Yield of Vinegar, Alcohol and Sugar from the Sap of *Nypa fruticans*

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Abstract

Two sap samples of *Nypa fruticans* were collected in November 1996 and in April 1997 from the Chakaria Sundarbans for the production of sugar, vinegar and alcohol. The samples were first analysed to determine the sugar contents by hand refractometer. The first sample contained around 10.0% sugar, while the second contained about 8.0%. Experiments were also conducted to produce vinegar trom the first sample and alcohol from the second, each with and without addition of sugar. Various amounts of yeast, such as 0.2%, 0.6% and 1.0% (w/v) were added to determine the optimum yield of vinegar and alcohol by fermentation process. It was observed that, in both the cases, addition of 0.6% (w/v) yeast yielded the highest amount of acetic acid (4.3%) and alcohol (~5.0%) as azeotropic mixture. There was about 0.2% increase in acetic acid yield with 0.6% (w/v) yeast and further addition of 2.0% sugar to the original sample. The highest yield of alcohol, 5.5% (as azeotropic mixture) was obtained when the sap solution contained 13.0% sugar and 0.6% (w/v) yeast. This shows that *N. fruticans* sap may be a prospective source for production of sugar, vinegar and alcohol in the country.

সারসংক্ষেপ

নিপা রস হতে চিনি, ভিনেগার ও এ্যালকোহল উৎপাদনের জন্য ১৯৯৬ সালের নভেম্বর ও ১৯৯৭ সালের এপ্রিল মাসে চকরিয়া সুন্দরবন থেকে দু'টি নমুনা সংগ্রহ করা হয়। প্রথমে চিনির পরিমাণ নির্ণয়ের জন্য নমুনাদ্বয় হ্যান্ড রিফ্রাক্টোমিটারের সাহায্যে বিশ্লেষণ করা হয়। প্রথম ও দ্বিতীয় নমুনায় চিনির পরিমাণ ছিল যথাক্রমে ১০.০% ও ৮.০%। অতঃপর মূল নমুনায় অতিরিক্ত চিনি মিশিয়ে এবং না মিশিয়ে প্রথম নমুনা থেকে ভিনেগার ও দ্বিতীয়টি থেকে এ্যালকোহল উৎপাদনের জন্যও পরীক্ষা চালানো হয়। ফার্মেন্টেশন প্রক্রিয়ার মাধ্যমে সর্বাধিক পরিমাণ ভিনেগার ও এ্যালকোহল প্রস্তুতের জন্য বিভিন্ন পরিমাণ ঈষ্ট, যেমন ০.২%, ০.৬% ও ১.০% (w/v) মূল নমুনার সাথে মিশানো হয়। দেখা যায়, উভয় ক্ষেত্রেই নমুনার সাথে ০.৬% (w/v) ঈষ্ট মিশানো হলে সর্বাধিক পরিমাণ এ্যাসেটিক এ্যাসিড (৪.৩%) এবং এ্যাজিওট্রপিক মিশ্রণ হিসেবে এ্যালকোহল (~৫.০%) পাওয়া যায়। মূল নমুনার সাথে ০.৬% (w/v) ঈষ্ট এবং অতিরিক্ত ২.০% চিনি মিশানোর ফলে এ্যাসেটিক এ্যাসিডের উৎপাদন ০.২% বৃদ্ধি পায়। রসের দ্রবণে চিনি ও ঈষ্ট-এর পরিমাণ যখন যথাক্রমে ১৩.০% ও ০.৬% (w/v) তখন এ্যালকোহলের (এ্যাজিওট্রপিক মিশ্রণ) উৎপাদনের পরিমাণ ছিল সর্বোচ্চ (৫.৫%)। এতে প্রতীয়মান হয় যে, দেশে নিপা রস থেকে চিনি, ভিনেগার ও এ্যালকোহল উৎপাদনের উজ্জ্বল সম্ভাবনা রয়েছে।

Key words : Alcohol, azeotropic mixture, *Nipa fruticans*, sugar, vinegar, yeast

Introduction

Nypa fruticans (Wurmb), locally known as golpata, is one of the important forest produces abundantly available in the tidal estuaries in the coastal belt of Bangladesh. It is a gregarious palm with a large number of pinnate leaves arising in tufts from a stout creeping rhizome. There are varied uses of *N. fruticans*. Its leaves are traditionally harvested for thatching roof and making walls of cottages (Hamilton and Murphy 1988). Leaves are also used for making shingles, coarse mats, baskets and bags (Anon. 1966). Unexpanded young leaflets are used for wrapping cigarettes, older ones to weave hats, umbrellas, raincoats, etc. (Burkill 1935, Rabinson 1911).

Literature reval that the leaflets contain about 10.0% tannin and 15.2% hard tans and, therefore, these may be used for direct tanning of light leather (Anon. 1966). The palm is much valued, particularly in the Philippines for the sweet sap tapped from the stalk of the spadix. Large quantities of sweet juice can be extracted if the flower-stalks are cut off at proper time. The sap has the potential to be used for making sugar, alcohol and vinegar. Tapping starts sometime after or just before fruit formation, and sap collection is continued for about three months (Anon. 1966). The sap is a source of sugar where there is around 14-17% sucrose (Halos 1981). Freshly collected sap of *N. fruticans* can be fermented to make a good beverage. If acetic fermentation of the sap is allowed to follow, vinegar is produced (Melana 1980). In Malaysia, there is a scope for utilization and manufacture of sugar from *N. fruticans* sap to make alcohol (Chai and Lai 1984). Vinegar production from the sap is practised in Papua New Guinea and the Philippines (Hamilton and Murphy 1988).

However, no work has so far been done in Bangladesh on the utilization of *N. fruticans* sap. It was, therefore, considered important to undertake a study for the determination of sugar content in and production of alcohol and vinegar from the sap of *N. fruticans*.

Materials and methods

Sap samples of *N. fruticans* were collected twice, one in November 1996 and another in April 1997, from Dingakata of Chakaria Sundarbans, Cox's Bazar, Bangladesh for determination of sugar content and production of vinegar and alcohol. Production of vinegar and alcohol (azeotropic mixture) from the sap were determined separately with and without further addition of sugar.

Sugar content of the sap was determined by hand refractometer. The sap was pasteurized and then cooled. Yeast was added to the pasteurized sap to make concentrations of 0.2%, 0.6% and 1.0% (w/v) to determine optimum yield of vinegar and alcohol. The method followed was that of Anon. (1984). Additional quantities of sugar were also added to the samples for yield optimization of alcohol and vinegar. The samples with added sugar contained 13.0% and 18.0% sugar for alcohol production (Batch 1) and 12.0%, 15.0% and 18.0% sugar for vinegar production (Batch 2) respectively. Each treatment comprised of three replications.

Sap samples with and without addition of sugar (Batches 1 and 2) were allowed to stand for seven days for fermentation. At the end of this period, the alcoholic fermentation of the sap was thought to be completed as no further bubbles of carbon dioxide were observed to emerge. The samples were then strained through a clean cloth to remove the yeast and other solid materials. Alcohol was then prepared from the solution (Batch 1) by distillation.

The samples with alcoholic solution (Batch 2) were again pasteurized and cooled. To start acetic acid fermentation mother vinegar was added to the samples, which were allowed to stand for a month to complete the fermentation. They were then filtered and again pasteurized to kill the microorganisms. To make the vinegar clear, it was stirred with well-beaten egg white and heated until egg white was coagulated. The percentage of acetic acid as vinegar was determined by standard method (Vogel 1961).

Yield data for both vinegar and alcohol were analyzed statistically using factorial analysis. Analyses of variance (ANOVA) were done accordingly. LSD tests were then made to compare the treatment means.

Results and discussion

N. fruticans sap samples, collected in November 1996 and in April 1997, contained 10.0% and 8.0% sugar respectively (Tables 1 and 2). It appears that there is a seasonal variation in the sugar content of the sap. In the Philippines, sugar content of the sap was seen to be in the range of 14-17%. Sugar content of *N. fruticans* sap compares well with those of other sugar crops, such as sugarcane with 15-20% sucrose and sugar beat with 10-15% sugar (Halos 1981).

The results obtained on the yield of vinegar and alcohol with and without addition of sugar are shown in Tables 1 and 2 respectively. Results show that there are significant differences (P=0.01) among the mean yields of different sugar contents as well as three yeast contents in both the experiments. Interactions between the two treatments (sugar and yeast) are also highly significant in both the experiments.

Table 1 shows that without addition of extra sugar in the sap and with 0.2%, 0.6% and 1.0% yeast gave the mean yields of 3.04%, 4.30% and 2.16% acetic acid as vinegar respectively. It is noted that acetic acid percentage was maximum (4.30%) with 0.6% of yeast. After making the sap having 12.0%, 15.0% and 18.0% sugar, the grand mean yields of acetic acid obtained were 2.32%, 4.08% and 2.06% respectively for 0.2%, 0.6% and 1.0% of yeast, where acetic acid yield was maximum (4.08%) with 0.6% of yeast. The difference was statistically significant (LSD value = 0.033 at 1% probability level). Similarly, with three different yeast percentages, the mean yields of acetic acid obtained were 3.17%, 3.87%, 2.37% and 1.87% for 10.0%, 12.0% 15.0% and 18.0% sugar contents respectively in the sap, where 3.87% ranked the highest for 12.0% sugar. The

Table 1. Mean yield (%) of vinegar from *Nypa fruticans* sap collected in November 1996.

Sugar	Yeast			Grand mean	F-ratio	LSD value
	0.2%	0.6%	1.0%			
10.0% (as present in the original sample)	3.04	4.30	2.16	3.17		
12.0%	3.72	4.48	3.41	3.87**	8236.7[a]	0.039
15.0%	1.30	4.39	1.42	2.37		
18.0%	1.23	3.16	1.23	1.87		
Grand mean	2.32	4.08**	2.06			
F-ratio	17312.9[b]				1091.2[c]	
LSD value	0.033					

Note : a, b and c indicate highly significant differences among different sources of variations like sugar contents, yeast contents and the interaction; ** indicates the highest treatment mean and significant at 1% probability level.

Table 2. Mean yield (%) of alcohol (azeotropic mixture) from *Nypa fruticans* sap collected in April 1997.

Sugar	Yeast			Grand mean	F-ratio	LSD value
	0.2%	0.6%	1.0%			
8.0% (as present in the original sample)	3.91	4.95	2.50	3.79		
13.0%	4.86	5.50	2.59	4.32**	449.2[a]	0.27
18.0%	4.36	5.04	2.86	4.09		
Grand mean	4.38	5.16**	2.65			
F-ratio	1199.2[b]				14.7[c]	
LSD value	0.17					

Note : a, b and c indicate highly significant differences among different sources of variations like sugar contents, yeast contents and the interaction; ** indicates the highest treatment mean and significant at 1% probability level.

difference was statistically significant (LSD value = 0.039 at 1% probability level). The sap yielded the highest percentage of acetic acid (4.48%) when it contained 12.0% sugar and 0.6% yeast (Table 1).

From Table 1, it reveals that addition of extra sugar is not necessary when the sap sample itself contains around 12.0% sugar for optimum yield of acetic acid as vinegar. The cause of such reduction in acetic acid yield with more than 12.0% sugar in the sap solution might be due to some unknown reactions taking place between the molecules of acetic acid and sugar. This reaction is supposed to disturb the equilibrium position of acetic acid and sugar at 12.0%. However, such reaction still remains unexplained. For this, further investigation may be necessary to clarify the situation. The study also shows that, by fermentation of *N. fruticans* sap, vinegar of around 4-5% acetic acid can be obtained.

Table 2 shows that before addition of extra sugar in the sap, the mean yields of alcohol (azeotropic mixture) were 3.91%, 4.95% and 2.50% with 0.2%, 0.6% and 1.0% of yeast respectively, where alcohol percentage was maximum (4.95%) with 0.6% of yeast. After making the sap 13.0% and 18.0% sugar solution, the grand mean yields of alcohol obtained were 4.38%, 5.16% and 2.65% with 0.2%, 0.6% and 1.0% of yeast respectively, where alcohol yield was maximum (5.16%) with 0.6% of yeast. The difference was statistically significant (LSD = 0.17 at 1% probability level). Here alcohol yield was maximum (5.50%) with 13.0% sugar and 0.6% yeast and the yield reduced with further addition of sugar and yeast. The cause behind such reduction of the yield of alcohol may be something like that stated in the case of acetic acid (vinegar).

Based on the yeast contents (0.2%, 0.6% and 1.0%), the mean yields of alcohol were 3.79%, 4.32% and 4.09% with 8.0%, 13.0% and 18.0% sugar respectively, where 4.32% yield being the highest which was statistically significant

(LSD value = 0.27 at 1% probability level). *N. fruticans* sap yielded the highest percentage of alcohol (5.50%) as azeotropic mixture when the solution contained 13.0% sugar and 0.6% yeast (Table 2). Yield of alcohol did not increase with further addition of sugar and yeast. It may be inferred that when the sap solution becomes saturated with sugar, further addition of sugar plays no role to increase the yield of alcohol.

Conclusion

N. fruticans sap could be a good source of vinegar as well as alcohol to meet the consumers' demand in Bangladesh. But considering the cost involved in adding extra sugar and their corresponding yield, large-scale production of vinegar and alcohol from *N. fruticans* sap could be suggested without addition of extra sugar in the sap.

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