C/b	1.03 ± 0.02C/c	0.91 ± 0.02 B/b	0.97 ± 0.01 B/b	0.68 ± 0.10 A/a
	HT	1.00 ± 0.05 B/b	0.85 ± 0.03 B/a	0.88 ± 0.02 B/a	0.90 ± 0.01 B/ab	0.95 ± 0.05 B/b	0.92 ± 0.05 B/ab

[a] The above number represnsents for lactic acid calculation. Values are means ± standard deviations from at least three independent tests. Values with different letters in the same row or column are significantly different ($P < 0.05$) from each other. Capital letters represent the differences between different temperatures of the same strain; small letters represent the differences between different strains at the same temperature.

nitrogen) were determined by the method used for *huangjiu* (Liu et al., 2021). The 3,5-dinitrosalicylic acid method was used to detect reducing sugar in *huangjiu* samples (Miller, 1987). The concentration of urea was determined using diacetyl monoxime reactions (Zhao et al., 2014). Absorbance at 525 nm was measured using SpectraMax 190 Microplate Reader (MD Electronics, USA). The reducing sugar and urea concentration were calculated by using a standard curve.

2.6. Quantitative analysis of non-volatile substances

The concentration of BAs, OAs and AAs were determined by HPLC according to the described methods of *huangjiu* (Liu et al., 2021b; Wang et al., 2014). All the samples were filtrated through a 0.22 mm microporous membrane before determination. BAs concentration was analyzed by HPLC, using Agilent 1100 equipped with a X Bridge C18 column (250 mm × 4.6 mm, 5 μm) and UV detection set at 254 nm. In terms of mobile phase with a flow rate of 0.8 mL/min. For the determination of OAs. The separations were carried out on Agilent 1100 (250 mm × 4.6 mm and 5 μm ODS HYPERSIL column). The column temperature was set at 30. The speed of flow was set at 1.0 mL/min and the detective wavelength was set at 338 nm and 262 nm. A mixture of pH 2.3 phosphate buffer (0.01 mol/L KH2PO4), adjusted with 5% (w/v) acetic acid solution to pH (2.3 ± 0.05) was used as the mobile phase with a flow rate of 0.8 mL/min. The detection wavelength was 210 nm. The content of AAs was determined by RP-HPLC with pre-column derivation. The column (250 × 4.6 mm and 5 μm ODS HYPERSIL column) temperature was maintained at 40 °C. The speed of flow was set at 1.0 mL/min and the detective wavelength was set at 338 nm and 262 nm.

2.7. Quantitative analysis of volatile flavor compounds

For the rapid determination of volatile flavor compounds in *huangjiu*, a headspace solid phase microextraction (HS-SPME) technique was used to extract the flavor compounds, a dispersive liquid-liquid microextraction (DLLME) was used for determination of main HAs with 4-methyl-2-pentanol (0.4536 g/L in ethanol) as internal standardization, sample preparation followed the described method (Zhou et al., 2020). In order to determine the key aroma compounds and eliminate variations in extraction efficiency caused by small differences in the sample matrix, 2-octanol (0.1018 g/L in ethanol) as dardization were applied to quantify the analytes by GC-MS (Liu et al., 2019).

2.8. Quantitative analysis of EC

The content of EC was quantified using GC-MS after extraction by HS-SPME, and nPC (0.1500 g/L in ethanol) was internal standardization (Zhao et al., 2014). Simulated *huangjiu* solution was prepared with 140 mL ethanol and 5 g lactic acid dissolved in 300 mL deionized water, adjusted pH to 4.0 with 1.0 mol/L NaOH solution, transferred to 1 L volumetric bottle, and used deionized water to constant volume to scale for reserve. A total of 8 mL of sample, 8 μL nPC internal standard solution and 3.1 g sodium chloride were added into 20 mL headspace bottle. Then inserting into the extraction head, at 70 °C, 250 r/min constant temperature stirring extraction for 45 min. The injection method is non-split injection, injection volume (1 μL). The detection mode is selected ion detection (SIM) and EC mode characteristic ion (M/Z, 62). The EC content in the samples was calculated according to the internal standard curve method, and each sample was determined independently in triplicate and the data were presented as the average.

2.9. Statistical analysis

Statistical analysis was performed by using GraphPad Prism 8. All experiments were performed in triplicate. Results were expressed as the mean ± standard deviation (SD) and analyzed with one-way analysis of variance (ANOVA) to establish the significant difference between groups. The contribution of temperatures and different strains to the difference in flavor of *huangjiu* samples were analyzed by heat maps made online (Metsalu and Vilo, 2015).

3. Results and discussion

3.1. Effect of temperature on the growth of different S. cerevisiae strains

Different temperatures can affect the growth and metabolic proliferation of *S. cerevisiae*, which determine the performance of strain during the process of fermentation. We studied the growth of the six *S. cerevisiae* strains at the range 5–50 °C by steps of 5 °C to determine the suitable range of FT of *S. cerevisiae in huangjiu* fermentation. The cell growth profile of six *S. cerevisiae* strains with temperature was similar, too higher (40 °C) or too lower (10 °C) temperature seriously inhibited the growth (Fig. 1). *S. cerevisiae* strains had the better growth at the range 15–40 °C, the cell population (OD_{600}) of the two strains (SYH-607 and TP-555) were lower values than that of the other strains under the same culture conditions, which indicated the lower of the cell proliferation. The FT varied from 20 to 34 °C during the pre-fermentation process and the FT were usually 10–15 °C in the post-fermentation stage of *huangjiu*. The progress of *huangjiu* brewing was multiple species, temperature-variable and parallel fermentation, containing pre-fermentation and post-fermentation (Shuang et al., 2012; Wang et al., 2014). The FT varied from 20 to 34 °C during the pre-fermentation process and the FT were usually 10–15 °C in the post-fermentation stage of *huangjiu*. The FT in pre-fermentation stage was first increased (Metabolic heat generation) and then decreased (Cooling water circulation to cool down), which was better to the combination of progressive saccharification of starch and alcoholic fermentation of *S. cerevisiae*. The FT in post-fermentation stage was long-term and low temperature (anaerobic), aiming to promote the participation of other brewing microorganisms in the fermentation to stabilize the fermentation and make the flavor more balanced. The primary fermentation were carried out at 20 °C, 30 °C and 35 °C in *huangjiu* fermentation to investigate the combined effects of the temperature (LT, MT and HT) and *S. cerevisiae* strains in the follow-up study.

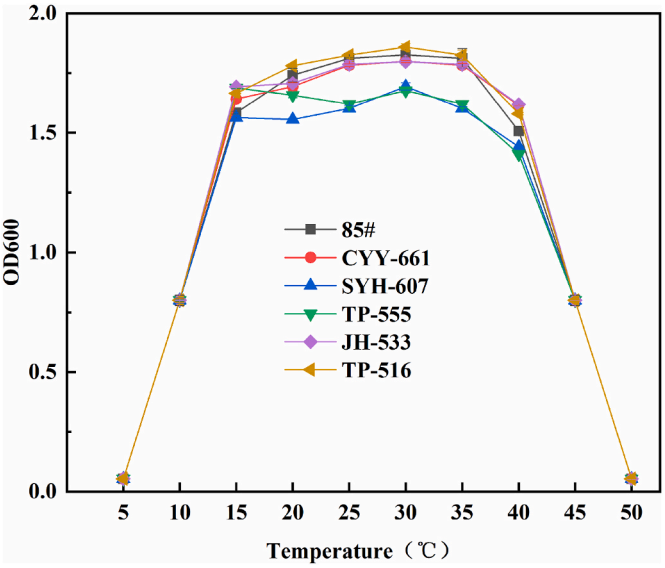

Fig. 1. Effects of temperature on the growth of different *S. cerevisiae* strains.

3.2. Effect of temperatures and strains on fermentation completion

All the fermentation experimental samples were finished and evaluated after 20 days. The same pre-fermentation time (5 days) was guaranteed to explore the effects of FT and strains on *huangjiu*, although different *S. cerevisiae* strains had differentiated characteristics. Furthermore, post-fermentation were under the same conditions (15 °C, 15 days). Basic physical and chemical indicators related to the *huangjiu* quality meet the national standard "*Huangjiu*" (GB/T 13,662–2018). The production of alcohol of different *S. cerevisiae* strains at different temperature during *huangjiu* fermentation were shown in Fig. S1. The alcohol content was the basic and the key indicator for the ability of *S. cerevisiae* strains used in *huangjiu* fermentation (Table 1). There was no significance difference between LT and MT, while HT was significantly decrease in alcohol content (1–1.5 %vol). For different strains, the temperatures for the best alcohol production capacity were strain-dependent. Residual sugar content was negatively correlated with alcohol content, fermentations at HT had higher residual sugar and subsequently lower alcohol content. HT accelerated *S. cerevisiae* proliferation under the condition of the same raw materials and inoculum amount, causing arrest of yeast metabolism. However, accidental error occurred in raw materials were also the factors that cause differences in residual sugar content. The pH and total acid (lactic acid) were the results of acid-producing bacteria. An increase in temperature results in an increase in total acid content, not exceed the limit requirements in *huangjiu*, which was strain-dependent and also the reason for the low alcohol content at HT. The range of pH for all the fermentation is 4.2–4.6, less affected by temperature in normal fermentation. Amino nitrogen represented the amino acid content in *huangjiu,* which indicated that LM had a reduced content (Table 1). Hence, fermentation completion was included as an essential variable, more fermentation characteristics should be discussed and compared between samples (Deed et al., 2017).

3.3. Effect of temperatures and strains on volatile compounds of huangjiu

Yeast strains contribute to the volatile flavor profiles of FABs has been extensively studied, while FT affects the production of flavor substances (Chen and Xu, 2010; Romano et al., 2015). Forty-nine volatile compounds were detected in all the fermentation treatments by HS-SPME/GC-MS, including 17 esters, 15 alcohols, 2 aldehydes, 10 acids, 4 phenols and 2, 3-dihydrobenzofuran, which arose from yeast metabolism (Fig. 2). Heat map analysis was carried out on volatile compounds obtained by fermentation of six strains at three different FT, 18 different fermentation treatments were separated by temperature, six strains at HT, MT, and LT showed similar flavor characteristics cluster together, respectively (Fig. 2). Analysis of the effects of temperature on fermentation characteristics of strains, the flavor profile of two strains (TP-516 and SYH-607) at MT were more skewed towards HT (Cluster 1), while that of the other strains were more skewed towards LT (Cluster 2). The alcohols in HT group were relatively higher, while esters and volatile acids were higher in LT group (Fig. 2). Under the premise of satisfying the fermentation completion, the effects of temperatures on the flavor were greater than that of *S. cerevisiae* strains in *huangjiu* fermentation. A study had discussed the effects of FT and different strains of yeast on Aurore wine composition, indicating that FT did not significantly affect ester content by contrast of yeast strain (Samoticha et al., 2019). In other studies, temperature affected not only the fermentation kinetics (rate and length of fermentation) but also the yeast metabolism, which determined the chemical composition of the wine, although it was strain-dependent (Torija et al., 2003). Studies also found that the ability of some strains to finish the fermentation was most important, since any delay would allow undesirable compounds to appear (Tronchoni et al., 2012). The effects of temperatures and strains

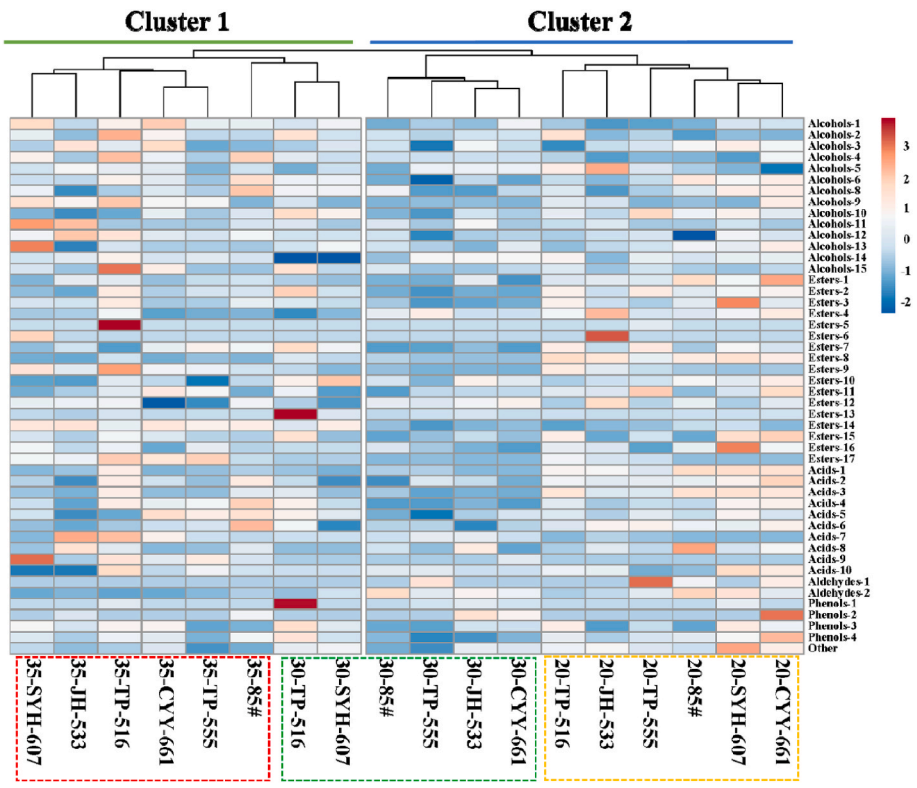

Fig. 2. The production of volatile compounds of different *S. cerevisiae* strains at different temperature in *huangjiu* fermentation. Alcohols-1: 1-propanol, 3-(methylthio)-; Alcohols-2: n-Propanol; Alcohols-3: isobutanol; Alcohols-4: ethoxyethanol; Alcohols-5: 1-octene-3-ol; Alcohols-6: 2-phenylethanol; Alcohols-7: 2-nonanol; Alcohols-8: farnesol; Alcohols-9: 2, 3-butanediol; Alcohols-10: 4-hydroxyphenethyl alcohol; Alcohols-11: isooctanol; Alcohols-12: isoamyl alcohol; Alcohols-13: D-geraniol; Alcohols-14: n-amyl alcohol; Alcohols-15: n-butyl alcohol; Esters-1: ethyl butyrate; Esters-2: ethyl acetate; Esters-3: ethyl caprate; Esters-4: ethyl oenanthate; Esters-5: ethyl lactate; Esters-6: ethyl isovalerate; Esters-7: ethyl caproate; Esters-8: ethyl caprylate; Esters-9: phenylethyl acetate; Esters-10: ethyl succinate; Esters-11: ethyl valerate; Esters-12: ethyl benzoate; Esters-13: monoethyl succinate; Esters-14: ethyl palmitate; Esters-15: ethyl phenylacetate; Esters-16: ethyl laurate; Esters-17: isoamyl acetate. Acids-1, hexanoic acid; Acids-2, dodecanoic acid; Acids-3, octanoic acid; Acids-4, nonanoic acid; Acids-5, n-hexadecanoic acid; Acids-6, tetradecanoic acid; Acids-7, 2-methylhexanoic acid; Acids-8, butanoic acid; Acids-9, 2-methylbutyric acid; Acids-10, heptanoic acid, Aldehydes-1: nonanal; Aldehydes-2: benzaldehyde. Phenols-1: 4-ethylphenol; Phenols-2: 4-ethyl guaiacol; Phenols-3: 2-methoxy-4-vinylphenol; Phenols-4: 2, 4-di-*tert*-butylphenol. Other: 2,3-Dihydrobenzofuran.

on two key substances, high alcohols (HAs) and acetate esters (AEs), needing further analysis.

3.4. Analysis of ethyl acetate, high alcohols and their corresponding acetate esters

3.4.1. Effect of temperature and S. cerevisiae strains on high alcohols

As the key substances of *huangjiu* aroma, ethyl acetate, HAs and their corresponding AEs were further quantitated and analyzed. Four HAs were detected in all the fermentation treatments by DLLME/GC–MS (n-propanol, isobutanol, isoamyl alcohol and 2-phenethyl alcohol). Clearly, production of n-propanol in the same strain was higher at higher temperatures (MT and HT) than that at LT, while the significant difference ($P < 0.5$) during different strains at same temperature was strain-dependent (Table 3 and Table 4). Some studies had found that isobutanol (solvent and wine) was the only HAs shown to be a driver of variation between wine samples and the concentrations were significantly and positively influenced by FT (Deed et al., 2017; Rollero et al., 2015; Torija et al., 2003). The production of isobutanol in the same strain was highest at MT (Table 3). Strain 85# produced the lowest amounts of isobutanol at HT, while that of the other five strains were at LT. Strain 85# produced the greatest amounts of isobutanol at MT, following LT and HT, due to genetic differences between the yeast strains in amino acid pathways (Styger et al., 2011). All strains had the lowest amounts of isoamyl alcohol and 2-phenethyl alcohol (1 %vol alcohol corresponds to the content) at MT, suggesting that the change of temperature (increase or decrease) would cause the content to change. It had been reported that the production of isoamyl alcohol was higher at higher FT, but the production of isobutanol and n-propanol did not change significantly (Ough et al., 1966). Considering the alcohol content and total HAs content (1 %vol alcohol corresponds to the content) in *huangjiu* fermentation, HT produced the highest total HAs, some strains produced lower amounts of HAs at LT than that at MT, while some strains produced the least HAs at MT (Table 3). Whether fermentation was inhibited might be the reason for the lower content of HAs at MT or LT. The results above were different from the conclusion of reducing FT could reduce the content of HAs (Molina et al., 2007; Ough et al., 1966). Where differences in HAs during different FABs could be best explained by the combination of yeast strain and FT.

3.4.2. Effect of temperature and S. cerevisiae strains on acetate esters

Another important aroma substance, AEs (fruity and floral aromas) are generated from acetyl-CoA and HAs or ethanol, by the action of the alcohol acetyl transferases Atf1p and Atf2p in *S. cerevisiae* influenced by FT (Molina et al., 2007). Like HAs production, their corresponding AEs were also affected by temperature. Ethyl acetate, isoamyl acetate and 2-phenylethyl acetate were the key and yeast-derived aroma compounds in *huangjiu* (Mo et al., 2010). Three kinds of AEs (Ethyl acetate, isoamyl acetate and 2-phenylethyl acetate) were also detected and quantified, while isobutyl acetate was not detected in *huangjiu* fermentation. Three strains (85#, TP-555 and CYY-661) had the highest amounts of ethyl acetate at LT, following HT and MT, while two strains (SYH-607 and JH-533) were higher at LT than MT and HT (Table 4). Unlike other strains, TP-516 produced the greatest ethyl acetate at MT, which might be the reason for the different tolerance of strains to temperature. The production of isoamyl acetate was higher at HT than that at the lower temperature (LT and MT) in the same strain, while the least amount of isoamyl acetate was at MT, expect SYH-607. Study found that only 2-phenylethyl acetate had shown a linear regression with its related alcohol, indicating that higher 2-phenylethanol content yield higher 2-phenethyl acetate amount in the same strains (Antonelli et al., 1999). In this study, we obtained another conclusion that higher production of 2-phenylethanol in some strains (CYY-661, SYH-607, and JH-533) were corresponding to the higher of 2-phenylethyl acetate content in *huangjiu* fermentation, while the opposite results were occurred in some strains (85#, TP-555, and TP-516) (Table 4). Regardless of higher temperatures (HT), AEs were higher at LT than at MT found in some other studies, whereas the opposite effects were also found in some studies (Molina et al., 2007; Ntuli et al., 2022; Papathanasiou et al., 2006). The differential results in different FABs may be partially due to variation in

Table 3
The production of higher alcohols and ethanol of different *S. cerevisiae* strains at different temperature in *huangjiu* fermentation.

Parameters (mg/L)	Temperature	85#	TP-555	CYY-661	SYH-607	JH-533	TP-516
[a] Ethanol	LT	16.10 ± 0.57 B/a	15.36 ± 0.45 B/a	16.45 ± 0.78 B/a	16.14 ± 0.10 B/a	14.76 ± 0.94 AB/a	15.02 ± 0.38 B/a
	MT	14.99 ± 0.72 AB/a	15.63 ± 0.60 B/a	15.83 ± 0.32 B/a	16.38 ± 0.57 B/a	15.40 ± 0.22 B/a	15.11 ± 0.26 B/a
	HT	14.49 ± 0.20 A/b	14.35 ± 0.25 A/b	14.45 ± 0.27 A/b	15.20 ± 0.44 A/c	14.42 ± 0.12 A/b	13.80 ± 0.25 A/a
N-propanol	LT	29.62 ± 1.52 A/a	41.54 ± 1.58 A/c	36.47 ± 1.25 A/b	36.55 ± 1.45 A/b	36.44 ± 1.23 A/b	74.30 ± 2.03 A/d
	MT	62.48 ± 2.41 B/c	44.77 ± 1.65 A/a	67.40 ± 2.54 B/cd	59.98 ± 1.04 B/b	75.44 ± 2.23C/e	70.90 ± 2.21 A/de
	HT	62.83 ± 2.05 B/a	75.11 ± 3.21 B/b	87.81 ± 2.55C/c	76.36 ± 2.01C/b	66.33 ± 1.56 B/a	101.45 ± 4.58 B/d
Isobutanol	LT	102.63 ± 2.85 B/cd	90.56 ± 2.58 A/b	91.73 ± 1.05 B/b	97.28 ± 1.87 A/c	102.93 ± 2.01 A/d	66.61 ± 0.85 A/a
	MT	111.91 ± 1.25C/d	102.84 ± 1.25C/b	102.65 ± 1.85C/b	107.27 ± 2.05 B/c	119.06 ± 2.32 B/e	88.41 ± 1.08 B/a
	HT	85.62 ± 1.01 A/a	96.09 ± 1.01 B/c	88.41 ± 1.25 A/ab	96.26 ± 1.47 A/c	120.20 ± 2.68 B/d	90.20 ± 1.50 B/b
Isoamyl alcohol	LT	240.13 ± 1.25 B/d	231.84 ± 0.86 A/c	230.69 ± 2.96C/bc	225.85 ± 1.50 A/b	225.88 ± 1.54 A/b	189.89 ± 1.25 A/a
	MT	217.82 ± 1.01 A/b	231.67 ± 1.54 A/d	213.58 ± 1.63 A/ab	231.25 ± 0.98 B/d	225.69 ± 1.23 A/c	209.89 ± 1.54 B/a
	HT	240.31 ± 0.98 B/c	249.82 ± 1.58 B/d	219.28 ± 0.92 B/a	224.11 ± 1.02 A/b	256.28 ± 2.01 B/e	221.54 ± 2.03C/ab
2-phenylethanol	LT	188.62 ± 1.20 B/e	185.83 ± 1.89C/e	168.83 ± 2.03 B/d	162.27 ± 1.26 B/c	147.75 ± 1.54 A/b	141.09 ± 1.04 A/a
	MT	157.35 ± 1.85 A/b	153.80 ± 1.85 B/b	148.58 ± 1.25 A/a	155.43 ± 1.05 A/b	155.00 ± 2.41 B/b	148.26 ± 1.50C/a
	HT	153.24 ± 1.63 A/c	147.41 ± 2.01 A/ab	148.86 ± 1.41 A/b	162.27 ± 1.63 B/d	172.52 ± 2.23C/e	145.47 ± 0.85 B/a
Total content of HAs	LT	565.10 ± 6.82 B/d	553.21 ± 6.91 B/c	531.72 ± 7.29 A/b	525.32 ± 6.08 A/b	516.54 ± 6.32 A/b	474.00 ± 5.17 A/a
	MT	551.82 ± 6.52 A/c	535.87 ± 6.29 A/b	535.68 ± 7.27 A/b	556.96 ± 5.12 B/c	578.74 ± 8.19 B/d	520.09 ± 6.33 B/a
	HT	544.00 ± 5.67 A/a	571.02 ± 7.81 B/c	546.52 ± 6.13 B/ab	561.49 ± 6.13 B/bc	620.02 ± 8.48C/d	562.19 ± 8.96C/bc
[b] Total content of three HAs	LT	535.48 ± 5.30 B/e	511.67 ± 5.33 B/d	495.26 ± 6.04C/c	488.77 ± 4.63 A/bc	480.10 ± 5.09 A/b	399.69 ± 3.14 A/a
	MT	489.35 ± 4.11 A/c	491.10 ± 4.64 A/c	468.28 ± 4.73 B/b	496.98 ± 4.08 B/c	503.30 ± 5.96 B/d	449.18 ± 4.12 B/a
	HT	481.17 ± 3.62 A/b	495.91 ± 4.60 A/c	458.71 ± 3.58 A/a	485.14 ± 4.12 A/b	553.69 ± 6.92C/d	460.74 ± 4.38C/a

[a] The above number represnsents for (% vol).
[b] The above number represnsents for total content without n-propanol. Values are means ± standard deviations from at least three independent tests. Values with different letters in the same row or column are significantly different ($P < 0.05$) from each other. Capital letters represent the differences between different temperatures of the same strain; small letters represent the differences between different strains at the same temperature.

Table 4
The production of acetate esters of different *S. cerevisiae* strains at different temperature in *huangjiu* fermentation.

Parameters	Temperature	85#	TP-555	CYY-661	SYH-607	JH-533	TP-516
Ethyl Acetate (mg/L)	LT	170.49 ± 2.58C/b	196.72 ± 10.94C/cd	225.31 ± 6.51C/e	188.93 ± 1.52C/c	129.63 ± 1.54C/a	201.77 ± 2.49 A/d
	MT	134.93 ± 1.85 A/b	155.72 ± 2.61 A/c	110.09 ± 3.57 A/a	162.06 ± 3.48 B/c	115.58 ± 2.79 A/a	244.9 ± 3.88C/d
	HT	159.16 ± 2.02 B/c	176.70 ± 2.80 B/d	158.55 ± 2.36 B/c	127.16 ± 1.46 A/b	116.85 ± 2.64 B/a	216.8 ± 3.75 B/e
Isoamyl acetate (μg/L)	LT	144.06 ± 2.80 B/c	124.53 ± 2.51 B/b	300.37 ± 14.62 B/e	114.99 ± 1.87 A/a	273.43 ± 11.76 B/d	482.31 ± 20.27 B/f
	MT	84.36 ± 1.31 A/a	116.64 ± 1.85 A/d	109.94 ± 3.56 A/c	149.96 ± 2.44 B/e	102.54 ± 2.15 A/b	250.51 ± 3.30 A/f
	HT	285.35 ± 5.27C/a	847.59 ± 29.87C/d	858.29 ± 23.66C/d	508.39 ± 19.56C/c	461.96 ± 15.48C/b	876.25 ± 22.55C/d
2-Phenylethyl acetate (μg/L)	LT	92.73 ± 1.60 A/b	87.67 ± 1.43 B/a	228.05 ± 13.07C/d	89.07 ± 1.46 B/ab	124.96 ± 1.70 B/c	262.31 ± 24.58 B/d
	MT	91.91 ± 2.22 A/d	73.42 ± 2.04 A/c	64.95 ± 1.80 A/b	58.01 ± 1.83 A/a	66.04 ± 1.20 A/b	154.10 ± 5.01 A/e
	HT	116.12 ± 11.80 B/a	238.36 ± 5.28C/c	171.72 ± 4.07 B/b	275.13 ± 11.93C/d	255.37 ± 17.09C/cd	364.60 ± 17.72C/e

Values are means ± standard deviations from at least three independent tests. Values with different letters in the same row or column are significantly different ($P < 0.05$) from each other. Capital letters represent the differences between different temperatures of the same strain; small letters represent the differences between different strains at the same temperature.

carbon and nitrogen ratios of the different raw material, as which is known to impact the production of HAs and AEs (Saerens et al., 2008). The amino acids (AAs) in the raw material directly correlated to the formation of AEs and HAs. Strains *S. cerevisiae* variation in AEs and HAs were largely strain-dependent and also found in other studies, differences in the activity of alcohol acetyltransferases, nitrogen catabolism and AAs use during different yeast strains (Lilly et al., 2000; Rollero et al., 2015). The obtained strains with lower HAs and higher AEs were strain-dependent, which were more superior than that of the control strain, suggesting that the *huangjiu* quality could be improved by industrial production with specific temperature and strains.

3.5. Effect of temperatures and strains on non-volatile compounds in huangjiu

3.5.1. Effect of temperature and S. cerevisiae strains on amino acids

As one of the rich indispensable components and flavor precursor substances of *huangjiu*, AAs provide various tastes, such as umami, acerbity, sweet, bitter, and briny (Wang et al., 2014). Seventeen kinds of AAs were determined, including seven kinds of essential AAs in *huangjiu* (Fig. 3A). In the same strain, FT significantly affected the content of AAs ($P < 0.5$), the highest amount of AAs were at HT, following MT and LT, except SYH-607 with the opposite amount of AAs. AAs derived mainly from the protease enzymatic degradation of the protein in the raw material, while autolysis of yeast and some other microorganisms were also the source of AAs in *huangjiu* fermentation. Some AAs were used by yeast as nutrients, some (isoleucine, leucine, valine and phenylalanine) could be transformed into corresponding HAs, and the residual became part of composition improved *huangjiu* quality (Ough et al., 1966; Pires et al., 2014). What needed illustration was that the total HAs content (without n-propanol) showed the opposite results impacted by FT compared to AAs (Table 3), whether there was a certain correlation between the two substances needed further research in the future.

3.5.2. Effect of temperature and S. cerevisiae strains on organic acids

Organic acids (OAs) played a very important role in *huangjiu*, the content and type of OAs could influence the final quality. Furthermore, OAs affected the color, flavor and biological stability, appropriate amount of OAs could reduce the sweet tasting and enhance the strong flavor and promote the formation of esters during storage (Mato et al., 2005). Eight kinds of OAs were detected and analyzed (Fig. 3B). Lactic acid was the highest amount of OAs, following the acetic acid, accounting for more than 75% of the total content. In the same strain, temperature significantly affected the content of OAs ($P < 0.5$), the lowest content was at LT, while MT as the optimal FT had the highest amount of OAs. These acids originated from the raw materials released or developed during alcoholic fermentation, malolactic fermentation, and oxidation of the ethanol (Ugliano and Moio, 2005). OAs (Lactic acid) produced during the rice soaking process by lactic acid bacteria (LAB) and brought into the fermentation with raw materials as the ingredients were also important sources, and this provided an acidic environment for the subsequent fermentation process of yeast and LAB (Liu, Sun, et al., 2021a). Strains *S. cerevisiae* also produced OAs during alcoholic fermentation or by LAB genera during fermentation influenced by strains and FT, which were the possible reasons for these differences.

3.6. Effect of temperatures and strains on undesirable secondary metabolites in huangjiu

3.6.1. Effect of temperatures and strains on ethyl carbamate

Yeast (*S. cerevisiae*) was the dominant flavor-producing microorganism, emerging the final aroma-active compounds. However, some hazardous substances also produced in *huangjiu* fermentation. Controlling the FT of *S. cerevisiae* strains in the production of FABs was considered to increase the production and retention of desirable secondary metabolites, while undesirable compounds such as ethyl carbamate (EC) and biogenic amines (BAs) were usually ignored. EC was a potentially carcinogenic compound, which was formed by the reaction of urea with ethanol in FABs. As a precursor of EC, higher amount of urea further transformation produced more EC in *huangjiu*. In the same strain, FT significantly affected the production of urea, suggesting that LT could decrease the content of urea (Fig. 4A). The concentration of EC was detected in all the fermentation treatments by HS-SPME/GC-MS (Fig. 4B), showing that EC was lower at LT than that of higher temperature (MT and HT) in some strains (TP-555, CYY-661, SYH-607 and JH-533), the same effects as urea. Different from the above, two strains (85# and TP-516) had the least amount of EC at MT, but the higher content of urea at MT. In our current study, the production of EC by

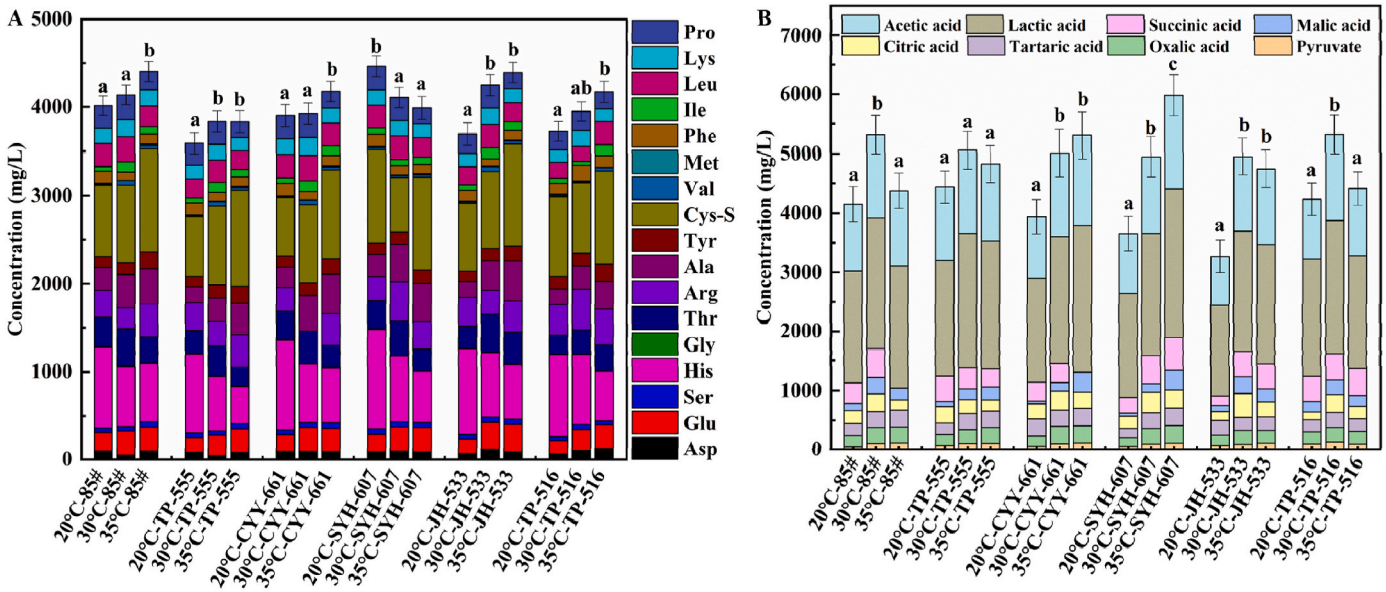

Fig. 3. The production of amino acids and organic acids of different *S. cerevisiae* strains at different temperature in *huangjiu* fermentation. A: Effects of temperature and *S. cerevisiae* strains on AAs. Proline, Pro; Lysine, Lys; Leucine, Leu; Isoleucine, Ile; Phenylalanine, Phe; Methionine, Met; Valine, Val; Cysteine, Cys-S; Tyrosine, Tyr; Alanine, Ala; Arginine, Arg; Threonine, Thr; Glycine, Gly; Histidine, His; Serine, Ser; Glutamic acid, Glu; Aspartic acid, Asp. B: Effects of temperature and *S. cerevisiae* strains on OAs. Values with different letters are significantly different ($p < 0.05$) from each other. Error bars represent the standard errors of the mean.

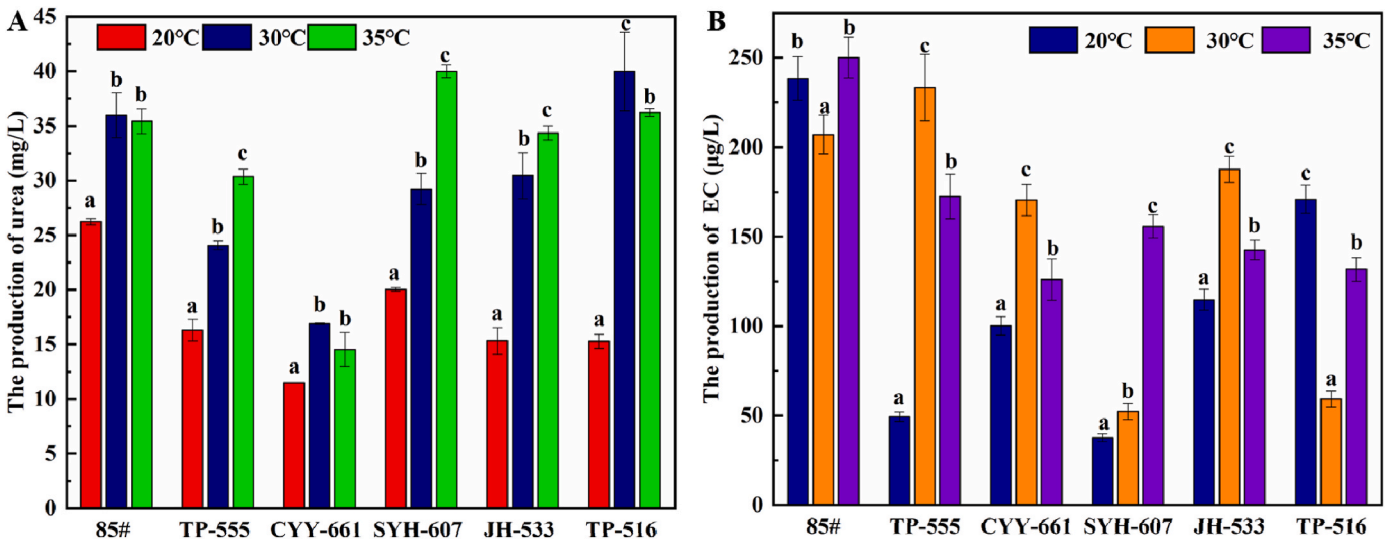

Fig. 4. The production of urea and ethyl carbamate of different *S. cerevisiae* strains at different temperature in *huangjiu* fermentation. A: Effects of temperature and *S. cerevisiae* strains on urea in *huangjiu* fermentation. B: Effects of temperature and *S. cerevisiae* strains on EC in *huangjiu* fermentation. Values with different letters are significantly different ($p < 0.05$) from each other. Error bars represent the standard errors of the mean.

S. cerevisiae in *huangjiu* fermentation was not only the accumulation of urea, but also the change of temperature and the differential ability of yeast or other microorganism to metabolize EC. The fortified starter culture with *S. cerevisiae* strains and LAB reduced the urea and EC content in *huangjiu* significantly, which had been found in the latest research (Tian et al., 2022). EC was the most common one of the harmful components found in *huangjiu*, the average concentration of which was 160 μg/kg in *huangjiu* (in excess of the limit in sake, 100 μg/kg in America) (Zhao et al., 2013). To control the concentration of EC was urgent to improve the drinking safety of *huangjiu*, although there was yet no limitation standard in *huangjiu*. Several methods had been proposed to reduce EC content, including physical (Low temperature), chemical (Copper catalysts), enzymatic (Urease), and metabolic engineering routes (Disruption related genes) (Saerens et al., 2008; Zhao et al., 2013). It has also been found that *S. cerevisiae* strains with lower urea production could control the urea and EC contents in *huangjiu* production effectively, due to 90% of EC in *huangjiu* is produced by the reaction of the urea and the alcohol of *S. cerevisiae* (Liang et al., 2022). However, controlling FT of different *S. cerevisiae* strains may also be an effective way to reduce EC.

3.6.2. *Effect of temperatures and strains on biogenic amines*

BAs are formed mainly by microbial decarboxylation of AAs, which are low molecular organic nitrogenous compounds widely present in FABs (Gardini et al., 2016). Seven kinds of BAs were detected in all the fermentation treatments by HPLC, three kinds of BAs had the content less than 1 mg/L. The majority of BAs in *huangjiu* was putrescine (PUT), cadaverine (CAD), tyramine (TYR), and histamine (HIS), the proportion of which were significant difference between different FT (Fig. 5). Five *S. cerevisiae* strains produced highest amount of BAs at LT, while TP-555 produced least amount of BAs at LT. TP-555 was chosen due to screening of fermentative ability at LT, owning the ability of low-yeild BAs especially at LT, which might explain the above result. At the same temperature, the production of BAs was significantly different among different strains. BAs in *huangjiu* were predominantly produced during rice soaking process (Liu et al., 2021b). *PEP4* gene (encoding proteinase A) of *S. cerevisiae* had been found related to the ability of the strain to produce BAs during fermentation process (Guo et al., 2015). In this study, the difference of BAs content was mainly the result of the influence of different temperature on *S. cerevisiae* metabolism due to the short rice soaking time (3–5 days) and the same experimental materials. The short rice soaking time produced small amounts of BAs. Therefore,

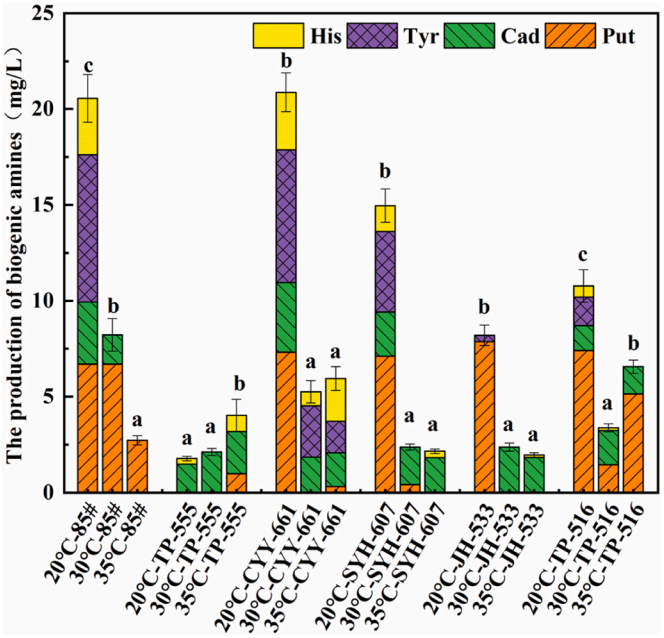

Fig. 5. The production of biogenic amines of different *S. cerevisiae* strains at different temperature in *huangjiu* fermentation. Histamine, His; Tryptamine, Try; Cadaverine, Cad; Putrescine, Put. Values with different letters are significantly different ($p < 0.05$) from each other. Error bars represent the standard errors of the mean.

the differences between different strains may be the result of *PEP4* gene expression. The metabolisms of non-preference by-products (Urea, EC and BAs) of the obtained strains were lower than that of the control strain, suggesting that the drinking safety and *huangjiu* quality could be improved in industrial production by controlling FT of different *S. cerevisiae* strains.

4. Conclusion

FT can clearly affect the development of the different *S. cerevisiae* strains. LT fermentation generally has a more positive effect (higher ester, lower HAs, or lower acid) than higher temperature (MT and HT).

However, some strains may have the opposite effects fermented in *huangjiu*. If fermentations are conducted at restrictive temperatures (LT or HT), which may proceed with difficulty and a higher probability of stuck fermentations, unless a resistant strain appeared, as the six strains in this study. It seems that the better temperature-resistant strains (From LT to HT) are more likely to have a positive effect on fermentation. Three different strains (TP-555, CYY-661, and SYH-607) show good performance in ethanol yield at different temperatures, and have good ability to produce lower HAs and higher AEs in this study. Moreover, TP-555 has minimal concentration of BAs, and CYY-661 has minimal concentration of urea and EC, while SYH-607 has relatively low levels of undesirable secondary metabolites, and maximal concentration of ethanol at 35 °C. *S. cerevisiae* strains with higher temperature tolerant can fill the defect of rancidity of *huangjiu* fermentation at HT in summer. Controlling FT and the industrial *S. cerevisiae* strains can meet the requirements for *huangjiu* fermentation and increase the variety of styles. In conclusion, the quality and characteristics of *huangjiu* are strongly affected by the *S. cerevisiae* strains and their different endurance to temperatures, which can be a criterion for indigenous *S. cerevisiae* strains selection for further purposes such as fermentation starters in future *huangjiu* production.

Credit author statement

Yuzong Zhao: Conceptualization, Investigation, Methodology, Project administration, Writing - original draft. **Shuangping Liu**: Data curation, Formal analysis, Funding acquisition, Writing – review & editing. **Xiao Han:** Supervision, Project administration. **Zhilei Zhou**: Investigation, Methodology, Visualization. **Jian Mao**: Resources, Project administration, Funding acquisition, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fm.2022.104091.

References

Antonelli, A., Castellari, L., Zambonelli, C., Carnacini, A., 1999. Yeast influence on volatile composition of wines. J. Agric. Food Chem. 47, 1139–1144.
Bisson, L.F., 1999. Stuck and sluggish fermentations. Am. J. Enol. Vitic. 50, 107–119.
Chen, S., Xu, Y., 2010. The influence of yeast strains on the volatile flavour compounds of Chinese rice wine. J. Inst. Brew. 116, 190–196.
Deed, R.C., Fedrizzi, B., Gardner, R.C., 2017. Influence of fermentation temperature, yeast strain, and grape juice on the aroma chemistry and sensory profile of Sauvignon blanc wines. J. Agric. Food Chem. 65, 8902–8912.
Gardini, F., Özogul, Y., Suzzi, G., Tabanelli, G., Özogul, F., 2016. Technological factors affecting biogenic amine content in foods: a review. Front. Microbiol. 7, 1218.
Guo, X., Guan, X., Wang, Y., Li, L., Wu, D., Chen, Y., Pei, H., Xiao, D., 2015. Reduction of biogenic amines production by eliminating the PEP4 gene in Saccharomyces cerevisiae during fermentation of Chinese rice wine. Food Che 178, 208–211.
Liang, Z., Su, H., Ren, X., Lin, X., He, Z., Li, X., Zheng, Y., 2022. Analysis of key genes responsible for low urea production in Saccharomyces cerevisiae JH301. Front. Microbiol. 13, 894661.
Lilly, M., Lambrechts, M., Pretorius, I., 2000. Effect of increased yeast alcohol acetyltransferase activity on flavor profiles of wine and distillates. Appl. Environ. Microbiol. 66, 744–753.
Liu, S., Chen, Q., Zou, H., Yu, Y., Zhang, S., 2019. A metagenomic analysis of the relationship between microorganisms and flavor development in Shaoxing mechanized huangjiu fermentation mashes. Int. J. Food Microbiol. 303, 9–18.
Liu, S., Ma, D., Li, Z., Sun, H., Mao, J., 2021. Assimilable nitrogen reduces the higher alcohols content of huangjiu. Food Control 121, 107660.
Liu, S., Sun, H., Liu, C., Zhou, Z., Mao, J., Hu, Z., Xu, X., Han, X., Zhang, S., Mao, J., 2021b. Reducing biogenic amine in seriflux and huangjiu by recycling of seriflux inoculated with Lactobacillus plantarum JN01. Food Res. Int. 150, 110793.
Mato, I., Suárez-Luque, S., Huidobro, J.F., 2005. A review of the analytical methods to determine organic acids in grape juices and wines. Food Res. Int. 38, 1175–1188.
Metsalu, T., Vilo, J., 2015. ClustVis: a web tool for visualizing clustering of multivariate data using Principal Component Analysis and heatmap. Nucleic Acids Res. 43, W566–W570.
Miller, L., 1987. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. 31.
Mo, X., Xu, Y., Fan, W., 2010. Characterization of aroma compounds in Chinese rice wine Qu by solvent-assisted flavor evaporation and headspace solid-phase microextraction. J. Agric. Food Chem. 58, 2462–2469.
Molina, A.M., Swiegers, J.H., Varela, C., Pretorius, I.S., Agosin, E., 2007. Influence of wine fermentation temperature on the synthesis of yeast-derived volatile aroma compounds. Appl. Microbiol. Biot. 77, 675–687.
Ntuli, R.G., Saltman, Y., Ponangi, R., Jeffery, D.W., Bindon, K., Wilkinson, K.L., 2022. Impact of fermentation temperature and grape solids content on the chemical composition and sensory profiles of Cabernet Sauvignon wines made from flash détente treated must fermented off-skins. Food Chem. 369, 130861.
Ough, C., Guymon, J., Crowell, E., 1966. Formation of higher alcohols during grape juice fermentations at various temperatures. J. Food Sci. 31, 620–625.
Pérez, D., Assof, M., Bolcato, E., Sari, S., Fanzone, M., 2018. Combined effect of temperature and ammonium addition on fermentation profile and volatile aroma composition of Torrontés Riojano wines. LWT–Food Sci. Technol. 87, 488–497.
Papathanasiou, I., Selvagini, R., Servili, M., Vaughan-Martini, A., Roussis, I., 2006. Winemaking ability of wild yeast strains and comparative volatile profiles of wines fermented at 12°C or 20°C. Food Sci. Technol. Res. 12, 194–198.
Pires, E.J., Teixeira, J.A., Brányik, T., Vicente, A.A., 2014. Yeast: the soul of beer's aroma—a review of flavour-active esters and higher alcohols produced by the brewing yeast. Appl. Microbiol. Biot. 98, 1937–1949.
Robinson, A.L., Boss, P.K., Heymann, H., Solomon, P.S., Trengove, R.D., 2011. Influence of yeast strain, canopy management, and site on the volatile composition and sensory attributes of cabernet sauvignon wines from western Australia. J. Agric. Food Chem. 59, 3273.
Rollero, S., Bloem, A., Camarasa, C., Sanchez, I., Ortiz-Julien, A., Sablayrolles, J.-M., Dequin, S., Mouret, J.-R., 2015. Combined effects of nutrients and temperature on the production of fermentative aromas by Saccharomyces cerevisiae during wine fermentation. Appl. Microbiol. Biot. 99, 2291–2304.
Romano, P., Pietrafesa, R., Romaniello, R., Zambuto, M., Calabretti, A., Capece, A., 2015. Impact of yeast starter formulations on the production of volatile compounds during wine fermentation. Yeast 32, 245–256.
Saerens, S., Delvaux, F., Verstrepen, K., Van Dijck, P., Thevelein, J., Delvaux, F., 2008. Parameters affecting ethyl ester production by Saccharomyces cerevisiae during fermentation. Appl. Environ. Microbiol. 74, 454–461.
Saison, D., Schutter, D.P.D., Uyttenhove, B., Delvaux, F., Delvaux, F.R., 2009. Contribution of staling compounds to the aged flavour of lager beer by studying their flavour thresholds. Food Chem. 114, 1206–1215.
Samoticha, J., Wojdyo, A., Chmielewska, J., Nofer, J., 2019. Effect of different yeast strains and temperature of fermentation on basic enological parameters, polyphenols and volatile compounds of Aurore white wine. Foods 8.
Styger, G., Jacobson, D., Bauer, F.F., 2011. Identifying genes that impact on aroma profiles produced by Saccharomyces cerevisiae and the production of higher alcohols. Appl. Microbiol. Biot. 91, 713–730.
Tian, S., Zeng, W., Guan, X., Zhou, J., Du, G., 2022. Effects of fortified starter culture containing Saccharomyces cerevisiae and Lactobacillus fermentum on microbial community structure and ethyl carbamate. Food Control 137, 108890.
Torija, M.J., Beltran, G., Novo, M., Poblet, M., Rozès, N., 2003. Effects of fermentation temperature and Saccharomyces species on the cell fatty acid composition and presence of volatile compounds in wine. Int. J. Food Microbiol. 85, 127–136.
Tronchoni, J., Rozès, N., Querol, A., Guillamón, J., 2012. Lipid composition of wine strains of Saccharomyces kudriavzevii and Saccharomyces cerevisiae grown at low temperature. Int. J. Food Microbiol. 155, 191–198.
Ugliano, M., Moio, L., 2005. Changes in the concentration of yeast-derived volatile compounds of red wine during malolactic fermentation with four commercial starter cultures of Oenococcus oeni. J. Agric. Food Chem. 53, 10134–10139.
Wang, P., Mao, J., Meng, X., Li, X., Liu, Y., Feng, H., 2014. Changes in flavour characteristics and bacterial diversity during the traditional fermentation of Chinese rice wines from Shaoxing region. Food Control 44, 58–63.
Zhang, W., Cheng, Y., Li, Y., Du, G., Xie, G., Zou, H., Zhou, J., Chen, J., 2018a. Adaptive evolution relieves nitrogen catabolite repression and decreases urea accumulation in cultures of the Chinese rice wine yeast strain Saccharomyces cerevisiae XZ-11. J. Agric. Food Chem. 66, 9061–9069.
Zhang, W., Li, Y., Chen, Y., Xu, S., Du, G., Shi, H., Zhou, J., Chen, J., 2018b. Complete genome sequence and analysis of the industrial Saccharomyces cerevisiae strain N85 used in Chinese rice wine production. DNA Res. 25, 297–306.
Zhao, X., Du, G., Zou, H., Fu, J., Zhou, J., Chen, J., 2013. Progress in preventing the accumulation of ethyl carbamate in alcoholic beverages. Trends Food Sci Tech 32, 97–107.

Zhao, X., Zou, H., Fu, J., Zhou, J., Du, G., Chen, J., 2014. Metabolic engineering of the regulators in nitrogen catabolite repression to reduce the production of ethyl carbamate in a model rice wine system. Appl. Environ. Microbiol. 80, 392–398.
Zheng, H., Wei, P., Zhang, G., Xu, W., Li, Y., 2021. The impact of different Saccharomyces cerevisiae strains on microbial composition and quality of Chinese rice wine fermentations. Yeast 38, 147–156.
Zhou, Z., Ni, W., Ji, Z., Liu, S., Han, X., Li, X., Mao, J., 2020. Development of a rapid method for determination of main higher alcohols in fermented alcoholic beverages based on dispersive liquid-liquid microextraction and gas chromatography-mass spectrometry. Food Anal Method 13, 591–600.