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Different factors affecting the Mango (*Mangifera Indica*) Wine Fermentation

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Abstract.

Mango *(Mangifera indica)* is is a popular fruit due to its sweet taste and high nutrient ccontent. Mango possesses favourable nutritional characteristics as a source of phenolic compounds, carotenoids and vitamin C, excellent flavour, aroma and colour. Mangoes are fresh during the harvesting season but perishable under the prevailing conditions of temperature and humidity as well as lack of adequate storage facilities. An alternative way of preserving surplus mangoes could be to ferment the juice to fruit wine. Therefore we explored a wine fermentation from mango by focusing on the effect of different parameters such as pectinase concentration and time of treatment for juice extraction, yeast inculate for wine fermentation, and secondary fermentation to wine quality**.** Our results proved that 0.20% pectinase was used for juice extraction, 1.5% *sacchromyces cerevisiae* was used for the main fermentation at 12^{o}C, and 3 weeks of sencondary fermentation in dark bottle at 10^{o}C was applied to get a pleasant mango quality.

Keywords: *Mango, wine, fermentation, sacchromyces cerevisiae, pectinase*

1. INTRODUCTION

Mango (*Mangifera indica L.)* is one of the most important fruits in the tropics and subtropics. Mango *(Mangifera indica L.)* is rich in bioactive molecules that protect human cells against the detrimental effect of free radicals. The phytochemical analysis revealed the presence of alkaloids, terpenoids, saponins, tannins, phenolics and flavonoids. The antioxidant activity of mango fruit extracts is even greater than that of avocado.[1] Mango fruit is rich in antioxidants and, therefore, reduces the risk of cardiac disease, anti-diabetic, anticancer, anti-inflammatory and antiviral activities.[2, 3, 4] Mango peel had high flavonoids and tocopherols content and showed significant antioxidant activity. Beta-carotene content of mango peels significantly ranged from 9.14 to 11.98 µg/g while the vitamin C content ranged from 21.66 µg/g to 51.54 µg/g.[5] It could be used as a value added ingredient or functional food and may contribute considerably to promote consumer health.[6]

A study was to evaluate the physicochemical parameters of mango jellies with different concentrations (0% to 3.5%) of mango peel powder obtained from residues.[7] The probiotification of mango juice was carried out by lactic acid bacteria fermentation. Mango juice fermentation was performed at 30^{o}C for 72 h under micro-aerophilic conditions.[8] Production of ethanol from mango (*Mangifera indica* L.) fruit juice fermentation was investigated.[9] A study was to address the problem of large post harvest losses of mangoes by employing yeast fermentation technology to produce a more stable, value-added product in this case fruit wine.[10] The preparation of mango wine by yeast-mango peel immobilised biocatalyst system by repeated batch fermentation was conducted and compared to free cells fermentation at 15, 20, 25, and 30°C.[11] Production of ethanol from mango (*Mangifera indica* L.) peel by *Saccharomyces cerevisiae* was examined.[12] A study was to determine the effects of temperature and yeast concentration on the fermentation kinetics and chemical properties of Apple mango fruit wine through process optimization.[13] Production and Evaluation of Fruit Wine from *Mangifera indica* was examined.[14] It was found that the mango juices were similar to grape juice in terms of sugar and acidity. After fermentation, the ethanol concentration was 7–8.5% w/v, the methanol concentration was slightly higher than that of grape wines and other volatile compounds were present in comparable amounts. From the physicochemical characteristics of the mango wine produced, it was observed that aromatic components were comparable in concentration to those of grape wine.[15] Mango is an underutilized fruit crop and still now there is very limited research available regarding to processing of this fruit into value added product. The mango fruit, which typically has high fermentable sugar composition when mature and ripe, could be exploited as a substrate for alcoholic fermentation.[16] 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 inculate for wine fermentation, and secondary fermentation to wine quality.

2. MATERIAL & METHOD

2.1 Material

We collected mango in Ke Sach district, Soc Trang province, Vietnam. They must be cultivated following VietGAP without pesticide and fertilizer residue to ensure food safety. After harvesting, they must be conveyed to laboratory within 8 hours for experiments. Apart from collecting mango, we also used other materials such as pectinase, yeast. Lab utensils and equipments included knife, weight balance, fermentation tank, refractometer,

viscometer, flow UV system, pH meter, ethanol meter, buret.

Figure 1. Mango *(Mangifera indica)*

2.2 Research method

2.2.1 Effect of pectinase concentration and time for juice extraction

Mango extract was treated with pectinase enzyme with different concentration (0.1, 0.15, 0.20, 0.25%) in different duration (10, 15, 20, 25 minutes). We analyzed the extract recovery (%), viscosity (cP) and turbidity (mJ/cm^2).

2.2.2 Effect of yeast inculate for wine fermentation

Mango wort after being treated by pectinase would be inoculated with *Saccharomyces cerevisiae* at different ratio (0.5, 1.0, 1.5, 2.0%). After 10 days of fermentation at 12^{o}C, we analyzed the soluble dry matter (^{o}Brix), ethanol (%v/v), acidity (g/l), and sensory characteristics (score) in wine.

2.2.3 Effect of secondary fermentation to wine quality

We preserved mango wine at 8^{o}C in dark bottle by different time (1, 2, 3, 4 weeks) as the secondary fermentation. We monitored soluble dry matted (^{o}Brix), ethanol (% v/v), acidity (g/l), and sensory characteristics (score) in wine.

2.2.4 Statistical analysis

Data were statistically summarized by Statgraphics Centurion XVI.

3. Result & Discussion

3.1 Effect of pectinase concentration and time of treatment for juice extraction

Alcoholic fermentation is a combination of complex interactions involving must variety, micro biota and winemaking technology. Some factors strongly affect alcoholic fermentation, and consequently the quality of the wine. The most important factors are the clarification of the juice, the temperature of fermentation, the composition of the juice, inoculation with selected yeasts and the interaction with other microorganisms.[13] Mango extract was treated with pectinase enzyme with different concentration (0.1, 0.15, 0.20, 0.25%) in different duration (10, 15, 20, 25 minutes). Our results were depicted in table 1, 2 and 3. We clearly found that 0.2% pectinase in 20 minutes treatment was optimal for mango 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 (%)			
	10 minutes	15 minutes	20 minutes	25 minutes
0.10	56.22±0.01^b	57.31±0.03^{ab}	58.29±0.01^{ab}	58.43±0.02^a
0.15	58.49±0.02^b	60.11±0.01^{ab}	61.18±0.02^{ab}	61.39±0.01^a
0.20	61.09±0.00^b	62.80±0.02^{ab}	64.74±0.01^{ab}	64.75±0.00^a
0.25	61.15±0.03^b	63.18±0.01^{ab}	64.75±0.03^{ab}	64.80±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 2. Viscosity (cP) by diffferent pectinase concentration (%) and time of treatment (minutes)

Pectinase concentration (%)	Viscosity (cP)			
	10 minutes	**15 minutes**	**20 minutes**	**25 minutes**
0.10	1.01±0.01^a	0.90±0.04^{ab}	0.78±0.02^{ab}	0.77±0.03^c
0.15	0.90±0.02^a	0.85±0.01^{ab}	0.77±0.01^{ab}	0.75±0.04^c
0.20	0.85±0.01^a	0.79±0.02^{ab}	0.73±0.03^{ab}	0.73±0.01^c
0.25	0.77±0.03^a	0.76±0.00^{ab}	0.73±0.00^{ab}	0.73±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%).				

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

Pectinase concentration (%)	Optical density (mJ/cm^2)			
	10 minutes	**15 minutes**	**20 minutes**	**25 minutes**
0.10	73.73±0.02^a	71.32±0.03^{ab}	69.15±0.03^{ab}	69.03±0.03^b
0.15	71.40±0.01^a	69.14±0.01^{bb}	67.27±0.01^{ab}	66.44±0.01^b
0.20	69.18±0.01^a	67.24±0.00^{ab}	65.74±0.02^{ab}	65.64±0.01^b
0.25	69.09±0.02^a	67.28±0.02^{ab}	65.71±0.01^{ab}	65.68±0.04^b
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 4. Effect of yeast ratio to soluble dry matter (°Brix) in wine

Fermentation time (days)	Soluble dry matter in wine (oBrix)			
	Yeast ratio 0.5%	Yeast ratio 1.0%	Yeast ratio 1.5%	Yeast ratio 2.0%
1	18.55±0.01^{a}	17.35±0.03^{b}	16.21±0.02^{c}	15.01±0.03^{d}
2	17.26±0.03^{a}	16.34±0.02^{b}	15.49±0.01^{c}	14.78±0.00^{d}
3	16.11±0.02^{a}	15.29±0.00^{b}	14.06±0.01^{c}	13.65±0.01^{d}
4	14.78±0.01^{a}	13.87±0.01^{b}	13.19±0.01^{c}	12.39±0.02^{d}
5	13.38±0.03^{a}	12.78±0.02^{b}	12.36±0.02^{c}	11.17±0.02^{d}
6	12.01±0.00^{a}	11.47±0.03^{b}	10.75±0.02^{c}	9.84±0.01^{d}
7	11.95±0.00^{a}	11.42±0.03^{b}	10.69±0.02^{c}	9.79±0.01^{d}
8	11.15±0.01^{a}	10.88±0.03^{b}	10.01±0.02^{c}	9.64±0.03^{d}
9	10.69±0.03^{a}	10.11±0.00^{b}	9.45±0.03^{c}	8.21±0.01^{d}
10	10.04±0.01^{a}	9.48±0.02^{b}	8.77±0.01^{c}	7.48±0.02^{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 5. Effect of yeast ratio to ethanol formation (%v/v) in wine

Fermentation time (days)	Ethanol in wine (%v/v)			
	Yeast ratio 0.5%	Yeast ratio 1.0%	Yeast ratio 1.5%	Yeast ratio 2.0%
1	1.04±0.01^{d}	1.58±0.01^{c}	2.39±0.00^{b}	2.43±0.01^{a}
2	1.25±0.01^{d}	1.89±0.02^{c}	2.42±0.01^{b}	2.77±0.02^{a}
3	2.29±0.03^{d}	2.35±0.01^{c}	2.96±0.01^{b}	3.05±0.02^{a}
4	2.31±0.00^{d}	2.79±0.02^{c}	3.03±0.00^{b}	3.07±0.01^{a}
5	2.89±0.01^{d}	3.01±0.03^{c}	3.27±0.00^{b}	3.29±0.01^{a}
6	3.15±0.03^{d}	3.23±0.00^{c}	3.41±0.02^{b}	3.42±0.02^{a}
7	3.18±0.01^{d}	3.25±0.03^{c}	3.45±0.01^{b}	3.47±0.01^{a}
8	3.28±0.02^{d}	3.32±0.04^{c}	3.48±0.01^{b}	3.50±0.01^{a}
9	3.35±0.01^{d}	3.40±0.01^{c}	3.53±0.02^{b}	3.64±0.02^{a}
10	3.43±0.03^{d}	3.47±0.02^{c}	3.60±0.01^{c}	3.77±0.04^{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. Effect of yeast ratio to acidity (g/l) in wine

Fermentation time (days)	Acidity in wine (g/l)			
	Yeast ratio 0.5%	Yeast ratio 1.0%	Yeast ratio 1.5%	Yeast ratio 2.0%
1	1.12±0.01^{c}	1.23±0.02^{b}	1.98±0.01^{ab}	2.01±0.01^{a}
2	1.14±0.03^{c}	1.29±0.01^{b}	2.02±0.01^{ab}	2.09±0.01^{a}
3	1.45±0.01^{c}	1.96±0.00^{b}	2.13±0.01^{ab}	2.22±0.03^{a}
4	1.78±0.02^{c}	2.04±0.00^{b}	2.27±0.01^{ab}	2.38±0.02^{a}
5	2.01±0.00^{c}	2.18±0.01^{b}	2.31±0.03^{ab}	2.40±0.01^{a}
6	2.19±0.01^{c}	2.32±0.00^{b}	2.40±0.02^{ab}	2.98±0.04^{a}
7	2.22±0.01^{c}	2.35±0.03^{b}	2.42±0.01^{ab}	3.01±0.01^{a}
8	2.29±0.02^{c}	2.55±0.02^{b}	2.74±0.02^{ab}	3.12±0.03^{a}
9	2.32±0.01^{c}	2.80±0.03^{b}	2.95±0.01^{ab}	3.24±0.01^{a}
10	2.49±0.03^{c}	2.88±0.01^{b}	3.07±0.01^{ab}	3.30±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%).				

Table 7. Effect of yeast ratio to soluble dry sensory characteristics (score, 1-5) in wine

Fermentation time (days)	Sensory score of wine (1-5) by different yeast ratio			
	Yeast ratio 0.5%	Yeast ratio 1.0%	Yeast ratio 1.5%	Yeast ratio 2.0%
1	2.45±0.02^{c}	3.01±0.01^{b}	4.17±0.01^{ab}	4.65±0.01^{a}
2	2.48±0.00^{c}	3.15±0.01^{b}	4.22±0.00^{ab}	4.67±0.01^{a}
3	2.95±0.01^{c}	3.26±0.02^{b}	4.35±0.01^{ab}	4.72±0.01^{a}
4	3.16±0.01^{c}	3.79±0.00^{b}	4.41±0.00^{ab}	4.72±0.03^{a}
5	3.69±0.00^{c}	3.98±0.03^{b}	4.42±0.03^{ab}	4.72±0.01^{a}
6	4.15±0.01^{c}	4.29±0.02^{b}	4.69±0.04^{ab}	4.80±0.02^{a}
7	4.17±0.00^{c}	4.33±0.04^{b}	4.70±0.02^{ab}	4.81±0.02^{a}
8	4.30±0.04^{c}	4.56±0.02^{b}	4.76±0.01^{ab}	4.85±0.02^{a}
9	4.46±0.01^{c}	4.62±0.01^{b}	4.81±0.02^{ab}	4.91±0.01^{a}
10	4.54±0.02^{c}	4.70±0.03^{b}	4.88±0.00^{ab}	4.92±0.00^{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 8. Effect of the sencondary fermentation to wine quality

Criteria	Secondary fermentation (weeks)			
	1	2	3	4
Soluble dry matter (^{o}Brix)	10.68±0.02^a	10.53±0.01^{ab}	10.14±0.02^{ab}	10.01±0.04^c
Ethanol (%v/v)	3.85±0.01^b	3.87±0.01^{ab}	3.93±0.01^{ab}	3.98±0.01^a
Acidity (g/l)	2.43±0.04^c	2.44±0.00^{ab}	2.50±0.01^{ab}	2.52±0.00^a
Sensory score	4.70±0.00^b	4.77±0.01^{ab}	4.80±0.01^a	4.80±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%).				

A research developed a method of mango juice extraction with pectinase and characterized ethanol and some volatile contents of mango wine.[17] Efficiency of enzymatic complex of *Trichoderma* sp. as crude enzymatic extract in the extraction of mango juice was evaluated, improving the yield up to 79%, representing an alternative to give an added value of mango peels improving the yields of production of mango juice.[18]

Enzyme produced by *Aspergillus oryzae* was utilized to express juice from mango. Various concentrations of enzyme, 1%, 2%, 5% and 10% (v/v) were used to facilitate juice extraction. The enzyme when used at 10% level and incubated at 30±2^{o}C for 18h produced maximum juice (68%) yield with increase in free flowing characteristic of the juice.[19]

In another research, mango pulp was incubated at 60^{o}C for 1 hr to destroy natural enzymes present in the juice. Prepared mango pulp and separately prepared orange juice were formulated into various mixes in the ratio mango: orange (v/v) 100: 0, 50: 50 and 0: 100. A portion of the mango pulp or the one with equal volume of orange was then treated with 0.1 per cent (v/w) pectinase enzymes at 40^{o}C for 24 hrs. All the treated and untreated mixes were separately packed inside a bottle and pasteurized at 80^{o}C for 10 mins. The use of enzyme (pectinase) in juice production from mango was found beneficial and profitable since it increases the yield of juice extracted.[20]

3.2 Effect of yeast inculate for wine fermentation

Mango wort after being treated by pectinase would be inoculated with *Saccharomyces cerevisiae* at different ratio (0.5, 1.0, 1.5, 2.0%). After 10 days of fermentation at 12^{o}C, we noticed the change of soluble dry matter (^{o}Brix), ethanol (%v/v), acidity (g/l), and sensory characteristics (score) in wine as in table 4, 5, 6 and 7. We found that the approprate yeast inculate should be 1.5% to get the highest wine quality.

A study was to address the problem of large post harvest losses of mangoes by employing yeast fermentation technology to produce a more stable, value-added product in this case fruit wine. Results showed that two of the yeast types namely; Red Star Pasteur and Red Star Montrachet displayed superior fermentation characteristics and produced mango wines that were acceptable by both descriptive and affective sensory panels.[10]

The preparation of mango wine by yeast-mango peel immobilised biocatalyst system by repeated batch fermentation was conducted and compared to free cells fermentation at 15, 20, 25, and 30°C. The operational stability of the biocatalyst was good as the ethanol concentrations (76.0–96.0 g/l) and productivities (1.53–3.29 g/l/h) were high, showing the suitability of the biocatalyst for even low temperature winemaking. The concentration of ethyl acetate was not above 40 mg/l in all cases, and higher alcohols were low (< 330 mg/l) in wine with immobilised cells indicating an improvement in the product compared to free cells fermentation. Amyl alcohols were proved to be temperature dependent and decreased with the decrease in temperature (262.48–146.83 and 239.74–184.34 mg/l) in the case of fermentation batches with immobilised and free cells, respectively, from 30°C to 15°C. Sensory evaluation revealed fruity aroma (7.9 ± 0.73), fine taste (7.7 ± 0.24), and the overall improved quality of the wines produced by the immobilised system.[11]

An investigation was aimed to investigate the suitability of dried mango peel for ethanol production. The mango peel contained good amount of reducing sugars up to 40% (w/v). Direct fermentation of mango peel extract gave only 5.13% (w/v) of ethanol. The rate of the fermentation was very slow. Nutrients such as yeast extract, peptone and wheat bran extract were tested for the supplementation of mango peel medium and it was observed that the nutrient supplementation increased the ethanol production significantly up to 7.14% (w/v).[8]

A study was to determine the effects of temperature and yeast concentration on the fermentation kinetics and chemical properties of Apple mango fruit wine through process optimization. The fermentation conditions were optimized by varying temperature at 20°C, 25°C, 30°C and 35°C and the yeast concentration at 0.0065%, 0.01%, 0.05% and 0.1%. The increase in temperature and yeast concentration increased the fermentation kinetics significantly ($p<0.05$). However, at high temperature (35°C) and yeast concentration (0.1%) the sugars were not completely utilized during fermentation. At low temperature of 25°C, the alcohol yield was highest (9.44%) relative to high temperature of 35°C that gave the lowest yield (6.93%). Yeast concentration of 0.05% and fermentation temperature of 25°C gave the optimal characteristics for Apple mango wine using wine yeast (*Saccharomyces cerevisae*).[13]

3.3 Effect of secondary fermentation to wine quality

We preserved mango wine at 8^{o}C in dark bottle by different time (1, 2, 3, 4 weeks) as the secondary fermentation. We monitored soluble dry matted (^{o}Brix), ethanol (% v/v), acidity (g/l), and sensory characteristics (score) in wine. Our results were elaborated in table 8. 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 3rd and 4th week so we choosed 3 weeks of secondary fermentation for economy.

Production of ethanol from mango (*Mangifera indica* L.) fruit juice fermentation was investigated. The mango juice from selected varieties contained 18-20% Total Soluble Solids (TSS) and 5-18.5% of reducing sugars. Finally 8.5-10% (w/v) of ethanol was obtained from the fermentations which were conducted without adding any nutrients. The fermentation was completed within 72 h in all variety juices. Fermentation process optimized and pH 5.0, 30°C temperature, 3% (v/v) inoculum density and 3 days incubation was found be good for maximal ethanol production from mango juice.[8]

4. CONCLUSION

Mangifera indica (Mango) is a fruit with good nutritional attributes but has short shelf-life under the prevailing weather conditions in tropical countries. Mature and ripe mangoes with their high composition of fermentable reducing sugars such as glucose, sucrose and fructose could serve as substrates for fruit wine production using wine yeast (*Saccharomyces cerevisiae*), thus transforming a perishable products to more stable and value added product. Therefore, production of wine from this fruit can help increase wine variety and reduce post-harvest losses.

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