EFFECTS OF YEAST STRAINS, pH AND FERMENTATION TEMPERATURE ON WINE MADE FROM *Rhodomyrtus tomentosa* FRUIT (MANG DEN, KONTUM PROVINCE)

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Received date: 24.10.2013 Accepted date: 24.02.2014

ABSTRACT

The effects of yeast strains, fermentation temperature and pH on quality of *Rhodomyrtus tomentosa* wine were examined. At ambient temperature (28±2°C), the fermentation was induced by inoculation with *Saccharomyces cerevisiae* strains isolated, purified and screened from sugar palm (*Borassus flabellifer)* and pineapple juice in comparison with commercial yeast (initial populations of yeast raging from $10^4 \div 10^7$ cells/ml). The medium was adjusted before fermentation to five different pH values (3.4÷4.2). The effect of fermentation temperature (20 and 28±2°C) on strain population was also studied. The resulting wines were chemically analyzed. Pure cultures of *Saccharomyces cerevisiae* isolated from sugar palm significantly yielded in ethanol production higher than other strains in the fermentation at 28±2°C.. Yeast strains performed better at low temperatures with high alcohol yield. At 20±2°C, the fermentation was dominated by the growth of *S. cerevisiae* in *Rhodomyrtus tomentosa* juice with maximum ethanol concentrations (13.43%Vol.) The methanol and SO_2 concentrations met the Vietnamese Standards (QCVN 6-3 2010/BYT). In addition, the total acid, ester and aldehyde concentration were also low.

Keywords: Alcohol quality, pH, *Rhodomyrtus tomentosa* fruit, *Saccharomyces cerevisae*, temperature.

Ảnh hưởng của dòng nấm men, pH và nhiệt độ lên men đến quá trình sản xuất rượu vang sim

TÓM TẮT

Rượu vang sim rừng Măng Đen (vang đỏ) được lên men từ trái sim chín tím đỏ với nấm men phân lập và thuần chủng. Ảnh hưởng của dòng nấm men, nhiệt độ lên men và pH đến chất lượng rượu vang sim đã được nghiên cứu. Quá trình lên men ở nhiệt độ phòng (28±2°C) sử dụng nấm men *Saccharomyces cerevisiae* được phân lập, tuyển chọn từ nước thốt nốt và nước khóm so sánh với nấm men thương mại (mật số nấm men dao động trong khoảng $10^4 \div 10^7$ tế bào/ml). Dịch lên men được điều chỉnh ở 5 mức độ pH khác nhau (3,4÷4,2). Ảnh hưởng của nhiệt độ (20 và 28±2°C) đến quá trình lên men cũng được nghiên cứu. Các phân tích hóa học trên rượu vang thành phẩm đã được thực hiện.

Dòng nấm men thuần chủng phân lập từ nước thốt nốt thể hiện khả năng sinh ethanol vượt trội so với các dòng nấm men khác (nấm men phân lập từ nước khóm và nấm thương mại) khi lên men ở nhiệt độ 28±2°C, pH 3,6 và mật số nấm men 10^6 tế bào/ml (với nồng độ ethanol thu được từ 11,85 và 12,35%v/v). Hàm lượng ethanol thu được cao hơn khi lên men ở nhiệt độ thấp. Ở 20±2°C, nấm men *S. cerevisiae* thể hiện khả năng lên men tốt hơn trong nước sim và nồng độ ethanol thu được tối đa (13,43% v/v). Các chỉ tiêu hóa học của rượu vang như hàm lượng methanol và SO_2 đạt yêu cầu Quy chuẩn Việt Nam (QCVN 6-3 2010/BYT). Ngoài ra, hàm lượng acid tổng số, ester và aldehyde trong rượu cũng ở mức thấp.

Từ khóa: Chất lượng, nhiệt độ, pH, *Saccharomyces cerevisae*, trái sim.

1. INTRODUCTION

Rhodomyrtus tomentosa (Ait.) Hassk, commonly known as rose-myrtle, mainly distributes in South-East Asian countries, especially Southern parts of Vietnam, China, Japan, Thailand, Philippines, and Malaysia (Saising et al., 2011). In Vietnam, the *Rhodomyrtus tomentosa* grows on the highland and mountains regions. Especially, there are over 700 hectares of the *Rhodomyrtus tomentosa* growing in Mang Den, Kon Plong district, Kontum province. The *Rhodomyrtus tomentosa* fruit is sweet and slightly sour. The main pigments which are responsible for *Rhodomyrtus tomentosa* color are anthocyanin compounds (Tung et al., 2009) including hydrolysable tannins, flavones, triterpenes and steroids (Hui et al., 1976).

Red wine has long been thought to be heart healthy. The alcohol and certain substances in red wine called antioxidants may help prevent heart disease. Resveratrol might be a key ingredient in red wine that helps prevent damage to blood vessels, reduces "bad" cholesterol and prevents blood clots. The *Rhodomyrtus tomentosa* fruit from Phu Quoc Island was also reported to be used for red wine fermentation (Thuy, 2010). However, study on using isolated yeast for *Rhodomyrtus tomentosa* wine fermentation to improve wine quality has not been conducted. Moreover, maintaining *Rhodomyrtus tomentosa* wine color is still a problem. Red wine quality is affected by complex interactions involving yeast strain, must condition and winemaking technology (Torija et al., 2002). Some factors, such as *Saccharomyces cerevisiae* species, different sources of yeast temperature, and pH of the must strongly affect fermentation and wine quality (Fleet and Heard, 1993; Ribéreau-Gayon et al., 2000). Therefore, the objectives of this study were to determine whether the pH of must effects on the *Rhodomyrtus tomentosa* wine quality, especially wine color, and to select high activity yeast strains, yeast population as well as fermentation temperature to improve the *Rhodomyrtus tomentosa* wine quality.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Yeast strains

Three yeast strains (isolated from palm juice, pineapple juice, and commercial yeast) were used for this investigation. The isolated strains that were selected from palm juice and pineapple juice with highest fermentation capacity were identified as *Saccharomyces cerevisiae* (Thuy et al., 2011a; Thanh et al., 2013). A mixture of isolated yeast strains including isolates from palm juice and from pineapple juice was also applied for wine fermetation. Commercial yeast (Saf-instant, France) as commercial *S. cerevisiae* was bought from CEMACO company.

Yeast culture and propagation: Pure culture of each strain wase propagated to obtain the required fresh yeast (10^6cell/ml). Yeast cells were cultured in nutritional medium (20% of potato, 2% glucose , 0,2% $(NH_4)_2SO_4$, 0,2% KH_2PO_4 in 100 ml distilled water) which was sterilled for 15 minutes at 121°C. Then, the cultured medium was incubated at 30°C for 1 days in shaker (140 rpm).

2.1.2. Rhodomyrtus tomentosa fruits

The *Rhodomyrtus tomentosa* fruits were harvested from Mang Den village, Kom Tum province and transported to laboratory of Department of Food Technology, Can Tho University.

2.2. Methods

2.2.1. Wine fermetation

The fruits were selected, and, washed with water and drained before crushing with warm water (45°C). The fruit paste was treated with pectinase (0.075% of Pectinex Ultra SPL, China) for 30 minutes. The fruit juice, afterwards, was extracted and filtered by hydraulic press. Sucrose and citric acid were added to serve as additives to the must [total soluble solid content (TSS) 23°Brix with the corresponding sugar content of 219 g/l and five different pH values

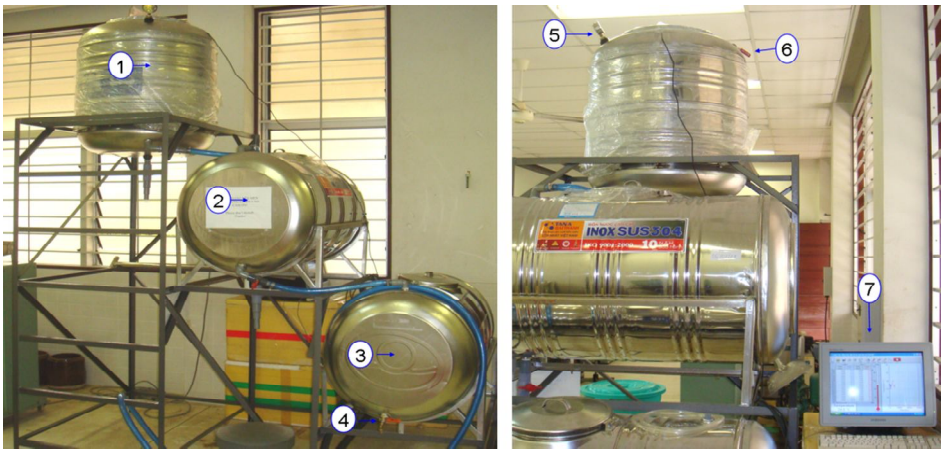

Figure 1. Fermentation system

Note: ①: Primary fermentation tank; ② and ③: Secondary fermentation tank ④: Exhaust valve; ⑤: Pressure gauge; ⑥: Gas exhaust valve; ⑦: Controlling temperature system

from 3.4 to 4.2], followed by the addition of Sodium metabisulfite (120 mg/L) for 2 hours to inhibit bacterial growth. For primary fermentation, the yeast cultures (from palm juice, pineapple juice, and mixture of both isolates (from palm juice and pineapple juice) and commercial yeast were inoculated into the must with different populations ($10^4 \div 10^7$cells/ml). The fermentation process was conducted by using the fermentation tanks shown in figure 1. The effect of temperature on fermentation efficiency was investigated by performing primary fermentation at two temperatures, including ambient temperature 28±2°C and controlled temperature (20±2°C). Secondary fermentation process was followed for 3 months before transferring final wine product to bottles.

2.2.2. Temperature mornitoring

During the primary fermentation step, temperature was kept track by using thermosensor connectingd to the computer and using Logger Lite Software version 4.0.

2.2.3. Quality analysis

Aliquot samples were taken after primay fermentation for analysis of alcohol content (%Vol.), total soluble solid content (Brix degree), residual sugar (g/l), titratable acidity (mg/l), methanol (g/l of 100% ethanol), aldehyde (mg/l), sulfite (mg/l), ester (mg/l), and tannin content (g/l) using assays as described by Mai et al. (2009).

Absorbance: The absorbance (A) of red wine was measured at 550nm by a UV-Vis spectrophotometer model U-2800A (Hitachi High Technologies America, Inc) to evaluate color difference. The absorbance was calculated by the equation:

$$A = \log\left(\frac{I_o}{I}\right) x\lambda$$

where A is the absorbance, I_o and I are the light intensity before and after transmission through the cuvet, λ is the wave length of the light (at 700 nm).

Total anthocyanin measurement: The total anthocyanin content was determined according to the spectrophotometric pH-differential method (Lee et al., 2005). The total anthocyanin content was calculated as cyanidin-3-glucoside equivalents as the following equation:

$$C = \frac{A \times M \times DF \times V \times 10^3}{\varepsilon \times L \times m} (mg / L)$$

where A is the absorbance, MW is the molecular weight of cyanidin-3-glucoside (449.2 Da), DF is the dilution factor, V is the final

volume (mL), 10^3 is the factor for conversion from g to mg, ε is the cyanindin-3-glucoside molar absorbance (26,900), L is the cell path length (1 cm), and m is the sample weight (g).

2.2.4. Sensory analysis

Sensory analysis was done on color, taste and odor of the wine. The sensory evaluations were carried out by a panel of 10 fixed panellists. For QDA analysis, each panel was requested to evaluate the wine quality for various attributes using 5-point hedonic scale (0 = unacceptable, 1 = moderately unacceptable, 2 = neither good nor bad, 3 = moderately good, 4 = good).

2.3 Data analysis

Significant differences between mean of parameters were determined by ANOVA and the Multiple Range Test at 95% confidence interval by using Statgraphic software (version 15.2.11).

3. RESULTS AND DISCUSSIONS

3.1. Effect of pH on *Rhodomyrtus tomentosa* wine quality

3.1.1. Physicochemical properties

Several factors affecting to yeast fermentation rate such as temperature, pH and nutritional compounds of the must (Torija et al., 2003). The quality of *Rhodomyrtus tomentosa* wine which were fermented from different pH media were shown in table 1. A similar alcohol content (11,6%) was obtained in all the samples in which pH was initially adjusted from 3.6 to 4.2, while a significantly lower ethanol content was shown in the sample of low initial pH medium (pH value of 3.4). Samples with low initial pH media, corresponding to high acid content, indicated high anthocyanin and tannin content in the final product and vice versa. The anthocyanin content of *Rhodomyrtus tomentosa* wine was in range of 11.2 to 16.1 mg/l and tannin content varied from 0.47 to 0.66g/l, depending on pH value. According to Roobha et al. (2011) the intensity and stability of the anthocyanin pigments is dependent on various factors including concentration of the pigments, pH, temperature, light intensity and so on. The concentrations of anthocyanin and tannin are responsible for the colour of *Rhodomyrtus tomentosa* wine. As a consequence, high anthocyanin and tannin contents, resulting in higher absorbance value (0.71 and 0.51 at 550 nm) were observed when using low initial pH media (pH value of 3.4 to 3.6). Figure 2 showed a deep-red color of *Rhodomyrtus tomentosa* wine samples which had pH value of 3.4 or 3.6 whereas by stepwise pH increase until 4.2, the color gradually changed toward slight reddish color.

3.1.2. Sensory evaluation

Sensory quality of final wine was also evaluated in three attribute parameters (odor, color and taste). Odor average scores from the panel ranged from 2.6 to 2.9 ("quite good" to "good") for all the wine samples (Figure 3).

Table 1. Effect of pH on the quality of *Rhodomyrtus tomentosa* wine (after 18 days of fermentation at ambient temperature)

pH	Ethanol (% Vol.)	Tritratable acidity (mg/l)	Anthocyanin (mg/l)	Tannin (g/l)	Absorbance at 550 nm
3.4	10.94^{b*}	6616^{a}	16.11^{a}	0.65^{a}	0.71^{a}
3.6	11.65^{a}	4680^{b}	14.61^{a}	0.66^{a}	0.56^{b}
3.8	11.61^{a}	4520^{b}	12.75^{b}	0.43^{bc}	0.46^{c}
4.0	11.6^{a}	4120^{c}	11.81^{b}	0.40^{c}	0.40^{d}
4.2	11.67^{a}	4000^{c}	11.18^{b}	0.47^{b}	0.37^{d}

Note: Mean of triplicates values in the same column with similar superscript letters are not significantly different (P > 0.05).

Figure 2. Colour of *Rhodomyrtus tomentosa* wine with different initial pH value (a) pH 3.4, (b) pH 3.6, (c) pH 3.8, (d) pH 4.0, (e) pH 4.2

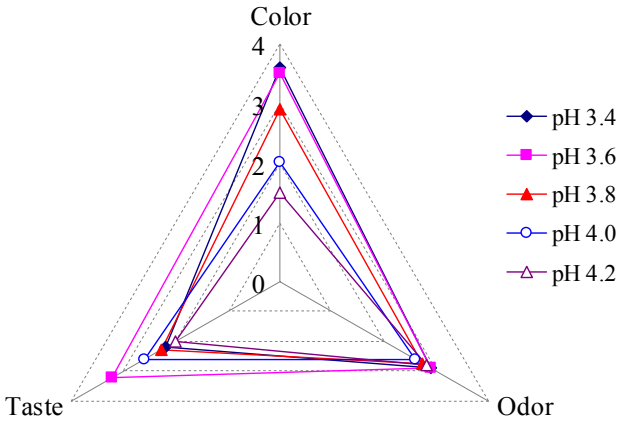

Figure 3. Sensory evaluation of *Rhodomyrtus tomentosa* wine with different initial pH value

Increase in pH value resulted in a decrease in color acceptance. At pH 3.4 to 3.6, the wine has the most favorite color, however, the taste represented low score (2.4) while good taste is noticed for wine fermented in pH 3.6. In general, at pH value of 3.6, the *Rhodomyrtus tomentosa* wine has good quality with high ethanol content, high concentration of anthocyanin and tannin, leading to favorable flavor and color.

3.2. Effect of different yeast strains and their population on *Rhodomyrtus tomentosa* wine quality

3.2.1. Ethanol content

The effect of different yeast strains and their populations on ethanol content was observed. By using isolated yeast from palm juice, the must had the highest fermentation and achieved highest ethanol content (approximate 12.08 % Vol.) after 18 days. The mean of ethanol yields by isolates from pineapple juice, isolate mixtures and commercial yeast in wine showed a lower levels (around 10.8% Vol.) (Table 2).

Different yeast cell populations for each type of yeast strain were also inoculated for wine fermentation. After 18 days, the primary fermetation (at ambient temperature) of all samples stopped and the quality of these wine products were determined. The statistical results showed that high alcohol yield reached similar maximal levels (11.85 and 12.18% Vol.) by using yeast populations of 10^6 to 10^7 cells/ml in the initial media while for the samples using

Table 2. Alcohol degree (% Vol.) of *Rhodomyrtus tomentosa* wine using different yeast strains and their population (after 18 days of fermentation at ambient temperature)

Yeast strains	Yeast cell population (cell/ml)				Means
	10^4	10^5	10^6	10^7	
Isolates from pineapple juice	10.77	10.77	11.63	12.16	11.33[b]
Isolates from palm juice	11.60	11.90	12.52	12.28	12.08[a]
Mixture of isolates	10.87	11.17	11.6	12.63	11.57[b]
Commercial yeast	10.80	11.10	11.66	11.67	11.31[b]
Means	11.01[b]	11.24[b]	11.85[a]	12.18[a]	

Note: Values in the same column or row with similar superscript letters are not significantly different (P > 0.05).

lower initial yeast populations of 10^4 and 10^5 cells/ml showed less alcohol contents in wine (11.01 and 11.24% Vol., respectively). It could be explained by the consuming nutrients in the fermentation environment to increase biomass of yeast cell that caused sugar content loss and low alcohol content in the final product. However, according to Nagodawithana et al. (1974), the higher the initial yeast count (8.10^8 cells/ ml), the greater the rate of cell death occurred even though the nutrients and oxygen were not apparently limiting. The above results suggested that it might be possible to get a high ethanol yield by selecting an initial yeast count from 10^6 to 10^7cell/ml. This result was in agreement with previous finding (Thuy et al., 2011b) that the *S. cerevisiae* (isolated from palm juice) performed better than commercial yeast in fermentation plam juice wine and obtained highest alcohol content of 13.67% Vol.

3.2.2. Residual sugar content

Rhodomyrtus tomentosa wine which was fermented with the *Saccharomyces cerevisiae* isolated from palm juice, has low residual sugar content (4.43g/l) and significant differences from the wine using other yeast strains (Table 3). Moreover, higher yeast cell populations led to higher sugar consumption during fermentation process and as a consequence the lower residual sugar content in the final product was obtained. *Rhodomyrtus tomentosa* wine which used cell populations of 10^6 to 10^7 cells/ml has lower residual sugar content (5.5 and 3.43 g/l, respectively) compared to the sample fermented from yeast population of 10^4 and 10^5 cells/ml (around 6.5 - 6.8 g/l of residual sugar).

3.3. Effect of fermentation temperature on *Rhodomyrtus tomentosa* wine quality

Temperature affects not only the fermentation rate and length of fermentation but also the yeast metabolism, which determins the chemical compositions and flavor of the wine. The quality parameters of *Rhodomytus tomentosa* wine which were fermented at two different temperatures were determined.

Table 3. Residual sugar content (g/l) of *Rhodomyrtus tomentosa* wine using different yeast strains and their population (after 18 days of fermentation at ambient temperature)

Yeast strains	Yeast cell population (cell/ml)				Means
	10^4	10^5	10^6	10^7	
Isolated from pineapple juice	6.86	6.81	6.04	3.58	5.82[a*]
Isolated from palm juice	5.52	5.57	4.04	2.59	4.43[b]
Mix of isolated yeast	7.49	7.10	5.79	3.23	5.82[a]
Commercial yeast	7.26	6.46	6.49	4.32	6.13[a]
Means	6.78[a]	6.49[a]	5.50[b]	3.43[c]	

Note: Mean of triplicates values in the same column or row with similar superscript letters are not significantly different (P > 0.05).

3.3.1. Ethanol content, residual sugar content and total fermentation days

Controlling fermentation temperature is very important for high quality wine production (Zamora, 2009). In this study, different temperature [ambient temperature (28±2°C) and controlled temperature (20±2°C)] were applied for primary fermentation periods. The fermentation time, TSS and ethanol content of *Rhodomyrtus tomentosa* wine which were fermented at different temperatures are shown in table 4. At controlled temperature (20±2°C), the fermentation took place longer but produced good wine product with higher ethanol content (13.43% Vol.) and low residual sugar content. These results are in the line with previous study of Torija et al. (2002) which reported that at low temperature, fermentation started slowly, but consumed faster all the sugars because the high biomass of yeast was maintained throughout the process, as a result the high ethanol yield was obtained at lower fermentation temperature.

In contrast, a shorter time of fermentation was observed at ambient temperature (28±2°C). In fact, this result agreed with previous reports (Nagodawithana et al., 1974; Casey et al., 1984) that illustrated a shorter fermentation period, lower ethanol content and high sugar content product was obtained throughout high temperature fermentation. These results have been explained in literatures as a decrease of yeast viability due to a greater accumulation intracellular ethanol at higher temperatures that produce cell toxicity, alter the structure of the membrane, and decrease its functionality (Lucero et al., 2000).

3.3.2. Methanol

Methanol content in *Rhodomytus tomentosa* wine was not effected by different fermentation temperature. The methanol content obtained from the wine fermented at ambient temperature and low temperature took account of 0.163 and 0.153 g/l ethanol 100% (Table 5), respectively. The result of Gnekow & Ough (1976) also indicated that a temperature difference between 60 and 70°F made little difference in the methanol content of the wine.

3.3.3. Titratable acidity

Titratable acid content of the wine fermented at low temperatures (5736 mg/l) was significantly different and higher than that at ambient temperature (4920 mg/l) (Table 5). According to Zamora (2009), the delay or fermentation can produce a greater amount of acetic acid. Thus, *Rhodomyrtus tomentosa* wine fermented at low temperatures may contain high amount of acetic acid which was, produced during long fermentation period.

Table 4. Effect of fermentation temperature on ethanol content, residual sugar content of *Rhodomyrtus tomentosa* wine and total fermentation days

Fermentation temperature	Ethanol content (%Vol.)	Residual sugar content (g/l)	Total fermentation days
Ambient temperature (28±2°C)	12.52[a]	6.9[a]	18
Controlled temperature (20±2°C)	13.43[b]	4.6[b]	32

Note: Mean of triplicates values in the same column with similar superscript letters are not significantly different (P > 0.05).

Table 5. Quality parameters of *Rhodomyrtus tomentosa* wine after primary fermentation period (30 days at low temperature and 18 days at ambient temperature)

Fermentation temperature	Methanol (g/l 100% ethanol)	Titratable acidity (mg/l)	Ester (mg/l)	Aldehyde (mg/l)	SO_2 (mg/l)
Ambient temperature (28±2°C)	0.163[a]	4920[b]	2200[b]	473.73[a]	29.01[a]
Controlled temperature (20±2°C)	0.153[a]	5736[a]	2769[a]	271.33[b]	24.75[a]

Note: Mean of triplicates values in the same column with similar superscript letters are not significantly different (P > 0.05).

Ester, aldehyde, and sulfite content

In order to evaluate the effect of temperature on the production of secondary metabolites, the concentration of ester, aldehyde, and sulfite content were recorded. Low temperature is considered as an extra cellular stress, this could explain a higher ester production such as increasing floral (fatty acid ethyl esters) and fruity (fusel alcohol acetates) yeast aromas and maintained a high level of varietal aromas (terpens) (Beltran et al., 2008). The statistical analysis illustrated that the concentrations of ester were higher in wine fermented at low temperature (2769 mg/l) than that at ambient temperature (2200 mg/l) (Table 5).

Acetaldehyde contributes positive effect on the aroma of wines (Etievant, 1991). However, a beyond threshold of acetaldehyde was described as creating bad smell (Jackson, 1994). Aldehyde content in the *Rhodomyrtus tomentosa* wine fermenting under controlled temperatures (271.33 mg/l) was lower than at ambient temperature (473.73 mg/l) (Table 5).

Sulfur compounds evolution during fermentation was not effected by temperature of environment. Sulfite concentration in wine fermented at ambient and under controlled temperature has similar level and not significantly different among them (29.01 and 24.15 mg/l, respectively) (Table 5).

The sulfite and metathol content in the *Rhodomyrtus tomentosa* wine derived from fermentation process at both temperatures met the requirement of Vietnamese standard for red wine (QCVN 6-3 2010/BYT). However, controlled temperature at 20±2°C is recommended for fermentation *Rhodomyrtus tomentosa* wine to obtain not only high ethanol content but also greater flavor of the final product.

4. CONCLUSION

Yeast s isolated from palm juice showed greater ethanol yield than isolates from pineapple juice, mixture of both isolated yeasts and commercial yeast during fermentation *Rhodomyrtus tomentosa* wine. At pH 3.6 and inoculation with yeast populations of 10^6 cells/ml, the ethanol concentration produced ranged between 11.85 and 12.52% Vol. In addition, the wine obtained in these conditions had favorable colour.

Low temperature fermentation (20±2°C) took longer time and produced high ethanol content (13.43% Vol.). The chemical criteria of wine such as methanol and sulfite concentrations met Vietnamese standards for red wine (QCVN 6-3 2010/BYT). Moreover, the total acid, ester and aldehyde contents were kept at low levels.

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