Romanian Biotechnological Letters *Vol. 17, No.3, 2012*

ORIGINAL PAPER

Utilization of cassava wastes in the production of fructosyltransferase by *Rhizopus stolonifer* LAU 07

Received for publication, June 10, 2010
Accepted, September 11, 2011

A. LATEEF*[,1], AND E. B. GUEGUIM KANA[2],
[1]*Biotechnology Group, Microbiology Unit,*
Department of Pure and Applied Biology, Ladoke Akintola University of Technology, PMB 4000, Ogbomoso. Nigeria.
[2]*School of Biochemistry, Genetics and Microbiology, University of KwaZulu-Natal, Pietermaritzburg, Scottsville, South Africa.*
**Corresponding author: agbaje72@yahoo.com; Tel.: +234 803 7400 520*

Abstract

A newly isolated strain of Rhizopus stolonifer LAU 07 was evaluated for the production of fructosyltransferase (FTase), the enzyme responsible for the conversion of sucrose to fructooligosaccharide (FOS), a heathful food ingredient using locally available cassava wastes in both solid state (SSF, cassava peel) and submerged fermentations (SmF, cassava steep liquor). High titers of enzyme of more than 20 U/g were obtained when 5-15 % inoculum sizes were used with minimal supplementation of cassava peels with yeast extract. Maximum FTase yield of 32.87 U/ml was obtained after 96 h of fermentation in cassava steep liquor, that was not supplemented with any nutrient. The systems are advantageous and economical for the production of FOS, in that the complex substrates do not require extensive supplementation to produce the enzyme with acceptable titer. The FTase yielded 34 % of FOS (1-kestose, GF_2; nystose, GF_3) using 60 % (w/v) sucrose as the substrate. As far as we know, this is the first contribution towards the production of FTase and FOS by a species of Rhizopus using low-cost complex media obtained from cassava peel and cassava steep liquor. The large-scale availability of these materials can make the process industrially relevant especially in Nigeria, where these agricultural wastes/residues are generated in abundance.

Keywords *Rhizopus* . Submerged fermentation . Solid state fermentation . Cassava wastes . Fructosyltransferase . Fructooligosaccharides

Introduction

Fructooligosaccharides (FOS) are important food ingredients that are rapidly becoming popular because of the numerous health benefits, which include lowering of the blood cholesterol and glucose levels, enhancement of mineral absorption in the body, maintenance of healthy intestinal microbiota, promoting the growth of beneficial lactobacilli and bifidobacteria in the colon, prevention of the growth of pathogenic bacteria in the colon and prevention of colonic cancer [1]. The physicochemical attributes of FOS particularly the relative sweetness to sucrose, bulk density and viscosity have also increased their use in a number of food and pharmaceutical applications. FOS are primarily produced industrially using fructosyltransferase (FTase) (E.C.2.4.1.9), which catalyzes the formation of fructooligosaccharides from sucrose. Several fungi have been reported to possess transfructosylating activity thereby producing FOS from sucrose [23]. These include *Aspergillus niger* AS0023 [14], *Penicillium citrinum* [10], and *Aspergillus japonicus* [5]. *Aureobasidium pullulans* has been a major source of fructosyltransferase (EC 2.4.1.9) for the industrial production of fructooligosaccharides from sucrose, and several strains of the organism have been extensively studied [11, 24, 28, 29, 32-35].

There are several reports on the use of submerged fermentation (SmF) for the production of FTase by different microorganisms [10, 11, 14, 29, 30, 32, 35]. In all these

works, chemically defined media were used for the microbial fermentation. Research work on the use of SSF for the production of FTase lags behind that of SmF. Until now, there are only two of such reports in literature. Hang et al. [9], reported the use of apple pomace for FTase production using *Aspergillus foetidus* NRRL 337, while Sangeetha et al. [24] screened an array of agro wastes such as cereal brans, corn products, sugarcane bagasse, cassava bagasse (tippi), and by-products of tea and coffee processing for the production of FTase by *Aspergillus oryzae* CFR 202.

However, the use of SSF for the production of different enzymes has increased tremendously due to the advantages it has over SmF. These include simplicity in operation, high productivity fermentation, concentrated product formation and less favorable growth of contaminants [4]. It is interesting to note that more agro-industrial wastes are been evaluated on continuous basis for their suitability to produce enzymes. Recently, De Azevedo et al. [6] reported the use of feather meal in SSF to produce protease by *Streptomyces* sp. 594.

In this work, we attempt to extend the frontiers in the production of FOS, by utilizing a new source of FTase, *Rhizopus stolonifer* LAU 07 (13), grown in SmF and SSF using cassava steep liquor and cassava peels respectively. As far as we know, these two substrates have not been reported for the production of FTase. In fact, there is no report on the use of cassava steep liquor for the production of any enzyme in the literature. The two agro wastes are abundant in Nigeria, the World's largest producer of cassava. They are the major wastes of cassava processing in Nigeria; the peels are obtained from the peeling process, which is the first stage in the processing of cassava, while the steep liquor can be obtained during the processing of cassava to produce fermented products such as 'Lafun' and 'Fufu' [16, 26, 27]. As a rough estimate, about 10 million tons of cassava are processed into 'garri' alone in Nigeria [19]. Since the peels could make up to 10 % of the wet weight of cassava roots, they constitute a major agro waste. While cassava peels can be partially used to supplement animal feeds, albeit limited in doing this because of high cyanide content, the cassava steep liquor is of little use. The liquor is simply discharged into the environment thereby causing pollution.

Therefore, the present work seek to find cheap sources of producing FTase and high valued product (FOS), using an indigenous strain of *R. stolonifer*. In this regard, we have considered two important and abundant agrowastes obtained from the processing of cassava; the cassava peels and cassava steep liquor. Our work, as far as we know represents the first mention of the use of these substrates to produce FTase by a species of *Rhizopus.*

Materials and methods

Isolation of microorganism and preparation of inoculum

Rhizopus stolonifer LAU 07 (black bread mold) was isolated from spoilt orange fruit using potato dextrose agar (PDA) that was supplemented with 20 % sucrose. Dark patches were scrapped with sterile scalpel to inoculate the medium and incubated at room temperature (30 ±1 ^{o}C) for 48h. This was purified to obtain the pure culture. The pure culture was subsequently stored on PDA slants at 4 ^{o}C, and sub-cultured every three months. It was identified conventionally according to its macroscopic and microscopic features following the scheme of Domsch et al. [7]. The combination of rhizoids (root-like hyphae), long sporangiophores and striations on sporangiospores are the most easily identified characteristics of the species. The inoculum was developed by transferring a loopful mycelium into the inoculum medium (1 % sucrose, 0.2 % yeast extract, pH 5.50, 50ml/250ml flask) as previously reported [11, 12, 25]. The Flasks were incubated at 30±1 ^{o}C on a shaker, at 100 rpm for 24 h.

Preparation of substrates

Both cassava peels and cassava steep liquor were obtained from fresh cassava tubers procured from a local market in Ogbomoso, Nigeria. The peels were removed from washed tubers, and sundried for 8h, after which they were further dried at 60 ^{o}C for 12h. The dried peels were pulverized to 50-mesh size, and the moisture content was determined by drying to constant weight at 110 ^{o}C in a hot-air oven [1].

Cassava steep liquor was obtained following the traditional method of natural fermentation. Peeled cassava tubers (1.5 kg), were cut into cylindrical pieces (5-7 cm length), before being steeped in borehole water (2: l) in a plastic container covered with muslin cloth for 96 h at room temperature (30 ± 1 ^{o}C). The course of pH was monitored daily throughout the period of fermentation. At the end of 96 h, the liquor was decanted and filtered using Watman No. 2 filter paper. The liquor was diluted to 25 and 50 % (v/v) using borehole water. The liquors were dispensed into flasks (50ml/250ml flask), sterilized at 121 ^{o}C for 15minutes and then used for the fermentation for up to 96 h. The final pH of both substrates were not adjusted prior to fermentation, while the cassava peels (25 g in 100 ml of water) gave pH of 6.8, the final pH of cassava steep liquor was 4.8.

Submerged fermentation

Submerged fermentation was carried out in duplicate by inoculating 20 % (v/v) of 24 h-old inoculum into the cassava steep liquor (50ml/250ml flask). Incubation was done at room temperature (30 ± 1 ^{o}C) at 100 rpm for up to 96 h. At the end of fermentation (24 h-interval), corresponding flask was taken out, the broth was filtered using Whatman No. 2 filter paper, and the filtrate served as crude extracellular FTase, and used without further purification. When not used immediately, the crude enzymes were stored under refrigeration at 4 ^{o}C. Both the inoculum and culture samples were routinely observed under the microscope to ascertain purity that is devoid of contamination.

Solid state fermentation

SSF was carried out by investigating the effects of supplementation of cassava peels with sucrose and yeast extract, and varying inoculum sizes on the production of FTase by *R. stolonifer* LAU 07 using an in-house genetic algorithm software developed by our research group. The software is the optimization search engine module of Biopro_optimizer used to control bioprocesses [8]. The parameters were discretized as follows:

Sucrose concentration (0-15 % w/w) at interval of 5 %

Yeast extract concentration (0-0.4 % w/w) at interval of 0.2 %

Inoculum size (5-15 % v/w) at interval of 5 %

Twenty five profiles were generated at a crossover rate of 70 %, and ten of these were evaluated in duplicate in the laboratory to study the interactions among these components, and the effects on the production of FTase by the fungus in SSF (Table 1). The experiment was conducted twice. SSF was carried out in 250ml capacity bottles containing 25 g of cassava peels. The peels were moistened to a final moisture content of 65 %, before sterilizing at 121 ^{o}C for 1h. After cooling, the bottles were inoculated using appropriate inoculum size, vigorously shaken to ensure homogenization, and then fermented for 5 days. At the end of fermentation, samples of cultured substrates were observed under the microscope to ascertain purity of the fungus. Contents of each bottle were mixed with 50 ml of water and agitated at 100 rpm for 1h, and the filtrate squeezed out in a muslin cloth. This was followed with centrifugation at 5000 rpm at 10 ^{o}C for 25 minutes. The pH of filtrates were recorded and served as the crude FTase from SSF.

Analytical methods

FTase activity

The FTase activity of the crude extracellular enzyme was determined as previously described [11] by incubating 250 µl of the enzyme with 750 µl of sucrose (60 % w/v) in 0.1 M citrate buffer (pH 5.15) at 55 ^{o}C for 1 h in a shaking water bath at 100 rpm. At the end of incubation, reaction was arrested by keeping the reaction mixture in boiling water for 15 minutes. The enzyme activity was determined based on the amount of glucose released using glucose-oxidase kit (Quimica Clinica Aplicada S.A, Spain). Fifty fold dilution of the reaction mixture was allowed to react with the test reagent according to the manufacturer's instruction, and absorbance of the resulting product was measured at 505 nm on a UV/Vis spectrophotometer model 6405 (Jenway Ltd. Essex, UK). FTase activity is defined as the amount of enzyme required to liberate 1µmol of glucose under the specified conditions herein described [34]. The residual amount of cyanide in the crude enzymes was determined by the silver nitrate method [20].

Production and analysis of FOS

The production of FOS was carried out by incubating 250 µl of the crude extracellular enzyme with 750 µl of sucrose (60 % w/v) in 0.1 M citric acid/K_2HPO_4 buffer (pH 5.15) at 55 ^{o}C for 18 h in a shaking water bath at 100 rpm. At the end of incubation, reaction was arrested by keeping the reaction mixture in boiling water for 15 minutes, and then membrane filtered. The products formed were analyzed by HPLC with a quaternary pump (Delta 600, Waters) coupled to a Lichrosorb-NH_2 column (250 mm × 4.6 mm, Merck, Spain). The mobile phase was acetonitrile : water (75:25 v/v) conditioned with helium and used at a flow rate of 0.7ml/min. The column was kept at a temperature of 25 ^{o}C. A different refractometer (model 9040, Varian) was used and set to a constant temperature of 30 ^{o}C. The data obtained were analyzed using the millenium software, using external standards for calibration in the range of 0-100g/L. A fructooligosaccharide standard mixture (Actilight 950P, Beghin-Meiji) was used for reference purpose.

Results and discussion

Solid state fermentation

The result obtained in this study showed that cassava peel not only supported the growth of *R. stolonifer* LAU 07, but it also led to the production of FTase, with high enzyme activity in the range of 5.82-22.13 U/g, and 60 % of them yielding more than 15 U/g (Table 1). The cyanide content of the crude enzyme was about 1.4 ppm; having reduced the cyanide content of cassava peels through fermentation by 90. 6 % from the initial value of 14.7 ppm. The cyanide content is far lower than the reported cyanide contents of a number of fermented cassava products [20].

Table 1 The profiles generated by the genetic algorithm software and the corresponding FTase activity in SSF.

*Profile no.	Inoculum size	Sucrose	Yeast extract	FTase activity (U/g ± SD) n = 4
1	5	0	0.2	21.28 ± 1.8
2	15	10	0.2	17.32 ± 2.1
3	10	10	0.4	14.38 ± 2.1
4	10	4	0.2	11.3 ± 1.2
5	5	6	0.2	5.82 ± 0.9
6	15	10	0	15.85 ± 3.2
7	15	2	0.4	20.47 ± 3.1
8	10	0	0.4	21.33 ± 2.6
9	15	8	0.2	12.0 ± 1.6
10	15	10	0.4	22.13 ± 3.8

*, all the substrates were fermented for 5 days.

The microscopic examination of cultured substrates showed that the purity of the fungus was maintained and devoid of contaminants, while profuse growth patterns were generally noticed. The pattern of enzyme activity indicates that probably, the solid substrate does not need to be supplemented with sucrose, however the presence of yeast extract contributed to higher enzyme activity. Despite the absence of sucrose in profiles 1 and 8, higher enzyme titers of 21.28 and 21.33 U/g were obtained respectively, whereas in profiles 2, 3 and 6 with 10 % sucrose supplementation, lower enzyme titers of 17.32, 14.38 and 15.85 U/g were obtained. However, higher titer of 22.13 U/g in profile 10, could be as a result of high levels of inoculum (15 %) and yeast extract (0.4 %). It seems that high enzyme titer can be obtained using any of the inoculum size studied in this work, but considering the cost of production, 5 % level inoculum size is acceptable. We verified this claim in a number of validation experiments in profiles 1 and 8 using inoculum size of 5 % (w/v), where FTase titers of 19.55-21.80 U/g were obtained. Therefore, this work concludes that FTase can be produced using cassava peels with only supplementation with 0.4 % yeast extract. Sangeetha et al. [25] previously reported FTase activity of 15, 18, 18 and 22 U/g for *Aspergillus oryzae* CFR 202 grown on oat bran, wheat bran, corn germ and rice bran respectively.

The use of cassava peels for different purposes have been reported. It contributes to the agro wastes that are used to feed sheep and goats [22], however its low protein contents and high cyanide content have been limiting factors. Asagbra et al. [3], also reported the evaluation of cassava peels to produce tetracycline by some species of *Streptomyces*. It was found out that cassava peels supported the growth of the organisms to produce 2.16 mg/g of tetracycline ranking third after peanut shells (4.36 g/mg) and corncorb (2.64 mg/g).

However, there are only few reports on the use of cassava peels to produce enzyme. Sani et al. [28] reported that when grown on cassava peel, the highest amylase activity in the culture filtrate of *Aspergillus flavus* was 170-times higher than that on soluble starch, while that of *Aspergillus niger* was 16-times higher. The study concluded that cassava peel might be a better substrate for the production of amylase by *A. flavus* and *A. niger* than commercial soluble starch. In a recent study, Oboh [19] reported the use of squeezed liquid from cassava pulp fermented with strains of *Saccharomyces cerevisiae, Lactobacillus coryneformis* and *Lactobacillus delbruckii* to ferment cassava peels for 7 days to improve the nutritional quality. The study concluded that fermented cassava peels could be a good protein source in livestock feed. The present study proposes another use for the cassava peels, that is solid state fermentation by *R. stolonifer* LAU 07 for the production of fructosyltransferase, and ultimately, a functional food ingredient, FOS. We have recently reported that *R. stolonifer* LAU 07 improved both the nutritional quality and antioxidant activity of cassava peels as a result of fermentation for the production of fructosyltransferase [12], and was capable of producing FTase in chemically defined medium (13).

Submerged Fermentation

The steeping of cassava in water witnessed changes in pH during the course of 96 h of fermentation. The pH drastically dropped from 7.1 at 0 h to 5.1 within 24 h, thereafter, it fluctuated between 4.8 and 4.4 during the last 72 h of fermentation. This is in agreement with reported changes in the pH of cassava steep liquor. Obadina et al. [17] reported that the pH of cassava steeping water during 'fufu' production decreased from 6.95 at 0 h to 3.78 after 96 h of fermentation. The marginal difference might be attributed to the type of cassava tuber, and the quantity steeped in specified amount of water.

The activity of FTase produced by *R. stolonifer* LAU 07, grown in 25 % (v/v) cassava steep liquor increased gradually during the period of fermentation. The FTase activity rose from low-level of 2.34 to reach 32. 87 U/ml at 96h of fermentation (Fig 1), which was

comparable to the results obtained in 50 % (v/v). The FTase activity fall within the range of 12.31-45.70 U/ml obtained for the organism when grown in a chemically defined reference medium. During the period of fermentation, little changes were observed in the pH of the fermenting liquor from initial value of 4.8 to 4.1 at the end of 96 h of fermentation (Fig 1). There was no trace of cyanide in the fermenting liquor.

The cassava steep liquor from all indications should be rich in nutrients. A similar waste (cassava waste water) obtained from processing of cassava to produce 'garri', have been studied for the production of gin [31], due to the presence of high levels of fermentable sugars and essential elements, which equally supported the growth of different bacteria and fungi. The squeezed out liquid from the fermented cassava pulp (waste water) had been previously assessed as an important source of microbial enzymes [21]. Oboh [18] reported the presence of amylase in the cassava waste water. Most recently, a number of microorganisms obtained from cassava waste water from 'gari' processing were found to produce amylase and cellulase [2]. There seems to be a basis for using cassava steep liquor for the fermentation of *R. stolonifer* LAU 07 to produce FTase.

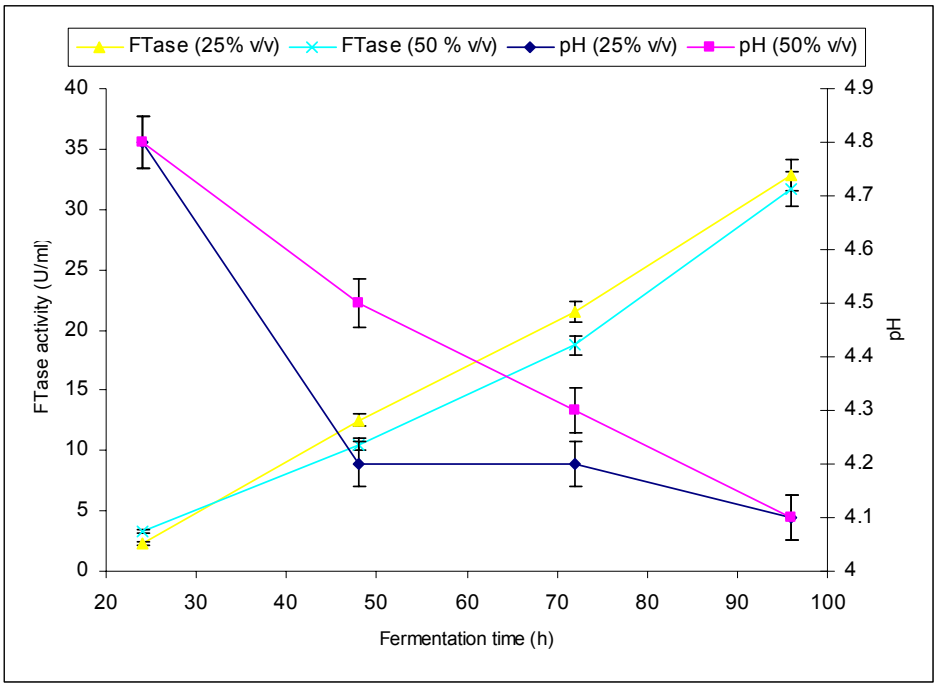

Fig. 1 The course of pH and FTase activity of *R. stolonifer* LAU 07 grown in submerged fermentation (25 and 50% (v/v) cassava steep liquor).

Production of FOS

The FTase was used to produce FOS, which gave average yield of 34 % (29 % GF_2, 1-Kestose and 5 % GF_3, Nystose). Although, the amount of FOS is lower compared to a range of 44-60 % that have been reported for several organisms by different authors [11, 24, 25, 29, 30], efforts are underway to manipulate the growth parameters and reaction conditions to optimize the production of FOS by this new isolate. The ability of the fungus to produce FTase in complex media of cassava peels and cassava steep liquor, and subsequently the production of FOS, may open a new way in the industrial production of FOS using agrowaste materials for the production of FTase.

Conclusion

In the present study, *R. stolonifer* LAU 07 was able to utilize cassava wastes in both SmF and SSF to produce FTase, which was subsequently used to produce a high valued product, FOS. As far as we know, our work is the first contribution towards the production of FTase by a strain of *Rhizopus* using low-cost wastes from cassava (cassava peels and cassava steep liquor). The only related report was the use of cassava bagasse (tippi) for the production of FTase by *A. oryzae* CFR 202 [25]. These indications offer an interesting potential for enzymatic or microbial production of FOS, including cheap and readily available substrates, such as cassava peels and cassava steep liquor at the industrial level.

References

1. AOAC (1984) Official methods of analysis, 14th edn. Association of Official Analytical Chemists, Washington, D.C.
2. AROTUPIN DJ (2007) Evaluation of Microorganisms from Cassava Waste Water for Production of Amylase and Cellulase. Res J Microbiol 2:475-480
3. ASAGBRA AE, SANNI AI, OYEWOLE OB (2005) Solid-state fermentation production of tetracycline by *Streptomyces* strains using some agricultural wastes as substrate. World J Microbiol Biotechnol 21:107-114
4. BALASUBRAMANIEM AK, NAGARAJAN KV, PARAMASAMY G (2001) Optimization of media for β-fructofuranosidase production by *Aspergillus niger* in submerged and solid state fermentation. Process Biochem 36:1241-1247
5. CHIEN CS, LEE WC, LIN TJ (2000) Immobilization of *Aspergillus japonicus* by entrapping cells in gluten for production of FOS. Enzyme Microb Technol 29:252-257
6. DE AZEVEDO LAI, DE LIMA MB, COELHO RRR, FREIRE DMG (2006) Thermophilic protease production by *Streptomyces* sp. 594 in submerged and solid-state fermentations using feather meal. J Appl Microbiol 100: 641-647
7. DOMSCH KH, GAMS W, ANDERSON TH (1980) Compendium of soil fungi. 865 p. London, England: Academic Press. ISBN 978-0122204012
8. GUEGUIM KANA EB, OLOKE JK, LATEEF A, ZEBAZE KANA MG (2007) Novel optimal temperature profile for acidification process of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* in yoghurt fermentation using Artificial Neural network and Genetic Algorithm. J Ind Microbiol Biotechnol 34: 491-497
9. HANG YD, WOODAMS EE, JANG KY (1995) Enzymatic conversion of sucrose to kestose by fungal extracellular fructosyltransferase. Biotechnol Letters 17:295-298
10. HAYASHI S, YOSHIYAMA T, FUJI N, SHINOHARA S (2000) Production of a novel syrup containing neofructooligosaccharides by the cells of *Penicillium citrinum.* Biotechnol Letters 22:1465-1469
11. LATEEF A, OLOKE JK, PRAPULLA SG (2007) The effect of ultrasonication on the release of fructosyltransferase from *Aureobasidium pullulans* CFR 77. Enzyme Microb Technol 40: 1067-1070
12. LATEEF A, OLOKE JK, GUEGUIM KANA EB, OYENIYI SO, ONIFADE RO, OYELEYE AO, OLADOSU CO, OYELAMI AO (2008) Improving the quality of Agro-wastes by solid-state fermentation: enhanced antioxidant activities and nutritional qualities. World J Microbiol Biotechnol. 24: 2369-2374
13. LATEEF A, OLOKE JK, GUEGUIM KANA EB, OYENIYI SO, ONIFADE RO, OYELEYE AO, OLADOSU CO (2008) *Rhizopus stolonifer* LAU 07: a novel source of fructosyltransferase. Chemical Papers 62: 635-638
14. L'HOCINE L, WANG Z, JIANG B, XU S (2000) Purification and partial characterization of fructosyltransferase and invertase from *Aspergillus niger* AS0023. J Biotechnol. 81: 73-84
15. NAKAKUKI T (2003) Development of functional oligosaccharides in Japan. Trends in Glycosci Glycotechnol 15: 57-64
16. NWABUEZE TU, ODUNSI FO (2007) Optimization of process conditions for cassava (*Manihot esculenta) lafun* production. African J Biotechnol 6:603-611
17. OBADINA AO, OYEWOLE OB, SANNI LO, TOMLINS KI (2006) Bio-preservative activities of *Lactobacillus plantarum* strains in fermenting Casssava 'fufu'. African J Biotechnol 5: 620-623

18. OBOH G (2005) Isolation and characterization of amylase from fermented cassava waste water. African J Biotechnol 4:1117-1123
19. OBOH G (2006) Nutrient enrichment of Cassava peels using a mixed culture of *Saccharomyces cerevisiae* and *Lactobacillus* spp solid media fermentation techniques. Electronic J Biotechnol 9:46-49
20. OBOH G, AKINDAHUNSI AA, OSHODI AA (2002) Nutrient and anti-nutrient content of *Aspergillus niger* fermented cassava products (flour and gari). J Food Composition Analysis 15: 617-622
21. OKAFOR N (1998) An integrated Bio-system for the disposal of cassava wastes. Integrated Bio-system in Zero Emission Applications. In: Proceedings of the international conference on integrated Bio-systems. http://www.ias.unu.edu/proceedings/icibs
22. ONWUKA CFI, ADETILOYE PO, AFOLAMI CA (1997) Use of household wastes and crop residues in small ruminant feeding in Nigeria. Small Ruminant Res 24:233-237
23. PRAPULLA SG, SUBHAPRADA V, KARANTH NG (2000) Microbial Production of Oligosaccharides: a review In: NEIDLEMAN, S.L., LASKIN, A.I, BENNET, J.W., AND GADD, G (eds). Advances in Applied Microbiology. New York, Academic Press. Pp 299-337
24. SANGEETHA PT, RAMESH MN, PRAPULLA SG (2004a) Production of fructooligosaccharides by fructosyltransferase from *Aspergillus oryzae* CFR 202 and *Aureobasidium pullulans* CFR 77. Process Biochem 39: 753-758
25. SANGEETHA PT, RAMESH MN, PRAPULLA SG (2004b) Production of fructosyl transferase by *Aspergillus oryzae* CFR 202 in solid-state fermentation using agricultural by-products. Appl Microbiol Biotechnol 65:530-537
26. SANNI LO, AKINGBALA JO, OGUNTUNDE AO, BAMBRIDGE ZA, GRAFFHAM AJ WEITHY A (1998) Processing of fufu from cassava in Nigeria: Problem and prospects for development. Sci Technol Dev 16:58–71.
27. SANNI LO, ADEBOWALE AA, FILANI TA, OYEWOLE OB, WESTBY A (2006) Quality of flash and rotary dried fufu flour. J Food Agric Env 4 : 74-78
28. SANI A, AWE FA, AKINYANJU JA (1992) Amylase synthesis in *Aspergillus flavus* and *Aspergillus niger* grown on cassava peel. J Ind Microbiol Biotechnol 10:55-59
29. SHIN HT, BAIG SY, LEE SW, SUH DS, KWON ST, LIM YB, LEE JH (2004a) Production of fructooligosaccharides from molasses by *Aureobasidium pullulans* cells. Bioresource Technol 93: 59-62
30. SHIN HT, PARK KM, KANG KH, OH DJ, LEE SW, BAIG SY, LEE JH (2004b) Novel method for cell immobilization and its application for production of oligosaccharides from sucrose. Letters Appl Microbiol 38: 176-179
31. UZOCHUKWU SVA, OYEDE R, ATANDA O (2001) Utilization of gari industry effluent in the preparation of a gin. Nigerian J Microbiol 15:87-92
32. VANDÁKOVÁ M, PLATKOVÁ Z, ANTOSOVÁ M, BÁLES V, POLAKOVIĆ M (2004) Optimization of cultivation conditions for production of fructosyltransferase by *Aureobasidium pullulans.* Chemical Papers 58: 15-22
33. YUN JW (1996) Fructooligosaccharides-Occurrence, preparation, and applications. Enzyme Microb Technol 19: 107-117
34. YUN JW, JUNG KH, JEON YJ, LEE JH (1992) Continuous production of fructooligosaccharides by immobilized cells of *Aureobasidium pullulans.* J Microbiol Biotechnol 2: 98-101
35. YUN JW, KIM D, MOON H, SONG C, SONG S (1997) Simultaneous formation of fructosyl-and glycosyltransferase in *Aureobasidium pullulans.* J Microbiol Biotechnol 7: 204-208