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Technical Parameters Influencing to the Fermentation of Seeded Banana (*Musa Balbisiana*) Wine

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Abstract.

Banana fruit has flesh not only rich in starch which changes into sugars on ripening but is also a good source of resistant starch. Banana is known to be rich in carbohydrates, dietary fibres, certain vitamins, and minerals. Banana is one of fruits with high level of potassium. Due to its high nutrient values, bananas are nutritious food recommended for people at all ages, especially for baby, also diet food for adults. In order to accelerate the added value of this valuable fruit, we investigated the wine fermentation from ripen banana. Substrate concentration, pH, and soluble dry matter content in Banana juice were an important parameters strongly affecting to wine fermentation. We used bentonite and isinglass as coagulant supporting for the clarification. Our results showed that banana juice must be diluted at ratio (1.0: 1.0, juice: water) ready for the fermentation. The fermentation process could be accomplished at the 14th day at pH = 4.2, ^{o}Brix = 20. Turbidity in the fermented fluid could be removed effectively by treating with 1.0 gram of bentonite per 1 litter of fermented fluid in 6 weeks to get a good appearance of wine bottle.

Keywords: *Banana, fermentation, coagulant, bentonite, isinglass, clarification*

1. INTRODUCTION

Banana is an edible fruit and is herbaceous flowering plant belonging to the genus Musa and the family *Musaceae*.[1] Banana belongs to the tropical fruits as it grows more profusely in tropical rain forest areas . It is a widespread species and available in Vietnam. The majority of edible bananas had their origins from two wild seeded banana species i.e. *Musa acuminata* Colla and *Musa balbisiana* Colla.[2] It is a good source of minerals. Composition and nutrient values of banana fruits are varies of each cultivar. It is indicated by the increase of carbohydrates level in form of starch when the fruit turn mature.[3] It may be incorporated as functional ingredient of diet and useful as therapeutic agents in treating free radical related pathological damages.[4] *Musa balbisiana* seeds have their activity against *Cryptolestes pusillus*.[5] *Musa balbisiana* root extract has antidiabetic and antilipidemic effect.[6] The lipophilic extract of ripe banana pulp from several cultivars of the M. acuminata and M. balbisiana species has been found to be a source of ω-3 and ω-6 fatty acids, phytosterols, long-chain aliphatic alcohols, and α-tocopherol, thus offering well-established nutritional and health benefits.[7, 8, 9]

There were several studies mentioned to banana wine production. Different factors affecting to banana wine (*Musa chiliocarpa & Musa basjoo sieb*) fermentation were investigated.[10] A mixed fruit (pawpaw, banana and watermelon) wine using *Saccharomyces cerevisiae*isolated from palm wine was produced.[11] A study was aimed to improve production process of alcoholic beverage based maize and banana extract and to evaluate sensory parameters of the obtained alcoholic beverage.[12] So we investigated a wine fermentation from this valuable source. We focused on examining the effect of soluble dry matter and pH in banana juice before fermentation, effect of bentonite and isinglass as coagulant.

2. MATERIAL & METHOD

2.1 Material

We collected banana fruits in Soc Trang province, Vietnam. They must be cultivated following VietGAP to ensure food safety. After harvesting, they must be conveyed to laboratory within 8 hours for experiments. Beside Banana fruits, we also used other materials during the research such as citric acid, ascorbic acid, NaOH, HCl, $KMNO_4$, $K_2Cr_2O_7$, KI, $Na_2S_2O_3$, $NaHSO_3$, starch, sacharose, *Saccaromyces cerevisiase*, bentonite, isinglass. Lab utensils and equipments included grinder, pH meter, spectrophotometer, refractometer, weight balance, electrical oven, fermentation tank.

2.2 Research method

2.2.1 Investigate nutritional contents in the raw banana fruit

Ripen banana fruits must be washed under tap water thoroughly before being mashed and filtered to get juice for fermentation. In the juice, we analyzed soluble dry matter, moisture, acidity, sugar and pH.

2.2.2 Investigate the effect of juice concentration for fermentation

Our experiment focused on different dilution ratios of juice with water (1:0, 1: 0.5, 1:1, 1:1.5) in three replications. Then we added more sugar to 18^{o}Brix, pH=4.2. After that, the fluid was sterilized by $NaHSO_3$ in 100 mg/l by 20 minutes. The yeast addition with 0.05% was applied. The fermentation process was monitored in 14 days. We analyzed the remaining sugar and ethanol to find the end of fermentation. After the primary fermentation, the fermented wine was kept intact in 6 weeks ready for coagulation and filtration. Sensory evaluation was carried out to find out the best sample with the appropriated dilution ratio or juice concentration.

2.2.3 Investigate the effect of pH and sugar content before fermentation

We examined different pH values (4.0, 4.2, 4.4) and sugar concentration (18, 20, 22^{o}Brix). The fluid was sterilized by $NaHSO_3$ in 100 mg/l by 20 minutes. The yeast addition with 0.05% was applied. The fermentation process was monitored in 15 days. After the primary fermentation, the fermented wine was kept intact in 6 weeks ready for coagulation and filtration. Sensory evaluation was carried out to find out the optimal values of pH and sugar content.

2.2.4 Investigate the effect of coagulating agents for filtration

Ripen Banana fruits must be washed under tap water thoroughly before being mashed and filtered to get juice for fermentation. Dilution ratios of juice with water (1:1.0), sugar to 20^{o}Brix, pH=4.2 were applied. After that, the fluid was sterilized by $NaHSO_3$ in 100 mg/l by 20 minutes. The yeast addition with 0.05% was applied. The fermentation process was monitored in 15 days. After the primary fermentation, the fermented wine was kept intact in 1, 2, 3, 4, 5, 6 weeks ready for coagulation and filtration on diatomit (0 g/l, 0.5 g/l, 1 g/l, 1.5 g/l) and isinglass (0 g/l, 0.5 g/l, 1 g/l, 1.5 g/l) as wine fining and clarifying agents. Sensory evaluation and tubidity (spectrophotometer) were carried out to find out the optimal concentration of wine fining and clarifying agent.

2.2.5 Physical, chemical and sensory analysis

We collected 100 ml of each sample for testing. We analyzed different parameters: sugar content (Bectran), acidity (pH meter), vitamin C (titration), ethanol (distillation), soluble dry matter (refractometer), turbidity (spectrophotometer). Sensory acceptance was evaluated by consumer satisfaction in score range from 1 to 5 for wine.

2.2.6 Statistical analysis

Data were statistically summarized by Statgraphics.

3. RESULT & DISCUSSION

3.1 Nutritional contents in the raw banana fruit

Testing analysis results in raw banana fruit were depicted in table 1. We saw that soluble dry matter in Banana juice was quite high, so it's quite convenient for fermentation.

Nutrient analysis revealed that mature banana pulp contain of high carbohydrates (16.72-35.24 g/100g), total sugar (12.12-20.82 g/100g), vitamin C (16.45-30.27 g/100g) and potassium (275-375 g/100g); moderate protein (1.48-1.78 g/100g) and low fat (0.03-0.08 g/100g). About 100 g edible portion of banana fruit produce73.43 to 148.80 calories. It is one of fruits with high level of potassium. Potassium content in bananas were varies between 275 mg to 375 mg.[2]

3.2 The effect of juice concentration for fermentation

Fermentation involves a reaction that converts the sugars in the juice into alcohol and carbon dioxide. Yeasts utilise the sugars during the fermentation period. From table 2, 3 and 4 we clearly noticed that dilution ratio of banana juice: water (1:0.5) was optimal for wine fermentation in 10 days so we selected these values for further studies.

The quality properties of alcoholic beverages made from ripe banana were examined. Long and short hand banana (LHB and SHB) of the genus *musa* were used. The extracted juices contained 15 0brix, pH ranged between 4.28 and 4.58, titratable acidity (TA) ranged between 0.23 and 0.27g/100ml. LHB extract was light yellow while SHB extract was dark yellow in color.[13]

Table 1. Nutritional contents in the raw banana fruit

Description	Value
Moisture (%)	83.21±0.01
Soluble dry matte (^{o}Brix)	13.44±0.03
pH	4.0±0.02
Reducing sugar (%)	10.85±0.00
Total acidity (%)	0.38±0.01
Vitamin K (%mg)	281.38±0.02

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

Table 2. Sugar and ethanol in the fermented Banana wine

Dilution ratio (juice:water)	Remaining sugar (g/100ml)	Ethanol (%v/v)
1:0	4.13±0.01^a	8.21±0.02^c
1:0.5	3.84±0.03^b	9.15±0.01^b
1:1	2.44±0.02^c	9.94±0.01^a
1:1.5	1.35±0.01^d	7.06±0.00^d

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

Table 3. Fermentation by time on the effect of juice concentration

Fermentation time (days)	Remaining sugar (g/100ml)	Ethanol (%v/v)
2	18.44±0.01^f	3.48±0.01^e
4	15.35±0.02^e	6.79±0.02^d
6	9.42±0.01^d	8.14±0.03^{cd}
8	6.31±0.01^c	9.22±0.01^c
10	2.01±0.01^{ab}	10.38±0.02^b
12	1.43±0.02^{ab}	11.16±0.00^a
14	0.85±0.01^a	11.20±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 (α = 5%).

3.3 Effect of pH and sugar content for wine fermentation

Fermentation proceeds under anaerobic conditions and may be boosted with di-ammonium phosphate (DAP) to supplement nitrogen required for yeast growth in non-traditional approach of winemaking.[14] Spoilage bacteria do not grow well below pH 3.6. Wine yeasts and some lactic acid bacteria can still metabolize in a pH range of 3.3–3.6. The low pH can prolong the fermentation process due to slow growth of microorganisms involved .[15] They demonstated pH as an important factor for the fermentation of fruit juice into a good quality wine. Low pH inhibits the growth of unwanted microflora and thus can improve the quality of final product.

Table 4. Sensory evaluation on the Banana fermentation by different dilution ratio

Dilution ratio	Color	Flavor	Taste	Turbidity
1:0	3.84±0.02^{a}	3.52±0.01^{a}	2.62±0.03^{b}	4.31±0.01^{a}
1:0.5	3.1±0.00^{b}	3.07±0.02^{b}	3.54±0.03^{a}	3.52±0.01^{b}
1:1	2.83±0.01^{c}	2.83±0.00^{c}	3.21±0.01^{a}	3.24±0.02^{bc}
1:1.5	2.14±0.01^{d}	2.31±0.00^{d}	2.81±0.02^{b}	2.94±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 (α = 5%).

Table 5. Effect of pH and sugar content for wine fermentation

Description	Demonstrated value	Remaining sugar (g/100ml)	Ethanol (%v/v)
pH	4.0	5.84±0.01^{b}	10.57±0.02^{b}
	4.2	5.75±0.01^{c}	11.69±0.01^{a}
	4.4	5.91±0.02^{a}	10.03±0.01^{c}
^{o}Brix	18	4.62±0.01^{b}	10.93±0.02^{b}
	20	4.83±0.03^{ab}	11.32±0.01^{ab}
	22	4.90±0.02^{a}	11.47±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 (α = 5%).

Table 6. Fermentation by time on the effect of pH and sugar content

Fermentation time (days)	**Remaining sugar (g/100ml)**	**Ethanol (%v/v)**
2	17.14±0.01^{a}	2.68±0.03^{d}
4	14.29±0.03^{b}	6.11±0.01^{cd}
6	9.47±0.01^{c}	7.42±0.01^{c}
8	6.35±0.01^{d}	8.55±0.04^{bc}
10	3.26±0.02^{e}	9.37±0.00^{b}
12	1.40±0.01^{ef}	10.58±0.01^{ab}
14	0.95±0.03^{f}	11.20±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 (α = 5%).

Table 7. Sensory evaluation on the Banana fermentation by different pH and sugar content

Dilution ratio	Color	Flavor	Taste	Turbidity
pH: Sugar (4.0:18)	3.03±0.3^{b}	3.06±0.02^{b}	3.12±0.01^{c}	2.92±0.02^{bc}
pH: Sugar (4.0:20)	3.17±0.01^{ab}	3.17±0.01^{ab}	3.41±0.01^{ab}	3.04±0.01^{b}
pH: Sugar (4.0:22)	3.21±0.00^{ab}	3.20±0.01^{ab}	3.43±0.02^{ab}	3.15±0.00^{ab}
pH: Sugar (4.2:18)	3.55±0.02^{a}	3.58±0.02^{a}	3.70±0.01^{a}	3.80±0.03^{a}
pH: Sugar (4.2:20)	3.26±0.01^{ab}	3.38±0.01^{ab}	3.36±0.02^{b}	3.32±0.01^{ab}
pH: Sugar (4.2:22)	2.92±0.01^{b}	2.82±0.02^{bc}	2.94±0.03^{cd}	2.73±0.01^{bc}
pH: Sugar (4.4:18)	2.51±0.01^{c}	2.50±0.01^{c}	2.55±0.02^{d}	2.51±0.02^{c}
pH: Sugar (4.4:20)	2.92±0.00^{b}	2.81±0.01^{bc}	2.86±0.01^{cd}	2.83±0.01^{bc}
pH: Sugar (4.4:22)	3.14±0.02^{ab}	3.14±0.02^{ab}	3.25±0.01^{ab}	3.14±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 (α = 5%).

Fermentable sugars consist mainly of hexoses and are normally found at varying concentrations. One important reason is that a higher amount of ethanol is produced during alcoholic fermentation which will lead to higher toxicity towards the end of fermentation. From table 5, 6 and 7, we saw the optimal parameter for wine fermentation with the pH 4.2 and 20^{o}Brix so we selected these values for further experiments.

The quality properties of alcoholic beverages made from ripe banana were examined. Long and short hand banana (LHB and SHB) of the genus *musa* were used. Part of each extract was fermented at (30 ± 2)°C by adding wine yeast *(Saccharomyces cerevisiae)* and another, a combination of both wine yeast and lactic acid bacteria (LAB). It took 7 to 10 days for complete fermentation. Soluble solids (SS) and pH decreased while (TA) increased with Time. The banana beverage produced had values that ranged from 3.45 to 4.04 for pH, 0.77 to 1.1 g/100ml for TA, 6 0Brix, 5.5 to 6.45 for alcohol (v/v) percentage (%).[13]

Different factors affecting to banana wine (*Musa chiliocarpa & Musa basjoo sieb*) fermentation were investigated. The percentage of sugar 8% was added to the fermented mixture. Amount of yeast added to the fermentation batch was 20 g/l; pH 3.6 was appropriate. Fermentation time was 11 days.[10]

A study was aimed to improve production process of alcoholic beverage based maize and banana extract and to evaluate sensory parameters of the obtained alcoholic beverage. De-pectinized banana pulp was added to wort and the mixture was fermented in anaerobic condition, at ambient temperature of about 25 °C for 72 h in presence of *Saccharomyces cerevisiae*.[12]

3.3 Effect of coagulating agents for clarification

Clarification may be achieved by racking, filtration and/or centrifugation. In table 8, we saw the possitive effect of bentonite at 0.5 g/l in wine fining and clarifying. This result was similar to the research to demonstrate the effect of

bentonite fining at different stages of white winemaking on protein stability. Bentonite fining to remove protein is the most widely used treatment to prevent protein haze in white wines. Adding bentonite during fermentation or fining finished wines was the most efficient option in terms of amounts of bentonite required. Fermenting with bentonite present also may increase fermentation rates.[16]

A study was to apply enzyme preparations to banana pulp to improve wine clarity, alcohol yields and to assess sensory properties. The ultimate goal was to produce quality and safe banana wine with a stable shelf life. Commercial enzymes were applied to banana (*Musa* spp.) pulp. The materials used in the study included four selected commercial enzyme preparations and banana juices extracted from pulps of three banana cultivars. Compared with the control, the wine turbidity was lowered significantly in the wines prepared from juices that were extracted from commercial enzyme treated pulps. The effect of proteases on protein hazes was also investigated after periods of one week and four weeks. The addition of proteases had a significant effect on the protein hazes. A longer incubation period resulted in greater reduction in turbidity.[17]

Table 8. Effect of coagulating agents for clarification

Clarifying agent	Concentration (g/l)	Optical density
Bentonite	0	0.445 ± 0.01^{a}
	0.5	0.283 ± 0.03^{b}
	1.0	0.278 ± 0.02^{b}
	1.5	0.271 ± 0.01^{b}
Isinglass	0	0.420 ± 0.03^{a}
	0.5	0.119 ± 0.01^{c}
	1.0	0.116 ± 0.02^{c}
	1.5	0.110 ± 0.01^{c}

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

4. CONCLUSION

Banana plants are very adaptive to environment especially during drought condition. Banana is known to be rich not only in carbohydrates, dietary fibres, certain vitamins and minerals, but is also rich in many health-promoting bioactive phytochemicals. Utilization of banana fruit as the wine is expected to increase the economic value of the banana fruit. Therefore, manufacturing of wine from banana as a ready to serve value added product to Vietnamese beverage industry will be a good solution for the enormous wastage of local banana fruit.

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