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HYDROGEN SULPHIDE FORMATION IN FERMENTING TODDY*

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1. INTRODUCTION

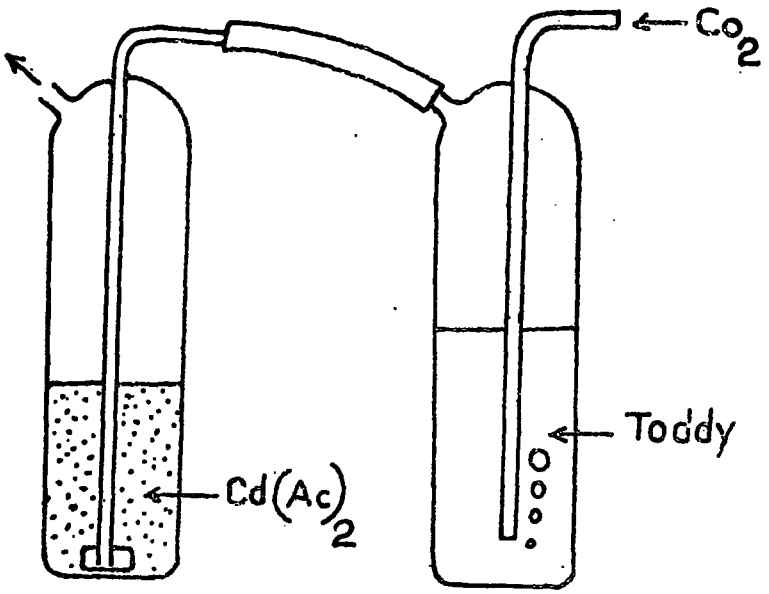
Coconut toddy is the sap exuded from the tender coconut floral spadix obtained by the process known as 'tapping'. The product, 'sweet toddy' contains 16-20% sugar (mainly sucrose). Sweet toddy, on fermentation with wild yeast present in the collecting vessel, gives an alcoholic product called toddy which contains up to 9% alcohol. Fermented toddy, itself a popular drink, also forms the starting material for the preparation of coconut arrack by distillation.

Traditionally copper stills have been used for the distillation of fermented toddy, but recently a stainless steel continuous still constructed for the purpose was found to produce distillates with an objectionable odour. The compound responsible for this odour was found to be hydrogen sulphide.

Our studies were directed towards determining the reason for H_2S formation and finding methods by which H_2S can be removed from toddy-based products.

2. ASSAY FOR H_2S IN TODDY

The fermentation was carried out in gas washing bottles (500 ml.). Toddy was fermented in one bottle and gases formed were led into a trap of cadmium acetate in a second gas washing bottle (figure 1). After the fermentation was complete the H_2S formed was aspirated with CO_2. A second trap may be attached but this was found to be unnecessary with the specialised system to break up bubbles used for this study.



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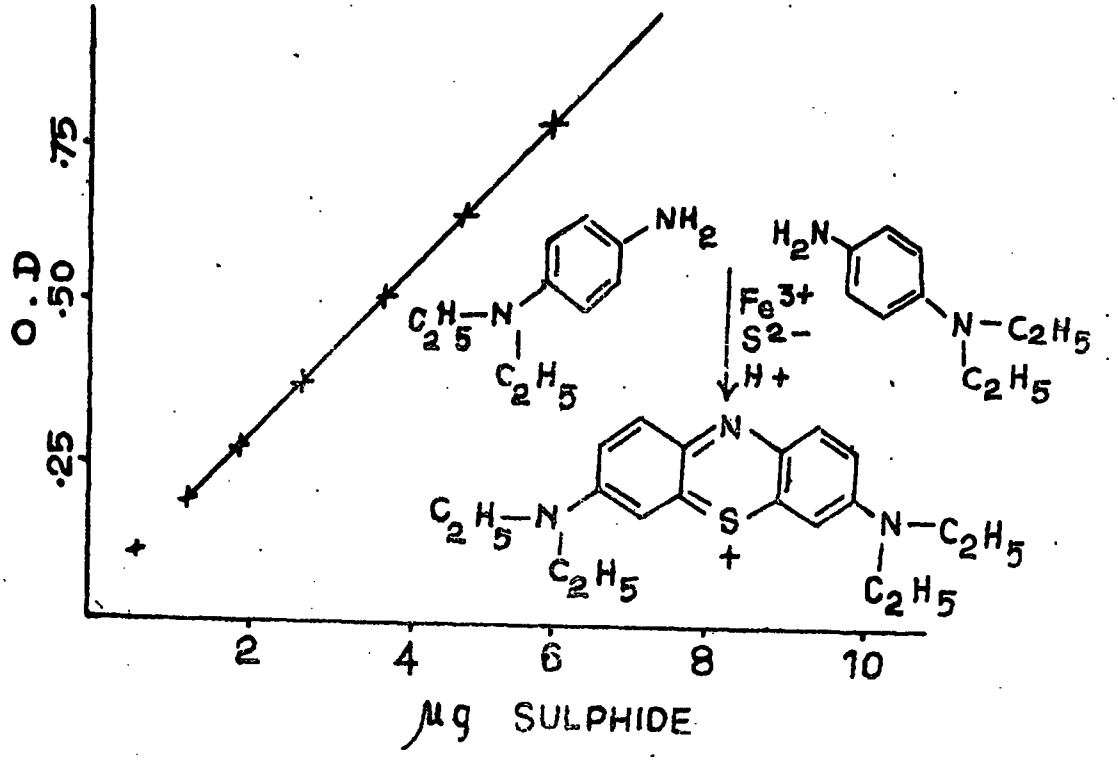
Trial experiments showed that release of H_2S was maximum when: (1) 2ml. of conc. H_2SO_4/ 100 ml. toddy was added prior to aspiration (2) aspiration was continued for 45 min. (rate 250 ml./min.).

The validity of the procedure adopted was confirmed by recovery experiments using ZnS added to H_2S free toddy. These experiments showed that H_2S could be recovered as CdS at more than 90% yield. Reproducibility of the fermentation, recovery and estimation were generally within 10% error.

Sulphide was determined by converting it to diethyl homologue of methylene blue and using the following standard curve (Figure 2):

Fig. 2

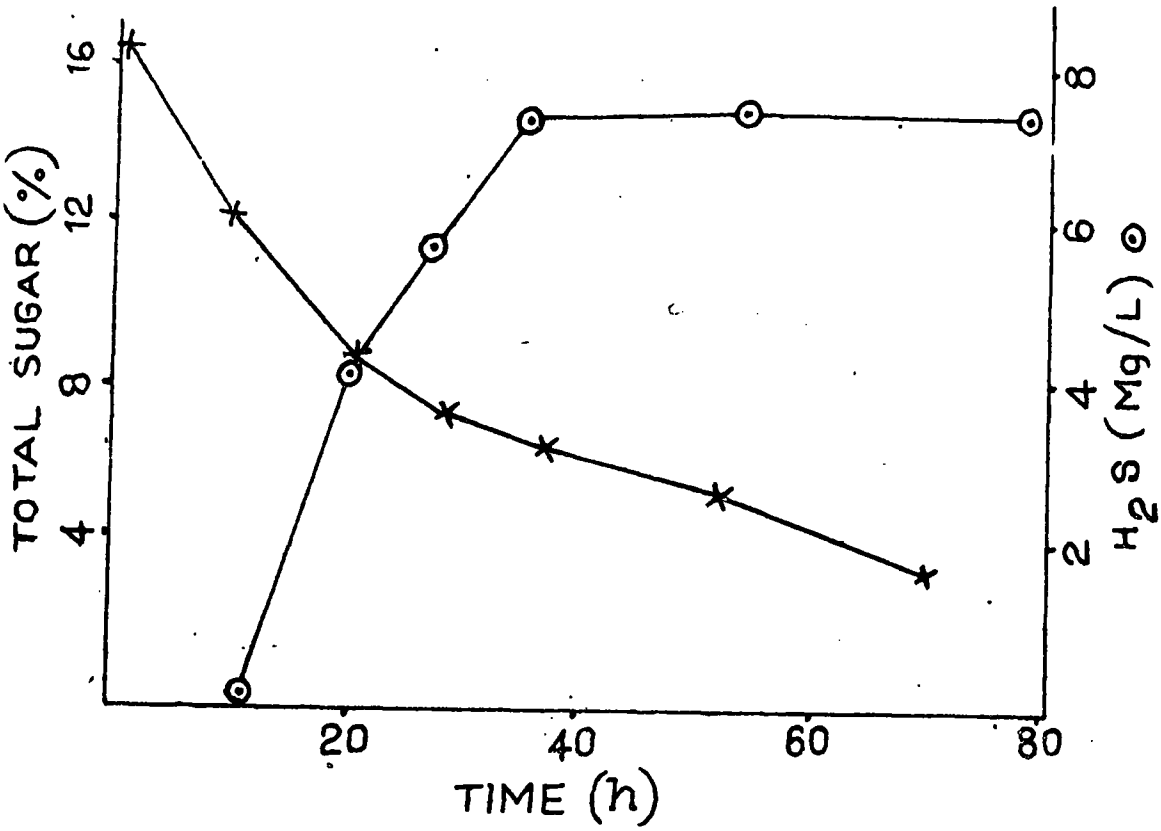


3. VARIATION IN EXTENT OF H_2S FORMATION

Study of several samples of toddy showed that H_2S formed varied from sample to sample. The minimum and maximum levels obtained with wild yeast was 0.04 mg. and 5 mg. per litre. of toddy respectively. However the most frequently obtained values lay between 0.5 and 1.0 mg per litre.

On studying a time course for H_2S formation it was found that most of the H_2S formed during the middle phase of sugar utilization (figure 3) showing that H_2S was produced during the fermentation phase and that this was not a post-fermentative phenomenon.

Fig. 3



4. EFFECT OF STRAIN OF YEAST

The variation is H_2S liberation from toddy could have been due to either (1) differences in population of yeast strains or (2) factors dependent on the toddy.

On testing 34 pure strains of yeast, most of them isolated from coconut toddy it was found that more than half of them could liberate > 1 mg H_2S/litre of toddy. Nearly all the top yeasts did not produce H_2S while the majority of bottom yeasts were H_2S-producers. However there were exceptions in both types. We could not relate any other known characteristic of the yeast cells with H_2S production. Some of the results of these studies are shown in Table 1.

TABLE 1

Effect of yeast strain on H_2S formation

Yeast culture No.	*Source of yeast*	*Sulphide formed (mg/l)*
2	Coconut toddy	1.9
15	Coconut toddy	4.1
17	Coconut toddy	< 0.03
19	Coconut toddy	0.18
20	Coconut toddy	3.4
21	Coconut toddy	3.7
28	Palmyrah toddy	1.9
32	Mysore wine yeast	< 0.03
40	Coconut toddy	N.D.

These studies clearly showed that yeasts are responsible for H_2S formation in toddy, and that H_2S formation could be controlled by use of selected strains of yeast.

There is some variation in the extent of H_2S produced depending on the toddy sample used. This is probably due at least in part to the sulphoamino acid content of the toddy samples.

5. CONTROL OF H_2S FORMATION

It was found that the addition of NH_4^+ ion resulted in reduction of H_2S formation. In most cases .01% NH_4^+ was sufficient to prevent H_2S formation. In a few cases however as much as 0.3% NH_4^+ was necessary. However in all cases 0.06% NH_4^+ was sufficient to reduce the H_2S level to $<$ 0.3 mg/litre.

Results of a typical experiment are shown in Table 2.

TABLE 2

Effect of NH_4^+ on H_2S formation

Conc. of NH_4^+ added (%)	*Total sulphide formed (mg/l)*
0	4.8
0.005	0.60
0.01	$<$ 0.03
0.03	$<$ 0.03
0.1	$<$ 0.03

6. OTHER EFFECTS OF THE NH_4^+ ION

In addition to preventing H_2S formation the NH_4^+ ion has several other effects: (1) increase in the rate of fermentation (2) increase in sugar utilization (3) reduction of off-odours NH_4^+ addition has no significant effect on efficiency of fermentation i.e. alcohol formed per unit of sugar utilized. Some of these effects are shown in Table 3.

TABLE 3

Effect of NH_4^+ on the fermentation

	NH_4^+ added	*Control*
Sugar (21 h.) (%)	8.5	11.7
Alcohol (21 h.) (%)	3.96	2.52
Sugar (48 h.) (%)	0.6	3.9
Alcohol (48 h.) (%)	7.52	5.64
Alcohol formed: sugar utilized (48 h.)	0.47	0.45

The NH_4^+ ion however tends to reduce the intensity of the flavour of the toddy. This is probably due to the sparing of amino acids.

7. ORIGIN OF H_2S

Fermentation of synthetic media containing methionine or cysteine with selected yeast strains led to the following results (Table 4).:

TABLE 4

Effect of Methionine and Cysteine on H_2S formation

Yeast culture	*Met.*	*Cys.*	NH_4^+	$\frac{S^{2-}}{mg/l}$
32	+	—	—	0.03
32	+	—	0.7	N.D.
32	+	—	0.2	N.D.
20	+	—	—	0.38
20	+	—	0.2	0.19
32	—	+	—	3.9
32	—	+	0.7	0.13
20	—	+	—	6.7
20	—	+	0.7	0.03
20	—	—	—	N.D.

N.D.= not detected

This showed that:

(1) cysteine produced H_2S more readily than methionine

(2) the yeast strains producing higher H_2S from toddy produce more H_2S from cysteine.

(3) H_2S formation from the sulphoamino acids was also inhibited by the NH_4^+ ion.

Chromatographic study of the free sulphoamino acids of toddy showed that the sulphoamino acid content was sufficient to account for the H_2S produced. Other experiments showed that addition of the SO_4^{2-} ion resulted in no increase of H_2S formation.

All this strongly suggests that the amino acids cysteine and methionine are the source of H_2S in toddy (Figure 4).

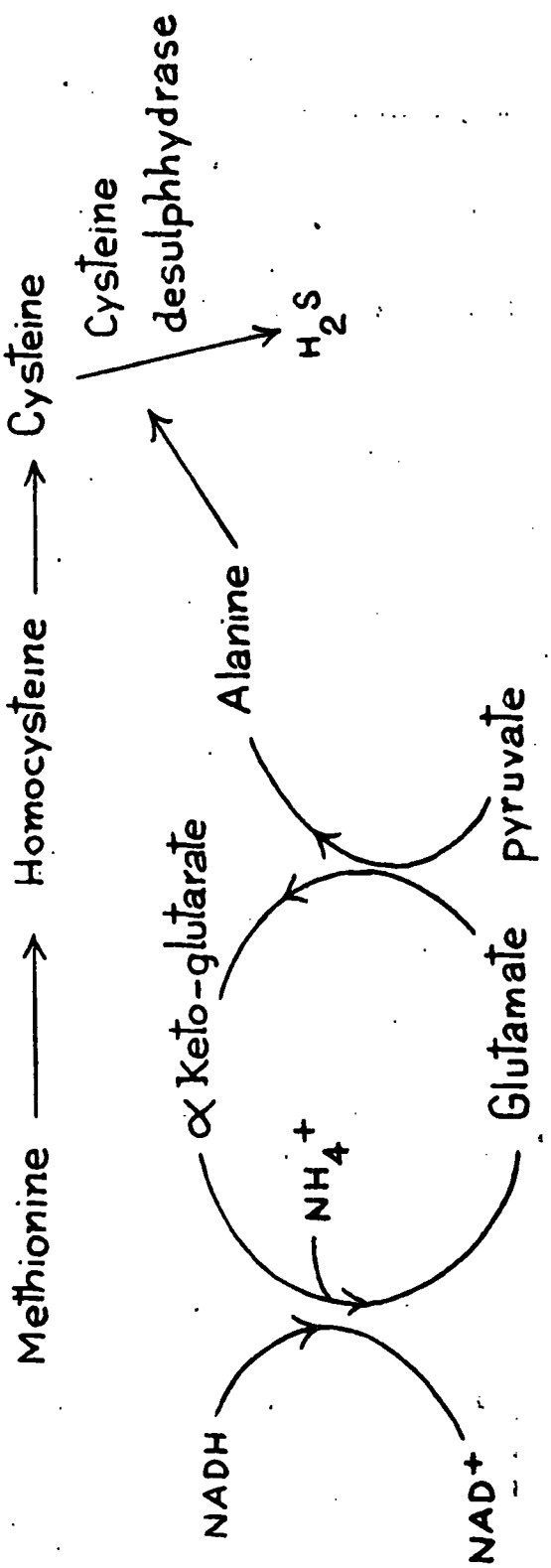

Methionine
Homocysteine
Cysteine
Cysteine desulphhydrase
H_2S
Alanine
Pyruvate
α Keto-glutarate
Glutamate
NH_4^+
NADH
NAD^+

Fig. 4

The effect of NH_4^+ may be due either to a repression of synthesis of cysteine desulphhydrase or to inhibition of the enzyme, possibly by alanine which is known to inhibit the enzyme from *Escherichia coli*.

There also seems to be an inverse relationship between invert sugar and H_2S formation; this needs further investigation.

8. EFFECT OF METALS ON H_2S CONTENT OF TODDY

Metals have a marked effect on free H_2S in toddy. Free H_2S in toddy decreases markedly in the presence of copper turnings the sulphide being trapped as CuS. Iron and mild steels increase H_2S levels markedly, while several other metals have no effect. (Table 5).

TABLE 5

Effect of metals on free sulphide content

Conditions		*Free sulphide (mg/l)*		
		Expt. 1	*Expt.* 2	*Expt.* 3
	Control	0.30	0.70	1.1
Type A	+ Cu	0.14		< 0.03
	+ Fe	2.6	1.7	
Type B	+ Mild steel	0.96		
	+ Bright steel		3.6	
	+ Al	0.42		
Type C	+ Sn	0.31		
	+ Stainless steel		0.56	

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