International Scholars Journals

Frontiers of Agriculture and Food Technology ISSN 2736-1624 Vol. 10 (7), pp. 001-006, July, 2020. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Raw starch degrading amylase production by mixed culture of *Aspergillus niger* and *Saccharomyces cerevisae* grown on sorghum pomace

E.A. Abu[1]*, S.A. Ado[2] and D. B. James[1]

[1]Department of Biochemistry Ahmadu Bello University Zaria-Nigeria.
[2]Department of Microbiology Ahmadu Bello University Zaria-Nigeria

Accepted 14 June, 2020

Production of raw starch degrading amylase by a mixed culture of *Aspergillus niger* and *Saccharomyces cerevisae* grown on sorghum pomace as nutrient source was investigated. Effect of mineral nutrient supplementation of sorghum pomace on raw starch degrading amylase activity was also determined. Sorghum pomace medium significantly (P<0.05) induced higher level of raw and extracellular amylase than soluble starch medium. Mixed culture media recorded higher (P<0.05) level of raw starch degrading amylase than monoculture media. However, mineral nutrient supplementation significantly (P<0.05) suppressed raw starch degrading amylase production. The crude enzyme solution degraded both cereal and tuber or root starches significantly (P<0.05). Sources of crude enzyme significantly (P<0.05) influenced raw starch digesting activity. Optimum pH for the raw starch degrading amylase which varied between 3.0 and 8.0 depended on the source of the crude enzyme.

Key words: Raw starch, Amylase, *Aspergillus niger*, *Saccharomyces cerevisae,* Sorghum pomace.

INTRODUCTION

Micro-organisms had made significant contribution to the production of foods and beverages in the last three decades. Various industries, such as food, brewing, textile pharmaceutical and confectionaries depend largely on the various products especially extra-cellular enzymes produced by these micro-organisms (Ibukun and Akindumila, 1998). An extra-cellular amylase, specifically raw starch digesting amylase has found important application in bioconversion of starches and starch- based substrates (Forgarty, 1983; Okolo et al., 1995).

Industrial conversion of starch with raw starch saccharifying amylase has been reported to represent an economically superior alternative to the conventional process which uses pregelatinised starch as substrate based on energy utilization and process simplicity (Forgarty, 1983; Achi and Njoku, 1992; Okolo et al., 1995). Only few micro-organism including *Aspergillus* species have been reported to posses ability to produce raw starch degrading amylase (Abe et al., 1988; Hayashida et al., 1988; Okolo et al., 1995). Production of this enzyme largely depends on substrates (cereal and tuber starches) that are heavily competed for as staple food, especially in the developing countries like Nigeria. These countries depend on these starches as major source of energy and other nutrients. Exploitation of alternative substrate such as pomace for the production of this enzyme could immensely reduce the level of competition for these starches in the developing countries.

Sorghum pomace obtained as by-product of starch extraction during “ogi” production from sorghum is currently a waste and constitute environmental pollution (Effiuwerwere and Akoma, 1995). Almost every household among low or middle income earners in Nigeria, infact, other African countries depend on “Ogi” as breakfast diet. Hence the production of associated pomace is very high, accounting for 25-30% of the entire production (Adeyemo et al., 1999). Adequate utilization and proper disposal of this waste will constitute great environmental remediation.

*Corresponding Author E-Mail: mmnlabu@yahoo.com.

Therefore, in the present study, an attempt has been made to produce raw starch digesting amylase from sorghum pomace compared to soluble starch, using a mixed culture of *Aspergillus niger* and *Saccharomyces cerevisae*. We also evaluated the effect of mineral nutrient supplementation on raw starch degrading amylase production.

MATERIALS AND METHOD

Micro-organisms

The fungus, *A. niger* sl.1 and *S. cerevisae* were isolated from soil and rotting cassava, respectively. They were purified, characterized and identified in the Department of Microbiology, Ahmadu Bello University, Zaria-Nigeria.

Starch

Cassava, potato, sorghum, maize and yam starches were prepared in our laboratory according to standard procedures (Corbishley and Miller, 1984; Watsom, 1984). Soluble starch from potato (*Solanum tuberosum*) was obtained from Merck chemicals (Germany).

Media and cultures

Innocula were prepared by growing the fungus, *A. niger* in Yeast Peptone Soluble starch (YPS) agar medium containing (per litre) 5 g yeast extract, 10 g peptone, 10 g soluble starch and 10 g agar. The medium was incubated for 96 h at 30°C. Medium for *S. cerevisae* consisted of (g/l), sucrose, 50; yeast extract, 10; peptone, 5; KH_2P0_4, 1; $(NH_4)_2$ SO_4, 2; and $MgSO_4.7H_20$, 1. The medium was incubated for 96 h at 30°C.

Fermentation medium comprised (g/l) of soluble starch, 50; yeast extract, 0.5;
KH_2PO_4, 10; $(NH_4)_2SO_4$, 10.5; $MgSO_4.7H_2O$, 0.3; $CaCl_2$, 0.5; $FeSO_4.7H_2O$, 0.013; $MnSO_4.7H_2O$, 0.004; $ZnSO_4.H_2O$., 0.004 and $CoCl.6H_2O$, 0.0067. For studies on the use of sorghum pomace and mineral nutrient supplementation, soluble starch was substituted with sorghum pomace (50 g) in mineral salt media while it was used as the sole nutrient source in non-mineral nutrient supplemented media.

The media were sterilized in an autoclave for 15 minutes at 121°C and pH was adjusted to 5.0 after cooling. Monoculture media (including that of soluble starch) were inoculated with a spore suspension (3.62 x 10^5 spores) *of A. niger* while those for mixed culture were inoculated with 1.81 x 10^5 each of *A. niger* and *S. cerevisae.* The media were then incubated at 30°C in an orbital shaker (CAT NO: 14460; APPNO.1b, 2621 CUO Gallen KAMP) set at 100 rpm for 72 h. Three replicate fermentations were carried out for each culture medium.

The media were centrifuged at 5,000 g for 15 min to obtain the crude enzyme solution. Protein in the crude enzyme solution was determined by Lowry et al. (1951) method.

Enzyme assays

Starches used for enzyme assays were completely reduced using $NaBH_4$ as described by Abdel-Akher et al (1959) until no reducing activity was noticed with Fehling's solution. Amylase activity was assayed as described by Wood and Bhat (1988) using a reaction mixture (4 ml) comprising 1 ml of enzyme solution, 2 ml of soluble starch (10 g l^{-1}) and 1 ml of 0.1 M acetate buffer pH 5.0. The mixtures were incubated for 10 min at 30°C. Total reducing sugars was determined by dinitrosalicylate method (Miller, 1959). One unit of amylase activity was defined as the amount of enzyme which released 1 μmole glucose $min^{-1}.mg^{-1}$ protein.

The reaction mixtures for raw starch digesting activity contained 0.3 g of raw starch, 30 ml deionised water, 5.5 ml enzyme solution (2.5 units ml^{-1}) and 4 ml of 0.1 M acetate buffer (pH 5.0). The mixtures were incubated for 1 h at 30°C.

Acetate buffer (0.1 M) of pH 3.0, 4.0, 5.0, 6.0, 7.0 and 0.1 M phosphate buffer of pH 8.0 and 9.0 were used to study the effect of pH on amylase digesting activity.

Statistical analyses

Statistical analyses were by analyses of variance (ANOVA). Turkey test was used to identify means that differed significantly ($P<0.05$).

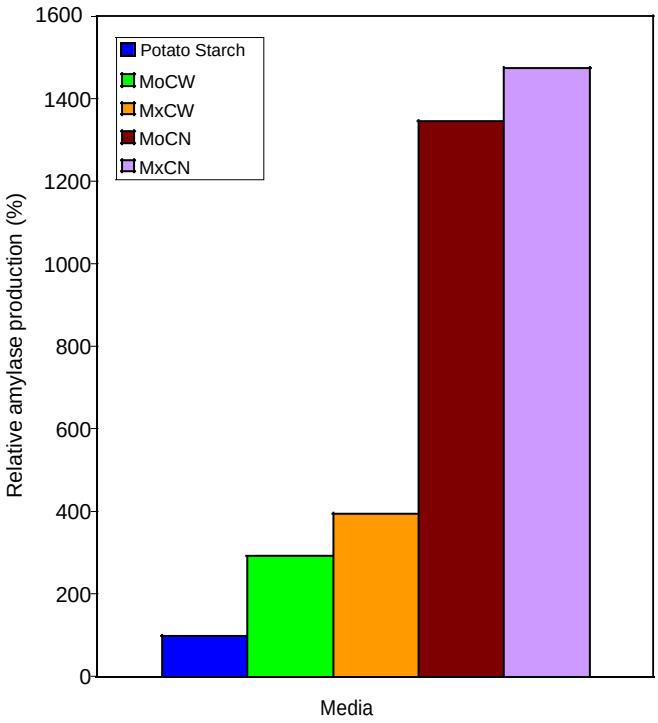

Figure 1. Effect of mixed culture fermentation and mineral supplementation of pomace on extracellular amylase production. MoCW, mono culture of *A. niger* with mineral supplementation; MxCW, mixed culture of *A. niger* and *S. cerevisae* with mineral supplementation; MoCN, mono culture of *A. niger* without mineral supplementation; MxCN, mixed culture of *A. niger* and *S. cerevisae* without mineral supplementation.

RESULTS AND DISCUSSION

Figure 1 shows the relative amylase production in the different media. All the media significantly ($P<0.05$) induced higher level of extra-cellular amylase than the soluble starch medium. Mixed culture media recorded 9.34 to 35.10% increase in amylase production above the monoculture media. Enhanced production of

Table 1. Digestibility of raw starches with crude enzymes.

Starches	Crude enzyme relative activities (%)				
	Starch	M_oCW	M_xCW	M_oCN	M_xCN
Soluble starch	100.00^{b}	439.56^{b}	564.18^{ab}	346.59^{bc}	671.87^{ab}
Cassava starch	79.12^{b}	415.38^{b}	336.26^{bc}	395.60^{b}	593.41^{b}
Potato starch	178.02^{a}	692.31^{a}	692.31^{a}	454.95^{a}	810.98^{a}
Sorghum starch	138.46^{ab}	474.73^{b}	217.58^{c}	514.29^{a}	731.87^{a}
Maize starch	118.68^{b}	712.08^{a}	652.75^{a}	276.92^{c}	336.26^{c}
Yam starch	197.80 ^{a}	731.87 ^{a}	474.73 ^{b}	415.38 ^{b}	652.75^{ab}
SEM	18.63	60.81	75.45	33.82	66.56

Values are means of three replicate fermentations.

Values not followed by the same superscript in the same column are significantly different (P<0.05).

MoCW, mono culture of *A. niger* with mineral supplementation; MxCW, mixed culture of *A. niger* and *S. cerevisae* with mineral supplementation; MoCN, mono culture of *A. niger* without mineral supplementation; MxCN, mixed culture of *A. niger* and *S. cerevisae* without mineral supplementation.

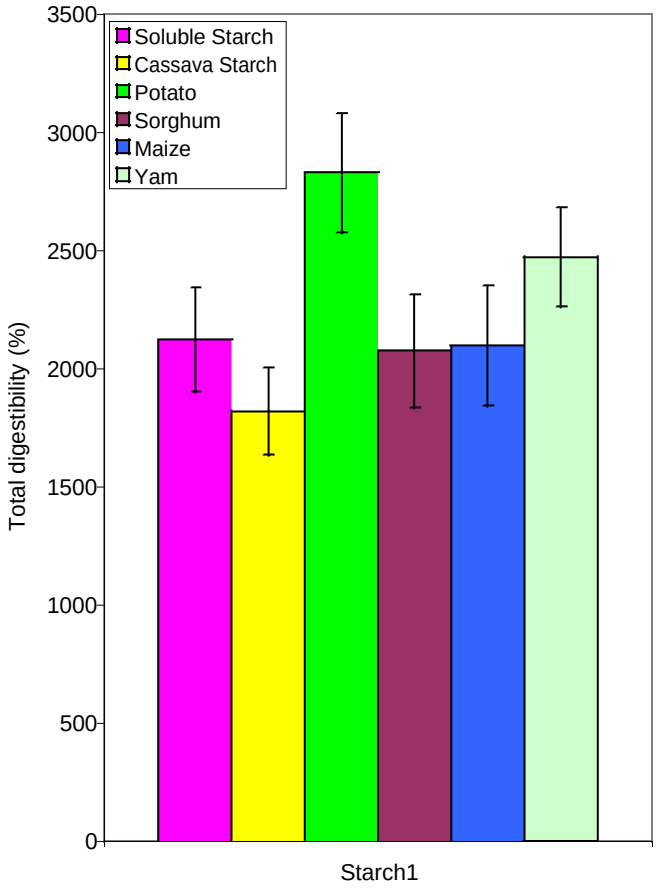

Figure 2. Total digestibilities of the various starches.

amylase in the mixed culture media may not be unconnected with the kinetic advantage in the media due to reduced inhibition of amylase production as a result of further conversion of glucose to ethanol by yeast (Gregg and Sadder 1995; Ladish et al., 1983).Mineral supplementation significantly (P<0.05) inhibited amylase production in both monoculture and mixed culture media, respectively. This suggests that sorghum pomace contained all the nutrients required for optimum growth and production of enzymes by the organisms. Hence mineral supplementation may not be necessary. Inhibition of amylase production and activity by the excess of some mineral elements has been previously reported (Mbaneto 1991; Cooke and Whipps, 1993).

The digestibility of raw starches with cell-free filtrates is shown in Table 1. The susceptibility of starches to raw starch digesting amylase depends on the source of crude enzyme. Highest level of total digestibility (810.95%) was recorded in potato starch by culture filtrate from mixed culture of *A. niger* and *S. cerevisae* without mineral supplementation. It was observed that crude enzyme from different sorghum pomace media were significantly (P<0.05) more active on the starches than that from soluble starch medium. Surprising though, it demonstrates the suitability of sorghum pomace for raw starch digesting amylase production when compared with soluble starch. The use of sorghum pomace and other agro-industrial wastes for extra-cellular amylase and cellulase production has earlier been reported (Abu et al., 2002, 2003; Abu, 2004). “Ogi” starch production from which pomace was obtained does not involve prior heat treatment (Effiuweuwere and Akoma, 1995). This could explain the suitability of residual starch in pomace as substrate for raw starch amylase production. Potato derived starch demonstrated the highest susceptibility to crude enzymes based on results shown in Figure 2. This was contrary to the findings of Taniquchi et al. (1982) and Okolo et al. (1995), who had earlier reported high resistance of potato starch to amylase digestion. Susceptibility of potato derived starch to the crude enzyme in the present study could be due to media

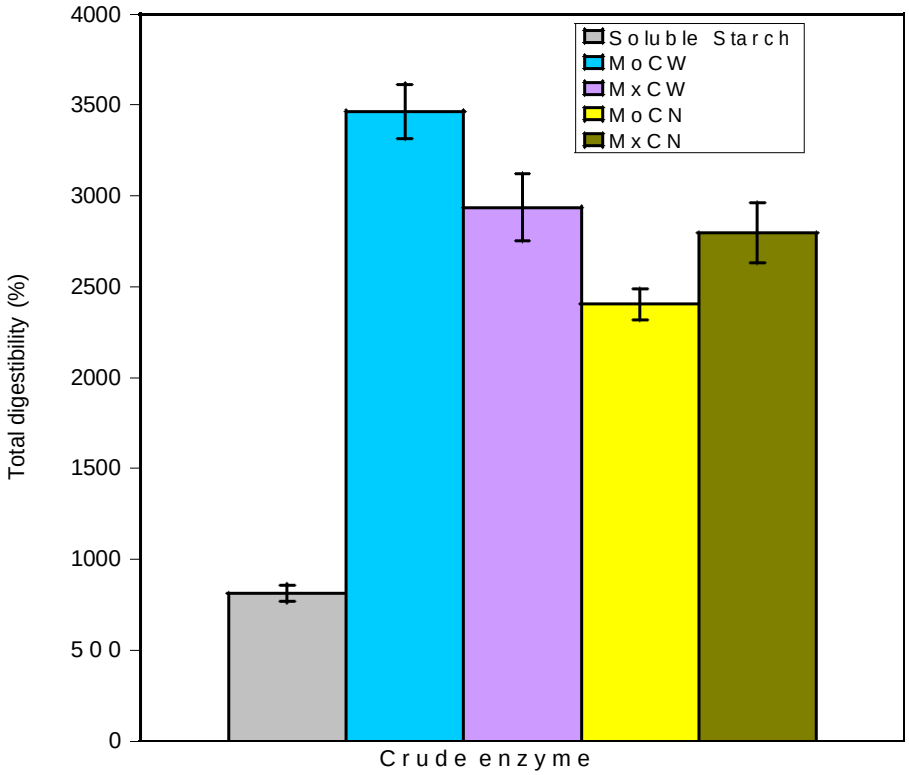

Figure 3. Total digestibilities recorded by crude enzymes. MoCW, mono culture of *A. niger* with mineral supplementation; MxCW, mixed culture of *A. niger* and *S. cerevisae* with mineral supplementation; MoCN, mono culture of *A. niger* without mineral supplementation; MxCN, mixed culture of *A. niger* and *S. cerevisae* without mineral supplementation.

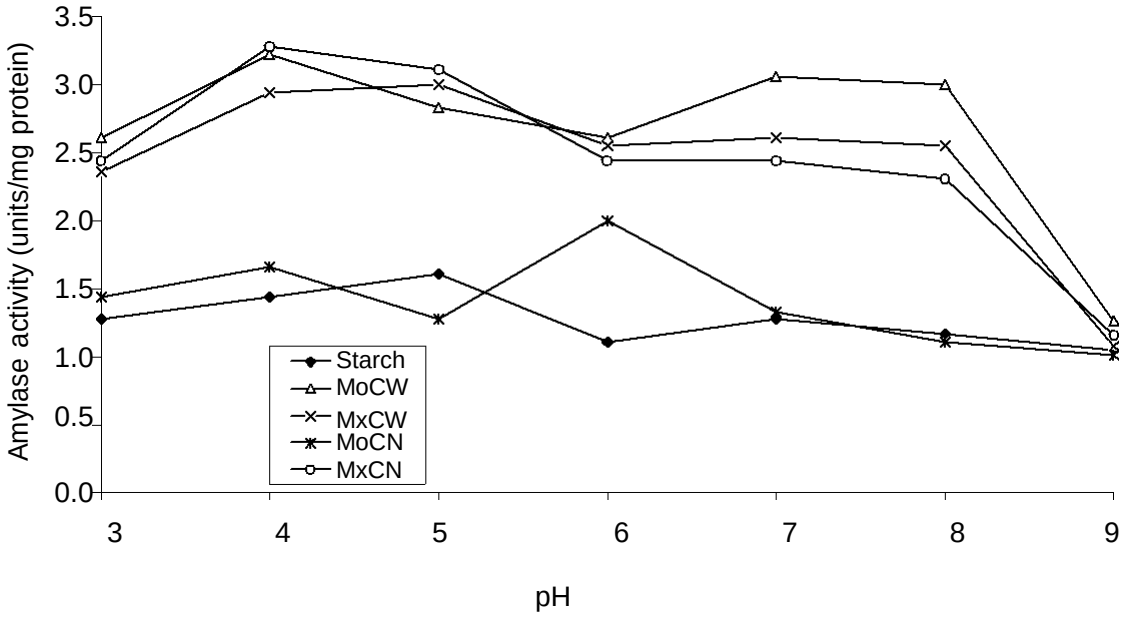

Figure 4. Effect of change of pH on crude amylase activity (units/mg protein). MoCW, mono culture of *A. niger* with mineral supplementation; MxCW, mixed culture of *A. niger* and *S. cerevisae* with mineral supplementation; MoCN, mono culture of *A. niger* without mineral supplementation; MxCN, mixed culture of *A. niger* and *S. cerevisae* without mineral supplementation.

composition and the nature of substrate. Media composition has been reported to significantly influence enzyme production and activity (Iwakolun et al., 2001; Ibukun and Akindumila, 1998) . Several *Aspergillus* species are known to produce raw starch digesting amylase for cereal starches (Maih and Ueda, 1975; Ishigami et al., 1985) but few have been known to be active towards root or tuber starches (Abe et al., 1988; Okolo et al., 1995). The current study reveal that all the cell-free filtrates from sorghum pomace were active on both cereal and root or tuber starch (Figure 3). This clearly demonstrates a unique characteristic of the isolate. This also suggests that with manipulation of media composition and a combination of mixture of isolates, raw starch digesting amylase with broad spectrum of activity can be produced using sorghum pomace as substrate.

The result of the activity of crude enzyme evaluated at various pH is shown in Figure 4. The two pH optima observed for most of the crude enzymes suggests the presence of at least two amylolytic activities in the preparation. This is in agreement with the observation of previous authors (Yamasaki et al., 1977, Ueda, 1981; Bergmann et al., 1988; Hayashida et al., 1988) who reported that crude amylase preparation from fungal species consists of at least two different amylases; an alpha amylase and a glucoamylase. Some of these enzymes act synergistically in starch degradation (Ueda, 1981). For all the culture media, the difference between the optimum pH and other pH values are very little in absolute terms, which suggests a possible pH insensitivity of enzyme over a pH range of 3.0-8.0 investigated. This may be a reflection of pH relationship of the synergistic interactions of various amylases in our crude enzyme preparations. It has been reported that the existence of strong synergistic interaction between certain enzyme components cocktails when acting on raw starch (Ueda, 1981; Okolo et al., 1995).

Conventional substrates for raw starch digesting amylase production has been based on soluble starch which serve as staple food for humans in developing countries like Nigeria. Data presented in this paper indicate that crude amylase with raw starch digesting characteristics can be produced from sorghum pomace, a by-product of 'ogi' preparation, using *A. niger.* Mixed culture fermentation using *A. niger* and *S. cerevisae* can enhance raw starch digesting activity of the crude extract. A special feature of this by-product, sorghum pomace, is that it does not require supplementation with additional mineral nutrients. The enzyme extract was active both on cereal starches and root or tuber starches with broad pH range of 3.0- 8.0 which is a very remarkable characteristic of the isolate and the mixed culture. This study is important in upgrading the value of sorghum pomace from waste to wealth in Nigeria.

REFERENCES

Abdel-Akher M, Hamilton JK, Smith F (1959). The reduction of sugars with sodium borohydride. J. Am. Chem. Soc. 73: 469-4692.

Abe J, Bergmann FW, Obeta K, Hizukuri S (1988). Production of the raw starch digesting amylase of *Aspergillus* spp. K-27. Appl. Microbiol. Biotechnol. 27: 447-450.

Abu EA, Ado SA, Adeleye IO (2002). Time course of amylase and cellulase production from sorghum pomace by mixed culture of *A.niger* and *S.cerevizae.* A paper present at the 22nd Annual Conference of Nig. Soc. Biochem. Mol. Biol. 18th-21st Sept. 2002. Zaria-Nigeria Abstract CI:18.

Abu EA, Ameh DA, Ibrahim S, Agbaji AS, Ameh JB (2003). Cellulase and Amylase production by *A.niger* sl.1 in ammonia treated agrowastes and evaluation of some kinetic parameters of the enzymes. Biosci. Res. Commun. 15: 49-57.

Abu EA (2004). Enzymatic Saccharification of some agro-industrial cellulosic wastes by cellulase produced from a mixed culture of *Aspergillus niger* and *Saccharonyces cerevisae* grown on sorghum pomace. Ife J. Sci. 6(1): 84 – 87.

Achi OK, Njoku-Obi ANU (1992). Production of raw starch saccharification amylase by *Bacillus alvei* grown on different agricultural substrates. World J. Microbiol. Biotechnol. 8: 206-207.

Adeyemo SO, Akoma O, Adeyeye AA (1999). Improvement of nutritional value of pomace from Kurun-zaki production by fermentation with *Candida tropicalis.* Nig. J. Biotechnol. 10: 55-59.

Bergmann FW, Abe J, Hizukuri S (1988). Selection of micro-organims which produce raw starch degrading amylases. Appl. Microbiol. Biotechnol. 27: 443-446.

Cooke RC, Whipps JM (1993). Resource acquisition and utilization In: Eco-physiology of fungi. Blackwell Scientific publications. University press, Cambridge. Great Britain. pp. 46-50.

Corbishley DA, Miller W (1984). Tapioca, arrow root and sago starches: production In: Starch chemistry and technology, ed. Whistler RL, Be Miller JW, Paschal EF. Academic Press, New York, U.S.A., pp. 469-778.

Effiuvwewere BJO, Akoma O (1995). The Microbiology of "Kunun-zaki", a beverage from Northern Nigeria during the fermentation (production) process. World J. Microbiol. Biotechnol. 11: 491-493.

Forgarty WM (1983). Microbial amylase. In: Microbiol. And Biotechnol. Ed. Forgarty WM. Applied Science Publishers, Barking, U.K. pp. 1-92.

Gregg D, Saddler JN (1995). A techno-economic assessment of the pretreatment and fractionation steps of a biomass to ethanol process. Appl. Biochem. Biotechnol. 58: 1261 – 1282.

Hayashida S, Teramoto Y, Inove T (1988). Production and characteristics of raw-potato starch digesting α-amylase from *Bacillus substilis* 65. Appl. Environ. Microbiol. 54:1516-1522.

Ibukun EO, Akindumila F (1998). Extracellular amylase production by isolates of Bacilli micro-organism cultured on different starchy food broths. Nig. J. Biochem. Mol. Biol. 13: 91-95.

Ishigami H, Hashinmoto H, Kanunma K (1985). Determination of optimum culture conditions for chalara enzyme production. J. Jpn. Soc. Starch Sci. 32:189-196.

Iwalokun BA, Akinside KI, Adeola SA (2001). Extracellular protease and amylase secretion by four strains of *Bacillus cereus* isolated from fermented melon seeds *(Citrullus vulgaris).* Nig. J. Biochem. Mol. Biol. 16:113-118.

Laddish MR, Lin KW, Voloch M, Tsao GT (1983). Process consideration in the enzymatic hydrolysis of biomass. Enzyme Microbial. Technol. 5: 8 – 16.

Maih MNN, Ueda S (1975). Multiplicity of glucoamylase of *Aspergillus oryzae.* Starke 29: 253-259.

Mbanefo CB (1991). Comparative biochemical studies of α-amylase from malted sorghum, pearl millet and micro-organims M.Sc. Thesis. ABU Zaria. pp. 36-45.

Okolo BN, Ezeogu LI, Mba CI (1995). Production of raw starch digesting amylase by *Aspergillus niger* grown on native starch sources. J. Sci. Food Agric. 69:109-115.

Taniguchi H, Odashima F, Igarashi M, Maruyama Y, Nkamura M (1982). Characterisation of potato starch digesting bacterium and its production of amylase. Agric. Biol. Chem. 46: 2107-2115.

Ueda S (1981). Fungal gluco-amylases and raw starch digestion. TIBS March pp. 89-90.
Watson SA (1984). Corn and sorghum starches production. In: Starch: Chemistry and Technology, ed. Whistler RL, Bemiller JW, Paschal EF. Acad. Press N/Y, U.S.A. pp. 417-464.
Wood TM, Bhat KM (1988). Methods for measuring cellulases activites. In: Aselson JN, Simon M. (Series eds). Methods enzymology series. Biomass part A. Cellulose and Hemicellulose. Acad. Press N/Y pp. 87-117.
Yamasaki Y, Tsuboi A, Suzuki Y (1977a). Two forms of glucoamylases from *Mucor rouxianus:* properties of two glucoamylases. Agric. Biol. Chem. 41: 2139-2148.