Nigerian Journal of Biochemistry and Molecular Biology
23 (2): 40 - 45, 2008. ISSN 0189-4757
Available online at http://www.njbmb.researchjournals.org

Availability of Baker's Yeast: A Survey of Propagation.

*Rose .M. Gidado[1], Nnenna R. Isu[1], and G.H. Ogbadu[2]
[1]National Biotechnology Development Agency, Wuse, Abuja, Nigeria
[2] Biotechnology Advanced Laboratory, Sheda Science & Technology Complex, Garki, Abuja, Nigeria.
*Corresponding Author. e-mail: roxydado@yahoo.com

ABSTRACT

The potential of Rhizga (*Plecthantrus esculentus*) for the propagation of baker's yeast in different starch sources such as cassava (*Utillisima esculentus*), sweet potato (*Ipomea batatas*), rhizga *(Plectanthrus esculentus)* and tacca (*Tacca leontopetaloides*) was investigated. This was done by an enzyme hydrolysis of the substrates using alpha/beta amylases from *Bacillus utilis*, Termamyl (a commercial thermostable alpha amylase from *Bacillus licheniformis)* and amyloglucosidase (a commercial preparation from NOVO). The hydrolysates were employed as feedstock for the aerobic cultivation of *Saccharomyces cerevisiae* DSM2155. From the results, there was a significant difference ($p<0.05$) between the hydrolysate yield (expressed as dextrose equivalent, DE) of the substrates. Alpha and beta - amylases from *Bacillus subtilis* produced consistently lower yields of 25DE, 28DE, 89DE and 88DE for sweet potatoes, cassava, rhizga and tacca respectively as compared with the termamyl and amyloglucosidase combination which resulted in higher hydrolysate yields of 82DE, 90DE, 100DE and 75DE respectively. The results of the aerobic growth studies showed that the hydrolysates from all the substrates supported the growth of *Saccharomyces cerevisiae* DSM 2155 with. biomass concentrations of $1.82x10^5$cells/ml for cassava, $4.86x10^4$ cells/ml for sweet potatoes, $1.84x10^4$ cells/ml for tacca and $1.4\ x10^4$ cells /ml for rhizga; while molasses (used as control) yielded $3.0\ x10^2$ cells/ml after an incubation period of 72hrs.

Key Words: Baker's yeast, propagation, enzyme hydrolysis.

INTRODUCTION

Yeast is a unicellular organism that has a very long history in industrial fermentation. It constitutes a tiny form of plant-like microorganism that exists in or on all living matter (Giorilli & Lauri, 1996). There are hundreds of different species of yeast identified in nature, but the genus and species most commonly used for baking is *Saccharomyces cerevisae*. The scientific name *Saccharomyces cerevisiae* means 'a mold which ferments the sugar in cereal *(saccharo-mucus cerevisiae)* to produce alcohol and carbon dioxide' (Giorilla & Lauri, 1996).

In recent years, baker's yeast (*Saccharomyces cerevisiae*) is considered the most cultivated and commercial microorganism, that has been used extensively for the production of single-cell protein (SCP) for human and animal consumption, and ethanol (industrial and potable alcohol) from fermentable sugars because of its 'Generally Regarded As Safe' (GRAS) status (Solomon *et al.*, 1997). In addition, it is widely used in leavening of dough because of its ability to produce carbon dioxide and ethanol from sugars (i.e. maltose and sucrose) present in the dough (Chen and Chiger, 1985; Jørgensen *et al*., 2002; Reed and Nagodawithana, 1991). Furthermore, *S. cerevisiae* is also employed in the leavening process because of its contribution to the aroma and flavour of bread (Hoek *et al.*, 1999).

The international baker's yeast market is expanding at about 4% per annum as a result of population growth and dietary changes as reported by Evans (1990). Bread has long been a staple food in Western cultures, but there is now increase in its consumption throughout Asia and Africa.

One of the main constraints in baker's yeast production is the raw material (generally sugar molasses). Molasses are not present in large quantities in Nigeria because of the problems besetting the sugar industry. Any sugar-containing raw materials or any material that can be hydrolyzed to fermentable sugars using enzymes may as well serve as a carbon and energy source for the production of baker's yeast. These sugars namely, sucrose, maltose, glucose and fructose are fermented only very slowly. Such sugar-containing substrates may be grape juice concentrates, root and tuber starch e.g. (cassava, tacca, rhizga and sweet potato), cereals, wood hydrolysates, or waste sulfite liquor (Prescott & Dunns, 1982).

Starch hydrolysing enzymes, analogous to catalyst used in petroleum industries, are produced in small amounts when compared to the amount of material produced by their reaction. Amylases are more common enzymes which can hydrolyse the

glycosidic bonds in starch. They are one of the largest industrial enzymes in the market (Aunstropcon, 1977) and are utilized in numerous and varied processes. Alpha-amylases are widely used for the liquefaction of the starch to allow the efficient production of dextrose by the saccharifying enzymes which are mainly beta-amylase and glucoamylases. The sources of amylases are numerous.

In Nigeria, the yeast used (both brewer's and baker's) are imported. Nigerian Yeast and Alcohol Manufacturing Company (NIYAMCO), a company that has the mandate to produce yeast in Nigeria, was more involved in alcohol (ethanol) production than yeast probably because molasses are not always available. There are only few sugar industries in the country and consequently cannot meet the demands of baker's yeast producers. Furthermore, considering the enormous amount of foreign exchange used in the importation of yeast and industrial enzymes (Bello, 2002) and the consequent effect on the price of the products, the local production of baker's yeast in commercial quantity using indigenous substrates (tacca, rizga, cassava and sweet potato) will go a long way to improve our economy by conserving the foreign exchange; and providing jobs (both skilled and unskilled) for the teeming unemployed population. This work is therefore aimed at expanding the feedstock for producing highly efficient, cheap, readily available as well as affordable yeast to the rural bakers.

MATERIALS AND METHODS

Sample Collection

Cassava (*Mannihot utilissima)* tuber cultivar TMS 30572- batch no.5643 and Sweet potatoes (*Ipomea batatas)* tuber cultivar T.I.S.-OP-batch no.5641were both obtained from Agricultural Development Programme Farm (ADPF at Gwagwalada, while t*acca (Tacca leontopetaloides)* batch no.5642 was harvested from the wild in Sheda village.

Rhizga (*Plectanthrus esculentus) t*uber cultivar N.E.Br of batch no. 5640 was obtained from Rhizga Community Farm in Barikin Ladi- Plateau State and Molasses were obtained from the brown sugar firm set up by National Office for Technology Acquisition and Promotion (NOTAP) and the Raw Materials Research and Development Council in Kona Mada along Lokoja Road.

Experimental Design

The experimental design was completely randomized Design (CRD) with triplicates used per sample treatment.

Enzymes:

The enzymes used included amyloglucosidase AMG (300U/kgDS) {Novo 300U/ml} and alpha-amylase Termamyl(0.125kg/tonne DS){Novo} both of which were obtained from the Jos International Brewery. Also a crude enzyme preparation containing a mixture of alpha and beta amylases from *Bacillus subtilis* was obtained from the Sheda Science & Technology Complex (SHESTCO) Biotech Advanced Laboratory., Sheda, along Abuja-Lokoja Road.

Reagents:

All the reagents used were of analytical grade except otherwise stated.

Microbiological Media:

The media used included Potato Dextrose Agar (Oxoid) and Peptone powder (Oxoid) which were prepared according to the manufacturer's specification and sterilized by autoclaving at 121^0C, 15psi for 15minutes

Methods

Raw material preparation and formulation

Freshly harvested cassava, tacca, sweet potato and rhizga tubers were sorted, washed with water, peeled, sliced using knife and sundried. The dried tuber chips were dry-milled in a blender mill and sieved using 500 microns sieve to obtain the flour needed for the research.

Slurry preparation

Twenty gram of the flour of each tuber was weighed in Explorer Ohaus weighing scale and slurred in 200 ml of distilled water at room temperature before finally homogenizing by stirring using a Jenway 1000 hotplate & stirrer giving a slurry concentration of 20g /200ml. The pH of each sample was adjusted from 7.5 to 6.0-6.5 by the addition of 0.05 M H_2SO_4.

Hydrolysis of Cassava, sweet potato, rizga and tacca.

Gelatinization/liquefaction of tuber starch was carried out according to the method of Nagodawithana and Reed (1993). Hydrolysis was carried out using crude enzyme preparation (with a dilution factor 1:200) containing alpha/beta amylase enzyme by taking 1 mg/ml of the enzyme and adding to each of the gelatinized samples and incubated for 24 hours in a water bath maintained at 65 ^{0}C which is the optimum temperature for beta amylase (Solomon *et al*,1990). Samples were withdrawn in quadruplets at the interval of 4 hours. The samples were analyzed

interval of 4 hours. The samples were analyzed for reducing sugar concentration. The experiment was repeated using Termamyl enzyme only and maintained at 95 ^{0}C. In this case, samples were withdrawn every thirty minutes for a period of 90 minutes. The third set of experiment was carried out using AMG alone. Hydrolysis was carried out at 60^{0}C with sampling as in the case for Termamyl.

Enzyme hydrolysis of Cassava, sweet potato, rizga and tacca

Gelatinization/liquefaction of tuber starch was carried out according to the method of Nagodawithana and Reed (1993). Four milliliters of Termamyl (0.125kg/tonne DS) [Novo] enzyme was added to each of the gelatinized samples and slowly heated with constant agitation to 105^{0}C for 7 minutes. The samples were later cooled in a GFL 1083 water bath to 95^{0}C and maintained at this temperature for 90 minutes. The samples were again taken at 30, 60 and 90 minute intervals to assay for reducing sugar.

Saccharification

Saccharification of tuber starch was carried out according to Nagodawithana and Reed (1993). The dextrinised starch solution was adjusted to pH 4.0-4.5 using 0.05 M H_2SO_4 and temperature of 60^{0}C maintained. Then, 4 mls of glucoamylase AMG (300U/kgDS) [Novo, 300U/ml] was added to each sample solution and then placed in GFL 1083 water bath maintained at 60^{0}C , pH 4.0-4.5 for 40-50 h. During this period, reducing sugar content was monitored by DNS method (Miller, 1959) by withdrawing 2 ml of the samples (each sample was diluted with distilled water before reducing sugar assay for Dextrose Equivalent (DE) determination) every 24 hr until hydrolysis was completely achieved. The enzymes were later denatured to prevent excessive reversion of glucose to isomaltose by heating the syrups to 120^O C for 15 minutes and the solutions filtered using Whatman's filter paper. The filterate was collected in 250 ml Erlenmeyer flasks. The syrups obtained were stored in the refrigerator at 10^{o}C ready for culture of Baker's yeast.

DE = (82.6×Enzyme concentration) + (0.359×Hydrolysis température) + (0.0635×Hydrolysis time)-45.1

Microorganism

S. cerevisiae, (DSM 2155), collected from the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria was used.

Fermentation

Fermentation was carried out according to the method of Dunns & Prescott, (1987).Two hundred millilitres of each of the stored filtrates obtained from the hydrolysis of all the tubers (cassava, sweet potato, rhizga and tacca) as well as molasses which became the carbon sources were sterilized by autoclaving at 121^{o}c for 15 minutes and later cooled to 30^o C in a water bath. Inoculation of the cooled filtrates were carried out as thus: 1ml of the inoculum was taken with a sterile pipette and put into 99ml of peptone water and then using a sterile pipette 1ml was again taken from this 10^{-2} dilution and added to another 99ml of peptone water to get a 10^{-4} dilution which was then plated out to count the cell load. Each of the flasks was inoculated with 1ml of the inoculum.

Fermentation was carried out at 30^{o}C in a Stuart Scientific Orbital incubator S1 shaker for 72 h in 250 ml Erlenmeyer flasks with samples withdrawn for microbial load analysis at every four hour interval.

Yeast concentration was determined by Beckman Life Science DU 530 UV/VIS spectrophotometer reading of the culture to assay for yeast cells.

Determination of Biomass or Growth Yield

The method of determination of Biomass yield is according to (Murray *et al*, 1998).

$$\text{No of Yeast cells} = \frac{\text{No of colonies X Absorbance}}{\text{Dilution factor}}$$

No of colonies on the plated culture media were 15 colonies for cassava, 6 colonies for sweet potato, 2 colonies for both tacca and rhizga while molasses had 3 colonies.

Statistical Analysis:

The various data obtained from the experiments were analyzed using the F test (ANOVA) and the one tailed Student t- test at 95% confidence limit.

RESULTS AND DISCUSSION

The results obtained in the study of hydrolysis of cassava, sweet potato, rhizga and tacca showed partial successful application of these three different sets of enzymes which were the crude enzyme preparation from *Candida subtilis*, and commercially available enzymes Termamyl and Amyloglucosidase (AMG) in the tuber starch hydrolysis processes (Tables 1-3). Starting with initial 10 %(w/v), cassava, sweet potato, rhizga and tacca starch slurry, hydrolysis with amyloglucosidase gave the highest concentration of sugar occurring in rhizga. This was followed by termamyl which gave high yield in cassava. Considering Table 1, it can be seen that after 24hours of hydrolysis using crude enzyme preparation, Tacca gave the DE of 109 indicating complete

hydrolysis, followed by Rhizga with a DE of 89 while the Cassava and Sweet potato having the DE of 28 and 25 respectively were not significantly hydrolysed.

The results in Table 2 which demonstrate the activity of Termamyl which is a commercial thermostable alpha amylase produced from *Bacillus licheniformis.* It can be seen from this Table that after 90 minutes of hydrolysis, Cassava, Sweet potato and Tacca gave DE of 53.9, 53.7 and 50 respectively indicating partial hydrolysis which is expected since termamyl is a liquefying enzyme. Rhizga gave a very low DE of 39.

On Table 3 are the results of hydrolysis using Amyloglucosidase. It can be seen that generally, the extent of the hydrolysis is significant for all the substrates with Rhizga giving DE of 145, Cassava DE of 128, Sweet potato DE of 108 and Tacca DE of 96. The high extent of hydrolysis is expected since glucosidase is a saccharifying enzyme with ability to break both alpha1-4 glycosidic bonds and the 1-6 glycosidic bonds.

ity of starch granules to digestion by amylase is dependent on starch source and length of amylase treatment. Contrary to previous reports (Taniguich *et al*., 1982; Okolo *et al,* 1995) that potato starch is not easily hydrolysed, the amylase of *Actinomyces* was able to hydrolyze the potato starch used in this study and at 24 hr incubation at 40^{o}C. This study shows further that cassava starch was not susceptible to hydrolysis by the crude *Actinomyces* enzyme while rhizga and tacca starches were susceptible to this crude enzyme.

Results obtained during the fermentation of *S.cerevisiae* (bakers yeast) on the hydrolysates of the various substrates are shown in Table 4. This shows the growth yield of the yeast DSM2155 on the various hydrolyzates in comparison with molasses. It can be noticed that at increased time from 24– 72hr, absorbance at 540 nm of all substrates including molasses increased with time. It was observed that Cassava had the highest concentration of $5.15x10^4$ cells/ml in 24hr,

Table 1: Concentration of reducing sugar (mg/ml) using a locally produced alpha/ beta amylase enzyme at SHESTCO Laboratory 1:200 dilution.

Time/hrs	Cassava		Sweet Potato		Tacca		Rhizga	
	RS	DE	RS	DE	RS	DE	RS	DE
0-4	1.31± 0.03		1.75± 0.04		3.23± 0.06		2.46± 0.05	
8	1.21± 0.02		1.21± 0.02		2.30± 0.05		1.37± 0.03	
12	1.59± 0.03		1.21± 0.02		2.35± 0.05		1.37± 0.03	
16	1.26± 0.03		1.10± 0.02		3.01± 0.06		3.67± 0.37	
20	1.21±0.02		1.26±0.03		3.01±0.06		3.45±0.17	
24	1.42±0.03		1.26±0.03		5.48±0.01		4.44±0.09	

Values are means ± standard deviation. of triplicates determinations. DE =Dextrose Equivalent. RS= Reducing Sugar

The use of amyloglucosidase (AMG) and termamyl enzymes for the hydrolysis of tuber starch agrees with earlier reports of Aransiola *et al* (2006), Rehana (1989), Adebiyi and Akinyanju (1998) that AMG and termamyl amylolytic enzymes can be used to hydrolyse tuber starches.

Hydrolysis of Cassava has been reported using enzymatic method by Gorinstein (1993), Paolucc-Jeanjean *et al*, (1997b) and Aransiola *et al, (*2006). All authors reported high efficiency of hydrolysis of Cassava.

This study showed that the susceptibility of the cassava, sweet potato, tacca, and rhizga tuber starches to the crude enzyme of *Actinomyces* was significantly dependent on the starch source and time of incubation. This agrees with earlier reports of Okolo *et al.*, (1995) that the susceptibil-

$1.09x10^5$cells/ml in 48 hr and $1.8x10^5$ cells/ml in 72 hr. Table 4 clearly shows Cassava to be the best substrate for yeast growth. Sweet potato was next. Molasses had the least support for biomass production of the yeast used.

S. cerevisiae has been grown on several substrates like molasses, cashew apple juice for production of single cell protein, (Solomon *et al*, 1991; Solomon *et al* 1988 and Aransiola *et al,* 2006). From Table 3, it was expected that since Rhizga gave the highest DE of 145, it should also give the highest yield of *S.cerevisiae*. However, this was not the case. The reason for this is not known but it was thought to be due to lower concentration of glucose which favours the growth of *S. cerevisiae* higher than fructose.

However, the result of cassava supporting the

Table 2: Concentration (mg/ml) of reducing sugar in gelatinization/liquefaction of the substrates after addition of Termamyl enzyme at 1:1000 dilution.

Time/min.	Cassava		Sweet Potato		Tacca		Rhizga	
	RS	DE	RS	DE	RS	DE	RS	DE
30	3.50±0.04		3.40±0.03		2.35±0.02		2.41±0.12	
60	4.77±0.05		4.55± 0.15		3.78± 0.04		2.90± 0.03	
90	5.31 ±0.0 5		5.37± 0.05		5.04± 0.05		3.94± 0.04	

Values are means ± standard deviation. of triplicates determinations. DE= Dextrose Equivalent. RS= Reducing sugar

Table 3: Reducing sugar concentrations (mg/ml) of saccharification (after addition of AMG) at 1:2000 dilution.

Time(hrs)	**Cassava**		**Sweet Potato**		**Tacca**		**Rhizga**	
	RS	**DE**	**RS**	**DE**	**RS**	**DE**	**RS**	**DE**
8	4.44 ± 0.09		4.27± 0.09		3.83± 0.34		4.93± 0.01	
24	4.98± 0.01		4.43± 0.09		3.89± 0.08		5.20± 0.01	
36	5.09± 0.01		4.93± 0.01		4.22± 0.08		6.57± 0.11	
48	6.41± 0.01		5.42± 0.01		4.82± 0.01		7.24± 0.02	

Values are means ± standard deviation. of triplicates determinations. DE =Dextrose Equivalent. RS= Reducing Sugar

growth of *S. cerevisiae* agrees with the report of Aransiola *et al,* (2006) that showed Cassava as a good source of substrate for propagation of bakers yeast *(S.cerevisiae)*. The observation of concomitant biomass and ethanol production during cultivation of the bakers yeast is supported in literatures. To corroborate this observation, Petrik *et al* (1983) reported that oxidative and fermentative glucose catabolism take place simultaneously. Even under fully aerobic conditions, a mixed respiro-fermentative metabolism is observed when sugar concentration exceeds a certain threshold value as reported by Aransiola *et al* (2006).

Altogether, this research has shown alternative sources of substrates to molasses for the production of baker's yeast. Cassava did better than all the other substrates. It is therefore recommended that more research be carried out especially on the chemical composition of the substrate sources to determine the anti-nutritional factors. It has been suggested that in order to increase volumetric production of yeast concentration or biomass, it is better to carry out the cultivation of the microorganism using fed-batch process (Aransiola *et al,* 2006). This will help in preventing formation of ethanol which reduces biomass production.

That is a worthwhile venture which will need to be surveyed for a proper utilization of the indigenous starchy substrates as alternatives for the propagation of baker's yeast in the tropics.

REFERENCES

Adebiyi Cab, Akinyanju, J.A. (1998). Thermophillic amylase producers from the soil. *Nigerian Journal Science Technology* 11 (1) 30 – 38.

Aransiola, E. F., Betiku, O.K. Adetunyi And Solomon, B.O. (2006) Production of Baker's Yeast (*Saccharomyces cerevisiae*) from Raw Cassava Starch Hydrolyzates in a Bioreactor under batch Process. *Biotechnology* 5 (400):1-6

Bello, G. H., And Gallo, M.(1971). Effect of impurities on oxygen transfer. *Process Biochem.* 6 4):33-35.

Chen, S.l, Chiger, M. (1985). Production of bakers' yeast. In: Moo- Young M (Ed). *Comprehensive Biotechnology*, 3. Oxford: Pergamon Press, pp 429-455.

Giorilli, P., Lauri, S. (1996). *"Il Pane: Un'arte una Tecnologia",* Zanichelli, Franco Lucisano Editore, Milano,

Gorinstein, S., 1993. Kinetics studies during enzyme hydrolysis of potatoand cassava starch. *Starch/Starke*, 45**:** 91-95

Hoek P. Van, D.E. Hulster E, Van Dijken J, Pronk JT (2000).Fermentative capacity in high-cell-density fed-batch cultures of baker's yeast.

Table 4: Growth yield of DSM2155 on the Various Substrates

Time	Cassava		Sweet potato		Tacca		Rhizga		Molasses	
	Absorbance (nm)	Biomass Yield(cells/ml)	Absorbance (nm)	Biomass Yield(cells/ml)	Absorbance (nm)	Biomass Yield (cells/ml)	Absorbance (nm)	Biomass Yield (cells/ml)	Absorbance (nm)	Biomass Yield(m/l)
24	1.03±0.0	51500	0.7±0.1	7000	0.78±0.0	0000	0.6±0.0	0000	0.08±0.1	800
48	1.09±0.0	109000	0.73±0.1	21900	0.81±0.0	8100	0.65±0.0	6500	0.09±0.1	1800
72	1.21±0.0	181500	0.81±0.0	48600	0.92±0.0	18400	0.70±0.0	14000	0.10±0.0	3000

Values are means ± standard deviation. of triplicates determination.

Biotechnol. Bioeng. 68: 517-523.

Jorgensen H., Olsson L., Rønnow B., Palmqvist E.A (2002). Fed-batch cultivation of baker's yeast followed by nitrogen or carbonstarvation: effects on fermentative capacity and content of trehalose and glycogen. *Appl. Microbiol. Biotechn*., 59: 31317.

Miller, G.L., (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar, *Analytical Chemistry*., 31, 426.

Nagodawithana, T.W. and Reed, G. (1993) Enzymes in Food Processing; 3rd edition, Academic Press [Library reference 664,024]

Okolo BN, Ezeogu LI, and Mba C.N. (1995). Production of raw starch- digesting Amylase by *Aspergillus niger* and *Bacillus alvei* grown on native starch sources. *Journal Science Food Agriculture* 69:109-115.

Paolucci-Jeanjean , D, M-P., Belleville, Zakhia ,N. and Rios, G.M. (2000). Kinetics of cassava starch hydrolysis with termamyl enzyme. *Biotechnolology Bioengineering*, 68: 71-77

Petrik, M.O, Kappeli and Fetcher A., (1983). An expanded concept for the glucose effect in the yeast Saccharomyces uvarium: Involvement of short and long-term regulation. *Journal of General Microbiology*, 129: 43-49.

Reed G, Nagodawithana TW (1991). Baker's yeast production. In: Reed G, Nagodawithana TW (eds) *Yeast technology*. Van Nostrand Reinhold, New York, pp 261-314.

Reed, G. and Nagodawithana, T.W. (1991). Yeast-derived products. In: *Yeast technology*. New York, AVI Van Nostrand Reinhold, 369-412

Rehana F, Venkatsubbiah, and Naud K. (1989).Preliminary studies on the Production of thermostable □-amylase by a mesophillic strain of *B.licheniformis*. *Chemical Microbiology Technology Leberism.* 12: 8 – 13.

Solomon, B. O., L. E. Erickson and J. E. Hess, (1981) Application of Data Consistency Tests and New parameter Estimation Methods to Microbial Growth on Corn Dust in Batch Culture" *Biotechnology and Bioengineering*, 23, 2333 - 2360.

Solomon B. O., S.K. Layokun and T. O. Omobuwajo, (1991). Nigeria's blackstrap molasses as substrate for the production of single cell protein with reference to baker's yeast. Ife *J.Technology,* 3: 25-29.

Solomon, B. O., S. Oladimeji and S. K. Layokun (1990) "Effect of Kilning Temperature on the Diastatic activity of malted sorghum". *JNSChE* 9(1&2), 62 - 65.

Solomon, B.O, Odeseye Or, Betiku E, Pretorius IS (1997). Investigation of starch degradation ability of *Saccharomyces cerevisiae* strain ZC89 in batch processes. *JNSChE*. 16: 69-76.

Taniguchi H, Odashima F, Igarashi M, Maruyama Y, Nkamura M. (1982).Characterization of a potato starch digesting bacterium and its Production of amylase. *Agriculture Biology Chemistry*. 46: 2107-2115.