Production of Vinegar from Nipa (Nypa fruticans Wurmb) Sap Using Rapid-tray-culture Method for Community Use

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Abstract

The objectives of this research were to 1) study the vinegar fermentation from nipa sap using the rapid-tray-culture method and 2) train the production technology of nipa sap vinegar to community enterprises in Laem Fha Pa subdistrict, Phra Samut Chedi district, Samut Prakarn province. The results showed that during alcohol fermentation, the pH was rather consistent while the TSS decreased and the alcohol content increased. After 9 days, the alcohol content reached 12.1% with 11°Brix TSS and a pH of 4.10. The acid fermentation via rapid-tray-culture method took 7 days and yielded 5.7% acetic acid with 8°Brix TSS and a pH of 3.15. The quality of the nipa sap vinegar passed all requirements of Thai Community Product Standard (TCPS 326-2547), except small excessive amounts of sulfur dioxide. The vinegar production training course achieved a high satisfaction on overall aspects.

Keywords : Nipa sap, vinegar fermentation, rapid-tray-culture method

Introduction

Nipa (Nypa fruticans Wurmb) is the only one palm that grows well in the mangrove forests. It has a wide diversity of use. Nipa palm forest is useful for both agriculture and ecology because it is the habitat for a variety of fish, crab, shrimp, including mollusk and also helps to protect river bank erosion. The long leaves of the nipa can be used as roof material and woven walls. The young leaves are used as cigarette wrapper. Flowers and immature fruits (the sweet, translucent and gelatinous young fruits) are used as food ingredient. The fruit shell and leaf stalks are used as fuel (Bamroongrugsa, 1997). The nipa sap or juice obtained from the fruit stalks is a good source of sugar. The sugar content in nipa sap was claimed to be 10.6 to 28.6% (Matsui et al., 2014). In southern Thailand, the nipa sap is used to produce sugar, vinegar and

alcohol. Compared to the coconut palm sap and toddy palm sap, the nipa palm sap is easier and safer to be collected since there is no need to climb up high.

Pholawat (2001) reported that the traditional vinegar fermentation from nipa sap by local people in Trang province is simple. The sugar-rich sap is poured into earthen jars, covered with straws and exposed to the sun up to 10 days for natural acid fermentation. However, the vinegar production by this method still remains home use. Since there is no control of the fermentation conditions, the quality of the vinegar is inconstant and the acetic content is usually found to be lower than standard requirement at 4% for both Thai Community Product Standard (TCPS 326-2547) and Notification of the Ministry of Public Health No.204 B.E. 2543 (NMPH 204-2543). Likewise, Jantima et al., (2015) reported that the natural processed vinegar from banana was not qualified the NMPH standard because the acetic acid of the vinegar was less than 4% and the residual alcohol also exceeded 0.5%.

Recently, the new technique on vinegar fermentation in a stainless steel known as rapid-tray-culture method was reported (Institute of Food Research and Product Development, 2009). The research showed that the key factors of this technique were the control of fruit wine volume in the fermentation vessel and a proper ratio of fruit wine and vinegar starter. It could yield the vinegar product with 6.5 – 7% acetic acid after fermentation at ambient temperature for 6 -7 days.

In Laem Fha Pa subdistrict, Phra Samut Chedi district, Samut Prakarn province, there are several nipa palm clusters growing naturally in many areas. The local people have made many uses of nipa palm, for example, nipa leaves for basketry, thatching and wrapping tobacco for smoking; flower stalks for making mosquito whisk; and young fruits for dessert and drink. However, the use of nipa sap in this area is not noted. This research attempted to study the vinegar fermentation from nipa sap using rapid-tray-culture method. The results were propagated to the community enterprises in this area aiming to encourage more utilization on local nipa palms. The vinegar product could also be sold as community product. Lastly, it could be a way to enlighten local people to preserve the nipa palm forest in their district.

Research Objectives

1. To study the vinegar fermentation from nipa sap using rapid-tray-culture method;

2.To train the production technology of nipa sap vinegar to community enterprises in Laem Fha Pa subdistrict, Phra Samut Chedi district, Samut Prakarn province.

Research Methodology

1. The study of the composition and chemical properties of nipa sap

Since only small amount of local nipa sap was obtained and it was not enough for vinegar fermentation, the nipa sap used in this research was bought from Nakhon Si Thammarat province. To support that the same method could be implied for the local nipa sap, both samples were analyzed for total soluble solid (TSS) using a hand refractometer and pH using a pH meter.

2. The study of the vinegar fermentation

In this study the vinegar fermentation was performed as follows:

2.1 Alcohol fermentation stage

The nipa sap was prepared to have a desired TSS of 22-25°Brix and a pH of 3.5-4.0. Approximately 0.1% of diammonium phosphate (DAP) was added as yeast nutrient. About 5% of the liquid was taken to make the wine starter by adding commercial wine yeast and shaking for 24 hours at room temperature. KMS (potassium metabisulfite) was added into the remaining sap to kill microorganisms. After 24 hours, the starter was mixed with the sap and the mixture was kept at room temperature under still condition. The fermentation reaction was continued for 2 weeks and the samples were taken every day for the analysis of pH, TSS and alcohol content (Ebulliometer method).

2.2 Acid fermentation stage

The acetic acid fermentation was conducted using the rapid-tray-culture method as described by Muangnoi and Srichayes (2014) with some modification. Firstly, the nipa sap was prepared to have the TSS of 3–4°Brix. After boiling for 5 minutes to kill microorganisms, 600 ml of nipa sap was poured into a sterile stainless steel tray together with 300 ml nipa wine from step 3.2.1 and 100 ml of vinegar starter bought from the Institute of Food Research and Product Development, Kasetsart University. The fermentation proceeded for 2 days allowing the increase of the acetic acid bacteria population. After that, 1,000 ml of nipa wine were added. The fermentation was continued for further 5 days. Every day, the samples were taken for the analysis of pH,

TSS and acetic acid content (titratable acidity, AOAC 2000). The vinegar was then filtrated, pasteurized at 70°C for 2 min and bottled. The qualities of the vinegar product were analyzed and compared to the requirements of Thai Community Product Standard (TCPS 326-2547).

3. The training on the production technology of nipa sap vinegar

The training course on the production technology of nipa sap vinegar was held at Dhonburi Rajabhat University on 17 September 2015. The convenience sampling technique was used to get the participants who can and were willing to join the training course from the target area, Laem Fha Pa subdistrict, Phra Samut Chedi district, Samut Prakarn province. The course content covered three topics which were 1) nipa sap utilization, 2) how to collect nipa sap, and 3) vinegar production. At the end of the course, the participants' satisfaction was evaluated using an evaluation form. The questionnaire consisted of three parts; 1) respondent information, 2) satisfaction questions with 5-point Likert scales and 3) recommendations. The data were analyzed in terms of percentage, mean and standard deviation. The criteria for the interpretation were based on the mean scores as follows: 4.50-5.00 = very high satisfied; 3.50 – 4.49 = high satisfied; 2.50 – 3.49 = moderate satisfied; 1.50 – 2.49 = low satisfied; and 1.00 – 1.49 = very low satisfied.

Research Results

Firstly, the researcher surveyed the area in Laem Fha Pa subdistrict, Phra Samut Chedi district, Samut Prakarn province and found that the local wisdom of nipa sap collection in this area has been lost. Only some middle-aged adults could recognized that they had seen their parents doing this while some interviewees did not exactly know what nipa sap is. Some of them misunderstood that the nipas sap was made by boiling the young fruits with light syrup. Therefore, the nipa sap used for vinegar fermentation in this study was obtained from Nakhon Si Thammarat province. The sap was boiled to prevent spoilage during delivery to Dhonburi Rajabhat University, the research site. The hot-fill bottled nipa sap was then kept frozen at -18°C for further use. The TSS and pH of this sample were found to be 23.5°Brix and 6.10, respectively while the TSS and pH of local nipa sap were 21.0°Brix and 5.71, respectively.

The vinegar fermentation was divided into 2 stages; alcohol fermentation and acetic acid fermentation. At the start of the alcohol fermentation stage, a small amount of citric acid was added to bring the pH of the sap from 6.10 down to the appropriate pH range for the activity of wine yeast (3.5-4.0). During the alcohol fermentation, the pH was observed to be rather consistent while the TSS decreased and the alcohol content increased (Figure 1 and 2). From day 5–9, the alcohol content had slowly raised so the activity was stopped on day 9 using KMS. The fermented product was analyzed for the alcohol content, TSS and pH. The results showed that the nipa wine contained 12.1% alcohol, 11°Brix TSS and had a pH of 4.10.

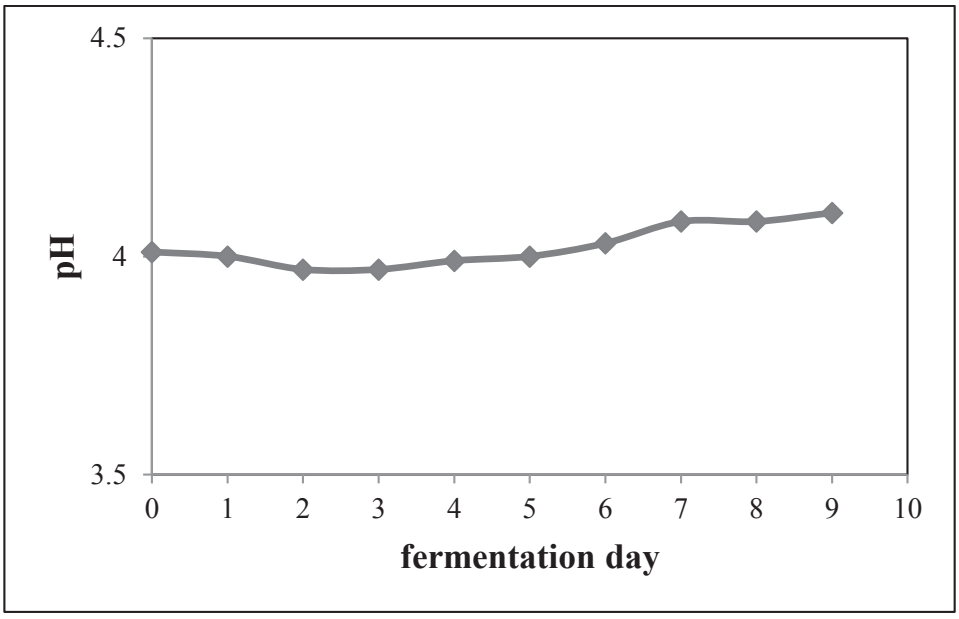

Figure 1 pH change during alcohol fermentation stage

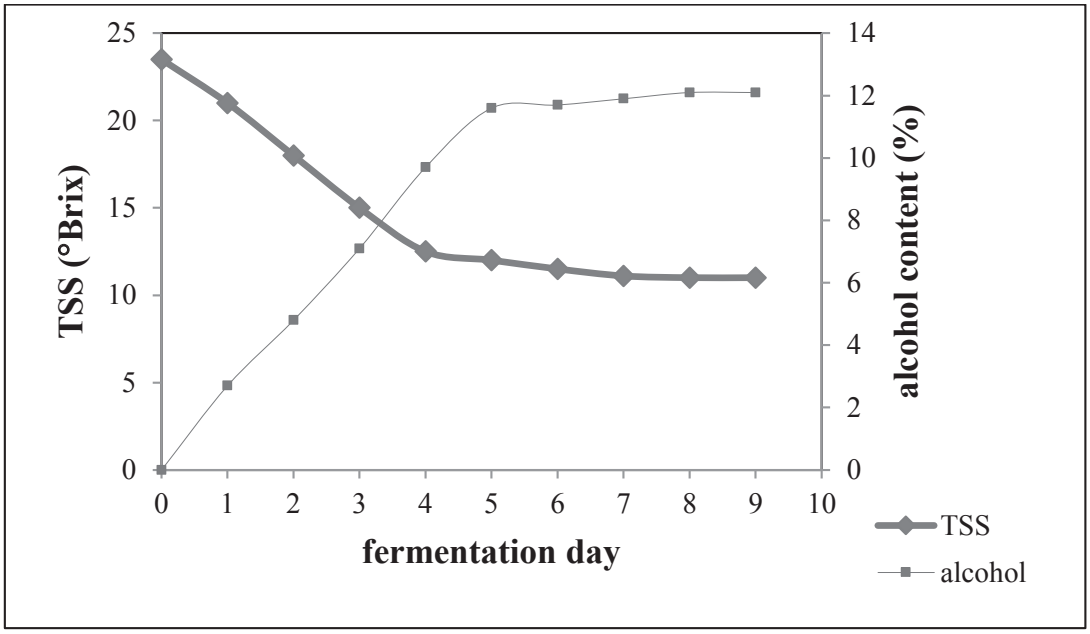

Figure 2 TSS and alcohol change during alcohol fermentation stage

At the acid fermentation stage, the first 2 days was intended to multiply the number of acetic acid bacteria from the vinegar starter. Figure 3 showed that there were many small water droplets hanging the plastic covered the tray on day 2 indicating the growth and active of acetic acid bacteria. After adding the same amount of nipa wine, the samples were analyzed for pH, TSS and acetic acid content. The results showed that the TSS increased, pH gradually decreased and acetic acid content increased during fermentation (Figure 4). On day 7, the fermented vinegar was found to have 5.7 g acetic acid /100 ml vinegar, 8°Brix TSS and a pH of 3.15.

(a)

(b)

Figure 3 Appearance of acetic acid fermentation in the trays.
(a) clear covered plastic at day 0;
(b) water droplets on covered plastic at day 2

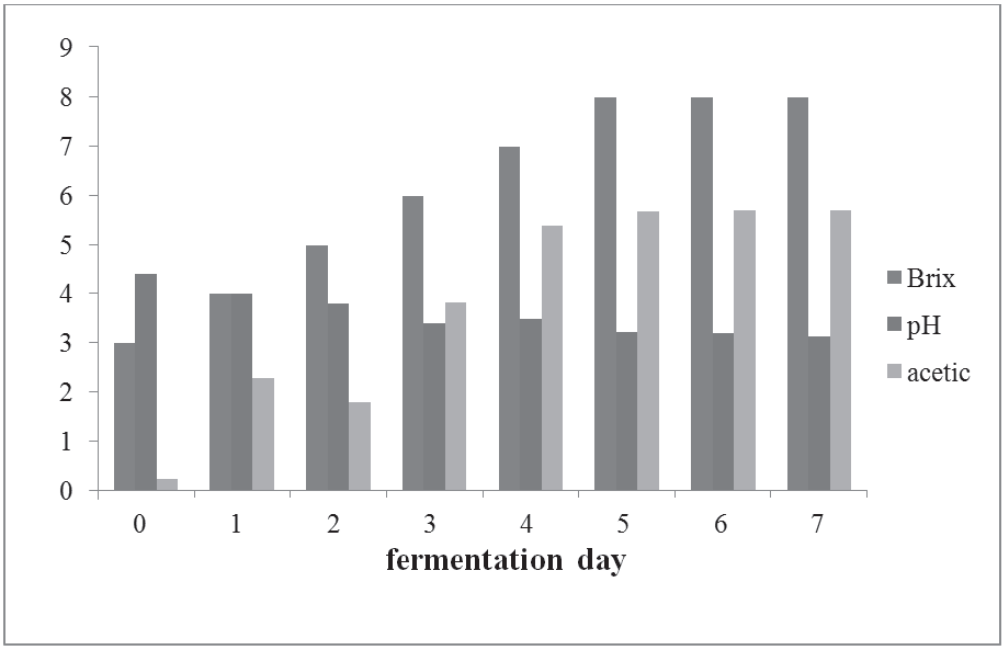

Figure 4 TSS, pH and acetic acid change during acetic fermentation stage

The filtrated and pasteurized nipa vinegar was hot-filled in a glass bottle (Figure 5). The qualities of the product were analyzed and compared to the requirements of Thai Community Product Standard (TCPS 326-2547) (Table 1).

Figure 5 Nipa sap vinegar product

Table 1

The characteristics of nipa sap vinegar product compared to the TCPS 326-2547

Characteristics	TCPS 326-2547 (2004) (Thai) : FERMENTED VINEGAR	Nipa sap vinegar	Remark
1. Overall	clear liquid precipitation may be found	clear liquid with some precipitation at the bottom	Pass
2. Color	natural color	light brown	Pass
3. Smell	pungent smell of acetic acid Smell of raw material may be detected.	sour and pungent smell of vinegar with some trace of sweet smell of nipa sap	Pass
4. Foreign material	Foreign material such as vinegar eels, human hair, animal hair, soil, sand, etc. must not be found.	Not detected	Pass
5. Contaminant			
5.1 Arsenic (As)	must not exceed 1 mg/kg	0.189 mg/kg	Pass
5.2 Lead (Pb)	must not exceed 1 mg/kg	0.29 mg/kg	Pass
5.3 Copper (Cu)	must not exceed 10 mg/kg	Not detected	Pass
5.4 Zinc (Zn)	must not exceed 10 mg/kg	Not detected	Pass
5.5 Iron (Fe)	must not exceed 10 mg/kg	<3.60 mg/kg	Pass
6. Food Additives			
6.1 colorant	no artificial colors Caramel coloring is allowed.	Not detected	Pass
6.2 sulfur dioxide	Sulfur dioxide (SO_2) (if used) must not exceed 70 mg/kg	104.09 mg/kg	Fail
7. acetic acid	must not lower than 4 g/ 100 cm^3	5.7 g/ 100 cm^3	Pass
8. sulfuric acid or free mineral acid	must not be found	Not detected	Pass
9. methyl alcohol	must not exceed 420 mg/l	Not detected	Pass

Table 1 showed that the quality of the product passed all requirements of the standard except the sulfur dioxide.

For the training course on the production technology of nipa sap vinegar, there were 26 participants including 24 females and 2 males. The percentage by age at 16-25 years, 36-45 years, 46-55 years and 56 years or older were 19.23%, 7.69%, 30.77% and 42.31%, respectively. Most of them were members of the community enterprises in Laem Fha Pa subdistrict, Phra Samut Chedi district, Samut Prakarn province (65.38%). The satisfaction with the training course was shown in Table 2.

Table 2

The satisfaction with aspects of the training course

Aspects	$\bar{x}$	S.D	Interpretation
1. Place, equipment and facilities	4.62	0.57	very high satisfied
2. The training content	4.31	0.55	high satisfied
3. The training material	4.54	0.51	very high satisfied
4. The instructor communication and response to the questions	4.58	0.50	very high satisfied
5. The obtained knowledge after training	4.50	0.58	very high satisfied
6. The application of the knowledge for further use	4.27	0.67	high satisfied
7. Overall aspects	4.46	0.51	high satisfied

The findings showed the high satisfaction on overall aspects. Considering each aspect, it was found that the training content and the application for further use were at high satisfaction while place, equipment and facilities, the training material, the instructor communication and response to the questions and the obtained knowledge after training were at very high satisfaction.

Discussions

Based on the results of this study, there were some points to be discussed as follows:

1. The TSS and pH of the local nipa sap was analyzed and compared to the distant nipa sap. It was found that their properties were similar. So the vinegar fermentation via rapid-tray-culture method could be implied to the local nipa sap.

2. To be able to get the vinegar product that reaches the minimum requirement of acetic acid for both standards (TCPS 326-2547 and NMPH 204-2543) via the rapid-tray-culture method, the sugar content expressed in terms of TSS in the material for alcohol fermentation is one of the key factors. As described by Muangnoi and Srichayes (2014), the appropriate alcohol amount for vinegar fermentation by this method is 10%. Therefore the combination of nipa sap, nipa wine and vinegar starter at the ratio of 6:3:1 would contain approximately 3% alcohol in the mixture. This is suitable for the bacteria in the vinegar starter to convert ethyl alcohol into acetic acid. From the oxidation mechanism "$C_2H_5OH \rightarrow$ CH3CHO $\rightarrow$ CH_3COOH" (Nakano and Fukaya, 2008), the 3% alcohol would yield 3% acetic acid. After adding the one-fold amount of wine (10% alcohol), the acetic in the mixture will be 1.5% and the alcohol in the mixture will be 5%. At the end of the fermentation process, 5% alcohol will be converted to 5% acetic acid. This reaction will ideally bring the total acetic acid in the vinegar product up to 6.5%. From this reason, the sugar content in the material for alcohol fermentation should be at least 20% to get the wine with at least 10% ethyl alcohol. The excess sugar at 22-25% in this study was to make sure that the final alcohol content would reach 10% or over since the action of the microorganism is still uncontrollable.

3. From the analysis of the nipa vinegar characteristics, it was found that the amount of sulfur dioxide exceeded the limitation of the TCPS standard. The possible cause may come from the KMS used to kill microorganisms after alcohol fermentation. Also, the nipa sap may contain natural sulfur or the free sulfite which can be detected by the SO_2 test method based on AOAC (2012), 990.28. This method measures both free sulfite and reproducible portion of bound sulfites in food.

Conclusion

The nipa sap sample used in this study contained 23.5°Brix TSS and had a pH of 6.10. During alcohol fermentation, the pH was rather consistent while the TSS decreased and the alcohol content increased. After 9 days, the alcohol content reached 12.1% with 11°Brix TSS and a pH of 4.10. The acid fermentation via rapid-tray-

culture method took 7 days and yielded 5.7% acetic acid with 8°Brix TSS and a pH of 3.15. The quality of the nipa sap vinegar passed all requirements of Thai Community Product Standard, except small excessive amounts of sulfur dioxide. The vinegar production training course achieved a high satisfaction on overall aspects.

Recommendations

1. The vinegar fermentation via rapid-tray-culture method is suitable for community production since it can yield high acetic acid content within a short time and the production cost is considerably lower than the vinegar fermenter used in vinegar industry.

2. The phytochemical constituents, antioxidant activities, antimicrobial activities and medicinal properties of the fresh nypa sap including fermented products should be further studied.

3. For Laem Fha Pa subdistrict in Samut Prakarn province, the nipa fruits are more easily acquired than the nipa sap. Therefore, a study on the vinegar fermentation using nipa fruits at different stages such as mature and immature fruits may be conducted.

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