African Journal of Biotechnology Vol. 4 (11), pp. 1290-1296, November 2005
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2005 Academic Journals

Full Length Research Paper

The alcoholic fermentative efficiency of indigenous yeast strains of different origin on orange juice

Wahab. O. Okunowo*, Rufus. O. Okotore and Akinniyi. A Osuntoki

Department of Biochemistry, College of Medicine, University of Lagos. P.M.B. 12003. Lagos. Nigeria.

Accepted 24 June, 2005

The alcoholic fermentative ability of yeast strains; *Saccharomyces cerevisiae* (isolated from yam), *S. cerevisiae* (from sugarcane molasses), *S. carlsbergensis* (from sugarcane molasses) and *S. cerevisiae var. ellipsoideus* (from orange juice) were examined on orange juice (Citrus sinensis). The quality of the wine produced on the basis of the acidity, ash content, vitamin C and the alcohol content were assayed. The fermentation efficiency varied between 48.05% with *S. cerevisiae var. ellipsoideus* and 99.46% with *S. carlsbergensis*. The highest ethanol concentration, yield and productivity were 6.80 ± 0.07% (w/v), 0.46 gg^{-1} and 0.57g $l\text{-}1h^{-1}$, respectively. The rate of sugar utilization was least, (2.76 g/day) with *S. carlsbergensis* and highest (3.07 g/day) with *S. cerevisiae* from yam. The total alcohol produced was least (3.19 ± 0.21%, w/v) with *S. cerevisiae var. ellipsoideus* and highest (6.80 ± 0.07%, w/v) with *S. carlsbergensis*. The optimum pH ranged between 3.81 for *S. cerevisiae var. ellipsoideus* and 3.71 for *S. cerevisiae* (from yam). The Vitamin C level was highest (9.02 mg/100 g) with *S. cerevisiae var. ellipsoideus* and lowest (6.65 mg/100 g) with *S. carlsbergensis.*

Key words: *Saccharomyces,* yeast strains, fermentation, orange wine.

INTRODUCTION

Oranges are produced for internal market and export in most parts of the world. About 20 per cent of the total crop of oranges is sold as whole fruit; the remainder is used in preparing orange juice, extracts, and preserves (Microsoft® Encarta® Reference Library, 2002). Oranges are highly perishable products, susceptible to bacteria and fungal attacks. A number of measures are taken for the storage and preservation; amongst these measures are refrigeration of the fruits, extraction, concentration of juice to about 4 or more times and dried concentrate preparation. Fermentation of the fruit sugar and formation of ethanol is another way of preservation and preventing wastage of the citrus fruit during harvesting (Afe, 1995; Fernando and Loreto, 1997)).

*Corresponding author. E-mail: modelprof@yahoo.com.

Yeast species are useful in many industrial processes, such as the production of alcoholic beverages, biomass and various metabolic products (Remize et al., 1999; Ueno et al., 2003; Wartmann and Kunze, 2000; Stephen et al., 2003; Journoud and Jones, 2004). The production of wines from some tropical fruits using yeast strains has been reported (Maldonado et al., 1975; Obisanya et al., 1987; Ndip et al., 2001; Ezeronye, 2004). Several approaches has been documented for the production of quality wines, which includes: the use of immobilized yeast cells (Wada et al, 1981; Nigam et al., 1998; De Vasconcelos et al., 2004), increased rate of agitation, increased nutritional supply, increased inoculum concentration and fermentation at optimum temperature of yeast strains (Anuna and Akpapunam, 1995; Buescher, 2001).

The alarming wastage associated with the orange fruit coupled with its low level of industrial utilization in the developing countries calls for a great concern. This work

aims to redress the problem by exploiting the ability of yeast species to produce wines. In this study, we investigated the efficiency of various yeast strains from indigenous sources in the production of quality wine from orange juice.

MATERIALS AND METHODS

Microorganisms

The yeast strains used in this study were obtained from the stock cultures maintained at the Federal Institute of Industrial Research Oshodi (F.I.I.R.O), Lagos, Nigeria. They were identified as *Saccharomyces cerevisiae* (isolated from sugarcane molasses), *S. cerevisiae* (isolated from yam), *S. carlsbergensis* (isolated from sugarcane molasses) and *S. cerevisiae var. ellipsoideus* (isolated from orange juice). The organisms were subcultured aerobically for reactivation and increased biomass concentration at pH 4.5, 30°C for 24 h in a medium containing (g/l): yeast extract, 3; peptone, 5; malt extract, 5 (Nigam et al., 1998). The cells were harvested by centrifugation at 1600 g for 5 min and washed with 0.85% NaCl solution. These steps were carried out under sterile conditions. The cells obtained were used as the starter cultures.

Preparation of the fermentation medium

The orange fruits (*Citrus sinensis*) were purchased at a local market in Lagos. They were thoroughly washed with 0.1% of sodium metabisulphite solution, cut into pieces and pressed manually to obtain the juice. The juice was sterilized with 200 mg/L sodium metabisulphite and allowed to clarify at –5°C for 24 h. The supernatant was analysed for the total soluble solid and the pH. The total soluble solid was fortified with sucrose from 12.5 to 18°Brix to give enough fermentable sugar.

Fermentation of the wort

Fermentation experiments were performed in 1nL glass batch reactors system equipped with an agitator. After cleaning by steam sterilization at 121°C for 15 min, the fermenter was filled with 750 ml of the fermentation medium with the addition of the following nutrients (g/l): diammonium hydrogen phosphate, 0.5 g; magnesium sulphate ($MgSO_4.7H_2O$), 0.2 g and urea, 0.5 g (Nigam et al., 1998). One drop of antifoam was added to each reactor unit to prevent foaming. Each reactor was inoculated with 1% (w/v) of the yeast strains. The fermentation was allowed to proceed at room temperature (25 ± 2°C) for five days. The agitation speed was maintained through out the experiment at 200 rpm for even distribution of the yeast and the nutrient respectively. The wine was clarified with 0.1% bentonite, racked and stored at 2°C until analyzed.

Analyses

The pH of the fermentation medium and the total soluble solid were measured using a pH meter (Model 3305, Jenway, United Kingdom) and a Abbe hand refractometer (model RG 701, Officine Galileo, Italy), respectively. Other parameters such as total titrable acidity (T.T.A.), total acidity (T.A.), fixed acidity (F.A.), volatile acidity (V.A.) and vitamin C were determined by established methods (A.O.A.C, 2000). The total alcohol (g) and the percentage ash of the wines were determined by the specific gravity and dry ash method respectively (A.O.A.C., 2000). The percentage fermentation efficiency of the yeast strains was calculated on the basis of the relationship between the sugar consumed and alcohol produced following the fermentation stoichiometry, where 1 g of total reducing sugar produces 0.461 g ethyl alcohol.

Data analysis

Data are given to 2 decimal places and are reported as means ± SEM of measurements from three separate reactor tanks containing the same strain of organism. Significant difference between time points were determined by Fisher's protected least significant different t-test with two-tail probabilities of less than 0.05 considered significant. Significant differences between yeast strains (as compared to *S. cerevisiae* from sugarcane molasses) were assessed by a one-way analysis of variance and the Student's t-Test.

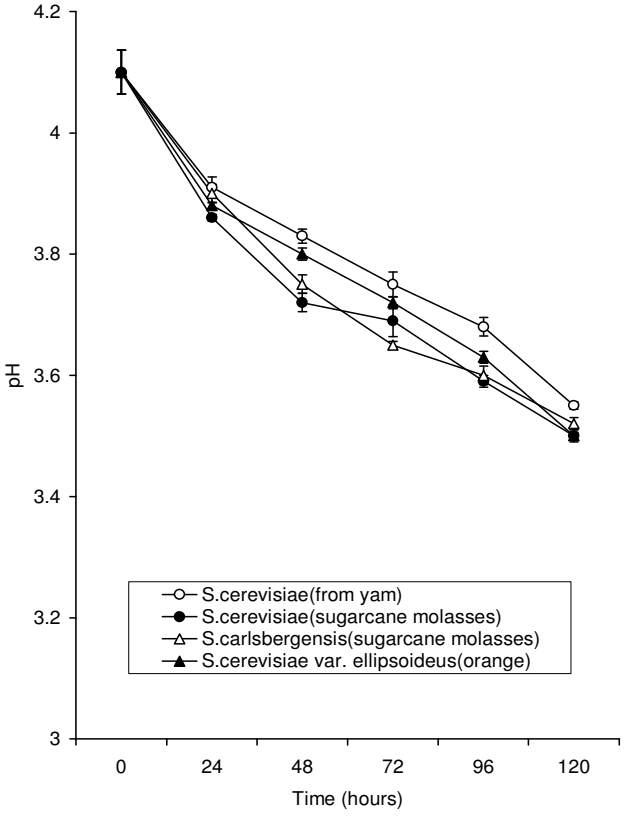

Figure 1. Yeast strains and the pH changes in the fermentation medium.

RESULTS AND DISCUSSION

pH change

The pH change in the fermentation medium followed the same pattern (Figure 1) and this was not significantly different between the yeast strains ($P > 0.05$). It was

Table 1. Rate of pH change, total titratable acid (T.T.A.) change and sugar utilization of yeast strains.

Parameters	*S. cerevisiae*[a]	*S. cerevisiae*[b]	*S. carlsbergensis*[b]	*S. cerevisiae var. ellispsoideus*[c]
Rate of pH change/day	0.10	0.11	0.11	0.11
Rate of T.T.A change/day	0.08	0.08	0.08	0.07
Rate of % sugar utilization/day	3.07	2.98	2.76	2.82

a = from yam.
b = from sugarcane molasses.
c = from orange juice.

observed that the pH value decreased significantly with the fermentation period ($P < 0.05$). At the end of the process, the pH was 3.55 with *S. cerevisiae* (from yam), 3.52 with *S. cerevisiae* (from sugarcane molasses) and approximately 3.50 each with *S. carlsbergensis* and *S. cerevisae var. ellipsoideus*. The rate of pH change was determined from the gradient ($\delta pH/\delta t$) in Figure 1. The result obtained (Table 1) shows that rate of pH change was approximately the same in the media.

Total titrable acids (TTA)

The percentage TTA per day in the fermentation media increased significantly ($P < 0.05$). The pattern of increase (Figure 2) as compared to *S. cerevisiae* (sugarcane molasses) was not significantly different ($P > 0.05$). The %TTA was highest (0.85%) with *S. cerevisiae* (sugarcane molasses) and *S. carlsbergensis*, 0.84% with *S. cerevisiae* (from yam), and least (0.79%) with *S. cerevisiae var. ellipsoideus*. Moreso, the rate of change in %TTA (as obtained from the slope; δ%TTA/δt in Figure 2) was approximately the same for all the organisms (Table 1).

Sugar utilization

The level of sugar in the reactors decreased with the progress of the fermentation process. The sugar consumption pattern as compared to *S. cerevisiae* (sugarcane molasses) show no significant difference ($P > 0.05$). Although, each of the strains reduced the sugar level significantly from the initial concentration with increasing number of days ($P < 0.05$). The residual sugar % (w/v) was least (2.88 g) with *S. cerevisiae* (from yam) and highest (3.6 g) with the *S. cerevisiae var. ellipsoideus*. This indicates that the sugar consumption was highest with the former and lowest with the latter. However, the rate of sugar utilization (obtained from the slope; δ%sugar/δt in Figure 3) differed between organisms (Table 1) was 3.07 g/day (highest) with *S. cerevisiae* (from yam), 2.98 g/day with *S. cerevisiae* (from sugarcane molasses), 2.82 g/day with *S. cerevisiae var. ellipsoideus* and 2.76 g/day (least) with *S. carlsbergensis*. However, this does not account for their alcoholic fermentative ability since it is a heterolactic fermentation process coupled with the possibilities of substrate or product inhibition (Nigam et al., 1998).

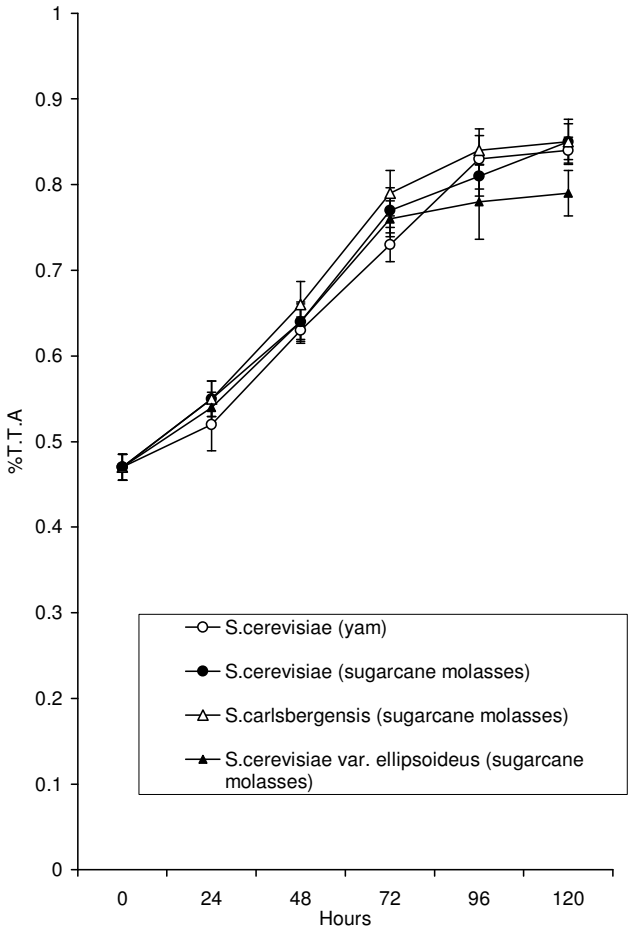

Figure 2. Yeast trains and the percentage changes in the total titrable acidity of the wine over the fermentation medium.

Total alcohol

The percentage total alcohol produced by the yeast strains was significantly different ($P < 0.05$). This was 6.80 ± 0.07% (w/v) with S. carlsbergensis, 5.61 ± 0.11% with *S. cerevisiae* (from yam), 4.80 ± 0.06% with

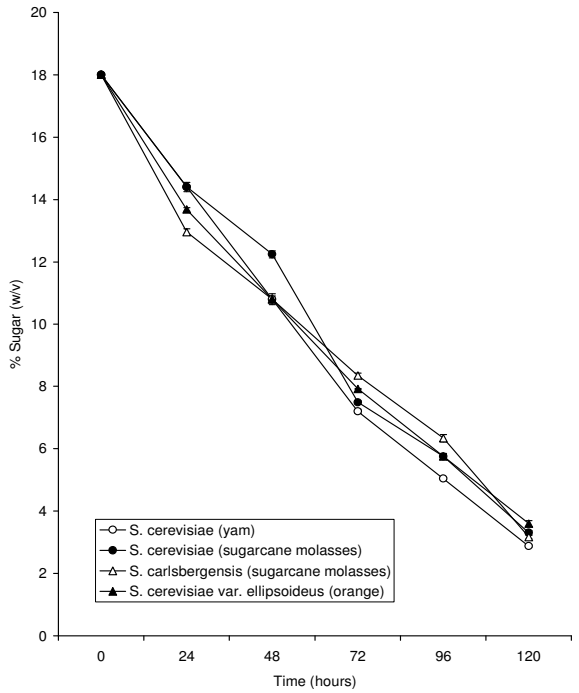

Figure 3. Yeast strains and their pattern of sugar utilization over fermentation period.

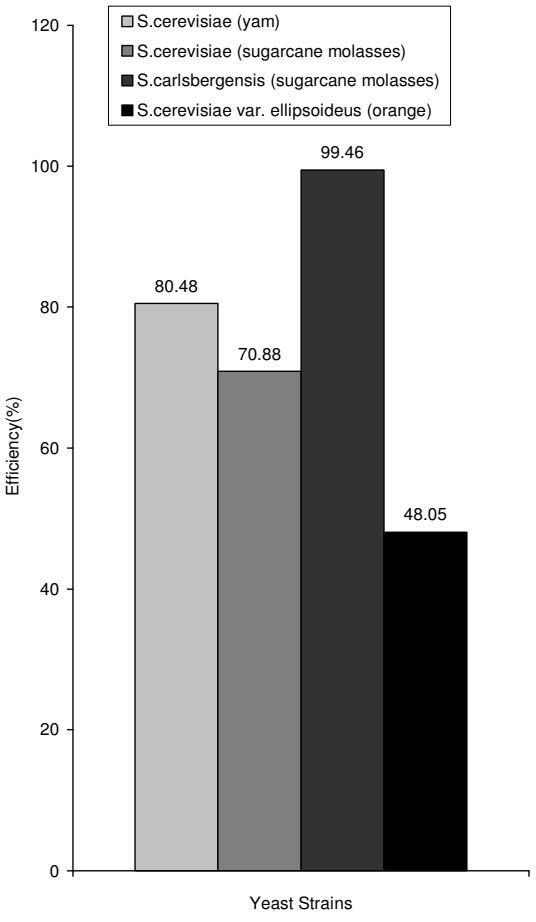

Figure 4. Yeast strains and their fermentation efficiency.

S. cerevisiae (from sugarcane molasses) and lowest, 3.19 ± 0.21% (w/v) with *S. cerevisiae* var. ellipsoideus. The variation in alcohol levels could be due to the difference in their optimal physico-chemical conditions; temperature or pH. It has been reported that temperature affects the gene expression in yeast (Staci et al., 2003). Cook (1958) reported also that the optimum growth temperatures for S. carlsbergensis, *S. cerevisiae* and *S. cerevisiae var. ellipsoideus* are 25, 28-30 and 30-35°C, respectively.

However, the fermentation was carried out at 25–27°C, thus favouring *S. carlsbergensis* in total alcohol production. The lowest percentage of total alcohol formed by *S. cerevisiae var. ellipsoideus* may not only be due to the physico-chemical condition at which the process was carried out but may also be due to product or feedback inhibition of alcohol. *S. cerevisiae var. ellipsoideus* has a low alcohol tolerance (Cook, 1958). The difference in the total alcohol level produced by *S. cerevisiae* (from yam) and *S. cerevisiae* (from sugarcane molasses) could not have been due to the physico-chemical condition since they both have the same optimal temperature and pH; but could be due to the difference in their source of origin. Fermentation conditions and the source of yeast wine (Anuna et al., 1990; Anuna and Akpapunam, 1995).

Fermentation efficiency

The fermentation efficiency was 99.46% (highest) with *S. carlsbergensis*, 80.48% with *S. cerevisiae* (from yam), 70.88% with *S. cerevisiae* (sugarcane molasses) and least (48.05%) with *S. cerevisiae var. ellipsoideus* (Figure 4). This is directly proportional to the percentage total alcohol produced (Table 2), and independent of the rate of fermentation by the strains. This could be due to the utilization of sugar for the formation of other products apart from alcohol. However, it will be interesting to see the efficiency of individual strains if the fermentation is carried out at their respective optimal pH and temperature. The removal of alcohol from the medium as the fermentation progresses may also increase the efficiency of low alcohol tolerant strains. The ethanol productivity and yield were highest (0.57 g $l^{-1}h^{-1}$ and 0.46 g $g^{-1,}$ respectively) with *S. carlsbergensis* and least (0.27 g $l^{-1}h^{-1}$ and 0.22 gg^{-1}, respectively) with *S. cerevisiae var. ellipsoideus* (Table 2).

Effect of pH on the activity

The effect of pH on the activity of the yeast strains was examined. The activity was determined from the ratio of the percentage sugar consumed to the fermentation period (days). The optimal pH was determined from the corresponding maximum activity value in Figure 5. The

Table 2. Sugar utilized, total alcohol, ethanol productivity and yield, efficiency, vitamin C and percentage ash of yeast strains.

Parameters	*S. cerevisiae* (yam)	*S. cerevisiae* (sugarcane molasses)	*S. carlsbergensis* (sugarcane molasses)	*S. cerevisiae var. ellispsoideus* (orange juice)
Sugar utilized (g)	15.12	14.69	14.83	14.4
Total alcohol % (w/v)	5.61 ± 0.11↑	4.80 ± 0.06	6.80 ± 0.07↑	3.19 ± 0.21↑
	P = 0.0028*		*P* = 0.0000*	*P* = 0.0020*
Ethanol Productivity, P (g l^{-1} h^{-1})	0.47	0.40	0.57	0.27
Ethanol yield, Y (gg^{-1})	0.37	0.33	0.46	0.22
Fermentation Efficiency, E (%)	80.48	70.88	99.46	48.05
Vitamin C (mg/100 g)	8.95 ± 0.20↑	7.46±0.25↑	6.65 ± 0.05↑	9.02 ± 0.27↑
	P = 0.0008**	*P* = 0.0007**	*P* = 0.0007**	*P* = 0.0015**
	P = 0.0097*		*P* = 0.0342*	*P* = 0.0134*
% Ash	0.32 ± 0.02↑	0.38 ± 0.02↑	0.44 ± 0.02↑	0.26 ± 0.02↑
	P = 0.0808*		*P* = 0.05*	*P* = 0.0051*

↑Mean ± SEM of the mean from three fermentation tanks.
*Significant difference at 5% level of yeast as compared to *S. cerevisiae* (sugarcane molasses).
**Significant difference at 5% level of values as compared to the initial concentration in the reactor.
P = P_E/t (g l^{-1} h^{-1}), P_E = Ethanol concentration (g l^{-1}), t = Fermentation time (h) = 120 h
Y = g ethanol per g sugar utilized.
E = Actual alcohol produced/theoretical alcohol from sugar consumed X 100.

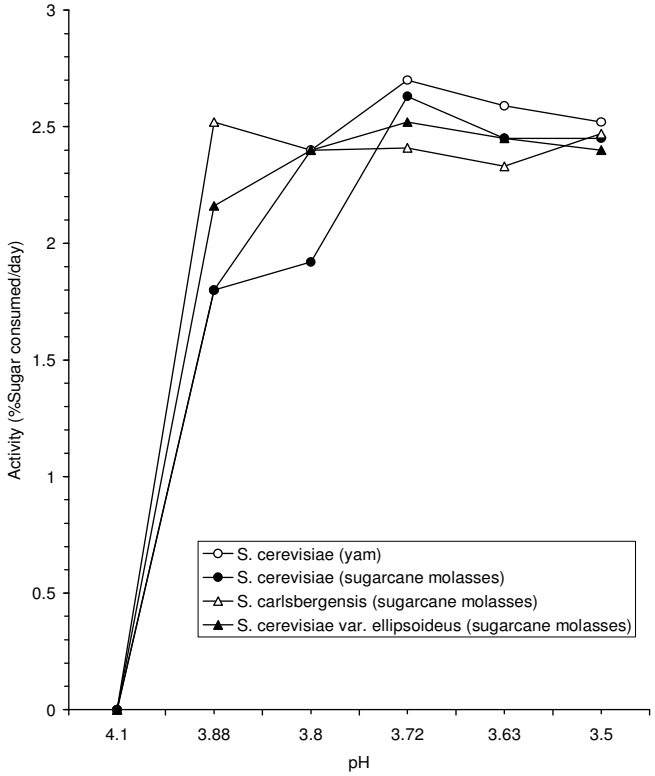

Figure 5. Effects of pH on the activity of the yeast strains.

pH of the fermentation medium affected the activity of the various strains. All the strains gave the same optimal pH (3.72) except *S. carlsbergensis* with an optimal pH of 3.88. Thus, the constant maintenance of the organisms at their respective optimal pH may improve the rate of fermentation, fermentation efficiency and ethanol yield in a wine making process.

Vitamin C level

The initial level of vitamin C in the orange juice was measured as 35.47 ± 0.45 mg/100g. This was reduced significantly by the yeast strains ($P < 0.05$). The vitamin C utilization was highest, 6.65 ± 0.05 mg/100 g, with *S. carlsbergensis* and least, 9.02 ± 0.27 mg/100 g with *S. cerevisiae* var. ellipsoideus. This reduction in the amount of the vitamin C in the wine samples suggests that it is needed for the metabolism and growth of the yeast strains. Yeast contains little or no ascorbic acid but may absorb it from the medium to be used as possible sources of carbon (Cook, 1958; Fleet and Heard, 1993), also ascorbic acid helps to reduce molecular oxygen from the medium thereby promoting yeast growth (B. Zoecklein, Department of Food Science and Technology VPI and SU – 0418 Blacksburg, VA 24061, personal communication). *S. cerevisiae* var. ellipsoideus, which gave the highest level of vitamin C in the wine, may be considered as the best strain the for production of wines with appreciable amount of vitamin C, owing to the paramount importance of vitamin C in human health.

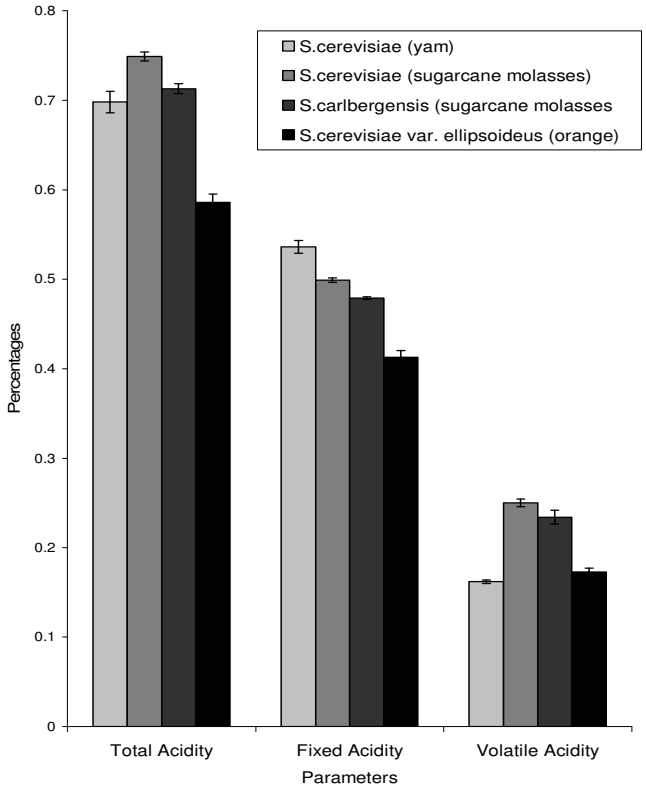

Figure 6. Total, fixed and volatile acidity of wine produced by the different yeast strains.

Acidity

There was a significant difference ($P < 0.05$) in the acids produced by the yeast strains. Total acidity (as tartaric acid) was highest (0.75%) with *S. cerevisiae* (from sugarcane molasses) and least (0.59%) with *S. cerevisiae var. ellipsoideus* (Figure 6). The fixed acidity was highest, 0.54% with *S. cerevisiae* (from yam), 0.50% with S.cerevisiae (from sugarcane molasses), 0.48% with *S. carlsbergensis* and least, 0.4% with *S. cerevisiae var. ellipsoideus*. Moreso, the volatile acidity (as acetic acid) was highest, 0.25% with *S. cerevisiae* (sugarcane molasses), 0.23% with *S. carlsbergensis*, 0.17% with *S. cerevisiae var. ellipsoideus* and least, 0.16% with *S. cerevisiae* (from yam). This study suggests that *S. cerevisiae* (from sugarcane molasses) may be employed in the production of total and volatile acids, which may be used as acidulants or preservative in carbonated drinks, effervescent tablets, fruit juices etc. Moreso, this may also increase the shelf life of the wine produced by the organism.

Ash content

The percentage ash content of the wine samples was highest, 0.44 ± 0.0153%, with *S. carlsbergensis* and least, 0.26 ± 0.0153% with *S. cerevisiae var. ellipsoideus* (Table 2). The mineral uptake of *S. cerevisiae var. ellipsoideus* when compared to *S. cerevisiae* (sugarcane molasses) was significantly different ($P < 0.05$). *S. carlsbergensis* appears to be the best organism for the production of wine of low mineral depletion. Ash content in wine is of paramount importance since wine contains lots of minerals in a natural concentration. It offers a way in which they are easily absorbed, and also in a favourable mixing ratio.

CONCLUSION

The quality of wine produced greatly depends on the types and source of yeast strains employed in the fermentation process. *S. cerevisiae var. ellipsoideus* which produced a moderate level of alcohol and an appreciable amount of vitamin C appears to be the best organism for orange wine production. On the other hand, *S. carlsbergensis* may be more economical in the production of high alcohol wine and also other industrial processes where a high percentage of alcohol is needed. This strain may also be employed in the production of wines with appreciable amount of mineral. *S. cerevisiae* (from cane molasses) is most effective in the production of acids such as tartaric acid and acetic acid. Our study of wine production from orange juice by various non-commercial yeast isolates has shown the potential use of indigenous yeast isolates as starters for wine production. Moreover, it would be interesting to investigate the amount of oil, types and amount of vitamins and minerals in the wine samples produced by the different yeast strains.

ACKNOWLEDGEMENT

The authors wish to thank Mr. Olatope of the Federal Institute of Research Oshodi (F.I.I.R.O), Lagos, Nigeria for providing the yeast strains and making their Biotechnology Laboratory available for this research work.

REFERENCES

AOAC (2000). Official Methods of Analysis of AOAC INTERNATIONAL 17th Edition. Vol. II, Gaithenburg, MD, USA, Official Method 920.57, 920.67, 955.24, 962.12, 968.08, 967.21. ISBN 0-93558-67-6.

Anuna MI, Akpapunam MA (1995). Quantitative analysis of alcohol types in Pineapple (Ananas comosus) L (L).Merr.) wine fermented by two strains of *Saccharomyces cerevisiae*. Nig. Food J. 13: 12-17.

Anuna MI, Sokari TG Akpapunam MA (1990). Effect of source of yeast (*Saccharomyces* spp.) on alcohol content and quality of pineapple (Ananas comosus) wine. Discovery and Innovation 2(2): 8-84.

Buescher WA, Siler CE, Morris JR, Threlfall RT, Main GL, Cone GC (2001). High alcohol wine production from grape juice concentrates. AJVEC. 52 (4): 345-351.

Cook AH (1958). The chemistry and biology of YEASTS. The Brewing Industry Research Foundation Redhill, England. 83: 160-167.

Academic Press Inc. Publishers. New York. Library of Congress Card Number: 57-8374.
De Vasconcelos JN, Lopes CE, De Franca FP (2004). Continuous ethanol production using yeast immobilized on sugar-cane stalks. Brazilian J. Chem. Engr. 21(3): 357-365.
Ezeronye OU (2004). Nutrient utilization profile of *Saccharomyces* c*erevisiae* from palm wine in tropical fruit fermentation. In: Antonie van Leeuwenhoek. (ed.) IngentaConnect. Kluwer Academic Publishers, 86(3): 235-239.
Fernando F, Loreto R (1997). Technical manual on small-scale processing of fruits and vegetables. Food and Agriculture Organization of the United Nations. http://www.fao.org/documents/show_cdr.asp?url_file=/DOCREP/x0209e/x0209e00.htm.
Fleet GH, Heard GM (1993). Yeasts- Growth during fermentation in Wine Microbiology and Biotechnology. In: (GH Fleet, ed.). Harwood Academic Pubs. Australia, pp. 27-54.
Journoud M, Jones PJ (2004). Red yeast rice: a new hypolipidemic drug. Life Sci. 74(22): 2675-83.
Microsoft® Encarta® Reference Library (2002). Orange (fruit). Encyclopeadia article.
Ndip RN, Akoachere JF, Dopgima LL, Ndip LM. (2001). Characterization of yeast strains for wine production: effect of fermentation variables on quality of wine produced. Appl. Biochem. Biotechnol. 95(3): 209-20.
Nigam JN, Gogoi BK, Bezbaruah RL (1998). Short communication: Alcoholic fermentation by agar-immobilized yeast cells. World J. Microbiol. Biotechnol. 14(3): 457-459.
Obisanya MO, Aina JO, Oguntimehin GB (1987). Production of wine from mango (Mangifera indica L.) using *Saccharomyces* and *Schizosaccharomyces* species isolated from palm wine. J. Appl. Bacteriol. 63: 191-196.
Remize F, Roustan JL, Sablayrolles JM, Barre P, Dequin S (1999). Glycerol Overproduction by Engineered *Saccharomyces cerevisiae* Wine Yeast Strains Leads to Substantial Changes in By-Product Formation and to a Stimulation of Fermentation Rate in Stationary Phase. Appl. Environ. Microbiol. 65(1): 143-149.
Staci N, Jeff M, Linda B (2003). Effect of Temperature on Genomic Expression in Wine Yeast. ASEV 54th Annual Meeting Reno, Nevada. p.16.
Stephen R. Hamilton PB, Beata B, Robert CD, Huijuan L, Teresa M, Juergen HN, Sebastian R, Terrance AS, Harry W, Stefan W, Tillman UG (2003). Production of Complex Human Glycoproteins in Yeast. Science 301: 1244-1246.
Ueno R, Hammada-Sato N, Urano N (2003). Fermentation of molasses by several Yeasts from hot spring drain and phylogeny of the unique isolate producing ethanol at 55oC. J.Tokyo University of Fisheries. 90: 23-30.
Wada M, Kato J, Chibata I (1981). Continuous production of ethanol in high sugar concentration using immobilized growing yeast cells. Eur. J. Appl. Microbiol. Biotechnol. 11: 67-71.
Wartmann T, Kunze G (2000). Genetic transformation and biotechnological application of the yeast Arxula adeninivorans. Appl. Microbiol. Biotechnol. 54(5): 619-24.