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Optimization of Red Wine Brewing Processing Condition by using Taguchi Method

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Abstract

Brewing red wine is one of the most studied topics in recent years. In this study, we used Taguchi designed experiment to explore the effect of fermentation temperature (T_f), sugar content (X_s), yeast content (X_y), and fermentation duration (t_f) on alcohol concentration, alcohol yield rate and antioxidant capacity. Data were analyzed with Minitab software. Experimental and analysis results suggested that the degree of influence on the maximum alcohol concentration is, in the order of importance: X_s at 14 wt.% > t_f for 13 days > T_f at 25 °C > X_y at 0.029 wt.%. The optimal brewing condition that producing the highest alcohol yield rate is, in the order of importance, X_s at 14 wt.% > t_f of 13 days > T_f at 30 °C > X_y at 0.029 wt.%. Condition that producing the highest antioxidant capacity is: T_f at 30 °C > X_y at 0.021 wt. % > X_s at 14 wt. % > t_f for 10 days. These results were also verified with a second order polynomial equation that equation parameters of this equation were fitted with experimental data obtained from Taguchi experiment.

Keywords: *Red Wine, Brewing, Fermentation, Kyoho Grape, Taguchi Designed Experiment.*

1. Introduction

1.1 Red Wine Brewing from Grape

The Kyoho grape is an improved grape breed by Japan especially for wine producing. Kyoho grapes can be cultivated under subtropical environment. The skin of Kyoho grape has a thick layer of fruit powder. The shape is round and full, while the color is purple black. The flesh texture is soft and tender with a flexible and refreshing juicy. The Kyoho is a high quality fresh grape.

Grapes by far are the most fermented fruit, although any fruit can be turned into wine. The yeast *Saccharomyces cerevisiae* (*S.cerevisiae*) is commonly used during wine production. Most yeast can produce wine with an alcohol content of 6 to 14 vol. % [1]. Phenols and volatile aroma in the wine are the important ingredients of the wine sensory. Wine aroma compounds are generated during fermentation and cooking. Those compounds are monoterpene, isoprene, higher alcohols, fatty acids, esters, aldehydes, and ketones. Phenolic compounds are produced from grape skin and seeds in brewing process. Phenolic compounds can affect wine taste and color. These compounds like phenolic acid, flavonols, and tannins will produce astringency and bitter taste. Anthocyanins will affect the color of wine [2]. While brewing, the glucose in the grape is converted to ethanol and carbon dioxide by *Saccharomyces cerevisiae*, which is called alcohol fermentation (AF). In order to change the taste of wine and decrease the level of acid, we usually work on a secondary fermentation. The secondary fermentation is also known as apple lactic acid fermentation (MLF). *Saccharomyces cerevisiae* is affected by alcohol content and other stimulants in the first or primary fermentation. High sugar content, low pH is favorable in the first fermentation [3]. The secondary fermentation is very critical, the adverse factors are high ethanol content, low pH, low temperature, high total acidity, high glycerol content and others [4~6]. White wine fermentation temperature is relatively low, usually at 15 ~ 20 °C. Red wine fermentation temperature should be in 20 ~ 25 °C [7].

The optimum temperature for growth and reproduction of yeast is about 30 °C. The yeast will be constrained if temperature is above 32 °C. Therefore, some breweries have a fermentation temperature being controlled within 18 to 32 °C. As the temperature rise, yeast growth and enzyme reaction will accelerate. In addition, due to increased cell membrane fluidity, cell sensitivity to alcohol toxicity will also be increased [8]. In case of brewing other fruit wines, some water will be added to dilute the high acidity, such as currant, or too strong taste, such as elderberry taste [9].

1.2 Important Composition in Red Wine

Red wine is rich in polyphenols. Phenolic compounds are usually divided into two categories: flavonoids and non-

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flavonoids. The main flavonoids present in the wine are flavanols, flavonols, and anthocyanins. While the non-flavonoids in the wine are phenolic, phenol, and stilbene. Flavanols are mainly composed of catechins, epicatechin and epicatechin gallate, which are often aggregated with oligomeric or polymeric forms. Many different types of proanthocyanidins are also the main flavonoid compounds of red wine [10]. On the other hand, by heating acidic material can produce anthocyanins. Anthocyanins and carbohydrates with glycosidic are natural antioxidants. They are the major compounds contribute to the color of flowers and leaves. The color of anthocyanin will vary with the environmental pH value. They are red or purple in the acidic environment and blue in the alkaline environment. That is why anthocyanins are also used as pH indicator. Although anthocyanins can absorb light energy, they have nothing to do with photosynthesis. Quercetin has attracted considerable attention as an antioxidant and can prevent or delay the cardiovascular disease (CVD) and cancer [11].

Nonflavonoids include phenolic acids, phenol, and stilbene (or resveratrol). Resveratrol can be either in cis- or trans-areomeric structure. Resveratrol can directly inhibit cAMP degradation of phosphodiesterase, increase cAMP and activate AMPK. So AMPK pathway activation is considered to be a treatment for metabolic syndrome and anti-aging strategy [12]. Resveratrol is also responsible for regulating the oxidation of human low density lipoprotein (LDL) and for producing a series of protective effects on atherosclerosis. But the direct antioxidant capacity of resveratrol is very weak and the bioavailability is also very low [11]. Drinking of red wine has a good effect on the performance and survival of familial rectal cancer. From the meta-analysis study, the appropriate amount of wine consumption is good on protection of kidney cancer [12].

1.3 Ethanol Fermentation Rate and Their Kinetics

There are several ethanol fermentation kinetics proposed. Lainioti and Karaiskakis [13] gave a brief review on alcohol fermentation kinetics. Atala et. al. [14] developed a rate equation of ethanol fermentation at high biomass and the effect of temperature was evaluated. Liu and Li [15] proposed an ethanol fermentation kinetics based on adsorption process. Zinnai et. al. [16] proposed an ethanol fermentation kinetics based on two yeast strain. Ariyajaroenwong et. Al. [17] proposed a kinetic model of ethanol fermentation from sweet sorghum juice. So far, no kinetic model specifically applicable for the fermentation of red wine from Kyoho grape. The optimal condition for red wine brewing from this grape is unknown.

1.4 Purpose of Present Study

In this study, the brewing condition of red wine was explored by using Taguchi method. Taguchi orthogonal array is used to optimize process parameters [18]. This is a simple and useful tool for planning industrial experiments because it requires few experiments and is widely used in manufacturing process and in academic fields [19]. This study considered the influences of four fermentation parameters on the objective function, alcohol content, liquor yield and antioxidant. Parameters are fermentation temperature (T_f), the amount of sugar (X_s), the amount of yeast (X_y), and the fermentation duration (t_f). Through the conversion of the signal-to-noise ratio, the degree of influence of each factor and the degree of change of each factor level on the response variable is also discussed.

An empirical reaction kinetic was proposed based on second-order polynomial. Parameter values of this polynomial equation were fitted with experimental data obtained from Taguchi experiments.

2. Materials and methods

2.1 Experimental steps

The Kyoho grape was from Xinyi township, Nantou county, Taiwan. Grape was first washed with deionized water. After the grape was naturally air-dried, the grapes were crushed and was filled into several fermentation tanks.This study used the $L_9 3^4$ Taguchi orthogonal table design experiment. There were 3 levels for each of the 4 factors. Based on the literature survey result, level values of these factors were: the fermentation temperature (T_f at 20 °C, 25 °C, and 30 °C), sugar addition (X_s of 0 wt.%, 14.29 wt.%, and 28.57 wt.%), yeast amount (X_y of 0.014 wt.%, 0.021 wt.% and 0.029 wt.%), and fermentation duration (t_f for 7 days, 10 days, and 13 days). Taguchi experimental factor and the level values are shown on Table 1. According to the selected factors and values, the $L_9 3^4$ orthogonal table was shown on Table 2.

When fermentation was complete, the samples were removed from the tanks and was filtered. Above experiments was repeated four times. Average value of three objective functions (alcohol content, yield rate, and antioxidant capacity) were then analyzed and calculated. Taguchi analysis was carried out to find the optimal conditions.

Table 1 Taguchi experiment factor and level

	Factor	Level 1	Level 2	Level 3
A	Fermentation temperature (T_f), °C	20	25	30
B	Sugar content (X_s), wt. %	0	14.29	28.57
C	Yeast content (X_y), wt. %	0.014	0.021	0.029
D	Brewing duration (t_f), days	7	10	13

Table 2 Taguchi experiment direct table $L_9 3^4$

Number of Exptl.	Factor			
	A (T_f, °C)	B (X_s, wt%)	C (X_y, wt%)	D (t_f, day)
1	20	0	0.014	7
2	20	14.29	0.021	10
3	20	28.57	0.029	13
4	25	0	0.021	13
5	25	14.29	0.029	7
6	25	28.57	0.014	10
7	30	0	0.029	10
8	30	14.29	0.014	13
9	30	28.57	0.021	7

2.2 Determination of alcohol content

After fermentation, a 25 mL of de-ionized (DI) water was mixed with 50 mL of the filtrate solution. Solution was heated in a set of distillation glassware. A 45 mL of the distillate was collected. The DI water was added to make a total volume of 50 mL. The solution specific gravity was measured by filling solution into the pycnometer battle at 20 °C. The alcohol content (vol.%) was then calculated [20, 21].

2.3 Determination of alcohol yield rate

The alcohol yield rate (L/Kg %) was calculated from the alcohol content (vol. %) as follows:

$$\text{alcohol yield rate}\left(\frac{\text{L}}{\text{kg}}\%\right) = \text{alcohol content (vol \%)} \times \frac{\text{wine volume (L)}}{\text{grape weight (kg)}} \quad (1)$$

2.4 Determination of antioxidant activity

In order to test the antioxidant activity, the distillate was diluted 10 times with DI water. A 1 mL of the diluted solution was mixed with 2 mL of 2,2-diphenyl-1-picrylhydrazyl, DPPH (Sigma-Aldich) and 2 mL of methanol (Mallinckrodt, 100%). This mixture was then sited in a dark room for 30 minutes. The absorbance (A_{sample}) of the mixture at a wavelength of 517 nm was measured [10, 22-24]. The absorbance (A_{DPPH}) of a DPPH solution containing 2 mL of DPPH and 3 mL of methanol was measured as the reference.

The antioxidant activity of the distillate was then expressed as a percentage of inhibition of DPPH radical. It was calculated as following:

$$\text{Inhibition}(\%) = \left[\frac{A_{DPPH} - A_{sample}}{A_{DPPH}}\right] \times 100\% \quad (2)$$

2.5 Fitting of Taguchi Experimental Data with Empirical Equation

Taguchi design of experiment can evaluates the significant of individual factor and reaches the optimal condition with few experiments. However, the dependence of objective function on each factors are not clear. An empirical equation was used to exam the dependence of objective function on each factor.

A second order polynomial (Eq. 3) was used for this purpose. The Here x_i is factors under studied. They were T_f, X_s, X_y, and t_f, (A, B, C, and D on Table 1). The "y" was the objective function, such as alcohol content, alcohol yield rate, and antioxidant capacity. Values of parameter α_{i0}, α_{i1}, and α_{i2} for factor "i" can be calculated by fitting the equation with Taguchi experimental data.

$$y_i = \sum_{i=0}^{4} \alpha_{i0} + \alpha_{i1}\, x_i + \alpha_{i2}\, x_i^2 \quad (3)$$

3. Results and discussion

Experiments were carried out according to the $L_9 3^4$ Taguchi orthogonal array as listed on Table 2. Three objective functions (alcohol concentration, liquor yield rate and antioxidant capacity) were measured and calculated. Each experiment was repeated four times and average value of these objective functions were calculated as shown on Table 3. The signal-to-noise (S/N) ratio of objective function was analyzed by the Minitab software statistical module. The larger the objective function represented the better brewing condition. The S/N ratio was calculated by Large-the-Better criterion.

Table 3 Average value of objective functions obtained from the $L_9 3^4$ Taguchi experiment

	Factor				Alcohol content (%)	Alcohol yield rate (L/Kg %)	Antioxidant capacity (%)
	A (T_f, °C)	B (X_s, wt%)	C (X_y, wt%)	D (t_f, day)			
1	20	0	0.014	7	7.22	3.45	72.38
2	20	14.29	0.021	10	13.58	7.76	82.09
3	20	28.57	0.029	13	11.46	7.46	79.44
4	25	0	0.021	13	9.80	4.82	85.64
5	25	14.29	0.029	7	13.15	7.76	87.40
6	25	28.57	0.014	10	11.17	8.12	83.99
7	30	0	0.029	10	8.97	5.57	88.89
8	30	14.29	0.014	13	15.23	9.82	83.89
9	30	28.57	0.021	7	8.52	5.99	91.52

Parameter values of equation (3), α_{i0}, α_{i1}, and α_{i2} were calculated by fitting the equation with the objective function value (alcohol content, alcohol yield rate, and antioxidant capacity) at each experimental conditions listed on Table 3. The calculated result was listed on table 4.

Table 4 Calculated parameter value by fitting equation (3) with Taguchi experimental data.

Alpha	Objective Function		
	Alcohol Content	Alcohol Yield Rate	Antioxidant Capacity
10	-0.419	-0.415	-0.384
11	0.178	0.095	5.326
12	-0.002	-0.002	-0.086
20	15.405	20.378	671.292
21	0.449	0.679	0.213
22	-0.013	-0.022	-0.004
30	3163.862	3956.035	-20038.818
31	2.132	2.133	8.043
32	-0.328	-0.328	-0.325
40	-3189.901	-3979.492	19361.738
41	2.292	1.884	2.288
42	-0.101	-0.073	-0.119

Figure 1 was comparing calculated results from equation (3) with parameter value listed on Table 4 with experimental data obtained from Taguchi experiments. Reasonable agreement was obtained. Equation (3) with parameter value listed on Table 4 was used to calculate the dependence of objective function on individual factors.

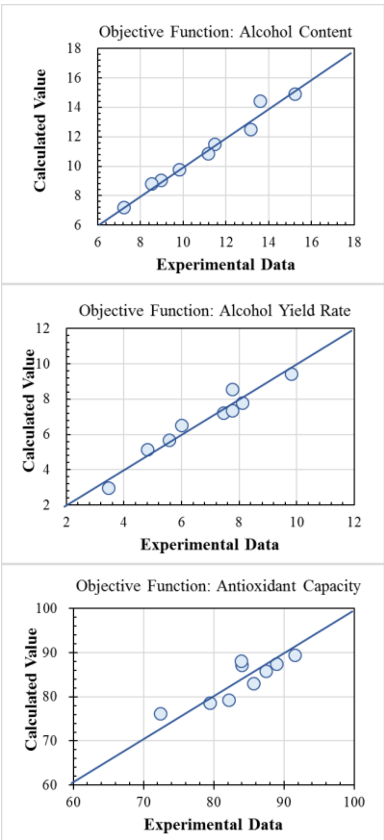


Figure 1 Comparing calculated results from equation (3) with parameter value listed on Table 4 with experimental data obtained from Taguchi experiments.

3.1 Alcohol content

The average alcohol content S/N ratio of these factors obtained from Taniguchi L_93^4 result was plotted on Fig. 2. Based on the S/N ratio criteria the Larger-the-better, the optimal average S/N ratio of the fermentation temperature was 20.72 at 25 °C (level 2), the S/N ratio was 22.7 at sugar content of 14.29 wt% (level 2), the S/N ratio was 20.66 at yeast content of 0.029 wt% (level 3), and the S/N ratio was 21.35 at brewing duration of 13 days (level 3). This represent the optimal brewing condition was $A_2B_2C_3D_3$. Table 5 is the list of Delta value and ranking of these four factors. The Delta value is the difference between the maximum S/N ratio and the minimum S/N ratio. The larger the Delta value, the greater influence of that factor on the objective function. Rank represented the importance of the factor. The most important factor was marked as 1. The analysis result from the S/N ratio of alcohol content showed that the optimum brewing conditions and the importance of factor were: sugar content at 14.29 wt. % > fermentation duration at 13 days > fermentation temperature at 25 °C > yeast content at 0.029 wt.%.

Table 5 Delta and ranking calculated from the S/N ratio of alcohol content obtained from the L_93^4 Taguchi experiments

	Factor			
	A	B	C	D
Delta	0.72	4.27	0.52	2.20
Rank	3	1	4	2

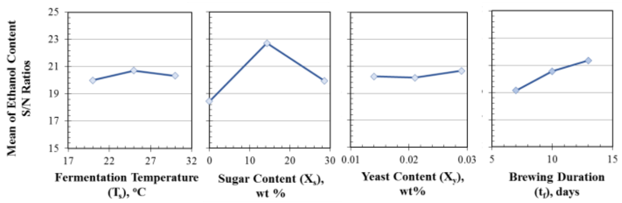


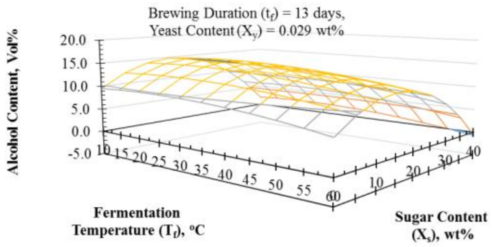
Figure 2 Response plots of S/N ratios from ethanol content analysis. S/N ratio criteria is Larger is better.

The optimum condition for brewing was $A_2B_2C_3D_3$ (fermentation temperature at 25 °C, sugar content at 14.29 wt.%, yeast content at 0.029 wt %, and fermentation duration at 13 days). Two experiments were carried out at this condition. Alcohol content of 15.68 ± 2.17 vol. % at this optimal condition was calculated by Minitab statistical software. The experimental results and the prediction were compared on Table 6. This result demonstrated that the prediction of Taguchi experimental design was accurate.

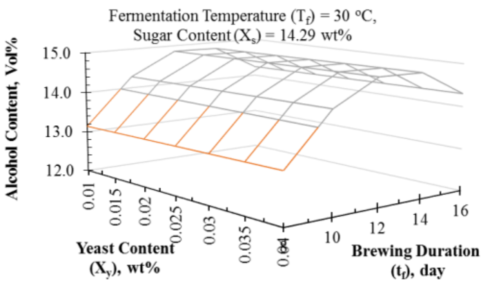
Table 6 Comparison of brewing result of alcohol content between prediction and experimental data

	Best setting	Alcohol content (vol. %)	
predictive value	$A_2B_2C_3D_3$	15.68±2.17	
experimental verification	$A_2B_2C_3D_3$	16.83	16.99

The dependence of alcohol content on fermentation temperature (T_f) and sugar content (X_s) was plotted on Fig. 3a where the brewing duration was 11 days and the yeast content was 0.029%. Maximum value of alcohol content was appeared for T_f and X_s over the testing range of 10 °C ≥ T_f ≥ 60 °C, 0 wt% ≥ X_s ≥ 40 wt%. However, the alcohol content was more sensitive to X_s than T_f. The dependence of alcohol content on yeast content (X_y) and brewing duration (t_f) was plotted on Fig. 3b where the fermentation temperature was 30 °C and sugar content was 14.29 wt%. A maximum alcohol content was observed as a function of brewing time. Over the testing range of 0.01 wt% ≥ X_y ≥ 0.04 wt%, 8 days ≥ t_f ≥ 16 days, alcohol content was more sensitive to t_f than X_y.



(a)



(b)

Figure 3 Dependence of alcohol content on (a) fermentation temperature (T_f) and sugar content (X_s) and (b) yeast content (X_y) and brewing duration (t_f).

3.2 Alcohol yield rate

The average S/N ratio of alcohol yield rate obtained from Taguchi experiment L_93^4 was plotted on Fig. 4. Based on the Larger-the-better criteria, optimal condition was the S/N ratio of 16.55 at fermentation temperature of 30 °C (level 3), the S/N ratio of 18.02 at sugar content of 14.29 wt% (level 2), the S/N ratio of 16.46 at yeast content of 0.029 wt% (level 3), the S/N ratio of 16.72 at the brewing duration of 13 days (level 3). The results showed that $A_3B_2C_3D_3$ was the best brewing condition based on alcohol yield rate. Table 7 is the list of Delta value and ranking of these four factors. The analysis result from the S/N ratio of alcohol yield rate showed that the optimum brewing conditions and the importance of factor were: sugar content at 14 wt.% > brewing duration at 13 days > fermentation temperature at 30 °C > yeast content at 0.029 wt.%.

Table 7 The Delta and ranking of the alcohol yield S/N ratio obtained from L_93^4 Taguchi experiment

Level	A	B	C	D
Delta	1.87	5.11	1.29	2.29
Rank	3	1	4	2

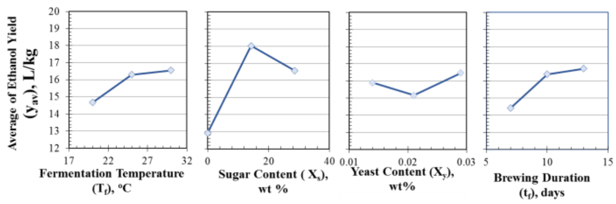


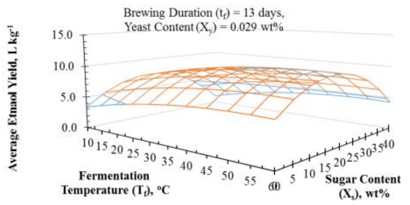
Figure 4 Response plots of S/N ratios from ethanol yield analysis. S/N ratio criteria is Larger is better.

A similar statistical analysis as mentioned on section 3.1 indicated that the alcohol yield at brewing condition of $A_3B_2C_3D_3$ should be 9.62 ± 1.12 L/kg %. Two experiments at this optimum conditions were carried out (fermentation temperature at 30 °C, sugar content at 14.29 wt.%, yeast content at 0.029 wt. %, and fermentation at 13 days). The comparison of brewing result of alcohol yield rate between prediction and experimental data on Table 8.

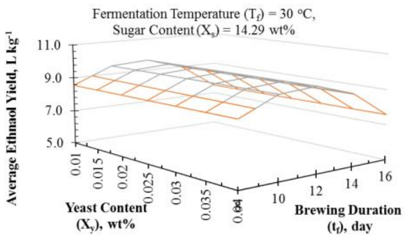
Table 8 Comparison of brewing result of alcohol yield rate between prediction and experimental data

	Best setting	Alcohol yield rate (L/Kg %)	
predictive value	$A_3B_2C_3D_3$	9.62±1.12	
experimental verification	$A_3B_2C_3D_3$	9.89	9.84

The dependence of alcohol yield rate on fermentation temperature (T_f) and sugar content (X_s) was plotted on Fig. 5a where the brewing duration was 11 days and the yeast content was 0.029%. Maximum value of alcohol yield rate was appeared for T_f and X_s over the testing range of 10 °C ≥ T_f ≥ 60 °C, 0 wt% ≥ X_s ≥ 40 wt%. However, the alcohol yield rate was more sensitive to X_s than T_f. The dependence of alcohol yield rate on yeast content (X_y) and brewing duration (t_f) was plotted on Fig. 5b where the fermentation temperature was 30 °C and sugar content was 14.29 wt%. A maximum alcohol yield rate was observed as a function of brewing time. Over the testing range of 0.01 wt% ≥ X_y ≥ 0.04 wt%, 8 days ≥ t_f ≥ 16 days, alcohol yield rate was more sensitive to t_f than X_y.



(a)



(b)

Figure 5 Dependence of alcohol yield rate on (a) fermentation temperature (T_f) and sugar content (X_s) and (b) yeast content (X_y) and brewing duration (t_f).

3.3 Antioxidant capacity

The average S/N ratio of antioxidant capacity obtained from Taguchi experiment L_93^4 was plotted on Fig. 6. The optimal condition was the S/N ratio of 38.83 at fermentation temperature of 30 °C (level 3), the S/N ratio of 38.20 at sugar content of 14.29 wt% (level 2), the S/N ratio of 38.46 at yeast content of 0.021 wt% (level 2), the S/N ratio of 38.18 at the brewing duration of 10 days (level 2). The results showed that $A_3B_2C_2D_2$ was the best brewing condition based on alcohol yield rate. Table 9 is the list of Delta value and ranking of these four factors. The analysis result from the S/N ratio of alcohol yield rate showed that the optimum brewing conditions and the importance of factor were: > brewing duration at 13 days > fermentation temperature at 30 °C > yeast content at 0.021 wt.% > sugar content at 14.29 wt.% > brewing duration 10 days

Table 9 Delta and ranking calculated from the S/N ratio of antioxidant capacity obtained from the L_93^4 Taguchi experiments

	A	B	C	D
Delta	1.88	0.36	1.02	0.20
Rank	1	3	2	4

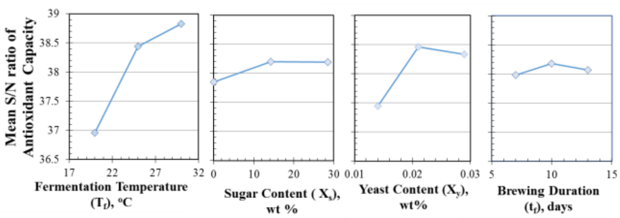


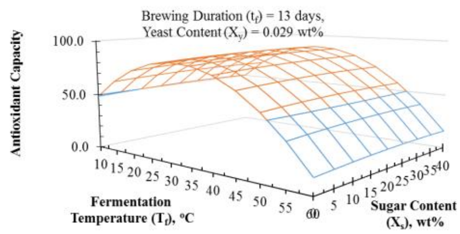
Figure 6 Response plots of S/N ratios from antioxidant capacity analysis. S/N ratio criteria is Larger is better.

A similar statistical analysis as mentioned on section 3.1 indicated that the antioxidant capacity at brewing condition of $A_3B_2C_2D_2$ should be 92.22 ± 4.01 L/kg %. Two experiments at this optimum conditions were carried out (fermentation temperature at 30 °C, sugar content at 14.29 wt.%, yeast content at 0.021 wt. %, and fermentation at 10 days). The comparison of brewing result of alcohol yield rate between prediction and experimental data on Table 10.

Table 10 Antioxidant capacity ideal condition prediction and experimental verification

	Best setting	Antioxidant capacity (%)	
predictive value	$A_3B_2C_2D_2$	92.22±4.01	
experimental verification	$A_3B_2C_2D_2$	93.76	93.85

The dependence of antioxidant capacity on fermentation temperature (T_f) and sugar content (X_s) was plotted on Fig. 7a where the brewing duration was 13 days and the yeast content was 0.029%. Maximum value of alcohol content was appeared for T_f and X_s over the testing range of 10 °C ≥ T_f ≥ 60 °C, 0 wt% ≥ X_s ≥ 40 wt%. However, the alcohol content was more sensitive to T_f than X_s. The dependence of antioxidant capacity on yeast content (X_y) and brewing duration (t_f) was plotted on Fig. 7b where the fermentation temperature was 30 °C and sugar content was 14.29 wt%. A maximum antioxidant capacity was observed as a function of brewing time. Over the testing range of 0.01 wt% ≥ X_y ≥ 0.04 wt%, 8 days ≥ t_f ≥ 16 days, antioxidant capacity was not so sensitive to t_f or X_y.



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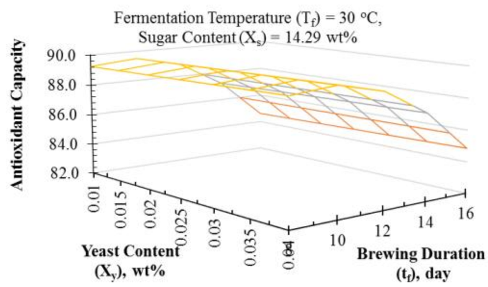

(b)

Figure 7 Dependence of alcohol yield rate on (a) fermentation temperature (T_f) and sugar content (X_s) and (b) yeast content (X_y) and brewing duration (t_f)

Conclusions

In this study, we try to locate the optimal fermentation condition of red wine brewing by using Taguchi experimental design method. Four important parameters of fermentation temperature, sugar content, yeast content, and fermentation duration were investigated by experiments based on $L_9 3^4$ orthogonal array Taguchi method. Experiment result showed the effect that optimal settings placed on alcohol content, alcohol yield, and antioxidant capacity. The study found out that the optimum experimental conditions with the sequence of degree of influence were as follows: sugar content at 14.29 wt. % > fermentation duration of 13 days> fermentation temperature at 25 °C > yeast content at 0.029 wt. %. The optimum condition for maximum alcohol yield was: sugar content at 14.29 wt. %, the fermentation duration of 13 days, fermentation temperature at 30 °C, and yeast content at 0.029 wt. %. The optimum condition for the best antioxidant capacity was: fermentation temperature at 30 °C > yeast content at 0.021 wt.% > sugar content at 14.29 wt. % > fermentation duration of 10 days. These results were also verified with a second order polynomial equation that equation parameters of this equation were fitted with experimental data obtained from Taguchi experiment.

Statistical analysis predicted that the alcoholic content of brewing in the optimum condition $A_2B_2C_3D_3$ was 15.68 vol. % ± 2.17 vol. %. And the best condition of $A_3B_2C_3D_3$ for maximum alcohol yield was 9.62 ± 1.12 kg/L%. The predicted antioxidant capacity was 92.22% ± 4.01% under the optimum condition $A_3B_2C_2D_2$. Experimental results by using those optimal settings were all agree with the Taguchi predictions.

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