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Research Article
Effect of Solid or Liquid Fermentation State, Yeast Strain, Fermentation Temperature and Time on the Flavor Content of Jujube (*Ziziphus jujuba*) Brandy

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Abstract
Background: Jujube brandy is characteristic distilled fruit liquor in China, which lack mature production technology until now. **Materials and Methods:** The conditions of fermentation way (solid and liquid state), starter culture (single-yeast, mixed-yeast and jiuqu), fermentation temperature (15,18, 23, 28, 32) and fermentation time (6,10,14, 20, 24, 28 days) were optimized based on one-factor tests and Response Surface Methodology (RSM). **Results:** From one factor flavor content result, solid state fermentation (K-3790.63, J-3250.22, DK-3553.64 mg L^{-1}) was about 8 times of liquid state fermentation (Z, W). Jujube brandy fermented with single-yeast GH and PH have the best flavor (4232.64, 4125.46 mg L^{-1}, respectively), followed by mixed-yeast PHGHSX and GHSX, J Jiuqu has the worst (1670.48 mg L^{-1}). Flavor content increased with temperature rising before 28, reach the peak at 28 (3790.63 mg L^{-1}), then decreased. At 20 and 24 days, jujube brandy got the better flavor (4442.71, 4303.66 mg L^{-1}), significantly higher than 6 days (3790.63) and 28 days (2373.71 mg L^{-1}). **Conclusions:** Combined with RSM result, the optimal conditions of brewing were: Choosing solid-state fermentation, yeast GH (*Saccharomyces cerevisia* cctcc-M94055), fermentation temperature of 18°C, fermentation time of 24 days and the content of flavor compounds was 4525.934 mg L^{-1}, which is 1.19 times of normal brewing, almost accords with the predicted data, has great practical values.

Key words: Jujube brandy, flavor compounds, response surface method, alcohol, CO_2 loss weight

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Jujube (*Ziziphus jujuba*) is one of the characteristic fruit in China. By the end of 2012, the total cultivating area of jujube in China has reached 3200000 ha, annual output of 4.683 million t[1,2]. Hebei is a major produce place of jujube, but the development of processing technology and high value-added products is not increasing. Studies have shown that jujubes are rich in sugar[3,4] and contain similar components as grapes, which are used for producing brandy[5,6].

Fermentation conditions are the decisive factor of quality and flavor of liquor[7,8]. The main factors influencing the liquor aroma components include fermentation way, yeast strains, fermentation temperature and time[9,10]. In Western countries, brandy is produced with grape juice or hide trimmings and different kinds of yeasts[11-13]. A number of researchers and winemakers have found that spontaneous fermentations (*Saccharomyces cerevisiae* mix with non-*Saccharomyces cerevisiae*) are associated with greater wine body, unusual or odd aromas and flavours, creamy texture and greater complexity.

Britain liquor brewster think that the best temperature for brewing fruit wine is between 22-25°C, because low fermentation temperature could reduce the generation of higher alcohols[14]. But for the French and German winemaker, 15-18°C is considered the best temperature for fermentation for a long time[15]. Daqu and solid-state fermentation are characteristic of Chinese traditional liquor production techniques[16-18] and have recently been used in the brewing of fruit wine, bringing unique flavors and improving the quality of production[19,20]. Most white wines in China have long fermentation time at the temperature of 25-30°C, maybe as long as 3 months[21-24].

Until now, improved processing technology and high quality are needed for jujube brandy to have a larger market share. In this study, fermentation way, yeast strains, fermentation temperature and time were selected for one-factor experiment, followed by the response surface test. The purpose of this study was to find out the optimal fermentation parameters, get the maximum flavor content and obtain high quality jujube brandy, which would prompt the development of jujube and jujube brandy industry in China.

MATERIALS AND METHODS

Samples: Jujube: dried Ziziphus jujube (Hebei, Fuping).
Single yeast: (Anqi yeast company, China).

PH : Active dry wine yeast (RW), *Saccharomyces cerevisia*
PZ : Active dry wine yeast (SY), *Saccharomyces cerevisia*
GH : Alcohol active dry yeast (Thermal resistant), *Saccharomyces cerevisia* cctcc-M94055
SX : Smell improve dry yeast, *Pichia pastoris*, non-*Saccharomyces cerevisiae*

Mixed yeast: GHSX(GS): GH mixed SX with the ratio of 1:1.
PHGHSX(HGS): PH, GH, SX mixed with the ratio of 3:3:2.
Jiuqu:

N : Chinese strong-flavor liquor daqu
J : Chinese maotai-flavor liquor daqu
Q : Chinese faint-scent liquor daqu

N, J and Q are main kinds of daqu in China (Guangxi Jinhua Jiuqu company, China).

AQ : Starter of liquor-making (Anqi yeast company, China).

Yeast and jiuqu activation: Take 1.5% yeast or jiuqu in 100 mL of 2% glucose water, 40°C water bath for 30 min.

Brewing process of jujube brandy: Brewing process of jujube brandy show as Fig. 1.

Alcohol and CO_2 loss weight test: Alcohol content is tested with alcohol meter.

CO_2 loss weight test: Whole weight of fermentation container is tested at 10 am everyday, CO_2 loss weight is obtained by minusing the first weight (before fermentation), which is also a parameter determining the degree of fermentation.

SPME-GC-MS parameters: Jujube brandy was diluted by distilled water (10% alcohol content). Sodium chloride (1 g) was added to 7.5 mL of sample solution in a 20 mL sealed glass vial. The sample was exacted at 40°C for 40 min with 50/30 μm DVB/CAR/PDMS fiber, then used to GC-MS analysis.

Flavor compounds of jujube brandy were detected by GC–MS. The contents of flavor compounds were quantified using an internal standard (3-octanol, 99%, Sigma-Aldrich). Wine volatile compounds were analyzed using an Agilent 5975 Mass Spectrometer coupled to an Agilent 7890A Gas Chromatograph (Agilent, Santa Clara, USA). A DB-WAX column (60 m×0.25 mm ID and 0.25 μm film thickness) was used for

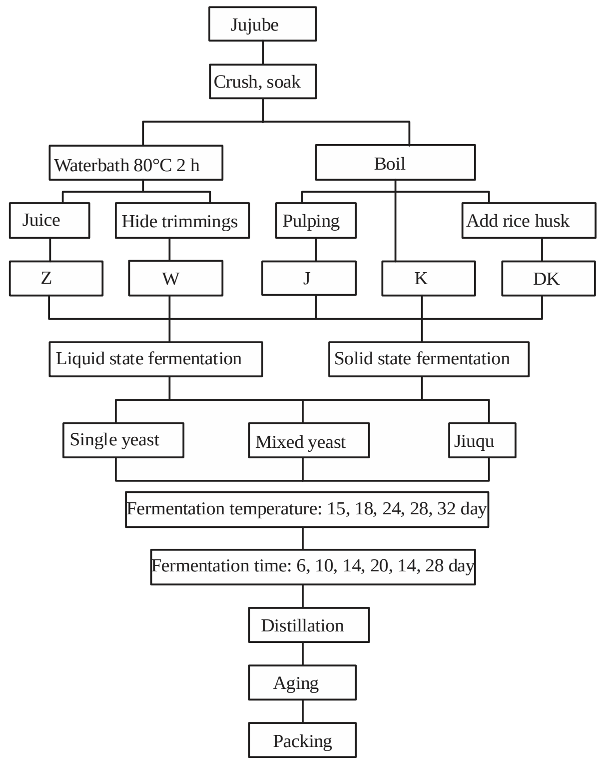

Fig. 1: Brewing process of jujube brandy

Table 1: Experimental parameters of one-factor test

Level	1	2	3	4	5	6
Fermentation way	Liquid (Z)	Liquid (W)	Solid-state (J)	Solid-state (K)	Solid-state (DK)	-
Yeast	Single-PH	Single-PZ	Single-GH	Single-SX	Mixed-GHSX	Mixed-PHGHSX
Jiuqu	N	J	Q	AQ	ZJ	-
Fermentation temperature (°C)	15	18	24	28	32	-
Fermentation time (day)	6	10	14	20	24	28

separation. The working parameters were as follows: Injector temperature of 250°C, EI source of 230°C, MS Quad of 150°C and transfer line of 250°C. The initial temperature was 50°C for 3 min, which was increased to 80°C at a rate of 3°C min^{-1}. The temperature was further raised to 230°C at 5°C min^{-1} and maintained at 230°C for 6 min. The carrier gas had a flow rate of 1.0 mL min^{-1}. Samples were injected using the splitless mode. A mass range of 50-550 m/z was recorded at one scan per second.

Qualitative and quantitative analysis: Flavor compounds were identified by Nist 2005 library of GC-MS. The contents of flavor compounds were quantified using an internal standard (3-octanol, 99%, Sigma-Aldrich).

$$m_i = (f^*A_i)/(A_s/m_s),\ f = (A_s/m_s)/(A_r/m_r)$$

m_i, m_s, m_r represent contents of determinand, internal standard, contrast, A_i, A_s, A_r represent peak area or peak height of determinand, internal standard, contrast and f represent correction factor.

Table 2: Independent variables and their levels used in the response surface design

Level	X_1 (yeast strains)	X_2 (temperature) (°C)	X_3 (time) (days)
-1	Single-yeast	18	8
0	Mixd-yeast	24	16
1	Jiuqu	30	24

Experimental: One-factor test was performed as Table 1.

Box-Behnken design: Response surface methodology was applied to determine the brewing conditions jujube brandy. On the basis of the preliminary single factor experiment, a Box-Behnken Design (BBD) with 3 independent factors (X_1, yeast strains; X_2, fermentation temperature; X_3, fermentation time) set at three variation levels was carried out (Table 2). And +1, 0, -1 encoded factors represent variables[25]. The flavor content of jujube brandy was selected

Table 3: Variable levels and responses of flavor content based on yeast, fermentation temperature and time

Run	Yeast strains (X_1)	Temperature (X_2, °C)	Time (X_3 day)	Observed (Y_0, mg L^{-1})	Predicted (Y, mg L^{-1})
1	3	30	16	1646.772	1750.870
2	2	18	24	4338.867	4305.921
3	2	30	8	1456.794	1489.740
4	3	18	16	1966.980	1991.251
5	1	24	8	1450.520	1441.845
6	2	24	16	1855.934	1964.511
7	2	24	16	2061.225	1964.511
8	2	24	16	2254.669	1964.511
9	0	0	0	1736.894	1964.511
10	2	18	8	1806.913	1919.686
11	1	24	24	3686.072	3823.116
12	3	24	8	1863.416	1726.371
13	2	30	24	3561.602	3448.829
14	2	18	16	1913.834	1964.511
15	1	30	16	1296.085	1271.814
16	1	18	16	2422.570	2318.472
17	3	24	24	3681.750	3690.424

as the responses for the combination of the independent variables shown in Table 3.

Experiment design and statistical analysis: Experimental dates were analyzed using Design-Expert 8.0.6.1 (State-Ease Inc. Minneapolis, MN, USA) statistical package. A Box-Behnken response surface experiment design with 3 factors was to optimize and investigate the individual and interactive effects of process variables on the flavor content of jujube brandy. The experiments were conducted in a randomized order and the data were analyzed by multiple regression analysis in order to develop an empirical second order egression polynomial mathematical model, which exhibits the relationships between response and independent variables. The construction and analysis of the experimental design, Analysis of Variance (ANOVA) to obtain the interaction between the process variables and the response, quality of the fit of the polynomial model (coefficient of determination (R^2), adjusted coefficient of determination (adj-R^2) and predicted coefficient of determination (pre-R^2) and optimization of process condition were obtained using Design-Expert 8.0.6.1. After optimization, triplicate experiments were performed under the optimal conditions and the average value of the experiments was compared with the predicted values of the developed model equation. All experiments were performed at least in triplicate and results were expressed as Means±SD. Data obtained were analyzed using one-way Analysis of Variance (ANOVA, $p<0.05$, SPSS, version17.0) and p-value of <0.05 were considered to be statistically significant.

RESULTS AND DISCUSSION

Influence of fermentation way on the quality of jujube brandy: Choosing mixed-yeast GHSX, fermentation at 28°C for 6 days, alcohol, CO_2 loss weight and flavor content of jujube brandy were analyzed to determine the proper fermentation way. Significant difference of alcohol, CO_2 loss weight and flavor content appeared with different fermentation ways ($p<0.05$). Jujube brandy has higher alcohol, CO_2 loss weight and flavor content with solid-state fermentation (J, K, DK) than liquid-state fermentation (Z,W, Fig. 2).

Jujube brandy with solid-state fermentation adding auxiliary materials (rice husk) has the highest alcohol (35.38% vol). Jujube brandy fermented with different crush degree of jujube (K and J) have similar alcohol content, means crush degree of jujube has no big influence on alcohol of jujube brandy. The W (hide trimmings) fermentation way obtained the lowest alcohol (15% vol) because of its low reducing sugar.

The CO_2 loss weight is also a parameter determining the degree of fermentation. Jujube brandy fermented with J has the highest The CO_2 loss weight, followed by DK (rice husk), W has the lowest. From change trend of CO_2 loss weight, liquid fermentation occurs mainly in first 2 days and solid-state fermentation occurs mainly in first 3-4 days. Jujube brandy fermented with K and auxiliary materials have the best flavor, W has the worst. Therefore, the proper fermentation way for jujube brandy is solid-state fermentation adding with rice husk, which was used for the follow-up test.

Influence of yeast strains on the quality of jujube brandy: Fermentation is mainly carried out with *Saccharomyces cerevisiae* inocula, non-*Saccharomyces* yeasts have been proved to contribute significantly to the aromatic quality of the final beverage[26,27]. Besides, fermentation with a single yeast may lead to aroma deficiencies, many brewers have used mixed yeast to enrich the flavor of wine. Especially, some distilleries used daqu, which contain not only yeast, but also mold and a variety of bacteria, much more advantageous to generate a lot of aroma[28-30].

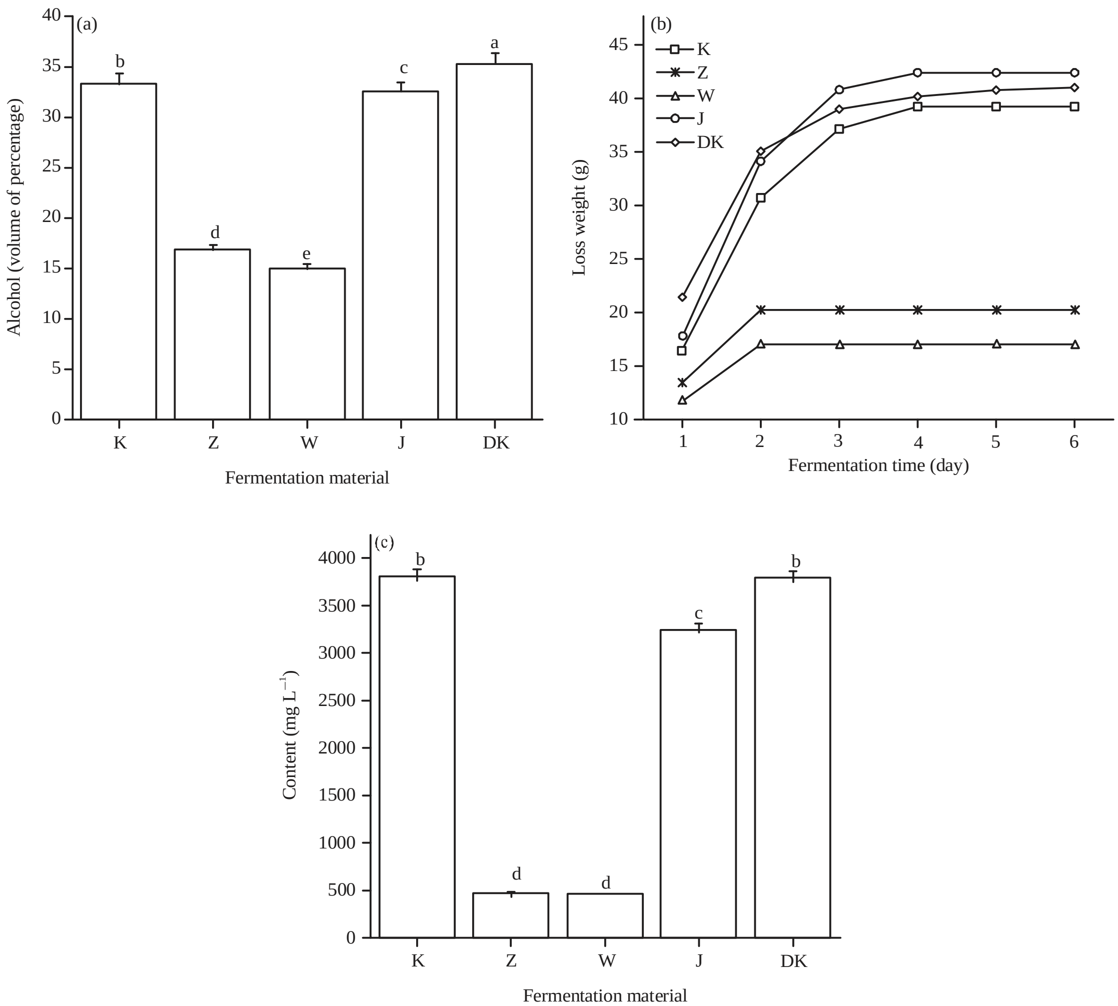

Fig. 2(a-c): Influence of fermentation way on the (a) Alcohol, (b) CO_2 loss weight and (c) Flavor content of jujube brandy

Besides ZJ Jiuqu, alcohol of jujube brandy maintain between 33-36% vol. Jujube brandy fermented with single-yeast PH and mixed-yeast GHSX have higher alcohol than others. The CO_2 loss weight of jujube brandy with SX, N, Q and ZJ Jiuqu was significantly lower than other group ($p<0.05$), which have similar CO_2 loss weight. Significant difference of flavor content appeared with different yeast strains ($p<0.05$). Jujube brandy fermented with single-yeast GH and PH have the best flavor, followed by mixed-yeast PHGHSX and GHSX, J Jiuqu has the worst (Fig. 3). Therefore, single-yeast GH, PH and mixed-yeast GHSX are proper yeast strains for brewing jujube brandy.

Influence of fermentation temperature on the quality of jujube brandy: It is reported that chromaticity value is greatly affected by fermentation temperature[31]. Peng[32] believed the proper fermentation temperature of dry white wine is 15~18°C, when has a pleasing fresh fruit flavor and fresh, soft taste, higher fermentation temperature may produce rough wine. Lin[33] found that fermentation of grape juice with 23~25°C would improve the quality of wine products.

Significant difference of alcohol and flavor content appeared with different fermentation temperatures ($p<0.05$). Jujube brandy got the highest alcohol at 28°C, then at 18°C, the least at 15°C. Flavor content and CO_2 loss weight both increased with temperature rising before 28°C, reach the peak at 28°C (Fig. 4). Therefore, the proper temperature for brewing jujube brandy is 28°C.

Influence of fermentation time on the quality of jujube brandy: The flavor compounds produced in the process of brewing constitute the skeleton of wine aroma, when the style of wine is determined[34-36]. Significant difference of alcohol and flavor content also appeared with different fermentation time ($p<0.05$). Jujube brandy got the highest alcohol at 6 days, then decreased gradually, which means jujube brandy got fully

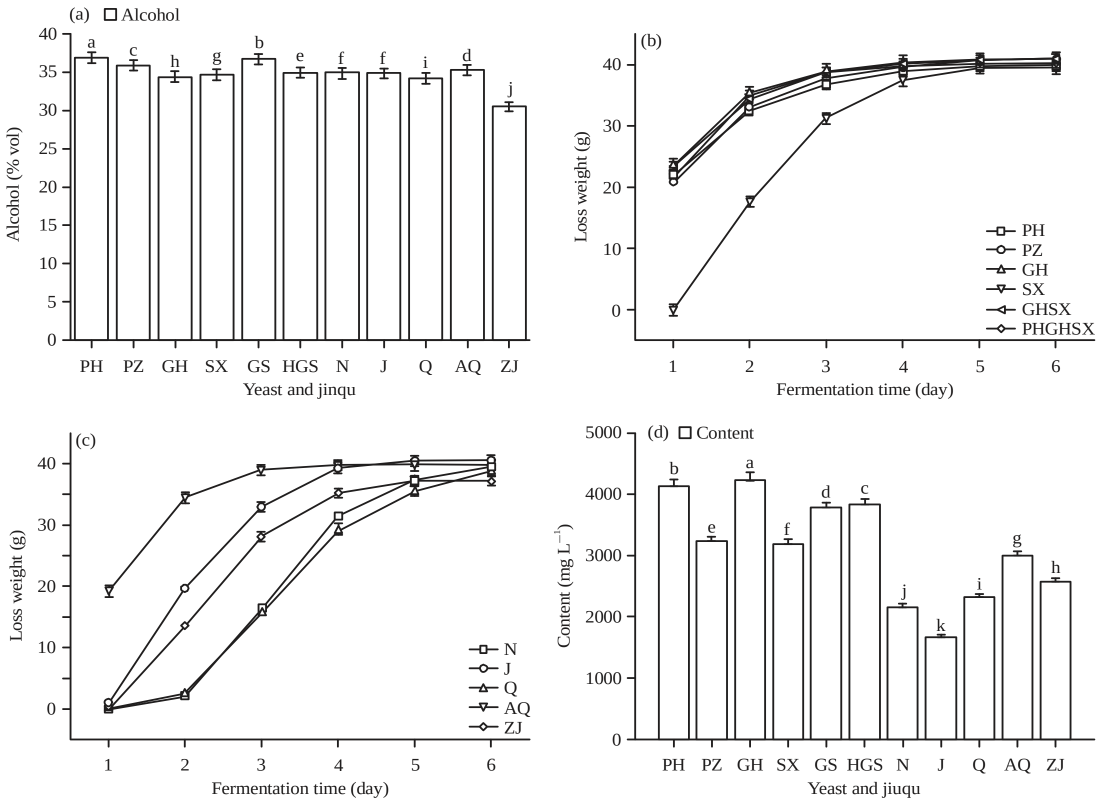

Fig. 3(a-d): Influence of yeast and Jiuqu on the alcohol, CO_2 loss weight and flavor content of jujube brandy

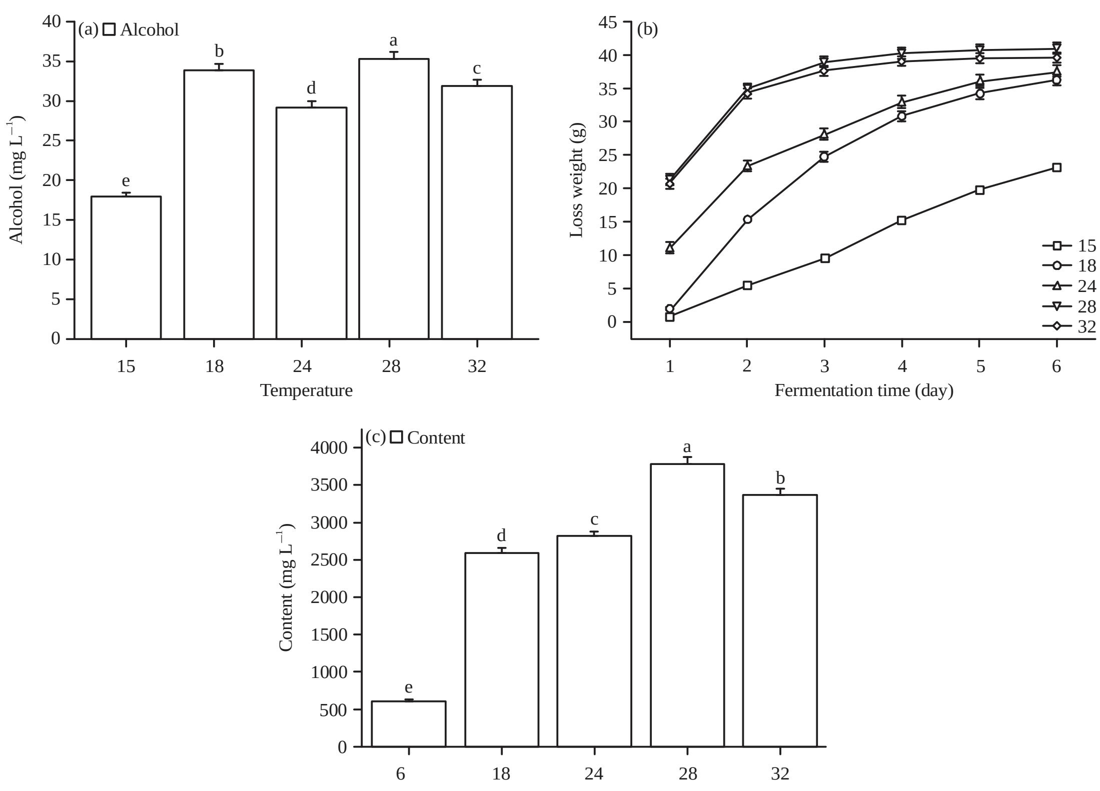

Fig. 4(a-c): Influence of fermentation temperature on the (a) Alcohol, (b) CO_2 loss weight and (c) Flavor content of jujube brandy

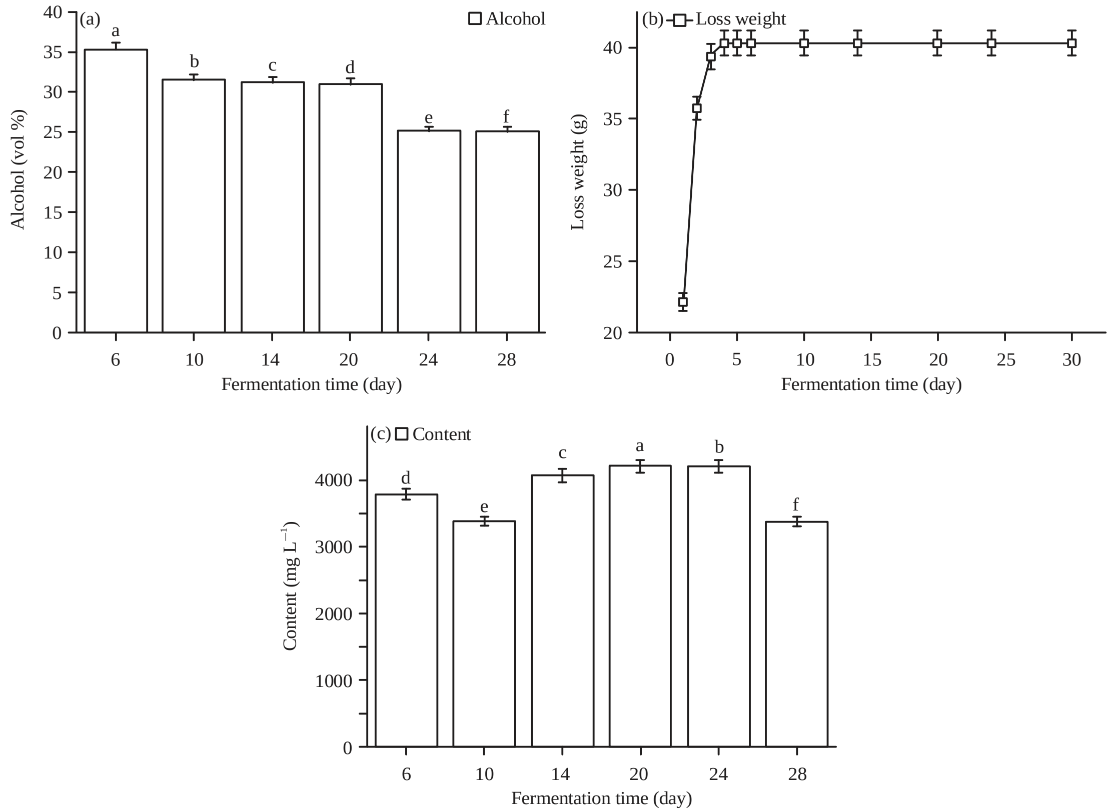

Fig. 5(a-c): Influence of fermentation time on the (a) Alcohol, (b) CO_2 loss weight and (c) Flavor content of jujube brandy

Table 4: Analysis of Variance (ANOVA) for response surface quadratic model for flavor content of jujube brandy and independent variables (X_1, X_2, X_3, X_4)

Factor	Coefficient estimate	Sum of squares	df	Standard error	F-value	p-value
Model		1.348E+007	9	1.498E+006	42.30	< 0.0001
A-yeast	37.96	11527.01	1	66.53	0.33	0.5862
B-temperature	-321.76	8.282E+005	1	66.53	23.39	0.0019
C-time	1086.33	9.441E+006	1	66.53	266.60	< 0.0001
AB	201.57	1.625E+005	1	94.09	4.59	0.0694
AC	-104.30	43517.71	1	94.09	1.23	0.3042
BC	-106.79	45613.43	1	94.09	1.29	0.2938
A^2	-126.01	66853.72	1	91.71	1.89	0.2118
B^2	-5.40	122.89	1	91.71	3.470 E-003	0.9547
C^2	831.94	2.914E+006	1	91.71	82.29	< 0.0001
Residual		2.479E+005	7	35411.72		
Lack of fit		88170.16	3	29390.05	0.74	0.5828
Pure error		1.597E+005	4	39927.97		
Cor total		1.373E+007	16			
Standard Devation		188.18		R^2	0.9819	
Mean		2294.17		adj R^2	0.9587	
CV (%)		8.20		Pre R^2	0.8791	
PRESS		1.660E+006		Adeq precision	21.022	

fermentation during 6 days, then went on flavor generation reaction. At 20 days, jujube brandy got the best flavor. The CO_2 loss weight increased sharply during first 4 days, then remain stable, which means main fermentation reaction happened in first 4 days (Fig. 5). Therefore, although alcohol fermentation finished at 6 days, for obtaining high-quality-flavor jujube brandy, 20 days should be chosen to be the proper fermentation time.

Box-Behnken result

Statistical analysis and model building: The 17 experiments were carried out according to the conditions indicated in Table 3. Response values (flavor content) were reported in the last column of this table. Regression analysis (Table 4) was made to the experimental data aiming at an optimal region for the responses study. The analyses of variance were used to determine the coefficient of determination, lack of fit and the

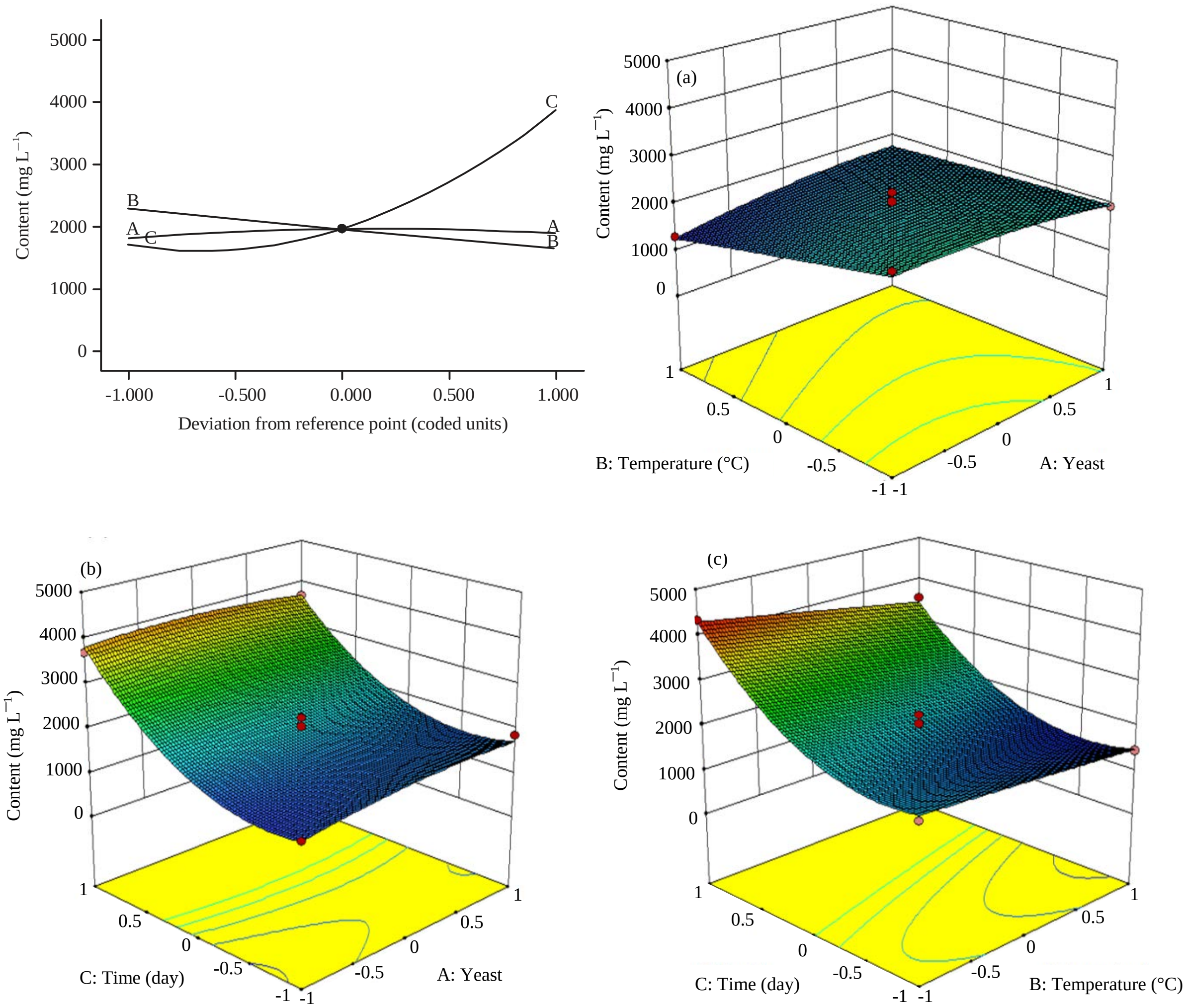

Fig. 6(a-c): Perturbation plot showing the effect of process variables and surface plots for flavor content of jujube brandy, (a) figure plot to show yeast strains and temperature, (b) figure plot to show yeast strains and time and (c) figure plot to show temperature and time

significance of the linear, interaction effects and quadratic of the independent variables on the response.

The significance of each coefficient was determined using the F-test and p-value in Table 3. The p-value represents the significance of the corresponding coefficients in terms of flavor content, with a smaller p-value indicating more significant impact of the corresponding coefficient. The results of regression coefficient analysis showed that the variable with the largest effect was the quadratic term of fermentation time (X_3^2), followed by liner term of fermentation time (X_3), the fermentation temperature (X_2), which were extremely significant ($p<0.01$). However, the interaction effects of yeast strains and fermentation temperature (X_1X_2), yeast strains and fermentation time (X_1X_3), fermentation temperature and time (X_2X_3), the quadratic term of fermentation temperature (X_1^2) and fermentation time (X_2^2), liner term of yeast strains (X_1) were not significant ($p>0.05$).

An empirical quadratic polynomial model corresponding to the BBD was fitted to correlate the relationship between independent variables and the responses to predict the optimized conditions. The quadratic model is following as:

$$Y = 4081.28755 - 55.68122X_1 - 78.01755X_2 - 200.70689X_3 + 33.59487X_1X_2 - 13.03806X_1X_3 - 2.22472X_2X_3 - 126.00698X_1^2 - 0.15007X_2^2 + 12.99899X_3^2$$

where, Y is the predicted response (flavor content of jujube brandy) and X_1, X_2, X_3 are coded values of yeast strains, fermentation temperature and fermentation time, respectively.

The analysis of variance (F-test) shows that the second order model matches well with the experimental data. The Coefficient of Variation (CV) indicates the degree of the precision. Here, a lower value of CV (8.20) indicates the experiments are more precise and reliable[37]. The precision of a model can be represented by the determination coefficient (R^2). The determination coefficient (R^2) implies that the sample variation of 98.19% for the flavor content of jujube brandy is attributed to the independent variables. Adjusted coefficient of determination (adj R^2) and predicted coefficient of determination (pre-R^2). Meanwhile, the high R^2 (0.9819), adj-R^2 (0.9587) and pre R^2 (0.8791) clearly demonstrated that the experiment and the theoretical values predicted by polynomial model had a very close agreement. From the analysis, the F-value of 42.30 and p-value<0.0001 indicates the response surface quadratic model was significant. Furthermore, results of the ANOVA indicated that the lack of fit of 0.5828 was insignificant.

Analysis of response surface

Perturbation plot: In generating the perturbation plots, all the factors were plotted on the same response graph. This graph could be used to find factors that most affect the response. A steep slope or curvature in a factor shows that the response is sensitive to that factor. A relatively flat line shows insensitivity to change in that particular factor[38]. The response (Y) was plotted against the deviation from the reference point by changing only one factor over its entire range while holding all other factors constant as shown in Fig. 6 (Actual factors: A-yeast = 2, B-temperature = 24, C-time = 16). The relationship between the responses and the experimental variables can be illustrated graphically by plotting 3-dimensional response surface plots. Fermentation temperature and time have great influence on the flavor content compared with yeast strains.

Validation of the model: The objective of optimization was to find out the conditions which give the maximum flavor content of jujube brandy. The desirability function approach was applied in the optimization process. This numerical optimization technique evaluates a point that maximizes the desirability function. The optimum brewing conditions and the maximum flavor content were obtained desirability function approach was single-yeast GH, fermentation temperature of 18°C, fermentation time of 24 days and the maximum flavor content of jujube brandy was 4447.824 mg L^{-1} with a desirability value of 0.851. The suitability of the optimized conditions for predicting the optimum response values was tested experimentally using the selected optimal conditions. Triplicate experiments were performed under the optimized conditions and the mean values (4525.934±0.062 mg L^{-1}) obtained from real experiments, which agree well with the expected value of 4447.824 mg L^{-1}, demonstrating that the optimized conditions agree well with the real experiments.

CONCLUSION

In this present study, evaluation system was built for jujube brandy, which take flavor compounds as main evaluation criteria, alcohol, CO_2 loss weight and higher alcohols as supplementary. It is interesting to find that solid-state fermentation adding with rice husk is the proper fermentation way for jujube brandy with higher alcohol and flavor compounds, when comparing Western liquid-state fermentation and Chinese traditional solid-state fermentation way. Futhermore, the fermentation function of different kinds of yeasts, single and mixed yeasts and Chinese daqu was compared and find that single-yeast GH, PH and mixed-yeast GHSX are proper yeast strains for brewing jujube brandy. The comparison of fermentation way and single or mixed yeast can also give instruction for grape brandy and Chinese rice liquor about improvement of quality and processing technology.

The brewing conditions of jujube brandy were optimized with a three factor three level Box-Behnken response surface design coupled with desirability function methodology. The results showed that, fermentation temperature and time had significant effect on the flavor content of jujube brandy and a high correlated quadratic polynomial mathematical model was developed. The optimal conditions were determined to be: single-yeast GH, fermentation temperature of 18°C, fermentation time of 24 day. Under the optimal conditions, the experimental values (4525.934±0.062 mg L^{-1}) agreed with the predicted values (4447.824 mg L^{-1}), which is 1.19 times of normal brewing, has great practical values.

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