Comparative studies on the effect of organic and inorganic nitrogen supplementation of millet and sorghum pomace on the production of three industrial enzymes by *Aspergillus niger* SL.1

Emmanuel A. ABU¹ and Saleh A. ADO²

¹*Department of Biochemistry, Ahmadu Bello University, Zaria. Email: emmakeshi@yahoo.com*

²*Department of Microbiology, Ahmadu Bello University, Zaria. Email: salehado@yahoo.com*

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Abstract

Effect of nitrogen source and supplementation of millet and sorghum pomace on the production of cellulase, amylase and laccase by *Aspergillus niger* SL.1 was investigated using a submerged culture fermentation. The nitrogen sources (ammonium sulphate, yeast extract groundnut cake and neem seed cake) were supplemented at 5 – 20% level in the pomace medium and fermentation was carried out for 72 hours. Supplementation of both millet and sorghum pomace with 10 – 20% groundnut cake and also 5 – 20% neem seed cake in sorghum pomace media significantly ($P < 0.05$) recorded higher level of cellulase production than the control. Amylase production significantly ($P < 0.05$) decreased with increase in the level of supplementation of the pomace with ammonium sulphate and yeast extract. The pomace media containing 10 – 20% supplementation with groundnut cake recorded higher ($P < 0.05$) amylase activity than the control. Sorghum pomace media significantly ($P < 0.05$) induced higher laccase production than that of millet pomace. However, supplementation of millet pomace with ammonium sulphate and groundnut cake significantly ($P < 0.05$) recorded higher laccase production than the control. The study revealed that nitrogen supplementation of the pomace is important and enhanced the production of the three industrial enzymes.

Key words: Nitrogen source, supplementation, enzyme production

¹Author to whom all correspondence should be addressed.

INTRODUCTION

Millet and sorghum are major cereal crops grown in tropical region. They constitute major source of energy especially among the average and low income groups. In Northern part of Nigeria, for instance, millet or sorghum is used in preparation of food, beverages and breakfast snacks. By-products of these processing of food items include brans and residue commonly referred to as pomace^{8, 14}.

Few of these food processing by-products are fed to livestock especially ruminants with greater proportion being discarded, which constitute environmental pollution. Recent research efforts have been directed toward nutritional improvement of the pomace using micro-organism. Protein content of apple pomace was increased from 6 - 20% by fermentation using co-culture of cellulolytic mould (*Aspergillus niger* and *Candida utilis*) and used as cattle feed⁹. Increase in protein content of cereal pomace from 3.68 – 15.75% over 5-day fermentation with *Candida tropicalis* has been reported⁵. Chick growth study with the dried fermented pomace showed an increase in weight gain and feed efficiency which compared favourably with Growers-mash⁵.

One of the major problems that would arise from large scale production of “ogi” or “kunu zaki” is the management of solid waste referred to as pomace, which is cellulosic in nature and account for about 25 – 30% of the entire production⁷. Industrial production of enzymes using this pomace as an in-expensive carbon source could serve as another alternative to the utilization of this pomace and could reduce the degree of environmental pollution. Bioconversion of other similar agrowastes for industrial enzymes and chemical production is being actively investigated^{6, 27, 22, 1}.

Cellulase, amylase and laccase are three industrial enzymes whose need is on the increase. Cellulase and Amylase are used in paper, textile, pharmaceutical, food industries, brewing and chemical industries^{11, 15}. In recent years, there has been an increasing interest in the use of laccase in the pulp and paper industry, stabilization of fruit and vegetable juices and detoxification systems²⁵. The enzyme, laccase, is one of the major lignin degrading enzymes

and produced mainly by the white-rot fungi such as Basidiomycetes,^{25, 13}. Lignin degrading potential has also been associated with some *Aspergillus* species^{18, 19, 26, 2}.

Most of these enzymes are imported into Nigeria. Local production of these enzymes from food processing waste will enormously help in conserving foreign exchange while reducing environmental pollution caused by wastes accumulation. One of the variables that influence the rate of growth and activity of microorganism is nitrogenous constituent and its source^{23, 22, 6}.

The present study was therefore carried out to investigate the effect of nitrogen supplementation of pomace (Millet and Sorghum) on the production of three industrial enzymes (namely; cellulase, amylase and laccase) by *Aspergillus niger* Sl. 1

MATERIALS AND ME THODS

Micro-organism Used: *Aspergillus niger* S. 1. 1 isolated from a compost soil at the Ahmadu Bello University, Zaria was characterized and maintained on potato dextrose agar (PDA) slants at 4°C.

Pomace: This was prepared from millet and sorghum grains according to the method previously described¹⁴.

Nitrogen Sources: The nitrogen sources used include ammonium sulphate, yeast extract, groundnut cake and neem seed cake.

Enzyme Production: Growth was carried out using a submerged culture technique. The media consisted of 5% pomace mixture for control or nitrogen sources supplemented at 5 – 20% of pomace concentration in 250ml conical flask. The flasks were inoculated with 4.2×10^6 spores of *Aspergillus niger* Sl. 1 following sterilization. The media were incubated at 30°C in an orbital shaker set at 100rpm for 72 hours.

Enzyme Assays

Cellulase: Cellulase activity was determined by measuring the amount of reducing sugar released as glucose⁶. The reaction mixture

(3.0ml) contained 1ml each of 0.1M acetate buffer (pH 5.0), 1% carboxymethylcellulose and culture supernatant and incubated at 30°C for 10 minutes. Reducing sugar was determined by dinitrosalicylate method²⁰. One unit of enzyme activity was defined as the amount of enzyme which released 1μmole glucose min⁻¹.

Amylase: Amylase activity was determined according to the method already described³⁰. The reaction mixture consisted of 1ml each of 0.1M acetate buffer pH(5.0), 1% starch and culture supernatant. This was incubated at 30°C for 10 minutes. Reducing sugar was measured by dinitrosalicylate (DNS) method²⁰. One unit of amylase activity was defined as the amount of enzyme which released 1μmole glucose min⁻¹

Laccase: Laccase activity was determined by using 2,2¹-azino-bis-ethylthiazoline sulphonate (ABTS)¹². The reaction mixture consisted of 1ml each of 0.1M acetate buffer (pH 5.0), culture supernatant and 0.05% ABTS. This was incubated at 37°C for 10 minutes. Oxidation of ABTS was measured by monitoring the increase in absorbance at 420nm. One unit of enzyme activity was defined as the amount of enzyme required to oxidize 1μmole ABTS per minute using a Σ420 value for oxidized ABTS of 3.6 x 10⁴ (mol/cm³)¹⁰.

Statistical Analysis: Statistical analysis was by analysis of variance (ANOVA).

RESULTS AND DISCUSSION

The effect of supplementation of some inorganic and organic nitrogen sources in millet and sorghum pomace on cellulase production is shown in Figure I. All the samples tested induced cellulase production. Millet pomace supplemented with groundnut cake, however, induced the highest level of cellulase production (11.09 units/ml), followed by the control, ammonium sulphate then the least being yeast extract and neem seed cake. Cellulase production significantly (P<0.05) increased with the levels of nitrogen supplementation in millet pomace media. Increase in cellulase production with the level of supplementation was observed

in sorghum pomace supplemented with groundnut cake. There was no significant difference (P>0.05) in cellulase production between 10 and 20% supplementation with yeast extract or neem seed cake. Similar trend in cellulase production by *A.fumigatus* when different nitrogen sources were supplemented at 0.2 to 1.0% in rice husk and wheat straw media has been reported²². However, the cellulase values obtained in the present study was significantly higher (P<0.05) than that previously reported²². This could be due to difference in the level of supplementation, nature of agrowaste or the species of *Aspergillus* used^{6, 11}. Groundnut cake supplementation (10 – 20%) was observed to enhanced cellulase production by 15.65 – 47.08% in millet pomace medium and 34.86 – 50.81% in sorghum pomace medium but other nitrogen sources depressed cellulase production by 25 to 53.85% compared to the control in millet pomace media. Supplementation with neem seed cake induced highest cellulase production (7.10 units/ml) in sorghum pomace media though significantly lower (P<0.05) than the highest value (11.09 units/ml) obtained in millet pomace media. It is interesting to note that 20% supplementation of sorghum pomace with neem seed cake enhanced cellulase production by 91.89%. Groundnut cake and neem seed cake are both organic nitrogen sources and were found to support better cellulase yields. This finding is very relevant because of their local availabilities, thereby making incorporation into fermentation processes relatively cheaper^{6, 22}.

In a similar set of experiment, the effect of medium nitrogen source and supplementation of the millet and sorghum pomace on expression of *A. niger* amylase is shown in figure II. Amylase production decrease significantly (P<0.05) with increase in the levels of supplementation of both millet and sorghum pomace with ammonium sulphate. Amylase activity recorded for all levels of supplementation with ammonium sulphate and yeast extract in the two pomace media and millet pomace-neem seed cake were significantly lower (P<0.05) than that of the control. This suggests that supplementation of pomace with these nitrogen sources (ammonium sulphate and yeast extract) may not be important

Figure I: Effect of Nitrogen Source and Supplementation of Millet and Sorghum Pomace on Cellulase Production (units/ml) by *Aspergillus niger* SL 1

Key:

AS-MP = Ammonium Sulphate - Millet Pomace, AS - SP = Ammonium Sulphate - Sorghum Pomace
 YE - MP = Yeast Extract - Millet Pomace, YE-SP = Yeast Extract - Sorghum Pomace
 GC - MP = Groundnut Cake - Millet Pomace, GC - SP = Groundnut Cake - Sorghum Pomace
 NC - MP = Neem Seed Cake and Millet Pomace, NC - SP = Neam Seed Cake and Sorghum Pomace

Figure II: Effect of Nitrogen Source and Supplementation of Millet and Sorghum Pomace on Amylase Production (units/ml) by *Aspergillus niger* SL 1

Key:

AS-MP = Ammonium Sulphate - Millet Pomace, AS - SP = Ammonium Sulphate - Sorghum Pomace
 YE - MP = Yeast Extract - Millet Pomace, YE-SP = Yeast Extract - Sorghum Pomace
 GC - MP = Groundnut Cake - Millet Pomace, GC - SP = Groundnut Cake - Sorghum Pomace
 NC - MP = Neem Seed Cake and Millet Pomace, NC - SP = Neam Seed Cake and Sorghum Pomace

for amylase production in these media. Some enzymes productions are specially repressed by some type and levels of nitrogen^{23, 6}. This could be responsible for the reduction in amylase activity in the pomace media supplemented with ammonium sulphate and yeast extract. It was observed that 10 – 20% supplementation of both pomace with groundnut cake recorded significantly ($P < 0.05$) higher level of amylase activity than the control. All levels of supplementation of sorghum pomace with neem seed cake recorded significantly ($P < 0.05$) higher amylase activity than the control. The use of sorghum pomace and other agro-industrial wastes for extracellular amylase production has earlier been reported^{3, 4}. However, the amylase activity recorded in the present study was higher than the previous reports. This could be due to media composition^{16, 17}.

The effect of nitrogen source and supplementation of millet and sorghum pomace on laccase production (units/ml) is shown in figure III. Sorghum pomace media generally induced higher level of laccase than millet pomace media. In the control medium, sorghum pomace recorded a 10-fold increase in laccase over millet pomace. All levels of supplementation of sorghum pomace with ammonium sulphate, yeast extract and groundnut cake significantly ($P < 0.05$) reduced laccase production. However, supplementation

of sorghum pomace with 10 – 20% seed cake as well as ammonium sulphate and groundnut cake in millet pomace significantly ($P < 0.05$) induced higher level of laccase activity. The bitter taste associated with neem seed cake has been a major concern for its utilization in animal feeds²¹. Hence the incorporation of neem seed cake into the growth media for industrial enzyme production will help in no small measure in beefing the data on neem seed cake utilization. This is the first documentation on the use of neem seed cake in growth media for industrial enzyme production to best of the knowledge of the authors. Laccase is one of the major lignin degrading enzymes associated with fungal species^{24, 13, 26, 2}. Fungi have extremely active lignolytic enzymes compared to bacteria and therefore have potential for successful application in large scale biological treatment processes. However, very few fungal species, almost all of which very often require expensive growth supportive substrates such as glucose, have been exploited for application to the treatment of pulp and paper mill effluents²⁶. Thus to date, fungal treatment processes have remained economically unfeasible for industrial application due to requirement for expensive growth factor. Recent studies which revealed the production of laccase and other lignin degrading enzymes from *Aspergillus* species could provide a major break through and add to

the data bank of the new fungal strains for industrial applications^{18, 26, 2}. The lignolytic potential (based on laccase) of our isolate, *A. niger* Sl. 1 is similar to that reported for *A. foetidus* and *A. fumigatus*¹⁸. There is also the tendency of our isolate to grow on a wide spectrum of substrate².

In conclusion, this study revealed that supplementation of both millet and sorghum pomace with 10 – 20% groundnut cake and 5 – 20% neem seed cake in sorghum pomace significantly ($P<0.05$) enhanced higher level of cellulase production by *A. niger* Sl. 1. Amylase production significantly ($P<0.05$) decrease with increase in the level of supplementation of both millet and sorghum pomace with ammonium sulphate and yeast extract. Sorghum pomace media generally recorded higher ($P<0.05$) levels of laccase than millet pomace media. The use of neem seed cake as growth substrate in the media for industrial enzyme production was encouraging and reported for the first time to best of the knowledge of the authors. Practical application of this result could constitute an economic benefit in industrial enzyme production.

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