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Fermentation conditions, total polyphenol content, and antioxidant activity of threeleaf cayratia (*Cayratia trifolia* L.) wine prepared using thermotolerant yeast *Saccharomyces cerevisiae* HG1.3

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Abstract

The biological activity and optimum conditions for threeleaf cayratia (*Cayratia trifolia* L.) wine fermentation using *Saccharomyces cerevisiae* HG1.3 were studied. The tests on the effect of fermentation conditions, including initial total sugar content (20, 22, 24 °Brix), pH (4.0, 4.5, 5.0), inoculum concentration (10^3, 10^5, 10^7 cells/mL), and temperature (30, 35, 37, 39, 41 °C) indicated that the ethanol contents of threeleaf cayratia wine were 5.19–12.82% (v/v). The results showed that appropriate fermentation conditions were 35 °C, 20 °Brix, 10^5 cells/mL yeast cells, and 6 days. The ethanol content of the final product was 11.68% (v/v) and the methanol, SO_2, hydrocyanic acid, and aldehyde concentrations were acceptable according to Vietnam National Standard 7045:2002. The total polyphenol content and antioxidant activity of threeleaf cayratia juice (0.66 mg gallic acid equivalents (GAE)/mL and 54.7%, respectively) and wine (0.60 mg GAE/mL and 57.4%, respectively) were not significantly different.

Keywords: Antioxidant capacity, *Cayratia trifolia*, *Saccharomyces cerevisiae,* Threeleaf cayratia wine

1. Introduction

Fox grape or threeleaf cayratia (*Cayratia trifolia* L.) is an important ingredient in the Vietnamese traditional cuisine and a medicinal plant. It is rich in bioactive compounds such as phenolic acid, flavonoid, stilbene, and anthocyanidin [1]. This plant also contains yellow waxy oil, alcohol, amino acid, and phenol. The berry skin contains cayratinin, delphinidin 3-p-coumaroyl-sophoroside-5-monoglucoside. Indians use this plant in traditional medicine [1]. Additionally, since it is widely distributed throughout the Mekong Delta in Vietnam, it is an abundant source for wine production.

Wine has long been intertwined with the history of Georgia and Iran. Traditionally, wine was produced from grape juice using commercial yeasts as starters. *Saccharomyces cerevisiae*, which ferments sugars to produce up to 16% ethanol [2], plays a major role in wine fermentation with abundant living cells presented in surfaces of wineries [3]. Currently, winemaking is being researched and improved for quality, yield, size, and diversified fermentation product in industrial production to meet the demand. The required temperature for white wine fermentation is 10–20 °C and that for red wine fermentation is up to 28 °C [4]. However, simultaneous alcoholic and malolactic low alcohol wine fermentation occurs at up to 45 °C [5]. Also, wine fermented at high temperature is of good quality [5] with good flavor and aroma [6]. Besides, due to climate change and global warming, the continuous rising temperature impacts the capability of yeast to ferment wine as well as the energy required to maintain temperature stability in fermentation systems [7]. The selection of thermotolerant yeast for wine production has many significant benefits such as fermentation at high temperature and reduced investment costs for cooling equipment, which is economical [7,8]. This study aims to show the genetic diversity and application of useful thermophilic microorganisms in fermentation technology.

In recent studies, *S. cerevisiae* HG1.3 is isolated from threeleaf cayratia berries that could produce 9.9% (v/v) ethanol concentration and could tolerant up to the temperature of 43 °C [9,10]. Therefore, this study was addressed to optimize the fermentation conditions for threeleaf cayratia wine using *S. cerevisiae* HG1.3 and to assess the quality and change of bioactive compounds of the final wine product.

2. Materials and methods

2.1 Materials

Juicy, shiny, ripe, and dark black threeleaf cayratia berries were collected from 13 provinces in Mekong Delta, Vietnam, brought to the Laboratory of Food Biotechnology, Can Tho University, and immediately processed. The un-crushed ripe berries were washed several times under tap water and finally rinsed with distilled water. After draining for an hour, the juice was collected using a blender (Philips HR1811; Amsterdam, The Netherlands) and filtering through a filter cloth. Juice samples were directly used for the next experiments. Thermotolerant yeast *S. cerevisiae* HG1.3 with fermentative ability at 41 °C was isolated from threeleaf cayratia. This strain tolerates 43 °C temperature and 9% (v/v) ethanol concentration [9,11].

2.2 Physical and chemical characteristics of threeleaf cayratia juice

The pH was measured using digital pH meter (WTW pH 525, Expotech; Houston, TX, USA) and the °Brix level was measured using a manual refractometer (FG102/112, Euromex; Arnhem, The Netherlands). Reducing sugar contents were determined using dinitrosalicylic acid (DNS) method by measuring the absorbance at 540 nm wavelength.

2.3 Determination of temperature and pH values for threeleaf cayratia fermentation

S. cerevisiae HG1.3 pure cells were sub-cultured on Petri dish and incubated at 35 °C for 2 days. The separated colony was added to 100 mL Yeast peptone dextrose (YPD) broth (1% w/v yeast extract, 2% w/v peptone, 2% w/v glucose) (10^8 cell/mL) sterilized at 121 °C for 15 min and shaken at 120 rpm in 36 h at room temperature (28±2 °C). The total sugar level of extracted juice was adjusted at 22°Brix using ≥ 99.8% pure saccharose and pH was adjusted to 4.0, 4.5, and 5.0 using 1 mol/L sodium carbonate and 0.5 mol/L citric acid. The adjusted juice was then sterilized with $NaHSO_3$ (140 mg/L for 2 h). Then, 1 mL *S. cerevisiae* HG1.3 (10^8 cells/mL) was inoculated into 99 mL juice and fermented at 35, 37, 39, and 41 °C and 28±2 for 5 days [12]. Changes in pH, °Brix, and ethanol contents were monitored.

2.4 Factors affecting threeleaf cayratia wine fermentation

A factorial experimental design was developed to study the effects of inoculation level (10^3, 10^5, 10^7 cells/mL), initial total sugar (20, 22, 24 °Brix) and time period (5, 7 and 9 days) on fermentation [13]. The pH of threeleaf cayratia juice and temperature was employed based on the selective results of the previous screening tests. Each treatment had triplicates. The ethanol content was measured using the distillation method according to AOAC 920.57.

2.5 Evaluation of threeleaf cayratia wine quality

C. trifolia wine was analyzed for microbiological and chemical quality parameters including coliforms (TCVN 6848:2007), *Escherichia coli* (TCVN 7924:1-2008), methanol (FAO-1986 14/8, P301), SO_2 (AOAC 892.02-2000), hydrocyanic acid (TCVN 6181-96), aldehyde (TCVN 8009:2009), and ethanol contents (AOAC 920.57).

The clarity, color, aroma, taste, and the overall confidence of the wine was evaluated according to Vietnam National Standard 3217:79 [14] by a sensory board of 10 members.

2.6 Total polyphenol content (TPC) and antioxidant activity of C. trifolia juice and wine product

The TPC and antioxidant activity of threeleaf cayratia juice and final wine product were investigated. The TPC was determined by the Folin-Ciolcateau method [15] as follows: 1.25 mL 10% (v/v) folin solution was added to 0.5 mL sample diluted with methanol with appropriate concentration (60 µg/µL) and incubated for 5 min. Then, 1 mL 2% (w/v) Na_2CO_3 was added to the solution for 45 min in dark and the optical density (OD) was measured at 760 nm. The antioxidant activity was determined according to the method reported by Tabart et al. [16] as follows: 1 mL reagent sample (in methanol) was mixed with 2 mL 2,2-diphenyl-1-picrylhydrazyl

(DPPH, 100 µmol/L) solution. The final solution was mixed thoroughly and incubated in dark for 30 minutes and the OD was measured at 517. Negative control (Ac) was prepared by mixing 2 mL DPPH with 1 mL methanol and 3 mL methanol was used as the blank. The results were calculated and expressed as the DPPH free radical scavenging activity percentage using: $\frac{Ac-}{Ac}$ ×100 (where, As and Ac indicate the absorbance of DPPH radical in the presence and absence of sample, respectively).

2.7 Statistical analysis

Statgraphics Centurion version XVI (Statpoint Technologies Inc.; Warrenton, VA, USA) was used for data analysis. The analyzed data were graphed using Excel 2013 (Microsoft Inc.; Redmond, WA, USA). The optimum fermentation conditions were calculated based on the regression model of correlation models between the fermentation conditions and the alcohol contents of the product.

3. Results and discussion

3.1 Characteristics of threeleaf cayratia juices

A total of 53 threeleaf cayratia berry samples were collected from 13 provinces in the Mekong Delta. The pH and °Brix values of threeleaf cayratia juices were different depending upon the geographical location and climate conditions of each area. The pH values ranged from 3.01 to 4.75 and total sugar contents were quite low (3.5-10 g/100 mL).

Table 1 The pH, total sugar, and reducing sugar content of threeleaf cayratia juice samples.

Province	Sample	pH	Total sugar (°Brix)	Reducing sugar (g/100 mL)
	BL1	3.28	5.0	0.26±0.01^{ijkl}
Bac Lieu	BL2	3.23	4.0	0.26±0.01^{ijkl}
	BL3	3.32	4.0	0.30±0.01^{ghijkl}
	BL4	3.30	5.0	0.27±0.00^{ijkl}
Soc Trang	ST1	3.52	5.0	0.27±0.01^{hijkl}
	ST2	3.39	7.0	0.41±0.01^{defghijkl}
	ST3	3.38	4.0	0.26±0.01^{ijkl}
	ST4	3.21	5.0	0.33±0.01^{fghijkl}
Tra Vinh	TV1	3.51	6.0	0.25±0.00^{ijkl}
	TV2	3.25	5.0	0.25±0.01^{ijkl}
	TV3	3.14	4.0	0.25±0.00^{ijkl}
	TV4	3.01	4.0	0.26±0.01^{ijkl}
Ben Tre	BT1	3.36	6.0	0.26±0.01^{ijkl}
	BT2	3.41	6.0	0.25±0.00^{ijkl}
	BT3	3.44	7.0	0.55±0.01^{cde}
	BT4	3.44	7.0	0.36±0.01^{efghijkl}
Ca Mau	CM1	3.27	5.0	0.30±0.01^{ghijkl}
	CM2	3.56	6.5	0.34±0.05^{efghijkl}
	CM3	3.41	5.0	0.23±0.04^{kl}
	CM4	3.55	5.0	0.42±0.05^{defghijkl}
Vinh Long	VL1	4.08	4.5	0.23±0.00^{kl}
	VL2	4.16	5.0	0.25±0.00^{ijkl}
	VL3	4.71	8.0	0.56±0.01^{cde}
	VL4	4.75	7.5	0.28±0.01^{ghijkl}
Tien Giang	TG1	3.35	6.0	0.27±0.01^{hijkl}
	TG2	3.29	5.0	0.25±0.01^{ijkl}
	TG3	3.70	8.0	0.81±0.05^{ab}
	TG4	3.70	6.0	0.26±0.01^{ijkl}
Hau Giang	HG1	3.35	6.0	0.32±0.01^{ghijkl}
	HG2	3.21	6.0	0.24±0.00^{jkl}
	HG3	3.12	5.0	0.24±0.00^{jkl}
	HG4	3.35	4.0	0.30±0.00^{ghijkl}
Can Tho	CT1	3.72	8.5	0.55±0.01^{cdef}
	CT2	3.60	6.0	0.45±0.00^{defghijk}
	CT3	3.78	6.5	0.63±0.01^{bcd}
	CT4	4.71	10	0.96±0.03^{a}
Dong Thap	DT1	3.61	7.0	0.39±0.03^{efghijkl}
	DT2	3.60	7.0	0.46±0.02^{defghi}
	DT3	3.77	8.0	0.49±0.00^{cdefgh}
	DT4	3.46	6.0	0.29±0.00^{ghijkl}
An Giang	AG1	4.07	6.5	0.50±0.02^{cdefg}
	AG2	4.18	9.0	0.39±0.03^{fghijkl}
	AG3	4.04	6.5	0.39±0.00^{efghijkl}
	AG4	4.30	7.0	0.46±0.01^{defghij}

Table 1 The pH, total sugar, and reducing sugar content of threeleaf cayratia juice samples (Continued).

Province	Sample	pH	Total sugar (°Brix)	Reducing sugar (g/100 mL)
Long An	LA1	3.35	6.5	0.25 ± 0.01^{ijkl}
	LA2	3.50	5.5	0.25 ± 0.01^{ijkl}
	LA3	3.90	8.5	0.71 ± 0.01^{bc}
	LA4	3.47	7.0	0.62 ± 0.06^{bcd}
Kien Giang	KG1	3.23	3.5	0.23 ± 0.01^{jkl}
	KG2	3.26	4.0	0.24 ± 0.00^{jkl}
	KG3	3.10	4.0	0.22 ± 0.01^{l}
	KG4	3.13	5.0	0.25 ± 0.00^{ijkl}
	KG5	3.10	5.0	0.23 ± 0.01^{jkl}

Note: The average values in a group with the same letter were not significantly different at the 95% confidence level. BL, ST, TV, BT, BM, CL, etc. are the abbreviation of provinces where the samples were collected; 1-5: number of collected samples of each province.

Reducing sugar is a carbohydrate with a free aldehyde or ketone group. All monosaccharides, such as glucose and fructose, which are utilized by yeast in fermentation because of their oxidizing ability, are reducing sugars [2]. The ratio of glucose–fructose is different between fruits and may be affected by climatic conditions during the growth season or geographical conditions. The reducing sugar content of threeleaf cayratia juice ranged between 0.22±0.01 g/100 mL (KG3) and 0.96±0.03 g/100 mL (CT4) (Table 1) and did not differ significantly between treatments (p<0.05). However, the reducing sugar content of CT4, TG3, and LA3 samples (0.96±0.03, 0.81±0.05, and 0.71±0.01 g/100 mL, respectively) was significantly higher than that of other samples. The glucose utilization rate was high at 17–20% reducing sugar content, while the fructose utilization rate was high at >25% reducing sugar content and both sugars were fermented equally at 20–25% reducing sugar content [17]. The remaining fermentable sugar quantity after complete fermentation may play an important role in microbial stability and potential blend preparations [2], thus reducing sugar content was analyzed in this study.

3.2 Temperature and pH values for threeleaf cayratia fermentation

The results of fermentation using *S. cerevisiae* HG1.3 at different temperatures and pH levels are presented in Table 2. The ethanol content after fermentation at room temperature and pH 4.5 was the highest (12.82% v/v), whereas that after fermentation at 41 °C and pH 5.0 was the lowest (5.19% v/v). The ethanol contents were high after fermentation at 35, 37, and 39 °C and pH 4.5 (12.53, 10.78, and 8.45% v/v, respectively). On the contrary, the ethanol content after fermentation at 41 °C and pH 4.0 and 4.5 were equal (5.98% v/v). Interestingly, fermentation at the same pH level produced less ethanol when the temperature increased from 39 to 41 °C. Fermentation at 35 °C produced more ethanol than that at 37 °C, it was not significantly different (p<0.05).

Table 2 The effects of temperature and pH on ethanol production.

Treatments	Testing factors		Results of alcoholic fermentation		
	Incubation temperature (°C)	pH	pH	Total sugar (°Brix)	Ethanol content (% v/v)
1	28±2	3.47	3.57	8.17	11.68^{c}
2	28±2	4.00	3.95	6.33	11.85^{bc}
3	28±2	4.50	4.42	7.33	12.82^{a}
4	28±2	5.00	4.64	7.50	10.53^{ef}
5	35	3.47	3.55	9.00	11.55^{cd}
6	35	4.00	3.95	9.00	11.68^{c}
7	35	4.50	4.24	9.00	12.53^{ab}
8	35	5.00	4.62	9.00	9.79^{fg}
9	37	3.47	3.55	11.17	9.57^{g}
10	37	4.00	3.93	11.00	9.87^{fg}
11	37	4.50	4.31	10.67	10.78^{de}
12	37	5.00	4.90	12.00	9.14^{gh}
13	39	3.47	3.46	13.00	7.98^{ij}
14	39	4.00	3.84	12.00	7.99^{ij}
15	39	4.50	4.22	12.00	8.45^{gh}
16	39	5.00	4.07	11.00	7.21^{ij}
17	41	3.47	3.23	14.67	5.98^{k}
18	41	4.00	3.41	15.00	5.98^{k}
19	41	4.50	4.04	16.00	5.98^{k}
20	41	5.00	4.22	16.00	5.19^{k}

Note: The values in this table were the average of triplicate values. The average values in the same column with the same letters were not significantly different at the 95% confidence level.

The ethanol contents after fermentation at room temperature and 35 °C and pH 4.5 were the highest (12.82% and 12.53% v/v, respectively), though the difference is not statistically significant at the 95% confidence level. In contrast, the ethanol content decreased when fermented at temperature ≥ 35 °C and pH ≥ 5, and the difference

was statistically significant from that at 30 and 35 °C with 95% confidence. According to the results, the *p*-value of the interaction of temperature and pH factors is $p< 0.05$, indicating that temperature and pH interact with each other. Therefore, the optimum ethanol content should be accounted for by combining the effects of these two factors (Table 3). The minimum (T_{min}), optimal (T_{opt}), and maximum (T_{max}) temperature ranges of thermotolerant yeasts are 20–26 °C, 26–35 °C and 37–45 °C, respectively, and these yeasts also grow above 45 °C. However, the above temperature ranges are not suitable for many yeasts such as *Candida macedoniensis* (T_{min} 5 °C, T_{max} 45 °C) and *Saccharomycopsis guttulata* (T_{min} 34 °C, T_{max} 42 °C) [17]. Accordingly, *S. cerevisiae* strains capable of fermenting above 35 °C have been mainly isolated from tropical regions [18]. The results of fermentation with pH 4.5 at 35 °C were selected for the next experiments.

Table 3 Analysis of variance for temperature and pH.

Source	Sum of Squares	Degrees of Freedom (Df)	Mean Square	F-Ratio	*p*-value
Main effects					
A: temperature	297.6810	4	74.42030	1005.47	0.0000
B: pH	23.1889	3	7.72964	104.43	0.0000
Interactions					
AB	4.69137	12	0.3909480	5.28	0.0000
Residual	2.96062	40	0.0740154		
Total (Corrected)	328.522	59			

Note: All F-ratios are based on the residual mean square error.

3.3 Optimization of threeleaf cayratia wine fermentation

The results in Table 4 show that °Brix level affects the fermentation process at all treatments. The ethanol content after fermenting samples with the highest total sugar contents, such as treatments 26 and 27 (24 °Brix), were the lowest (8.05% v/v). In contrast, the ethanol content in samples with low total sugar content, such as treatments 2 and 5 (20 and 22 °Brix, respectively), were high (13.47 and 12.30% v/v, respectively). During fermentation, the ethanol content is related to the sugar concentration in the media. Nutritional deficiency of yeast due to low sugar content decreases the ethanol concentration; however, extremely high sugar content also decreases the ethanol concentration [19]. The physiological state of yeast is out of balance because of the high soluble solid content, which inhibits yeast growth, further affecting fermentation, as seen in treatments 24, 25, and 26. In addition, the ethanol levels of treatment 1 (20 °Brix), treatment 5 (22 °Brix), treatment 11 (20 °Brix), and treatment 14 (22 °Brix) were not significantly different at 5%. Therefore, 20 °Brix was chosen to perform the optimal fermentation equation (Equation 2).

Table 4 Effects of total sugar content, inoculum concentration, and incubation time on threeleaf cayratia wine production.

Treatments	Tested factors			Results of alcoholic fermentation	
	Incubation period (day)	Total sugar (°Brix)	Inoculum concentration (cells/mL)	Total (°Brix)	Sugar Control content (% v/v)
1	5	20	10^3	8.67	11.40^{bc}
2	5	20	10^5	7.33	13.47^{a}
3	5	20	10^7	7.00	12.00^{bc}
4	5	22	10^3	8.67	11.40^{bc}
5	5	22	10^5	8.33	12.30^{ab}
6	5	22	10^7	8.67	11.67^{bc}
7	5	24	10^3	11.67	11.40^{bc}
8	5	24	10^5	10.76	11.07^{bc}
9	5	24	10^7	11.33	10.83^{cd}
10	7	20	10^3	7.67	11.40^{bc}
11	7	20	10^5	8.33	12.30^{ab}
12	7	20	10^7	9.33	11.07^{bc}
13	7	22	10^3	9.00	11.40^{bc}
14	7	22	10^5	9.00	12.30^{ab}
15	7	22	10^7	11.67	10.83^{cd}
16	7	24	10^3	10.33	10.77^{cd}
17	7	24	10^5	10.33	11.07^{bc}
18	7	24	10^7	11.67	10.83^{cd}
19	9	20	10^3	8.33	10.83^{cd}
20	9	20	10^5	8.67	10.77^{cd}
21	9	20	10^7	9.67	10.77^{cd}
22	9	22	10^3	9.33	10.83^{cd}
23	9	22	10^5	10.00	10.87^{c}
24	9	22	10^7	10.67	9.53^{de}
25	9	24	10^3	9.00	8.86^{ef}
26	9	24	10^5	10.33	8.05^{f}
27	9	24	10^7	12.67	8.05^{f}

Note: The values in this table were the average values of triplication. The average values in a group with the same letter were not significantly different at the 95% confidence level.

All three factors tested significantly impacted the fermentation process (Table 5). The lowest ethanol content (8.05% v/v) was produced after treating for 9 days with 10^5 or 10^7 cells/mL. Treatments 3, 12, and 21 (10^7 cells/mL and 20 °Brix) produced 12.00, 11.07%, and 10.77% (v/v) ethanol after 5, 7, and 9 days, respectively. Interestingly, treatments 4, 5, and 6 (22 °Brix) produced 11.40, 12.30, and 11.67% (v/v) ethanol, respectively, after treatment with 10^3, 10^5, and 10^7 yeast cells/mL for 5 days. As shown in Table 4, treatments 5 (5 days and 22 °Brix), 11 (7 days and 20 °Brix), and 14 (7 days and 22 °Brix) with 10^5 yeast cells/mL produced the highest ethanol (12.30% v/v). The ethanol level after treatment with 10^3 cells/mL was low, which increased after increasing the inoculum concentration to 10^5 cells/ mL and reduced after treatment with 10^7 cells/mL. Increased inoculum concentration decreases the soluble content as the yeast utilized sugar for alcoholic fermentation, thus increasing the ethanol content [2]. Nevertheless, the ethanol content does not increase, but decreases if the fermentation time is prolonged after the optimal fermentation period [19]. Furthermore, low inoculum concentration and prolonged fermentation time that affects the progress, cost, and time for fermentation.

Table 5 Analysis of variance for ethanol content.

Source	Sum of Squares	Df	Mean Square	F-Ratio	*p*-value
Main effects					
A: °Brix	31.4858	2	15.742900	37.80	0.0000
B: Inoculation level	7.3578	2	3.678940	8.83	0.0004
C: Time	52.1956	2	26.097800	62.66	0.0000
Residual	30.8218	74	0.416511		
Total (Corrected)	121.8610	80			

Note: All F-ratios are based on the residual mean square error.

The non-linear multiple regression equation (1) identifying the effects of time period, total sugar level (°Brix), and yeast inoculum concentration (log cell/mL) on ethanol content, established by Statgraphics Centurion XV.I software at 95% confidence, was employed to select the optimum fermentation conditions for *S. cerevisiae* HG1.3.

$$H= -27.4471 + 1.58638\times Z + 3.66409\times Y + 5.10075\times X - 0.019213\times Z^2 - 0.124219\times Z\times X - 0.0769965\times Z\times Y - 0.124352\times Y^2 - 0.181007\times Y\times X - 0.174352\times X^2 + 0.00619792\times Z\times Y\times X \quad (1)$$

(H = ethanol, X = day, Y = inoculum concentration, Z= total sugars)

The sugar content was then fixed at 20 °Brix (Z = 20) and the regression equation was reduced to:

$$H= -3.4047 + 2.12416\times Y + 2.61837\times X - 0.124352\times Y^2 - 0.0570486\times Y\times X - 0.174352\times X^2 \quad (2)$$

The derivative was calculated according to each variable of equation (2) and the system of equations was solved. The relationship between ethanol content and independent variables gives the equation of the fitted model, which indicates the response surface and contour plot of the appropriate conditions for wine production (Figure 1). The optimum conditions for threeleaf cayratia juice fermentation using thermotolerant yeast *S. cerevisiae* HG1.3 were statistically determined as follows: 6 days fermentation period, 10^5 cells/mL inoculum, 20 °Brix initial total sugar level, and 4.5 pH. Under these defined fermentation conditions, the ethanol content was predicted to be 12.37% v/v.

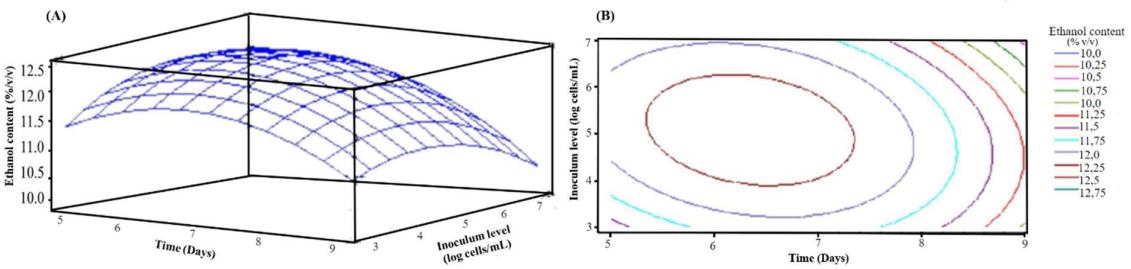

Figure 1 (A) The response surface and (B) contour plotting showing the effects of fermentation time and inoculum concentration on threeleaf cayratia juice fermentation. Statgraphics Centurion XV.I program was used to determine the equation with 95% confidence. Regression equation (1) was set up and °Brix (Z = 20) was fixed to define the other variables.

Fermenting 1 L threeleaf cayratia juice using *S. cerevisiae* HG1.3 under the optimum conditions (inoculum concentration 10^5 cells/mL, total sugar content 20 °Brix, pH 4.5, and fermentation time 6 days at 35°C) produced 11.68% (v/v) ethanol, which is not significantly different from the ethanol content (12.37% v/v) previously determined by the multiple regression model of Statgraphics program. Thus, it was demonstrated that the optimum condition suggested by the statistical equation was feasible and reasonable. Also, the results show similarity in optimal equations when compared with those of previous studies on watermelon wine yeast [20] and pineapple wine [21].

The available sugar concentration directly impacts the ethanol content during fermentation. The product (ethanol) and material (sugar) concentration interact, along with the inhibitory effect of ethanol. This interaction clarifies that yeast viability and fermentative capacity reduces due to the accumulation of intracellular ethanol (ethanol is toxic to the yeast cells [1]), while the osmotic pressure of the medium increase [19]. The quality of threeleaf cayratia wine was acceptable based on suitable fermentation conditions in this study. As shown in the previous reports, where the optimum conditions were 35 °C and 10^5 cells/mL, fermentation using *S. cerevisiae* CM3.2 at 21.09 °Brix/ pH 4.5 and *S. cerevisiae* AG2.1 at 22.06 °Brix/ pH 4.0, shows the consistency of the statistics [10,22].

3.4 Evaluation of the quality of threeleaf cayratia wine

The wine produced satisfied the Vietnamese standard requirements with 12.0% (v/v) ethanol content, 2.534 mg/L methanol content, 1.4 mg/L SO_2 content, and the absence of hydrocyanic acid (Table 6). The coliform and *E. coli* counts of threeleaf cayratia wine were acceptable based on the requirements of microbiological safety standards.

Table 6 The physicochemical and microbiological results of the threeleaf cayratia wine

Criteria	Results	Vietnam National Standard 7045:2002 [23]
Ethanol	12.0% v/v at 20 °C	6-18% v/v at 20 °C
Methanol	2.534 mg/L in 100° alcohol	3000 mg/L in 100° alcohol
SO_2	1.4 mg/L	350 mg/L
Acid hydrocyanic	0 g/L	0.1 mg/L
Alcohol (according to methyl 2-propanol)	2425.4 mg/L (100° of ethanol)	
Aldehyde	638.6 mg/L (100° of ethanol)	
E. coli	0 CFU/mL	0 CFU/mL
Coliform	0 CFU/mL	≤ 10 CFU/mL

Ten examiners specialized in wine sensory analysis participated in the evaluation. The color, clearness, flavor, and taste of wine were assessed on a scale of 0–5. Scores for a certain attribute given by all examiners were averaged, which was corrected using the corresponding weight factor. Subsequently, the weighted average scores were totaled. The results of organoleptic analysis of threeleaf cayratia wine are described in Figure 2, including clarity and color (4.9 points), aroma (4.6 points), taste (4.0 points), and overall confidence (4.12 points).

Furthermore, the examiners also assessed the acceptability of threeleaf cayratia wine. The preliminary trial assessments were consistent and reproducible. Also, the sensory outcomes indicated that threeleaf cayratia wine received adequate and acceptable evaluation.

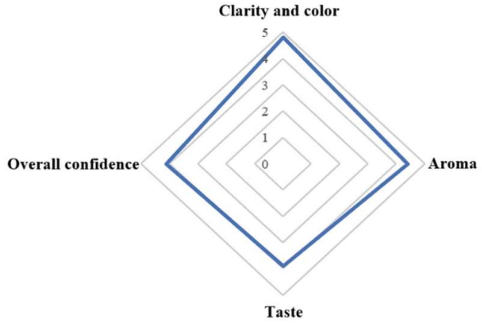

Figure 2 Sensory evaluation of threeleaf cayratia wine.

3.5 TPC and antioxidant activity of threeleaf cayratia juice and wine

The TPC of three types of plum wine including Crvena ranka, Požegača, and Trnovača are 0.124, 0.145, and 0.158 mg gallic acid equivalents (GAE)/mL, respectively, and the antioxidant capacity levels are 0.94, 1.33, and 1.40 mg Trolox/L, respectively [24]. This study reports that the TPC of threeleaf cayratia wine was higher (0.60 mg GAE/mL), with the antioxidant capacity being 57.3%. The higher values of TPC were reported in grapes collected from different regions including Cabernet Sauvignon (1216.17 mg GAE/L), Kalecik Karasi (1336.21 mg GAE/L), and Narince (217.06 mg GAE/L) [25].

The TPC and the antioxidant activity of threeleaf cayratia berries were 0.66 mg GAE/mL and 54.7%, respectively. Rabeta and Lin have determined the highest antioxidant ability of freeze-dried berries using methanol extraction to be 45.1 mg GAE/g sample, which is higher than that reported in this study. The antioxidant property of methanol extracts of fresh fruit sample (4.6 mg GAE/g sample) was higher than that of

water extracts (2.9 mg GAE/g sample) [26]. The TPC of methanol extracts and dried samples were the highest due to decreased polyphenol oxidation in the berries. The different drying and extraction methods can affect the estimation of antioxidant activity in a sample; however, the TPC and antioxidant activity did not change significantly after fermentation.

4. Conclusion

The findings of this study suggest the promising application of the tested yeast-*S. cerevisiae* HG 1.3-for fruit wine fermentation at high temperature. At the optimum fermentation conditions, including initial total sugar level 20 °Brix, pH 4.5, and 10^5 cells/mL yeast suspension, at 35 °C for 6 days, the produced ethanol content could reach 11.68% (v/v).

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