

Control of Hydrogen Sulphide Formation and Enhancement of the Ethanol Yield in Coconut Toddy — Field Trials

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Abstract: Field trials were carried out to test the finding that the addition of ammonium salts, as a source of nitrogen for the metabolism of toddy yeast, suppresses completely the formation of hydrogen sulphide and enhances the ethanol yields. Four field trials carried out, under normal conditions of tapping, showed that the addition of ammonium salts at a concentration of 0.08% (w/v) of NH_4^+ ions to the collection pot prior to tapping increased the ethanol content of toddy by an average of 12.5% and the total yield of ethanol by an average of 26.5%. At this concentration of NH_4^+ ions the formation of hydrogen sulphide was also completely suppressed. Statistical analysis showed that the results observed are of high statistical significance.

1. Introduction

Coconut palm wine (toddy) is a traditional beverage of Sri Lanka and other coconut growing countries. Toddy is the fermented sap of the young inflorescence of the coconut palm (*Cocos nucifera*). The unfermented sap (sweet toddy) is the exudate obtained from the young inflorescence by subjecting it to a skilled process called tapping. The unfermented sap contains about 15-20% (w/v) sugars (mainly sucrose) which are fermented to ethanol and a number of minor components by a mixture of wild yeast and bacteria. The fermented coconut palm wine which contains about 7% (v/v) ethanol is drunk fresh or bottled (pasteurized) or is distilled to produce a palm brandy (arrack).

The natural fermentation of toddy caused by the various types of wild yeast and bacteria produces not only ethanol but also many by-products. The formation of these by-products is responsible for the low ethanol yields and the off-flavours of coconut toddy.^{2,4} It has been reported that the ethanol content of naturally fermented coconut toddy is about 30% less than the theoretical yield.⁴

The main contributory factor for the off flavour of coconut toddy has been traced to the formation of hydrogen sulphide during the natural fermentation. Jansz *et al*³ who carried out a detailed study on this subject attributed the formation of hydrogen sulphide in toddy to the metabolic activities of some wild yeasts where the sulphur containing amino acid cysteine is utilized with the release of hydrogen sulphide. The mechanism of this process as proposed by Hough *et al*¹ is given in figure 1.

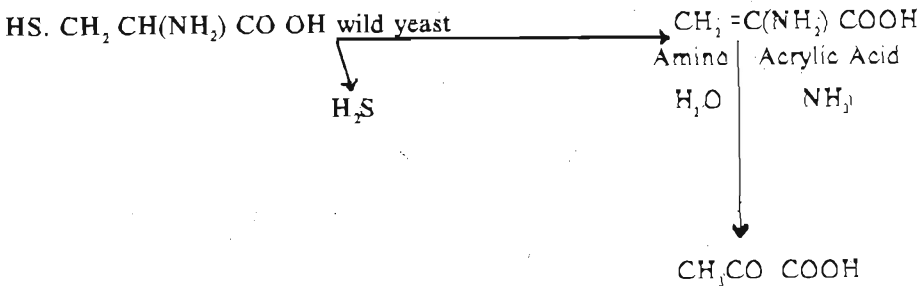


Figure 1. Cysteine metabolism by yeast.

Subsequent work on the control of hydrogen sulphide formation in toddy carried out by Jansz *et al*² and Kalyananda *et al*⁴ resulted in the discovery of a number of methods for this purpose. Out of these methods the addition of small amounts of ammonium salts to the sweet toddy before the commencement of the fermentation process appeared to be the simplest and the most feasible on a commercial scale. The most attractive feature of this process was the increase in the ethanol yield in the resulting toddy, which was around 25%.⁴

Though coconut toddy has been a popular alcoholic beverage for several centuries and also served as the base for the distillation of coconut arrack, the problems of undesired flavours and low ethanol yields have remained unsolved. The methods reported earlier such as the use of pure culture fermentation for increasing the ethanol yield^{3,6} are impracticable on a commercial scale. Therefore, the findings of Jansz *et al*² and Kalyananda *et al*⁴ after extensive research were considered to be of paramount importance to the coconut toddy industry in solving the above mentioned problems.

These findings were important as they indicated that the low yields of ethanol and off flavour of coconut toddy could be solved by the simple operation of adding ammonium salts to the collecting pot during the normal tapping process used on a commercial scale.

The field trials reported in this paper were carried out to test the applicability of these findings on a commercial scale. A detailed account is presented of four field trials carried out in four different locations and a statistical analysis of the results.

2. Experimental

2.1 Tapping and Fermentation of Coconut Toddy

Tapping of coconut palms for toddy was carried out by the regular tappers using the normal tapping procedure and schedule. The total number of coconut palms in each field trial were grouped into batches for convenience, and one sample (200 ml) from each batch was drawn for analysis. Control (without added NH_4^+) and experimental (with added NH_4^+) trials were carried out on alternate days. The same collection pot was used for both the control and the experimental trials for each inflorescence. In the experimental trials a calculated amount of ammonium chloride was added (as a solution or in the form of tablets) to the collecting pot at the time of tapping. Each ammonium chloride tablet weighed about 0.6 g and contained 0.20 g of NH_4^+ . The amount of ammonium chloride to be added was calculated based on the volume of toddy collected on the previous day. The number of tablets added was one for every 250 ml of toddy. The volume of toddy collected per pot ranged from 250 ml to 2000 ml. In order to complete the fermentation, toddy samples collected 24 hours after tapping were allowed to stand for another 8-10 hours before analysis. However the evolution of hydrogen sulphide was monitored right from the time the samples were collected i.e. from 8.00 a.m. to 3.00 p.m. on the same day of collection.

2.2 Analytical Methods

2.2.1 *Estimation of Ethyl Alcohol* - The ethyl alcohol content of samples of toddy was estimated by the use of an Ebulliometer and was expressed as a percentage by volume.

2.2.2 *Estimation of Residual Sugar* - The total residual sugar after inversion was estimated by the Lane and Eynon method and was expressed as a percentage by weight.

2.3 **Detection of Hydrogen Sulphide** — Hydrogen sulphide was detected qualitatively by the use of lead-acetate paper.

2.4 Ammonium chloride powder (technical grade) was obtained from British Drug House Limited, Overseas Division, U.K.

3. Results

3.1 Field trials Ia and Ib — Conducted at the Bandirippuwa Estate Coconut Research Institute Lunuwila (From 15th March to 20th March 1978)

For these field trials a total number of seventy seven (77) trees were used, which were grouped into 9 batches. The effect of adding NH_4^+ on the yield of ethanol and the formation of hydrogen sulphide was studied using two concentrations of ammonium chloride. The ammonium chloride was added in the form of a solution and the two concentrations used were 0.05% (w/v) and 0.08% (w/v) of NH_4^+ based on the volume of toddy.

The results of this field trial are given in Table 1.

Incorporation of NH_4^+ at a concentration of 0.05% (w/v) only partly suppressed the formation of hydrogen sulphide. In some batches there was no suppression at all. However, the ethanol yield was found to increase appreciably and the results showed an average increase of 28% in total yield of ethanol per batch and an average increase of 13.0% in the ethanol content of the toddy. With the increase of the NH_4^+ concentration to 0.08% (w/v) a complete suppression of the hydrogen sulphide formation was observed in all batches. At the same time the total yield of ethanol per batch increased by an average of 46% while the ethanol content in toddy increased by an average of 32%. The residual sugar contents of fermented toddy was found to be very much less in samples treated with NH_4^+ (at both concentrations) as compared with untreated (control) samples.

3.2 Field Trial 2 — Conducted at the Galawatte Estate, Land Reform Commission, Bandirippuwa (From 13th February to 20th February 1979)

In this field trial, ammonium chloride was incorporated into toddy in the form of tablets to give the required concentration of NH_4^+ ions. The concentration of NH_4^+ ions used was 0.08% (w/v) based on the volume of toddy expected. The results are given in table 1.

Table 1. — The effect of NH_4^+ (as an alternative source of nitrogen for yeast metabolism) on the ethanol yield and the formation of hydrogen sulphide in coconut toddy.

Field trial	No. of trees	NH_4^+ conc. (%)	Volume of toddy (ml)		Avg. Ethanol content of toddy % (v/v)		Avg. Residual sugar (% w/v)		H_2S^* formation		Total yield of ethanol (ml)		Avg. % increase in the yield of ethanol	Increase in the ethanol content %
			C	E	C	E	C	E	C	E	C	E		
1a	77	0.05	65350	74750	7.6	8.6	1.4	0.4	+++	—+	5032	6450	28.3	12.5
1b	77	0.08	152770	165500	7.0	9.2	3.0	0.2	+++	—	10604	15276	46.3	32.0
2	360	0.08	—	—	7.1	7.5	0.34	0.08	+++	—+	—	—	—	7.0
3	21	0.08	64925	70847	7.8	8.4	0.76	0.33	+++	—	5122	5976	17.1	6.6
4	100	0.08	752200	820650	7.7	8.1	1.65	0.66	+++	—	51163	58554	14.1	4.4
Total	635	—	1,035,245	1,131,747	7.4	8.4	1.43	0.33	—	—	71921	86256	26.5	12.5

* Key — C — control, without added NH_4^+
 E — Experimental, with added NH_4^+
 H_2S formation : +++ high
 —+ traces
 — absent

Three hundred and twenty four (324) trees were used in this trial which were grouped into nine batches. In this trial the effect of adding NH_4^+ ions on the ethanol content of the toddy and the formation of hydrogen sulphide was studied. The effect of NH_4^+ on the total yield of ethanol per batch was not studied.

The results showed that the suppression of the formation of hydrogen sulphide in the NH_4^+ treated samples was not complete and also the increase in the ethanol content of toddy was around 7%.

The results of this field trial was affected by the changing weather conditions mainly by the intermittent fall of rain.

3.3 Field Trial 3 — Conducted at the Kiripallagahawatte Estate, Molligoda, Wadduwa (From 30th November to 5th December 1979)

In this field trial, NH_4^+ (0.08% w/v) were added to the pot in the form of NH_4Cl tablets as in the case of the 2nd field trial. The results are given in Table 1.

At this concentration of NH_4^+ in the toddy an average increase of 17.1% in the total yield of ethanol per batch and an average increase of 6.6% in the ethanol content of the toddy was observed. In addition the hydrogen sulphide formation in toddy was also completely suppressed.

3.4 Field Trial 4 — Conducted at the Molligoda Estate, Molligoda, Wadduwa (From 29th October to 7th November 1980)

In this field trial too NH_4Cl was added to the pot in the form of tablets to give a concentration of 0.08% (w/v) of NH_4^+ based on the expected yield of toddy. As a result, an average increase of 14.1% in the total yield of ethanol per batch and an average increase of 4.4% in the ethanol content of the toddy were observed. The hydrogen sulphide formation in toddy was also completely suppressed (Table 1).

3.5 Statistical Analysis of the Results

A statistical analysis of the results obtained with respect to the total yield of ethanol and the ethanol content of toddy is given in Table 2. The average increase in the total yield of ethanol and the ethanol content of the toddy were statistically analysed to determine the significance of the observed increases. In this analysis the 'Paired-sample Test' was applied. This analysis showed that the observed mean increases in both the ethanol content of toddy and the total yield of ethanol were very highly significant.

Table 2.—Statistical Analysis of Results of the Five Field Trials on the Effect of Adding NH_4^+ Salts on the Ethanol content and the Total yield of Ethanol in Toddy

Field Trial	No of samples	Average increase in ethanol content	Average increase in total yield of ethanol	t	Degrees of freedom	Probability	Significance of the Avg. increase*
Field Trial 1a	9	12.5%	—	7.5757	8	p 0.001	VHS
	—	—	28.0%	1.8182	8	p 0.1	NS
Field Trial 1b	9	31.7	—	10.5000	8	p 0.001	VHS
	—	—	46.3%	6.4076	8	p 0.001	VHS
Field Trial 2	10	7.0%	—	2.5080	9	p 0.02 p 0.05	S
	—	—	—	—	—	—	—
Field Trial 3	7	—	17.1%	3.5330	6	p 0.01 p 0.02	S
	—	4.4%	—	3.3613	9	p 0.002 p 0.01	HS
Field Trial 4	10	—	14%	9.6932	9	p 0.001	VHS
All Trials	45	12.5%	—	6.9800	44	p 0.001	VHS
	35	—	26.5%	5.5439	34	p 0.001	VHS

* Key : VHS — Very Highly Significant
 HS — Highly Significant
 S — Significant
 NS — Not Significant.

4. Discussion

In the natural fermentation of coconut toddy it appears that organic nitrogen in the form of amino acids acts as the main source of nitrogen for the yeast metabolism.¹ During the utilization of cysteine hydrogen sulphide is formed as a by-product which contaminates both toddy and the distilled product 'Arrack' giving rise to off-flavours. Furthermore, the ethanol yields obtained by the natural fermentation are usually far below the theoretical yields. The low efficiency of sugar utilization under normal conditions appears to be the main reason for this which is apparent by the high residual sugar contents observed. The supply of an alternative and an easily digestible source of nitrogen in the form of NH_4^+ , as recommended by Jansz *et al*² appears to suppress the utilization of amino nitrogen by the wild yeast. As a result the formation of hydrogen sulphide is avoided, and the utilization of sugar is increased resulting in higher yields of ethanol.

In the field trials carried out to test this finding on a commercial scale as reported in this paper a mean increase of 26.5% in the total yield of ethanol and a mean increase of 12.5% in the ethanol content were observed. The significance of these increases on statistical evaluation showed to be very high. It was necessary in this study to express the increases in the ethanol yields in terms of both the total yield per field trial and the percentage ethanol content of the toddy. This was because the percentage ethanol content itself was inadequate to give a true picture due to dilution of the toddy by rain water on many occasions.

The high statistical significance of the observed trends in these experiments are important when the highly variable experimental conditions under which the field trials were conducted are considered. The higher increases in the alcohol yields observed in trials 1a and particularly in 1b may be attributed to the use of ammonium chloride in the form of a solution in these trials. However, comparative studies on the solubility and mixing of ammonium chloride in the toddy, when it is used as a solution or as a tablet, revealed that even in the form of a tablet ammonium chloride dissolved and mixed adequately in the toddy. Some of the main problems encountered in this study were:

- (a) Inaccuracies caused by uneducated tappers in the estimation of the number of tablets or the volume of solution of NH_4Cl to be added to the collection pot on the basis of the expected volume of toddy.
- (b) Adverse weather conditions mainly rainfall, which cause variation of sugar content of the sap, dilution of the toddy, etc.

The most attractive feature of the findings of this study is the financial benefits that could be achieved by the commercial implementation of the process. From preliminary calculations it has been shown that the successful commercial implementation of this process would bring about the following:

- (a) An increase of 0.36 million proof gallons of ethanol per annum from the coconut toddy based distillation industries in Sri Lanka.
- (b) A saving of Rs. 6.2 millions per annum in foreign exchange spent for the import of potable spirit.
- (c) An increase of Rs. 6,350/- per annum per acre in the income from coconut toddy production.

The above figures were calculated on the following data and assumptions:

- (i) Total production of coconut spirits/annum
= 1.47 million proof gallons
- (ii) Increase in the ethanol yield by the use of the reported
process = 25%
- (iii) Total imports of rectified spirits = 1.5 million proof gallons/year (at the cost
of Rs. 22/- per proof gallon)
- (iv) Approximate volume of toddy/acre/day = 96 litres
price of NH_4Cl (Technical)/kg = Rs. 12.00
Cost of NH_4Cl tablets/acre/day = Rs. 6.60
Price of a gallon of coconut toddy (7% v/v alcohol) = Rs. 6.00

References

1. HOUGH, J. S., BRIGGS, D. E. & STEVENS, R., (1975). *Making and Brewing Science*, pp. 474-475 Chapman & Hall Ltd.
2. JANSZ, E. R., JEYARAJ, E. E., ABEYRATNE, D. J. & PREMARATNE, I. G., (1975). *J. Vain. Sci. Coun., Sri Lanka*, 3 (1) 1.
3. KALYANANDA, M. K. G. S., (1977). *Some aspects of the fermentation of coconut toddy*. M.Sc. thesis, University of Sri Lanka, Colombo, Sri Lanka.
4. KALYANANDA, M. K. G. S., JEYARAJ, E. E. & JANSZ, E. R., (1977). *Proc. Inst. Chem., Sri Lanka, Ann. Sess.*
5. PARANAVITHANE, S., KALYANANDA, M. K. G. S., JEYARAJ, E. E. & JANSZ, E. R., (1975). *Proc. Inst. Chem., Sri Lanka, Ann. Sess.*
6. THEIVENDIRARAJA, K., JAVASEELAN, K. & PUVIRAJASINGHAM, V., (1976). *Proc. Ceylon Assoc. Adv. Sci., Sri Lanka*.