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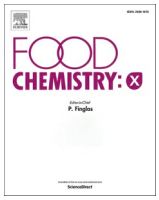

RSM optimization of fermentation technology of yellow wine produced from Millets rice (containing Daylily and Agaricus blazei Murr) and analysis of volatile aroma constituents and amino acid contents by GC–MS and HPLC

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ABSTRACT

The fermentation process was carried out with varied input variables, and all the models showed significant *p*-values for interaction of variance (<0.05). Millet rice yellow wine with ethanol yield of 15.68%, *v*/v was produced at an optimized temperature of 32 °C, yeast addition quantity of 9%, and time of 41 days. The volatile compounds in millet rice wine were analyzed by gas chromatography coupled with mass spectrometry (GC/MS). Amino acid components in yellow wines were analyzed by high performance liquid chromatography (HPLC). Ten, twelve and sixteen volatile compounds were detected in wines in fermentation time of 41, 55 and 70 days, respectively. HPLC shows that the content of amino acids in rice wine is not only the highest, but also has 20 kinds, including 8 kinds of essential amino acids for human body. The difference in volatile components and amino acids composition greatly contribute to the flavor of the wine.

1. Introduction

Millets contain proteins, vitamins, minerals and dietary fiber content (Jaybhaye, Pardeshi, Vengaiah, & Srivastav, 2014). Millet is also a healthy grains that can be effective in patients with celiac disease (Sarita & Singh, 2016). Because millet has low glycemic index and good prebiotic activity, it can promote intestinal health and digestion (Cordelino et al., 2019). The studys showed that protein extract from millet seed could inhibit the growth of Rhizoctonia solani, Coleoptera Fabricius and Fusarium oxysporum (Radhajeyalakshmi, Yamunarani, Seetharaman, & Velazhahan, 2003).

Daylily (*Hemerocallis lilioasphodelus* L.), a kind of perennial herbs of Liliaceae, widely cultivated in all over China and some other countries (Korea, Japan, and Europe). For hundreds of years, chinese people have been eating it as a vegetable and traditional medicine (Liu et al., 2017).

Agaricus blazei Murr is a kind of grass rotting and aerobic fungus occurring in summer and autumn, which originated from Brazil and is currently cultivated in many countries. Its chemical components contain agaritine, steroids,(Kawagishi et al., 1988), lipids, lectin, and various polysaccharides (Mizuno et al., 1990). Studys found that polysaccharide–protein complexes in the mushroom has antitumor activity (Fujimiya et al., 1998).

Response surface methodology (RSM) is a set of statistical and mathematical techniques for developing, improving and optimizing the process (Hatambeygi, Abedi, & Talebi, 2011). Through a series of experiments, a high precision regression equation describing the relationship between response and parameters is obtained. This allows us to plot the best response and characterize the experimental parameters. Response surface methodology can also effectively evaluate multiple factors and their interactions (Switzar, Giera, Lingeman, Irth, & Niessen, 2011), and is widely used in food analysis, chromatographic condition optimization and prescription screening (Huang, Kuo, Chen, Liu, & Shieh, 2017).

A few studies have applied RSM to fermentation technology (Rani, Rastogi, & Appaiah, 2011). The Box–Behnken design (BBD) is easy to perform experiments and interpret in comparison to other models (Box & Behnken, 1960; Ferreira et al., 2007). Yellow rice wine is the oldest beverage wine in China, with a brewing history of >4000 years. It contains sugar, dextrin, organic acids, amino acids, esters, glycerol, vitamins and other nutrients. Thus, the present study was designed to

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determine the optimum fermentation condition for maximizing ethanol yield from millets rice (containing small proporation of Daylily and Agaricus blazei Murr) using the Box–Behnken design. The volatile compounds and amino acid contents from the yellow wine samples were analyzed by Gas Chromatography and Mass Spectrometry (GC–MS) and high performance liquid chromatography (HPLC).

2. Material and methods

2.1. Materials

Commercial millet, Agaricus blazei Murr and Daylily were obtained from DaTong city (ShanXi province, China). Fermentation Hong Qu starter (yeast) was purchased from a wine product company (Taiyuan city, ShanXi province, China). Methanol, 2,6-dichloroindophenol were purchased from Shanghai YuanMo Biotechnology Ltd. (Shanghai, China). Sodium dihydrogen phosphate, sodium carbonate, sodium chloride were purchased from Tianjin Damao Chemical Reagent Company (Tianjin city, china). All other chemicals were reagent grade and commercially available.

2.2. Wine fermentation

At the beginning of the fermentation process, every 2.0 kg millet was washed and soaked in water at room temperature for 4–8 h, then cooked and steamed at 100 °C for 30 min. After the steamed millet rice was cooled to room temperature, it was well mixed with 0.4 kg Daylily (fine powder), 0.3 kg Agaricus blazei Murr (fine powder) to obtain fermentation raw material mixture. Then, the fermentation raw material mixture is equally divided into 7 groups. Different quantity (4%, 5%, 6%, 7%, 8%, 9% and 10%) of fermentation Hong Qu starter (yeast) was added into 7 groups, respectively. Every group was transferred to a traditional Chinese wine jar. Finally, sterilized water was added to make a total volume of 2.0 L (every group), and the wine underwent fermentation at different temperature (20, 23, 26, 29, 32, 35 and 38 °C) for different (15, 20, 25, 30, 35, 40, and 45) days. Effect of different quantity (4%, 5%, 6%, 7%, 8%, 9% and 10%) of fermentation Hong Qu starter (yeast), different fermentation temperature (20, 23, 26, 29, 32, 35 and 38 °C) and different (15, 20, 25, 30, 35, 40, and 45) fermentation days on ethanol and reducing sugar contents in yellow wine was investigated.

2.3. Response surface methodology (RSM)

RSM is used to model and analyse problems whose response is affected by multiple variables. In millet yellow rice wine fermentation optimization, the independent variables were the fermentation temperature (A, ^{o}C), and the yeast addition quantity (B, %) and fermentation time (C, day). The responses (dependent variables) were the ethanol yield (R1, % *v*/v), and reducing sugar level (R2, mg/ml). All extraction experiments were performed in accordance with a Box-Behnken design with 3 factors and 3 levels. These variables were coded to three levels as −1, 0, and + 1 (Table 1).

2.4. Headspace solid phase microextraction (HS-SPME)

According to the methods established by Schmarr, Keiser, and Krautwald (2016), before introducing the sample, the air in the sampling device was evacuated for 1 min, using a MD 4C diaphragm vacuum pump (7 mbar = 0.007 atm ultimate vacuum without gas ballast) manufactured by Vacuubrand GmbH & Co. KZ (Wertheim, Germany). The samples were stirred at 30 °C for 20 min (500 RPM), and then extracted by headspace at the same temperature for 40 min. The analyte was then desorbed directly in the split //splitless inlet (250 °C; SPME liner, 0.75 mm i.d.; Supelco) of the GC–MS-O system for 1 min. After desorption, the fibers were heated in a fiber conditioning station at 270 °C for 20 min.

Table 1
Values of factors in Box–Behnken design (BBD).

Level	Independent variable		
	Temperature (°C)	Yeast addition quantity (%)	Time (d)
−1	29	8	35
0	32	9	40
1	35	10	45

2.5. GC/MS analysis

According to the methods established by by Xian et al. (2019), GC/MS analysis was used. For the analysis of the volatile compounds mentioned above, GC–MS 5975 Series MSD (Agilent Technologies, Palo Alto, California, USA) instrument was employed. The injection of the wine extract was made in splitless mode, the injector temperature was 250 °C. The column oven temperature was initially maintained at 40 °C and then programmed to 200 °C at a rate of 4 °C / min for a final holding time of 20 min. The working conditions of the mass spectrometer are as follows: electron collision mode, ionization energy of 70 EV, mass range of 30–500 amu, scanning speed of 3.2 times / s, solvent delay time of 6–10 cycles. The volatile compounds were identified by comparing the retention index with the mass spectra of commercial standards. The volatile compounds were also identified using the software library of mass spectra database Willey 6.1 (NY, USA).

2.6. Derivatisation

According to Alaiz, Navarro, Girón, and Vioque (1992) and Gómez-Alonso, Hermosín-Gutiérrez, and García-Romero (2007) et al.'s method with some modifications, the derivatization was carried out. The reacting mixture was composed of 430 μL of 300 μL methanol, 1 M borate buffer (pH 9.0), 400 μL sample, 12 μL DEEMM and 10 μL internal standard (2-aminoadipic acid, 1.00 g/L). The derivatization reaction was performed for >30 min in a screw-cap test tube in ultrasonic bath. The mixture was then heated at 70–80 °C for 2 h to completely degrade the excess DEEMM and other by-products.

2.7. HPLC analysis

According to the methods established by Perestrelo, Bordiga, Locatelli, Silva, and Câmara (2020), analysis was carried out on a Thermo Finnigan Surveyor HPLC fitted with an Alltima C18 HPLC column (250 × 2.1 mm i.d., 5 μm, 100 Å, Grace Davison Discovery Sciences, Rowville, VIC, Australia) connected to a Thermo Finnigan LCQ Deca XP Plus mass spectrometer using electrospray ionization in positive ion mode. The injection volume was 2 μL. For detection, a photodiode array detector (G1315d) is used to monitor at 280 nm. The target compounds were identified according to the retention time of the standard samples. A calibration factor programmed by MembraPure was used to quantify the amino acids.

3. Results and discussion

3.1. Effect of different independent variable (temperature, yeast addition quantity and time) on ethanol yield and reducing sugar level in millet rice yellow wine

As shown in Fig. 1, the ethanol content in yellow wine increased with the increasing fermentation temperature and reached the maximum value (15.59, % *v*/v) when fermentation temperature at the level of 32 °C. Beyond this level, the ethanol content in yellow wine started to decline. By contrast, the reducing sugar content in yellow wine decreased with the increasing fermentation temperature and reached

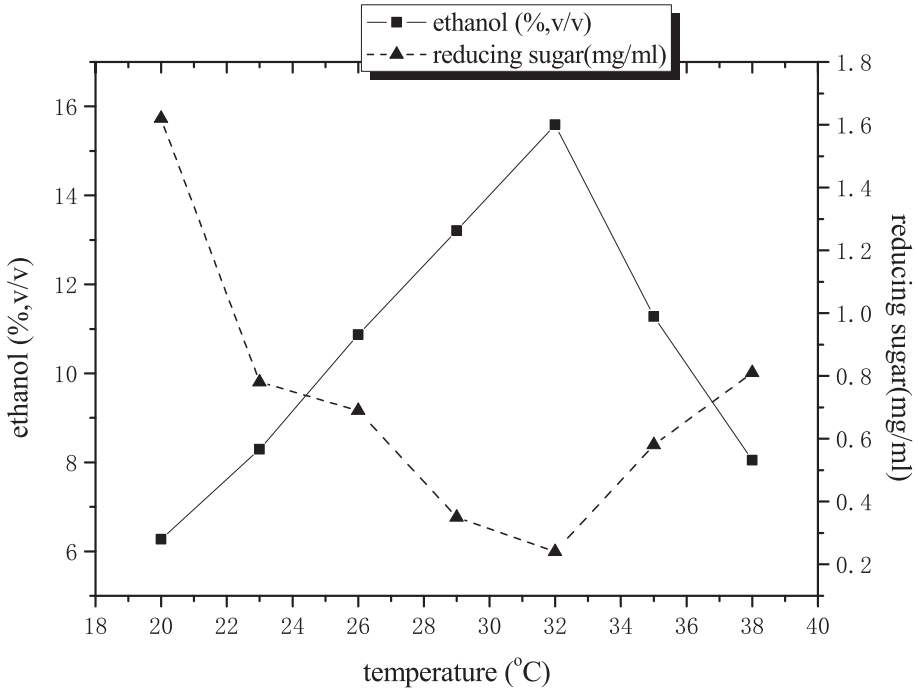

Fig. 1. Effect of fermentation temperature on ethanol yield and reducing sugar level in yellow wines. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the minimum value (0.24 mg/ml) when fermentation temperature at the level of 32 °C. Beyond this level, the reducing sugar content in yellow wine started to increase. At higher temperature, yeast viability decreased, ethanol yield reduced and reducing sugar accumulated (García-Ríos & Guillamón, 2019).

Fig. 2 showed that the ethanol content in yellow wine increased with the increasing yeast addition quantity and reached the peak value (15.84, % *v*/v) at 9%. After this level, the ethanol content in yellow wine maintained almost constant. By contrast, the reducing sugar content in yellow wine decreased with the increasing yeast addition quantity and reached the minimum value (0.25 mg/ml) at 9%. After this level, the reducing content in yellow wine mildly increase. In some literatures, yeast addition quantity affected ethanol production. Beyond optimal addition quantity, ethanol production no longer significantly increased (Chiva, López-Malo, Salvadó, Mas, & Guillamón, 2012; Vu & Kim, 2009). These are in agreement with results of our experiment.

As shown in Fig. 3, the ethanol content in yellow wine increased with the extending fermentation time and reached the maximum value

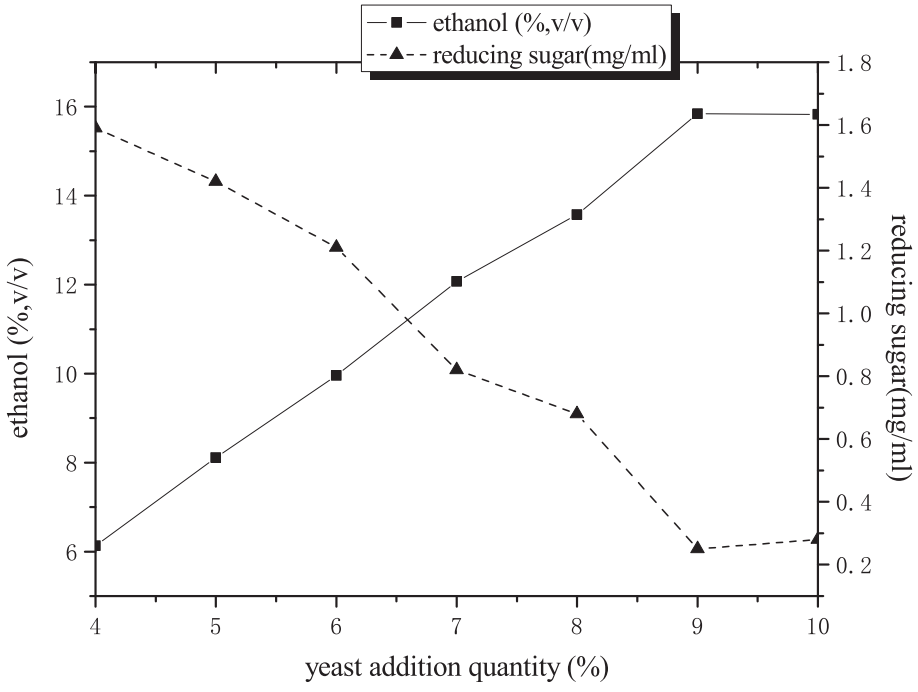

Fig. 2. Effect of yeast addition quantity on ethanol yield and reducing sugar level in yellow wine. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

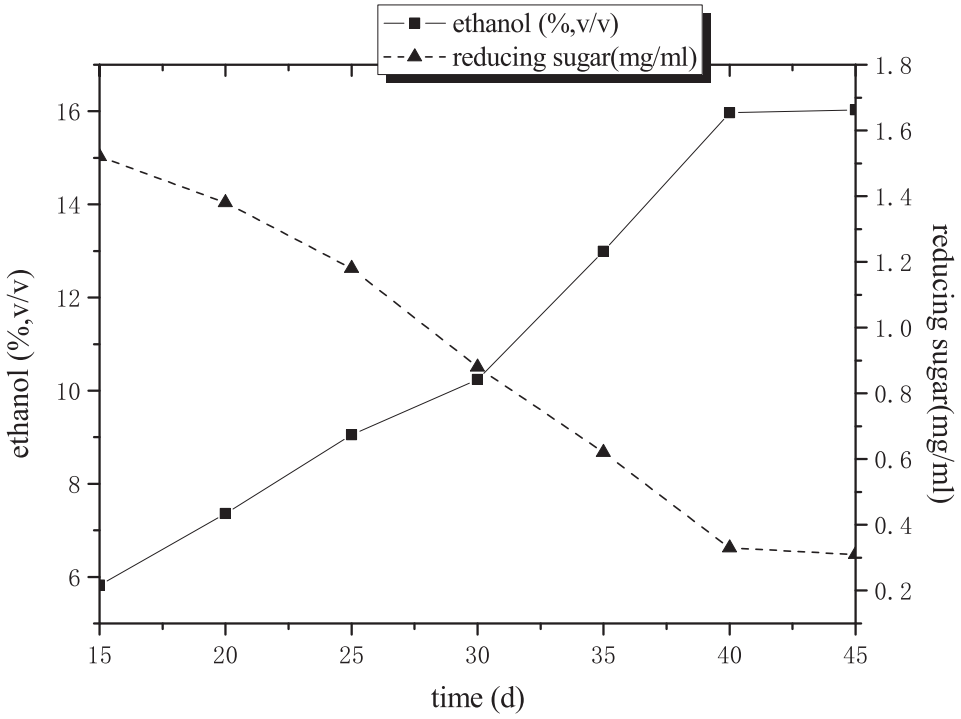

Fig. 3. Effect of fermentation time on ethanol yield and reducing sugar level in yellow wine. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(15.97, % v/v) at 40 day. Beyond this level, the ethanol content in yellow wine no longer increased. By contrast, the reducing sugar content in yellow wine decreased with the extending fermentation time and reached the minimum value (0.33 mg/ml) at 40 day. The reducing sugar content in yellow wine mildly no longer obviously changed. At this moment, the production of alcohol and the consumption of sugar reached a dynamic equilibrium state.

3.2. Fermentation experiment optimization analysis

3.2.1. Optimization analysis (Regression model, ANOVA) of ethanol yield and interaction between fermentation variables

Results of target responses, obtained from millet yellow rice wine fermentation performed using Box-Behnken configuration, are shown in Tables 2 and 3. The second-order quadratic model equation of the target response (ethanol) was used as the coding function of the independent variable, and was fitted by multiple regression analysis. They are represented by Eqs. (1).

$$R1\ (\text{ethanol},\%v/\text{v}) = -115.69500 + 3.93500^{*}\ (\text{temperature},\ ^{\circ}\text{C}) + 7.63500^{*}\ (\text{Yeast addition},\%) + 1.89967^{*}\ (\text{time},\text{d}) - 5.00000\text{E}-003^{*}\ (\text{temperature},\ ^{\circ}\text{C})^{*}\ (\text{Yeast addition},\%) - 5.33333\text{E}-003^{*}\ (\text{temperature},\ ^{\circ}\text{C})^{*}\ (\text{time},\text{d}) + 0.037000^{*}\ (\text{Yeast addition},\%)^{*}\ (\text{time},\text{d}) - 0.060000^{*}\ (\text{temperature},\ ^{\circ}\text{C})\text{^}2 - 0.50000^{*}\ (\text{Yeast addition},\%)\text{^}2 - 0.026200^{*}\ (\text{time},\text{d})\text{^}2 \quad (1)$$

As shown in Tables 2 and 3, p-value was used to evaluate the significance of the model. The higher F value (623.14) and lower probability value ($p < 0.0001$) indicated that the model was significant. The higher R^2 value (0.9988) indicates that the statistical significance of the formula regression model is higher. The lack of fit value (0.0932) is not significant (> 0.05), which suggests that the quadratic model has good reliability and accuracy. The correction coefficient $R^2_{\text{Adj}} = 0.9972$ indicates that the equation can explain 99.72% of the response value changes, which can well reflect the accuracy and universality of the model.

Through the regular analysis of response surface, three response surface graphs are obtained. Interpretation of the response surface 3D model and contour plot were the graphical representations of regression equation. They provide an intuitive explanation of the relationship between the response of each variable and their experimental levels, as well as the type of interaction between the two test variables. It has been reported that RSM can be successfully applied to the modeling and optimization of fermentation system for ethanol production recovery in similar experimental fields (Chandra, Oro, Ferreira-Dias, & Malfeito-Ferreira, 2015) which has been also confirmed in present study.

It is reported that alcohol content may be the decisive factor of product quality, and increasing alcohol content can improve the perceived fullness of drinks (Nyanga, Nout, Smid, Boekhout, & Zwietering, 2013). R^2 of the model was 0.9988 (Table 3), which implied that variability of millet yellow rice wine fermentation independent process variables could explain 99.88% of the variations in the corresponding ethanol yield. Highest total ethanol yield (16.15%, v/v) was observed in experimental run number 15 under the fermentation conditions: fermentation temperature 32 °C, yeast addition quantity 9%, and fermentation time 40 day.

The response surface plots of influencing parameters of ethanol yield and reducing sugar level are shown in Fig. S1, Fig. S2, respectively. Fig. S1 i-iii shows the interactive effects of two variables on the ethanol yield presented in response surface and contour plots, while the other

Table 2
BBD matrix and response.

Run	A (temperature, °C)	B (Yeast addition, %)	C (time, d)	Ethanol (%, v/v)	reducing sugar (mg/ml)
1	−1.00 (29 °C)	−1.00 (8%)	0.00 (40 d)	15.6	1.48
2	1.00 (35 °C)	−1.00	0.00	14.67	1.5
3	−1.00	1.00 (10%)	0.00	15.52	1.45
4	1.00	1.00	0.00	14.53	1.61
5	−1.00	0.00 (9%)	−1.00 (35 d)	15.54	1.46
6	1.00	0.00	−1.00	14.7	1.57
7	−1.00	0.00	1.00 (45 d)	15.31	1.49
8	1.00	0.00	1.00	14.15	1.68
9	0.00 (32 °C)	−1.00	−1.00	15.33	1.53
10	0.00	1.00	−1.00	14.89	1.59
11	0.00	−1.00	1.00	14.67	1.56
12	0.00	1.00	1.00	14.97	1.62
13	0.00	0.00	0.00	16.12	1.41
14	0.00	0.00	0.00	16.13	1.37
15	0.00	0.00	0.00	16.15	1.38
16	0.00	0.00	0.00	16.09	1.38
17	0.00	0.00	0.00	16.11	1.4

variable being fixed at level "0". Higher F-value of 1563.44 and lower *p*-value of <0.0001 showed that fermentation temperature was the most influential factor in affecting the ethanol yield, followed by fermentation time (F-value of 188.19 and *p*-value of <0.0001) and yeast addition quantity (F-value of 13.19 and *p*-value of =0.0084).

By drawing the response surface curve and studying the interaction between variables, the optimal level of each variable is determined and the maximum response is obtained. The three-dimensional response surface contour map is used as the graphical representation of the regression equation. Fig. S1 (i) shows the effect of temperature (A), yeast addition quantity (B), and their reciprocal interaction on ethanol yield (% *v*/v), when time (C) was fixed at 40 day. The result revealed that the ethanol yield increased with the increase in temperature and yeast addition quantity. With a further increasing in temperature and yeast addition quantity, the yield began to decrease. As shown in Fig. S1 (ii), the ethanol yield increased with increasing temperature (A) and time (C). With a further increase in temperature (A) and time (C), the yield showed slight decrease. As shown in Fig. S1 (iii), the ethanol yield increased with an increase in yeast addition quantity (B) and time (C). With a further increase of yeast addition quantity (B) and time (C), the yield showed a slight decrease.

3.2.2. *Optimization analysis (Regression model, ANOVA) of reducing sugar level and interaction between fermentation variables*

The analysis of variance of response surface model in supplied Table 1 shows that the regression model $p < 0.0001$, suggesting that the model is very significant (Eqs. (2)). If the properties of the evaluated system do not belong to the second-order polynomial regression, the RSM method may have some shortcomings (Ameer et al., 2017). The Lack of fit *p* (0.1310) was higher than 0.05, which indicated that the predicted value of regression model was consistent with the actual value within the test range.

The coefficient of determination of coefficient of variation R^2 was 0.9732, indicating that the regression model could explain 97.3% variability of experimental data, and the predicted value was highly correlated with the actual value, which could be used to analyse and predict the corresponding reducing sugar level. The correction coefficient $R^2_{\text{Adj}} = 0.9387$ indicates that the equation can explain 93.87% of the response value changes, which can well reflect the accuracy and universality of the model.The second-order quadratic model equation of the target response (reducing sugar) was used as the coding function of the independent variable, and was fitted by multiple regression analysis. They are represented by Eq. (2).

$$R^2\ (\text{reducing sugar, mg/ml}) = +24.12539–0.48322^{*}\ (\text{temperature, }^{\circ}\text{C}) - 1.67133^{*}\ (\text{Yeast addition, \%}) - 0.40087^{*}\ (\text{time, d}) + 0.011667^{*}\ (\text{temperature, }^{\circ}\text{C})^{*}\ (\text{Yeast addition, \%}) + 1.33333\text{E}-003^{*}\ (\text{temperature, }^{\circ}\text{C})^{*}\ (\text{time, d}) + 0.000000^{*}\ (\text{Yeast addition, \%})^{*}\ (\text{time, d}) + 5.38889\text{E}-003^{*}\ (\text{temperature, }^{\circ}\text{C})\text{ˆ}2 + 0.073500^{*}\ (\text{Yeast addition, \%})\text{ˆ}2 + 4.54000\text{E}-003^{*}\ (\text{time, d})\text{ˆ}2 \quad (2)$$

Fig. S2(i) shows the effect of temperature (A), yeast addition quantity

Table 3
Analysis of variance (ANOVA) for the experimental results (ethanol yield).

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	6.89	9	0.77	623.14	< 0.0001	significant
A-(temperature, °C)	1.92	1	1.92	1563.44	< 0.0001	
B-(Yeast addition, %)	0.016	1	0.016	13.19	0.0084	
C- (time, d)	0.23	1	0.23	188.19	< 0.0001	
AB	9.000E−004	1	9.000E−004	0.73	0.4204	
AC	0.026	1	0.026	20.84	0.0026	
BC	0.14	1	0.14	111.43	< 0.0001	
Aˆ2	1.23	1	1.23	999.36	< 0.0001	
Bˆ2	1.05	1	1.05	856.79	< 0.0001	
Cˆ2	1.81	1	1.81	1470.34	< 0.0001	
Residual	8.600E−003	7	1.229E−003			
Lack of Fit	6.600E−003	3	2.200E−003	4.40	0.0932	not significant
Pure Error	2.000E−003	4	5.000E−004			
Cor Total	6.90	16				
Std. Dev.		0.035		R-Squared		0.9988
Mean		15.32		Adj R-Squared		0.9972
C.V. %		0.23				
PRESS		0.11				

(B), and their reciprocal interaction on reducing sugar level (mg/ml), when time (C) was fixed at 40 day. The result revealed that the reducing sugar level decreased with the increase in temperature and yeast addition quantity. As shown in Fig. S2(ii), the reducing sugar level decreased with increasing temperature (A) and time (C). With a further increase in temperature (A) and time (C). As shown in Fig. S2(iii), the reducing sugar level decreased with an increase in yeast addition quantity (B) and time (C). With a further increase of yeast addition quantity (B) and time (C), the level showed a slight increase.

As shown in Fig. S1, the optimum conditions for millet ethanol fermentation are as follows: temperature 33 °C, yeast addition 9%, fermentation time 41 days. The influence order of the factors on ethanol level was as follows: temperature > time > yeast addition quantity according to the regression coefficients significance of the quadratic polynomial model (Table 3) and gradient of slope in the 3-D response surface plot (Fig. S1).

As shown in Fig. S2, optimal fermentation condition of reducing sugar level from the millet rice were as followings: temperature 32.2 °C, yeast addition quantity 9%, and time 40 days. Among the three fermentation parameters investigated, temperature was the most significant factor to affect the level of reducing sugar, followed by time and yeast addition quantity according to the regression coefficients significance of the quadratic polynomial model (Table S1) and gradient of slope in the 3-D response surface plot (Fig. S2).

Basing on comprehensive consideration results (ethanol yield and reducing sugar level), we concluded that when highest ethanol yield is 15.68%, *v*/v, optimal fermentation condition of the millet rice were temperature 32 °C, yeast addition quantity 9%, and time 41 days.

3.2.3. Verification of predictive model

The suitability of the model equations for predicting optimum response values was validated for the three variables within the design space. The result shows that under the following conditions: temperature 32 °C, yeast addition quantity 9%, and time 41 days, the concentration of ethanol (15.67 ± 1.26 ($N = 3$)) nearly reached the optimized concentration, 15.68. The predicted values and actual experimental values were compared, indicating that the model was adequate for the fermentation process (Table S2).

3.3. Analysis of volatile compounds in millet rice yellow wine in different fermentation time

Ten volatile compounds were detected in millet rice yellow wine (41 days). Millet rice yellow wine presented significantly higher concentration for the following eight compounds (Fig. S3, and Table S3): Ethyl alcohol (84.79%), Ethyl Acetate (2.60%), Ethane, 1,1-diethoxy- (1.33%), 1-Butanol, 3-methyl- (2.09%), Cyclotrisiloxane, hexamethyl- (3.13%), and Cyclopentasiloxane, decamethyl- (1.07%). Lower concentration for the following five compounds in wine were Acetaldehyde (0.71%), 1-Butanol, 2-methyl- (0.72%), Cyclotetrasiloxane, octamethyl- (0.73%), Butanedioic acid, diethyl ester (0.6%) and Naphthalene (0.6%).

Twelve volatile compounds were detected in millet rice yellow wine (55 days). Millet rice yellow wine presented significantly higher concentration for the following eight compounds (Fig. S4 and Tables S4): Ethyl alcohol (84.63%), Ethyl Acetate (7.12%), Benzoyl bromide (2.09%), and Cyclotrisiloxane, hexamethyl- (2.15%). Lower concentration for the following nine compounds in wine were Ethylene oxide (0.35%), Oxirane, 2-(1,1-dimethylethyl)-3-ethyl-, cis- (0.54%), Styrene (0.7%), Benzoic acid, 4-methyl-2-trimethylsilyloxy-, trimethylsilyl ester (0.92%), Cyclopentasiloxane, decamethyl- (0.41%), 10-Bromodecanoic acid, ethyl ester (0.34%), Cyclohexasiloxane, dodecamethyl- (0.25%), Decanoic acid, ethyl ester (0.28%) and Cycloheptasiloxane, tetradecamethyl- (0.23%).

Sixteen volatile compounds were detected in millet rice yellow wine (70 days). Millet rice yellow wine presented significantly higher concentration for the following eight compounds (Fig. S5, Tables S5): Ethyl alcohol (64.13%), Ethyl Acetate (19.43%), (Methoxymethyl)trimethylsilane (1.35%), 1-Butanol, 3-methyl-(2.52%), 1-Butanol, 2-methyl-, (.+/−.)- (1.28%), Cyclotrisiloxane, hexamethyl- (2.26%), Phenylethyl Alcohol (1.01%) and Benzoic acid, ethyl ester (1.41%). Lower concentration for the following eight compounds in wine were Acetaldehyde (0.86%), Hexanal (0.5%), Benzaldehyde (0.49%), Cyclotetrasiloxane, octamethyl- (0.62%), Hexanoic acid, ethyl ester (0.64%), Benzene, (ethoxymethyl)- (0.92%), 1H-Indene, 1-methylene- (0.74%), and Hexadecanoic acid, ethyl ester (0.43%).

Different kinds and quantities of metabolites of yeast cells, such as esters and higher alcohols, can lead to the change and loss of flavor (Echeverrigaray, Scariot, Menegotto, & Delamare, 2020). Pathways of biosynthesis of flavor compounds are many, such as carbohydrates, fatty acids, amino acids and terpenoids (Cheng et al., 2015; Zhang et al., 2013). Generally speaking, the mixed flavor compounds released during fermentation are caused by a variety of biochemical pathways (Matias-Guiu, Rodríguez-Bencomo, Pérez-Correa, & López, 2018).

From Fig. S3–5, it can be observed for yellow wines made from millet rice when the permentation period was extended from 40 days to 70 days, the relative amounts of alcohols decreased with longer permentation. Other chemical components have caused many change. Some new volatile components can be detected. This means that a series of physical and chemical changes take place in the process of extended fermentation. Sensory flavor of wine can be affected by these new volatile components.

3.4. Variation in amino acid content in yellow wines

The amino acid contents in three yellow wines samples of 41, 55 and 70 days of fermentation were analyzed. From Table S6–8 and Fig. S6, it could be found that L-Asparagine (5.54 mg/L), and Alanine (5.50 mg/L) were the major amino acid components after 41 days of fermentation. L-Asparagine (7.49 mg/L), Alanine (6 mg/L), methionine (4.39 mg/L), and glutamic acid (4.38 mg/L) were the major amino acid components after 55 days of fermentation. L-Asparagine (7.98 mg/L), Alanine (7.33 mg/L), Proline (4.33 mg/L), methionine (4.31 mg/L), Leucine (4.19 mg/L), and Valine (4.01 mg/L) were the major amino acid components after 70 days of fermentation.

Histidine, Arginine and Cysteine weren't all detected in three yellow wines samples. Experiment results indicated that fermentation time clearly affected amino acid content in wine. Yellow wine has six flavors: sour, sweet, bitter, spicy, astringent and fresh, which is due to its abundant flavor substances. Amino acid is one of the important flavor substances in yellow wine, and different amino acids have different taste. For example, alanine and threonine have sweet taste; tyrosine, amino acid and leucine have astringent taste; glutamic acid, aspartic acid and lysine have fresh taste; in addition, nine kinds of amino acids are bitter. The content of amino acids in rice wine is not only the highest, but also has 20 kinds, including 8 kinds of essential amino acids for human body. Amino acids not only provide abundant nutrients for microorganisms in the brewing process of rice wine, but also are the precursors of many flavor substances (Hu et al., 2014). In addition, amino acids endow rice wine with full taste levels, making it delicious, mellow, rich and refreshing (Xie et al., 2020). Therefore, in production, it is of great significance to master and control the amount of amino acid nitrogen and fermentation time by detecting the amount of amino acid nitrogen in production.

4. Conclusions

The fermentation conditions of millets rice (containing small proporation of Daylily and Agaricus blazei Murr) were successfully optimized by employing RSM and Box–Behnken design. The obtained quadratic polynomial equations could well described the changes in alcohol yield and reducing sugar content of fermented yellow wine. The

optimal conditions for yellow wine fermentation were determined as follows: temperature 32 °C, yeast addition quantity 9, and time 41 days. Under the optimal fermentation condition, the highest ethanol yield (15.68) from the millet rice (containing small proporation of Daylily and Agaricus blazei Murr) was obtained. GC–MS and HPLC methods were employed to analyse volatile components and amino acids in fermented millets rice (containing small proporation of Daylily and Agaricus blazei Murr) yellow wines. It could be found that volatile components, amino acids composition and contents in fermented yellow wine were different at different fermentation times. Ethyl alcohol and Ethyl Acetate had been major volatile components in wine during fermentation. Other major volatile components contents (Ethane, 1,1-diethoxy-; 1-Butanol, 3-methyl-; Cyclotrisiloxane, hexamethyl-; Cyclopentasiloxane, decamethyl-; Benzoyl bromide; (Methoxymethyl)trimethylsilane; 1-Butanol, 2-methyl-, (+/−)-;,Phenylethyl Alcohol; Benzoic acid; ethyl ester) had varied during 70 days fermentation. This, in turn, enhances the content of aromatic compounds in wine. L-Asparagine and Alanine had been the major amino acid in wine during fermentation. Other components (contents of methionine, glutamic acid, Proline, Leucine, and Valine) had varied during 70 days of fermentation. This is very important to the flavor of the wine.

CRediT authorship contribution statement

XinMing Li: Writing – review & editing, Writing – original draft, Software, Methodology, Investigation, Formal analysis. **YaJun Zhang:** Writing – original draft, Software, Methodology, Investigation, Data curation. **Shang Guo:** Supervision, Project administration, Funding acquisition. **RunFang Dai:** Funding acquisition.

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.

Data availability

The authors do not have permission to share data.

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Appendix A. Supplementary data

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