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KINETICS OF PALM WINE FERMENTATION: THE EFFECT OF HYDROGEN PEROXIDE PRESERVATION

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Abstract
Kinetic studies of Elais sap dosed with hydrogen peroxide, in order to inhibit bacterial activities during fermentation of Elais sap showed that a dose 0.1% H_2O_2 did not inhibit bacterial activity, suggesting that bacterial inhibition was not achieved at this dose rate but activates bacterial activity significantly, producing more alcohol. The similar fermentation results obtained with the sap dosed with 0.4% H_2O_2 and 0.8% H_2O_2 suggested that Elaeis sap dosed with 0.8% H_2O_2 will produce a better effect on palm wine fermentation. In this regard, sap dosed with 0.8% H_2O_2 will offer the most promising way of preserving palm wine by bottling

Keywords: Kinetics, Elais sap, Fermentation, Hydrogen peroxide, Bacterial inhibition.

Introduction

Palm wine is the collective name for a group of alcoholic beverages produced from the sap of various palms, (Elaeis and Raffia palms) and contains a heavy suspension of live yeasts and bacteria. These organisms give the drink a milky-white appearance and are responsible for the initial fermentation. (Okafor, 1975a).

To be acceptable to most consumers of palm-wine, it must only be whitish in appearance and have a pleasant sugary taste, but must also exhibit vigorous effervescence. The presence of the micro-organisms, result in the disappearance of the pleasant sugary taste of the fresh wine within 36-48 h and production of various organic acids and alcohol (Bassir, 1962; Okafor, 1975a).

Palm wine is consumed in various parts of the tropical world including South America, Asia, and Africa (Chandrasekhar et al., 2012). In Africa, the production of palm wine appears to have been known for the chronicle of European travellers recorded as early as 1591. Figures for the production of palm wine in Nigeria do not exist but a conservative estimate would be a daily production of 80-120 thousand litres. (Okafor, 1975a).

Although attempts have been made towards the inhibition of the microbial activities and extend the shelf-life of the palm wine through bottling, use of chemical additives and addition of plant extracts have greatly affected the quality and quantity of the product (Orimaiye, 1997; Iheonu, 2000; Nwokeke, 2001; Obire, 2005; Chime et al., 2007). No suitable method of preservation have been evolved, much of the wine is wasted or distilled for alcohol-the so-called "illicit gin" (Okafor, 1975a). A successful and safe preservation method giving a shelf-life of at least 6 months is desirable since production peak is during the rainy season and much reduced in the drier periods of the year.

Hydrogen peroxide is used as antimicrobial agent and oxidizing agent. The interest in nontoxic and degradable yet potent biocides such as hydrogen peroxide (H_2O_2) has never been so high. The nontoxic and biodegradability of H_2O_2 solutions has meant that it hadalso found extensive use in food industry (Ezra et al., 2012).

Hydrogen peroxide an oxidative biocide remove electrons from susceptible chemical groups, oxidizing them and become reduced in the process (Russell, 2003). Oxidizing agents are usually low-molecular-weight compounds and pass easily through cell walls/membranes and react with internal cellular components, leading to cell death. Mechanism of action may differ between oxidative biocides, but the physiological actions are often similar (Denyer and Stewart, 1998). The best understood mechanism act to kill microorganisms is via a respiratory bust and in particular H_2O_2 (Klebanoff, 1980).

Generally Recognized As Safe (GRAS) for the use of H_2O_2 in food are at the Limit of Detection (LOD) in the final product. These residual levels may be zero, due to high reactive nature of H_2O_2. Thus actual exposure to $H_2O_2$from its use in food is likely to be much lower (Clausen, 1999). Therefore this study was primarily carried out to assess the effect of various concentrations of hydrogen peroxide (H_2O_2), on 24hrs fermented palm wine (*Elaeis guineensis*).

Materials and Methods

Sample Collection

Palm sap sample (*Elaeisguineensis*) was collected by special commissioned tapper to avoid adulteration and dilution from Amuri, Nkanu East Local Government Area in Enugu State.

Chemicals

Hydrogen peroxide and other chemicals used in this fermentation studieswere of analytical grade and purchased from FinLab Nigerian Ltd.

Fermentation Studies of Palm Wine

The experimental procedure of Agu et al; 2000 was used. Exactly 100mL of. *E guineneus* sap were dosed with 1mL 0f 0.0, 0.1, 0.2, 0.4 and 0.8% of hydrogen peroxide (H_2O_2) and the rate of fermentation was determined by monitoring the volume of carbon dioxide evolved during fermentation and reading taken every 1hr.

Determination of Percentage Alcohol

Hundred milliliter each of fresh fermented palm wine and those dosed with various concentrations of hydrogen peroxide (H_2O_2) were distilled with 50mL of distilled water respectively. The volume of 95mL of the distillate was made up to 100mL with distilled water and the percent alcohol determine using Gay Lussac alcohol meter (Model No. 6181).

Test for other fermentation products

Test for methanol

Two milliliter of reagent A (3g potassium permanganate and 40% Phosphoric acid solution) was added to 5mL of each distillate and allowed to stand for 10minutes. Then 2mL of reagent B (5% Oxalic acid in sulphuric acid solution) was added followed by 5mL of Schiff's reagent and observed for violet colouration for presence of methanol.

Test for Iso-propanol

Five milliliter of each distillate was added to 5mL of acidified mercuric sulphide (5g mercuric oxide dissolved in 40mL of water and stirred then 20mL of concentrated sulphuric acid was added gradually and the volume made up to 100mL) and heated in boiling water bath for 3 minutes. The appearance of white precipitate is a positive test.

3. Result and Discussions

Tables 1 shows the result obtained when 0.0, 0.1, 0.2, 0.4, and 0.8% of hydrogen peroxide were dosed to Elaeis guineensis sap prior to fermentation. There was noticeable effect on the volume of CO_2 produced

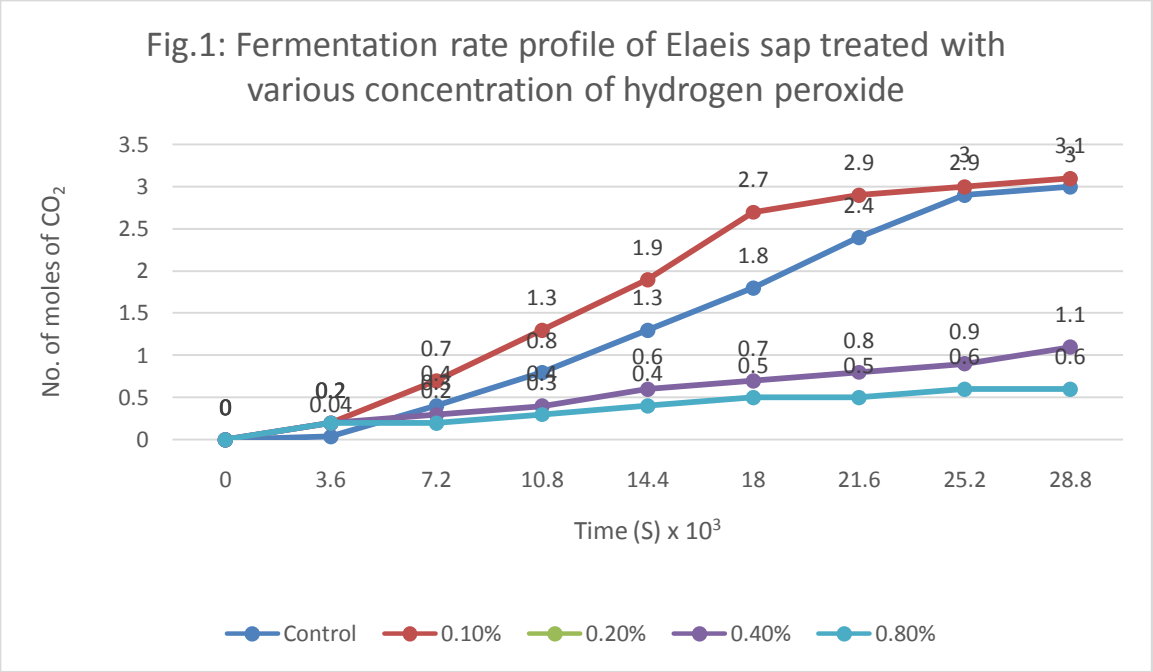
Table 1. Volume of $C0_2$ (cm^3) evolved during fermentation of *E. guineenus*sap treated with various concentrations (%) of H_2O_2 /100mL sap

S/N	Fermentation Period ($Sx10^3$)	0.0 % H_2O_2 (Control)	0.1% H_2O_2	0.2% H_2O_2	0.4% H_2O_2	0.8% H_2O_2
1	0.0	0(0.0)	0(0.0)	--	0(0.0)	0(0.0)
2	3.6	10(0.04)	50(0.2)	--	40(0.2)	40(0.2)
3	7.2	90(0.4)	180(0.7)	--	70(0.3)	50(0.2)
4	10.8	190(0.8)	310(1.3)	--	100(0.4)	70(0.3)
5	14.4	310(1.3)	460(1.9)	--	140(0.6)	90(0.4)
6	18.0	450(1.8)	650(2.7)	--	170(0.7)	110(0.5)
7	21.6	590(2.4)	705(2.9)	--	195(0.8)	123(0.5)
8	25.2	700(2.9)	740(3.0)	--	220(0.9)	140(0.6)
9	28.8	730(3.0)	760(3.1)	--	270(1.1)	150(0.6)

Values in parenthesis are moles of CO_2 produced

At the different concentrations of H_2O_2. There was a remarkable increase in the volume of CO_2 produced from 0.1% $H_2O_2$when compared with the control. At a concentration as low as 0.1% of H_2O_2there was increased in the volume of CO_2 produced as a result of enhancement of the microbial activity at that concentration.

However, the volume of CO_2 evolved in pure *Elaeis* saps (control) varied remarkably from 0.1% H_2O_2dosed saps. The observed increase in the rate of fermentation of the sap dosed with 0.1% of H_2O_2might be attributed to the increased activities of microbes in the sap (Faparusi and Bassir, 1971; Okafor, 1968). It is likely that 0.1%H_2O_2/100mL of *E. guineenus* sap is the optimum concentration of the H_2O_2 that activates the microbes and hence speed up the fermentation process. Figs 1 and 2 show the result obtained for the two saps, dosed with various amount of of H_2O_2including the control. The number of moles of CO_2 liberated over time during fermentation was plotted against time (Agu et al., 2000). It was clear that the control produced the less amount of CO_2 (Fig. 2) than 0.1% of H_2O_2dosed sap.



The addition of 0.4-0.8% H_2O_2decreased the rate profile against that of the control (in which no H_2O_2were dosed). The decrease in the volume of CO_2 evolved showed that H_2O_2acts as inhibitor to the fermentation microbes at that concentration range. The observed general decrease in the rate of fermentation of the saps with the addition of 0.4-0.8% H_2O_2might be attributed to the decreased activities of microbes in the saps (Fig. 1)

It is likely that H_2O_2seem to be inhibiting the microbes and hence slowing down the fermentation process at the 0.4-0.8% H_2O_2 concentrations studied. This is suggesting that the inhibition will be optimal as lower concentration. However, with respect to the amount of alcohol produced, it was found that the control and 0.4% H_2O_2dosed experiments generated the same amount of alcohol more than the o.8% dosed samples (Table 2). It is possible that 0.8% H_2O_2 reduces the rate of fermentation by making the microbes to convert the sugar at a slower rate into alcohol and other products by inhibiting microbial activities (Fig. 1).

The volume of CO_2 evolved and alcohol produced during the fermentation processes were recorded (Table 1 and 2). It is clear from the volume of CO_2 evolved that the rate of fermentation was remarkably enhanced with the dose of 0.1% H_2O_2. However, with respect to the amount of alcohol produced, it was found that 0.1% H_2O_2 generated more alcohol (5%) followed by 0.4% H_2O_2 and 0.0 % H_2O_2 (control) (3%). It is possible that the 0.1% H_2O_2 enhances the rate of fermentation and alcohol production by making the microbes to convert the sugar into alcohol and other products by microbial activities and oxidation (Agu et al., 2000; Okafor, 1974; Illao, 1981; Vanpee and Swing, 1971).

This study has shown that hydrogen peroxide (H_2O_2) enhances the rate of fermentation and alcohol from *E. guineenus* sap at a concentration of 0.1%. The fermentation rate results of the control, 0.4% H_2O_2 with 0.8% H_2O_2werecompared. The rate of fermentation, of thecontrol experiment was higher than that of sap dosed with 0.4% H_2O_2 and 0.8% H_2O_2(Table 1). The similar percent alcohol obtained for thesap dosed with 0.4% H_2O_2 are difficult to explain.These results, however, suggest that when the sap dosed with 0.8% H_2O_2inhibiting bacterial activity would be achieved. In this regard, the sap, dosed with 0.8% H_2O_2, would be a better option in controlling the fermentation rate of palm sap than treating the sap with other chemical inhibitors such as an antibiotic as long as hydrogen peroxide an oxidative biocide remove electrons from susceptible chemical groups, oxidizing them and become reduced in the process (Russell, 2003).

Table 2. Properties and some fermentation products of *E. guineenus* sapand wine dosed various concentration (%) of H_2O_2 /100mL sap

Parameters	**0.0 % H_2O_2 (Control)**	**0.1% H_2O_2**	**0.2% H_2O_2**	**0.4% H_2O_2**	**0.8% H_2O_2**
Percent alcohol (%)	3	5	--	3	2
Specific gravity	0.9940	0.9908	--	0.9932	0.9922
Presence of Methanol	—ve	—ve		—ve	—ve
Presence of Iso- propanol	+ve	+ve		+ve	+ve

Key;- +ve = positive, —ve = negative

Other fermentation products studied revealed that Iso-propanol was present in both the control and those dosed with various concentration (%) of H_2O_2/100mL sap.Methanol as fermentation product was absent in both control and dosed saps. The absence of methanol as fermentation product revealed that palm wine is nontoxic and safe to human (Table 2).

Conclusion

At doses higher than0.1 % H_2O_2 ,$CO_2$and alcohol production decreased, showing that the fermentation process was inhibited. It therefore follows that H_2O_2 has a potential for use in the preservation of palm wine by bottling.

References

Agu, R. C., Ogbuanu, C. C., Ezema, P. O., Onyia, A. I, Ude, C. M. & Nwankwo, Y. I. (2000). Effect of bacteria inhibition on the fermentation kinetics of Elaeisguineensis. *J. Food Sci and Technol 37*(6): 648-650.

Agu, R. C., Okendii, M. U., Ude, C. M., Onyia, A. I., Onwumelu, A. H., & Ajiwe, V.I.E. (1999). Fermentation kinetics studies of Nigerian palm wine *Eleaisquineenisi* and *Raphiahookeri* for preservation by bottling. *J. Food Sci. Technol 36*: 205-209.

Bassir O. (1962)."Observation of the fermentation of palm wine", West African Journal of Biological Chemistry 6: 20-25

Chandrasekhar K, Sreevani S, Sesphapani P., Pramodhakumari J. (2012). A review on Palm wine. *Int. J Res Biol. Sci.* 2(1): 33-38

Chime C. C., Ogbuanu C. C. and Attah J. C. (2007). The effect of Hausa kola-nut (kola nitida) on the fermentation of Elaeisguineensis and Raphiahookeri. *J. Sci. Engr. Tech. 14*(1): 7217-7225.

Clausen, E. Jr. (1999). Generally regarded as safe for use of H_2O_2 in preparation of dehydrated onions. Center for FoodSafety and Applied Nutrition. Food and Drug Administration. Pp 7-10

Denyer, S.P. and Stewart, GSAB.(1998). Mechanisms of action of disinfectants. Int. Biodeter Biodegrad. 41:261-268.

Ezra L., Stephen P.D, Gerald McDonnell, Claire s and Jean-Yres M. (2012). Uses of hydrogen peroxide as a biocides: New consideration of its mechanism of biocidal action. Journal of Antimicrobial Chemotherapy 67(7):1589-1596.

Iheonu R.T. E. (2000). Effect of local preservatives of plant origin on microbial and shelf life stability of palm wine. B.sc. Thesis submitted to Abia State University, Uturu, Nigeria.

Klebanoff, S.J.(1980). Oxygen intermediates and the microbicidal event. In R. Van Furth (ed.), Mononuclear phagocytes, functional aspects, Part 2. Martins Nijhoff, Dordrecht, The Netherlands. Pp 1105-1141.

Nwokeke N. V. (2001). Palm wine preservation using traditional plants that have preservative bases. B.Sc. Thesis submitted to Imo State University Owerri, Nigeria.

Obire, O. (2005). Activity of zygomonas species in palm sap obtained from three areas in Edo State Nigeria. *Journal of Applied Sciences Environmental Management, 9*: 25-30

Obire, O. (2005). Activity of zygomonas species in palm sap obtained from three areas in Edo State Nigeria. *Journal of Applied Science, environmental management*. 9: 25-30.

Okafor .N. (1968). Microbiology and Biochemistry of oil palm. *Adv in Appl. Microbiology* 24: 237-256

Okafor N. (1974). Preliminary microbiological studies on the preservation of palm wine. *J. Appl. Bact.* 36:1-7

Okafor, N. (1975). Microbiology of Nigeria palm wine with particular reference to bacteria. *J. Appl Bact., 38* (2): 81-88.

Orimaiye D.O (1997). Isolation and characterization of yeast from palm wine (Elacis guineesis and Raphia hookereri for industrial producton. Biotechnology of alcoholic beverage proceedings of 1997 international conference on biotechnology for the development in Africa, Enugu Nigeria: 196-203 Faparusi S.I and Bassir O. (1971). Micro flora of fermenting palm wine sap. *J. Food Sci technol*. 8: 206-210.

Russell, A.D. (2003). Similarities and differences in the responses of microorganism to biocides. J. Antimicrob Chemother 52750-763.

Ulao, M.C (1981). Global impacts of Applied Microbiology, academic press London, UK pp. 56-59.

Vanpee, W and Swing J.G (1971). Soil Chemistry and productivity of palm plants. *East African Agric Forestry J*. 36:311.