GSJ: Volume 10, Issue 12, December 2022
ISSN 2320-9186

Global Scientific JOURNALS

GSJ: Volume 10, Issue 12, December 2022, Online: ISSN 2320-9186
www.globalscientificjournal.com

STUDIES ON THE THERMO- ,OSMO- AND pH- TOLERANCES OF SOME PALMWINE YEASTS

Okpalla J [1*]

[1*] Department of Microbiology, Chukwuemeka Odumegwu Ojukwu University, Uli Anambra State

Email address:Judyzuby@yahoo.com Gsm:08035474139

Abstract

An investigation was carried out to determine the thermo-, osmo- and pH–tolerances of yeasts isolated from palm wines collected from various locations in Ihiala town in Ihiala Local Government Area, Anambra State. For the determination of thermo-tolerance, the isolates were inoculated separately into yeast extract, dihydrogen phosphate, sucrose (YEPS) medium and incubated at various temperatures. Osmo- and pH-tolerances were determined using the same medium and various sucrose concentrations and pH values respectively. All the yeast strains, showed very good level of growth at 35^0C, with *S. cerevisiae* OP1 recording the best result. At temperature higher than 40^0C the growth of all the selected yeasts decreased. All the yeasts isolated tolerated 10%w/v of sucrose concentrations. The yeasts did not tolerate 40% of the sucrose concentration, except *Saccharomyces cerevisiae* OP1 and *S. cerevisiae* OP2. None of the yeast isolates tolerated 50% w/v of sucrose concentration. Maximum yeast growth was recorded by *S.cerevisiae* at 15% w/v sucrose concentration. None of the yeast isolates grew below pH 4 and above pH 7. The growth of *S.chevalieri* OP4, *S.rosei* OP5 and *S.chevalieri* RP2 was observed to start at pH 5, while no growth was observed at pH 7 and 8 for *S.rosei* OP5, *S.pastorianus* RP1 and *S.rosei* RP5. Maximum yeast growth was recorded by *S.cerevisiae* OP1 at pH 6. It is concluded in this study that some of the yeasts isolated from the palm wines displayed thermo-, osmo- and pH–tolerances.

Keywords: yeasts, thermo- tolerance, osmo-tolerance, pH tolerance,

GSJ: Volume 10, Issue 12, December 2022
ISSN 2320-9186

Introduction

Palm wine is an important alcoholic beverage consumed in Nigeria and many other parts of the world. It appears as colorless juice containing about 10-12%(w/v) sugar, which is mainly sucrose(Bassir, 1962; Okafor, 1975;Ogbulie *et al.*2007). It is gotten (tapped) as sap from the *Raphia* palm trees *Raphia hookeri* or *Raphia vinifera* or oil palm trees *Elaeis guineensis* and is the major brand of wine in some parts of the world like the southern part of Nigeria(Isaac *et al.*, 2017). The sap is obtained from a variety of positions of the palm tree: the stem on the standing tree, the tip or trunk of the felled tree and the base of the immature male inflorescence(Oyegade *et al.*,2004).Palmwine contains nutritionally important components including amino acids, proteins, vitamins and sugars(Okafor, 1987).These make this wine a veritable medium for the growth of a consortium of microorganisms, whose growth in turn, change the physiochemical conditions of the wine, giving rise to competition and successions of organisms (Nwachukwu *et al.,* 2006).

Yeasts such as S. cerevisiae have been used in alcohol production, especially in the brewery and wine industries, for thousands of years. Obvious reasons being that this yeast gives high ethanol yield (90% theoretical yield), high ethanol productivity, and has a profound ability to withstand high ethanol concentration up to 40 g/L ethanol in the production milieu (Nigam and Singh, 2011). Yeasts must possess certain attributes if it could be efficiently employed in bioethanol production. The attributes include tolerance of the yeasts to its substrate (osmo-tolerance), fermentation product (ethanol- tolerance), temperature (thermo-tolerance), acids as well as possession of flocculating characteristics depending on process requirements. At the beginning of fermentation, cells are subjected to high substrate concentration and as the ethanol level increases, both the substrate and product causes stress to the organism (Guyot *et al.,*2005). During fermentation, heat is liberated due to exothermic reactions and if the environmental temperature is already high, the fermenter temperature tends to increase (Attfield *et al*., 1992). Therefore the yeast should have temperature-,osmotic pressure- and ethanol- tolerating capacities to perform efficiently in industrial scale. In tropical countries, maintaining the operating temperature at or around the optimum fermentation temperature requires cooling, which is expensive. (Sandrasegarampillai and Vasanthy, 2012). Significant cost savings become apparent if the fermenter can be kept at or above 40ºC. In addition, ethanol recovery cost shall also be low if the process is carried out at higher temperatures. This however would require a yeast strain that could produce high titre of ethanol at higher temperatures (Sandrasegarampillai and Vasanthy 2012).

Attempt has been made previously to isolate yeasts from *Raphia* palmwine and determine their ethanol tolerance level (Okpalla, *et al.,* 2014). The present study is a continuation of the work and the aim was to determine the thermotolerance, osmotolerance and pH tolerance of the yeasts isolated.

GSJ: Volume 10, Issue 12, December 2022
ISSN 2320-9186

Materials and Methods

Isolation of yeasts

The yeasts used in this study were earlier isolated from different *Raphia* palm wines in Ihiala town, Anambra State, Nigeria (Okpalla, *et al*. 2014). The Pure yeast isolates were identified using the method of Barnett *et al*. (1990) and Lodder (1971).

Inoculum preparation

Two (2) loopfuls of the palmwine yeast were collected from the agar slant and inoculated into a test tube containing 2 %(w/v) glucose solution. The solution was incubated for 24 h under room temperature.

Determination of Thermo-tolerance

Thermo-tolerance was conducted in various 250 ml Erlenmeyer flasks containing 50 ml YEPS medium which is composed of the following (g/l): Yeast extract,10; dihydrogen phosphate,2; sucrose,150 (Leveau and Bouix, 1979). The medium was sterilized and inoculated with the different yeast isolated (1.3x 10^7 cells/ml). Thereafter, the flasks were incubated at various temperatures of 30, 35, 40 and 45 ^{0}C for 72 h.The absorbance of the culture was recorded at 595nm using a spectrophotometer.

Determination of Osmo-tolerance

Osmo-tolerance was conducted in various 250ml Erlenmeyer flasks containing 50ml YEPS medium, which was added various concentrations of sucrose (10, 15, 30, 40 and 50 %w/v) separately. The medium was sterilized and inoculated with the different yeast isolated (1.3x 10^7 cells/ml) and incubated at 28^{0}C for 72h.The absorbance of t he culture was recorded at 595nm using a spectrophotometer.

Determination of pH tolerance

The pH tolerance was determined in various 250ml Erlenmeyer flasks containing 50ml YEPS medium and the pH of the medium was adjusted to values of 3.0 to 8.0. The medium was sterilized and inoculated with the different yeast isolated (1.3x 10^7 cells/ml) and incubated at 28^{0}C for 72h.The absorbance of the culture was recorded at 595nm using a spectrophotometer.

GSJ: Volume 10, Issue 12, December 2022
ISSN 2320-9186

Results and Discussion

The result for the effect of different temperatures on growth of the yeasts is shown in table 1. All the yeast strains, showed good level of growth at 35^0C (Table 1), with *Saccharomyces cerevisiae* OP1 observed to produce the best result. At temperature higher than 40^0C the growth of all the yeasts decreased. Cimpeanu *et al.* (2010), were able to obtain seven yeasts strains that showed good growth at 37^0C, at temperature higher than 40^0C their growth decreased.

Table 1: Effect of different temperatures on growth of yeasts

Yeast Strain	Temperature(^{0}C)				
	30	35	40	45	50
	Growth(OD595nm)				
Saccharomyces cerevisiae OP1	2.50	3.60	3.00	2.40	1.00
S. cerevisiae OP2	3.02	3.41	2.83	2.50	1.09
S. cerevisiae OP3	1.67	2.06	1.67	1.06	0.95
S. chevalieri OP4	1.42	1.90	1.20	0.96	0.62
S. rosei OP5	1.17	1.38	0.97	0.72	0.50
S. pastorianus RP1	2.00	2.30	1.90	1.50	1.30
S. chevalieri RP2	1.20	1.40	1.00	0.94	0.60
S. rosei RP3	2.16	2.20	1.96	1.50	1.30
S. cerevisiae RP3	1.98	2.82	1.50	1.00	0.67
S. cerevisiae RP4	3.04	3.40	2.90	2.50	2.00
S. rosei RP5	1.92	2.20	1.90	1.71	1.50

Table 2 shows the result of the effect of various concentrations of sucrose on growth of the yeasts is shown in table 2. All the yeasts isolated tolerated 10%w/v of the sucrose concentrations. The yeasts did not tolerate 40% of the sucrose concentration, except *Saccharomyces cerevisiae* OP1 and *S. cerevisiae* OP2. The yeasts *S.cerevisiae* OP3, *S.pastorianus* RP1, *S.chevalieri* RP2, and *S.rosei* RP3 were observed to tolerate only 10%w/v of sucrose concentration and did not survive at higher levels. None of the yeasts tolerated 50% w/v of sucrose concentration. Maximum yeast growth was recorded by *S.cerevisiae* at 15% w/v sucrose concentration. The study revealed that *S. cerevisiae* OP1 and *S. cerevisiae* OP2 grew at 40% w/v of sucrose, and this

GSJ: Volume 10, Issue 12, December 2022
ISSN 2320-9186

is similar to the observation of Bechem *et al*., (2007) who reported that two yeast isolates (Vip2 and Vip10) grew at 40% sucrose concentration. The result also showed that all the yeasts isolated could not grow at 50% sucrose concentration, this is also in agreement with the report of Bechem *et* al., (2007), but different with the finding of Bulawayo *et al*., (1996) whose yeast isolates showed growth at 50% sucrose concentration.

Table 2: Effect of Various Concentrations of Sucrose on Growth of Yeasts

Yeast Strain	Sucrose concentration % w/v				
	10	15	30	40	50
	Growth(OD 595nm)				
Sacharomyces cerevisiae OP1	3.98	4.24	3.30	2.94	-
S. cerevisiae OP2	4.10	4.70	4.01	3.12	-
S. cerevisiae OP3	3.60	-	-	-	-
S. chevalieri OP4	3.60	3.70	2.70	-	-
S. rosei OP5	2.80	2.50	2.00	-	-
S.pastorianus RP1	2.40	-	-	-	-
S. chevalieri RP2	3.00	-	-	-	-
S. rosei RP3	2.24	-	-	-	-
S. cerevisiae RP3	3.98	3.30	2.90	-	-
S. cerevisiae RP4	3.69	4.02	2.82	-	-
S. rosei RP5	3.20	2.95	2.00	-	-

The result of the effect of different pH values on growth of yeasts is as shown in table 3. None of the yeasts grew below pH 4 and above pH 7. This confirms the report of Bechem *et al*., (2007) who observed that none of the yeasts isolated could grow at pH 3.7. Again, Nwaga *et al*., (1998) reported that *S. cerevisiae* grew at pH 4.5 to 6.0 with the optimum around pH 6.0. The growth of *S.chevalieri* OP4, *S.rosei* OP5 and *S.chevalieri* RP2 was observed to start at pH 5, while no growth was observed at pH 7 and 8 for *S.rosei* OP5, *S.pastorianus* RP1 and *S.rosei* RP5. Maximum yeast growth was recorded by *S.cerevisiae* OP1 at pH 6.

GSJ: Volume 10, Issue 12, December 2022
ISSN 2320-9186

Table 3: Effect of Different pH values on Growth of Yeasts

Yeast strain	pH values					
	3	4	5	6	7	8
	Growth(OD 595nm)					
Saccharomyces cerevisiae OP1	-	2.20	3.80	4.06	3.00	-
S. cerevisiae OP2	-	2.40	3.10	3.92	2.80	-
S. cerevisiae OP3	-	1.50	2.00	2.50	1.60	-
S. chevalieri OP4	-	-	2.03	1.61	1.20	-
S. rosei OP5	-	-	2.32	2.00	-	-
S. pastorianus RP1	-	1.80	2.70	2.00	-	-
S. chevalieri RP2	-	-	3.20	2.90	1.60	-
S. rosei RP3	-	2.48	2.00	1.60	1.20	-
S. cerevisiae RP3	-	1.92	2.60	2.10	1.92	-
S. cerevisiae RP4	-	3.08	3.40	2.90	2.03	-
S. rosei RP5	-	1.62	2.80	2.17	-	-

Discussion

Conclusion

In conclusion, the study showed that some of the some of the yeasts isolated from the palm wines displayed thermo-, osmo- and pH–tolerances. These are some of the desirable properties that yeasts are expected to posses to be useful in alcohol production, especially in the brewery and wine industries,

GSJ: Volume 10, Issue 12, December 2022
ISSN 2320-9186

References

Attfield, P.V., Raman, A.and Northcott, C.J. (1992). Construction of *Saccharomyces cerevisiae* strains that accumulate relatively low concentrations of trehalose, and their application in testing the contribution of the disaccharide to stress tolerance. *FEMS Microbiol. Lett.* 94, 271-276

Barnett, J., Payne, R. and Yarrow, D. (1990). Yeasts. Characteristics and identification, 2nd ed. Cambridge University Press.

Bassir, O. (1962). Observation on the fermentation of palm-wine west. *Afri. J. Biol. Chem.* **6**: 20−25.

Bechem, E.E.T., Omoloko, C., Nwaga, D. and Titanji, V.P.K. (2007). Characterization of palm wine yeasts using osmotic, ethanol tolerance and Iso enzyme polymorphism of alcohol dehydrogenase. *Afri. Journal Biotech.* **6**(14): 1715−1719.

Bulawayo, B., Bvochora, J.M., Muzondo, M.I. and Zuauya, R. (1996).Ethanol production by fermentation of sweet stem sorghum juice using various yeast strains. *Wor. J. Microbiol. Biotech.* , 12: 57−360.

Cimpeanu, C., Campeanu, G., Begea, M., Vladescu, M. and Cornea, C.P. (2010). Bioethanol production by new thermotolerant Romanian yeast strains. *Rom Biotechnol Lett*. 15(3):5310–5316.

Guyot, S., Ferret, E. and Gervais, P. (2005). Responses of *Saccharomyces cerevisiae* to thermal stress. *Biotechnol. Bioeng*. 92(4): 403-409.

Isaac, U.E., Akpuaka, F.C. and Ndukwe, G.U.(2017). The effect of intake of palm wine on body weight and testicular microarchitecture in adult wistar rats. *J Exp Clin Anat*. 16:12-7

Leveau, J.Y. and Bouix, M. (1979).Etude des conditions extremes de croissance des levures osmophiles. *Ind. Alim. Agric.* **11** :1147−1151.

Lodder, J. (1971). The yeasts, a taxonomic study. 2nd Edition. North Holland Publishing Company Amsterdam, London, p 1385.

GSJ: Volume 10, Issue 12, December 2022
ISSN 2320-9186

Nigam, P.S., and Singh, A. (2011). Production of liquid biofuels from renewable resources. *Prog. Energy Combust. Sci*. 37: 52-68.

Nwachukwu, I.N., Ibekwe, V.I., Nwabueze, R.N. and Anyanwu, B.N. (2006). Characterization of palmwine yeast isolates for industrial utilization. *Afri. J. Biotech.,***5**(19) : 1725−1728.

Nwaga, D., Kouam, E. and Wu, E. (1998).Optimization de la production d'ethanola partir de hydrolyse de L'amidon de manioc et de la fermentation par lalevure de brere *Saccharomyces cerevisiae*. *Biologic. Sci. Proc.,***4** : 151−162.

Ogbulie, T.E., Ogbulie, J.N. and Njoku, H.O. (2007).Comparative study on the microbiology and shelf life stability of palm wine from *Elaeis guineensis* and*Raphia hookeri* obtained from Okigwe, Nigeria. *Afric. J. Biotechnol.* **6**(7): 914−920.

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

Okafor, N. (1987). Industrial microbiology.University of Ife Press Ltd. Ile Ife.

`Okpalla, J., Umeh, S.O., Onyeneto, T.C., Agu, K.C. and Ubajekwe, C.C. (2014). Isolation and ethanol tolerance of yeasts from palmwine obtained from Ihiala Town South Eastern Nigeria. *COOU Interdisciplinary Research Journal* 1(1):105-109

Oyagade, A.O., Famurewa, O.,Oyagade, J.O. and Aringbangba, J.O. (2004).Microbial population and survival of some pathogenic bacteria in fresh palmwine. *Nig. J. Microbiol.* **18**(1-2): 269−276.

Sandrasegarampillai B. and Vasanthy A.(2012). Osmo-, thermo- and ethanol- tolerances of *Saccharomyces cerevisiae* $S_{1.}$ *Braz. J. Microbiol*. 43(1)