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Full Length Research Paper

Effect of *Sacoglottis gabonensis* and *Alstonia boonei* on the kinetics of *Saccharomyces cerevisiae* isolated from palm wine

A. I. Elijah[1]*, P. C. Ojimelukwe[2], U. S. Ekong[3] and N. U. Asamudo[4]

[1]Department of Food Science and Technology, University of Uyo, PMB 1017, Uyo, Akwa Ibom State, Nigeria.
[2]Department of Food Science and Technology, Michael Okpara University of Agriculture, Umudike, PMB 7267 Umuahia, Abia State, Nigeria.
[3]Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Uyo, PMB 1017, Uyo, Akwa Ibom State, Nigeria.
[4]Department of Microbiology, University of Uyo, PMB 1017, Uyo, Akwa Ibom State, Nigeria.

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***Sacoglottis gabonensis* and *Alstonia boonei* are botanicals used for the preservation of palm wine in Southern Nigeria. This study investigated the effect of *S. gabonensis* (0.625%) and *A. boonei* (0.50%) on the kinetics of *Saccharomyces cerevisiae* isolated from palm wine (PW). Concentrations of the preservatives used in this study were optimal concentrations of the preservatives that had preservative effect on fermenting palm sap. The fermentation rate constant, k, of 2.79 × 10^{-4} mol^{-1} s^{-1} obtained for untreated PW was higher than the k values for PW treated with *A. boonei* (1.7 × 10^{-4} mol^{-1} s^{-1}) and *S. gabonensis* (1.1 × 10^{-4} mol^{-1} sec^{-1}). Both preservatives enhanced yeast growth. The specific growth rates (μ_{max}) for the yeast were 0.43, 0.76 and 0.88 for the control, samples treated with *A. boonei* and *S. gabonensis*, respectively. However, the sedimentation rate of the yeast was reduced by both preservatives, but *A. boonei* produced the greatest effect. The utilization of these botanicals for industrial fermentations involving yeast is promising.**

Key words: Palm wine, preservation, *Saccharomyces cerevisiae*, fermentation kinetic, growth kinetic, sedimentation rate, *Sacoglottis gabonensis*, *Alstonia boonei*.

INTRODUCTION

Palm wine (PW) is a generic name for a group of alcoholic beverages obtained by fermentation from the saps of palm trees (Agu et al., 1999). It is a refreshing beverage widely consumed in Southern Nigeria and other parts of the world particularly Asia and Southern America. Although PW may be presented in a variety of flavours, ranging from sweet (unfermented) to sour (fermented) and vinegary, it is mostly enjoyed by people when sweet. Fresh palm sap is a sweet, clear, colourless juice, which has high sugar content. Upon fermentation by the endogenous micro flora of the palm, the sugars are converted into ethanol and organic acids resulting in the rapid deterioration of the organoleptic quality of the juice. The wine becomes unacceptable to most consumers after about 24 h of production.

Successful attempts have been made to preserve palm wine using the tree barks of *Sacoglottis gabonensis* and *Alstonia boonei*. *S. gabonensis* is a tropical rainforest tree from Africa. It belongs to the family Humiriaceae and it is known to be inhibitory to *Lactobacillus plantarum*, *Leuconostoc mesenteroides*, *Escherichia coli and Sarcina lutea* isolated from palm wine (Ojimelukwe, 2001; Okafor, 1975). Neither the tree bark of *S. gabonensis* nor the crystalline C- glucoside, bergenin isolated from it has been shown to posses any significant mammalian toxicity (Okafor, 1975). It has been reported also that the tree bark extract of *S. gabonensis* delays the souring of palm

*Corresponding author. E-mail: aniplus_ai@yahoo.com. Tel: +2348037890522.

Abbreviations: PW, Palm wine; **SDA,** Saboraud dextrose agar; **MYGP**, malt extract yeast extract glucose peptone medium.

wine (Okafor, 1975), lowers its titratable acidity (Ojimelukwe, 2000), but the palm wine becomes more alcoholic on standing (Morah, 1995). In addition, Ojimelukwe (2000) reported that the optimal concentration of *S. gabonensis* required to preserve palm wine is 0.625%.

A. boonei, a rainforest tree indigenous to Africa, belongs to the family Apocynaceae. Omoregbe and Osaghe (1997) reported that ethanol extract from *A. boonei* possess antimicrobial properties against *E. coli*, *Salmonella typhi*, *Salmonella paratyphi*, *Shigella disentrae*, *Staphylococcus aureus*, *Klebsiella pneumonae* and *Proteus aureus*. It is also known to contain 2-mono substituted piperidines and pyrollidines (Edeoga and Eriata, 2001; Burkill, 1985). Elijah et al. (2007) reported that the pulverized stem bark of *A. boonei* reduces the rate of PW fermentation, the optimal concentration being 0.50%.

Presently, effort is being made towards exploiting other possible benefits of these botanicals for industrial fermentations. Ezeronye et al. (2005) reported that both *S. gabonensis* and *A. boonei* enhances ethanol production, ethanol tolerance and osmotolerance ability, while reducing the flocculation ability as well as invertase activity of *Saccharomyces cerevisiae* isolated from palm wine. *S. gabonensis* was superior to *A. boonei* in these respects. The present work, which reports on the effect of *S. gabonensis* and *A. boonei* on the growth and fermentation kinetics as well as the sedimentation rate of *S. cerevisiae* isolated from palm wine, is in line with the new trend. These characteristics are very important factors that control PW fermentation, which could be exploited for industrial fermentation involving yeasts.

MATERIALS AND METHODS

Sample collection

Fresh PW from *Raphia hookeri* was collected from a tapper in Ikot Ekpene, Akwa Ibom State, Nigeria. The tree bark of *S. gabonensis* and *A. boonei* were obtained from Umudike, Abia State, Nigeria, sun-dried, cleaned and pulverized to pass through 0.5 mm mesh size**.** The reference yeast (bottom fermented brewer's yeast) *S. cerevisiae* var *uvarum* was obtained from Champion Breweries PLC, Uyo, Akwa Ibom State, Nigeria.

Isolation and characterization of yeast

Yeast isolates were obtained from fresh PW using the method of Beech and Davenport (1971) after tenfold dilution of the sample. Appropriate dilutions were plated on Saboraud Dextrose Agar (SDA) fortified with 0.25 mg/l of chloramphenicol to suppress bacterial growth. The plates were incubated at 28°C for 48 h. The growth and maintenance medium was Yeast Extract Peptone Dextrose (YPD) agar containing the following (per litre): Yeast extract (Sigma) 10 g; peptone (Oxoid) 20 g; glucose (BDH) 20 g; and agar (Oxoid) 20 g.

Discrete colonies (identified on the basis of morphology) were aseptically collected with sterile loops and purified by sub-culturing until pure cultures were obtained. The yeast isolates were incubated at 4°C. Ability of the yeast to utilize certain sugars was the basis for identification (Barnett et al., 1983; Pelczer and Chan, 1977).

Fermentation kinetic studies

Fresh sugary Raphia palm sap was sterilized by membrane filtration (Millipore 0.45). A portion (150 ml) of the sterilized palm sap was dispensed into 3 sterile fermentation flasks (250 ml). *S. gabonensis* (0.625%) was added to one of the flasks while 0.5% of *A. boonei* was added to another. No preservative was added to the remaining flask which served as control. The content of the flasks were allowed to stand for 30 min, while shaking at intervals, to ensure proper extraction of the preservatives. A standard inoculum (10^6 cells/ml) of the yeast isolate (*S. cerevisiae*), was aseptically inoculated into the sterilized palm sap in the different flasks and allowed to ferment for 24 h at room temperature (32°C). The volume of CO_2 evolved (per hour), by downward displacement of water, was estimated using a 2000 cm^3 calibrated measuring cylinder on hourly basis. Mean values of CO_2 obtained from two replicates were used in the calculations.

Calculation procedures

The moles of CO_2 were obtained as described by Agu et al. (1999) from the following equations:

$$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + 2CO_2 \quad (1)$$

Hence the rate of disappearance of sugar is:

$$-r\,C_6H_{12}O_6 \longrightarrow \frac{d(C_6H_{12}O_6)}{dt} \quad (2)$$

and the rate of appearance of carbon dioxide is:

$$r\,CO_2 \longrightarrow \frac{d(CO_2)}{dt} \quad (3)$$

while the rate of appearance of ethanol is:

$$r\,C_2H_5OH \longrightarrow \frac{d(C_2H_5OH)}{dt} \quad (4)$$

Using carbon dioxide production as a model, then:

$$-d(C_6H_{12}O_6) \longrightarrow \frac{k\,d(CO_2)}{dt} \quad (5)$$

where k, is the fermentation rate constant for CO_2 evolved. Assuming an ideal gas behaviour of CO_2, then:

$$PV = nRT \quad (6)$$

where P = gas pressure, ~0.1 atm in this experiment; V = volume of CO_2 produced per hour; n = number of moles of CO_2 gas collected; R = gas constant, 0.08206 litre atm $mol^{-1}K^{-1}$; T = temperature of fermentation = 305K.

Since all the values except for n, in equation (6) are known, the number of moles of CO_2 gas may be obtained from the relation:

$$n = \frac{PV}{RT} : \frac{P}{RT} = 3.9955 \times 10^{-3} \quad (7)$$

Growth kinetic studies

The method described by Ezeronye and Okerentugba (2002) was adapted to investigate the effect of *S. gabonensis* and *A. boonei* on the growth kinetic of *S. cerevisiae* isolated from palm wine. The growth medium was Yeast Extract Peptone Dextrose (YPD) broth containing the following (per litre): yeast extract (Sigma) 10 g; peptone (Oxoid) 20 g; glucose (BDH) 20 g. Sterilized medium (200 ml each) was dispensed into 3 Erlenmeyer (250 ml) flasks. *Sacoglottis gabonensis* (0.625 %) was added to the growth medium in one of the flasks while *A. boonei* (0.50%) was added to another. The last flask contained no preservative and served as control. The media containing the preservatives were shaken vigorously and allowed to stand for about 30 min to ensure proper extraction of the preservatives. A standard inoculum (10^6 cells/ml) of the yeast isolate (*S. cerevisiae*), was inoculated aseptically into all the Erlenmeyer flasks. The initial Optical Density (OD) of each culture was taken at 540 nm using LKB-Biochrom Novaspec spectrophotometer (Model 4049, Cambridge, England). Un-inoculated medium corresponding to that of each culture was used as the blank. The flasks were then plugged with cotton wool and incubated at 30°C in a shaker incubator (Gallenkamp) at 200 g min^{-1}. Samples were taken aseptically from each culture after every 2 h for 12 h and the absorbance read in the same way. The experiment was carried out in duplicates. Mean values were used to determine the maximum specific growth rate (μ_{max}) using the Monod kinetic growth model (Monod, 1949).

Sedimentation rate

The effect of *S. gabonensis* and *A. boonei* on the sedimentation rate of *S. cerevisiae* isolated from palm wine was determined using the method reported by Ezeogu and Okolo, (1994). The medium, MYGP (malt extract yeast extract glucose peptone) containing malt extract 0.3%, yeast extract 0.3%, glucose 1% and peptone 0.5%, was used for growing yeast isolates (*S. cerevisiae* isolated from palm wine and the reference yeast-bottom fermented brewer's yeast, *S. cerevisiae var uvarum* obtained from the brewery), prior to sedimentation test. Sterilized medium (200 ml each) was dispensed into 4 Erlenmeyer (250 ml) flasks. To one flask was added *S. gabonensis* (0.625%) and to another, *A. boonei* (0.50%). The last two flasks contained no preservatives; one was inoculated with the reference brewing yeast, while the other one and the ones containing the preservatives, were inoculated with *S. cerevisiae* isolated from palm wine. Incubation was done at 30°C for 24 h. Yeast cells were harvested from each culture by high speed centrifugation at 16,000 g for 10 min and used for preparing a standard cell suspension of about 1×10^8 cells/ml in 0.9% saline. The Optical Density (OD) of each suspension was taken at 650 nm using LKB-Biochrom Novaspec spectrophotometer (Model 4049, England), and then after every 30 min for 2 h. Un-inoculated medium corresponding to that of each suspension was used as the blank. The experiment was carried out in duplicate. Mean values were used for the calculations.
The sedimentation rate (SR) was expressed as follows:

$$\% SR = \frac{\text{Total drop in Spectrophotometer reading}}{\text{Spectrophotometer at 0 h}} \times 100$$

RESULTS AND DISCUSSION

The morphological and fermentation characteristics of yeast isolated from palm wine are shown in Table 1. *Saccharomyces* species, *Candida* species and *Schizosaccharomyces* were isolated. 60% of the yeasts (estimated by percentage occurrence of colonies) were *Saccharomyces*. Earlier reports indicate that the predominant microflora involved in PW fermentation are *Saccharomyces* species (Owuama and Sanders, 1990; Okoli and Ezenkwe, 1989). Amanchukwu et al. (1989) isolated a hydrocarbon degrading strains of *Schizosaccharomyces pombe* from Nigerian PW.

Fermentation kinetic

The effect of treatment with *S. gabonensis* and *A. boonei* on the volumes of CO_2 released during the fermentation of PW by *S. cerevisiae*, and their corresponding numbers of moles are shown in Table 2. The fermentation kinetic study showed that although the fermentation patterns in treated and untreated (control) PW were similar, the fermentation vigour differed (Figure 1). The slopes of the graphs for the fermentation rate constant, k, of 2.79×10^{-4} mol^{-1} s^{-1} obtained for untreated PW was higher than the k values for PW treated with *A. boonei* (1.7×10^{-4} mol^{-1} s^{-1}) and *S. gabonensis* (1.1×10^{-4} mol^{-1} s^{-1}). This implies that the preservatives reduced CO_2 evolution. Gas production resulting from heavy contamination of palm sap is a major problem militating against successful bottling of palm wine (Agu et al., 1999). However, the fermentation rate constant in untreated PW was higher than the fermentation rate constant, k (7.4×10^{-5} mol^{-1} s^{-1}) obtained for *R. hookeri* (Agu et al., 1999).

Growth kinetic

The effect of *S. gabonensis* and *A. boonei* on the growth kinetic of *S. cerevisiae* isolated from palm wine is shown in Figure 2. Both preservatives enhanced yeast growth. The maximum specific growth rate (μ_{max}) obtained for cultures treated with *A. boonei* and *S. gabonensis* were 0.76 and 0.88, respectively, as against μ_{max} obtained for untreated culture which was 0.43. These values are also higher than the μ_{max} obtained for fusant yeasts (0.13 - 0.26) produced by the hybridization of palm wine and brewers' yeast (Ezeronye, 2003). The culture treated with *A. boonei* showed a typical batch culture growth profile with a lag phase, a sharp log phase and a stationary phase. However, the culture to which *S. gabonensis* was added showed actively growing cells with no lag phase. In this study, the period of fermentation was not extended to include the death phase. Although the control culture showed no lag phase, the growth rate of the cells was rather slow when compared with the ones treated with preservatives. The preservatives may have released into the medium, growth factors which may trigger off rapid growth of yeast cells. These observations are very significant especially for the production of single cell

Table 1. Morphological and biochemical characteristics of yeast isolates from palm wine.

Yeast isolate	Colony	Cell shape	Ft	Ab	Sb	Mn	Sc	Xl	Mt	Mb	Gc	Lt	Rf	Gt	Ls	Cl	Organism
1	SC	Round	AG	-	-	-	AG	-	AG	-	AG	-	A	AG	-	+	*S. cerevisiae*
2	EC	Elongated	AG	-	-	-	AG	A	A	-	AG	-	AG	-	+	+	*Candida utilis*
3	SC	Spherical	AG	-	-	-	AG	-	AG	-	AG	-	A	AG	-	+	*S. cerevisiae*
4	SC	Oval	AG	-	-	-	AG	-	AG	AG	AG	-	AG	AG	-	+	*S. uvarum*
5	EC	Spherical	AG	-	-	-	AG	-	AG	-	AG	-	A	AG	-	+	*Schizosaccharomyces pombe*

AG: Acid/gas production; A: acid production; -: no fermentation; +: fermentation; Ft: fructose; Ab: arabinose; Sb: sorbose; Mn: manitol; Sc: sucrose; Xl; xylose; Mt: maltose; Mb: melibiose; Gc: glucose; Lt: lactose; Rf: raffinose; Gt: galactose; Ls: lysine; Cl: catalase; S: Saccharomyces; Sch: Schizosaccharomyces SC: Smooth creamish; EC: Elevated creamish; ES: Elevated spherical.

Table 2. Effect of *S. gabonensis* and *A. boonei* on carbon dioxide production during palm wine fermentation by *S. cerevisiae*.

Time(x10^3) s	A		B		C	
	1	2	1	2	1	2
3.6	450	1.8	300	1.2	300	1.2
7.2	525	2.1	400	1.6	425	1.7
10.8	750	3.0	475	1.9	550	2.2
14.4	1076	4.3	625	2.5	700	2.8
18.0	1401	5.6	850	3.4	901	3.6
21.6	1626	6.5	1001	4.0	1201	4.8

A: untreated (control) palm wine; B: *S. gabonensis* treated palm wine; C: *A. boonei* treated palm wine; 1: Volume of CO_2 (cm^3); 2: Mole of CO_2; Moles of CO_2 = 3.9955 × 10^{-3} × volume of CO_2.

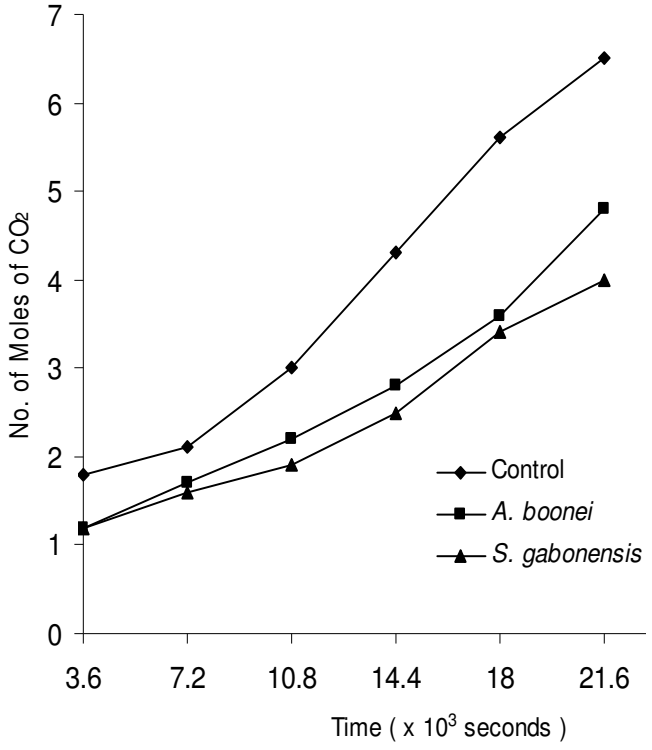

Figure 1. Effect of *S. gabonensis* and *A. boonei* on the fermentation kinetics of *S. cerevisiae* isolated from palm wine.

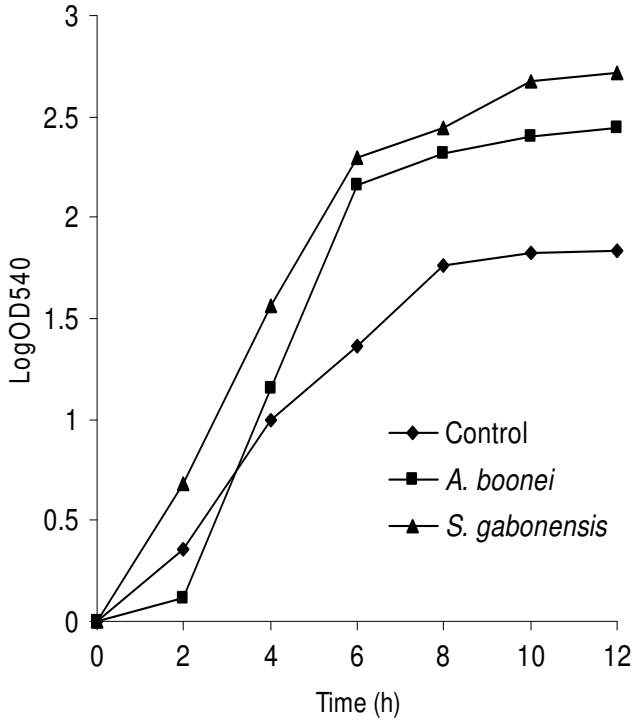

Figure 2. Effect of *S. gabonensis* and *A. boonei* on the growth kinetics of *S. cerevisiae* isolated from palm wine.

protein from yeasts using readily available and affordable materials.

Sedimentation rate

The effect of *S. gabonensis* and *A. boonei* on the SR of *S. cerevisiae* isolated from palm wine is presented in Figure 2. The result shows that both preservatives significantly reduced ($p < 0.05$) SR of the yeast isolate. The SR (88.91%) for the standard brewing yeast culture (*S. cerevisiae* var uvarum), which served as the reference, was significantly higher ($p < 0.05$) than the SR for all other cultures. This was followed by the SR (82.35%) for the untreated (control) culture which was significantly higher ($p < 0.05$) than that for the cultures treated with *S. gabonensis* and *A. boonei*. However, the SR for *S. gabonensis* treated culture (65.04%) was significantly higher ($p< 0.05$) than the SR for *A. boonei* treated culture (57.27%). In fermentations involving yeasts, sedimentation usually occurs after the formation of flocs which link yeast cells through salt bridges between calcium ions and the anionic polymer on the yeast surface (Ezeronye and Okerentugba, 2002). Adsorption of protein and polyphenolic materials on the surface of yeast during fermentation may play some part in modifying the rate of sedimentation (Hough et al., 1982). Therefore, the preservatives may have altered the rate of sedimentation of the yeast by releasing polyphenolic materials into the medium. Edeoga and Eriata (2001) report the presence of proanthocyanidine and procyanidine in *A. boonei*. The implication of this finding is that if used for industrial fermentations involving yeasts, the preservatives might not allow for rapid sedimentation of yeast cells, thereby making clarification of the final product difficult.

The results of this study indicate that *S. gabonensis* and *A. boonei* reduced the rate of fermentation of palm wine by *S. cerevisiae* as well as the sedimentation rate of the yeast, but increased the specific growth rate (μ_{max}) of the yeast. These findings provide useful information necessary for further utilization of these botanicals for industrial fermentations involving yeasts.

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