Journal of Biomedical Investigation, 2008; 6(2):24-28.

Original Article.

Isolation, Characterization and Propagation of Bakers Yeast from Local Palmwine *(Elaeis Guinensis).*

[1*]*Achufusi J.N,* [2]*Emelugo B. N,* [2]*Okonkwo J.C.*

1. Nwafor Orizu College of Education Nsugbe. 2. Production Technology Dept Unizik, Awka.

ABSTRACT

Bakers yeast was isolated, characterized, and propagated from local palmwine *Elaeis guinensis,* collected from two different stations, namely: Mgbakwu, in Awka North Local Government Area and Nchagbo, in Ihiala Local Government Area, both in Anambra State. This was carried out with the aid of Saubraud Dextrose Agar (SDA), using a Pour Plate Technique (PPT). The inoculation was carried out and incubated for three days at room temperature. The mixed yeast colonies obtained at the end of the incubation period were purified into pure cultures by sub-culturing into a fresh SDA, using Streaking Plate Technology (SPT). The characterization of yeast was carried out on the basis of the cultural, microscopic and biochemical examination of the isolates of yeast. The yeast finally isolated, characterized and identified include: *Saccharomyces cerevisiae, Schizosaccharomyce pombe* and *Saccharomyces exigus.* Pure cultures of *S. cerevisiae* were propagated and multiplied using cane molasses to obtain a good deal of biomass.

Keywords: Organisms, Isolation, Analysis.

INTRODUCTION

Yeast, a form of fungi, like other micro-organisms, exist in or on most organic living matter. A common example of yeast is the bloom observed on grapes. Yeasts have simple nutritional needs and are unable to carry out photosynthesis. Yeast are used in the fermentation industry especially in the anaerobic conversion of simple sugar to ethyl alcohol and carbon dioxide, otherwise called alcohol fermentation. Yeast belongs to the protascomycetes family characterized by building as a means of reproduction. The yeast family has two groups: Endomycatacaea and Saccharomycetacea; called the true yeasts. A typical example is *Saccharomyces,*[1] Bakers yeast is commonly used for baking flour and powder and *S cerevisiae* is the yeast of choice[2].

Bakers yeast is of three types: compressed yeast, active dry yeast and instant active yeast. Media for growth of yeast contains molasses and various salts including ammonium and phosphate salts. The medium is adjusted to P^H 4.5-5.0, sugar level between 0.5 to 1.5%, while temperature is 30^0C or less. Yeast reproduces by budding or sporulation.[3]

Yeasts are found in all habitats that contain fermentable sugar materials, liquid extracts and secretions. They have been isolated from places like human body, water, air, fruits, vegetable materials and the like[4] but for the purpose of this work, palmwine is the source of interest. Palmwine is a sweet milky white effervescence liquid with a sweet taste that was obtained from oil palm tree *Elaeis guinensis* and the raphia palm tree *Raphia hookeri*__or *Raphia verifera* respectively. Yeast and yeast like fungi are widely distributed in nature. True inhabitants are those that may reproduce in the habitat.[5] Fate of yeast is affected by factors like P^H, water content (Aw), oxygen, carbon, nitrogen and vitamin sources available as well as other living organisms like the plant and animal host, filamentous fungi; bacteria, algae and insects.[6] Nutritional needs of yeast range from simple sugar (carbon sources) to ammonia (Nitrogen source). Other nutritional needs of yeast include: growth factors, hormones (biotin) and trace elements.[7] Some of their uses include: bread making and other bakery product,[8] while derived food yeast is used as supplements for humans, production of B – vitamin, for

production of single cell protein (SCP). Some species are used to remove petroleum as a pollutant from environment; in fermentation, for research and studying eg. NAD, RNA, ATP. They are also a rich source of protein amino acid.[9] Some are infectious eg. *Candida albicans, Cryptococcus neoformans*. Bakers yeast is used mainly for baked foods,[2] and the yeast of choice is *S cerevisae* used as a leaving agent. It exhibits active growth in the dough medium; utilizing fermentable sugar in the medium to yield other compounds like CO_2 and alcohol. Thus the functions of yeast in the dough are leavening, maturation and development in dough. *S cerevisae* has been noted for their ability to ferment and tolerate high ethanol concentration. Thus, high tolerance, flavouring, aroma flocculation, food sugar utilization and acid tolerance are characteristic nature of bakers yeast.[10]

Palmwine analysis shows that palmwine extract contains about 57% of yeast, mostly *S cerevesiae.* The mass product of bakers yeast made locally from palmwine will reduce the amount spent on importation of this organism from other countries because it affects our foreign reserves. The production of this bakers yeast in large quantity will reduce drastically the use of chemical leavening agents like bromides and bicarbonates used as substrate bakers yeast; for these cause diseases like cancer, kidney failure among others.[11] Also, production of bakers yeast will reduce cost of raw materials for the bakery industry. The objective of this study is therefore to identify, isolate, concentrate and harvest the yeasts *S cerevesiae* which is the major yeast of the bakery industry. Comparative study was also carried out between the produced and the commercial yeast.

MATERIALS & METHODS

Media used include: Saubraud Dextrose Aga (SDA), Corn meal Agar, Presporulation medium, post ssporulation medium, peptone water, starch medium, non sugar dough and sugar dough.

Each media was prepared according to the directions given for preparation; they were autoclaved at 121^{0}C for 15 minutes and allowed to cool to 45^{0}C.

SAMPLE COLLECTION

Palmwine samples were collected from Mgbakwu in Awka North and Nchagbo in Ihiala Local Government Areas. Samples were collected in sterile containers from local palmwine tappers in the study area.

ISOLATION PROCEDURE

1ml of each wine sample was added to 9ml of sterile distilled water using a sterile pipette to make a 1 in 10 dilution,[8] Fowel (1969). Subsequent dilutions were made to obtain 10^{-5} and 10^{-6} dilutions of each sample. 1ml of 10^{-5} and 10^{-6} dilutions of each sample were inoculated into SDA, that was cooled to 45^{0}C in McCarthy bottles and poured aseptically into sterile petri dishes. Plates were incubated at room temperature for 3 days, after which the plates were observed for microbial growth. Growth colonies were picked at random and sub-cultured to obtain a pure culture. Colonial morphology, cellular characteristics were studied. Isolates were identified according to criteria described by[11] Lodder (1970).

IDENTIFICATION TESTS

Wet mount using lactophenol cotton blue test, sugar fermentation tests for the production of acid and gas, starch hydrolysis, ascospore production ie the sporulation test. Spore staining test using malachite green was done according to[8].

PREPARATION OF MEDIA FOR PROPAGATION

The molasses, containing about 50% sugar was heated and filtered, decanted and diluted with sterile water to provide a sugar concentration of 1.5%. Sugar level was deliberately low to favour cell multiplication. Hydrometer bulb was used to ascertain the sugar concentration. After dilution, P^H of medium was adjusted to 4.5 using dilution. Medium was finally sterilized at 121^{0}C for 10 minutes before it was allowed to cool.

CULTIVATION OF YEAST CELLS

After sterilization, the media was inoculated with 2 x 10^5 cells of *S. cerevisieae* in 250ml of molasses at 30^0C for 3 days. Fermented medium was centrifuged at 10,000rpm under aseptic conditions.

FERMENTATION ABILITY IN DOUGH

The fermentation ability of the bakers yeast isolated was investigated in various dough compositions on both sugar and non-sugar dough. This was compared with commercial bakers yeast.

STATISTICAL ANALYSIS

Statistical data was done using the students t – test, to know the relationship that exist between the palmwine bakers yeast and the commercial yeast.

RESULTS

Three yeast isolates were obtained from the palm wine samples. These are: *Saccharomyces cerevisiae, .S pombe* and *.S exigus.* These are coded PW_1, PW_2, and PW_3, respectively. They were isolated accordingly.[11] The result shows that the fermentative ability on sugar dough is also statistically not significant. Comparisms were also made in the CO_2 level produced by the commercial yeast and palm wine yeast on both sugar and non sugar dough. The result also showed no significant difference.

DISCUSSION

Palmwine in bakers yeast exhibited an impressive fermentative ability on both sugar and non sugar doughs as illustrated in table IV & V respectively.

Table I – Test for non sugar dough

Time	CBY	CBY^2	PWY	PWY^2
20	38	1444	30	900
30	50	2500	35	1225
40	55	3025	36	1290
50	62	3844	40	1600
60	71	5041	45	2025
70	75	5625	51	2601
80	78	6084	56	3136
90	79	6241	61	3721
100	82	6724	64	4096
110	84	7056	68	4624
120	87	7569	70	4900
	761	55153	556	30120

t = 2.913 Critical t value = 0.181

Table II – Test for sugar dough

Time	CBY	CBY^2	PWY	PWY^2
20	48	2304	40	1600
30	58	3364	42	1764
40	68	4624	48	2304
50	72	5184	50	2500
60	78	6084	56	3136
70	82	6724	58	3364
80	84	7056	62	3844
90	85	7225	68	4624
100	86	7396	70	4900
110	86	7396	74	5476
120	88	7744	78	6084
	853	65101	648	39596

t value = 3.1 Critical t value = 1.181

From table V, palmwine yeast and commercial bakers yeast performed well on dough containing mild quantity of sugar depending on weight of flour. Also, it was observed that there is drop in the rate of CO_2 produced after about 1 hr. 20 mins for commercial yeast and $1^1/_2$hrs for palmwine yeast. This happens when the free sugar in the dough is depreciated.[2] Looking at tables IV & V again, the rate of gas production by palmwine yeast and commercial yeast is different for both yeast types. This is due to the fact that the commercial bakers yeast has undergone some changes, purification and concentration, hence it cannot be said to be ordinary. Palmwine yeast may contain some impurities; hence higher technology is needed for the purification and concentration.

From the t-test analysis, it was observed that the t-value calculated is higher than the critical t-value. This is indicative that the commercial yeast is better than the palm wine yeast product

at (P>0.05). The same is true in non sugar and sugar dough's. This may be because the commercial bakers yeast must have undergone some modifications, concentrations and improvement, but the palm wine in bakers yeast produced is good enough for bakers and if commercialized, will reduce the bulk of money spent on importation of yeast. Also with the addition of little technology, the palm wine yeast produced will be concentrated and purified in which case it can compete favorably with the commercial yeast. Bakers yeast from the palm wine has excellent aroma which it produces when mixed with dough; which is lacking in commercial bakers yeast.

Table III – Shows the results and characteristics of the isolates.

Isolate Code	**PW1**	**PW2**	**PW3**
Colour of Colony	Creamy White	Creamy	Creamy
Cellular morphology	Oval or round	Cylindrical	Oval
Cultural morphology	Smooth, round smooth, large, raised entire, soft.	Smooth surface, convex	Rough surface, raised, circular surface.
Motility test	+VE	+VE	+VE
Ascopore formation	+VE	+VE	+VE
Nitrate reduction	+VE	+VE	+VE
Starch hydrolysis	-VE	-VE	-VE
Urease test	-VE	-VE	-VE
Glucose	+VE	-VE	-VE
Maltose	+VE	+VE	+VE
Sucrose	+VE	+VE	+VE
Arabinose	+VE	-VE	+VE
Melebiose	-VE	-VE	-VE
Galactose	+VE	+VE	+VE
Lactose	-VE	-VE	-VE
Culture on Corn meal Agar	Pseudomycelium not present	Pseudomyclium present	Pseudomycelum present
Probable identities	*Saccharomyces cerevisae*	*Saccharomyces pombe*	*Saccharomyces exgus*

Key: +ve = Positive result that is presence of acid and gas.

-ve = negative result that is no presence of acid and gas.

Table IV – Shows the fermentative ability of commercial and palm wine yeast on non sugar dough.

	Time	20	30	40	50	60	70	80	90	100	110	120	$\bar{X}$
CBY	Level	38	50	55	62	71	75	78	79	82	84	87	69.20
PWBY	Level	30	35	36	40	45	51	56	61	64	68	70	50.50

The result shows that the fermentative ability of both yeast in non sugar dough are not statistically significant.

Table V – Shows the fermentative ability of both types of yeast on sugar dough.

	Time	20	30	40	50	60	70	80	90	100	110	120	$\bar{X}$
CBY	Level	48	58	68	72	78	82	84	85	86	86	88	75.91
PWBY	Level	40	42	48	50	56	58	62	68	70	74	78	58.72

CONCLUSION

Due to the increase in the price and cost of importation of the commercial yeast, bakers now divert to the use of chemicals as alternatives. These chemicals have been proven to be disadvantageous to man. With the production of the bakers yeast from palmwine, and molasses, the problem seems to have a solution underway. The use of palmwine and molasses are economical, for both are cheap and readily available.

REFERENCES

1. Frizzre Weslhott DC (1997). Food Microorganism. New York. MacGraw Hill inc. 76-86.
2. Ponte J.G. and Tsen C.C (1977). Bakers Yeast in Food and Beverage Plycology. 2nd Ed. Benchat L.R (Ed). New York Van Nostrand Reinhold Coy. 233-247.
3. Buyers B.C. (1981). Cytology of Yeast Life cycle. The Molecular Biology of Yeast Saccaromyces. New York. Cold spring Hanbour. 59-96.
4. Faparusa S.I. (1973). origin of initial Microflora of Palmwine from oil Palm Tree, *Elaeis guieneses.* J.Apple Black J. Apple Black 36:559 – 565.
5. Devenport R.R (1980). Yeast and Yeast Like Organisms. Smiths Introduction to Industrial Mycology. 7th Ed. Onion London – Edward Amolo Publisher Ltd. 65-82.
6. Phaff H.J. (2002). Yeast. Encyclopaedia of Life Science. Vol 19.London. Nature Publishing Group. 625-635.
7. Eka O.U (1979). Effects of Yeast Fermentation in Nutrient status. Nigerian Journal of Science. 243-245.
8. Fowel R.R. (1969). Life Cycle of Yeast. Rose and Harison J.S. (Ed) The Yeast. London. Academic Press Inc. Ltd. 461-471.
9. Basire O, (1975). Microflora of fermenting palm sap. Journal of science and Tech. 206.
10. Rose A.H. and Harrison J.S. (1989).Metabolism and Physiology of Yeast. In. The Yeast Vol. 3 London. Academic Press . 234-235.
11. Lodder J. (1970). General Classification of Yeast. The Yeast. A taxonomic Study Lodder .J. (Ed) 2nd Ed. Armsterdam North Holland Publishing Coy.1-30.