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RESEARCH ARTICLE

Different Technical Variables Affecting Noni (*Morinda Citrifolia* L.) Wine Fermentation

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Abstract

Noni *(Morinda citrifolia L.)* is one of the most important fruit which was widely used for its health restorative properties. It has been used an herbal medicine in various ailments. This fruit is considered a natural antioxidant. It can prevent cancer, heart disease, diabetes, cognitive dysfunction, hypertension etc., and maintain overall good health. Over the years, its popularity has been diminished due to unpleasant smell from the ripened fruit. There is limited study mentioning to processing of this functional fruit. Therefore we explored a wine fermentation from noni fruit by focusing on the effect of different variables such as sugar supplementation, yeast ratio inculation, fermentation time and temperature in the primary fermentation, and fermentation time and temperature in the secondary fermentation to noni wine quality (%v/v alcohol; g/l acidity; ^{o}Brix residual sugar; sensory score). Our results proved that the primary fermentation should be conducted with 15% sugar supplementation, 0.15% *Sacchromyces cerevisiae* at temperature 29.0^{o}C in 10 days. The secondary fermentation was adequate in 9.5^{o}C for 3 weeks to get a pleasant flavor and aroma. Noni wine also evaluated the antioxidant capacity and total phenolic content. Noni wine could be considered as a good source of antioxidants and phenolics ideal for daily healthy consumption.

Keywords: *Noni, Wine, Fermentation, Sacchromyces cerevisiae, Antioxidant, Phenolic.*

Introduction

Noni (*Morinda citrifolia* L.) is a tropical and subtropical plant widely grown in the Vietnam. A yellowish-white ovoid lumpy bodied fruit approximately 12 cm in size composed of numerous, fused ripened ovaries each separate from white flower. The unripe fruit is dark green in color and the ripe fruit releases a strong butyric acid like decayed smell. The pulp is juicy and bitter, light dull yellowish white, gelatinous when the fruit is ripped. Noni juice is prepared from ripe Noni fruit which is having unpleasant odour and bitter taste [1].*Morinda citrifolia* (Rubiaceae) is an evergreen shrub whose ripe fruit has a strong butyric acid smell and flavor [2].

Octanoic acid and hexanoic acid were the major volatile acids, while malic acid, malonic acid and fumaric acid were the main non-volatile acids [3]. The unpleasant odor of *M. citrifolia* extract was reported to have been contributed by medium chain fatty acids such as capric, caproic and caprylic acids [4]. The fruit contains hydrophilic compounds like carbohydrates, proteins, minerals, vitamins and small amount of fat. The major micronutrients in noni (*Morinda citrifolia* L.) have been identified are phenolic acid, organic acid and alkaloids [5].Biological compounds such as glycosides, polysaccharides, iridoids, alkaloids, lignans, trisaccharide fatty acid esters, anthraquinones, scopoletin, morindin, vitamins, and minerals have been isolated from noni fruits, roots, and leaves [6,7]. Mostly *Morinda citrifolia* is consumed in the form of juice.

Sometimes people are also preferred to take *Morinda citrifolia* in the form of capsules which contains dehydrated *Morinda citrifolia* fruit [8]. The products obtained from the different parts of *M. citrifolia* plant viz. leaves, fruits, roots and barks are available

in the market as Noni juice, capsule, powder, Noni concentrates, and also tea in the market [1]. It has been used as a therapeutic remedy to various diseases as tumorous, anthelminthic, analgesic, viral, fungal, cardiovascular, hypotensive disorders. It even demonstrates beneficial consequences in the conditions like skin diseases, respiratory infections, gastritis, menstrual, diabetes and venereal diseases [8, 9].In afflictions like burns, headaches, arthritis, wounds and many more skin infections *Morinda citrifolia* fruit is proved very beneficial [10].

This fruit is considered a natural antioxidant and the daily consumption of its juice helps the immune system and increases the cells capacity of absorption [11]. An additive of herbal feed prepared from *Morinda citrifolia* fruit elevated the production and ameliorate the quality of egg Japanese quail [12]. Lactic acid bacteria such as *Lactobacillus plantrum, lactobacillus casei*, and *Bifidobacterium longumare* used in manufacturing probiotic *Morinda citrifolia* juice, always assessing a probability of *Morinda citrifolia* as raw substrate for manufacturing [13].

Adriana Bramorski et al [14]. Determined the total polyphenol content (TPC) and antioxidant capacity of a juice commercialized as noni juice. Commercial noni juice presented higher values of TPC (91.90 mg of gallic acid/100 mL juice) and antioxidant activity (5.85 mmol/L) compared to its 5% diluted constituents. Currently, it has been considered the supplement of low calorie most important negotiated in the international market [15].

A study was carried out to observe the fermentation process for noni (*Morinda citrifolia* L.) extract by *Saccharomyces cerevisiae* [16]. Behavior of polyphenol content and antioxidant activity of noni wine (*Morinda citrifolia* L.) during alcoholic fermentation was examined [17]. Nascimento, L.C.S. et al [18].Evaluated the chemical composition, nutritional properties and antioxidant capacity of noni's pulp and seeds.

Noni 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 technical variables such as sugar supplementation, yeast ratio inculation, fermentation time and temperature in the primary fermentation, and fermentation time and temperature in the secondary fermentation to noni wine quality

Material & Method

Material

Ripen noni fruits were naturally collected from Can Tho city, Vietnam. After harvesting, they must be conveyed to laboratory within 8 hours for experiments. Apart from collecting noni, we also used other materials such as sugar, yeast (*Saccharomyces cerevisae*). Lab utensils and equipments included weight balance, refractometer, pH meter, ethanol meter, thermometer, water bath, buret, erlenmeyer flask, glassware.

Figure 1: Noni *(Morinda citrifolia L.)*

Research Method

Effect of Sugar Supplementation in the Primary Fermentation

Noni fruits were set layer by layer with sugar in different sugar supplementation (5%, 10%, 15%, 20%).The primary fermentation was

conducted at ambient temperature (28°C) in 15 days with 0.05% *Saccharomyces cerevisiae*. Periodically (5 days) we took the wort to analyze alcohol content (% v/v), acidity (g/l), residual sugar (°Brix), and sensory score.

Effect of Yeast Ratio Inculation in the Primary Fermentation

Noni fruits were set layer by layer with sugar (15%). The primary fermentation was conducted at ambient temperature (28°C) in 15 days with *Saccharomyces cerevisiae* at different ratio (0.05%, 0.1%, 0.15%, and 0.2%).

Periodically (5 days) we took the wort to analyze alcohol content (% v/v), acidity (g/l), residual sugar (°Brix), and sensory score

Effect of Fermentation Time and Temperature in the Primary Fermentation

Noni fruits were set layer by layer with sugar (15%). The primary fermentation was conducted by *Saccharomyces cerevisiae* (0.15%) at different temperature (28.0°C, 28.5°C, 29.0°C, 29.5°C) by setting erlenmeyer flasks in water bath in 15 days. Periodically (5 days) we took the wort to analyze alcohol content (% v/v), acidity (g/l), residual sugar (°Brix), sensory score.

Effect of Fermentation Time and Temperature in the Secondary Fermentation

After completing the primary fermentation of noni fruits with supplementation of sugar (15%), *Saccharomyces cerevisiae* (0.15%), fermentation temperature (29.0°C) in 12 days; the wort should come to the secondary fermentation step by keeping noni wort at different temperature (9.0°C, 9.5°C, 10.0°C, 10.5°C) by different time (1, 2, 3, 4 weeks) to mild the noni wine quality. Weekly, noni wine would be monitored the sensory score.

Antioxidant Capacity and Total Phenolics Contents in Noni Wine

Noni wine quality was aslo evaluated on antioxidant capacity and total phenolic content. An antioxidant capacity was determined by DPPH (µM TE/g) and FRAPS (µM TE/g).

Total phenolics content was also examined by TPC (mg GAE/g).

Physico-chemical and Sensory Measurement

Alcohol content (%v/v) was analyzed by gas chromatography [19]. Acidity (g/l) was determined by using 5 ml of sample and titrated with 0.1N NaOH with phenolphthalein as indicator [20]. Residual sugar (°Brix) was analyzed by refractometer. Sensory score was performed by panelist of 15 members based on the Hedonic 9 points.

DPPH assay followed according to Rufino *et al.* (2010). The analysis of FRAP was performed according to Thaipong *et al.* (2006). Was determined using the Folin-Ciocalteu reagent [21].

Statistical Analysis

Data were statistically summarized by Statgraphics Centurion XVI.

Result & Discussion

Effect of Sugar Supplementation in the Primary Fermentation

Noni fruits were set layer by layer with sugar in different sugar supplementation (5%, 10%, 15%, 20%).The primary fermentation was conducted at ambient temperature (28°C) in 15 days with 0.05% *Saccharomyces cerevisiae*.

Periodically (5 days) we took the wort to analyze alcohol content (% v/v), acidity (g/l), residual sugar (°Brix), sensory score. Results were clearly presented in table 1-4. It's obviously noted that 15% sugar supplementation was optimal for noni wort.

Table 1: Effect of sugar supplementation (%) to alcohol content (% v/v) of noni wort

	Alcohol content (% v/v)			
Primary fermentation time (day)	5% sugar	10% sugar	15% sugar	20% sugar
5	1.63±0.04^{c}	2.84±0.03^{ab}	3.47±0.04^{a}	2.16±0.02^{b}
10	1.94±0.01^{c}	3.07±0.02^{ab}	3.85±0.01^{a}	2.35±0.05^{b}
15	2.00±0.03^{c}	3.10±0.02^{ab}	3.91±0.02^{a}	2.38±0.03^{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 2: Effect of sugar supplementation (%) to acidity (g/l) of noni wort

	Acidity (g/l)			
Primary fermentation time (day)	5% sugar	10% sugar	15% sugar	20% sugar
5	2.19±0.03^{b}	2.44±0.02^{ab}	2.63±0.04^{a}	2.35±0.01^{ab}
10	2.32±0.01^{b}	2.62±0.04^{ab}	2.75±0.01^{a}	2.47±0.02^{ab}
15	2.36±0.02^{b}	2.65±0.03^{ab}	2.77±0.02^{a}	2.50±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 (α = 5%)				

Table 3: Effect of sugar supplementation (%) to residual sugar (°Brix) of noni wort

	Residual sugar (°Brix)			
Primary fermentation time (day)	5% sugar	10% sugar	15% sugar	20% sugar
5	1.02±0.01^{d}	2.27±0.03^{c}	3.69±0.02^{b}	8.75±0.05^{a}
10	0.84±0.03^{d}	2.04±0.01^{c}	3.26±0.05^{b}	8.28±0.03^{a}
15	0.80±0.02^{d}	1.99±0.03^{c}	3.20±0.01^{b}	8.25±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 4: Effect of sugar supplementation (%) to sensory score of noni wort

	Sensory score			
Primary fermentation time (day)	5% sugar	10% sugar	15% sugar	20% sugar
5	4.59±0.04^{c}	6.40±0.01^{b}	7.38±0.06^{a}	5.28±0.01^{bc}
10	5.38±0.07^{c}	6.79±0.03^{b}	7.82±0.02^{a}	5.94±0.03^{bc}
15	5.41±0.03^{c}	6.84±0.02^{b}	7.87±0.05^{a}	6.03±0.02^{bc}
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%)				

Fermented fruit extract contain micronutrients, non-volatile and volatile components, ketones, lactones, beta-carotenoids, terpenoids, proxeronine [22]. A study was carried out to observe the fermentation process for noni (*Morinda citrifolia* L.) extract by *Saccharomyces cerevisiae*. The *M. citrifolia* extract was fermented with different combination of substrate concentration (40, 50, 60, 70 and 80%) (w/v).Titratable acidity and total polyphenol content, the effects of substrate concentration was significant. For total soluble solids, the effects of substrate concentration were found to be significant [16].

Effect of Yeast Ratio Inculation in the Primary Fermentation

Noni fruits were set layer by layer with sugar (15%). The primary fermentation was conducted at ambient temperature (28°C) in 15 days with *Saccharomyces cerevisiae* at different ratio (0.05%, 0.1%, 0.15%, 0.2%).Periodically (5 days) we took the wort to analyze alcohol content (% v/v), acidity (g/l), residual sugar (°Brix), sensory score. Results were comprehensively noted in Table 5-8.

Table 5: Effect of yeast ratio (%) to alcohol content (%v/v) of noni wort

	Alcohol content (%v/v)			
Primary fermentation time (day)	0.05% yeast	0.1% yeast	0.15% yeast	0.2% yeast
5	3.47±0.04^{b}	4.12±0.03^{ab}	5.23±0.04^{a}	5.26±0.02^{a}
10	3.85±0.01^{b}	4.49±0.02^{ab}	5.66±0.01^{a}	5.70±0.03^{a}

15	3.91±0.02[b]	4.54±0.06[ab]	5.71±0.02[a]	5.74±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: Effect of yeast ratio (%) to acidity (g/l) of noni wort

	Acidity (g/l)			
Primary fermentation time (day)	0.05% yeast	0.1% yeast	0.15% yeast	0.2% yeast
5	2.63±0.04[b]	2.75±0.03[ab]	2.85±0.03[a]	2.87±0.01[a]
10	2.75±0.01[b]	2.88±0.04[ab]	2.97±0.04[a]	3.00±0.02[a]
15	2.77±0.02[b]	2.91±0.02[ab]	3.00±0.05[a]	3.04±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 7: Effect of yeast ratio (%) to residual sugar (°Brix) of noni wort

	Residual sugar (°Brix)			
Primary fermentation time (day)	0.05% yeast	0.1% yeast	0.15% yeast	0.2% yeast
5	3.69±0.02[a]	2.71±0.04[b]	2.04±0.05[c]	1.98±0.03[c]
10	3.26±0.05[a]	2.47±0.02[b]	1.82±0.01[c]	1.79±0.04[c]
15	3.20±0.01[a]	2.40±0.05[b]	1.78±0.03[c]	1.75±0.06[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 8: Effect of yeast ratio (%) to sensory score of noni wort

	Sensory score			
Primary fermentation time (day)	0.05% yeast	0.1% yeast	0.15% yeast	0.2% yeast
5	7.38±0.06[b]	7.79±0.04[ab]	8.13±0.01[a]	8.15±0.06[a]
10	7.82±0.02[b]	8.02±0.05[ab]	8.39±0.05[a]	8.42±0.02[a]
15	7.87±0.05[b]	8.05±0.02[ab]	8.42±0.02[a]	8.46±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%)				

A study was carried out to observe the fermentation process for noni (*Morinda citrifolia* L.)Extract by *Saccharomyces cerevisiae*. The M. citrifolia extract was fermented with different inoculum size (0, 1.5, 3, 4.5 and 6%) (v/v).The effect of inoculum size was found to be signifcant for turbidity. For total soluble solids, the effect of inoculum size was found to be significant [16].

Effect of Fermentation Time and Temperature in the Primary Fermentation

Noni fruits were set layer by layer with sugar (15%). The primary fermentation was conducted by *Saccharomyces cerevisiae* (0.15%) at different temperature (28.0°C, 28.5°C, 29.0°C, 29.5°C) by setting erlenmeyer flasks in water bath in 15 days. Periodically (5 days) we took the wort to analyze alcohol content (% v/v), acidity (g/l), residual sugar (°Brix), sensory score. Results were depicted in table 9-12.

Table 9: Effect fermentation temperature (°C) to alcohol content (%v/v) of noni wort

	Alcohol content (%v/v)			
Primary fermentation time (day)	28.0°C	28.5°C	29.0°C	29.5°C
5	5.23±0.04[c]	5.88±0.03[b]	6.06±0.02[a]	5.94±0.04[ab]
10	5.66±0.01[c]	5.97±0.02[b]	6.24±0.04[a]	6.11±0.05[ab]

15	5.71±0.02^{c}	6.02±0.01^{b}	6.27±0.05^{a}	6.15±0.02^{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%)				

Table 10: Effect fermentation temperature (°C) to acidity (g/l) of noni wort

	Acidity (g/l)			
Primary fermentation time (day)	28.0°C	28.5°C	29.0°C	29.5°C
5	2.85±0.03^{c}	2.98±0.03^{b}	3.09±0.02^{a}	3.03±0.05^{ab}
10	2.97±0.04^{c}	3.02±0.04^{b}	3.21±0.04^{a}	3.09±0.03^{ab}
15	3.00±0.05^{c}	3.05±0.01^{b}	3.25±0.06^{a}	3.11±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 (α = 5%)				

Table 11: Effect fermentation temperature (°C) to residual sugar (°Brix) of noni wort

	Residual sugar (°Brix)			
Primary fermentation time (day)	28.0°C	28.5°C	29.0°C	29.5°C
5	2.04±0.05^{a}	1.61±0.02^{b}	1.33±0.04^{c}	1.42±0.01^{bc}
10	1.82±0.01^{a}	1.31±0.05^{b}	1.04±0.02^{c}	1.25±0.04^{bc}
15	1.78±0.03^{a}	1.27±0.03^{b}	0.99±0.05^{c}	1.12±0.03^{bc}
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 12: Effect fermentation temperature (°C) to sensory score of noni wort

	Sensory score			
Primary fermentation time (day)	28.0°C	28.5°C	29.0°C	29.5°C
5	8.13±0.01^{c}	8.28±0.04^{b}	8.43±0.01^{a}	8.36±0.05^{ab}
10	8.39±0.05^{c}	8.68±0.05^{b}	8.85±0.03^{a}	8.73±0.02^{ab}
15	8.42±0.02^{c}	8.72±0.01^{b}	8.89±0.06^{a}	8.81±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 (α = 5%)				

A study was carried out to observe the fermentation process for noni (*Morinda citrifolia* L.)Extract by *Saccharomyces cerevisiae*. The M. citrifolia extract was fermented with different temperature (30, 33.5, 37, 40.5 and 44oC) and fermentation time (0, 1.5, 3, 4.5 and 6 days). For pH, fermentation time was found to be not significant, while for titratable acidity and total polyphenol content, the effects of fermentation time was significant. Temperature level was found to be signifcant for turbidity [16].

Effect of Fermentation Time and Temperature in the Secondary Fermentation

After completing the primary fermentation of noni fruits with supplementation of sugar (15%), *Saccharomyces cerevisiae* (0.15%), fermentation temperature (29.0°C) in 10 days; the wort should come to the secondary fermentation step by keeping noni wort at different temperature (9.0°C, 9.5°C, 10.0°C, 10.5°C) by different time (1, 2, 3, 4 weeks) to mild the noni wine quality. Weekly, noni wine would be monitored the sensory score.

Table 13: Effect secondary fermentation to sensory score of noni wort

	Sensory score			
Secondary fermentation time (week)	9.0°C	9.5°C	10.0°C	10.5°C
1	8.92±0.01^{ab}	8.97±0.03^{a}	8.85±0.02^{b}	8.85±0.02^{b}
2	8.94±0.03^{ab}	8.98±0.01^{a}	8.85±0.02^{b}	8.85±0.02^{b}
3	8.96±0.04^{ab}	8.98±0.01^{a}	8.86±0.03^{b}	8.85±0.02^{b}
4	8.96±0.04^{ab}	8.99±0.04^{a}	8.89±0.01^{b}	8.86±0.03^{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%)				

Micronutrients, non-volatile and volatile components, ketones, lactones, beta-carotenoids, terpenoids, pro-xeronine occur in fermented fruit extract [22]. The organic acids present are cupric and caprylicacids [23]. In processing and storage, light, temperature, and oxygen can promote undesirable chemical reactions that can reduce the health benefits of noni products to consumers [24].

Antioxidant Capacity and Total Phenolics Contents in Noni Wine

Noni wine quality was aslo evaluated on antioxidant capacity and total phenolics contents. Antioxidant capacities were determined by DPPH (µM TE/g) and FRAP (µM TE/g). Total phenolics content was also examined by TPC (mg GAE/g). Results were expressed in Table 13.

Table 13: Antioxidant capacity and total phenolic content in noni wine

Parameter	DPPH (µM TE/g)	FRAP (µM TE/g)	TPC (mg GAE/g)
Value	428.59±1.25	37.36±0.18	83.46±0.23

Note: the values were expressed as the mean of three repetitions

Accodring to J. Yang et al [24].The fresh juice of noni (*Morinda citrifolia* L.) possessed free-radical-scavenging activity (RSA), 1,1-diphenyl-2-picrylhydrazyl (DPPH), at 140 mg equivalent ascorbic acid/100 ml and total phenols at 210 mg gallic acid/100 ml. Fermentation of noni fruit for 3 months resulted in a loss of more than 90% of RSA. Yanine Chan-Blanco et al [25].Demonstrated the ripening and aging of noni fruits (*Morinda citrifolia* L.): microbiological flora and antioxidant compounds.

At first, fermentation occurred and populations of molds, yeasts, and mesophilic bacteria increased significantly. After 2 weeks, microbial growth changed abruptly, stopping for yeasts, molds, and mesophilic bacteria, and decreasing suddenly for lactic bacteria. Analyses of pH, soluble solids, ethanol, and lactic acid in the fruits confirmed the microbial analyses, indicating initial sensitive variations, followed by values remaining comparatively steady during aging. Vitamin C and total phenol contents also remained constant at 300 ± 60 mg and 50 ± 20 mg GAE, respectively, per 100 g of pulp.

Antioxidant capacity likewise remained relatively high at 8 ± 1.5 µmol Trolox/g. According to Nascimento, L.C.S. et al. (2018), pulp of noni fruit showed higher antioxidant capacity (348.5 µM TE/g when compared to seeds (61.47 µM TE/g) and skins (294.9 µM TE/g) when antioxidant capacity was measured by DPPH. FRAP assays showed that pulp and seeds presented 38.1 and 34.8 µM TE/g of antioxidant capacity, respectively. They found Pulp presented TPC (79.6 mg GAE/100g); TPC of 6.09 mg GAE/ 100g for noni seeds, Skin presented TPC of 61.8 mg GAE/100g).

Conclusion

Morinda citrifolia (Noni) has been widely used as an alternative therapy owing to its potent antioxidant activity and healthy benefits. It contains many chemical components viz. Amino acids, anthraquinones, fatty acids, flavonoids, iridoids, lignans, polysaccharides, sterols etc. along with minerals, vitamins, micro and macro nutrients which are effective in many ailments. Literatures prove that Noni is pharmacologically active and is used in different forms of cancer, viz. colon, esophageal, breast, colorectal cancers; cardiovascular diseases, diabetes, arthritis, hypertension.

Noni can be found as pasteurized or fermented juice, powder, capsules and others. Many people avoid consuming *M. citrifolia* because of its odor. We have successfully utilized noni fruits as substrate for wine fermentation by investigating different parameters such as sugar supplementation, yeast ratio inculation, fermentation time and temperature in the primary fermentation, and fermentation time and temperature in the secondary fermentation to noni wine quality.These results were important because they could help wine makers to arrange proper processing method and storage.

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