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**FACTORS AFFECTING THE FERMENTATION PROCESS OF
VIETNAMESE TRADITIONAL WINE ("MEN LA" WINE) USING "BA
NANG" WINE STARTER**

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ABSTRACT

“Men la” wine is a traditional wine product of the upland people in Vietnam. The wine is of the cultural essence of the nation, reflecting the current trend of one village one product (OCOP). The “Ba Nang” wine starters are usually made from sticky rice and local herbs. In fact, the use of herbs in traditional wine production has become popular. However, the traditional methods used by the local producers are not standardized. The quality of the wines produced is therefore not stable and wine producers resort to laborious trials and personal experience. The aim of this study was to examine the factors affecting the fermentation process in the production of “Men la” wine using the wine starters of the Van Kieu people in Da Ban, Ba Nang commune, Dakrong district, Quang Tri province, Vietnam. The ratio of yeast to rice, solid fermentation time, temperature, and time of liquid fermentation, as well as some biochemical criteria, were investigated to determine the best parameters for “Men la” wine production. The results showed that the optimal parameters for obtaining the highest quality of wine were: a mixture ratio of wine starter and whole rice of 8g: 1000g; solid fermentation for 4 days at 30 – 32°C; and fermentation for 7 days at 25°C with a ratio of rice ingredients and water of 1:2. The final product contained 4.952% of total sugar, 0.08% of reducing sugar, 0.315% acid, 0.104 g/L amino acid, and 10.61g/100mL of ethanol. The analysis did not detect methanol in the distilled wine products. The finished wine produced on a laboratory scale had a sweet taste equivalent to that of wine, with a flavor that was thought to be superior to the local wine. The alcoholic fermentation time was shortened by 2 days compared with local wine. The resultant “Men la” wine met the National Technical Regulations for alcoholic beverage products (QCVN 6-3:2010/BYT).

Key words: Fermentation starter, wine starters, “men la” wine, Vietnam traditional wine

INTRODUCTION

In Vietnam, the craft of wine has existed for a long time in folk culture. In mountainous areas, ethnic minorities use rice, corn, and potatoes, cooked and fermented. Wine starters are cultured with rice and certain forest leaves including *Piper gymnostachyum* DC., *Adenosma caeruleum* R. Br, *Alpinia galangal* (L.) Willd., *Derris elliptica* (Roxb.) Benth., *Zanthoxylum nitidum* (Roxb.) DC that locals call leaf wine starter, or “banh men la”. After the alcoholic fermentation and distillation process is done, the product is white wine (ruou trang). If not distilled, it is called “ruou can,” which is a traditional product and is famous in the mountains of Vietnam. The traditional wine starters, which are passed down from generation to generation, are a unique secret of each locality. There are different types of wine starters in different regions. For example, in the Mekong Delta, there are traditional wine starters that are made of rice flour, cassava flour, some spices and herbs and in high mountainous areas like “Lang Son” province, there are wine starters that are a combination of rice, leaves, forest tubers. The wine from “Van” village, Bac Giang province, is famous for being cooked from glutinous rice combined with precious medicinal herbs. In different localities, there are different techniques and different recipes for making wine starters.

In general, the main ingredient of the wine starter is rice flour (glutinous rice), but other ingredients can also be used, such as Chinese medicine wine starter, or locally available leaves or berries. Thus, there is a typical wine flavor for each locality.

Besides wines produced by modern technology, people still want to consume traditional wines, and are often prompted in the direction of semi-manufacturing. Many studies have been conducted on the production of traditional wines. The comparison between wine products fermented from “medombae” wine starter (Cambodia) and “Phuong Trang” wine starter (Vietnam), revealed that “Phuong Trang” commercial wine starter for fermentation was better, as the alcohol content and aroma of the final product were better than in wine produced with the local “medombae” wine starter [1].

Additionally, the use of herbs in traditional wine production has become popular. Herbal use can be carried out in two ways: (1) adding herbal extracts to the mixture during fermentation or (2) adding them to the wine starter [2, 3, 4]. Both of these methods aim at enhancing aroma, antimicrobial and antioxidant properties. Both methods are very popular in Asian countries (India, China, Thailand, Vietnam, Philippines, Cambodia) and in the Americas (Mexico, Argentina). Wine starter is said to be one of the important determinants of the quality of wine products. However, wine starters have obvious regional characteristics in terms of the local herbal source. Therefore, the wine product (“men la” wine) is very diverse. Some herbs popular in Thailand for wine starters are species commonly used by people in everyday life, such as galangal (*Alpinia officinarum*), licorice (*Glycyrrhiza glabra*), and black pepper. The herbal wine starters such as Loog-pang are made from galangal, garlic, long pepper, licorice, and black pepper at a ratio of 0.5:8:1:4:1, respectively. Waste paddy has been used directly as a renewable resource for fuel ethanol production using solid-state fermentation (SSF) with Loog-pang [5]. The fermentation capacity of the yeast yielded

the maximum ethanol productivity (4.08 g/kg waste paddy/hour) and the highest ethanol concentration (149 ± 7.0 g/kg waste paddy) after 48 hours of incubation [5].

Consumer demand for different wines is constantly evolving. Thus, the new techniques and processes should focus on creating differentiated wine styles with high quality. "Men la" wines are usually mellow and fragrant, and are the unique cultural features of each ethnic community. This article assesses the factors that affect the fermentation and quality of "Men la" wine from "Ba Nang" wine starter of the ethnic minority in Da Ban village, Ba Nang commune, Dakrong district, Quang Tri province, Vietnam.

MATERIALS AND METHODS

Wine yeast starter samples were obtained from the "Van Kieu" people in Da Ban, Ba Nang commune, Dakrong district, Quang Tri province, Vietnam. The materials used for the preparation of wine yeast starter are leaves and other parts of local medicinal plants, starch, and sometimes people add yeast, mould and other microorganisms into the starter mixtures. Samples were stored at 4°C. Rice materials used by "Khang Dan" seeds were grown in Phu My commune, Phu Vang district, Thua Thien Hue province, Vietnam.

Determination of the ratio of yeast to rice and solid fermentation time

The traditional process was adjusted to make leaf yeast on a laboratory scale in the following manner. First, one kilogram of Khang Dan rice was crushed and cleaned in distilled water 3 - 4 times until water from the crumpled rice was clear. Then, 100g of rice was put into a 500mL conical flask, where it was soaked in distilled water, with a ratio of 1:1.2 rice and water for 3 hours at room temperature (25°C). The conical flask was covered with a cotton button. Next, rice was steamed in an autoclave for 1 hour at 100°C to gelatinize, and then cooled to 30 – 32°C. The rice was then mixed thoroughly with wine yeast starter at different ratios (CT1: 0.6g/100g, CT2: 0.7g/100g, CT3: 0.8g/100g, CT4: 0.9g/100g, CT5: 1.0g/100g, CT6: 1.1g/100g, CT7: 1.2g/100g) and the content of reducing sugar and total sugar was determined. Mixture was incubated at a temperature of 30 – 32°C for about 3 days for the mold to grow and develop and convert starch into sugar through enzymes secreted by molds. Acid-producing bacteria create a favorable pH environment for yeast to function [6]. The aerobic fermentation in solid state lasted for 4 days at 30°C. Then, 200ml of sterile water was added to flood the rice block after aerobic fermentation stage, and liquid fermentation was carried out for 9 days at 25°C. The content of reducing sugar, total sugar, alcohol, amino acid, and total acid in the product were then determined. Sensory evaluation was also conducted. Each data is the mean of three replicates.

Determination of temperature and time of liquid fermentation

After determining the ratio of leaven leaves and rice material, as well as the solid fermentation time, the temperature and time of liquid fermentation were determined, using the same procedure as above.

Analytical method

Content of reduced sugar and total sugar was determined by the Bertrand method [7], amino acid content by the Sorensen method, and total acidity by the AOAC Method No. 942.15 [8].

After liquid fermentation, all of the fermented rice mass was homogenized using a Stomacher Lab-blender, the mix was centrifuged at 3600 rpm for 20 minutes, the decanted liquid was analyzed for alcohol content. The supernatant was filtered using a 0.2 μm syringe filter (Sartorius Stedim Biotech, Goettingen, Germany). The alcohol content was determined using the Agilent Technologies 1260 infinity (USA) high performance liquid chromatograph (HPLC) with the Rezex ROA-Organic Acid H⁺ column (150 \times 7.8 mm) (Phenomenex, Torrance, CA, USA) and 0.005 N Sulfuric acid as the mobile phase. The methanol content in alcohol after simple distillation was determined according to AOAC 972.11 method (Methanol in distilled liquors by gas chromatography) [9].

Sensory evaluation

The sensory quality of "Men la" wine was evaluated by using the 9-point Hedonic scale, which is commonly used for testing consumer preference and acceptability of foods [10]. Three categories: color and clarity, odor, and taste were evaluated by 100 trained panelists.

Data processing methods

Experimental results were processed on Excel software, and analysis of one-factor variance ANOVA (One-way ANOVA) and comparison of the mean values by Tukey method (Tukey method) at 95% level of confidence were done using Minitab statistical package, version 18.0.

RESULTS AND DISCUSSION

Determination of the optimal ratio of yeast to rice and solid fermentation time

The wine yeast starter and cooked rice were mixed together for solid fermentation at 30 – 32°C for 5 days. Table 1 shows that the longer the solid fermentation time, the more the reducing sugar content, initially increasing slowly and then increasing faster, and then finally decreasing. In CT 1, after 2 days of fermentation, reducing sugar content increased slowly to 2.86%. From the 3rd to the 4th day of fermentation, the reduced sugar content increased dramatically, reaching 4.56% on day 3 and 8.64% on day 4, but on day 5, reducing sugar content came down to 7.3%. The increase and decrease in reducing sugar content were similar with each recipe. This was expected, because when the mold is under favorable environmental conditions, it takes time to adapt to the environment back to its active state. The temperature of the fermentation block increased gradually, and as molds grow and thrive, they secrete the enzymes α -amylase, β -amylase, and γ -amylase to convert starch into reducing sugars (glucose, maltose), creating a source of raw materials for them to use [11]. The total sugar content in each recipe decreased during 5 days of solid fermentation at 30 – 32°C. In CT 1, total sugar content in the first 3 days decreased rapidly. Through days 4 to 5, the sugar content decreased more slowly. The cause of this slower reduction was likely due

to the glycemic activity of the mold and to some of the glucose continuing to ferment anaerobically and convert to ethanol. The reduction in total sugar content during solid fermentation was similar in each recipe. From the above analysis, we chose the ideal time of solid fermentation to be 4 days at 30 – 32°C. The results of our study were similar to previous publications, where optimal solid fermentation time was found to be 2 - 3 days [12].

After solid fermentation, liquid fermentation was at 25°C for 9 days (traditional for indigenous peoples) with different ratios of yeasts to other ingredients, aiming to determine the criteria for fermentation, in terms of the appropriate alcohol mixing ratio. The results are shown in Table 2. When the ratio of wine yeast starter to the other ingredients increased, the total and reducing sugar content decreased after 9 days of liquid fermentation. The total acid content increased from 0.36% to 0.84% with increased percentage of wine yeast starter from 0.6g to 1.2g, with the highest total acid content from glaze 1.2g (CT7) and the lowest at 0.6g (CT1). As for the amino acid content, with increased percentage of wine yeast starters, the highest amino acid content was 0.198% in CT1, and the lowest at 0.15% in CT7. The active yeast was fungi such as *Aspergillus spp.* and *Rhizopus spp.*, which helped to create organic acids. During alcohol fermentation there was an increase in the total acid concentration and a change in organic acid composition of the fermentation medium, as well as production of common amino acids to confer flavor to the final products [13]. At high temperature, in an acidic environment, tryptophan will completely decompose, and threonine, serine, methionine, and cysteine will create both a sweet and bitter taste in the finished wine. When the ratio of wine yeast starters increase, it can create an acidic environment where amino acids can react to create other components that reduce amino acids, which is consistent with the known chemical properties of amino acids [3, 12].

Alcohol concentration is an important factor affecting the performance of the winemaking process. Besides, it could be that the residual sugar content at the end of the fermentation influenced the alcohol level. Table 2 shows that with increasing ratio of wine yeast starters from 0.6g to 0.8g (CT1, CT2, CT3), the alcohol concentration increased from 10.45% v/v to 11.15% v/v. However, increased ratio of wine yeast starters from 0.9g to 0.12g (CT4, CT5, CT6, CT7) caused a decrease in the alcohol concentration from 10.92% v/v to 10.05% v/v, likely due to the higher proportion of yeast causing the fermentation process to take place faster, resulting in a faster reduction of the substrate content. In addition, a small part of ethanol was likely oxidized by bacteria to acids, decreasing the alcohol content; whereas when the amount of yeast was less, the metabolism of sugar to create alcohol was less, producing a lower alcohol concentration [15].

On visual inspection, the color of the final product was ivory white (CT1, CT2, CT3, CT4) to opaque white (CT5, CT6, CT7). The taste of the fermented rice was recorded at the end of the fermentation. The end products had a clear wine flavor in all cases, and CT3, CT4 had a mellow aroma compared to the rest of the samples. From the above results, the optimal solid fermentation time chosen was 4 days, and used 0.8g wine yeast starters per 100g of culture for the following experiments.

Determination of liquid fermentation temperature

The solid fermentation was conducted with 0.8g wine yeast starter per 100g of the culture after 4 days at a temperature of 30 – 32°C. Then liquid fermentation was conducted with a ratio of rice to water of 1:2, at temperatures of 25°C, 30°C, or 35°C. The time of liquid fermentation was fixed at 9 days, in order to determine the parameters of the fermentation fluid and the alcohol level and to compare the fermentation quality at different temperature levels.

With the same time of fermentation, but at different temperatures, the residual sugar content of the fermentation solution in the samples was different. In general, the total and reducing sugar content were inversely proportional to the temperature. At 25°C, after 9 days of liquid fermentation, the total remaining sugar content (3.663%) showed a statistically significant difference ($p < 0.05$) from the sugar content at the other temperatures. Similarly, the reducing sugar content at 25°C also reached the highest value (0.052%), although there was no statistically significant difference from the reduced sugar content at 30°C. There was a significant difference in comparison with reducing sugar content at 35°C (0.034%). At 25°C, 30°C and 35°C, the acid content obtained was 0.537%, 0.656% and 0.746%, respectively. This increase in total acid content was likely due to the presence of lactic acid bacteria and acetic acid bacteria in the fermentation fluid [16]. The amino acid content with the lowest value was 0.14g/L at 25°C and the highest value was 0.28g/L at 35°C. The highest alcohol concentration was obtained at 25°C and decreased with increasing temperature of liquid fermentation. On visual inspection, the color of the final product was ivory white. The end products had a clear wine flavor in all cases, with products fermented at 25°C and 30°C giving a sweet smell, and products fermented at 35°C a slightly sour taste. From the above results, it could be that the appropriate temperature for fermentation was 25 - 30°C, but for the fermentation to be effective, generate less impurities, yield a high concentration of alcohol, and be easy to control, 25°C was the ideal fermentation temperature. This temperature can be used to investigate the effect of time on the fermentation process and on the quality of the finished wine.

Determination of liquid fermentation time

The solid fermentation was conducted with 0.8g wine yeast starter per 100g culture, then fermented for 4 days at 30 – 32°C. Next, the mixture underwent liquid fermentation with a ratio of rice: water of 1:2 at 25°C. The liquid fermentation took place for 1, 3, 5, 7, 9, 11, 13, and 15 days. The parameters determined during the fermentation process were: total sugar, reducing sugar, total acid, amino acid, and alcohol content.

Table 4 shows that the total and reducing sugar content of the fermentation solution tended to decrease gradually over the fermentation time, with the total sugar in the fermentation solution decreasing from 10.736% at Day 1, to 8.147% at Day 3, to 5.776% at Day 5. Along with the loss of the total sugar content, the reducing sugar also showed a marked decrease in the first 7 days of the fermentation. From 2.242% on Day 1 it decreased to 0.081% on Day 7, a statistically significant difference. From the 7th to the 11th day, the reducing sugar content decreased, but it was not statistically significant. At 13 - 15 days, the remaining reducing sugar content was quite low at

0.021%. From day 1 to day 5, the total acid content increased slightly but not statistically significant. After day 5, acid content increased significantly and reached the largest value on day 15 at 0.71%, an increase of 3.7-fold compared to the initial acid content. With amino acid content, there was no obvious change in the first 7 days, while after day 7, amino acid content increased rapidly and reached the highest value on the 15th fermentation day at 0.184%. In the fermentation of alcohol production, high amino acid content would inhibit the thorough fermentation of alcohol, affecting the taste, making it subjectively not pleasing; this was the major motive for using higher alcohols such as fusel oil. As just a small amount of fusel oil mixed in ethyl alcohol caused an unpleasant odor, higher alcohols were also called fusel alcohols [14,15].

When increasing the fermentation time from 5 to 11 days, the alcohol level increased over time. After 5 days of fermentation the wine was 10.10 g/100mL, and after 11 days of fermentation, 11.02 g/100mL, the maximum value. The differences between the alcohol levels at 7, 9, 11, and 13 days were not statistically significant. Although there was little difference in the alcohol level when fermented for a long time, many byproducts could arise during that time, such as alcohols (furfurol, methanol), aldehydes, and amino acids, which would make the taste of alcohol neither aromatic nor delicious and thus not economically viable; thus short-term fermentation should be favored [12,16]. Upon visual inspection, the color of the final product was ivory white. The final product had a clear wine flavor from day 5, with fermentation for 7 to 11 days still giving a sweet smell, but if fermented for 13 to 15 days yielding a slightly sour taste. It could clearly be seen that the best fermentation time in terms of economics, high flavor, and alcohol content was 7 days.

Product quality assessment

From the above results, alcohol was produced on a laboratory scale with 0.8g wine yeast starters per 100g of culture after 4 days of solid fermentation at 30 – 32°C, then proceeded to conduct liquid fermentation in the ratio of rice to water of 1:2 at 25°C after 7 days. The end product was distilled under laboratory conditions. According to this, around 20% of the first and last volume of distillation, was removed, and 60% of the volume was taken for the determination of methanol content and sensory evaluation compared to the indigenous wine product of Van Kieu people.

The final wine product did not contain methanol, meeting the international standards of methanol in distilled alcohol. The finished wine produced by the laboratory was transparent in color, with sweetness equivalent to, and an aroma that was more favored than, the indigenous wine of Van Kieu people.

CONCLUSION

This study determined the optimal technical parameters for the production of high quality "Men la" wine, meeting the provisions of the National Technical Regulations for alcoholic beverage products (QCVN 6-3:2010/BYT). This study demonstrated that wine produced using the "Ba Nang" starters of Van Kieu ethnic minority of Vietnam with a ratio of wine starter to rice of 0.8g:100g, solid fermented for 4 days at 30°C, and

liquid fermented for 7 days at 25°C with a ratio of rice ingredients to water of 1:2, gave the high quality final wine products.

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Table 1: Content of reducing sugar and total sugar according to the different ratios of wine yeast starter and rice material (%) after solid fermentation time

Sample	Days	1	2	3	4	5
	Content					
CT1	Reducing sugar	0,98 ^c	2,86 ^d	4,56 ^c	8,64 ^a	7,3 ^b
	Total sugar	37,5 ^a	34,2 ^b	28,6 ^c	25,4 ^d	24,33 ^c
CT2	Reducing sugar	0,93 ^c	2,63 ^d	4,46 ^c	8,47 ^a	7,18 ^b
	Total sugar	38,77 ^a	36,5 ^b	34,33 ^c	30,63 ^d	28,37 ^c
CT3	Reducing sugar	0,89 ^c	2,56 ^d	4,33 ^c	8,37 ^a	6,69 ^b
	Total sugar	36,53 ^a	35,4 ^b	31 ^c	23,7 ^d	22,77 ^c
CT4	Reducing sugar	0,88 ^c	2,45 ^d	4,23 ^c	8,27 ^a	6,46 ^b
	Total sugar	34,7 ^a	34,53 ^b	25,7 ^c	23,5 ^d	22,77 ^c
CT5	Reducing sugar	0,85 ^c	2,36 ^d	4,16 ^c	8,2 ^a	6,23 ^b
	Total sugar	37,67 ^a	36,47 ^b	35,8 ^c	27,6 ^d	26,07 ^c
CT6	Reducing sugar	0,85 ^c	2,26 ^d	3,97 ^c	7,86 ^a	6,2 ^b
	Total sugar	38,47 ^a	36,77 ^b	27,23 ^c	25,37 ^d	24,37 ^c
CT7	Reducing sugar	0,83 ^c	2,2 ^d	3,86 ^c	7,78 ^a	5,96 ^b
	Total sugar	33,47 ^a	25,03 ^b	23,93 ^c	21,6 ^d	20,33 ^c

Note: The different letter in the same column stated the statistical different at 95% confidence level ($p < 0.05$)

Table 2: Content of total sugar, reducing sugar, total acid, amino acid and alcohol content after 9 days of liquid fermentation at different ratios of yeast and rice materials

Samples	Total sugar content (%)	Reducing sugar content (%)	Total acid content (%)	Amino acid content (g/L)	Ethanol content (g/100mL)
CT1	4,88 ^a	0,13 ^a	0,36 ^g	0,198 ^a	10,45 ^d
CT2	4,76 ^b	0,1 ^b	0,46 ^f	0,175 ^a	10,78 ^{bc}
CT3	4,24 ^c	0,06 ^c	0,53 ^e	0,144 ^b	11,15 ^a
CT4	3,95 ^d	0,05 ^{cd}	0,63 ^d	0,134 ^b	10,92 ^b
CT5	3,65 ^e	0,04 ^{de}	0,66 ^c	0,125 ^{bc}	10,73 ^{bc}
CT6	3,46 ^f	0,04 ^{de}	0,72 ^b	0,115 ^c	10,70 ^c
CT7	3,18 ^g	0,03 ^e	0,84 ^a	0,105 ^c	10,05 ^e

Note: The different letter in the same column stated the statistical different at 95% confidence level ($p < 0.05$)

Table 3: Content of total and reducing sugar, total acid and amino acid, alcohol content at different fermentation temperatures with the time of liquid fermentation was 9 days

Temperature (degree Celsius)	Total sugar content (%)	Reducing sugar content (%)	Total acid content (%)	Amino acid content (g/L)	Ethanol content (g/100mL)
25	3,663 ^A	0,052 ^a	0,537 ^C	0,140 ^c	11.04 ^A
30	3,107 ^B	0,041 ^{ab}	0,656 ^B	0,251 ^b	10.83 ^B
35	2,099 ^C	0,034 ^b	0,746 ^A	0,276 ^a	10.05 ^C

Note: The different letter in the same column stated the statistical different at 95% confidence level ($p < 0.05$)

Table 4: Content of total and reducing sugars, total acid and amino acids, alcohol content at different fermentation temperatures with different liquid fermentation times

Time (Days)	Total sugar content (%)	Reducing sugar content (%)	Total acid content (%)	Amino acid content (g/L)	Ethanol content (g/100mL)
1	10,736 ^A	2,242 ^a	0,195 ^F	0,096 ^e	6,92 ^E
3	8,147 ^B	0,656 ^b	0,207 ^F	0,098 ^e	8,25 ^D
5	5,776 ^C	0,119 ^c	0,232 ^F	0,104 ^e	10,10 ^C
7	4,952 ^D	0,081 ^{cd}	0,315 ^E	0,104 ^e	10,61 ^B
9	4,310 ^E	0,071 ^{cd}	0,417 ^D	0,123 ^d	10,88 ^{AB}
11	3,671 ^F	0,053 ^{cd}	0,523 ^C	0,147 ^c	11,02 ^A
13	3,230 ^G	0,033 ^d	0,655 ^B	0,165 ^b	10,83 ^{AB}
15	2,689 ^H	0,021 ^d	0,710 ^A	0,184 ^a	10,61 ^B

Note: The different letter in the same column stated the statistical different at 95% confidence level ($p < 0.05$)

Table 5: Methanol content, sensory assessment of indigenous alcohol and wine produced by laboratory scale

Samples	Color and clarity	Odor	Taste	Methanol (%V/V Ethanol 100°)
Indigenous wine	7,66 ^a	7,75 ^b	5,81 ^a	Not detected
Alcohol produced on a laboratory scale	8,13 ^a	7,13 ^a	6,34 ^a	Not detected

Note: The different letter in the same column stated the statistical different at 95% confidence level ($p < 0.05$)

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