Research Article

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Developing an innovative raw wheat *Qu* inoculated with *Saccharopolyspora* and its application in *Huangjiu*

Donglin Ma,[a†] Shuangping Liu,[a,b,c†*] Haipo Liu,[d] Mujia Nan,[e] Yuezheng Xu,[c] Xiao Han[a,b,c] and Jian Mao[a,b,c*]

Abstract

BACKGROUND: Mechanized *Huangjiu* is a stable product, is not subject to seasonal production restrictions, and markedly reduces labor intensity compared to traditional manual *Huangjiu*. However, the bitterness of mechanized *Huangjiu* impedes its further development.

RESULTS: Based on process optimization, when the fermentation temperature was 45 °C and the fermentation time was 122 h, the inoculation amount of *Saccharopolyspora* was 5%, the amount of added water was 26%, and the glucoamylase and amylase activities of wheat *Qu* increased by 27% and 40% respectively, compared with those before optimization. *Huangjiu* fermented by raw wheat *Qu* inoculated with *Saccharopolyspora rosea* F2014 showed a significant ($P < 0.05$) decrease in bitter amino acid content (1.24 *vs.* 2.86 g L^{-1}, a decrease of 56%), which attenuated its bitterness.

CONCLUSION: An innovative fermentation process of inoculating *Saccharopolyspora* into raw wheat *Qu* was developed for the first time. Such a process could be used to control bitterness based on raw wheat *Qu* inoculated with *Saccharopolyspora rosea* F2014, instead of traditional wheat *Qu* in *Huangjiu* fermentation.

Supporting information may be found in the online version of this article.

Keywords: inoculated raw wheat *Qu*; *Saccharopolyspora*; response surface methodology; *Huangjiu* fermentation; bitterness

INTRODUCTION

Huangjiu, a traditional alcoholic beverage in China, is primarily made from glutinous rice, water, wheat *Qu*, and fermentation starters in an open environment.[1] Wheat *Qu* is produced by spontaneous fermentation with crushed wheat as the main raw material, and is mixed with water and naturally inoculated with mold, bacteria and yeast in an open environment.[2] Various enzymes are produced during the process of wheat *Qu* fermentation, including amylase, glucoamylase and protease. The metabolites that are broken down by these enzymes promote the growth of microorganisms and the formation of wheat *Qu* flavor compounds.[3-5] The inoculated raw wheat *Qu* is a new type of starter produced by inoculating specific functional microorganisms into raw wheat *Qu*. The raw materials, microbiota and manufacturing parameters, such as temperature, water addition and fermentation time, play a crucial role in the production of wheat *Qu*.[6] Many studies on wheat *Qu* have focused on enzymes and microorganisms and strategies to improve the activity of enzymes during manufacture.[7,8] Liu *et al*. employed *Aspergillus oryzae* su-16 to improve raw wheat *Qu* in the fermentation of *Huangjiu*. Based on their discovery, the glucoamylase, amylase and protease contents were 1.17-, 1.55- and 2.87-fold greater than those of the traditional wheat *Qu*, respectively.[9] Owing to a lack of understanding of key microorganisms in the division of metabolites and metabolism, similar studies on inoculated raw wheat *Qu* applied in *Huangjiu* are scarce.

Based on metagenome sequencing, *Saccharopolyspora* is the most abundant bacterial genus (18–21%) during *Huangjiu*

* *Correspondence to: Shuangping Liu or Jian Mao, National Engineering Research Center of Cereal Fermentation and Food Biomanufacturing, State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Jiangnan University, Wuxi, Jiangsu 214122, China. E-mail: liushuangping668@126.com (Liu); E-mail: maojian@jiangnan.edu.cn (Mao)*

† *Donglin Ma and Shuangping Liu contributed equally.*

a *National Engineering Research Center of Cereal Fermentation and Food Biomanufacturing, State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Jiangnan University, Wuxi, China*

b *Shaoxing Key Laboratory of Traditional Fermentation Food and Human Health, Jiangnan University (Shaoxing) Industrial Technology Research Institute, Zhejiang, China*

c *National Engineering Research Center of Huangjiu, Zhejiang Guyuelongshan Shaoxing Wine Co. Ltd, Zhejiang, China*

d *Famous Wine Collection Committee, China Alcoholic Drinks Association, Beijing, China*

e *Basic Department, University of Tibetan Medicine, Lhasa, China*

fermentation with Shaoxing wheat *Qu*.[10,11] Metagenome metabolic function predictions also indicated that, of all major bacterial genera, *Saccharopolyspora* annotated the most abundant enzymes associated with the production of *Huangjiu* flavor compounds, including esters, acids and phenolics.[12] During the production of Maotai *Daqu*, *Saccharopolyspora* is involved in the breakdown and metabolism of macromolecules, mainly through the production of enzymes, leading to the formation of flavor substances.[13]

Wheat *Qu*, fermentation time and yeast starter are well known to differ between traditional manual *Huangjiu* and mechanized *Huangjiu*.[14] Mechanized *Huangjiu* is superior to traditional manual *Huangjiu* based on its high production efficiency, consistent and safe product quality, production independent of seasonal restrictions and low labor intensity.[15] However, the bitterness of mechanized *Huangjiu* is stronger than that of traditional manual *Huangjiu*, thereby impeding its development.[15] The main objectives of this study were: (i) to establish a predictive model to regulate the amylase and glucoamylase activity in raw wheat *Qu* inoculated with *Saccharopolyspora*; (ii) to evaluate the performance of different raw wheat *Qu* inoculated with different *Saccharopolyspora* in *Huangjiu*; and (iii) to determine the prominent *Saccharopolyspora* for application in wheat *Qu* and *Huangjiu* fermentation.

MATERIALS AND METHODS

Strains and medium

Polished rice and wheat were purchased from Wuxi rice markets. Wheat *Qu* and cooked wheat *Qu* were obtained from *Huangjiu* Factories in Shaoxing, China. *Saccharomyces cerevisiae* HJ (GCA_015363515.1) was preserved on a YPD slope (glucose 2 g L^{-1}, peptone 2 g L^{-1}, yeast extract 1 g L^{-1}, agar 20 g L^{-1}). *Saccharopolyspora rosea* F2014 (GenBank: ON528575), *Saccharopolyspora rosea* F2017 (GenBank: ON527876), *Saccharopolyspora rosea* F2006 (GenBank: ON528175), *Saccharopolyspora hodei* F2009 (GenBank: ON528173) and *Saccharopolyspora hirsuta* F1909 (GenBank: ON528144) derived from *Huangjiu* wheat *Qu*, which were preserved on Actinomycetes medium (KNO_3 1.0 g L^{-1}, KH_2PO_4 0.5 g L^{-1}, $MgSO_4$ 0.5 g L^{-1}, $FeSO_4$ 0.01 g L^{-1}, NaCl 0.5 g L^{-1}, starch 20.0 g L^{-1}, agar 15.0 g L^{-1}). All reagents were analytically pure.

Strain culture and inoculated raw wheat *Qu*-making process

Culture of Saccharopolyspora

Saccharopolyspora strains were inoculated into Actinomycetes medium and incubated at 37 °C for 120 h. Individual colonies were then picked from the plates, inoculated into Actinomycete liquid medium, and incubated for 120 h at 37 °C on a shaker at 150 rpm to obtain the seed solution. The seed solution was transferred to Actinomyces liquid medium at a 3% (v/v) inoculum concentration and incubated for 72 h at 37 °C on a shaker at 150 rpm to obtain the *Saccharopolyspora* expansion solution. The initial concentration of *Saccharopolyspora* was 1×10^8 spores mL^{-1}.

Inoculated raw wheat Qu *synthesis process*

The process used to prepare inoculated raw wheat *Qu* is shown in Supporting Information Fig. S1. Briefly, the raw wheat without impurities was homogeneously crushed into three to four pieces, mixed with 21% water (v/m), inoculated with 5% (v/m) activated *Saccharopolyspora* expansion solution and mixed thoroughly. The inoculated wheat was then placed in a frame (length × width × height = 60 × 42 × 26 cm) with a small hole (8 mm in diameter) and transferred to a wheat *Qu* fermentation room (45 °C, 95% humidity) for 120 h of fermentation.

Huangjiu fermentation

The saccharification solution was prepared by mixing steamed rice and liquifying enzyme (700 U g^{-1} rice), glucoamylase (5 U g^{-1} rice) and wheat *Qu* (0.1 g g^{-1} rice); the mixture was then incubated at 60 °C. After saccharification, the rice saccharification liquid was packed and sterilized for 20 min at 115 °C.[16,17] The yeast starter of *Huangjiu* was prepared by transferring *Saccharomyces cerevisiae* HJ activated by YPD liquid medium to the previously prepared *Huangjiu* saccharification solution; the sample was then cultured at 28 °C for 36 h at 150 rpm. *Huangjiu* fermentation was performed by mixing 1125 g steamed rice, 937 mL water, 104 g *Saccharopolyspora*-inoculated raw wheat *Qu* and 86 mL *Saccharomyces cerevisiae*. The primary fermentation was conducted at 28 ± 1 °C for 4 days with stirring once per day. Post-fermentation was conducted at 15 ± 1 °C for 15 days with stirring once every 2 days. For the control group, the fermentation process was the same as that described previously, except that inoculated raw wheat *Qu* was replaced with 89 g factory wheat *Qu* and 15 g cooked wheat *Qu*.[17,18]

Optimization of inoculated raw wheat *Qu* synthesis process

Optimization of the fermentation parameters

To obtain the best process for inoculating wheat *Qu*, four conditions – fermentation temperature (37 °C, 45 °C and 50 °C), fermentation time (0–168 h), *Saccharopolyspora rosea* F2014 inoculum (1%, 3% and 5%), and the amount of added water (15%, 20%, 25% and 30%) – were employed to fabricate inoculated raw wheat *Qu*. The optimal process parameters were evaluated by determining the activities of glucoamylase and amylases.

Box–Behnken design

To model the effects of independent variables – temperature, fermentation time and amount of added water – a response surface methodology combined with the Box–Behnken design was employed. Based on single-factor experiments, the glucoamylase and amylase activities of wheat *Qu* were used as response values to design a response surface experiment with four factors and three levels; the levels of experimental factors are shown in Table 1.

Determination of enzymatic activity of wheat *Qu* and fermentation parameters of *Huangjiu*

Amylase activity and glucoamylase activity were determined according to a previously described method.[9] Briefly, a sample of wheat *Qu* (5.00 g) was soaked in 25 mL acetate buffer (pH 4.6) at 40 °C for 1 h, and then centrifuged at 8000 × *g* for 10 min. The supernatant was collected as the crude enzyme solution and used to determine amylase and glucoamylase activities. Total acid and amino nitrogen were determined by titration.[19] Ethanol content was determined using a previous method[20] and the reducing sugar content was determined using the dinitrosalicylic acid (DNS) method. The higher alcohol content was determined according to a previous method with modifications.[21] Briefly, 3.5 mL *Huangjiu* samples, 3.5 mL deionized water and a certain volume of disperse solvent (1 mL acetonitrile), extraction solvent (600 μL dichloromethane) and 50 μL internal standard

Table 1. Factor and level of the Box–Behnken experiment for determining glucoamylase and amylase activities

Variable	Symbol coded	Range and level		
		−1	0	1
Temperature (°C)	*A*	43	45	47
Fermentation time (h)	*B*	96	120	144
Amount of water added (%)	*C*	24	25	26

(18 640 mg L^{-1} 4-methyl-2-pentanol solution in ethanol) were added to a 15 mL screw-cap glass tube and shaken gently to ensure homogeneous mixing; the glass tube was gently shaken for 1 min and then centrifuged at 5000 rpm (2655 × *g*) for 5 min. The supernatant was withdrawn using a microsyringe and injected into a gas chromatograph–mass chromatograph for analysis.

High-performance liquid chromatography (HPLC) was used to analyze the free amino acids according to a previously described method, with modifications.[11] Briefly, the supernatant was analyzed using an Agilent series 1100 instrument (Agilent Technologies, Palo Alto, CA, USA) equipped with an ODS HYPERSIL column (250 × 4.6 mm, 5 μm) and a UV detector. The column temperature was maintained at 40 °C, the detection wavelengths were 338 and 262 nm, and the flow rate was 1.0 mL min^{-1}. The organic acid composition was analyzed by reversed-phase HPLC (Waters e2695, Milford, MA, USA) with an Athena C18-WP column (250 × 4.6 mm, 5 μm). The column temperature was maintained at 30 °C, the detection wavelength was 210 nm, the mobile phase was phosphate buffer (0.025 mol L^{-1} NaH_2PO_4, pH 3.1) and the flow rate was 0.7 mL min^{-1}.[22] The content of volatile flavor compounds was measured using headspace solid-phase microextraction (HS-SPME) and analyzed using a gas chromatograph–mass spectrometer (Trace 1300, ISQ LT, Thermo Scientific, San Jose, CA, USA), according to a previous method.[12]

Data analysis

All data were analyzed using one-way analysis of variance (ANOVA) on SPSS 15.0 (SPSS Inc., Chicago, IL, USA). R (version 3.6.3) and GraphPad Prism 7 were used for image processing. The Behnken combination of Design-Expert V8.0.6 software was used for response surface design and analysis. The coefficient of regression (R^2) was calculated to determine the goodness-of-fit of the model. An *F*-test was used to assess the significance of the model terms and equations. Three replicates were used for each experiment.

RESULTS

Effects of various fermentation parameters on glucoamylase and amylase activity

The activities of glucoamylase and amylase displayed an increasing trend followed by a decrease as the water content increased (Fig. 1A). When 25% water was added to wheat *Qu*, the maximum glucoamylase activity and amylase activity were 1019.50 ± 61.76 and 1.15 ± 0.01 U g^{-1}, respectively. Such findings indicate that the addition of 25% water was more favorable to the secretion of glucoamylase and amylase by microorganisms (Fig. 1A**)**. As the inoculation ratio increased, the glucoamylase and amylase activities of wheat *Qu* began to increase (Fig. 1B**)**. When 5% inoculum was employed, the glucoamylase activity of wheat *Qu* was 914.02 ± 49.98 U g^{-1} and its amylase activity was 1.18 ± 0.02 U g^{-1}. Therefore, this wheat *Qu* could be used for *Huangjiu* brewing.[23] The inoculation amount of *Saccharopolyspora* was 5% and no further optimization was performed. The effect of temperature on the glucoamylase activity was found to be significant ($P < 0.05$), with the lowest glucoamylase activity of 679.12 ± 72.12 U g^{-1} at 37 °C and the highest glucoamylase activity of 1119.58 ± 95.4 U g^{-1} at 45 °C (Fig. 1C). This finding indicates that the most robust glucoamylase-producing metabolism of microorganisms occurred at 45 °C. The difference in amylase activity between 37 °C and 45 °C was significant ($P < 0.05$), with the maximum amylase activity of 1.16 ± 0.04 U g^{-1} at 45 °C and the lowest amylase activity of 0.81 ± 0.08 U g^{-1} at 50 °C. As the fermentation time increased, the glucoamylase activity of wheat *Qu* increased and then decreased, with the highest activity of 1089.92 ± 73.56 U g^{-1} achieved at 120 h (Fig. 1D). Amylase activity reached a maximum value of 1.09 ± 0.03 U g^{-1} at 96 h and then began to decline.

A significant difference ($P < 0.05$) in glucoamylase activity was found between 96 h (711.21 ± 30.56 U g^{-1}) and 120 h of fermentation (1089.92 ± 73.56 U g^{-1}). As the amylase activity (0.96 ± 0.01 U g^{-1}) at 120 h was not significantly ($P > 0.05$) (Fig. 1D) different from that at 96 h (1.09 ± 0.03 U g^{-1}), 120 h was determined as the optimal fermentation time.

Response surface optimization to investigate the impact of the interaction of multiple factors on glucoamylase and amylase

The correlation coefficient (R^2) values were 0.976 and 0.9763, respectively, indicating the reliability of the regression model for predicting glucoamylase and amylase activities (Fig. 2A,B). Based on multiple regression fitting analysis of the data (Fig. 2C), the following empirical quadratic model was selected to derive the correlation between the response of glucoamylase activity and amylase activity and the independent variables:

$$Y1\left(\mathrm{U g^{-1}}\right) = 1081.67 + 48.23\times A + 41.76\times B + 62.33\times C - 38.45\times AB - 22.68\times AC - 30.98\times BC - 266.84\times A^2 - 181.98\times B^2 - 144.37\times C^2$$

$$Y2\left(\mathrm{U g^{-1}}\right) = 1.32 + 0.054\times A + 0.02\times B + 0.24\times C + 0.013\times AB + 0.022\times AC - 0.02\times BC - 0.19\times A^2 - 0.15\times B^2 - 0.31\times C^2$$

where *Y*1 represents glucoamylase activity, *Y*2 represents amylase activity, *A* is the fermentation temperature, *B* is the fermentation time and *C* is the amount of water added.

The *P*-value of the lack of fit ($P = 0.8147$, $P = 0.1222$) and the model ($P < 0.0001$, $P < 0.0001$) revealed that the actual corresponding glucoamylase activity and amylase activity exhibited a good fit with this model (Tables 2 and 3). Based on ANOVA

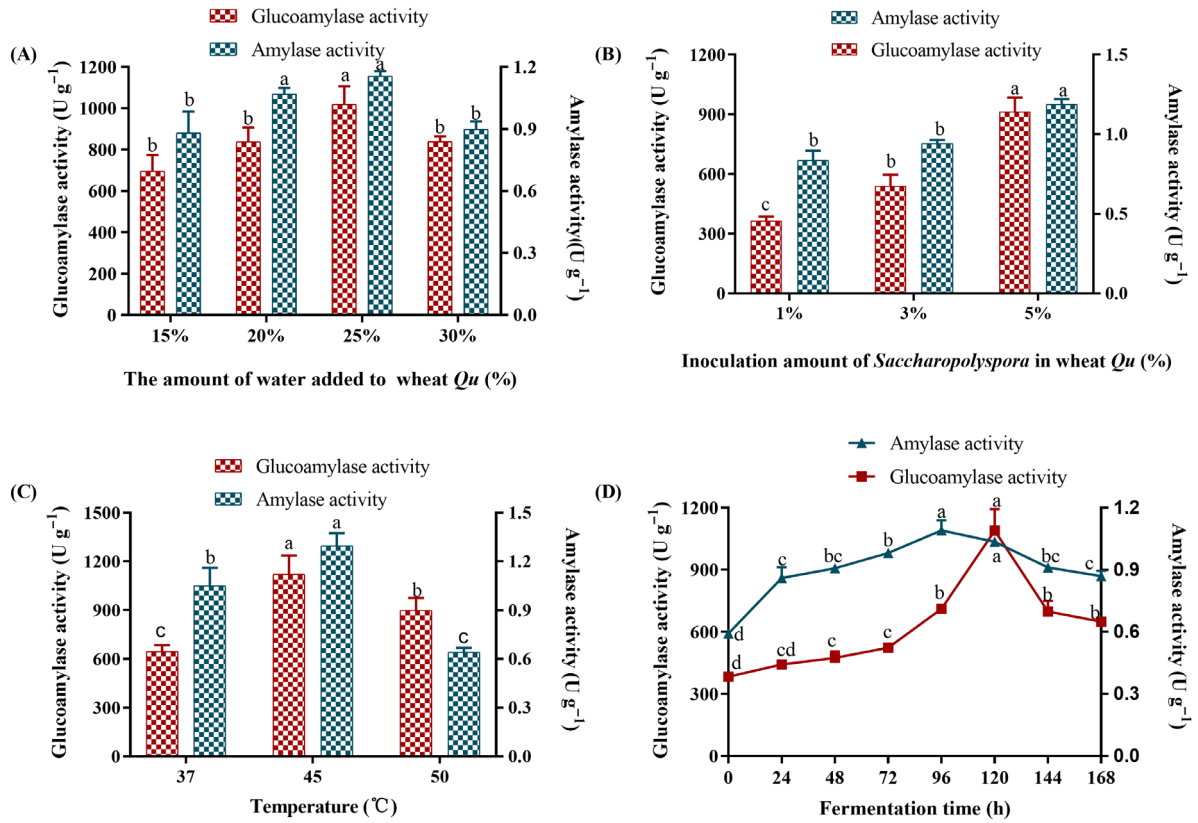

Figure 1. Effects of different fermentation parameters on the enzyme activity of wheat *Qu*: (A) amount of added water; (B) inoculation amount of *Saccharopolyspora*; (C) temperature; (D) fermentation time. Each parameter was tested in triplicate. Error bars represent the standard deviation of the mean.

(Table 2), the primary terms, *A* and *B*, and the secondary terms, A^2 and B^2, of the regression equation *Y*1 had a highly significant ($P < 0.0001$) effect on glucoamylase, indicating that temperature and fermentation time had a markedly greater effect on glucoamylase activity than the addition of water. The primary term *C* (water addition) in regression equation *Y*2 had a highly significant effect ($P < 0.0001$) on amylase activity, and the primary term *A* (fermentation temperature) had a significant effect ($P < 0.05$) on amylase activity. Such findings indicate that water addition and temperature are key environmental factors that affect amylase production in inoculated raw wheat *Qu* (Table 3). In summary, the *P*-values for the effect of each factor on glucoamylase were as follows: fermentation time > temperature > amount of added water; and those on amylase were as follows: amount of added water > temperature > fermentation time.

The glucoamylase and amylase activities of inoculated raw wheat *Qu* according to different combinations of independent preparation parameters were visualized through 3D response surface plots and contour plots (Figs 3 and 4). The curved contour lines demonstrated revealed a strong interaction between fermentation time, temperature and the amount of added water. Further, the maximum values appeared at the center of the response surface, indicating that the interaction of these factors had a significant ($P < 0.05$) effect on the glucoamylase and amylase activities of wheat *Qu*.

Validating the optimal process conditions

Under the best-predicted process conditions of 45.15 °C fermentation temperature, 122.15 h fermentation time and 25.20% water addition, the model predicted glucoamylase activity and amylase activity of 1091.61 and 1.36 U g^{-1}, respectively. Based on the practical operating conditions and costs, the optimal process conditions were adjusted to a fermentation temperature of 45 °C, fermentation time of 122 h and water addition of 26%. The activities of glucoamylase and amylase were 1119.53 ± 58.38 and 1.39 ± 0.01 U g^{-1}, respectively. Compared to the fermentation process (fermentation temperature of 45 °C, fermentation time of 120 h and water addition of 25%) before optimization, the activities of glucoamylase (1119 *vs.* 873 U g^{-1}) and amylase (1.39 *vs* 0.84 U g^{-1}) increased by 27% and 40%, respectively.

Comparison of the enzyme activity and physicochemical parameters of *Huangjiu* for different inoculated wheat *Qu*

The glucoamylase activity of the N-control (not inoculated with *Saccharopolyspora*) (366.5 ± 41.5 U g^{-1}), F2009 (974.5 ± 47.5 U g^{-1}) and F1909 (827 ± 19 U g^{-1}) were lowered by 67%, 35% and 26%, respectively, compared with those of F2014 (1126 ± 97 U g^{-1}) with the highest glucoamylase production (Fig. 5A). No significant difference ($P > 0.05$) was found between F2014, F2017 and F2006 in amylase activity, with F2017 having the highest amylase activity (1.18 ± 0.3 U g^{-1}) (Fig. 5B). Further, there was no significant difference ($P > 0.05$) in alcohol content between F2014 (15.3 ± 0.46% vol), F2017 (15.16 ± 0.21% vol) and the positive control (15.9 ± 0.17% vol) (P-control) (Fig. 5C). The reducing sugar content was below 10 g L^{-1} for all groups, except the P-control, indicating that reducing sugars derived from starch (glutinous rice) hydrolysis were mainly consumed by the microorganisms (Fig. 5D). Compared with the control, three types of inoculated raw wheat *Qu* –F2006, F2009 and F2017 – significantly ($P < 0.05$) reduced the content of amino nitrogen by 72%, 57% and 64%, respectively. Interestingly,

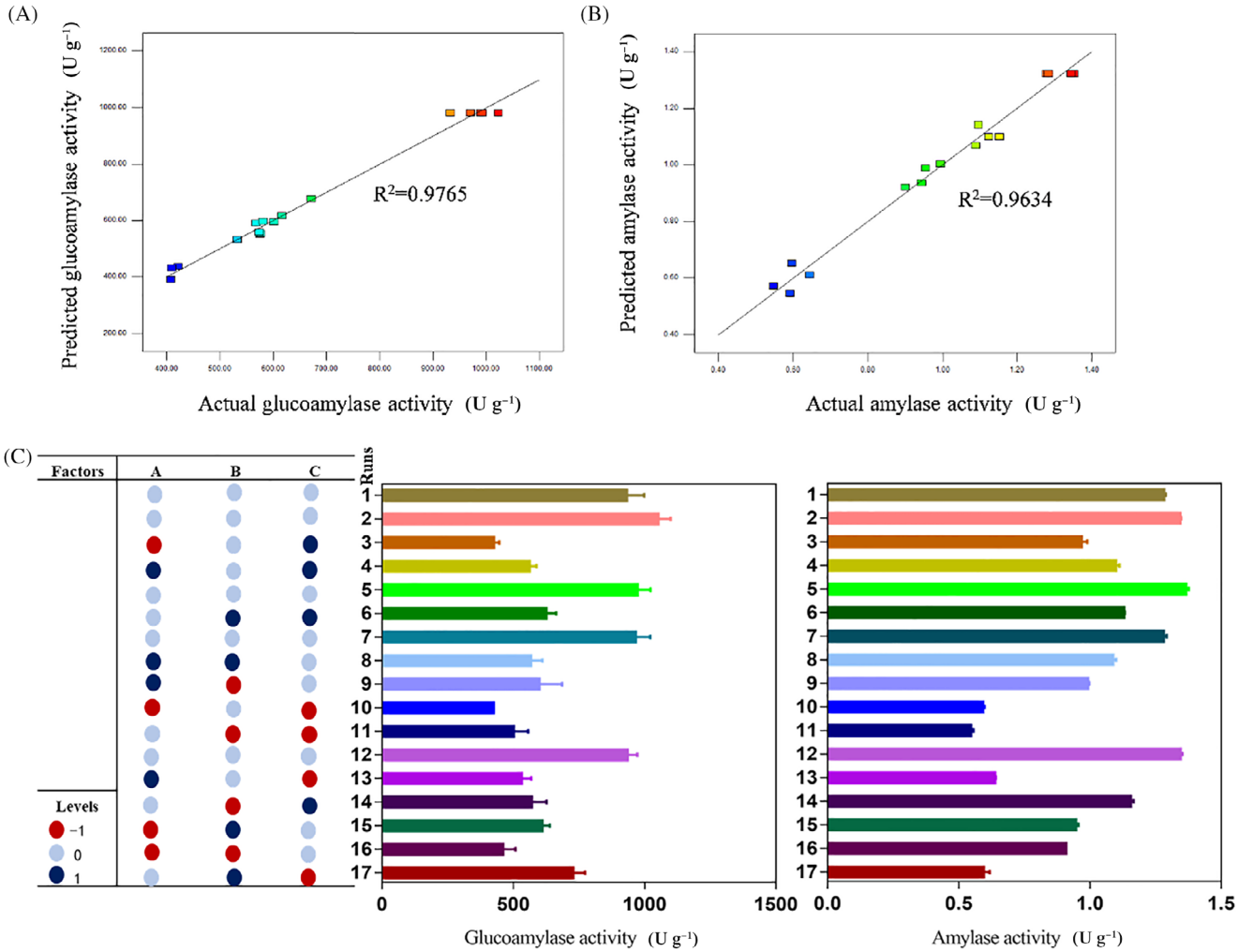

Figure 2. Predicted *versus* experimental values of (A) glucoamylase activity, (B) amylase activity, and (C) total glucoamylase activity and amylase activity in the 17 runs carried out using wheat *Qu*.

Table 2. ANOVA for the response surface quadratic model of glucoamylase activity

Source	Sum of squares	df	Mean squared	*F*-value	*P*-value prop > *F*	
Model	658 892.77	9	73 210.31	92.83	< 0.0001	Significant
A – temperature	18 610.99	1	18 610.99	23.60	0.0018	**
B – fermentation time	13 950.01	1	13 950.01	17.69	0.0040	**
C – amount of water added	31 078.98	1	31 078.98	39.41	0.0004	
AB	5914.92	1	5914.92	7.50	0.0290	*
AC	2056.85	1	2056.85	2.61	0.1504	
BC	3838.48	1	3838.48	4.87	0.0632	
A^2	299 800.36	1	299 800.36	380.13	< 0.0001	***
B^2	139 435.18	1	139 435.18	176.80	< 0.0001	***
C^2	87 763.13	1	87 763.13	111.28	< 0.0001	***
Residual	5520.74	7	788.68			
Lack of fit	1056.48	3	352.16	0.32	0.8147	Not significant
Pure error	4464.26	4	1116.07			
Corrected total	664 413.52	16				

*0.01 < *P* < 0.05;
** 0.001 < *P* < 0.01;
*** *P* < 0.001;
df, degrees of freedom.

Table 3. ANOVA for the response surface quadratic model of amylase activity

Source	Sum of squares	df	Mean squared	*F*-value	*P*-value prop > *F*	
Model	1.23	9	0.14	47.77	< 0.0001	Significant
A – temperature	0.02	1	0.02	8.17	0.0244	*
B – fermentation time	0.00	1	0.00	1.10	0.3300	
C – amount of water added	0.47	1	0.47	165.70	< 0.0001	***
AB	0.00	1	0.00	0.22	0.6514	
AC	0.00	1	0.00	0.69	0.4348	
BC	0.00	1	0.00	0.53	0.4890	
A^2	0.15	1	0.15	51.09	0.0002	***
B^2	0.10	1	0.10	34.38	0.0006	***
C^2	0.41	1	0.41	144.56	< 0.0001	***
Residual	0.02	7	0.00			
Lack of fit	0.01	3	0.00	3.64	0.1222	Not significant
Pure error	0.01	4	0.00			
Corrected total	1.25	16				

*$0.01 < P < 0.05$;
** $0.001 < P < 0.01$;
*** $P < 0.001$;
df, degrees of freedom.

only the F2014 inoculated raw wheat *Qu* induced a minimum decrease of 25% c compared to the P-control (Fig. 5F).

Flavor evaluation of *Huangjiu* fermented with different inoculated raw wheat *Qu*

In this study, the total higher alcohol content of the P-control (618.2 ± 13.9 mg L^{-1}) did not significantly ($P > 0.05$) differ from that of F2014 (573.2 ± 12.4 mg L^{-1}) and F2017 (582.8 ± 14.3 mg L^{-1}) (Fig. 6A). Except for the N-control, the lowest total higher alcohol content of F1909 was 530.2 ± 10.5 mg L^{-1}; however, its alcohol content (14.3% vol) was too low to be suitable for *Huangjiu* fermentation. The total amino acid content of the P-control group was 2.866 ± 0.09 (g L^{-1}), which was significantly ($P < 0.05$) higher than that of other groups (Fig. 6B). The total bitter amino acid (His, Arg, Val, Phe, Ile, Leu and Lys) content in the P-control was 5-, 2.64-, 6.67-, 24-, 2.64- and 2.85-fold greater than that in the experimental groups, respectively. Such findings indicate that raw wheat *Qu* inoculated with different *Saccharopolyspora* could significantly ($P < 0.05$) reduce the bitterness of mechanized *Huangjiu*. In addition, the total amino acid content of the raw wheat *Qu* inoculated with F2014 (1.24 ± 0.06 g L^{-1}) was 2.69-fold that of F2017 (0.46 ± 0.03 g L^{-1}), and could serve as a potential substitute for wheat *Qu* in mechanized *Huangjiu*. The total organic acid contents of F2014 (4.36 ± 0.61 g L^{-1}), F2017 (8.38 ± 0.65 g L^{-1}) and F2006 (6.04 ± 0.14 g L^{-1}) were lowered by 54%, 13% and 37%, respectively, compared with those of the control (9.6 ± 0.71 g L^{-1}) (Fig. 6C).

The total ester content (84.304 ± 4.213 mg L^{-1}) in the P-control group was significantly ($P < 0.05$) higher than that in the other six experimental groups (Table 4). The content of ester compounds in F2014 was the highest (65.002 ± 5.239 mg L^{-1}), and was 0.7-fold greater than that of the N-control. In this study, except for the positive control group, the F2014 group had the highest content of 4-vinyl guaiacol (24.995 ± 6.12 mg L^{-1}) and its total phenol content (25.411 ± 0.029 mg L^{-1}) was significantly ($P < 0.05$) higher than that of the other five experimental groups. Compared with F2006, F2009 and F2017, the total phenolic content of raw wheat *Qu* inoculated *Saccharopolyspora rosea* F2014 increased by 63%, 84% and 63%, respectively.

DISCUSSION

Wheat *Qu* fermentation is carried out in an open work environment. Thus environmental conditions are critical in the fermentation of wheat *Qu* and have a significant impact on the type and number of microorganisms.[24,25] Moisture is one of the essential factors for microbial growth and is indirectly linked to microbial enzyme production.[26] In this study, the activities of glucoamylase and amylase were found to fluctuate when the amount of added water ranged from 15% to 30% (Fig. 1A). Evidently, when 25% water was added to wheat *Qu*, the microorganism could favorably secrete glucoamylase and amylase. Temperature is an important environmental factor for microbial growth. In fact, temperature affects the secretion of enzymes by influencing the metabolism of microorganisms.[27] *Saccharopolyspora* is a heat-resistant microorganism, and its dominant position in wheat *Qu* is promoted by high-temperature fermentation (45 °C). Based on previous studies, higher temperatures are conducive to lipid oxidation and the formation of volatile compounds.[28] During wheat *Qu* fermentation, higher temperatures (45 °C) could provide strong environmental conditions for the formation of volatile compounds.[6]

Saccharopolyspora belongs to the Actinobacteria, which can produce enzymes, vitamins and cellulose degradation-promoting factors, and is a class of safe biological resource bacteria.[29] In this study, the glucoamylase and amylase activities of raw wheat *Qu* inoculated with *Saccharopolyspora rosea* F2014 and F2017 were higher than those inoculated with other *Saccharopolyspora*, which indicates that the differences in microorganisms are the main factors affecting the enzyme production of wheat *Qu*, in addition to culture composition, moisture content and culture temperature.[30] The glucoamylase and amylase provided by *Saccharopolyspora* can decompose starch for *Saccharomyces cerevisiae* to grow, producing alcohol and volatile flavor compounds during *Huangjiu* fermentation. Therefore, the

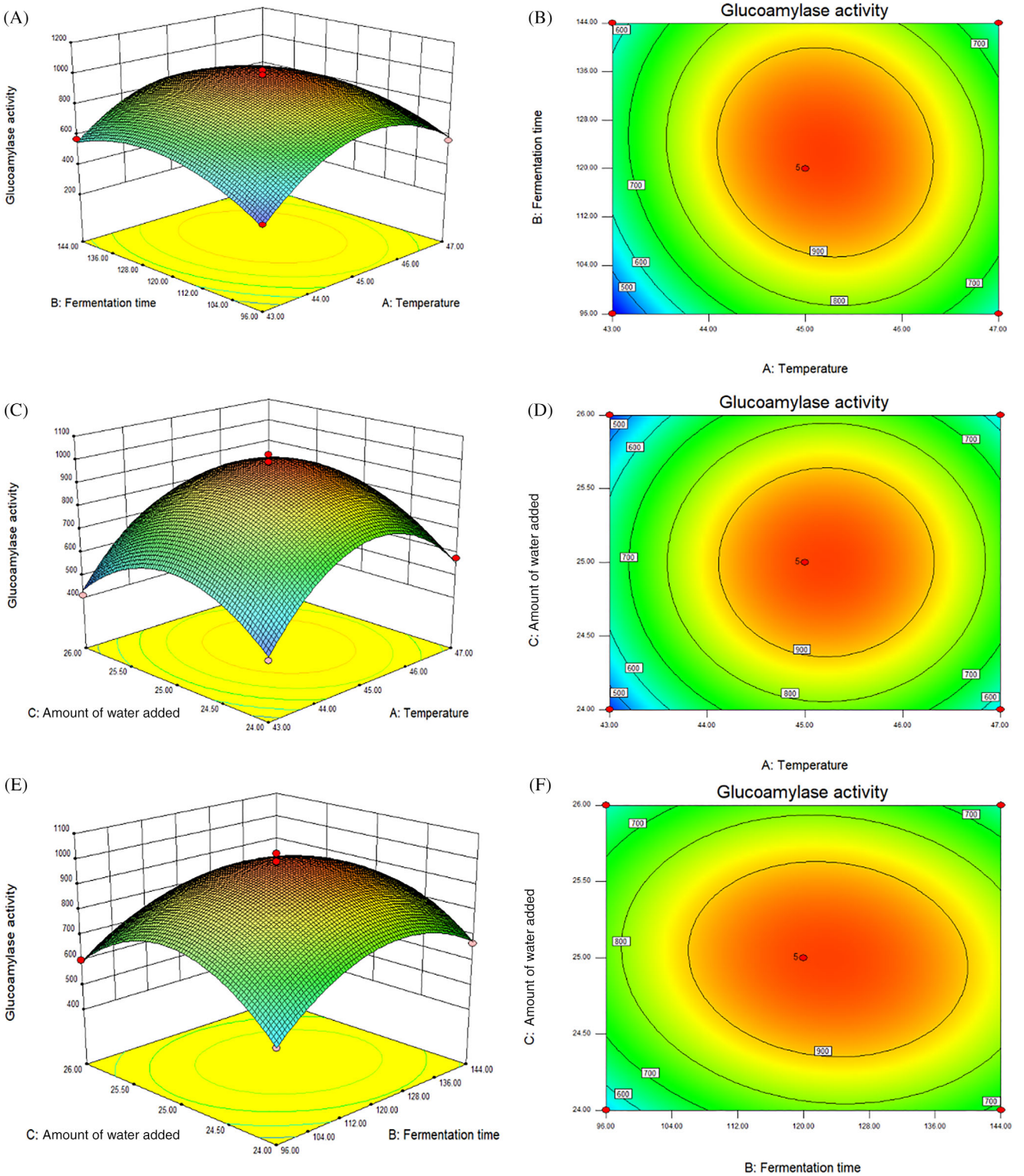

Figure 3. Response surface (3D) and corresponding contour (2D) of glucoamylase activity affected by the interaction of two factors. (A,B) Effect of the interaction of fermentation time and temperature on glucoamylase activity. (C,D) Effect of the interaction of the amount of added water and temperature on glucoamylase activity production. (E,F) Effect of the interaction of the amount of added water and fermentation time on glucoamylase activity.

alcohol content of *Huangjiu* fermented with raw wheat *Qu* inoculated with *Saccharopolyspora rosea* F2014 and F2017 was higher than that fermented with wheat *Qu* inoculated with other *Saccharopolyspora* (Fig. 5C).

Wheat *Qu* is the key saccharifying agent for *Huangjiu* fermentation.[31] The yields of glucoamylase and amylase secreted by different *Saccharopolyspora* in wheat *Qu* are based on the growth characteristics of *Saccharopolyspora*.[32] Wheat *Qu*

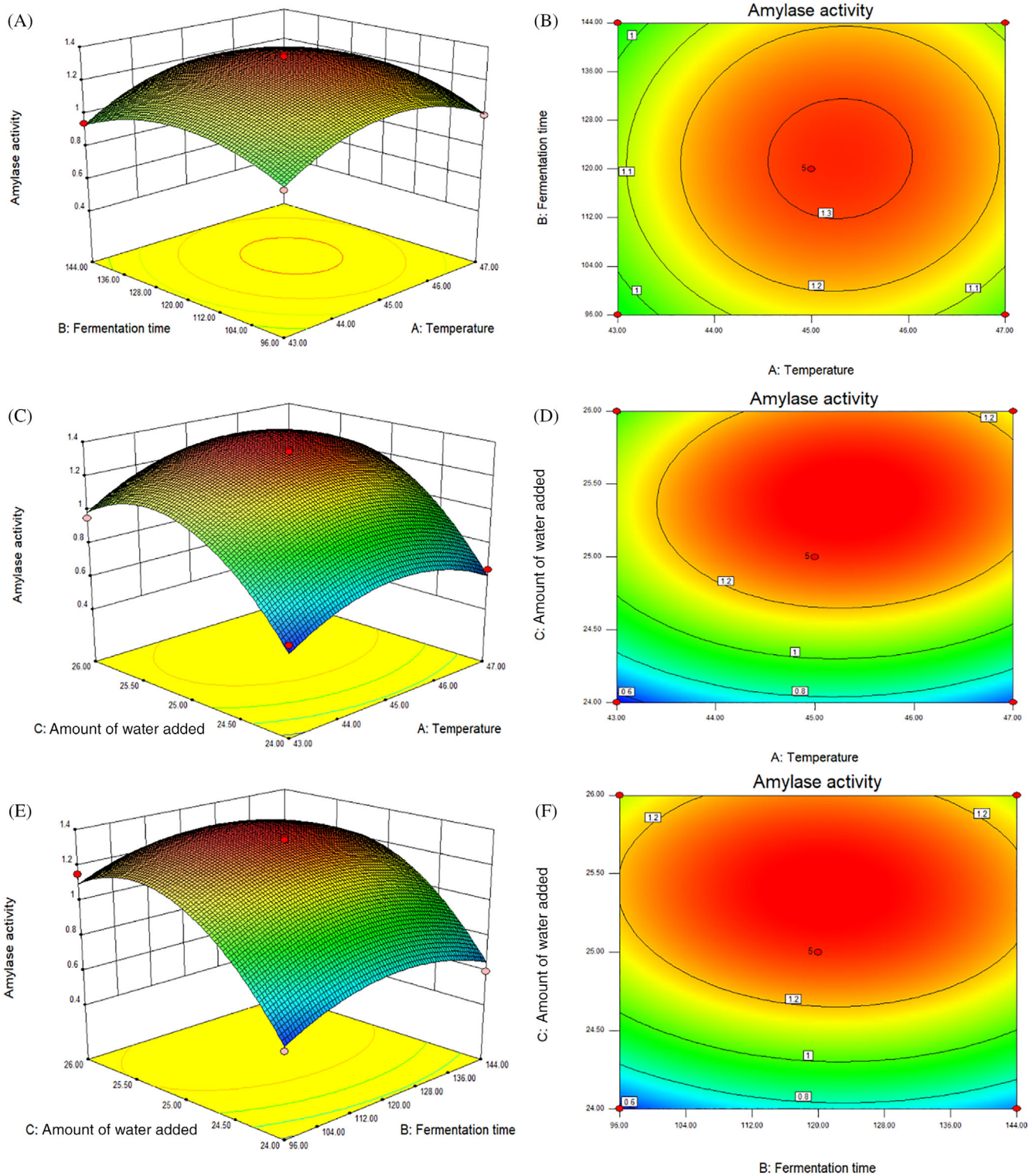

Figure 4. Response surface (3D) and corresponding contour (2D) of amylase activity affected by the interaction of two factors. (A,B) Effect of the interaction of fermentation time and temperature on amylase activity. (C,D) Effect of the interaction of the amount of added water and temperature on amylase activity. (E,F) Effect of the interaction of the amount of the added water and fermentation time on amylase activity.

indirectly contributes to the production of higher alcohols during *Huangjiu* fermentation by saccharify the grain starch to glucose for *Saccharomyces cerevisiae*, which can synthesize higher alcohols from glucose via the Harris pathway.[33] From a quantitative standpoint, higher alcohols are the most important volatile compounds produced during yeast (primarily *Saccharomyces cerevisiae*) fermentation in *Huangjiu*.[16] A moderate amount of higher alcohol will enrich the flavour, leading to a rounded and harmonious taste of alcoholic beverages; however, excessive amounts can cause a series of adverse

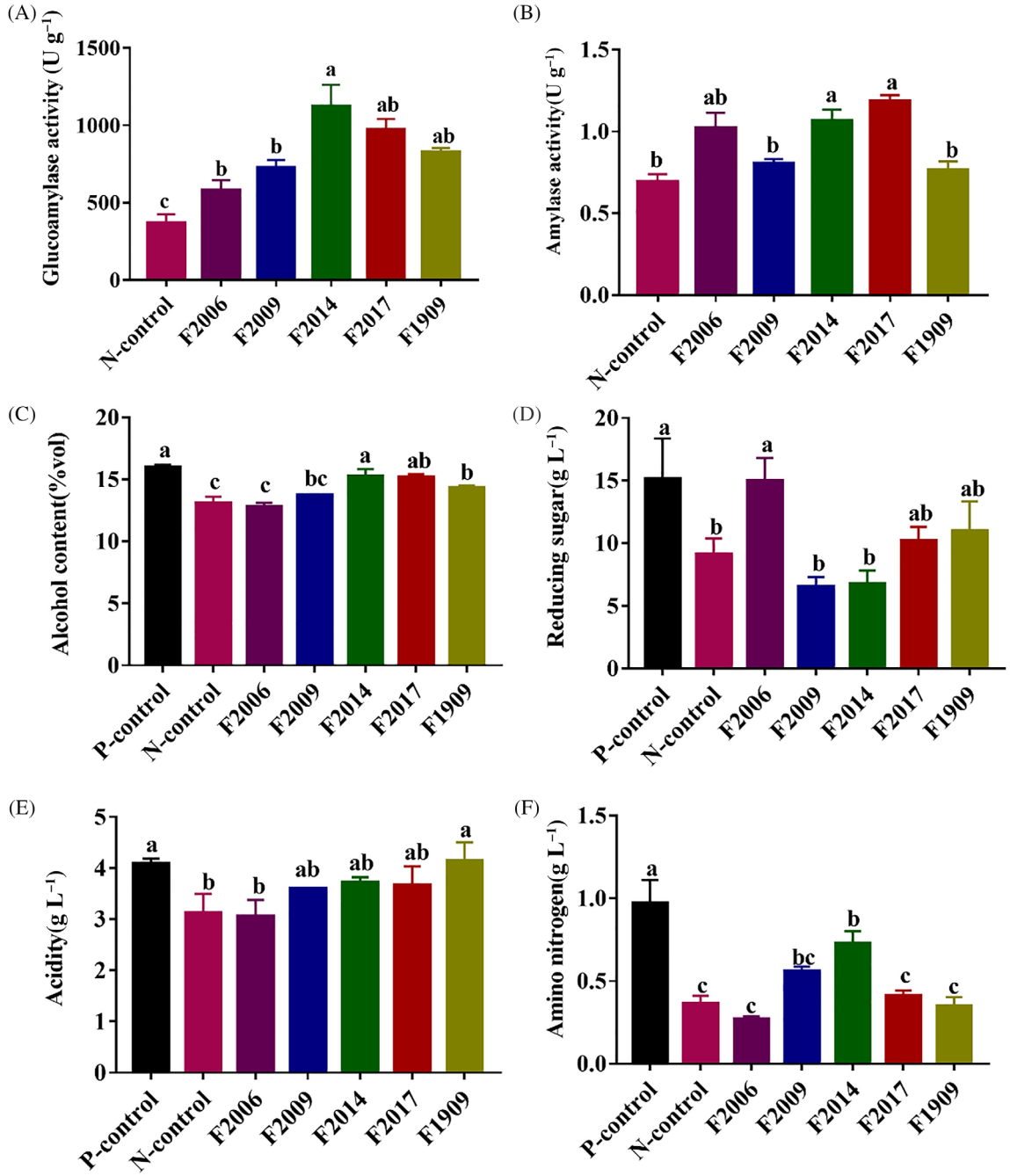

Figure 5. Enzyme activity of wheat *Qu* inoculated with different *Saccharopolyspora* and physicochemical parameters of *Huangjiu*. (A) and (B) indicate glucoamylase and amylase, respectively; (C), (D), (E) and (F) indicate the content of alcohol, reducing sugar, total acid and amino nitrogen, respectively.

symptoms, such as headache and nausea.[34] By employing a fish model to assess the intoxication of different higher alcohols in *Huangjiu*, phenylethanol and isoamyl alcohol were found to have the greatest positive effect on *Huangjiu* intoxication.[35] In this study, the content of isoamyl alcohol in F2014 and F2017 decreased by 18% and 19%, respectively, compared with that of the control (Fig. 6A). Such findings indicate that F2014 and F2017 can alleviate the hangover caused by higher alcohols in *Huangjiu* to some extent. The total content of higher alcohols in *Huangjiu* brewed with inoculated raw wheat *Qu* prepared with *Saccharopolyspora hirsuta* F1909 was significantly ($P < 0.05$) lower than that in *Huangjiu* brewed with inoculated raw wheat *Qu* prepared with the control (589 *vs.* 522) (Fig. 6A); however, its ethanol content was significantly ($P < 0.05$) lower than that brewed with the control (Fig. 6A). This phenomenon is due to the low enzyme yield (Fig. 5A,B) of inoculated raw wheat *Qu* prepared by F1909 and its inability to hydrolyze enough glucose to produce ethanol for the growth of *Saccharomyces cerevisiae*. Previous studies revealed that the content of higher alcohols is highly correlated with yeast strains and the types and addition of wheat *Qu*.[1,36] Additionally, the high protein content in wheat *Qu* has been demonstrated to be the main reason for the high concentration of higher alcohols in *Huangjiu* fermentation.

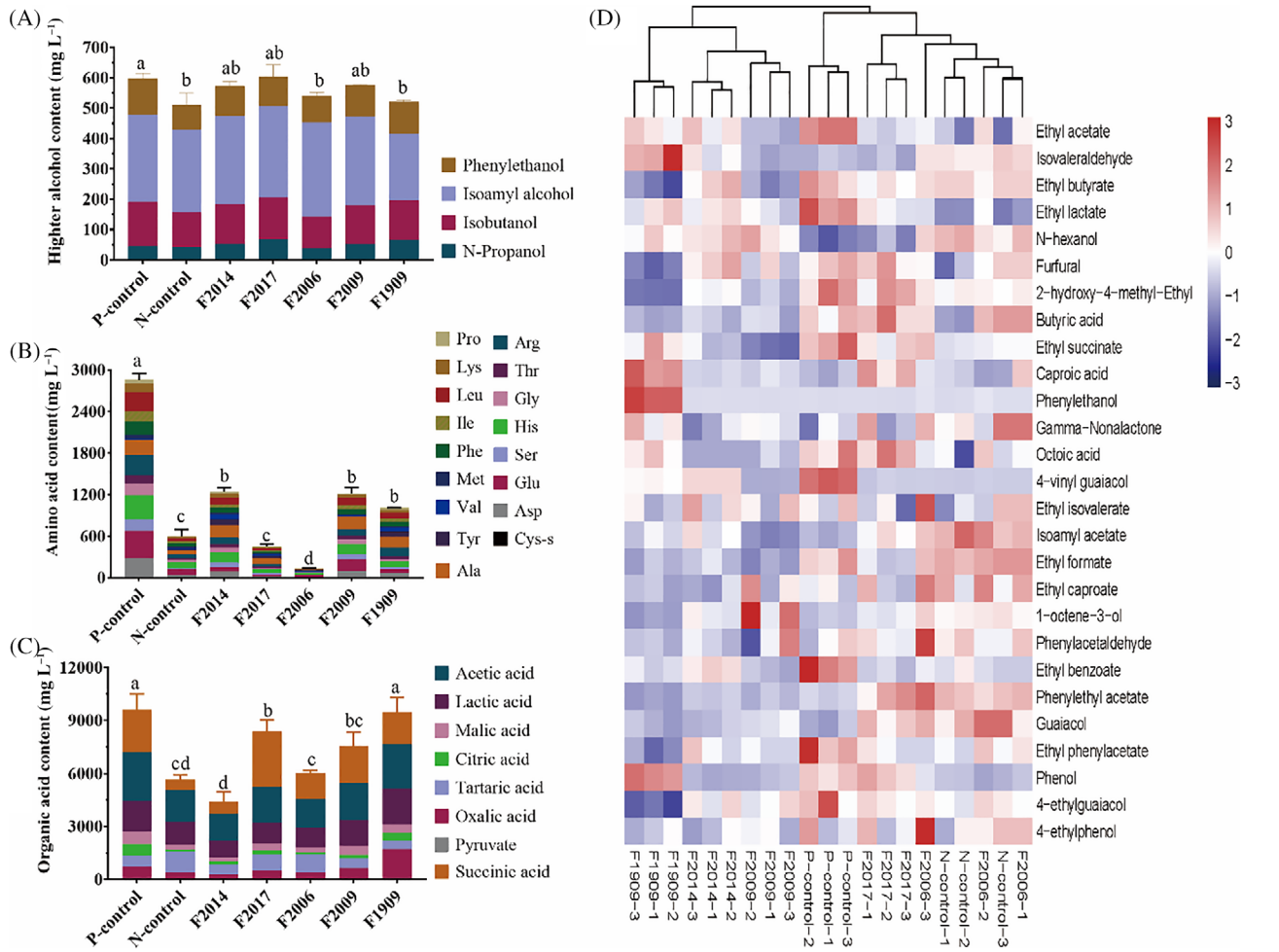

Figure 6. Flavor differences of *Huangjiu* fermented with raw wheat *Qu* inoculated with different *Saccharopolyspora*. (A), (B) and (C) indicate the contents of higher alcohols, amino acids and organic acids, respectively; (D) indicates the heat map of flavor compounds.

Free amino acids are not only an important nitrogen source for microbial growth in *Huangjiu* fermentation, but also precursors for the synthesis of flavor compounds, such as esters and alcohols.[37] The content of free amino acids (2.86 g L^{-1}) in the control group was significantly higher than that in other experimental groups (Fig. 6B); this is mainly because wheat *Qu* is rich in protease-producing *Aspergillus oryzae* and other carboxypeptidase-producing microorganisms, which hydrolyze the proteins in rice into peptides and amino acids via proteases and carboxypeptidases. The bitterness of mechanized *Huangjiu* is stronger than that of traditional manual *Huangjiu*, and the bitter amino acids, including leucine, isoleucine, valine, histidine, arginine, phenylalanine and lysine, contribute most of the bitter substances.[15] In this study, the raw wheat *Qu* inoculated with *Saccharopolyspora rosea* F2014 was applied to mechanized *Huangjiu* fermentation to ensure sufficient nitrogen source for microorganisms. F2014 significantly ($P < 0.05$) decreased the bitter amino acid content (1.24 g L^{-1}) by 56% compared with control (2.86 g L^{-1}), thereby attenuating the unique bitterness.

Similar to most alcoholic beverages, alcohols, esters and acids were identified as the main aroma-active compounds in *Huangjiu*. Of note, most of these aromatic compounds are the byproducts of yeast growth and ethanol fermentation.[34] All previous studies focused on the volatile aroma components of *Huangjiu*.[34,38,39] In this study, significant differences were found in the content of volatile flavor compounds in *Huangjiu* fermented by the raw wheat *Qu* inoculated with different *Saccharopolyspora* (Fig. 6D and Table 4). The total ester content (mainly ethyl acetate and ethyl lactate) (65 mg L^{-1}) in *Huangjiu* fermented with inoculated raw wheat *Qu* F2014 was higher than that in *Huangjiu* fermented with other inoculated raw *Qu*; this is mainly because *Qu* provided more precursor amino acids for the synthesis of esters (Fig. 6B). Guaiacol (odor intensity = 3.9) and 4-vinyl guaiacol (odor intensity = 3.4) contributed to the clove, spicy and smoky odors, which were mainly detected in wheat *Qu*, indicating that the two compounds in *Huangjiu* were mainly derived from wheat *Qu*.[1,40] The content of 4-vinyl guaiacol in *Huangjiu* fermented by wheat *Qu* inoculated with *Saccharopolyspora rosea* F2014 was significantly higher than that in *Huangjiu* fermented with other experimental *Saccharopolyspora* spp., indicating that *Saccharopolyspora rosea* secreted more 4-vinyl guaiacol in wheat *Qu* than the other strains. Interestingly, the content of γ-nonalactone was higher in *Huangjiu* fermented with raw wheat *Qu* inoculated with *Saccharopolyspora* than *Huangjiu* fermented with the control. Therefore, the inoculation

Table 4. Differences in the volatile flavor compounds in *Huangjiu* brewed with different wheat *Qu* inoculated with different *Saccharopolyspora*

Compound	P-control	F2014	N-control	F2006	F2009	F2017	F1909
Ethyl acetate	47.06 ± 1.414a	36.074 ± 3.36b	23.362 ± 3.639c	34.665 ± 1.939bc	26.599 ± 1.026c	29. 979 ± 1.139bc	35.758 ± 1.487b
Ethyl butyrate	0.704 ± 0.081a	0.636 ± 0.078a	0.645 ± 0.001a	0.601 ± 0.042a	0.316 ± 0.045b	0.551 ± 0.043a	0.242 ± 0.078b
Ethyl benzoate	0.015 ± 0.003a	0.008 ± 0.001b	0. 004 ± 0.002bc	0.004 ± 0.001bc	0.002 ± 0.001c	0. 003 ± 0.001bc	0.004 ± 0.001bc
Ethyl lactate	35.442 ± 2.57a	27.45 ± 1.715c	18.911 ± 0.548d	23.484 ± 2.501bc	24. 469 ± 1.684bc	26.065 ± 1.633b	25.596 ± 2.569b
Ethyl phenylacetate	0.009 ± 0.002a	0.006 ± 0.001ab	0.005 ± 0.001b	0.006 ± 0. 001ab	0.005 ± 0.001b	0.005 ± 0.001b	0.002 ± 0.001b
Ethyl isovalerate	0.015 ± 0.002a	0.015 ± 0.002a	0.014 ± 0.002a	0.017 ± 0.003a	0.015 ± 0.002a	0.012 ± 0.002a	0.013 ± 0.001a
Ethyl formate	0.141 ± 0.017a	0.085 ± 0.01b	0.153 ± 0.008a	0.153 ± 0.009a	0.061 ± 0.008b	0.087 ± 0.009b	0.073 ± 0.009b
Ethyl caproate	0.035 ± 0.003ab	0.031 ± 0.003b	0.04 ± 0.005ab	0.05 ± 0.002a	0.04 ± 0.008a	0. 036 ± 0.007ab	0.031 ± 0.002b
Phenylethyl acetate	0.07 ± 0.004b	0.065 ± 0.003b	0.147 ± 0.005a	0.164 ± 0.037a	0.055 ± 0.008b	0.152 ± 0.034a	0.036 ± 0.004b
Ethyl succinate	0.455 ± 0.054a	0.276 ± 0.031b	0.304 ± 0.012b	0. 346 ± 0.044ab	0.18 ± 0.013b	0. 35 ± 0.037ab	0. 373 ± 0.057ab
Isoamyl acetate	0.175 ± 0.01bc	0.247 ± 0.031bc	0.349 ± 0.048a	0. 336 ± 0.029ab	0.135 ± 0.008c	0.257 ± 0.022b	0. 208 ± 0.021bc
2-Hydroxy-4-methyl-pentanoic acid ethyl ester	0.185 ± 0.041a	0.109 ± 0.005ab	0.114 ± 0.008ab	0.126 ± 0.004ab	0.065 ± 0.012b	0.163 ± 0.043a	0.014 ± 0.002b
Esters	**84.304 ± 4.213a**	**65.002 ± 5.239b**	**44.048 ± 4.279c**	**59.953 ± 4.613b**	**51.942 ± 2.815c**	**57. 651 ± 2.970bc**	**57.661 ± 2.970b**
N-hexanol	0.691 ± 0.048b	1.117 ± 0.052a	1.149 ± 0.086a	1.129 ± 0.045a	1.112 ± 0.099a	0.776 ± 0.065b	1.05 ± 0.066a
1-Octene-3-ol	0.007 ± 0.001b	0.012 ± 0.002ab	0. 015 ± 0.001ab	0.016 ± 0.002ab	0.028 ± 0.012a	0.011 ± 0.001ab	0.003 ± 0.001b
Alcohols	**0.698 ± 0.049c**	**1.129 ± 0.054a**	**1.164 ± 0.087a**	**1.145 ± 0.047a**	**1.14 ± 0.11a**	**0.7878 ± 0.066b**	**1.053 ± 0.067a**
Butyric acid	45.129 ± 3.82ab	35.088 ± 2.002b	37.918 ± 9.803b	48.112 ± 3.026ab	35.345 ± 2.912b	49.796 ± 5.453a	32.649 ± 0.873b
Caproic acid	1.598 ± 0.124b	1.815 ± 0.041b	1.683 ± 0.162b	1.989 ± 0.491b	1.849 ± 0.086b	2.774 ± 0.335ab	3.262 ± 0.281a
Acetic acid	0.348 ± 0.034ab	0.254 ± 0.02b	0.268 ± 0.095b	0.31 ± 0.022ab	0.267 ± 0.012b	0.36 ± 0.029a	0.322 ± 0.017ab
Acids	**47.0759 ± 3.978a**	**37.157 ± 2.043a**	**39.862 ± 5.803a**	**50.412 ± 3.539a**	**37.461 ± 3.01a**	**52.935 ± 5.813a**	**32.918 ± 1.171a**
Furfural	0.473 ± 0.045a	0.471 ± 0.045a	0.337 ± 0.102ab	0. 423 ± 0.031ab	0. 372 ± 0. 063ab	0.476 ± 0.057a	0.235 ± 0.022b
Phenylacetaldehyde	0.041 ± 0.007a	0.036 ± 0.003a	0.045 ± 0.004a	0.053 ± 0.012a	0.04 ± 0.016a	0.042 ± 0.004a	0.034 ± 0.002a
Isovaleraldehyde	0.085 ± 0.008b	0.123 ± 0.019b	0.152 ± 0.012ab	0.148 ± 0.008b	0.073 ± 0.00b	0.071 ± 0.012b	0.237 ± 0.054a
Aldehydes	**0.598 ± 0.06a**	**0.629 ± 0.0.67a**	**0.533 ± 0.119a**	**0.625 ± 0.515a**	**0.486 ± 0.087a**	**0.589 ± 0.073a**	**0.506 ± 0.077a**
4-Vinyl guaiacol	47.635 ± 2.058a	24.995 ± 6.12b	8.379 ± 0.225d	8.907 ± 0.254d	3.505 ± 0.332e	8.399 ± 0.829d	18.945 ± 1.788c
Guaiacol	0.012 ± 0.002b	0.013 ± 0.002b	0.034 ± 0.00a	0.034 ± 0.008a	0. 018 ± 0.002ab	0. 029 ± 0.004ab	0.012 ± 0.002b
Phenol	0.78 ± 0.052b	0.356 ± 0.023b	0.406 ± 0.026b	0.424 ± 0.079b	0.396 ± 0.033b	0.831 ± 0.09b	1.053 ± 0.066a
4-Ethylguaiacol	0.018 ± 0.003a	0.015 ± 0.001a	0.014 ± 0.001a	0.015 ± 0.001a	0.015 ± 0.001a	0.015 ± 0.001a	0.008 ± 0.001b
4-Ethylphenol	0.037 ± 0.006a	0.033 ± 0.003a	0.037 ± 0.00a	0.045 ± 0.007a	0.034 ± 0.003a	0.037 ± 0.005a	0.033 ± 0.002a
Phenols	**48.481 ± 2.121a**	**25.411 ± 0.029b**	**8.87 ± 0.265d**	**9.426 ± 0.345d**	**3.968 ± 0.0371e**	**9.309 ± 0.928d**	**20.018 ± 1.859c**
γ-Nonalactone	0.342 ± 0.039a	0.332 ± 0.032a	0.432 ± 0.047a	0.435 ± 0.058a	0.381 ± 0.02a	0.374 ± 0.065a	0.41 ± 0.033a

of wheat *Qu* with *Saccharopolyspora* could promote the production of γ-nonalactone.

CONCLUSIONS

To the best of our knowledge, this is the first study to reveal a process for making raw wheat *Qu* inoculated with *Saccharopolyspora* and extend its application to *Huangjiu*. Based on single-factor results, the optimal conditions for improving the enzyme activity of inoculated wheat *Qu* were as follows: temperature, 45 °C; fermentation time, 120 h; amount of added water, 25%; and inoculation amount, 5%. Response surface optimization results showed that with a fermentation temperature of 45 °C, fermentation time 122 h and amount of added water 26%, glucoamylase activity of wheat *Qu* was 1119.53 ± 58.38 U g^{-1} and amylase activity was 1.39 ± 0.01; these activities increased by 27% and 40%, respectively, compared with those before optimization. Overall, the application of raw wheat *Qu* inoculated with *Saccharopolyspora rosea* F2014 to mechanized *Huangjiu* fermentation significantly ($P < 0.05$) decreased its bitter amino acid content (1.24 *vs.* 2.86 g L^{-1}, decreased 56%) and attenuated its unique bitterness, and thus could be used for the fermentation of mechanized *Huangjiu*.

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AUTHOR CONTRIBUTIONS

Donglin Ma: conceptualization, methodology, formal analysis, investigation, visualization, writing – original draft. Shuangping Liu: supervision, writing – review and editing. Haipo Liu: data curation, writing – review and editing. Mujia Nan: data curation, writing – review and editing. Yuezheng Xu: supervision, writing – review and editing. Xiao Han: supervision, writing – review and editing. Jian Mao: funding acquisition, supervision, writing – review and editing.

CONFLICT OF INTEREST

The authors declare no competing interests.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

REFERENCES

1 Chen S and Xu Y, Effect of 'wheat Qu' on the fermentation processes and volatile flavour-active compounds of Chinese rice wine (Huangjiu). *J Inst Brewing* **119**:71–77 (2013).

2 Shang YL, Chen LL, Zhang ZH and Lu J, A comparative study on the fungal communities of wheat Qu for Qingshuang-type Chinese rice wine. *J Inst Brewing* **118**:243–248 (2012).
3 Liu J, Chen J, Fan Y, Huang X and Han B, Biochemical characterisation and dominance of different hydrolases in different types of Daqu —a Chinese industrial fermentation starter. *J Sci Food Agric* **98**: 113–121 (2018).
4 Yu P, Du J, Cao C, Cai G, Sun J, Wu D *et al.*, Development of a novel multi-strain wheat Qu with high enzyme activities for Huangjiu fermentation. *J Sci Food Agric* **101**:4808–4817 (2021).
5 Chen S, Wang CC, Qian M, Li Z and Xu Y, Characterization of the key aroma compounds in aged Chinese rice wine by comparative aroma extract dilution analysis, quantitative measurements, aroma recombination, and omission studies. *J Agric Food Chem* **67**:4876–4884 (2019).
6 Zhang K, Li Q, Wu W, Yang J and Zou W, Wheat Qu and its production technology, microbiota, flavor, and metabolites. *J Food Sci* **84**:2373–2386 (2019).
7 Shou QH, Zhao GA, Wei TY, Song HY, Sui-Feng WU and Zhan LM, Study on Saccharifying properties of wheat starter of yellow rice wine. *Liquor Making Sci Technol* **8**:84–87 (2008).
8 Wang L, Cao Y, Jian LU and Xie GF, Study on a-amylase from wheat Qu of Shaoxing rice wine. *China Brewing* **5**:38–40 (2007).
9 Liu S, Hu J, Xu Y, Xue J, Zhou J, Han X *et al.*, Combined use of single molecule real-time DNA sequencing technology and culture-dependent methods to analyze the functional microorganisms in inoculated raw wheat Qu. *Food Res Int* **132**:109062 (2020).
10 Wang P, Mao J, Meng X, Li X, Liu Y and Feng H, Changes in flavour characteristics and bacterial diversity during the traditional fermentation of Chinese rice wines from Shaoxing region. *Food Control* **44**:58–63 (2014).
11 Zhang J, Liu S, Sun H, Jiang Z, Xu Y, Mao J *et al.*, Metagenomics-based insights into the microbial community profiling and flavor development potentiality of baijiu Daqu and huangjiu wheat Qu. *Food Res Int* **152**:110707 (2022).
12 Liu S, Chen Q, Zou H, Yu Y, Zhou Z, Mao J *et al.*, A metagenomic analysis of the relationship between microorganisms and flavor development in Shaoxing mechanized huangjiu fermentation mashes. *Int J Food Microbiol* **303**:9–18 (2019).
13 Li H, Lian B, Ding YH, Nie CC and Zhang QM, Bacterial diversity in the central black component of Maotai Daqu and its flavor analysis. *Ann Microbiol* **64**:1659–1669 (2014).
14 Hong XT, Chen J, Liu L, Wu H, Tan HQ, Xie GF *et al.*, Metagenomic sequencing reveals the relationship between microbiota composition and quality of Chinese Rice wine. *Sci Rep* **6**:26621 (2016).
15 Lu Z, Xie G, Wu D, Yang L, Jin Z, Hu Z *et al.*, Isolation and identification of the bitter compound from Huangjiu. *Food Chem* **349**:129133 (2021).
16 Liu SP, Ma DL, Li ZH, Sun HL, Mao JQ, Shi Y *et al.*, Assimilable nitrogen reduces the higher alcohols content of huangjiu. *Food Control* **121**: 107660 (2021).
17 Wei XL, Liu SP, Yu JS, Yu YJ, Zhu SH, Zhou ZL *et al.*, Innovation Chinese rice wine brewing technology by bi-acidification to exclude rice soaking process. *J Biosci Bioeng* **123**:460–465 (2017).
18 Liu SP, Yu JX, Wei XL, Ji ZW, Zhou ZL, Meng XY *et al.*, Sequencing-based screening of functional microorganism to decrease the formation of biogenic amines in Chinese rice wine. *Food Control* **64**:98–104 (2016).
19 Gump BH, Zoecklein BW, Fugelsang KC and Whiton RS, Comparison of analytical methods for prediction of prefermentation nutritional status of grape juice. *Am J Enol Vitic* **53**:325–329 (2002).
20 Di Egidio V, Sinelli N, Giovanelli G, Moles A and Casiraghi E, NIR and MIR spectroscopy as rapid methods to monitor red wine fermentation. *Eur Food Res Technol* **230**:947–955 (2010).
21 Zhou ZL, Ni W, Ji ZW, Liu SP, Han X, Li XT *et al.*, 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 Methods* **13**:591–600 (2020).
22 Gong M, Zhou ZL, Yu YJ, Liu SP, Zhu SH, Jian DZ *et al.*, Investigation of the 5-hydroxymethylfurfural and furfural content of Chinese traditional fermented vinegars from different regions and its correlation with the saccharide and amino acid content. *LWT--Food Sci Technol* **124**:109175 (2020).
23 Yang HY, Peng Q, Zhang HB, Sun JQ, Shen C and Han XY, The volatile profiles and microbiota structures of the wheat Qus used as traditional fermentation starters of Chinese rice wine from Shaoxing region. *LWT--Food Sci Technol* **154**:112649 (2022).
24 Jung JY, Lee SH, Kim JM, Park MS, Bae J-W, Hahn Y *et al.*, Metagenomic analysis of kimchi, a traditional Korean fermented food. *Appl Environ Microbiol* **77**:2264–2274 (2011).
25 Wolfe Benjamin E, Button Julie E, Santarelli M and Dutton Rachel J, Cheese rind communities provide tractable systems for in Situ and in vitro studies of microbial diversity. *Cell* **158**:422–433 (2014).
26 Fan GS, Du YH, Fu ZL, Chen M, Wang Z, Liu PX *et al.*, Characterisation of physicochemical properties, flavour components and microbial community in Chinese Guojing roasted sesame-like flavour Daqu. *J Inst Brewing* **126**:105–115 (2020).
27 Xiao C, Lu ZM, Zhang XJ, Wang ST, Ao L, Shen CH *et al.*, Bio-heat is a key environmental driver shaping the microbial community of medium-temperature Daqu. *Appl Environ Microbiol* **83**:e01550–e01517 (2017).
28 Tananuwong K and Lertsiri S, Changes in volatile aroma compounds of organic fragrant rice during storage under different conditions. *J Sci Food Agric* **90**:1590–1596 (2010).
29 Sayed AM, Abdel-Wahab M, Hassan HM and Abdelmohsen UR, Saccharopolyspora: an underexplored source for bioactive natural products. *J Appl Microbiol* **128**:314–329 (2020).
30 Chutmanop J, Chuichulcherm S, Chisti Y and Sirinophakun P, Protease production by Aspergillus oryzae in solid-state fermentation using agroindustrial substrates. *J Chem Technol Biotechnol* **83**:1012–1018 (2008).
31 Tian S, Zeng W, Fang F, Zhou J and Du G, The microbiome of Chinese rice wine (Huangjiu). *Curr Res Nutr Food Sci* **5**:325–335 (2022).
32 Spain AM, Forsberg CW and Krumholz LR, *Bergey's Manual of Systematics of Archaea and Bacteria*. John Wiley & Sons. Inc., New York (2015).
33 Avalos JL, Fink GR and Stephanopoulos G, Compartmentalization of metabolic pathways in yeast mitochondria improves the production of branched-chain alcohols. *Nat Biotechnol* **31**:335–341 (2013).
34 Chen S, Xu Y and Qian MC, Aroma characterization of chinese rice wine by gas chromatography-olfactometry, chemical quantitative analysis, and aroma reconstitution. *J Agric Food Chem* **61**:11295–11302 (2013).
35 Sun H, Liu S, Mao J, Yu Z, Lin Z and Mao J, New insights into the impacts of huangjiu compontents on intoxication. *Food Chem* **317**:126420 (2020).
36 Chen SA and Xu Y, The influence of yeast strains on the volatile flavour compounds of Chinese rice wine. *J Inst Brewing* **116**:190–196 (2010).
37 Gambetta JM, Cozzolino D, Bastian SEP and Jeffery DW, Exploring the effects of geographical origin on the chemical composition and quality grading of Vitis vinifera L. cv. chardonnay grapes. *Molecules* **22**:218 (2017).
38 Chen S, Wang D and Xu Y, Characterization of odor-active compounds in sweet-type Chinese rice wine by aroma extract dilution analysis with special emphasis on sotolon. *J Agric Food Chem* **61**:9712–9718 (2013).
39 Chen S, Xu Y and Qian MC, Comparison of the aromatic profile of traditional and modern types of Huang Jiu (Chinese rice wine) by aroma extract dilution analysis and chemical analysis. *Flavour Fragrance J* **33**:263–271 (2018).
40 Mo X, Xu Y and Fan W, 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 (2010).

