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Various factors influencing to the nipa (*Nypa fruticans)* wine fermentation

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Abstract
Fresh nipa sap, which has sweet and fruit-like odour, does not contain any higher alcohols and acetic acid. Fermentation of fresh nipa sap will lead to changes in aroma and taste, due to the highly fermentable nature of the sap. Fermented nipa sap contained high amount of ethanol, as well as higher alcohols, esters, diacetyl, and acetoin. In order to optimize the nipa **(***Nypa fruticans)* wine fermentation, we investigate different factors possibly affecting to its quality. Our results defined some major parameters: yeast *Saccharomyces cereviesiae* ratio 0.2%, initial soluble dry matter ^{o}Brix of fermentation juice 22%, initial pH of fermentation juice 4.5, fermentation time 6 days. Product is then supplemented with syrup 2% to get a better pleasant feeling.

Keywords: Nypa fruticans, saccharomyces cereviesiae, soluble dry matter, pH, fermentation, wine.

1. Introduction
Fresh palm sap, palm juice or air nira is a traditional beverage consumed by people in Vietnam. It has also been used in many Asian countries as sweetener in the form of palm sugar after going through heating processs (Ho *et al.*, 2007). Fermented palm sap on the other hand is usually called palm toddy, or simply palm wine and is widely consumed as a refreshing alcoholic beverage. Palm sap, which is sweet and translucent juice, is one of the important products from palm trees. Palm sap or palm toddy is a term commonly used by most of these countries (Nur Aimi *et al.*, 2013). Special name is given according to the origin of the sap. Coconut sap or neera is sap obtained from coconut palm (Borse *et al.*, 2007), while sap obtained from oil palm or Arenga palm is called palm sap or air nira (Ho *et al.*, 2007).
Nipa tree (*Nypa fruticans*) can be found in every part of Mekong river delta, Vietnam. Fermented palm sap or palm toddy is whitish with ethanol content in the range of 5 to 8%, and pH at about 3.6 depending on fermentation stage at which the toddy is consumed (Iwuoha and Eke, 1996; Lasekan *et al.*, 2007). Previous studies reported that the fermentation is dominated by yeasts (*Saccharomyces, Candida, Kloechera*) and lactic acid bacteria (Atputharajah *et al.*, 1986; Chanthachum and Beuchat, 1997; Amoa-Awua *et al.*, 2007).
Fermented palm sap has complex organic and inorganic compounds of which production depends on several factors, such as period of fermentation and microbial activity. In general, fermented palm sap contains low molecular mass carbonyls (C1-C6) as by-products of yeast fermentation, and alcoholic oxidation at various stages of fermentation (Anli *et al.*, 2007).
Ronald O. Ocampo, Normalina P. Usita. (2014) determined to improve the quality of nipawine using fruit additives. It sought to determine what fruit juices that can be added to nipa wine that improve its quality; to compare the sample treatments in terms of taste, color andaroma; determine the cost of production of the different samples.
Purpose of our research is to investigate various factors including pH, ^{o}Brix and yeast *Saccharomyces cereviesiae* supplementation to the nipa (*Nypa fruticans*) wine fermentation.

2. Material & Method
2.1 Material
Nipa sap was randomly collected from nipa trees in Tra Vinh, Soc Trang, Bac Lieu province, Mekong river delta, Vietnam. The sap was obtained by cutting the stalk of matured nipa fruit and sap was collected four times daily to avoid spontaneous fermentation. The end cut of the stalk was wrapped with sterile plastic bags to maintain hygiene. A thin slice of the stalk was cut away every four hours to reduce accumulation of microorganisms. The collected sap was then pooled together and transferred into 250 mL of laboratory bottles and immediately kept in cooler box with dried ice at 0 ºC in temperature.

Fig 1: Nipa (*Nypa fruticans*)

2.2 Research method

2.2.1 Investigate the effect of pH, Brix and yeast ratio to nipa wine fermentation

Experiments are randomly designed with three factors and two replications.

Factor A: Effect of pH
A1: pH = 4.5
A2: pH = 5.5
A3: pH = 6.5
Factor B: Effect of °Brix
B1: Brix = 18 %
B2: Brix = 20 %
B3: Brix = 22 %
Factor C: Effect of yeast ratio
C1: Yeast = 0%
C2: Yeast = 0.1%
C3: Yeast = 0.2%
Testing parameters
- Ethanol formation: two days/ one checking
- °Brix change: two days/ one checking

2.2.2 Syrup supplementation

Experiments are randomly designed with one factor and two replications.

Factor D: Syrup supplementation
D1 = 0%
D2 = 1%
D3 = 2%
D4 = 3 %
Nipa wine is supplemented syrup, pasteurized at 70 °C in 15 minutes, stable in 3 days before sensory evaluation.

2.3 Statistical analysis

All data are processed by ANOVA (Startgraphics) to check the significant difference via LSD.

3. Result & Discussion

3.1 Nutritional composition in fresh nipa sap

Table 1: Nutritional composition in fresh nipa sap

Criteria	Result
pH	5.25
Brix (%)	14
Total acidity (%)	0.126
Total sugar (%)	12.6

3.2 Effect of pH, °Brix and yeast supplementation to fermentation

Table 2: Effect of initial oBrix to ethanol formation

Brix, %	Ethanol, ml/ 100ml			
	Date 0	Date 2	Date 4	Date 6
18	0	6.639[a]	7.389[a]	7.556[a]
20	0	6.944[a]	7.694[a]	7.917[a]
22	0	8.361[b]	9.194[b]	9.444[b]

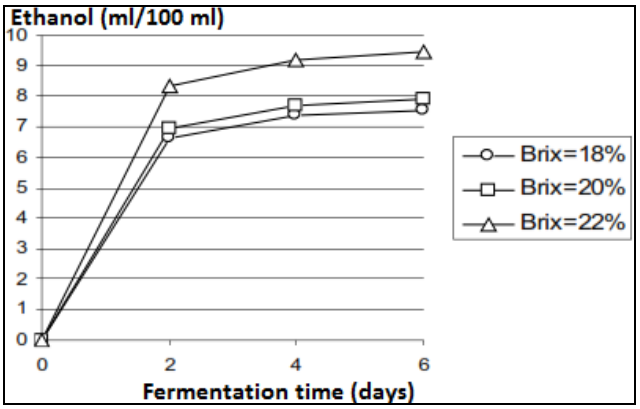

Fig 2: Effect of initial oBrix to ethanol formation

From table 2 and figure 2, the higher °Brix is, the more ethanol produces. At 22 °Brix, ethanol formation is maximum so we choose initial °Brix 22% for further experiments.

Table 3: °Brix change by fermentation time

Brix, %	Brix, %			
	Date 0	Date 2	Date 4	Date 6
18	18	9.211[a]	8.589[a]	8.478[a]
20	20	10.222[b]	9.689[b]	9.159[b]
22	22	12.322[c]	11.633[c]	11.478[c]

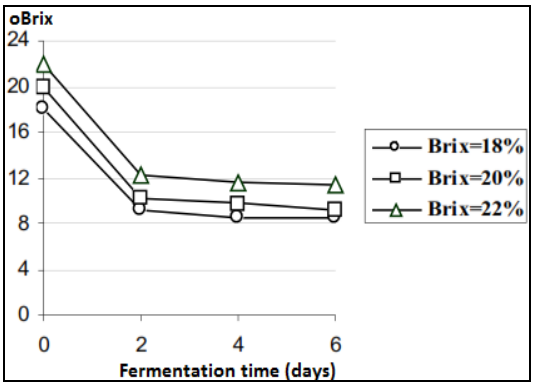

Fig 4: °Brix change by fermentation time

Table 5: Effect of yeast supplementation to ethanol formation

Yeast (%)	Ethanol, ml/100 ml			
	Date 0	Date 2	Date 4	Date 6
0	0	6.694[a]	7.417[a]	7.583[a]
0.1	0	7.25[b]	7.917[a]	8.250[b]
0.2	0	8.00[c]	8.917[b]	9.028[c]

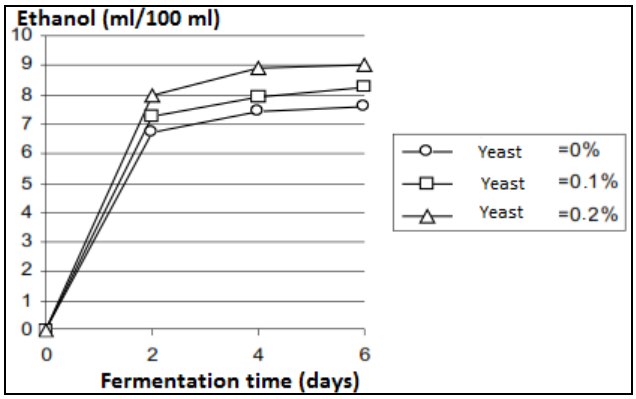

Fig 5: Effect of yeast supplementation to ethanol formation
During fermentation, yeast supplementation 0.2% gives the highest ethanol content so we choose this ratio for further experiments.

Table 6: °Brix changes by different yeast ratios during fermentation

Yeast, %	°Brix, %		
	Date 2	Date 4	Date 6
0	11.044[a]	10.500[a]	10.367[a]
0.1	10.578[b]	10.089[b]	9.956[b]
0.2	10.133[c]	9.322[c]	9.222[c]

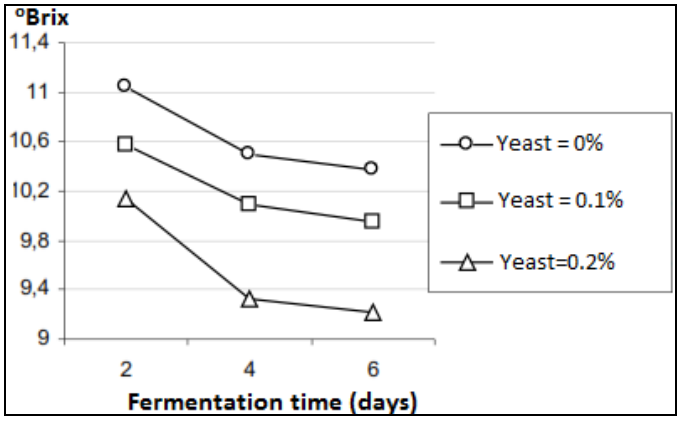

Fig 6: °Brix change by different yeast ratios during fermentation

Table 7: Effect of the initial pH to ethanol formation during fermentation

pH	Ethanol, ml/100 ml			
	Date 0	Date 2	Date 4	Date 6
4.5	0	7.556[a]	8.778[a]	8.917[a]
5.5	0	7.222[a]	8.000[b]	8.222[b]
6.5	0	7.167[a]	7.500[c]	7.722[c]

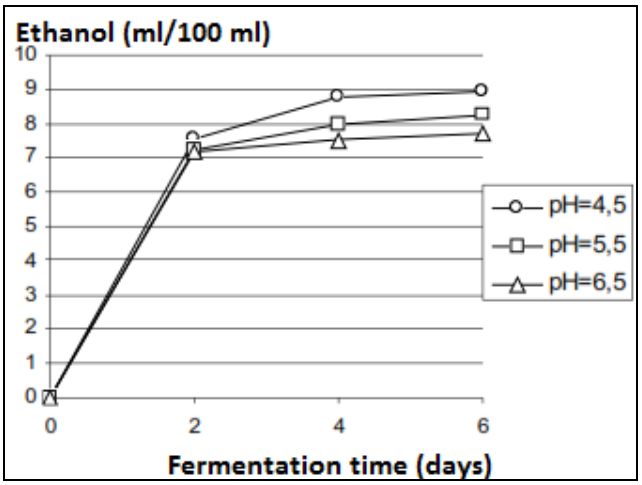

Fig 7: Effect of the initial pH values to ethanol formation during fermentation
At the initial pH 4.5, the ethanol formation is at the highest amount. So we choose pH 4.5 for nipa fermentation.

Table 8: Effect of the initial pH values to oBrix change during fermentation

pH	°Brix, %		
	Date 2	Date 4	Date 6
4.5	9.367[a]	8.622[a]	8.522[a]
5.5	11.011[b]	10.422[b]	10.266[b]
6.5	11.378[c]	10.867[c]	10.756[c]

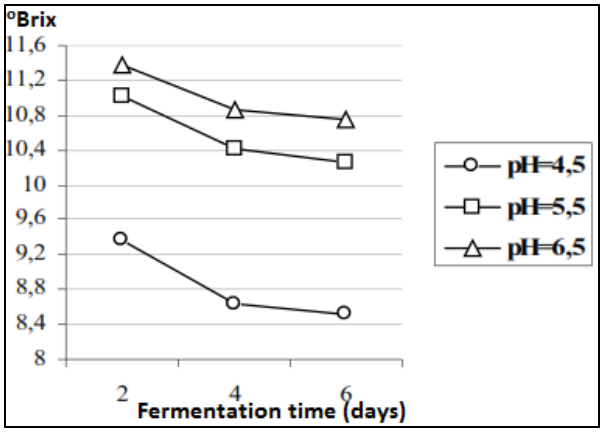

Fig 8: Effect of pH values to °Brix change during fermentation
At pH 4.5, the speed of fermentation happens quickly to avoid lactic bacteria and wild yeast.

3.3 Sensory score of nipa wine after being supplemented syrup

Table 9: Sensory score of nipa wine after being supplemented syrup

Syrup supplementation, %	Sensory score
0	3.05[ab]
1	2.90[a]
2	4.45[c]
3	3.45[b]

From table 9, we see very clearly that 2% syrup supplementation creates pleasant feeling for nipa wine.

3.4 Quality of the nipa wine

Table 10: Quality of the nipa wine according to TCVN 5013 – 89

Description	Recorded value	Standard TCVN 5013 - 89
Ethanol (%)	10	Not mentioned
Andehyt (mg acetaldehyt/litre wine)	40	≤ 50
Ester (mg etyl acetate / litre wine)	180	≤ 200
Furfural	Not detected	Trace

After checking the nipa wine quality, we notify all criteria are in limit allowance.

4. Conclusion

Nipa sap or air nira is a sweet natural beverage obtained from a type of palm tree, *Nypa fruticans*. It is readily and spontaneously fermented resulting in the development of alcoholic fermentation products. This study was aimed to improve the quality of nipa wine. Specifically, it was intended to determine pH, ^{o}Brix and yeast supplementation for nipa fermentation.

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