

# Optimization of fermentation technology of hawthorn-pear wine by uniform design and response surface design

Yanghui WANG<sup>1</sup>, Jianlou MU<sup>2</sup>, Jie WANG (✉)<sup>2,3</sup>

<sup>1</sup> College of Life Science, Agricultural University of Hebei, Baoding 071001, China

<sup>2</sup> Food Science and Technology College, Agricultural University of Hebei, Baoding 071001, China

<sup>3</sup> Agricultural Products Processing Engineering Technology Research Center of Hebei, Baoding 071001, China

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**Abstract** Uniform design methodology and response surface methodology were used to determine the optimum conditions for hawthorn-Yali pear wine. By using uniform design, the effects of fermentation temperature, sugar content, the ratio of hawthorn to pear, soaking time of hawthorn, additional volume of SO<sub>2</sub>, and yeast dosage on sensory quality were investigated, which indicated that the first three aspects were of great significance to the sensory quality. By using three-factor, three-level response surface methodology, a prediction model was established in the form of quadratic polynomial regression equation, with the best processing conditions hereby determined under the conditions of fermentation temperature (25.18°C), sugar content (22.00%), the ratio of hawthorn to pear (21.19:100), and the alcohol degree (11.05 (V/V %)).

**Keywords** hawthorn, pear wine, uniform design, response surface design

## Introduction

In Hebei Province, the output of Yali pear (*Pyrus. bretschneideri* Rehd) is immense, which has suffered from great difficulties in market sales in recent years, with relatively lower income of pear growers. To multiply processing approaches, take full advantage of Yali pear, and thus get rid of the dilemma of pear production, it is advisable that high-quality pear wine be produced. The phenolic compounds can affect the taste of wine (Careri et al., 2003), but Yali pear alone can produce light-flavored perry poor in taste quality (Yuan et al., 2003). Hawthorn (*Prunus salicina* Bge.) contains high-level flavonoid suited best for cardiovascular hygienic care (Rigelsky and Sweet, 2002; Xu et al., 2009). Our previous results proved that the brownish red hawthorn-Yali pear wine was excellent in color, aroma, and flavor. In the present study, we wanted to optimize the fermentation process to improve the quality of hawthorn-Yali pear wine.

Uniform design is an experimental method only emphasizing the uniformly distributed testing points, which is applied to multivariable and multilevel experiments to find out optimal experimental conditions as well as reduces the test frequency substantially (Tang and Cai, 2006; Xing and Li, 2008; Jin and Zhou, 2009). Response surface analysis is based on the polynomial fitting of dependent variables and independent variables (Ratnam et al., 2003; Martendal et al., 2007), which is widely used in wine researches (Yannam et al., 2009; Zhao and Song, 2009; Zhou et al., 2011). Jointly using the uniform design and the response surface design, working out secondary factors via uniform analysis, and then optimizing the experimental conditions according to the response surface design will reduce testing frequency, conduct data conveniently, and improve experiment efficiency.

This article explored the effect on sensory quality and alcohol degree with factors, such as fermentation temperature, sugar content, and the ratio of hawthorn to pear, by both uniform design and response surface design, in order to optimize the fermentation conditions of hawthorn-Yali pear wine, which will promote the development of Hebei Yali pear industry.

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Correspondence: Jie WANG

E-mail: wj591010@163.com

## Materials and methods

### Materials

Yali pears were provided by an orchard at Shunping County, Hebei Province. Hawthorns were purchased from market; Danbaoli high-activity dry yeast for wine was provided by Danbaoli Yeast Co., Ltd.

### Experimental methods

#### Sensory evaluation

A seven-member assessment panel graded the wines by color, aroma, taste, and typicality. The highest grade in four grades for these four aspects was 20, 30, 40, and 10, respectively. Grading standards are listed in Table 1 (Niu et al., 2009).

#### Alcohol degree

Gas chromatography (GC) is used to quantify alcohol degree by adding 0.2 mL 4-methyl-2-pentanol into 10 mL perry samples diluted 2 to 10 times as internal standard with column temperature 80°C rising to 180°C at 15°C/min. The temperature at injection port and detection port was 200°C and 220°C (GB/T, 2006), respectively.

#### Uniform design

The experiment factors consisted of fermentation temperature, sugar content, soaking time of hawthorn, the ratio of hawthorn to pear, SO<sub>2</sub> addition, and yeast inoculation at 5 × 10 × 10 × 10 × 10 level (Charoen et al., 1998; Torija et al., 2003; Serra et al., 2005; Wu et al., 2009). Hawthorn pulp was crushed into granular, and yeast was activated with 5% sugar water at 40°C for 30 min before SO<sub>2</sub>, hawthorn pulp granular, sugar, and activated yeast were added into 600 mL

extracted Yali pear juice in 1 L conical flasks for main fermentation. Seven days after main fermentation, the dregs were filtered out from the mixture followed by 20 days post-fermentation and 3 months aging when the wine was evaluated by the seven-member assessment panel, with the main effective factors of wine quality determined according to the results of sensory evaluation.

#### Response surface design

A three-factor, three-level response surface experiment was designed for optimizing the fermentation condition in order to obtain the high alcohol degree. Each experiment was performed twice.

## Results and analysis

### Initial screening of the fermentation factors by uniform design

To get the best sensory quality, uniform design experiments of 6 factors at 5 × 10 × 10 × 10 × 10 × 10 level were performed. The factors and levels are listed in Table 2. The sensory evaluation results in Table 3 showed that No. 3 treatment won the highest score (90.1) followed by No. 8 treatment (88.3). The various factors in the two treatments were fermentation temperature of 21°C and 29°C, sugar content of 19.8% and 21.3%, hawthorn soaking time of 8 h and 22 h, the hawthorn-to-pear ratio of 20:100 and 35:100, SO<sub>2</sub> of 50 and 95 mg/L, and yeast dosage of 0.3 and 0.8 g/L, respectively, which are more appropriate conditions for each factor.

According to the visual analysis, it was suggested that fermentation temperature, sugar content, and the ratio of hawthorn to pear were important impacts to the next response

**Table 1** Grading standards for fruit wine sensory evaluation

Term	Grading standard	Score
Color (20)	Clear, crystal, cheerful	18–20
	Clear, crystal, colored typically for fruit wine	15–17
	Clear, inclusion undetected, not so cheerfully colored	12–14
	Turbid, no luster, uncheerful	< 12
Aroma (30)	Fruity, wine aroma strongly fragrant and coordinated	26–30
	Fruity, fragrant, and still coordinated	22–25
	Less fruity, probably with other smells, not appealing	18–21
	Undesirable smell, disgusting	< 18
Taste (40)	Rich, strong, coordinated, and cheerful	36–40
	Coordinated, pure, and cheerful	30–35
	Either plain, bitter, sour, or astringent, unappealing	25–29
	Peculiar smell, disgusting	< 24
Typicality (10)	Typical, unique, and excellent	9–10
	Typical and unique	8
	Typical, no so elegant	7
	Nothing typical	< 6

**Table 2** Variables and levels of uniform experiment of filtered juice

No.	Temperature (°C)	Sugar content (%)	Hawthorn soaking time (h)	Ratio of pear juice to hawthorn (g/g)	Dosage of SO <sub>2</sub> (mg/L)	Dosage of yeast (g/L)
1	17	13.8	6	5:100	50	0.1
2	17	15.3	8	10:100	55	0.2
3	21	16.8	10	15:100	60	0.3
4	21	18.3	12	20:100	65	0.4
5	25	19.8	14	25:100	70	0.5
6	25	21.3	16	30:100	75	0.6
7	29	22.8	18	35:100	80	0.7
8	29	24.3	20	40:100	85	0.8
9	33	25.8	22	45:100	90	0.9
10	33	27.3	24	50:100	95	1.0

**Table 3** Design and results of uniform experiment of filtered juice

No.	Temperature (°C)	Sugar content (%)	Hawthorn soaking time/h	Ratio of hawthorn to pear juice (g:g)	Usage of SO <sub>2</sub> (mg/L)	Usage of SO <sub>2</sub> (mg/L)	Sensory evaluation
1	1	2	3	5	7	10	69.0
2	2	4	6	10	3	9	82.8
3	3	6	9	4	10	8	90.1
4	4	8	1	9	6	7	83.7
5	5	10	4	3	2	6	83.2
6	6	1	7	8	9	5	85.3
7	7	3	10	2	5	4	82.6
8	8	5	2	7	1	3	88.3
9	9	7	5	1	8	2	87.9
10	10	9	8	6	4	1	72.5

surface test, with the hawthorn soaking time, the dosage of SO<sub>2</sub>, and yeast as the less important impacts.

**Optimization of process conditions by response surface method**

*Experimental design*

According to the analysis of uniform experiments and available data (Constantinos and Olga, 1994), a three-factor, three-level response surface design was applied for optimizing the fermentation condition in order to obtain the high alcohol degree of hawthorn-pear wine, with the three independent variables of fermentation temperature (*A*, °C), sugar content (*B*, %), and ratio of hawthorn to pear juice (*C*, g:g) at three levels each (Table 4). A total of 15 experiments were designed (Table 5). The average alcohol degree (V/V %) was taken as the response, *Y*.

*Models*

On the basis of the response surface analysis, the predicted second-order polynomial model was

$$\begin{aligned}
 Y = & 9.16 - 0.19A + 0.88B - 0.22C + 0.042AB \\
 & + 0.015AC - 0.028BC - 0.51A^2 \\
 & + 0.048 B^2 - 0.3C^2.
 \end{aligned}
 \tag{1}$$

The results indicated that the sugar content (*B*) was the major factor contributing to the efficiency of alcohol degree (*Y*), while the fermentation temperature value and the ratio of hawthorn to pear juice were insignificant.

The analysis of variance for the response surface design is shown in Table 6. The model *P*-value of 0.0004 implied the significance of model (*P* < 0.01) (Li et al., 2011). The coefficient of determination (*R*<sup>2</sup>) of the model (0.9856)

**Table 4** Contrast of variables and levels

Levels	Variables		
	<i>A</i> (Fermentation temperature)(°C)	<i>B</i> (Sugar content)(%)	<i>C</i> (Ratio of hawthorn to pear juice) (g:g)
-1	21	18	15:100
0	25	20	25:100
+1	29	22	35:100

**Table 5** Design of response surface

Number	A	B	C	Response (Y, V/V %)
	-1	-1	0	
1	1	-1	0	7.61
2	0	1	1	9.43
3	-1	-1	0	7.92
4	0	-1	-1	8.34
5	1	0	-1	8.25
6	0	0	0	9.14
7	1	0	1	7.93
8	0	0	0	9.28
9	-1	0	-1	8.81
10	-1	0	1	8.43
11	0	1	-1	10.02
12	0	-1	1	7.86
13	1	1	0	9.57
14	-1	1	0	9.71
15	0	0	0	9.07

indicated that 98.56% of the variations could be explained by the structured model, which adequately represented the real relationship between the parameters chosen.

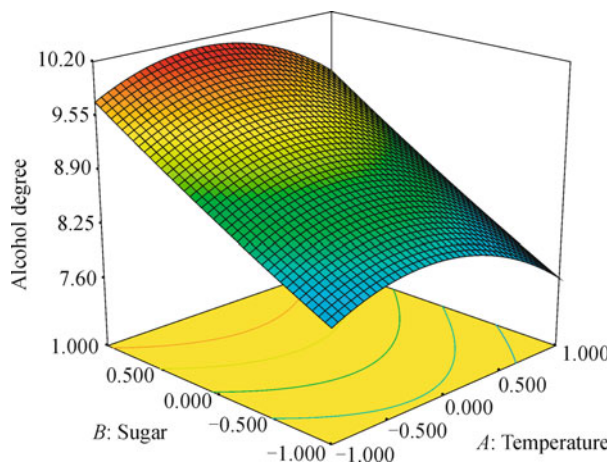
*Response surface plotting*

To determine optimal levels of the variables for the alcohol degree of hawthorn-pear wine, three-dimensional surface plots were constructed according to Eq. (1). Fig. 1 shows the effect of sugar content and fermentation temperature on the alcohol degree at a fixed ratio of hawthorn to pear of 25:100. Fig. 2 shows the effect of ratio of hawthorn to pear and fermentation temperature at a fixed sugar content of 20%. Fig. 3 shows the effect of ratio of hawthorn to pear and sugar content on the alcohol degree at a fixed fermentation temperature of 25°C.

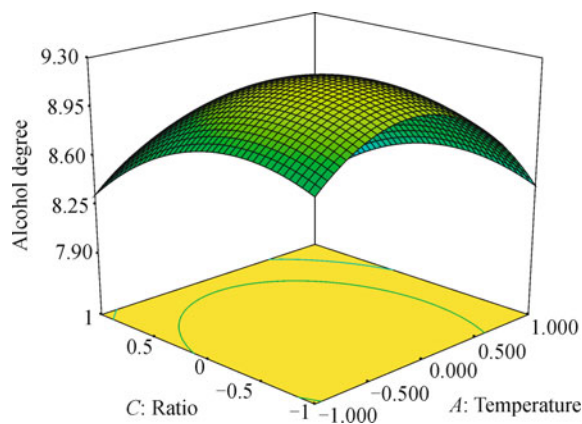
Higher sugar content was found beneficial to the alcohol degree. At the same time, the high temperature and the ratio of hawthorn to pear could lead to a decrease in the alcohol

**Table 6** Results of response surface design

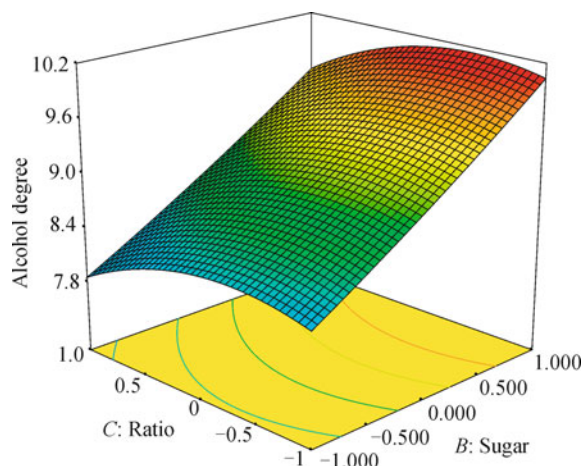
Variation source	Sum of square	df	Mean square	F-value	P-value
Model	8.063	9	0.896	38.045	4E-04
A	0.285	1	0.285	12.103	0.018
B	6.125	1	6.125	260.103	< 0.0001
C	0.392	1	0.392	16.630	0.010
AB	0.007	1	0.007	0.307	0.604
AC	9E-04	1	0.001	0.038	0.853
BC	0.003	1	0.003	0.128	0.735
A <sup>2</sup>	0.957	1	0.957	40.650	0.001
B <sup>2</sup>	0.009	1	0.009	0.366	0.571
C <sup>2</sup>	0.330	1	0.330	14.033	0.013
Lack of fit	0.095	3	0.032	2.766	0.277
Residual	0.118	5	0.024		
Cor Total	8.18	14			



**Figure 1** Alcohol degree response surface to sugar content and temperature. A and B represent fermentation temperature and sugar content, respectively.



**Figure 2** Alcohol degree response surface to ratio and temperature. A is fermentation temperature and C is ratio of hawthorn to pear juice.



**Figure 3** Alcohol degree response surface to ratio and sugar content. *B* is sugar content and *C* is ratio of hawthorn to pear juice.

**Table 7** Optimum condition, predicted and experimental values of response at optimum conditions

Optimum condition	Actual level		
Fermentation temperature (°C)	25.18		
Sugar content (%)	22.00		
Ratio of hawthorn to pear	21.19:100		
Responses	predicted value	experimental value	
		mean	range
Alcohol degree / (V/V %)	10.11	10.05±0.11	10.16–9.94

Note: <sup>a</sup> represents mean of three measurements.

degree. Therefore, the extraction temperature and the ratio of hawthorn to pear should be controlled strictly.

According to the actual production situation, the sugar content determined should be at 22.00%, the best condition determined by the software consisted of fermentation temperature 25.18°C, sugar content 22.00%, and the ratio of hawthorn to pear 21.19:100. Under the condition, the alcohol degree of 10.11 (V/V %) was predicted.

#### Verification results

The suitability of the model equation for predicting the optimum response values was tested using the recommended optimum conditions. The experimental value was 11.05 (V/V %), which was in agreement with the predicted ones (Table 7).

## Conclusions

In these experiments, the optimal fermentation conditions of hawthorn-pear wine were studied. Through uniform design experiment, the effects of fermentation temperature, sugar content, the ratio of hawthorn to pear, soaking time of

hawthorn, SO<sub>2</sub> addition, and yeast inoculation on sensory quality were investigated, and the first three factors were determined as major ones to sensory quality.

On this basis, the prediction model of fermentation temperature (*A*), sugar content (*B*), and the ratio of hawthorn to pear (*C*) to alcohol degree was performed with a three-variable, three-level response surface design. The experiment results showed that the sugar content was a major contributing factor to the alcohol degree. The best condition was at 25.18°C fermentation temperature, 22.00% sugar content, and 21.19:100 ratio of hawthorn to pear, with the optimal alcohol degree of 11.05%. The adequacy of this model needs to be confirmed by further experiments.

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