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Research Article

COMPARATIVE STUDY OF THE ACTIVITY OF AMYLASE PRODUCED BY *ASPERGILLUS NIGER* THOUGH SOLID STATE FERMENTATION (SSF) USING VARIOUS STARCHY MATERIALS

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ABSTRACT
Submerged fermentation holds tremendous fungal potentiality in high biomass yield of alpha-amylase. Isolation of fungi from bread sample and the rapid screening by plating on starch agar plates led to the finding of fungal strains capable of producing amylase. These strains were confirmed as *Aspergillus niger* by lacto phenol cotton blue staining. Different starchy substrates like jackfruit seed, cassava and coffee Husk, in dried and boiled forms were used as solid substrates for SSF. For 6 days of incubation under room temperature it was found that all substrates are good producers of amylase. The effect of carbon sources of the medium for the activity of α- amylase from *Aspergillus niger* utilizing jackfruit seed, cassava and coffee husk were investigated. The maximum activity of α-amylase was recorded as 0.875 x 10^{-3} µ mols/s after 6 days of incubation at room temperature 28^{o} C for dried jackfruit seed. Among the six substrate boiled jackfruit seed recorded as second (0.74 x 10^{-3} µ mols/s) and boiled cassava (0.63 x 10^{-3} µ mols/s) as third position in enzyme activity. The enzyme produced by *Aspergillus niger* could be used in industrial process after characterization. The present study suggest that the jackfruit seed may act as a potent substrate for industrial production of α-amylase and subjected for further explorations regarding industrial applications.

Keywords: *Solid State Fermentation, Jackfruit Seed, Amylase, Aspergillus Niger, α-Amylase*

INTRODUCTION
Microorganisms had significant contribution in production of various enzymes (Sivaramakrishnan *et al.*, 2006; Gupta *et al.*, 2003; Pandey *et al.*, 1999). Nowadays, the production of industrial many enzymes by using microorganisms is practiced (Sivaramakrishnan *et al.*, 2006; Singhania *et al.*, 2010). The global market for industrial enzymes estimated at $2 billion in 2010 and expected to rise at an average annual growth rate of 3.3% (Outtrup and Jørgensen, 2002; Cherry and Fidantsef, 2003). Starch degrading amylolytic enzymes are of great significance in biotechnological applications ranging from food, fermentation, textile, paper, pharmaceutical to sugar industry (Nigam and Singh, 1995; Fogarty and Kelly, 1979; Pandey *et al.*, 1999). Conversion of starch into sugar, syrup forms the major part of starch processing industry. Starch degrading enzymes like amylase have received great deal of attention because of their perceived technological and economic benefits (Pandey *et al.*, 1999; Sun *et al.*, 2010).
Amylase is one of the important and well-known industrial enzymes that can cause the breakdown of starch or glycogen (Gupta *et al.*, 2003; Nielsen and Borchert, 2000; Buléon *et al.*, 1998; Solomon, 1978). Use of microorganisms for the production of amylase is economical and less expensive (Demain and Vaishnav, 2009; Das and Mukherjee, 2007; Souza, 2010; Sodhi *et al.*, 2005). Bacteria and fungus is the main producer of these enzyme, however fungal amylase is more economical than the bacterial amylase (Souza, 2010; Gupta *et al.*, 2003; Haki and Rakshit, 2003; Pandey *et al.*, 1999; Sivaramakrishnan *et al.*, 2006; Couto and Sanromán, 2006). Amylase production is done by solid state fermentation (SSF) and submerged fermentation (SmF) (Souza, 2010; Gupta *et al.*, 2003; Haki and Rakshit, 2003). In submerged fermentation microorganisms are suspended on a liquid or partially liquid medium (Hixson and Gaden, 1950; Sandhya *et al.*, 2005; Bartholomew *et al.*, 1950). Microbes absorb their essential nutrients from that medium and the growth is resulted. In solid state fermentation the provided medium is solid or hard.
Amylases are widely distributed in microorganisms. Molds are capable of producing high amounts of amylase; *Aspergillus niger* is used for commercial production of α-amylase (Ramachandran *et al.*, 2004;

Suganthi *et al.,* 2011; Olempska-Beer *et al.,* 2006). Studies on fungal amylases especially in developing countries have concentrated mainly on *Aspergillus niger* (Ramachandran *et al.,* 2004; Gupta *et al.,* 2003; Pandey *et al.,* 1999).

In case of SSF the cost of the substrate also plays a key role in deciding the cost of production. Agro industrial wastes have been reported to be good substrate for the cost effective production of alpha amylases and are thus, attracting researchers for using starchy plant materials as a substrate for alpha amylase production (Ramachandran *et al.,* 2004; Gupta *et al.,* 2003; Pandey *et al.,* 1999; Bogar *et al.,* 2002). Fungal species have been studied a lot for the production of alpha amylase because of the low cost of substrates used for the production of alpha amylases. Thus, the present study was designed in the search of cheaper substrate for the production of alpha amylase enzyme by *Aspergillus niger*.

Given lacking qualitative and quantitative data on various starchy substrates in Kerala, objective of this study were to screens a variety of easily available and inexpensive starchy plant materials as substrate for the production of α-amylase using *Aspergillus niger* through solid state fermentation.

Review of Literature

Amylase is one of the most widely used enzymes in the world. It hydrolyses starch and used commercially for the production of sugar syrups from starch which consist of glucose, maltose, and higher oligosaccharides (Hagihara *et al.,* 2001). Amylases are of great significance in biotechnological applications ranging from food, fermentation, detergent, pharmaceutical, brewing and textile to paper industries (Miller, 1959; Kathireshan and Manivannan, 2006). To meet the higher demands of these industries, low cost production of amylase is required.

Amylase enzymes are important enzymes employed in starch processing industries for hydrolysis of polysaccharides such as starch into simple sugar constituents (Akpan *et al.,* 1999; Fogarty and Kelly, 1980; Nigam and Singh, 1995). Starch degrading enzymes like amylase have important in industrial scale because of their perceived technological significance and economic benefit (Ramachandran *et al.,* 2004; Gupta *et al.,* 2003; Pandey *et al.,* 1999). Amylase is also used for commercial production of glucose. In storage tissues such as seeds, starch, a polysaccharide of glucose is hydrolyzed by growing seedling to meet its energy requirement. Nowadays the new potential of using microorganism as biotechnological sources of industrially relevant enzymes has stimulated interest in exploration of extra cellular enzymatic activities in several microorganisms (Akpan *et al.,* 1999; Bilinski and Stewart, 1995; Buzzini and Martini, 2002). These enzymes are found in animals (saliva, pancreas), plants (malt), bacteria and molds (Abu *et al.,* 2005). Sources of amylases in yeast, bacteria and molds have been reported and their properties have been described (Akpan *et al.,* 1999; Buzzini and Martini, 2002). Amylase of fungal origin was found to be more stable than the bacterial enzymes on an industrial scale, Many attempts have been made to optimize culture conditions and suitable strains of fungi (Abu *et al.,* 2005).

Molds are capable of producing high amounts of amylase naturally; *Aspergillus niger* is used for commercial production of amylase. Studies on fungal amylases especially in developing countries have concentrated mainly on *Aspergillus niger*, probably because of their ubiquitous nature and non-fastidious nutritional requirements of these organisms (Abu *et al.,* 2005). The fungal amylase is more stable than the bacterial amylase and they can with stand stably if the environmental conditions are changed. Amylase production comprises of about 30% of the world enzyme production (Van der Maarel *et al.,* 2002).

Production of α amylases has been investigated through submerged (SmF) and solid-state fermentation (SSF) (Perez-Guarre *et al.,* 2003). However, the contents of a synthetic medium are very expensive and uneconomical, so they need to be replaced with more economically available agricultural and industrial byproducts, as they are considered to be good substrates for SSF to produce enzymes (Kunamneni *et al.,* 2005). In recent years the technique of solid-state fermentation (SSF) process has been developed and used more extensively. It has advantages over SmF like simple technique, low capital investment, cheaper production of enzyme having better physiochemical properties, lower levels of catabolic repression and better product recovery (Baysal *et al.,* 2003). The major factors that affect microbial synthesis of enzymes in a SSF system include selection of a suitable substrate and microorganism, particle size of the substrate, inoculums concentration and moisture level of the substrate, temperature and pH. Thus, it involves the

screening of a number of agro-industrial materials for microbial growth and product formation (Sodhi *et al.*, 2005). Temperature and pH are known to be important parameters in the production of enzymes from bacteria; hence, the thermal and the pH stability of the enzyme, which is a function of the exposure time, must also be taken into account

Before describing the action pattern and properties of amylolytic enzymes, it is essential to discuss the features of the natural substrate, starch. Starch is a major reserve carbohydrate of all higher plants. In some cases it accounts for as high as 70% of the undried plant material. It occurs in the form of water insoluble granules. The size and shape of the granules are often characteristic of the plant species from which they are extracted. When heated in water the hydrogen bonds holding the granules together begin to weaken and this permits them to swell and gelatinize. Ultimately they form paste or dispersion, depending on the concentration of polysaccharide. Starches are produced commercially from the seeds of plants, such as corn, wheat, sorghum or rice; from the tubers and roots of plants such as cassava, potato, arrowroot and the pith of sago palm. The major commercial source of starch is corn from which it is extracted by a wet milling process (Berkhout, 1976). Starch is a heterogeneous polysaccharide composed of two high molecular weight entities called amylose and amylopectin

There are mainly two methods which are used for production of α-Amylase on a commercial scale. These are: 1) Submerged fermentation and 2) Solid State fermentation. The latter is a fairly new method while the former is a traditional method of enzyme production from microbes which has been in use for a longer period of time.

Submerged fermentation (SmF) used free flowing liquid substrates, like molasses and broths. The products yielded by fermentation are secreted into the fermentation broth. The substrates are utilized quite rapidly; hence, the substrates need to be constantly added for the action. This fermentation technique is suitable for microorganisms such as bacteria that require high moisture content for their growth. SmF is primarily used for the extraction of secondary metabolites that need to be used in liquid form (Kunamneni *et al.*, 2005). This method has several advantages and SmF allows the utilization of genetically modified organisms to a greater extent than SSF. The sterilization of the medium and purification process of the end products can be done easily. Also the control of process parameters like temperature, pH, aeration, oxygen transfer and moisture can be done easily (Couto and Sanroman, 2006).

Solid state fermentation is a method used for microbes which require less moisture content and the absence of free flowing water for their growth. The main advantage of using solid substrate is that nutrient-rich waste materials can be easily recycled and used as substrates in this method. Unlike submerged fermentation, in this fermentation technique, the substrates are utilized very slowly and steadily. Hence, the same substrate can be used for a longer duration, thereby eliminating the need to constantly supply substrate to the process (Couto and Sanroman, 2006). Other advantages that solid state fermentation offers over submerged fermentation are simpler equipments, higher volumetric productivity, higher concentration of products and lesser effluent generation.

Fungal sources have been investigated for α-Amylase production through submerged and solid state fermentation. However, studies reveal that SSF is the most appropriate process in developing countries due to the advantages it offers which make it a cost effective production process (Couto and Sanroman, 2006). Also SSF provides a medium that resembles the natural habitat of fungal species, unlike SmF which is considered a violation of their habitat (Kenneth *et al.*, 1993).

Recently, it has gained importance in the production of microbial enzymes owing to several economic advantages over conventional submerged fermentation. The content of synthetic media is very expensive and uneconomical, so they need to be replaced with more economically available agricultural by-products to reduce the costs. Most agricultural wastes contain three major components; cellulose (30-50%), hemicellulose (20-35%) and lignin (4-35%). Starch based agro industrial residues are generally considered the best substrates for the SSF of α-amylases (Arasaratnam *et al.*, 2001).

However, the contents of a synthetic medium are very expensive and uneconomical, so they need to be replaced with more economically available agricultural and industrial byproducts, as they are considered to be good substrates for SSF to produce enzymes (Kunamneni *et al.*, 2005). In recent years the technique

of solid-state fermentation (SSF) process has been developed and used more extensively. It has advantages over SmF like simple technique, low capital investment, cheaper production of enzyme having better physiochemical properties, lower levels of catabolite repression and better product recovery (Baysal *et al.*, 2003). The major factors that affect microbial synthesis of enzymes in a SSF system include selection of a suitable substrate and microorganism, particle size of the substrate, inoculums concentration, moisture level of the substrate, temperature and pH. Thus, it involves the screening of a number of agro-industrial materials for microbial growth and product formation (Sodhi *et al.*, 2005). Temperature and pH are known to be important parameters in the production of enzymes from bacteria; hence, the thermal and the pH stability of the enzyme, which is a function of the exposure time, must also be taken into account.

Solid State Fermentation holds tremendous potentials for the production of enzymes. It can be of special interest in those processes where the crude fermented product may be used directly as the enzyme source (Canel and Moo, 1980; Tunga and Tunga, 2003). The free water is indispensable to the microorganism's growth and is adsorbed on a solid support or complexed into the interior of a solid matrix (Soccel, 1992). This method has economic value for countries with abundance of biomass and agro industrial residues, as these can be used as cheap raw materials (Tunga and Tunga, 2003).

The selection of suitable substrates for SSF has mainly been centered on agro industrial residues due to their potential advantages for filamentous fungi. Which are capable of penetrating into the hardest of these solid substrates, aided by the presence of turgor pressure at the tip of the mycelium (Tanyildizi *et al.*, 2005).

There are various factors that affect the solid state fermentation. Each factor that plays an important role to determines the activity of enzyme production. Temperature plays an important role in the fermentation process. Each microbial process requires a minimum temperature for the optimum activity. There are two temperatures for the optimum production of enzyme. They are temperature for the growth of the microbial source and optimum temperature at which maximum production of enzyme takes place. Optimum pH is a crucial factor for the stability of enzyme produced. Enzymes are pH sensitive and hence care must be taken to control the pH of the production process.

The substrate used for the production of amylase should contain starch granules, and they are suitable for the solid state fermentation. *Aspergillus*. *niger* also acts on the production of amylase take place according to the availability of the starch in the substrate. Addition of organic nitrogen sources such as casein, meat extract and beef extract to the medium resulted in a considerable increase in the production of amylase. Media supplemented with beef extract shows maximum activity of amylase compared to meat extract and casein. Various carbon sources are mainly supplemented with the media such as sucrose, glucose, maltose etc. The carbon source is the main energy source of microbes. Microbes are used these carbon sources for their metabolic functions.

Optimum levels of initial moisture content may vary depending on the microbial source used. For fungal sources the moisture content required is less whereas bacterial sources need more moisture content for high yield of the enzyme (Laveeque *et al.*, 2000). The optimum level can be determined by determining the enzyme yield within a range of initial moisture content.

This is a crucial factor in fermentation process. If the process is carried out for a time period shorter than the optimum duration the maximum yield cannot be obtained. The enzyme activity increases with increase in incubation time till it reaches the optimum duration. During the growth of fungi they utilizes the available nutrients in the medium the growth and release of various metabolic end products are take place. So, the time of culture will take place an important role in the production of amylase. Addition of another nutrient medium and control of various parameters depend upon the duration time.

Cassava (*Manihot esculenta*) is the third source of largest food carbohydrates in the tropics, after rice and maize (CasFauquet and Fargette, 1990). Cassava is a major staple food in the developing world, providing a basic diet for over half a billion people. It is one of the most drought-tolerant crops, capable of growing on marginal soils. Nigeria is the world's largest producer of cassava, while Thailand is the largest exporter of dried cassava.

Cassava is classified as either sweet or bitter. Like other roots and tubers, both bitter and sweet varieties of cassava contain anti-nutritional factors and toxins, with the bitter varieties containing much larger amounts. The high starch and carbohydrate content in the cassava may help the fungi to act on the substrate and convert them into useful product (Reade and Gregory, 1975; Ubalua, 2007). The microorganisms act on the starch to produce the enzyme amylase.

The cassava root is long and tapered, with a firm, homogeneous flesh encased in a detachable rind, about 1 mm thick, rough and brown on the outside. Commercial varieties can be 5 to 10 cm in diameter at the top, and around 15 to 30 cm long. A woody vascular bundle runs along the root's axis. The flesh can be chalk-white or yellowish. Cassava roots are very rich in starch and contain significant amounts of calcium (50 mg), phosphorus (40 mg) and vitamin C (25 mg) per 100 g. However, they are poor in protein and other nutrients. Cassava root is essentially a carbohydrate source (Reade and Gregory, 1975; Ubalua, 2007; Tewe and Egbunike, 1992). Its composition shows moisture (60–65%), carbohydrate (20–31%), crude protein (1–2%) and a comparatively low content of vitamins and minerals. However, the roots are rich in calcium and vitamin C and contain a nutritionally significant quantity of thiamine, riboflavin and nicotinic acid. Cassava starch contains amyl pectin (70%) and amylase (20%). Cooked cassava starch has higher digestibility (75%). Cassava root is a poor source of protein. Despite the very low quantity, the quality of cassava root protein is fairly good in terms of essential amino acids like Methionine and Cysteine. Cassava is attractive as nutritional source in certain ecosystems because cassava is one of the most drought-tolerant crops, can be successfully grown on marginal soils, and gives reasonable yields where many other crops do not grow well. The edible parts include flesh, as well as seeds. Seeds are roasted, turned into flour or boiled. Sometimes they're canned in curry, tomato sauce, a sweet syrup or brine. Jackfruit seeds can be a nutritious addition to your diet. A 100 g serving of jackfruit seeds, provides about 184 calories, 7 g of protein and 38 g of carbohydrates, including 1.5 g of fiber, but has less than 1 g of fat. This is approximately 6% of the daily value for fiber of 25 g. Fiber helps fill you up, making it easier to lose weight, and may lower your risk for heart disease, high cholesterol, high blood pressure and constipation. Jackfruit seeds also provide high starch content.

Jackfruit seeds are good sources of thiamine and riboflavin. Both of these B vitamins which gives energy. Riboflavin also acts as an antioxidant, helping prevent damage to your cells from free radicals. These seeds also provide at least small amounts of the minerals zinc, iron, calcium, copper, potassium and manganese. The dried and boiled jackfruit seeds are very good medium for the action of various microbes and resulted in the production of various metabolic products. The enzyme amylase is one of the major secondary metabolite produced by the *Aspergilles niger*.

MATERIALS AND METHODS

Sample Collection/Substrate Collection and Sterilization

Substrates were collected from different places in Kottayam district of Kerala state, India. Coffee husk is collected from the mill after the coffee bean is processed. The husks were collected in poly ethylene zipper bags, later washed two times with distilled water. The jackfruit seeds were collected from a local farm and were are sliced into smaller pieces and transported to the laboratory. Tapioca tubers were collected locally from a planatation and washed thoroughly with distilled water to remove the adhering dirt and soil particles. They were later finely chopped into pieces using surgical blades. All the samples are then oven dried (KOA4, KEMI lab equipments, Ernakulam, India) at 60°C for 24 h. The dried samples were powdered using a waring blender (Magic V2, Preethi Kitchen Appliances Pvt Ltd, Chennai, India) and stored in air-tight polyethylene bottles until further analysis.

Isolation of Aspergillus Niger

A piece of bread was kept in a moist condition at room temperature in dark for 2 days. The bread sample was serially diluted and different dilutions were inoculated on potato dextrose agar (PDA) medium. The slants were incubated at 30°C for 4 days. Fungal cultures were observed on PDA medium. The fungal strain was subjected to lactophenol cotton blue staining for studying the morphology. The fungal culture was confirmed as *Aspergillus niger* by studying the morphology and the spore colour.

Lacto Phenol Cotton Blue Staining

Place a drop of Lacto phenol Cotton Blue Solution on a slide. Using an inoculating needle carefully spread the fungal culture into a thin preparation. Place a cover slip edge on the drop and slowly lower it. Observe under low to high power objectives of microscope. Lactic acid acts as a preservative for fungi. The phenol portion kills the fungi. The cotton blue stains the fungal elements. Fungal elements are stained a deep blue; background is pale blue (Aneja, 2003).

Enzyme Production by Solid State Fermentation

The *Aspergillus niger* was subjected to solid state fermentation in different substrates like coffee husk (S1, S2, S3), jackfruit seed (S4, S5, S6), and tapioca (S7, S8, S9) which was used as solid substrates for SSF.

Each substrate was taken in about half inch thickness in all the fermentation trays and hydrated with 25ml of basal salt solution and adjusted with moisture content (43-81%). 1% of inoculums was inoculated after sterilization and incubated at room temperature for six days.

Enzyme Extraction

25 ml of 0.1M phosphate buffer saline (pH 7) was added to each of the inoculated substrate beds and was vigorously shaken in rotary shaker for 15 minutes at 120 rpm. The mixture was filtered through cheese cloth and centrifuged at 8000 rpm at 4°C for 15 min. The supernatant was filtered through cheese cloth and the filtrate was used as the crude enzyme preparation. Enzyme amylase was assayed by Dinitrosalicyclic acid method.

Determination of Amylase Activity

The *Aspergillus niger* isolate was tested for amylase production by starch hydrolysis. When starch agar medium was inoculated with the organism and subsequently flooded with iodine solution, the zone of clearance around the microbial growth indicated the production of amylase and the fungal isolate was taken for amylase production.

Determination of Amylase Activity

Enzyme assay was carried out by DNS method of (Miller, 1959) in which 0.5ml enzyme was reacted with substrate (1% starch in 100mM Tris buffer) under standard reaction conditions and the reaction was stopped by adding DNS reagent, amount of maltose released was determined by comparing the absorbance reading of the test enzyme at 540 nm with the standard graph plotted by reacting the known concentration of maltose ranging from 0.05mg/ml to 0.5mg/ml. One unit amylase activity was defined as amount of enzyme that releases 1 micromoles of maltose per minute under standard reaction conditions.

The culture supernatant was collected separately. 15 test tubes were taken and marked S1-S9, pure blank (PB), substrate blank (SB) and enzyme blank (EB). With the help of a micropipette, 2ml of phosphate buffer was transferred to all the tubes.

1ml of starch was added to all tubes except PB & SB. 1% Sodium Chloride was added to all the test tubes. 1ml of distilled water was added to PB & SB. The contents of the test tubes were mixed well and then incubated for 5mins at 37°C. After incubation crude enzyme was added to all the test tubes except PB & EB, and distilled water is added to PB & EB.

The contents of the test tubes were mixed well and incubated for 10mins at 37°C. After incubation 1ml of 2N NaOH were added to all the test tubes. The reducing sugars liberated were assayed calorimetrically by the addition of 1ml Dinitrosalicyclic acid (DNS) reagent. The contents of the test tubes were mixed well and incubated in boiling water bath for 10 mins. Intensity of the colour developed was read at 520 nm using a spectrophotometer.

A standard graph was plotted and the enzyme activity was calculated. One unit of enzyme activity was defined as the amount of enzyme required to liberate 1µmol of sugar per minute under the standard assay conditions and enzyme activity is expressed in terms of micromoles per second on fermented substrates.

Statistical Analysis

The survey results were analyzed and descriptive statistics were done using SPSS 12.0 (SPSS Inc., an IBM Company, Chicago, USA) and graphs were generated using Sigma Plot 7 (Systat Software Inc., Chicago, USA).

Figure 1: Production of Amylase Using Solid State Fermentation Using Various Substrates, Jackfruit Seeds (Top Left); Boiled Jackfruit Seeds (Top Right); Cassava Peels (Middle Left); Boiled Cassava Peels (Middle Right); Coffee Husks (Bottom Left); Boiled Coffee Husks (Bottom Right)

RESULTS AND DISCUSSION

Isolation of Fungi

Four different fungal isolates differentiated on the basis of colony morphology were obtained after spreading. All the four isolates were subcultures by point inoculation and used for further studies.

Screening of Fungal Isolates for Alpha Amylase Production

All the four fungal isolates were subjected to screening procedure and after completion of incubation period plates were flooded with iodine solution and observed for zone of hydrolysis.

Identification of the Isolate Showing Maximum Hydrolysis

Based on morphological studies, and Lactophenol cotton blue staining characteristics the isolate was identified as *Aspergillus niger*.

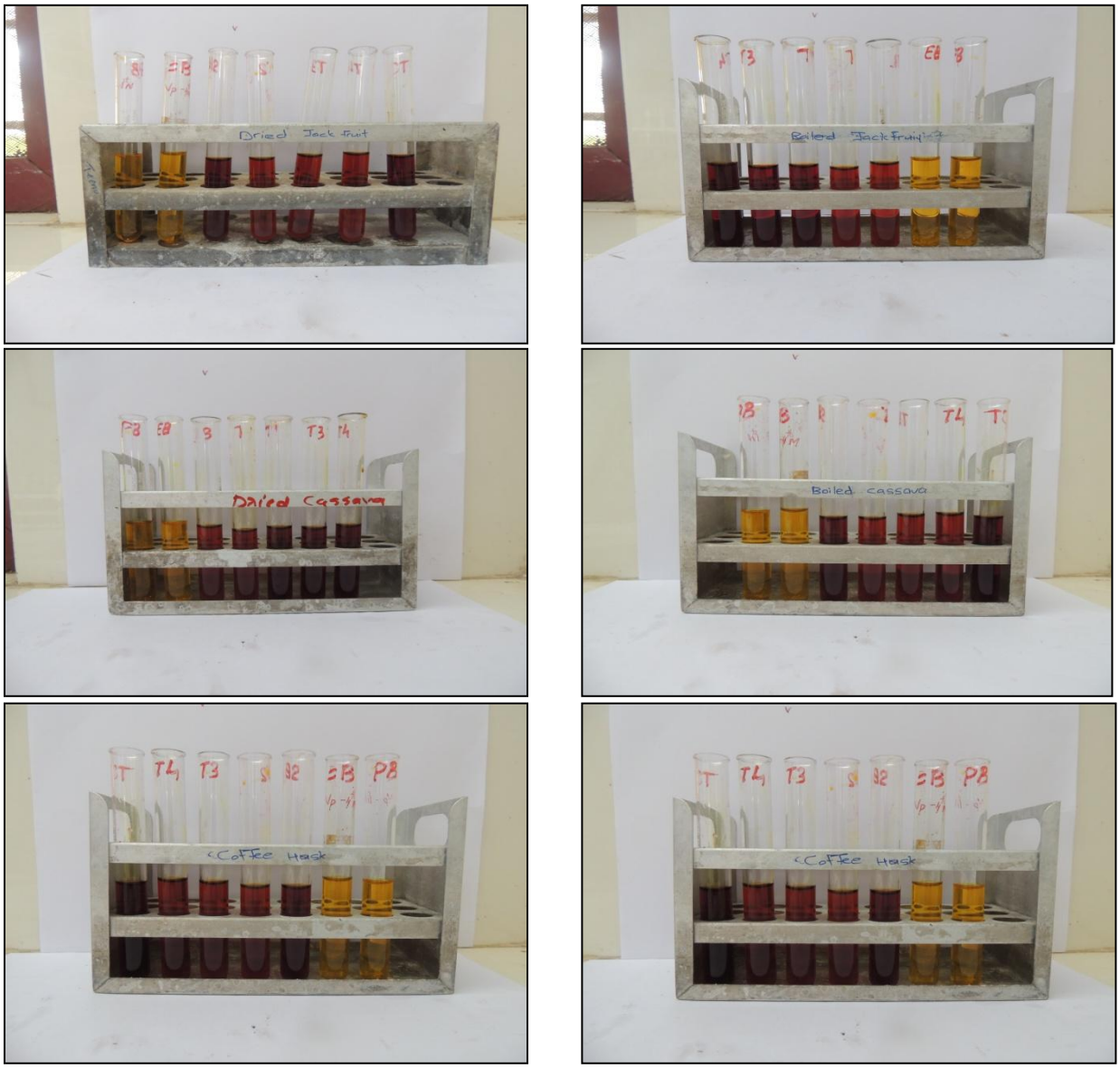

Figure 2: Amylase Produced by Solid State Fermentation Using Various Substrates and their Enzymatic Activity, Jackfruit Seeds (Top Left); Boiled Jackfruit Seeds (Top Right); Cassava Peels (Middle Left); Boiled Cassava Peels (Middle Right); Coffee Husks (Bottom Left); Boiled Coffee Husks (Bottom Right)

Evaluation of Starchy Food as Substrates for SSF

Enzyme activity in the extracted enzymes from different substrates was determined by DNS assay and the results of the same can be seen in table 1-6 and chart 1-6. The histogram in chart 7 shows a comparison between the alpha amylase activities in the boxes containing different substrates used in the present study. All the three were found to be good substrates as the alpha amylase activity was seen in all the five boxes. It can be seen that maximum amylase activity was seen when dried jackfruit seed was used as substrate followed by boiled jackfruit seed, boiled cassava, dried cassava and coffee husk and the enzyme activity was maximum in the box containing dried jackfruit seed as substrate and it was found to be (0.875 x10-3mols/s) followed by boiled jackfruit seed (0.737 x10-3 mols/s), boiled cassava (0.625 x10-3 mols/s), dried cassava (0.512 x10-3mols/s), Boiled coffee husk (0.38 x 10-3 mols/s) and dried coffee husk (0.3

x10-3mols/s). Dried jackfruit seed is the most efficient substrate which produced amylase under the culture condition.

Table 1: Activity of Enzyme Produced by *Aspergillus Niger* from Various Substrates; Dried Jackfruit, Boiled Jackfruit, Dried Cassava, Boiled Cassava, Dried Coffee Husk and Boiled Coffee Husk

Substrate	Trial 1	Trial 2	Trial 3	Trial 4	Average
Dried Jackfruit	$0.75x10^{-3}$	$0.80 x10^{-3}$	$0.95 x10^{-3}$	$1.00 x10^{-3}$	$0.89 x10^{-3}$
Boiled Jackfruit	$0.75 x10^{-3}$	$0.8 x10^{-3}$	$0.6 x10^{-3}$	$0.8 x10^{-3}$	$0.74 x10^{-3}$
Dried Cassava	$0.25 x10^{-3}$	$0.6 x10^{-3}$	$0.6 x10^{-3}$	$0.6 x10^{-3}$	$0.51 x10^{-3}$
Boiled Cassava	$0.55 x10^{-3}$	$0.6 x10^{-3}$	$0.7 x10^{-3}$	$0.65 x10^{-3}$	$0.63 x10^{-3}$
Dried Coffee Husk	$0.35 x10^{-3}$	$0.35 x10^{-3}$	$0.3 x10^{-3}$	$0.2 x10^{-3}$	$0.3 x10^{-3}$
Boiled Coffee Husk	$0.36 x10^{-3}$	$0.37 x10^{-3}$	$0.32 x10^{-3}$	$0.5 x10^{-3}$	$0.38 x10^{-3}$

Conclusion

The results obtained in the present study suggest that the Jack fruit seed may act as a potent substrate for industrial production of α-amylase and subjected for further explorations regarding industrial applications.

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