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Effect of metabisulphite on alcohol production in palm-wine

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This work reports on the effect of the metabisulphite ion on the concentration of ethanol in fermenting palm-wine. The work shows that the concentration of ethanol in untreated palm-wine reaches a maximum between three and four days and starts declining after this point. In the presence of the metabisulphite ion, the decline in ethanol content which takes place after three to four days no longer occurs. There was also an over 20% increase in the percentage of ethanol in palm-wine when the fermentation process was allowed to proceed in the presence of the metabisulphite ion. The work therefore recommends the use of sodium metabisulphite to increase the yield of alcohol (ethanol) during fermentation processes.

INTRODUCTION

Nigerian palm-wine is normally made from the sugary sap of *Elaesis guineensis* (oil-palm) and *Raphia vinifera* (Raphia palm). Palm-wine contains a lot of water-soluble vitamins (Bassir, 1967; Ankrah, 1973). Since vitamin B deficiency is wide spread amongst pregnant women and teenagers in Nigeria, palm-wine is considered an important additive to their diet (Bassir, 1967).

Raphia palm-wine is made from the sap of the Raphia palm tree. The sap contains many simple sugars. The sugars identified from Raphia palm-wine include sucrose, glucose, fructose and maltose (Faparusi, 1981). The sap is collected from slits along the trunks and the unexpanded flower spathes and is fermented by wild yeast into the palm-wine. Yeast cells, mainly *Schizosachromyces prombe* (Bassir, 1962), *Saccharomyces cerevisiae, Saccharomyces chevalieri, Saccharomyces pastriorianus, Saccharomyces ellipsoides* and *Saccharomyces vafer* (Okafor, 1972; Abalaka & Opara, 1990) accumulate in millions around the flower stalks and innoculate the sap naturally. The palm-wine yeasts have been shown by Abalaka and Opara (1990) to give low yields of biomass but high yields of alcohol (ethanol).

A motile rod-shaped and high alcohol-producing anaerobic bacterium, closely related to *Zymonomas mobilis*, has been reported (Van Pee & Swings, 1971) to occur in Congolese palm-wines. Different strains of *Zymonomas*, isolated from Zaire palm-wine, have also been shown to effect sucrose fermentation (Van Pee & Swings, 1972). Bacteria isolated from samples of Nigerian palm-wines produced from the oil-palm and Raphia-palm trees include *Micrococcus, Leuconostoc, Streptococcus, Lactobacillus, Acetobacter, Serratia, Aerobacter* (Klebsiella), *Racillus, Zymononas* and *Previbacterium* species. The observed lowering of pH from about 7 to about 4 during fermentation of palm-wine appeared to be due to lactic-acid-producing bacteria and certain gram-negative bacteria such as *Serratia* and *Aerobacter* species (Okafor, 1975*a,b*).

Sacaglottis gabonensis is traditionally added to fresh palm-wine to prevent souring. Stem bark extract of *Sacaglottis gabonensis* was shown by Faparusi and Bassir (1972) to appreciably inhibit the growth of certain bacteria isolated from palm-wine such as *Leuconostoc mesenteroides* and *Lactobacillus plantarum.* The palm-wine becomes more alcoholic on standing. The sour taste, which develops on standing, increases with time and is attributable to microbial activities resulting in the production and accumulation of organic acids. It has been reported (Morah, 1986) that the metabisulphite ion preserves the taste and flavour of palm-wine. Also, the usual increase in acidity of palm-wines with time has been shown to be drastically reduced by the presence of metabisulphite ion. The West African local gin is prepared by repeated simple distillation of three- to four-day-old palm-wine. The percentage of ethanol in the local gin varies, depending on the source and extent of distillation.

The presence of sulphur-dioxide-producing yeast strains has been reported by Wurdig and Schlotter (1971). This is responsible for certain finished wines containing considerably more sulphur dioxide than has been added before, during and after fermentation. Such

sulphur-dioxide-producing yeast strains are yet to be identified in palm-wine yeast cultures. The present work reports on the effect of added metabisulphite ion on the production of alcohol during fermentation of palm-wine. The amount of ethanol produced at different times during the fermentation process was estimated by the diffusion method (Morah, 1994).

MATERIALS AND METHODS

Unadulterated palm-wine, from *Raphia vinifera* P. Beauv (palmae), was tapped under special arrangement with local tappers from trees grown up land (dry land away from coastal swamps and river banks). The containers were rinsed with clean water in the evenings before using them to collect palm-wine overnight. The morning wines were collected around 10.00 a.m.

A solution of sodium metabisulphite in a wine sample was prepared by carefully weighing out a calculated amount of sodium metabisulphite and dissolving it in an appropriate volume of wine in a volumetric flask. These solutions were prepared within 30 min of collection of the palm-wine sample.

The analyses were done in the laboratory as follows: 25 cm^3 of a 0·1 M solution of potassium dichromate, acidified with H_2SO_4, was pipetted into a large container. A 1 cm^3 sample of the palm-wine was carefully measured into a 10 cm^3 conical flask. It was saturated with anhydrous potassium carbonate and placed inside the larger vessel containing the standard potassium dichromate solution. The large vessel was then carefully covered with a watch glass in such a way as to cut off outside air. This was left to stand at room temperature overnight to allow for complete diffusion of the ethanol into the dichromate solution.

After standing overnight, the dichromate solution was carefully washed into a 250 cm^3 volumetric flask. Then 20 cm^3 of 10% KI solution was added and the solution made up to 250 cm^3 with distilled water. This was left in the dark for about 3 h to allow for complete reaction between the unreacted dichromate and iodide ions. The liberated iodine was estimated by titrimetry using a 0·05 M solution of sodium thiosulphate with starch indicator. A blank experiment without palm-wine was set up each time, side-by-side with the main experiment. It was treated in the same way and also titrated with the standard sodium thiosulphate solution. The difference between the titres for the blank and the test sample was noted. From this, the quantity of ethanol in the 1 cm^3 of the palm-wine and hence the percentage of alcohol in the wine was calculated.

The above procedure was carried out for six different samples of palm-wine containing no sodium metabisulphite, then with additions of 80 mg, 110 mg, 320 mg, 480 mg and 640 mg of sodium metabisulphite per cubic decimetre of wine respectively. These samples were analysed between 7.30 p.m. and 8.00 p.m. each night for a period of 10 days. All the experiments were carried out in triplicate and replicated twice.

RESULTS AND DISCUSSION

During the analysis, samples of palm-wine were saturated with potassium carbonate to help expel the ethanol. The expelled ethanol was allowed to diffuse into a standard solution of acidified potassium dichromate and became oxidised to acetic acid. The difference between the amount of standard thiosulphate used for titrating the liberated iodine in the control and test titrations is directly related to the amount of dichromate that had reacted with the ethanol and hence the amount of ethanol that diffused out of the palm-wine.

The concentration of ethanol (alcohol) at zero time was 6·95% vol. The untreated palm-wine was shown from the analysis to contain about 7·61% of ethanol after 9 h of the experiment. This rose to 11·8% after 48 h. It then declined to 6·88% by the 225th hour. The decline after about four days is understandable as some other micro-organisms oxidise the produced ethanol to acetic acid, etc. This results in increased acidity of the stale palm-wine (Morah, 1986).

The batch fermentation process was used for the study and the simple sugars in solution constitute the substrate. The substrate was not replenished and the ethanol-producing bacteria and yeast grew at a maximum rate until the substrate was nearly exhausted. Since the growth of these micro-organisms is usually accompanied by the formation of ethanol, the production of ethanol also rose to a maximum and slowed down with depletion of the substrate until it eventually stopped. The acidity of palm-wine has been shown by Morah (1986) to increase continuously with time. This is mainly due to the activity of such micro-organisms as *Acetobacter, Micrococcus* and *Lactobacillus* species, producing acetic, glutamic and lactic acids, respectively. The *Acetobacter* species convert the ethanol into acetic acid. With the combined activity of the ethanol-producing micro-organisms and the *Acetobacter* species, the fermentation process of palm-wine becomes more or less converted to a consecutive reaction type (Deindoerfer, 1960) in which the produced ethanol, acting as the intermediate product, first acculates to some extent before being converted into the final product, acetic acid. As the growth of the *Acetobacter* culture continues, the ethanol is consumed while the concentration of acetic acid builds up. A certain critical period results in a maximum level of ethanol. After this point a decline in the concentration of ethanol sets in and this occurred between the 125th and 151st hours of the experiment. This observation explains why the indigenous inhabitants of the West African Coast distil three- to four-day-old palm-wine to obtain the West African local gin.

Table 1 shows that fermentation of palm-wine in the presence of the metabisulphite ion results in an increased concentration of ethanol in the wine. This could be attributable to the fact that the metabisulphite ion is a powerful bactericide and fungicide due to its action as an enzyme poison (Tolly, 1971). The bacteria which convert the produced ethanol to acetic acid are

Table 1. Effect of $Na_2S_2O_5$ on percentage of ethanol in palm-wine[a]

Time (h)	Concentration of $Na_2S_2O_5$					
	0·00 g dm^{-3} % C_2H_5OH	80 mg dm^{-3} ($4·21 \times 10^{-4}$M) % C_2H_5OH	160 mg dm^{-3} ($8·2 \times 10^{-4}$M) % C_2H_5OH	320 mg dm^{-3} ($1·68 \times 10^{-3}$M) % C_2H_5OH	480 mg dm^{-3} $2·52 \times 10^{-3}$M) % C_2H_5OH	640 mg dm^{-3} ($3·67 \times 10^{-3}$M) % C_2H_5OH
Zero time	6·95	6·95	6·95	6·95	6·95	6·95
9	7·61	8·44	9·24	10·5	10·7	7·96
33	10·0	10·8	11·2	11·8	11·8	11·2
57	11·0	11·9	12·4	12·8	12·8	12·5
81	11·8	13·0	13·4	13·8	13·4	13·4
105	11·7	14·3	13·7	13·9	13·4	13·4
129	10·4	14·2	13·8	13·7	14·1	13·0
153	9·65	14·1	13·9	14·1	13·7	12·8
177	7·04	14·0	14·0	14·5	13·5	12·8
201	6·97	13·8	13·7	13·9	13·3	12·6
225	6·88	13·6	13·7	13·9	13·2	11·7

[a]The data in the table are means of three determinations.

thus controlled and hence the concentration of ethanol becomes higher.

Table 1 also shows that the decrease in the concentration of ethanol which normally occurs in fermenting palm-wine after three to four days of collection, no longer occurs in the presence of the metabisulphite ion. With 80 mg dm^{-3} of sodium metabisulphite, the critical point of maximum concentration of ethanol in the palm-wine occurs at about 105 h from the zero time. After this, it declines slightly from 14·3 to 13·6% at the 225th hour. This shows that there is appreciable suppression of the growth of the micro-organisms responsible for the conversion of ethanol to acetic acid. Fermentation is almost reduced to a simple type. In this case, the rate of production of ethanol rises rapidly and slows down as the substrate becomes depleted. The trend is basically the same at other levels of metabisulphite ion in the palm-wine.

It is observed from Table 1 that an increase in concentration of sodium metabisulphite above 480 mg dm^{-3} (i.e. about 323 mg dm^{-3} of sulphur dioxide) results in a decline in the amount of ethanol in the palm-wine. The normal increase in acidity of fermenting palm-wine with time has also been shown (Morah, 1986) to be minimal at such a level of metabisulphite ion, indicating an appreciable suppression of the activity of *Acetobacter* species. It can therefore be inferred that the metabisulphite ion or sulphur dioxide has some effect on the palm-wine yeast. However, the level of metabisulphite ion required to appreciably suppress the growth and activity of the palm-wine yeast in the fresh wine is above the limit permitted in wine (i.e. over 200 mg dm^{-3}).

It is also observed from Table 1 that, in the presence of metabisulphite ion, the rise in ethanol in different test samples was from 13·4 to 14·5%. This implies that the yield of the local gin from palm-wine may increase significantly by first adding appropriate amounts of sodium metabisulphite to fresh palm-wine and then leaving it to stand for some days. At present, the local inhabitants use *Sacaglottis gabeneasis* shoots to achieve this. The stem of this plant has been shown to be a potent bactericide (Faparusi & Bassir, 1972). In this case, the wine could be distilled after four days. The metabisulphite ion suppresses the activity of certain micro-organisms which produce undesirable by-products, some of which are volatile, during the fermentation of palm-wine. Its presence in fermenting palm-wine may therefore improve the quality of the local gin made from the wine.

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