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RESEARCH ARTICLE

Different Parameters Affecting to *Rhodomyrtus tomentosa* Wine Fermentation

N. P. Minh[1*], T. T. Nghiep[2], B. V. Hung[3], T. V. Bay[4]

1. *Faculty of Food Technology - Biotech, Dong A University, Da Nang City, Vietnam.*
2. *Bac Lieu University, Bac Lieu Province, Vietnam.*
3. *Dong Thap University, Dong Thap Province, Vietnam.*
4. *Can Tho University, Can Tho City, Vietnam.*

***Corresponding Author: N. P. Minh**

Abstract

Fermentation is a relatively efficient, low energy preservation process which increases the shelf life, and decreases the need for refrigeration or other forms of food preservation technology. It is, therefore, a highly appropriate technique for use in developing countries and remote areas where access to sophisticated equipment is limited. *Rhodomyrtus tomentosa* plays an important holistic role in the daily lives of several ancient cultures, providing medicinal benefits. There is limited study mentioning to processing of this nutritional fruit. Therefore we explored a wine fermentation from *Rhodomyrtus tomentosa* by focusing on the effect of different parameters such as pectinase concentration and time of treatment for juice extraction, yeast inculate for wine fermentation, fermentation temperature and secondary fermentation to wine quality. Our results proved that 2.5% pectinase was used for juice extraction in 40 minutes, 2.5% *sacchromyces cerevisiae* was used for the main fermentation at 29.5°C in 15 days, and 6 weeks of aging in dark bottle at 10.5°C was enough to get a pleasant *Rhodomyrtus tomentosa* quality. Using *Rhodomyrtus tomentosa* having medicinal and nutritional value as a substrate for wine production, the health benefits of them can be improved widely.

Keywords: *Rhodomyrtus tomentosa, Wine, Pectinase, Fermentation, Sacchromyces cerevisiae, Aging.*

Introduction

Rhodomyrtus tomentosa, a member of the Myrtaceae family, is an evergreen shrub native to Southeast Asia , where it grows in abundance with rose-pink flowers and dark-purple edible bell-shaped fruits [1]. The 10-15 mm long blueberry-like fruits are edible and are well known for their sugar, vitamin, and mineral contents. The fruit is an ellipsoid berry that measures 1-1.5 cm in diameter, with a persistent calyx. Unripe fruits have green skin and an astringent taste. The berry turns to a purplish black when ripe, and the pulp is purplish, soft, and sweet.

The berries contain many deltoid seeds that measure 1.5 mm in diameter and are located in 6 pseudo-locules divided by thin false septa [2]. The sweet and ripe fruits are consumed fresh or made into pies, tarts, jellies, preserves, wine, tea and jams, or they are used in salads [3].

In Vietnam, the fruits are used to produce a wine called routusim [4]. The nutritional properties of *R. tomentosa* including proteins, amino acids, carbohydrates, lipids, vitamins, and minerals have been determined and reported [5,7].*R. tomentosa* has a similar total phenolic content as berries [7]. R. tomentosa exhibits a wide spectrum of pharmacological effects and has been used to treat colic diarrhea, wounds, heartburn, abscesses, gynecopathy, and as a pain killer.

They comprise phloroglucinol, flavonoid, terpenoid, anthracene glycoside, tannin, and other compounds. Stilbenes and ellagitannin, anthocyanins, flavonols, and gallic acid are major components in *R. tomentosa*. Piceatannol, a promising health-promoting stilbene component, was the major phenolic compound found in R. tomentosa fruits [8].

Several biological activities have been documented as antibacterial, antifungal, antimalarial, osteogenic, antioxidant, and anti-inflammatory. R. tomentosa has been studied extensively for alternative antimicrobial agents [2].Fermentation is a viable technique in the development of new products with modified physicochemical and sensory qualities, especially flavor and nutritional components. Fermentation requires very little sophisticated equipment, either to carry out the fermentation or for subsequent storage of the fermented product. It is a technique that has been employed for generations to preserve fruits in the form of drinks and other food for consumption at a later date and to improve food security. Basically, most fruits can be fermented if not all provided they are well prepared [9].

Wine is one of the functional fermented foods that have many health benefits. Commercially, wine is produced by the fermentation of yeast which involves the conversion of sugar to alcohol. They usually have an alcohol content ranging between 5 and 13%. Wine can act as a nutrient supplement for seasonal fruits and vegetables throughout the year. Using fruits and vegetables having medicinal and nutritional value as a substrate for wine production, the health benefits of them can be improved widely.

Fermentation is carried out with *Saccharomyces cerevisiae* commonly known as bakers yeast. The wine produced resembled the commercial wine in terms of its composition, taste and aroma. During the fermentation period the wines were analyzed for pH, titratable acidity, specific gravity, biomass content, alcohol and reducing sugar on a daily basis. PH show a decreased trend then attains minima and then increased. As the fermentation days proceed, the specific gravity increased and the alcohol percentage increased gradually [10].

The yeast is responsible for the production of ethanol in alcoholic drink. The process produces ethyl alcohol (ethanol) is the way of yeast to convert glucose into energy. Fermentation can extract valuable components from the raw materials used for production. Yeast is the magical ingredient that turns fruit juices into wine.In spontaneous fermentations, the 1st stages invariably being dominated by the alcohol-tolerant strains of *Saccharomyces cerevisiae*.

This species is universally known as the `wine yeast' and is widely preferred for initiating wine fermentations *S. cerevisiae* has adapted in several important ways and be able to break down their foods through both aerobic respiration and anaerobic fermentation. It can survive in an oxygen deficient environment for a period of time [11].The use of *S. cerevisiae* as starter culture is the most widespread practice in winemaking.Not many studies mentioned to wine fermentation of *Rhodomyrtus tomentosa*. The effects of yeast strains, fermentation temperature and pH on quality of *Rhodomyrtus tomentosa* wine were examined [12].

Harvested *Rhodomyrtus tomentosa* fruits may undergo rapid deterioration if proper processing and storage facilities are not provided, especially in the humid tropics where the prevailing environmental conditions accelerate the process of decomposition. It is an underutilized fruit crop and still now there is very limited research available regarding to processing of this fruit into value added product. Therefore, we utilized this fruit as subtrate for wine fermentation. We focused on the effect of different parameters such as pectinase concentration and time of treatment for juice extraction, yeast ratio for wine fermentation, fermentation temperature and secondary fermentation to wine quality.

Material & Method

Material

Rhodomyrtus tomentosa fruits were collected from Soc Trang province, Vietnam. After harvesting, they must be conveyed to laboratory within 8 hours for experiments. *Rhodomyrtus tomentosa* pulp was mixed with pectinase in different ratio (1.5, 2.0, 2.5, 3.0%) in different duration (20, 30, 40, 50 minutes) for juice extraction. Total soluble solid of *Rhodomyrtus tomentosa* was adjusted to diffrent concentration (15.0^{o}Brix, 16.0^{o}Brix, 17.0 ^{o}Brix, 18.0 ^{o}Brix) by sucrose. To avoid contamination and unpleasant odors in wine, everything that comes in contact with the wine must be very clean. This is, especially, critical when cleaning the fermenting vessel.

Rhodomyrtus tomentosa juice was then steriled by pasteurization at 72°C in 2.5 minutes. *Saccharomyces cerevisiae* was added into *Rhodomyrtus tomentosa* juice in different ratio (1.5%, 2.0%, 2.5%, 3.0%) and fermented in different temperature (28.5°C, 29°C, 29.5°C, 30.0°C) for 15 days.

Figure 1: Rhodomyrtus tomentosa fruit

Research Method

Effect of Pectinase Concentration and Time for Juice Extraction

Rhodomyrtus tomentosa extract was treated with pectinase enzyme with different concentration (1.5, 2.0, 2.5, 3.0%) in different duration (20, 30, 40, 50 minutes). We analyzed the extract recovery (%), viscosity (cP) and turbidity (mJ/cm²).

Effect of Soluble Solid Content in Rhodomyrtus Tomentosa Juice to Wine Quality

Rhodomyrtus tomentosa extract after being treated by pectinase would be formulated with sucrose into different soluble solid contents (15.0°Brix, 16.0°Brix, 17.0 °Brix, 18.0 °Brix). *Rhodomyrtus tomentosa* juice was then steriled by pasteurization at 72°C in 2.5 minutes. *Saccharomyces cerevisiae* was added into *Rhodomyrtus tomentosa* extract in 1.5%, and fermented in 28.5°C for 15 days. *Rhodomyrtus tomentosa* wine was periodically sampled in 3 days of interval for 15 days based on the residual soluble dry matter (°Brix), ethanol (%v/v), acidity (g/l), total phenolic compounds (mg/g), total flavonoids (mg/g) and sensory characteristics (score) in wine.

Effect of Yeast Inculate for Rhodomyrtus Tomentosa Wine Fermentation

Rhodomyrtus tomentosa extract after being treated by pectinase, formulated with sucrose to 17°Brix would be inoculated with *Saccharomyces cerevisiae* at different ratio (1.5, 2.0, 2.5, 3.0%), and fermented in 28.5°C for 15 days. *Rhodomyrtus tomentosa* wine was periodically sampled in 3 days of interval for 15 days based on the residual soluble dry matter (°Brix), ethanol (%v/v), acidity (g/l), total phenolic compounds (mg/g), total flavonoids (mg/g) and sensory characteristics (score) in wine.

Effect of Fermentation Temperature to Rhodomyrtus Tomentosa Wine Quality

Rhodomyrtus tomentosa extract after being treated by pectinase, formulated with sucrose to 17°Brix, inoculated with *Saccharomyces cerevisiae* at ratio 2.5% would be fermented in different temperature (28.5°C, 29°C, 29.5°C, 30.0°C) for 15 days. *Rhodomyrtus tomentosa* wine was periodically sampled in 3 days of interval for 15 days based on the residual soluble dry matter (°Brix), ethanol (%v/v), acidity (g/l), total phenolic compounds (mg/g), total flavonoids (mg/g) and sensory characteristics (score) in wine.

Effect of Secondary Fermentation to Wine Quality

We preserved *Rhodomyrtus tomentosa* wine at 10.5°C in dark bottle by different time (2, 4, 6, 8 weeks) as the secondary fermentation or aging. We monitored the residual soluble dry matted (°Brix), ethanol (% v/v), acidity (g/l), total phenolic compounds (mg/g), total flavonoids (mg/g) and sensory characteristics (score) in wine.

Analysis of Rhodomyrtus Tomentosa Wine

The viscosity of the samples was measured with Ostwalds's viscometer. Treated juices were kept overnight at room temperature (28°C) and were analysed for relative viscosity and turbidity, as a measure of clarification. Soluble dry matter (°Brix) was measured by refractometer. Ethanol (% v/v) was determined by megapore polar column with direct injection gas chromatography [13]. Acidity (g/l) was measured by potentiometry method (M. B. Rajković et al., 2007).

Total phenolic compounds (mg/g) in the extracts were determined using Folin–Ciocalteu reagent. The content of total phenolics was expressed as gallic acid equivalents (GAE). The spectrophotometer assay for the quantitative determination of flavonoid content (mg/g) was carried out. Total flavonoids (mg/g) of fruits were expressed as catechin equivalents. Sensory evaluation was carried out by a panel of 10 semi-trained judges.

Statistical Analysis

Data were statistically summarized by Statgraphics Centurion XVI.

Result & Discussion

Effect of Pectinase Concentration and Time of Treatment for Juice Extraction

Pectinase enzyme which includes pectin methyl esterase and depolymerising enzymes, finds extensive application in fruit processing industries for clarification of fruit juices and wines, in the extraction of fruit juices, in the manufacturing of pectin free starch, curing of coffees, cocoa and tobacco, refinement of vegetable fibres, scouring and as an analytical tool for the estimation of plant products [14, 16]. The enzymatic liquefaction process not only helped in increasing the overall yield of juice but also upgrading the quality features of the extracted juice leading to sparkling clarity [17].

Rhodomyrtus tomentosa extract was treated with pectinase enzyme with different concentration (1.5, 2.0, 2.5, 3.0%) in different duration (20, 30, 40, 50 minutes). Our results were depicted in table 1-3. We clearly found that 2.5% pectinase in 40 minutes treatment was optimal for *Rhodomyrtus tomentosa* extraction. So we selected these values for next experiments.

Table 1: Extract recovery (%) by diffferent pectinase concentration (%) and time of treatment (minutes)

Pectinase concentration (%)	Extract recovery (%)			
	20 minutes	30 minutes	40 minutes	50 minutes
1.5	52.69±0.01^{b}	52.95±0.01^{b}	53.14±0.02^{b}	53.20±0.02^{b}
2.0	53.37±0.02^{ab}	53.76±0.02^{ab}	53.97±0.03^{ab}	54.03±0.03^{ab}
2.5	53.84±0.01^{a}	54.02±0.01^{a}	54.45±0.01^{a}	54.51±0.03^{a}
3.0	53.90±0.00^{a}	54.10±0.02^{a}	54.51±0.03^{a}	54.62±0.01^{a}

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (a = 5%).

Table 2: Viscosity (cP) by diffferent pectinase concentration (%) and time of treatment (minutes)

Pectinase concentration (%)	Viscosity (cP)			
	20 minutes	30 minutes	40 minutes	50 minutes
1.5	1.38±0.02^{a}	1.33±0.03^{a}	1.25±0.03^{a}	1.24±0.03^{a}
2.0	1.20±0.03^{b}	1.16±0.01^{b}	1.10±0.01^{b}	1.08±0.01^{b}
2.5	1.12±0.00^{bc}	1.07±0.00^{bc}	1.02±0.00^{bc}	1.00±0.03^{bc}
3.0	1.09±0.01^{c}	1.05±0.02^{c}	0.97±0.02^{c}	0.95±0.02^{c}

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (a = 5%).

Table 3: Turbidity (mJ/cm^2) by diffferent pectinase concentration (%) and time of treatment (minutes)

Pectinase concentration (%)	Optical density (mJ/cm^2)			
	20 minutes	30 minutes	40 minutes	50 minutes
1.5	71.29±0.02^{a}	70.11±0.04^{a}	69.35±0.00^{a}	69.30±0.03^{a}
2.0	69.42±0.01^{b}	69.04±0.01^{b}	68.10±0.03^{b}	68.02±0.01^{b}
2.5	68.90±0.00^{bc}	68.73±0.00^{bc}	67.95±0.01^{bc}	67.90±0.02^{bc}
3.0	68.81±0.02^{c}	68.64±0.03^{c}	67.90±0.02^{c}	67.79±0.00^{c}

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (a = 5%).

The enzymatic liquefaction of pulp as a function of enzyme concentration, incubation time and hydrolysing temperature is standardized to obtain a desired yield of brilliantly cleared juice [18].

Effect of Soluble Solid Content in Rhodomyrtus Tomentosa Juice to Wine Quality

Sugar is the main substrate for fermentation of fruits juice into alcohol. Although other food nutrients such as protein and fats can be broken down by some microorganism in some cases where sugar is limited, as long as sugar is present, yeast cells will continue the process of fermentation until other factors that affect the growth of yeast become unfavorable. Sugars are the most common substrate of fermentation to produce ethanol, lactic acid, and carbon dioxide [19].

Although sugar is an important substrate of fermentation, higher sugar concentration inhibits the growth of microorganisms [20]. However, yeasts are fairly tolerant of high concentrations of sugar and grow well in solutions containing 40% sugar. At concentrations higher than this, only a certain group of yeasts - the Osmophilic type - can survive. There are only a few yeasts that can tolerate sugar concentrations of 65-70% and these grow very slowly in these conditions. A winemaker who wishes to make a wine with high levels of residual sugar (like a dessert wine) may stop fermentation early either by dropping the temperature of the must to stun the yeast or by adding a high level of alcohol (like brandy) to the must to kill off the yeast and create a fortified wine [19]. *Rhodomyrtus tomentosa* extract after being treated by pectinase would be formulated with sucrose into different soluble solid contents (15.0°Brix, 16.0°Brix, 17.0 °Brix, 18.0 °Brix). *Rhodomyrtus tomentosa* juice was then steriled by pasteurization at 72°C in 2.5 minutes. *Saccharomyces cerevisiae* was added into *Rhodomyrtus tomentosa* extract in 1.5%, and fermented in 28.5°C for 15 days. Results were depicted in table 4-9. It's obviously seen that 17.0°Brix of *Rhodomyrtus tomentosa* juice was adequate for *Rhodomyrtus tomentosa* wine fermentation.

Table 4: Effect of soluble solid content to soluble dry matter (°Brix) in wine

Fermentation time (days)	Residual soluble dry matter in wine (°Brix)			
	15.0°Brix	16.0°Brix	17.0°Brix	18.0°Brix
3	12.49±0.00^{b}	12.95±0.01^{ab}	13.14±0.02^{ab}	14.33±0.02^{a}
6	10.18±0.03^{b}	10.34±0.03^{ab}	10.45±0.00^{ab}	11.02±0.01^{a}
9	8.07±0.01^{b}	8.26±0.00^{ab}	8.58±0.01^{ab}	8.73±0.03^{a}
12	6.39±0.02^{b}	6.84±0.02^{ab}	7.02±0.04^{ab}	7.16±0.01^{a}
15	5.78±0.01^{b}	5.90±0.03^{ab}	6.12±0.02^{ab}	6.25±0.02^{a}
Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (a = 5%).				

Table 5: Effect of soluble solid content to ethanol formation (%v/v) in wine

Fermentation time (days)	Ethanol in wine (%v/v)			
	15.0°Brix	16.0°Brix	17.0°Brix	18.0°Brix
3	3.13±0.02^{b}	3.37±0.00^{ab}	3.60±0.04^{a}	3.65±0.02^{a}
6	4.11±0.00^{b}	5.40±0.02^{ab}	7.29±0.00^{a}	7.34±0.00^{a}
9	5.58±0.01^{b}	6.79±0.04^{ab}	8.35±0.03^{a}	8.40±0.01^{a}
12	6.84±0.02^{b}	7.32±0.01^{ab}	8.76±0.02^{a}	8.82±0.01^{a}
15	7.48±0.03^{b}	7.89±0.02^{ab}	9.05±0.01^{a}	9.11±0.03^{a}
Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (a = 5%).				

Table 6: Effect of soluble solid content to acidity (g/l) in wine

Fermentation time (days)	Acidity in wine (g/l)			
	15.0°Brix	16.0°Brix	17.0°Brix	18.0°Brix
3	1.08±0.02^{b}	1.11±0.01^{ab}	1.15±0.02^{a}	1.17±0.01^{a}
6	1.13±0.01^{b}	1.16±0.03^{ab}	1.22±0.01^{a}	1.25±0.00^{a}
9	1.19±0.00^{b}	1.24±0.01^{ab}	1.29±0.01^{a}	1.31±0.01^{a}
12	1.23±0.02^{b}	1.31±0.02^{ab}	1.37±0.02^{a}	1.39±0.03^{a}
15	1.29±0.03^{b}	1.38±0.00^{ab}	1.43±0.01^{a}	1.45±0.02^{a}
Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (a = 5%).				

Table 7: Effect of soluble solid content to total phenolic content (mg GAE/g) in wine

Fermentation time (days)	Total phenolic content (mg GAE/g)			
	15.0°Brix	16.0°Brix	17.0°Brix	18.0°Brix
3	354.47±0.01^{b}	429.48±0.03^{ab}	531.27±0.02^{a}	540.24±0.02^{a}
6	398.75±0.02^{b}	513.74±0.01^{ab}	645.14±0.01^{a}	651.28±0.01^{a}
9	457.69±0.04^{b}	629.36±0.02^{ab}	723.74±0.02^{a}	730.16±0.02^{a}
12	524.10±0.02^{b}	713.04±0.04^{ab}	811.20±0.03^{a}	820.41±0.04^{a}
15	530.21±0.03^{b}	720.29±0.02^{ab}	819.68±0.02^{a}	832.08±0.01^{a}
Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (a = 5%).				

Table 8: Effect of soluble solid content to total flavonoid (mg CE/g) in wine

Fermentation time (days)	Total flavonoid (mg CE/g)			
	15.0°Brix	16.0°Brix	17.0°Brix	18.0°Brix
3	45.69±0.03b	66.54±0.03ab	74.19±0.04a	80.02±0.03a
6	56.35±0.01b	78.55±0.01ab	89.55±0.01a	91.29±0.01a
9	69.38±0.02b	86.28±0.03ab	93.10±0.00a	95.20±0.01a
12	75.80±0.02b	94.50±0.02ab	104.28±0.01a	107.42±0.03a
15	80.13±0.04b	95.19±0.04ab	106.59±0.02a	108.31±0.02a

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (a = 5%).

Table 9: Effect of soluble solid content to sensory characteristics in wine

Fermentation time (days)	Sensory score of wine (1-5) by different yeast ratio			
	15.0°Brix	16.0°Brix	17.0°Brix	18.0°Brix
3	2.24±0.02b	2.34±0.03ab	2.49±0.02a	2.51±0.01a
6	2.87±0.00b	3.02±0.00ab	3.11±0.01a	3.15±0.00a
9	3.37±0.01b	3.51±0.02ab	3.83±0.03a	3.94±0.03a
12	3.75±0.02b	3.92±0.03ab	4.04±0.01a	4.08±0.02a
15	3.81±0.01b	3.97±0.01ab	4.09±0.02a	4.13±0.00a

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (a = 5%).

Effect of Yeast Inculate for Wine Fermentation

As a living organism, yeast primarily requires sugars, water, and warmth to stay alive. In addition, albumen or nitrogenous material is also necessary for yeast to thrive. Yeast is a unicellular fungus which reproduces asexually by budding or division, especially the genus *Saccharomyces* which is important in food fermentations has the ability to reproduce much faster. Cell division or cell reproduction generally takes place by budding. In the budding process, a new cell forms as a small outgrowth of the old cell, the bud gradually enlarges and then separates. In pure fermentation, the ability of inoculated *Saccharomyces cerevisiae* to suppress the wild microflora is one of the most important features determining the starter ability to dominate the process. During the winemaking process, various microorganisms coexist and interact influencing the dominance, the persistence of fermenting yeasts and the analytical profiles of wine [21]. *Rhodomyrtus tomentosa* extract after being treated by pectinase, formulated with sucrose to 17°Brix would be inoculated with *Saccharomyces cerevisiae* at different ratio (1.5, 2.0, 2.5, 3.0%), and fermented in 28.5°C for 15 days. *Rhodomyrtus tomentosa* wine was periodically sampled in 3 days of interval for 15 days based on the residual soluble dry matter (°Brix), ethanol (%v/v), acidity (g/l), total phenolic compounds (mg/g), total flavonoids (mg/g) and sensory characteristics (score) in wine. Results were revealed in table 10-15. We found that the appropriate yeast inculate should be 2.5% to get the highest wine quality.

Table 10: Effect of yeast ratio to soluble dry matter (°Brix) in wine

Fermentation time (days)	Soluble dry matter in wine (°Brix)			
	Yeast ratio 1.5%	**Yeast ratio 2.0%**	**Yeast ratio 2.5%**	**Yeast ratio 3.0%**
3	13.14±0.02a	12.84±0.03b	12.20±0.02bc	12.09±0.04c
6	10.45±0.00a	10.05±0.01b	9.74±0.04bc	9.68±0.01c
9	8.58±0.01a	8.01±0.02b	7.45±0.01bc	7.39±0.02c
12	7.02±0.04a	6.55±0.02b	6.11±0.03bc	6.06±0.01c
15	6.12±0.02a	5.24±0.04b	5.03±0.01bc	4.97±0.00c

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (a = 5%).

Table 11: Effect of yeast ratio to ethanol formation (%v/v) in wine

Fermentation time (days)	Ethanol in wine (%v/v)			
	Yeast ratio 1.5%	Yeast ratio 2.0%	Yeast ratio 2.5%	Yeast ratio 3.0%
3	3.60±0.04b	3.74±0.04ab	4.35±0.01a	4.40±0.02a
6	7.29±0.00b	7.43±0.01ab	7.67±0.00a	7.73±0.00a
9	8.35±0.03b	8.57±0.02ab	8.86±0.03a	8.90±0.03a
12	8.76±0.02b	8.89±0.01ab	8.98±0.01a	9.04±0.02a
15	9.05±0.01b	9.24±0.02ab	9.55±0.04a	9.61±0.01a

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (a = 5%).

Table 12: Effect of yeast ratio to acidity (g/l) in wine

Fermentation time (days)	Acidity in wine (g/l)			
	Yeast ratio 1.5%	Yeast ratio 2.0%	Yeast ratio 2.5%	Yeast ratio 3.0%
3	1.15±0.02[b]	1.18±0.02[ab]	1.21±0.03[a]	1.22±0.03[a]
6	1.22±0.01[b]	1.26±0.03[ab]	1.30±0.01[a]	1.31±0.01[a]
9	1.29±0.01[b]	1.35±0.01[ab]	1.38±0.02[a]	1.40±0.03[a]
12	1.37±0.02[b]	1.41±0.02[ab]	1.45±0.02[a]	1.46±0.02[a]
15	1.43±0.01[b]	1.51±0.04[ab]	1.57±0.01[a]	1.58±0.01[a]
Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (a = 5%).				

Table 13: Effect of yeast ratio to total phenolic content (mg GAE/g) in wine

Fermentation time (days)	Total phenolic content (mg GAE/g)			
	Yeast ratio 1.5%	Yeast ratio 2.0%	Yeast ratio 2.5%	Yeast ratio 3.0%
3	531.27±0.02[b]	553.17±0.01[ab]	569.30±0.03[a]	571.24±0.02[a]
6	645.14±0.01[b]	679.24±0.03[ab]	693.18±0.02[a]	695.21±0.01[a]
9	723.74±0.02[b]	749.42±0.02[ab]	784.92±0.00[a]	790.11±0.03[a]
12	811.20±0.03[b]	848.24±0.01[ab]	876.13±0.03[a]	880.24±0.02[a]
15	819.68±0.02[b]	854.58±0.04[ab]	890.29±0.01[a]	892.41±0.02[a]
Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (a = 5%).				

Table 14: Effect of yeast ratio to total flavonoid (mg CE/g) in wine

Fermentation time (days)	Total flavonoid (mg CE/g)			
	Yeast ratio 1.5%	Yeast ratio 2.0%	Yeast ratio 2.5%	Yeast ratio 3.0%
3	74.19±0.04[b]	81.35±0.03[ab]	85.37±0.04[a]	86.22±0.02[a]
6	89.55±0.01[b]	94.20±0.00[ab]	103.26±0.02[a]	104.14±0.03[a]
9	93.10±0.00[b]	105.38±0.01[ab]	124.33±0.00[a]	125.50±0.01[a]
12	104.28±0.03[b]	117.69±0.02[ab]	129.40±0.00[a]	130.11±0.03[a]
15	106.59±0.02[b]	123.42±0.03[ab]	131.46±0.02[a]	132.07±0.02[a]
Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (a = 5%).				

Table 15: Effect of yeast ratio to sensory characteristics (score, 1-5) in wine

Fermentation time (days)	Sensory score of wine (1-5) by different yeast ratio			
	Yeast ratio 1.5%	Yeast ratio 2.0%	Yeast ratio 2.5%	Yeast ratio 3.0%
3	2.49±0.02[b]	2.61±0.01[ab]	2.42±0.01[a]	2.48±0.01[a]
6	3.11±0.01[b]	3.33±0.03[ab]	3.42±0.02[a]	3.45±0.00[a]
9	3.83±0.03[b]	3.89±0.00[ab]	3.94±0.00[a]	3.96±0.02[a]
12	4.04±0.01[b]	4.09±0.02[ab]	4.14±0.03[a]	4.16±0.02[a]
15	4.09±0.02[b]	4.13±0.01[ab]	4.20±0.01[a]	4.22±0.03[a]
Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (a = 5%).				

Effect of different inoculum concentrations indicated that increased the inoculum concentration result in the increased of alcohol content. The result showed that when the concentration of yeast was increased, yeast cells converted more sugar to alcohol. However, at the higher inoculum concentration yeast cells grew not well because of the limited nutrient and were not able to convert more sugar in to it [22]. The results obtained were agreed with the report of Satav and Pethe [23] who studied wine production from banana fruits. In this study, 10% and 15% inoculum concentration gave similar alcohol content but 10% showed the better taste than 15%.

Effect of Fermentation Temperature to *Rhodomyrtus Tomentosa* Wine Quality

In winemaking, the temperature and speed of fermentation are an important consideration as well as the levels of oxygen present in the must at the start of the fermentation. Juice temperature must be warm for fermentation. However, yeast cells will die if temperature is too hot. The most notable is that of the internal temperature of the must. The biochemical process of fermentation itself creates a lot of residual heat which can take the must out of the ideal temperature range for the wine [24]. Thus, fermentation is an exothermic process. However, in winemaking, the temperature must not exceed 29.4°C for red wines or 15.3°C for white wines. Otherwise, the growth of yeast cells will stop. Therefore, a lower temperature is desirable because it increases the production of esters, other aromatic compounds, and alcohol itself. This makes the wine easier to clear and less susceptible to bacterial infection [25]. In general, temperature control during alcoholic fermentation is necessary to facilitate yeast growth, extract flavors and colors from the skins, permit accumulation of desirable by-products, and prevent undue rise in temperature that might kill the yeast cells. The low temperature and slow fermentation favor the retention of volatile compounds [26]. In most cases, fermentation at higher temperatures may have adverse effect on the wine in stunning the yeast to inactivity and even "boiling off" some of the flavors of the wines. Some winemakers may ferment their red wines at cooler temperatures more typical of white wines to bring out more fruit flavors [27]. *Rhodomyrtus tomentosa* extract after being treated by pectinase, formulated with sucrose to 17°Brix, inoculated with *Saccharomyces cerevisiae* at ratio 2.5% would be fermented in different temperature (28.5°C, 29°C, 29.5°C, 30.0°C) for 15 days. *Rhodomyrtus tomentosa* wine was periodically sampled in 3 days of interval for 15 days based on the residual soluble dry matter (°Brix), ethanol (%v/v), acidity (g/l), total phenolic compounds (mg/g), total flavonoids (mg/g) and sensory characteristics (score) in wine. Results were revealed in table 16-21. We found that the appropriate fermentation temperature should be 29.5°C to get the highest wine quality

Table 16: Effect of fermentation temperature to soluble dry matter (°Brix) in wine

Fermentation time (days)	Residual soluble dry matter in wine (°Brix)			
	28.5°C	29.0°C	29.5°C	30°C
3	12.20±0.02[a]	11.84±0.03[ab]	10.34±0.00[b]	10.29±0.02[b]
6	9.74±0.04[a]	9.13±0.01[ab]	8.68±0.03[b]	8.61±0.00[b]
9	7.45±0.01[a]	7.12±0.02[ab]	6.87±0.01[b]	6.79±0.03[b]
12	6.11±0.03[a]	5.97±0.02[ab]	5.75±0.02[b]	5.70±0.02[b]
15	5.03±0.01[a]	4.85±0.04[ab]	4.71±0.03[b]	4.68±0.01[b]

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (a = 5%).

Table 17: Effect of fermentation temperature to ethanol formation (%v/v) in wine

Fermentation time (days)	Ethanol in wine (%v/v)			
	28.5°C	29.0°C	29.5°C	30°C
3	4.35±0.01[c]	4.92±0.04[b]	5.44±0.01[a]	5.23±0.02[ab]
6	7.67±0.00[c]	7.94±0.00[b]	8.35±0.03[a]	8.18±0.03[ab]
9	8.86±0.03[c]	8.99±0.03[b]	9.15±0.03[a]	9.06±0.01[ab]
12	8.98±0.01[c]	9.25±0.04[b]	9.41±0.03[a]	9.32±0.02[ab]
15	9.55±0.04[c]	9.87±0.01[b]	10.04±0.01[a]	9.95±0.03[ab]

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (a = 5%).

Table 18: Effect of fermentation temperature to acidity (g/l) in wine

Fermentation time (days)	Acidity in wine (g/l)			
	28.5°C	29.0°C	29.5°C	30°C
3	1.21±0.03[c]	1.27±0.01[b]	1.33±0.02[a]	1.29±0.03[ab]
6	1.30±0.01[c]	1.38±0.02[b]	1.42±0.01[a]	1.40±0.00[ab]
9	1.38±0.02[c]	1.41±0.01[b]	1.46±0.01[a]	1.43±0.01[ab]
12	1.45±0.02[c]	1.49±0.03[b]	1.53±0.02[a]	1.51±0.02[ab]
15	1.57±0.01[c]	1.63±0.01[b]	1.66±0.03[a]	1.65±0.00[ab]

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (a = 5%).

Table 19: Effect of fermentation temperature to total phenolic content (mg GAE/g) in wine

Fermentation time (days)	Total phenolic content (mg GAE/g)			
	28.5°C	29.0°C	29.5°C	30°C
3	569.30±0.03[c]	578.25±0.01[b]	585.28±0.03[a]	581.13±0.02[ab]
6	693.18±0.02[c]	721.30±0.02[b]	749.46±0.02[a]	741.12±0.00[ab]
9	784.92±0.00[c]	798.45±0.00[b]	821.32±0.01[a]	817.49±0.03[ab]
12	876.13±0.03[c]	803.05±0.03[b]	837.56±0.04[a]	830.24±0.01[ab]
15	890.29±0.01[c]	911.42±0.04[b]	929.36±0.02[a]	925.67±0.04[ab]

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (a = 5%).

Table 20: Effect of fermentation temperature to total flavonoid (mg CE/g) in wine

Fermentation time (days)	Total flavonoid (mg CE/g)			
	28.5°C	29.0°C	29.5°C	30°C
3	85.37±0.04[b]	94.29±0.03[b]	98.40±0.00[a]	96.13±0.03[ab]
6	103.26±0.02[b]	118.49±0.02[b]	131.20±0.02[a]	122.38±0.01[ab]
9	124.33±0.00[b]	135.77±0.01[b]	143.54±0.01[a]	139.64±0.00[ab]
12	129.40±0.00[b]	139.75±0.03[b]	148.75±0.04[a]	142.23±0.01[ab]
15	131.46±0.02[b]	142.48±0.02[b]	151.69±0.01[a]	147.30±0.03[ab]

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (a = 5%).

Table 21: Effect of fermentation temperature to sensory characteristics in wine

Fermentation time (days)	Sensory score of wine (1-5) by different yeast ratio			
	28.5°C	29.0°C	29.5°C	30°C
3	2.42±0.01[c]	2.63±0.03[b]	2.84±0.02[a]	2.79±0.03[ab]
6	3.42±0.02[c]	3.58±0.00[b]	3.95±0.02[a]	3.64±0.01[ab]
9	3.94±0.00[c]	4.11±0.01[b]	4.25±0.04[a]	4.18±0.03[ab]
12	4.14±0.03[c]	4.22±0.03[b]	4.32±0.01[a]	4.28±0.00[ab]
15	4.20±0.01[c]	4.35±0.02[b]	4.46±0.02[a]	4.39±0.01[ab]
Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (a = 5%).				

The effects of yeast strains, fermentation temperature and pH on quality of *Rhodomyrtus tomentosa* wine were examined. At ambient temperature (28±2oC), 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 104÷107 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 SO2 concentrations met the Vietnamese Standards (QCVN 6-3 2010/BYT). In addition, the total acid, ester and aldehyde concentration were also low (Nguyen Minh Thuy et al., 2014)

Effect of Secondary Fermentation to Wine Quality

Alcoholic fermentation leads to a series of by-products in addition to ethanol. They include carbonyl compounds, alcohols, esters, acids, and acetals, all of them influencing the quality of the finished product. The composition and concentration levels of the by-products can vary widely [28]. Many of the polyphenols and other bioactive compounds in the source materials are bonded to insoluble plant compounds. The winemaking process releases many of these bioactive components into aqueous ethanolic solution, thus making them more biologically available for absorption during consumption [29].

During maturation, aging and storage of wine, coloured and noncoloured phenolics have an important role on the colour and taste of wine and they undergo a number of reactions during aging that result in changes of the sensory characteristics. We preserved *Rhodomyrtus tomentosa* wine at 10.5°C in dark bottle by different time (2, 4, 6, 8 weeks) as the secondary fermentation or aging. We monitored the residual soluble dry matted (°Brix), ethanol (% v/v), acidity (g/l), total phenolic compounds (mg/g), total flavonoids (mg/g) and sensory characteristics (score) in wine. Our results were elaborated in table 22. We noted that the longer of the secondary fermentation, the better of wine quality we got. However, there was not significant change of samples being preserved at the 6[rd] and 8[th] week so we choosed 6 weeks of secondary fermentation for economy.

Table 22: Effect of the sencondary fermentation or aging to wine quality

Criteria	Secondary fermentation (weeks)			
	2	4	6	8
Soluble dry matter (°Brix)	4.65±0.02[a]	4.59±0.03[ab]	4.35±0.03[ab]	4.21±0.02[b]
Ethanol (%v/v)	10.12±0.04[b]	10.29±0.01[ab]	10.42±0.01[ab]	10.48±0.01[a]
Acidity (g/l)	1.67±0.01[b]	1.68±0.02[ab]	1.70±0.02[a]	1.70±0.04[a]
Total phenolic content (mg GAE/g)	929.18±0.03[a]	928.16±0.00[a]	927.85±0.00[a]	927.74±0.03[a]
Total flavonoid (mg CE/g)	150.31±0.00[a]	149.86±0.01[a]	149.83±0.02[a]	149.78±0.00[a]
Sensory score	4.49±0.04[b]	4.53±0.03[ab]	4.62±0.01[a]	4.63±0.02[a]
Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (a = 5%).				

During fermentation, the pH of the wine reaches a value of 3.5–3.8, suggesting that an acidic fermentation takes place at the same time as the alcoholic fermentation. Final alcohol content was about 7-8% within a fortnight.

Acids present in wine enhance the taste, aroma, and preservative properties of the wine. As soon as the desired degree of sugar disappearance and alcohol production has been attained, the microbiological phase of winemaking is over. The wine was then pasteurized at 50°C–60°C. The temperature should be controlled so as not to heat it to about 70°C, since its alcohol content would vaporize at a temperature of 75°C–78°C [30].

Conclusion

Wine is an alcoholic beverage producing by fermentation of yeast, *Saccharomyces cerevisiae* in fruit juice. Yeast grows and converts sugar in fruit juices into alcohol and carbondioxide. Wine is a food with a flavor like fresh fruit which could be stored and transported under the existing conditions. Being fruit-based fermented and undistilled product, wine contains most of the nutrients present in the original fruit juice. The nutritive value of wine is increased due to the release of amino acids and other nutrients from yeast during fermentation. We have successfully utilized *Rhodomyrtus tomentosa* as substrate for wine fermentation by investigating different parameters such as pectinase concentration and time of treatment for juice extraction, soluble dry matter of the juice ready for fermentation, yeast ratio for wine fermentation, and secondary fermentation to wine quality. Wine has great health benefits similar to those of fruits from which they are derived. Fermentation is a cheap and energy efficient means of preserving perishable raw materials such as *Rhodomyrtus tomentosa* juice.

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