Shendi University

Production ,Purification and Characterization of Glucoamylase by Some Fungal Isolates

A thesis submitted for the fulfillment of the requirement for the degree of PhD in Microbiology (Applied Microbiology)

By

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DEDICATION

To soul of my Father and Mother

To my small family

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The Tables

Abstract

The Study was performed to Investigate the best Fungus isolate for producing Glucoamylase enzyme .125 soil samples were collected from various sources from six localities, Metema, Shandi, Damer, Atbara, Berber and, Abu hamad. Sources included corn stores, kitchens, agricultural land and desert. Each sample was taken from the soil, and one gram from each soil was dissolved in 10 ml sterile distilled water in test tubes capacity 20 ml. Tubes were incubated at 80 ° C for ten minutes. Equipped barley extract environment then inoculated from the previous pipe. And put the dishes in the Incubator at a temperature of 27 ° C for 48 hours.. Selected 55 colony from different sources and then transferred again to malt extract agar and placed in Incubator at a temperature of 27 ° C for 48 hours .Starch media was prepared and then vaccinated dishes above and incubated in the same grade the previous heat dishes flooded with a solution of iodine to test the biodegradable starch. 25 samples were selected based on the decomposition of starch and be Clean halo around the colony .We are choose 9 samples depending on higher production of the enzyme and then transferred to agar malt extract environment. Equipped with liquid amylase production environment and then vaccinated the previous samples. Edit the source of carbon in the environment above (starch) and other carbon sources, found that the highest production of the enzyme was found when using starch as the carbon source and concentration of 4.0 g / L. Edit the source of nitrogen in the environment above (concrete) other sources of nitrogen. Highest production of the enzyme log when you use malt extract carbon source and that at a concentration of 1.5 g / L. Replace the metal Magnesium (MgSO4) salts other metals, the best production of the enzyme record when using magnesium sulfate concentration of 10 g / L. Written different concentrations of sodium chloride and found that sodium

chloride negative impact on the production of the enzyme where the highest production of the enzyme at the lowest concentration of salt, is 10 g / L. Then change the PH values and the best production of the enzyme at 6.0 PH value when changing the temperature was found that the highest production of the enzyme has been recorded at a temperature of 55 m. The production of the enzyme was measured at different time intervals highest production of the enzyme record after 5 days of incubation.

Nine samples were selected according to the best conditions for the production of an enzyme and then used in the production of the enzyme in accordance with the conditions above. Were measured activity of the enzyme product according to special conditions included: the time period of 10-60 minutes, the highest activity recorded at 30 m temperature between 10-60 higher activity of the enzyme record temperature of 50 m, the values of the PH between 3-8, the highest activity of the enzyme log when the value of 4.0 PH use of different sources of nitrogen activity was highest when using malt extract a concentration of 2.0 g / L and finally carbon sources, where he found that the highest activity of the enzyme may log when we used starch as the action of the enzyme concentration of 4 g / L Chosen as the best samples of the enzyme 5 above, used in advanced trials, which included: purification of the enzyme and comparison with the enzyme product isolated from a sample of corn stores at Gadarif ..

ملخص البحث

تنقية وقياس نشاط إنزيم الجلوكواميليز المنتج

بواسطة بعض المعزولات الفطرية

تهدف هذه الدراسة إلى اختبار أفضل المعزولات الفطرية المنتجة لإنزيم الجلوكواميليز, كما تهدف إلى اختبار انسب الظروف لإنتاج هذا الإنزيم . جمعت 125 عينة تربة من مختلف محليات ولاية نهر النيل حيث شملت محليات : المتمة ' شندي ' الدامر ' عطبرة 'بربر و ابوحمد و قد شملت مصادر التربة : مخازن الذرة 'المطابخ 'التربة الزراعية والتربة الصحراوية . وقد تم إجراء التجارب في الفترة بين مارس 2012 و ابريل 2013

في العزل الأولي للعينات اختيرت أفضل 10 معزولات اعتمادا على النشاط العالي للإنزيم المنتج علي البيئات الصلبة .

عند اختبار إنتاج الإنزيم في البيئات السائلة سجلت كل العينات اعلي إنتاج لها عند استخدام النشا كمصدر للكربون وذلك بتركيز 4.0جرام/ لتر . و قد سجلت العينة *Aspergillus oryza* ABCH أعلى إنتاج حيث بلغ 9.1 ملجرام/لتر . عند استخدام مستخلص الشعير كمصدر للنيتروجين العضوي بتركيز 2.0 جرام/لتر , سجلت كل المعزولات اعلي إنتاج للإنزيم و قد سجلت العينة ADSS *Curvalaria sp* 11.2 ملجرام/لتر كأفضل معزولة . من بين كل المعادن المستخدمة في إنتاج إنزيم الجلوكواميليز كانت كبريتات الماغنيزيوم $Mg\ SO_4$ هي الأفضل وذلك بتركيز 1.0 جرام/لتر حيث سجلت العينة ATD *Aspergillus oryza* 8.9 ملجرام/لتر كأعلى إنتاج . عند درجة الحموضة 6.0 سجلت كل العينات اعلي إنتاج للإنزيم و عنده سجلت العينة SHSS *Aspergillus oryza* 10.8 ملجرام/ كأعلى إنتاج من بين باقي المعزولات . أعلى إنتاج للإنزيم سجل بعد 5 أيام من التحضين حيث سجلت العينة

Aspergillus oryza ATD 14.8 ملجرام/لتر كأعلى إنتاجية وذلك عند درجة حرارة 55°C .

في قياس نشاط الإنزيم المنتج بواسطة المعزولات الفطرية المختلفة استخدمت عدة عوامل شملت :الزمن بالدقائق ، الحموضة ، تركيزات مختلفة من مصدر النيتروجين (مستخلص الشعير) ، تركيزات مختلفة من مصدر الكربون (النشا) و درجة الحرارة . أعلى نشاط للإنزيم سجل عند 50 دقيقة ، درجة حموضة 4.0 ، درجة حرارة 50°C وذلك عند تركيز 2.0 جرام/لتر و 4.0 جرام/لتر من كل من مستخلص الشعير و النشا على التوالي .

عند تنقية الإنزيم بواسطة كبريتات الامونيوم (NH_4SO_4)ارتفع نشاط الإنزيم كثيرا حيث وصل 160.0 ملجرام/لتر للعينة *Aspergillus oryza* ATD و ذلك عند 50 دقيقة ، درجة حموضة 4.0 ، درجة حرارة 50°C و تركيز 2.0 جرام/لتر و 4.0 جرام/لتر من كل من مستخلص الشعير و النشا على التوالي .

عموما كانت المعزولة Aspergillus oryza ATD هي الأفضل في إنتاج إنزيم الجلوكواميليز من بين بقية المعزولات و ذلك تحت ظروف الإنتاج المختلفة .

CHAPTER ONE

Introduction

Amylases are a group of hydrolyses that can specifically cleave the O-glycosidic bonds in starch. Two important groups of amylases are Glucoamylase and α-amylase. Glucoamylase (exo-1,4-α-D-glucan glucanohydrolase, E.C. 3.2.1.3) hydrolyzes single glucose units from the nonreducing ends of amylose and amylopectin in a stepwise manner (Anto *et al.,* 2006). Whereas α- amylases (endo-1,4-α-D-glucan glucohydrolase, E.C. 3.2.1.1) are extracellular enzymes that randomly cleave the 1,4-α-D-glucosidic linkages between adjacent glucose units inside the linear amylose chain (Anto *et al.,* 2006, Castro *et al.,* 2010, Pandey *et al.,* 2005). Alpha-amylases are widely distributed in nature and can be derived from various sources such as plants, animals and microorganisms (Pandey *et al.* , 2005 Reddy *et al.,* 2003 3-4). However, fungal and, bacterial amylases have predominant applications in the industrial sector. Major advantage of using fungi for the production of amylases is the economical bulk production capacity and ease of manipulation. Many species of *Aspergillus* and *Rhizopus* are used as a source of fungal α-amylase(Pandey *et al.,* 2005). Usually amylase production from fungi has been carried out using well defined chemical media by submerged fermentation (SMF) and solid state fermentation (SSF) in recent times (Miranda *et al.,* 1999). The economics of enzyme production using inexpensive raw materials can make an industrial enzyme process competitive (Couto and Sanroman,2006).For the microbial α-amylase production, two types of fermentation methods are mainly used .submerged and solid state (Norouzian *et al.,* 2006).Submerged fermentation (SMF) is comparatively advanced and commercially important enzymes are traditionally produced by this method (Hashemi *et al.,* 2010). Whereas, solid state fermentation (SSF)is an old technology and has been used since 2600BC. However, in recent year SSF has emerged as a well developed biotechnological tool for the production of enzymes (Bhatnagar *et al.,*

2010).Nowadays, spectrum of applications of α-amylase is also extending in many other areas such as analytical chemistry, clinical and medicinal diagnosis e.g. diagnosis of acute inflammation of pancreas, macro amylasemia, perforated pelvic ulcer and mumps (Anto *et al.*, 2006), Muralikrishna *et al.*, 2005).

The aims of study :

1- Screening of Fungal species for production of Glucoamylase .
2- Optimization the production of enzyme
3- Characterization and Purification of the enzyme
4- Production Enzymes for Laboratory using

Chapter two

Literature review

2-1 General Description

Enzymes are substances present in the cells of living organisms in minute amounts and are capable of speeding up chemical reactions (associated with life processes), without themselves being altered after the reaction. They accelerate the velocity of the reaction without necessarily initiating it (Myrbrackk,1960). There are three major sources of enzymes (Burhan *et al*., 2003), that is derived from a variety of plants, e.g. pappain, animal enzymes (derived from animal glands), e.g. trypsin, pepsin .and microbial enzymes (derived from micro organisms)(fungal and bacterial) through the process of fermentation, e.g. amylase and proteinase . Microbial enzymes are preferred to those from both plants and animal sources because they are cheaper to produce, and their enzyme contents are more predictable, controllable and reliable (Burhan *et al*., 2003). Enzymes responsible for degradation of starch and related saccharides are produced either by prokaryotic or eukaryotic organisms. Hydrolyses of starch involves four groups of enzymes: endo- and exo-amylases, debranching enzymes attacking mainly the 1,6 linkages and cyclodextrins glycosyltransferase (Horváthova *et al*., 2000; Aiyer, 2005). α -Amylase (EC3.2.1.1) and glucoamylase (EC 3.2.1.3) are the endo and exo-amylases most studied respectively. These enzymes represent about 25-33% of the world enzyme market, second after proteases. Their main applications are in the production of high glucose syrup (HGS) from starch and in the production of high fructose corn syrup(HFCS) (Nguyen *et al*., 2002). Glucose from starch is used in the production of citric acid, glutamic acid, lactic acid, lysine, glucitol, ethanol and crystalline glucose(Pazur *et al*., 1990). Glucoamylase is an exo-enzyme that liberates D-glucose from the non-reducing chain ends of amylose, amylopectins and glycogen by consecutively hydrolyzing a-1,4; a-1,6 and rare

a-1,3 linkages. Usually these enzymes are produced from several microorganisms, but mainly *Aspergillus niger* and *Rhizopus oryzae* are used in biotechnology applications(Coutinho and Reilly, 1997). Thermostable enzymes from microorganisms have advantages in industrial biotechnological applications (Pandey *et al*., 2000) and Thermostable glucoamylases have been studied in the latter regard (Chen *et al*., 2005).

Amylase are Starch degrading enzyme . t hey are widely distributed in microbe , plant and animals kingdoms . They degrade starch and related polymers to yield product(Prasana.2005). Initially the term amylase was used originally to designate enzymes capable of hydrolyzing α-1,4- glucosidal bonds of amylose , amylopectins, glycogen and their degradation product (Bernfeld .1955; Fisher and Stein'1960;Myback and Neumuller'1950) In recent years a number of new enzymes associated with degradation of starch and related poly saccharides structures have been detected and studied (Boyer and Ingle ,1972; Griffin and Fogarty,1973 . According to Fogarty and Kelly,(1979) there are six classes of this enzyme 'One is enzyme that hydrolyze α-1,4 bonds and bypass α-1,6 linkages e.g. α- amylase that is namely endoacting amylase. One other is enzyme that hydrolyze α-1,4 and cannot bypass 1,6 linkages e.g. β- amylase that it's named exoacting amylase that is producing maltose as a major end product . Other one is enzyme that hydrolyzeα-1,4 and α-1,6 linkages e.g. glucoamylase namely amyloglucosidase. Other one is enzyme that hydrolyze onlyα-1,6 linkages e.g. pullulanase and other de branching enzymes. The fifth one is enzyme that hydrolyze preferentially α-1,4 linkages in short chains oligosaccharides produced by the action of other enzymes on amylose and amylopectins e.g. α- glucosidase. The last one is enzyme that hydrolyze starch to a series of non reducing cycilic D-glucosyl polymers called cyclodextrins or sachardinger dextrin e.g. *Bacillus macerans* amylase (cyclodextrins producing enzyme)

2-1-1 α- Amylases

α- Amylases (E.C.3.2.1.1) are starch degrading enzyme that catalyze the hydrolysis of internal α-1,4-O- glucosidal bonds in Polly saccharides with the reiteration of α- anomeric configuration in the products . Most of α- amylase are metallo enzymes that it is need Calcium ions (Ca^{+2}) for their activity (Bordbar *et al*,2005) . It is one of the most important industrial enzymes that have a wide variety of applications ranging from conversion of starch to sugar syrups ,to production of cyclodextrins for the pharmaceutical industry. These enzymes account for about 30% of world's enzymes produced by plant, animals and microbes. Where they play a dominant role in carbohydrates metabolism. Amylase from plants and microbial sources have been employed for centuries as food additive. Barley amylase have been used in the brewing industry. Fungal amylase have been widely used for the preparation of oriental foods(Burhan *et al*, 2003)

2-1-2 β- Amylases

Another form of amylase, β-amylase (EC 3.2.1.2, 1,4-_-D-glucan maltohydrolase) is also synthesized by bacteria, fungi and plants. Working from the non-reducing end, _-amylase catalyzes the hydrolysis of the second _-1,4 glycosidic bond, cleaving off two glucose units (maltose) at a time (Yashiyuki,1985). During the ripening of fruit, β-amylase breaks starch into sugar, resulting in the sweet flavor of ripe fruit. Both are present in seeds, β-amylase is present prior to germination whereas β-amylase and proteases appear once germination has begun. Cereal grain amylase is key to the production of malt. Many microbes also produce amylase to degrade extracellular starches. Animal tissues do not contain β-amylase, although it may be present in microorganisms contained within the digestive tract (Maton *et al.*, 1993).

2-1-3 Glucoamylase

Glucoamylase (GA) is a hydrolyzing enzyme. It can degrade both amylose and amylopectins by hydrolyzing both α-1,4 and α-1,6 glucosidic links of starch and produce glucose (Ono *et al*.,1964; Elegado and Fujio, 1993; Sasi *et al* ,2014). Hence glucoamylase can convert starch completely to glucose. Now a days, glucoamylase is one of the most important enzymes in food industries (Cook, 1982;; Soccol *et al*., 1992; Soccol, 1992), as it is used for the production of glucose and fructose syrup from liquefied starch(Dale and Langlois, 1940; Nigam and Singh, 1995; Nguyen *et al*., 2002). It is also employed in baking, juice, beverage pharmaceuticals, and many fermented foodstuffs industries for commercial production (Heseltine, 1965; Raimbault, 1981), in some cases textile, leather and detergents industries (Whister *et al*., 1984; Reed and Rhim, 1987). Due to its increasing demand, the production technique of glucoamylase and α amylase has been studied in detail. The enzyme was reported to produce by many fungi like *Aspergillus awamori*, *A. saitoi, A. oryzae*, *Rhizopus* sp, *Mucor sp*, *Penicillium sp*., and Yeast(Tsujisaka *et al*., 1958; and Chakarabarty, 1984). Among these, *Rhizopus* spp. Are considered good producers of amylolytic enzyme (Takahashi, *et al* , 1994;Jin *et al*., 1999).

2-2 Sources of Amylase :

Amylase s are widely distributed among animals, plants, and microorganism. α –Amylase occur in microorganism, plants and animals tissues whereas β – amylase occur in higher plants and microorganisms (Karlson, 1974) . Glucoamylase occur mainly in microorganism and some animals tissues (Wiseman , 1985) .

2-2-1 Animals:

α– Amylase of human saliva , human pancreas have been obtained in crystalline form (Meyer and Gibbos, 1951; Myrback and Newmiiler , 1951,Yashiyuki *et al*,1988 and Buzzini and Martini , 2002). α – Amylase of saliva initiates the hydrolytic attack on the dietary polysaccharides , producing malt oligosaccharides , while α– Amylase of pancreas is secreted as juice into the

small intestine and complex the digestion process yielding maltose which is hydrolysed by α –glycosidase to glucose (Laner and Nicke,1952) . Glucoamylase hydrolysis longer oligosaccharide and complex the degradation in the intestinal tract producing glucose . This enzyme also occur in muscle extract (Cori *et al* , 2000 ,Horri,1969,Mehrabadi and Bandani, 2009) . and at Insect(Campos and Fell,1995).

2-2-2 Plants:

Studies on the occurrence , properties and purification of amylase from higher plants are relatively few . Novellie (1982) reported that *Sorghum sp* . has ability to produce amylase on germination . α– Amylase of sorghum malt has been purified and characterized (Botes *et al* , 1976; Mundy, 1982 and Okon and Uwaifo, 1984). The un germinated barley grain contains α– Amylase whereas un germinated sorghum grain lacks it(Botes et al , 1976) . Amylases of barely and wheat are found in the aleurone layer of the grain , while those of sorghum are formed in the germ and spread outward through the endosperm (Daiber and Novellie, 1968, Stefan,2009) . The α– Amylase of sorghum malt is acid resistant and active over wide range of pH 4-7 (Botes *et al*, 1967); (Budair,1977) and (Ahmed,1988) studied amylase of Sudanese sorghum malt and showed that malt of (feterita) gave the highest amylolytic activity when compared to malt of other sorghum types . The richest source of β – amylase , apart from sweet potato , are the cereal, especially wheat and barely . (Hariss ,1968, Hanes,1932) reported that β – amylase together with other endogenous enzymes of barely malt , catalyzed the conversion of starch to maltose which constitutes 45% of the total carbohydrate content . The β – amylase of wheat and barely have been isolated and characterized by several workers (Kshipra *et al*, 2011) . At that time it was commonly accepted that sorghum malt contained little or no β – amylase activity . However contrary to this view , Novellie (1960) reported the isolation of β – amylase from kaffircorn (*Sorghum cafferorum*). Sorghum β – amylase have been isolated , purified and

characterized by several worker (Botes *et al*, 1967; Mundy, 1982 and Okon and Uwaifa, 1984) . Sweet potato and Soya been β – amylase have been isolated and optained in crystalline form by Ball *et al* (1948) and Fukunoto and Tsujsaka (1956) . Afiukwa *et al*,(2009) isolate β – amylase from mango .At same time Joana *et al* 2009 isolate β – amylase from *Zae maze* and. also El-Safey and Ammer, 2002 .

2-2-3 Bacteria:

Many bacteria are known to produce extracellular amylase . α- amylase from *Bacillus sp* have been found a considerable commercial application for several decades (Afiukwa *et al*,(2009) . Production of amylase by *bacillus polymexa* have been reported by Robyt and French (1964) *Bacillus lichenformis* is a known as the best producer of the rmostable α- amylase (Saitio, 1973, Gupta *et al* ; 2007,Vidyalakshmi *et al* ,2009, Sosmita and Mishra , 2008). Three the rmostable and acidophilic α- amylase of *Bacillus acidocaldarius* have been reported by Ingle and Boyer, (1976)and Uchino, (1981). Another the rmostable α- amylase from *bacillus acidocaldarius* strain was reported by Kanno, (1986). Yet another heat – stable α- amylase was isolated from *bacillus subtilis* by Yamane *et al* , (2002) and Uyar *et al* (2003) . In Sudan Dawood (1997) isolated α- amylase from several bacterial strain .Glucoamylase were produced by many species of bacillus (Srivastava and Baruah, 1963,Oyelek *et al*, 2010),(Hori, 1969) characterized and purified an amylase that produces glucose from *Lactobacillus brevis* . and also (Sekar *et al*, 2007,Cardeiro *et al* , 2002). Aiyer,(2004) and Tanaka and Hoskhin (2002) study the effect of nutrition on bacterial amylase .

2-2-4 Fungi :

Different types of amylase found in fungi were reviewed by some worker so that MacAllister,(1979); Reichelt,(1983) and Gupta and Gautan,(1995). α-Amylase have been isolated from *Aspergillus foestidus, Aspergillus oryza* and *Aspergillus niger* (Matsuura *et al*,1984, Imran *et al*, 2011, Imran *et al*, 2012,

Shah et al 2014, Abeer *et al* , 2014 , Ritesh and Barkha2011, Pederson and Nielson,200 and Boel *et al*, 1984) .Amylolytic activity of different Rhizopus isolates has been determined by several worker (Lim *et al*, 1987; Hang,1980 and Roch- Chuim and Hang, 1990) . Fungal β- amylases were isolated from *Aspergillus niger* and *Aspergillus oryzae* (Svensson *et al*, 1988) . Glucoamylase have also been isolated and purified from *Aspergillus niger* (Lineback *et al*, (1972), *Penicillium oxalicum* (Yamasaki *et al*, 1977,), *Penicillium fellutanum* (Katheresan and Manivannes,2006), *Penicillium citrinum* (Metin *et al* , 2010) ,*Aspergillus oryza* (Mitsue *et al*, 1979)and (Kita *et al*, 1982), and *Aspergillus awamori* (Bhumibhamon,1983) , Two form or isoenzyme of glucoamylase have been isolated and purified from *Aspergillus niger* (Flemingi and Stone, 1965; Pazur *et al*, 1971 and Lineback *et al* 1972). and three isoenzymes of glucoamylase have been isolated and separated from *Aspergillus oryza* (Mitsue *et al*, 1979) and from *Rhizopus sp* (Takahashi *et al*, 1978 and Kanlayakrit *et al*, 1987) . Also glucoamylase of yeast and *Sacharomyces sp* have been reported by Steverson *et al* (1984); Pestana and Casstillo, (1985) and Tani *et al*, (1986).

2-3 Applications of amylase :

Glucoamylase enzyme having many applications in food processing industry, fermentation biotechnology, paper making and fabric industries(Prassanna, 2005), microbial origin of starch hydrolyzing enzymes in the solid cultures of glucoamylase, uncover many applications in all types of industries(Meyer and Gibbons, 1951). This enzyme is used in dextrose production, in the baking industry, in the brewing of low-calorie beer and in whole grain hydrolysis for the alcohol industry(Selvakumar *et al*., 1994).And also in production of Glucose syrup (Akinola and Ayanle ,2004; Marc *et al* , 2002) .

Chapter Three

3- Materials and Methods

3-1 Materials

3-1-1Chemicals :

The chemicals used in this study such as sodium potassium tartarate, 3,5-Dinitro salicylic acid, phenol, sodium meta bisulphate, di hydrogen phosphate, manganese sulphate, yeast extract, ferrous sulphate, magnesium chloride, di ammonium sulphate, starch, ferrous sulphate, acryl amide, bi sacryleamide, trizmabase, Tris HCl, bromo phenol blue, β- mercaptoethanol, ammonium per sulphate, , etc were of analytical grade and obtained from Sigma(USA), BDH (UK), E-Merck (Germany), Acros (Belgium) and oxoid (U.K). All other chemicals were of the highest possible purity.

3-1-2 Selection of Isolates :

90 samples were collected from different locations at Nile River state . These samples were inoculated on malt extract agar media (MEA). The samples were chooses according to halo clear zone in the plates when we are adding the iodine . The clear zone mean that the starch had decomposed by enzyme which produced by the isolate . The activity of enzyme was measuring accepted to this equations:

Enzyme activity =

$$\frac{\text{The diameter of the clear zone – The diameter of culture}}{\text{The diameter of culture zone}}$$

according to(Hakin and Ang ,1975).

3-2 Screening of microorganism

Primary screening was done by starch agar plate method using Iodine reaction. The isolates were chosen by the maximum hydrolysis halo on medium, upon this result 10 isolates were chosen

3-3 Glucoamylase production

The culture medium used for Glucoamylase production contained (g/l): $NaNO_3$ 3.0, KCl 0.5, $MgSO_4.7H_2O$ 0.5, $FeSO_4.7H_2O$ 0.01, $KH_2PO_4$1.0, peptone 10 .; soluble starch 20. The pH was adjusted to5.4.The medium was sterilized by autoclaving at 121°C for 15 min. Approximately one full lopes from 5-day-old cultures were inoculated into 250 ml Erlenmeyer flasks containing 200 ml of the culture medium. The incubation was carried out at27°C for 5 days in a rotary shaker rotating (STUART - ENGLAND) at 200 rpm. The culture was filtered through a Whatman filter paper No. 1. and centerfugated and kept at -4℃ . To optimized the enzyme we used some factors .

3-3-1 Effect of Carbon Sources

Effect of substrate concentration on enzyme Production was measured at different concentrations of starch in the reaction mixture (0.1,0.2, 0.3, 0.4, 0.5and 0.6 %).Different Carbon Sources were used (Glucose, Fructose, Maltose and Sucrose.)

3-3-2 Effect of Nitrogen sources

The effect of nitrogen source on enzyme production was studied by replacing the nitrogen source in basal salt solution by Peptone ,Tryptone , Malt Extract and Meat Extract .

3-3-3 Effect of Minerals Ions

Various metal ions were added to the standard assay mixture and production of the enzyme were measured at different ions concentration.

3-3-4 Effect of NaCl Concentration

The Isolates were incubated in phosphate buffer (pH 6.0) containing various NaCl concentrations (1 to 6 M) for 24 h at 37C°.

3-3-5 Effect of pH:

To determine the optimum pH, Glucoamylase production was measured at different pH values3,4,5, 6, 7and 8.

3-3-6 Effect Time:

The effect of incubation time on enzyme optimization was examined at different incubation periods 2, 3, 4, 5, 6 and 7days .

3-3-7 Effect of Temperature:

To determine the optimum temperature, Glucoamylase production was measured at different temperatures 35, 40, 45, 50, 55 and 60ºC.

3-4 Enzyme assay:

Glucoamylase was assayed according to the method of (Youssef and Baraka, 2009),(Miller,1959) and (Lwrry,1951).One milliliter of enzyme extract was added to a test tube containing1.0ml of 1.0 % soluble starch solution, pH 7.0. The mixture was incubated at 30ºC for 10mins. Then 1.0 ml of DNS reagent was added to each tube . The tubes placed in boiling water for 5 min and cooled in cold water. The optical density (OD) the reaction mixture was determined at 540 nm using spectrophotometer.

3-5 Preparation of 3,5- Dinitrosalicylic acid (DNS) :

3,5-Dinitrosalicylic acid (DNS) used in this Experiments were prepared by dissolving 10g DNS, 182g Sodium Potassium Tartarate 3g Phenol, 0.5g Na_2SO_4, and 10g NaOH in liter Dis Water . according to(Miller,1959).

3-6 Assay of protein concentration:

The protein concentration was determined by the Lowry's method (Youssef *et al* ;2009) using bovine serum albumin as standard.

3-7 Characterization of Glucoamylase:

3-7-1 Effect of temperature on the activity of enzyme:

The enzyme activity was measured by incubating the partially purified enzyme preparation at various temperatures (35-65°C) with starch as substrate prepared in 20mM sodium phosphate buffer at pH 6.0 (Behal et al., 2006).

3-7-2 Effect of pH on activity of the enzyme:

The activity was measured at different pH values from 3-11 with starch as the substrate. The different buffers used included: 20mM acetate buffer (pH 3.0-5.0); 20mM sodium phosphate buffer (pH 6.0-8.0) and 20mM Tris/HCl (pH 9.0-11.0) a cording to (Reyed, 2007).

3-8 Enzyme purification:

Amylase produced was partially purified by precipitation with ammonium sulphate and followed by dialysis. Ammonium sulphate precipitation technique was performed by mixing culture filtrate and ammonium sulphate (75%, w/v) solution at 1:3 ratio (Nagim *at al*,2011). The mixture then stored in cold room for 24 h to precipitate all proteins present in the sample. Precipitate was removed by centrifuging sample in an ultra centrifuge at 10000 rpm for 10 min. The supernatant will discarded and precipitate obtained was dissolved in 5 ml of 1 M-citrate phosphate buffer (pH.5)(Nagim *et al*,2011) .Then the mixture was subjected to dialysis.

3-9 Paper chromatography:

Determination of Hydrolytic products on different time scales were assayed by paper chromatography. The reaction mixture contained 2% starch solution instead of the 1% used in the standard method. Hydrolytic reaction after commencement was stopped by boiling the mixture for 5 min at the intervals of 5, 15, 30, 60, and 120 min. The hydrolyzed solution was spotted on the sheet of Whatman 1 mm chromatographic paper. The chromatographic solution contained a 4:1:5 n-butanol: acetic acid: water mixture. The chromatograph was stained with silver nitrate (Robyt and French, 1964) and dried after 5 min. Sugar areas were developed in alkaline methanol.

3-10 Characterization and Identification of Isolates:

Fungal isolates was characterized and identified morphologically as described by (Ellis 1971).

3-11 Statistical analysis:

All experiments carried out in triplicates, and reaped three times. The samples collected from each replicate was tested for amylase production and activity. Means of amylase activity and production were calculated and significant differences was calculated by determining the standard error and standard deviation .

CHAPTER FOUR

4-RESULTS

4-1 Primary screening :

The samples give different results between 0.5-3.2 Mg/L . Some samples have no any results . Then we had choosing 9 samples according to the high activity . the 10^{th} sample (GDSS) choosing from Gadaref sorghum stores and we had choose it to comparison . (Table1) and (Table2) . The Chosen Isolate were Indented morphologically a cording to (Ellis .1971). And the summaries of characterization of its where description On Table(3) .

4-2 Production of Enzyme :

4-2-1 Effect of Carbon Sources on Enzyme Production :

The best production of enzyme was recorded when we used the starch as source of carbon at concentration 4.0 gm/l and the best product is 9.1Mg/l at the sample *Aspergillus niger* ABCH ,At this concentration of starch . At the same concentration the local samples *Aspergillus oryza* ATD and *Fusarium sp* ATSS accorded lower production (only 6.2 Mg/l). The sample *Aspergillus niger* GDSS record 4.2 Mg/L at this concentration of starch. The lower production of glucoamylase recorded when we used the starch as source of carbon is 1.8 Mg/L at the local sample *Aspergillus oryza* ATD and 1.3 Mg/L at the sample *Aspergillus niger* GDSS at concentration 1.0 G/L . The sample *Aspergillus niger* ABCH that it have record the higher product at 4.0 G/L, in this concentration recorded only 1.9 mg/L . At the higher concentrations of starch the production of enzyme began to be lower between 35-45% (Table 4).

Table (1) Sources and Activity of Isolates

NO	SYMBOL	SOURCE	ACTIVITY
1	MTCH	MATAMMA-CHETCHEN	2.4
2	MTDS	MATAMMA- DESERT	1.8
3	MTSS	MATAMMA SORGUM STORES	2.4
4	MTOS	MATAMMA-OUT OF STORES	2.9
5	MTFR	MATAMMA-FIELDS	2.8
6	MTBR	MATAMM-BAGROSI	2.9
7	SHCH	SHENDI-CHETCHEN	2.2
8	SHSS	SHENDI-SORGUM STORE	2.7
9	SHMH	SHENDI- MARKET	2.6
10	SHDH	SHENDI- DESERT	2.1
11	SHFA	SHENDI- FIELD	2,5
12	SHOS	SHENDI-OUT OF STORES	1.9
13	ADSS	ADDAMER-SORGUM STORE	3.1
14	ADCH	ADDAMER- CHTHCIN	2.8
15	ADFO	ADDAMER- FIELDS	2.9
16	ADBS	ADDAMER- AGRI BANK	2,3
17	ADFH	ADDAMER-FIELDS	1.7
18	ADOS	ADDAMER-OUT OF STORES	1.5
19	ATD	ATBARA KETCHEN	2.9
20	ATOS	ATBARA OUT OF STORES	1.3
21	ATSS	ATBARA-SORGUM STORES	3.4
22	ATBR	ATBARA-ATBARA RIVER	1.3
23	ATF	ATBARA-SCHOOL	2.1
24	ATMS	ATBARA-MARKET	1.2
25	BRCH	BARBAR-CHETCHEN	2.1
26	BROS	BARBAR-OUT OF STORES	1.3
27	BRSS	BARBAR-SORGUM STORES	2.6
28	BRFB	BARBAR-FIELD	2.2
29	BROC	BARBAR-OUT OF CHETCHEN	1.4
30	BRFB	BARBAR-FIELDS	1.3
31	ABSS	ABUHAMAD-SORGUM STORES	2.8
32	ABSG	ABUHAMAD-GOLD MARKET	1.3
33	ABCH	ABUHAMAD-CHETCHEN	2.9
34	ABFS	ABUHAMAD-FIELD	2.8
35	ABNS	ABUHAMAD-NILE SOIL	1.5
36	ABDS	ABUHAMAD-DESERT	2.5

37	ABOR	ABUHAMAD-OUT RAIL WAY STATION	1.2
38	ABMS	ABUHAMAD-SCHOOL	1.1
39	MAFR	MATAMMA – FRAHSEEN	1.1
40	MABD	MATAMMA-BAWALEED	0.9
41	MATT	MATAMMA-TABGA	1,0
42	MAST	MATANNA-STADIUM	1.2
43	MAFG	MATAMMA-FROM(GABAL)	0.8
44	MABS	MATAMMA-(BUSTAN)	1.8
45	SHMD	SHENDI-MARKET	0.0
46	SHKS	SHENDI-KABUSHIA	2.4
47	SHOM	SHENDI-OMALI	1.3
48	SHRN	SHENDI-RIVER NILE	2.0
49	SHTR	SHENDI-TRAGMA	2.1
50	SHFS	SHENDI-FIELD SOIL	2.3
51	ADZY	ADDAMER-ZAYDAB	1.7
52	ADNS	ADDAMER-NILE SOIL	2.1
53	ADSD	ADDAMER-SAGADI	0.7
54	ADMN	ADDAMER-MARKET	1.6
55	ADAK	ADDAMER-AKAD	0.7
56	ADMF	ADDAMER-MARKET	2.2
57	ATBR	ATBARA-BRED OVEN	2.4
58	ATSS	ATBARA-SORGUM STORE	3.2
59	ATCH	ATBARA-CHETCHEN	2.7
60	ATOS	ATBARA-OUT OF STORES	2.9
61	ATRS	ATBARA-RIVER SOIL	1.8
62	ATOB	ATBARA-OUT BANK	0.9
63	ATBS	ATBARA-BUS STATION	0.7
64	BRDR	BARBAR-DARMALI	1.1
65	BRSS	BARBAR-SORGUM STORE	2.6
66	BROT	BARBAR-HOUSE	1.8
67	BREB	BARBAR-ENAEBIS	0.5
68	BRSA	BARBAR-SAADABIA	1.5
69	BRNG	BARBAR-NADI	1.3
70	ABBS	ABUHAMAD-BUS STATION	0.3
71	ABMS	ABUHAMAD-MARKET	2.5
72	ABDS	ABUHAMAD-DESERT-1	1.3
73	ABDR	ABUHAMAD-DESERT-2	1.1
74	ABMS	ABUHAMAD-MUGRAT	2.3
75	ABRN	ABUHAMAD-RIVER SOIL	2.0
76	ABFB	ABUHAMAD-FOOT BALL.P.G	1.3

77	GDCH	GADARIF-CHETCHEN	2.4
78	GDSS	GDARIF SORGUM STORES	3.3
79	GDRS	GADARIF-SCHOOL	1.2
80	GDMS	GADARIF MARKET	2.1
81	GDHO	GADARIF-HOME	2.5
82	GDST	GADARIF-STATION	1.8
83	GDCA	GADARIF CAMP	1.5
84	GDFS	GADARIF FIELDS	2.1
85	GDDR	GADARIF DESERT	1.2
86	BIOLS	BIOLOGY LAB-1	2.1
87	BIOLT	BIOLOGY LAB-2	2.3
88	FEDSS	FACULTY OF EDUCATION 1	1.2
89	FEDUT	FACULTY OF EDUCATION 2	0.8
90	FEDUT	FACULTY OF EDUCATION 3	0.8

Table (2) The Isolates Samples

Sample	Source	Fungi	activity
ABCH	Abu hamad-kitchen	*Aspergillus niger*	2.9
ABSS	Abu hamad- sorghum store	*Aspergillus oryza*	2.8
BRSS	Barbar- sorghum store	*Penicillium notattum*	2.6
SHSS	Shandi- sorghum store	*Aspergillus oryza*	2.7
ATF	Atbara - school	*Rhizopus stolineffer*	2.1
MASS	Matamma- sorghum store	*Penicillium notattum*	2.4
ATD	Atbara -kitchen	*Aspergillus oryza*	2.9
ADSS	Addamar - sorghum store	*Curvalaria sp*	3.1
ATSS	Atbara - sorghum store	*Fusarium sp*	3.4
GDSS	Gadaref - sorghum store	*Aspergillus niger*	3.3

(Table 3) Identification of Choosing Isolate (on PDA medium)

Isolate	Conidia (µm)	Vesicle (µm)	Mycelium (mm)	Colony (mm)	Colony color	fungi
ABCH	3,5	75.0	2.5	7.7	Black	*Aspergillus niger*
ABSS	3.0	60.5	3.5	5.7	Yellow	*Aspergillus oryza*
BRSS	4.0	40.5	5.2	6.5	Green	*P. notattum*
SHSS	3.0	60.5	3.5	5.7	Yellow	*Aspergillus oryza*
ATF	4.0	0.0	7.7	20.0	White	*Rhizopus stolineffer*
MASS	4.0	40.5	5.2	6.5	Green	*P. notattum*
ATD	3.0	60.5	3.5	5.7	Yellow	*Aspergillus oryza*
ADSS	25.5	0.0	4.5	7.5	White	*Curvalaria sp*
ATSS	40.0	0.0	6.5	15.5	White	*Fusarium sp*
GDSS	3,5	75.0	2.5	7.7	Black	*Aspergillus niger*

Table(4) Effect of Starch Concentration

Sample	Concentration of Starch (g/L)					
	0.1	0.2	0.3	0.4	0.5	0.6
ABCH	1.9	2.4	6.7	9.1	8.2	7.4
ABSS	3.1	3.6	5.4	8.6	6.2	5.4
BRSS	3.8	4.2	5.0	8.8	6.1	5.7
SHSS	2.7	3.6	6.1	8.1	5.4	6.1
ATF	2.1	3.6	4.8	7.6	6.2	5.3
MASS	4.2	5.6	5.8	8.4	7.0	6.1
ATD	1.8	2.4	3.7	6.2	5.4	5.0
ADSS	3.6	4.6	6.2	8.6	6.4	5.7
ATSS	2.4	3.2	5.4	6.2	4.2	4.3
GDSS	1.3	2.7	2.9	4.2	3.5	3.0

Statistics

	ABCH	ABSS	BRSS	SHSS	ATF	MASS	TTD	ADSS	ATSS	GTSS
Std. Error of Mean	1.24706	.80433	.73166	.79106	.78895	.57759	.71294	.69845	.56711	.39384
Std. Deviation	3.05467	1.97019	1.79221	1.93770	1.93253	1.41480	1.74633	1.71085	1.38912	.96471

When we use the simple sugar so as Glucose is a course of carbon in glucoamylase production media we found that the enzyme was a lower . And we found that the higher enzyme had recorded at 4.0gm/l concentration and it is 7.6mg/l in the sample *Aspergillus oryza* ATD and the lower enzyme at the same sample is 6.0mg/lat concentration 6.0gm/l . The lower product of Glucoamylase when we had used the glucose as source of carbon is 4.2 mg/l at the sample *Aspergillus oryza* ABSS and 4.1mg/l at the sample *Aspergillus niger* GDSS and that at 1.0gm/l concentration of Glucose at same sample . In sample *Aspergillus niger* ABCH which it recognize the higher product when it had growing at starch as source of carbon, record 7.1mg/l at 4.ogm/l concentration when we used Glucose as carbon source and that is a higher product equal with a sample *Curvalaria sp* ADSS at same concentration .The sample *Aspergillus niger* ABCH record the lower enzyme 4.3 mg/l at the concentration of Glucose 1.0gm/l and at that same time the sample ADSS record 5.8mg/l at Same concentration (TABLE 5) When we had use the Fructose as source of carbon the production of glucoamylase had been a big lower, comparison at starch and glucose . Sample *Curvalaria sp* ADSS record the higher product 6.6mg/l when we used the fructose at concentration 4.0gm/l and the lower product at this sample is 4.7mg/l at concentration 6.0gm/l. at same concentration of fructose the tow local samples *Aspergillus oryza* ABSS and *Penicillium notattum* BRSS record 5.4mg/l as a lower product at this concentration. And the sample *Aspergillus niger* GDSS 5.1mg/l . The first lower product of enzyme when we used the fructose as source of carbon recorded by the sample SHSS at 6.0gm/l concentration and it's only 2.8mg/l. the same sample record 5.6mg/l at standard concentration (4.0gm/l) and this is a higher product for this sample when we used the fructose as source of carbon. The samples *Aspergillus oryza* ABSS and *Penicillium notattum* BRSS give a semi same results when the sample *Curvalaria sp* ADSS and *Aspergillus*

Table (5) Effect of Glucose Concentration

Sample	Concentration of Glucose(g/L)					
	0.1	0.2	0.3	0.4	0.5	0.6
ABCH	4.3	5.6	6.3	7.1	6.6	5.6
ABSS	4.2	4.8	5.6	7.0	6.1	5.8
BRSS	5.1	5.8	6.3	7.2	6.0	5.2
SHSS	5.8	6.2	6.7	7.5	6.7	5.8
ATF	4.8	5.3	6.0	6.8	6.1	5.7
MASS	5.0	5.1	5.8	6.4	5.7	5.6
ATD	6.2	6.8	7.1	7.6	6.8	6.0
ADSS	5.8	6.0	6.5	7.1	6.1	5.7
ATSS	4.8	5.0	5.7	6.6	6.0	5.3
GDSS	4.1	4.7	5.6	5.8	4.8	4.2

Statistics

	ABCH	ABSS	BRSS	SHSS	ATF	MASS	TTD	ADSS	ATSS	GTSS
Std. Error of Mean	.40118	.40201	.31588	.26677	.28215	.20817	.23910	.21292	.27406	.28713
Std. Deviation	.98268	.98472	.77374	.65345	.69113	.50990	.58566	.52154	.67132	.70333

oryza ATD give same result also. (Table 6) .When we had use Maltose as source of carbon instead of fructose we had found that the fructose is best than Maltose so that the production of enzyme is been a lower, but we find that when we had use the Maltose at concentration 2.0gm/l it can give a best result compression with other sources of carbon so that the best concentration of them is 4.0gm/l .The higher production of glucoamylase when we used the Maltose as source of carbon had recorded by sample ADSS at concentration 2.0gm/l and it' s 7.7 mg/l and at same time the same sample record the lower production at 6.0gm/l and it only 3.5mg/l this means it have hidden at more than 50% . At the best concentration (2.0gm/l) the samples BRSS and *Aspergillus oryza* ABSS and *Aspergillus oryza* ATD had recorded production more than 7.0mg/l when the lower product of enzyme at this concentration is 6.0 mg/l at the local samples *Aspergillus niger* ABCH and *Penicillium notattum* MASS . and 5.9mg/l at the sample *Aspergillus niger* GDSS . The lower production of glucoamylase the Maltose at concentration 2.0gm/l it can give a best result compression with other sources of carbon so that the best concentration of them is 4.0gm/l .The higher production of glucoamylase when we used the Maltose as source of carbon had recorded by sample ADSS at concentration 2.0gm/l and it' s 7.7 mg/l and at same time the same sample record the lower production at 6.0gm/l and it only 3.5mg/l this means it have hidden at more than 50% . At the best concentration (2.0gm/l) the samples BRSS and *Aspergillus oryza* ABSS and *Aspergillus oryza* ATD had recorded production more than 7.0mg/l when the lower product of enzyme at this concentration is 6.0 mg/l at the local samples *Aspergillus niger* ABCH and *Penicillium notattum* MASS . and 5.9mg/l at the sample *Aspergillus niger* GDSS . The lower product

Table (6) Effect of Fructose Concentration

sample	Concentration of Fructose (g/L)					
	0.1	0.2	0.3	0.4	0.5	0.6
ABCH	3.4	3.8	4.2	5.8	4.6	4.0
ABSS	4.0	4.6	5.0	5.4	4.7	4.3
BRSS	4.3	4.6	5.0	5.4	4.7	4.3
SHSS	3.7	3.8		5.6	3.1	2.8
ATF	4.6	5.0	3.6	6.0	5.2	4.1
MASS	3.7	4.2	4.5	5.8	5.2	4.4
ATD	4.7	5.8	5.1	6.2	5.6	4.7
ADSS	4.8	5.3	6.0	6.6	5.6	4.7
ATSS	4.6	5.2	5.8	6.0	5.4	5.0
GDSS	4.9	5.3	6.2	5.1	4.3	3.6

Statistics

	ABCH	ABSS	BRSS	SHSS	ATF	MASS	TTD	ADSS	ATSS	GTSS
Std. Error of Mean	.34157	.20276	.17401	.39889	.26802	.31029	.25133	.29665	.21082	.36240
Std. Deviation	.83666	.49666	.42622	.97707	.65651	.76004	.61563	.72664	.51640	769

of glucoamylase at generality and at all of samples had recorded when we used the Maltose sugar at concentration 6.0gm/l when the first lower of product is 2.8mg/l at the local sample *Penicillium notattum* MASS and 2.4mg/l at sample *Aspergillus niger* GDSS . Although of that using Maltose as carbon source at concentration2.0gm/l may be give much more product ' but that it is not good at all because that the product become too lower when the concentration of sugars be hidden at the production media . (Table 7) . The lower important source of carbon is Sucrose so that when we used it as source of carbon all of samples give a lower product at difference concentrations . although of that the concentration 4.0gm/l is a best compression with other concentrations but the given results are a lower. At this concentration the higher production of enzyme is 3.7mg/l at the sample *Aspergillus niger* ABCH and this is a higher product at all condition . this sample record 2.4 mg/l as a lower product at concentration 1.0gm/l . At the standard concentration (4.0gm/l) the sample *Fusarium sp* ATSS record 2.1 mg/l as a lower product at this standard concentration , so the lower product was given by the sample *Fusarium sp* ATSS at 1.0gm/l of sucrose and the product is only 0.9mg/l . At the same concentration the samples *Penicillium notattum* BRSS and *Penicillium notattum* MASS record same results . When we used Sucrose at 6.0gm/l concentration as a higher concentration the production was hidden 50% for the standard product at the sample *Aspergillus oryza* ATD ,*Rhizopus stolineffer* ATF , *Penicillium notattum* MASS , *Penicillium notattum* BRSS and *Aspergillus oryza* ABSS and 30% at other samples .(Table 8) . Generally we can say that the using of starch in 4.0 gm/l concentration is a best source of carbon for production Glucoamylase enzyme .

Table (7) Effect of Maltose Concentration

Sample	Concentration of Maltose (g/L)					
	0.1	0.	0.3	0.4	0.5	0.6
ABCH	3.3	6.0	5.4	4.8	5.0	3.7
ABSS	4.2	7.4	6.0	5.7	4.8	3.6
BRSS	5.4	7.3	6.1	5.8	5.0	4.8
SHSS	6.0	6.8	5.6	5.0	4.8	3.9
ATF	5.8	6.6	5.6	5.2	4.6	4.1
MASS	4.8	6.0	5.2	4.6	3.2	2.8
ATD	5.4	7.3	5.0	4.6	4.0	3.2
ADSS	6.1	7.7	6.0	5.5	4.0	3.5
ATSS	5.0	6.7	5.2	4.0	4.0	3.7
GDSS	4.9	5.9	4.2	3.8	2.8	2.4

Statistics

	ABCH	ABSS	BRSS	SHSS	ATF	MASS	TTD	ADSS
Std. Error of Mean	.41793	.56001	.37029	.41292	.36370	.49643	.57179	.62432
Std. Deviation	1.02372	1.37174	.90701	1.01143	.89088	1.21600	1.40060	1.52927

Table (8) Effect of Sucrose Concentration

Sample	Concentration of Sucrose (g/L)					
	0.1	0.2	0.3	0.4	0.5	0.6
ABCH	2.4	2.6	2.8	3.7	3.0	2.6
ABSS	3.0	3.1	2.8	3.2	2.4	1.9
BRSS	1.8	1.9	2.4	2.8	2.2	1.6
SHSS	2.7	2.4	2.8	3.2	2.7	2.0
ATF	1.9	2.3	2.0	2.4	2.0	1.7
MASS	1.8	2.2	2.6	3.0	2,4	2,0
ATD	2.1	2.8	3.0	3.6	2.6	2.2
ADSS	1.0	1.7	1.9	2.4	2.0	1.9
ATSS	0.9	1.1	1.8	2.1	1.7	1.4
GDSS	1.6	2.8	3.1	2.9	2.1	1.6

Statistics

	ABCH	ABSS	BRSS	SHSS	ATF	MASS	TTD	ADSS	ATSS	GTSS
Std. Error of Mean	.18930	.20276	.17966	.16465	.10567	.17638	.22571	.18871	.18439	.28107
Std. Deviation	.46368	.49666	.44008	.40332	.25884	.43205	.55287	.46224	.45166	.62849

4-2-2 Effect of Nitrogen source :

We had use a different nitrogen's compounds as source of nitrogen so that Peptone , Tryptone ,Urea ,Malt extract and Meat extract witch it is organic

source of nitrogen . The best production of enzyme has recorded when we used the malt extract at concentration 2.0gm/l . At this concentration the higher product of enzyme is 11.2 mg/l at the sample *Curvalaria sp* ADSS . at the same concentration the sample *Fusarium sp* ATSS record 9.6 as a lower product . The sample *Curvalaria sp* ADSS which record the higher production record 8.5mg/l at concentration 1.0 gm/l and this result mean that the malt extract is good nutrient substrate and we know that the fungi can growth in medially in media contain malt extract . At the lower concentration (1.0gm/l) the higher production of enzyme is 8.5mg/l at the sample *Curvalaria sp* ADSS and 8.4 mg/l at the sample *Fusarium sp* ATSS and 6.4 mg/l at local sample *Aspergillus oryza* ATD . The sample *Aspergillus niger* GDSS record 5.4 mg/l as a lower production. The higher production at same sample is 8.6 mg/l at concentration 2.0 gm/l (Table 9) . When we used the meat extract as source of nitrogen in the production media ' the results is not best than these one given when we use the malt extract . And it is same at these result recorded at Tryptone as source of nitrogen . When we use the malt extract the best results was recorded when we used it in concentration 1.0 gm/l At this concentration the sample *Fusarium sp* ATSS record 7.0 mg/l as a higher production at this concentration and the sample *Aspergillus oryza* ATD record a lower product at same concentration and it is only 4.0 mg/l . At the higher concentration of meat extract (3.0 gm/l) the production of enzyme had hidden about 50% at all of samples . At the lower concentration of meat extract (0.5 gm/l) the sample *Aspergillus oryza* ATD record the lower production is only 2.7 mg/l . and the sample *Fusarium sp* ATSS record 6.4 mg/l as a higher production at this concentration . The sample *Aspergillus niger* GDSS record 6.5 mg/l as a higher production at concentration 1.0 gm/l and 2.8 mg/l as a lower production at 3.0 gm/l concentration of meat extract (Table 10) . When we used Tryptone as source of nitrogen ' the best result was recorded generally at 1.5 concentration and the lower results at 3.o gm/l concentration of Tryptone . At 1.5 gm/l concentration the sample ATSS

record 5.7 mg/l as a higher production at local samples The sample *Aspergillus niger* GDSS record 6.4 mg/l as a higher production at this concentration of Tryptone . At same concentration the samples *Curvalaria sp* ADSS and *Aspergillus oryza* ABSS record the lower production of enzyme if it 3.9 mg/l at same one . At the lower concentration of Tryptone (0.5 gm/l) the sample *Aspergillus oryza* ATD record 4.7 mg/l as a higher production when the sample *Curvalaria sp* ADSS record 2.4 mg/l as a lower production of enzyme at same concentration of Tryptone . The lower production of enzyme is 1.7 mg/l witch recorded by sample *Curvalaria sp* ADSS at 3.0 gm/l concentration and the higher production at same concentration is 3.2 mg/l recorded by local sample *Penicillium notattum* MASS and 2.7 mg/l at local samples *Aspergillus niger* ABCH, *Fusarium sp* ATSS , *Aspergillus oryza* ATD and *Penicillium notattum* BRSS while the sample *Aspergillus niger* GDSS record 3.9 mg/l as a higher production at same concentration (Table 11) . The using of peptone as an organic source of nitrogen give best results than these given at Tryptone or Meat extract . The best results was recorded when we use peptone at 2.0 gm/l concentration and a lower ones at 0.5 gm/l concentration . The higher production of enzyme is 8.2 mg/l recorded by *Penicillium notattum* MASS sample when we used peptone at 2.0 gm/l concentration , At same concentration the local sample *Curvalaria sp*

Table(9) Effect of Malt Extract concentration

Sample	Concentration of Malt Extract (g/L)					
	0.5	1.0	1.5	2.0	2.5	3.0
ABCH	8.1	8.8	9.2	10.7	9.8	7.4
ABSS	7.0	8.6	9.0	9.8	8.2	6.8
BRSS	8.4	9.0	9.8	10.0	9.3	8.6
SHSS	8.2	8.8	9.7	10.2	9.5	8.7
ATF	7.3	8.0	8.4	9.7	8.0	7.4
MASS	8.2	9.0	9.6	10.7	9.3	8.3
ATD	6.4	7.2	8.8	10.9	10.0	9.2
ADSS	8.5	9.2	10.0	11.2	10.3	8.7
ATSS	6.3	7.5	8.1	9.6	8.4	7.3
GDSS	5.4	6.8	8.2	8.6	7.4	6.2

Statistics

	ABCH	ABSS	BRSS	SHSS	ATF	MASS	TTD	ADSS	ATSS	GTSS
Std. Error of Mean	.48236	.47446	.26130	.30267	.35559	.37720	.69077	.42328	.45656	.56427
Std. Deviation	1.18152	1.16218	.64005	.74140	.87101	.92394	1.69204	1.03682	1.11833	1.26174

Table (10) Effect of Meat Extract Concentration

Sample	Concentration of Meat Extract (g/L)					
	0.5	1.0	1.5	2.0	2.5	3.0
ABCH	5.1	6.0	5.8	4.9	4.7	3.9
ABSS	4.7	5.8	5.4	4.9	4.0	3.2
BRSS	5.3	6.4	6.0	5.7	4.3	3.7
SHSS	4.0	5.3	4.8	4.2	3.4	2.8
ATF	4.6	6.0	5.2	4.8	4.0	3.7
MASS	5.2	6.1	5.0	4.3	3.0	2.7
ATD	2.7	4.0	3.6	3.0	2.2	1.9
ADSS	4.6	6.8	6.0	5.0	4.6	3.8
ATSS	6.4	7.0	6.3	5.8	5.0	4.1
GDSS	6.0	6.5	5.3	4.2	3.8	2.8

Statistics

	ABCH	ABSS	BRSS	SHSS	ATF	MASS	TTD	ADSS	ATSS	GTSS
Std. Error of Mean	.31269	.38615	.42400	.37096	.33903	.54858	.32863	.44322	.43102	.57542
Std. Deviation	.76594	.94587	1.03859	.90866	.83046	1.34375	.80498	1.08566	1.05578	1.40949

Table(11) Effect of Tryptone Concentration

Sample	Concentration of Tryptone (g/L)					
	0.5	1.0	1.5	2.0	2.5	3.0
ABCH	3.1	3.2	4.6	4.3	3.9	2.7
ABSS	3.0	2.8	3.9	2.6	2.5	1.8
BRSS	4.0	4.6	5.0	4.2	3.6	2.7
SHSS	3.7	4.6	4.9	3.6	2.4	2.0
ATF	3.0	3.7	4.2	3.4	2.0	1.9
MASS	3.7	4.6	5.0	4.8	4.0	3.2
ATD	4.7	4.9	5.4	4.8	3.2	2.7
ADSS	2.4	3.0	3.9	2.7	2.0	1.7
ATSS	4.0	4.5	5.7	5.0	4.2	2.7
GDSS	3.8	4.4	6.4	5.3	4.3	3.9

Statistics

	ABCH	ABSS	BRSS	SHSS	ATF	MASS	TTD	ADSS	ATSS	GTSS
Std. Error of Mean	.30514	.28127	.32906	.47164	.37830	.28568	.43773	.31981	.41372	.45891
Std. Deviation	.74744	.68896	.80602	1.15528	.92664	.69976	1.07223	.78337	1.01341	1.02616

ADSS record 4.6 mg/l as a lower production in a local samples while the sample *Aspergillus niger* GDSS record 2.1 mg/l at this concentration . This sample record the higher production of enzyme 5.3 mg/l at 0.5 gm/l concentration of peptone , and the lower production is 1.2 mg/l at 3.0 gm/l as a lower one of all at peptone as source of nitrogen . At the lower concentration of peptone (0.5 gm/l) the higher production of enzyme was recorded by the local sample *Aspergillus oryza* ATD and it is 5.3 mg/l after it the sample *Penicillium notattum* MASS witch record 5.0 mg/l while the *Aspergillus niger* GDSS sample record 5.3 mg/l as a higher production for it . at same time the sample *Curvalaria sp* ADSS record 1.3 mg/l as a lower production at this concentration and it is a second lower of all . The third lower production is 1.9 mg/l record by sample *Penicillium notattum* BRSS addition to result recorded by *Curvalaria sp* ADSS sample at 1.o gm/l concentration and addition to reported by *Aspergillus niger* GDSS sample at 2.5 gm/l concentration (Table 12) . When we used Urea as an organic source of nitrogen the result will be best but not more than these ones given by malt extract . The best results recorded at 1.5 gm/l concentration and a lower record at 0.5 gm/l concentration . At 1.5 gm/l concentration the sample *Fusarium sp* ATSS record a higher production of Glucoamylase that is 8.0 mg/l and at same concentration the sample *Aspergillus niger* GDSS record 7.3 mg/l as a higher production for it while the sample *Aspergillus oryza* SHSS record 5.3 mg/l as a lower product at this concentration . after it come *Aspergillus oryza* ABSS and *Aspergillus oryza* ATD with 6.0 mg/l for etch one and the sample *Penicillium notattum* MASS that is record 6.1 mg/l at same concentration . At the lower concentration (0.5 gm/l) the local sample *Fusarium sp* ATSS record 5.9 mg/l as a higher production of enzyme at this concentration and at the second the sample *Curvalaria sp* ADSS record 5.7 mg/l while the local sample *Aspergillus oryza* ABSS

Table (12) Effect of Peptone Concentration

Sample	Concentration of Peptone (g/L)					
	0.5	1.0	1.5	2.0	2.5	3.0
ABCH	2.4	3.6	4.2	6.7	5.1	4.8
ABSS	3.7	4.0	4.4	5.8	5.0	4.6
BRSS	1.9	2.7	4.2	6.3	5.0	4.8
SHSS	2.4	3.6	4.3	6.0	5.0	4.7
ATF	4.2	5.6	6.3	7.1	6.7	6.0
MASS	5.0	5.5	6.8	8.2	7.0	6.9
ATD	5.3	5.8	6.0	6.4	5.9	3.7
ADSS	1.3	1.9	2.2	4.6	3.7	3.0
ATSS	4.8	5.4	6.0	8.0	7.3	6.8
GDSS	5.3	4.7	3.9	2.1	1.9	1.2

Statistics

	ABCH	ABSS	BRSS	SHSS	ATF	MASS	TTD	ADSS	ATSS	GTSS
Std. Error of Mean	.59423	.30596	.65663	.50442	.41587	.46952	.39108	.50028	.49154	.68431
Std. Deviation	1.45556	.74944	1.60842	1.23558	1.01866	1.15007	.95795	1.22543	1.20402	1.67621

record only 1.9 mg/l as a lower production at this concentration and at the same time the sample *Aspergillus niger* GDSS record 5.2 mg/l as a third higher product after the sample *Fusarium sp* ATSS and *Curvalaria sp* ADSS . A t the higher level of concentration of urea (3.0 gm/l) the production have lower about 20% at the all of samples witch the higher production is 6.0 mg/l recorded by the sample *Fusarium sp* ATSS and the lower product is 3.7 mg/l record by the sample *Aspergillus oryza* ATD (Table 13) .

4-2-3 Effect of Minerals:

Ions have a wide physiological effecting at all of living thing that it is regulated the body flouting and at other hand it is a Co factors of enzyme to complete her working . At this study we had use some salts in the production media so as Barium Chloride($BaCl_2$) , ferric Sulphate (Fe So_4) , Calcium Chloride (Ca Cl_2) , Zink Sulphate (Zn So_4) , Magnesium Sulphate ($MgSo_4$) and Manganese Sulphate ($MnSo_4$) .And at different concentrations ' 1.0 , 1.5 , 2.0 , 2.5 , 3.0 and 3.5 gm/l . When we used Barium Chloride the production had be too lower' and the best production record at the lower concentration (1.0 gm/l) that means this ion have a negative effect at the enzyme product and it is inhibiter for enzymes . A higher production of enzyme recorded when we use this Ions 2.8 mg/l record by the sample *Penicillium notattum* MASS at 1.0 gm/l concentration of Barium chloride while the lower production for this sample is 0.6 mg/l at the higher concentration (3.5 gm/l) . At the best concentration (1.0 gm/l) the lower production was recorded by the local sample *Fusarium sp* ATSS and it is 1.9 mg/l and the same sample record a lower product 3.0 mg/l at the higher concentration 3.5 gm/l. The sample *Aspergillus niger* GDSS record 2.0 mg/l at 1.0 gm/l concentration as a higher product for it and 0.7 mg/l at 3.5 gm/l as a lower production . The

Table (13) Effect of Urea Concentration

Sample	Concentration of Urea (g/L)					
	0.5	1.0	1.5	2.0	2.5	3.0
ABCH	2.6	4.2	6.8	5.8	5.0	4.8
ABSS	1.9	3.6	6.0	5.5	5.0	4.2
BRSS	4.3	5.0	7.4	6.6	5.7	4.8
SHSS	2.9	3.7	5.3	5.0	4.8	4.0
ATF	4.0	5.2	6.7	5.8	5.2	4.8
MASS	3.7	4.0	6.1	5.2	4.7	4.2
ATD	3.1	3.9	6.0	5.4	4.8	3.7
ADSS	5.7	6.1	7.7	6.8	6.0	5.6
ATSS	5.9	6.9	8.0	7.3	6.8	6.0
GDSS	5.2	6.4	7.3	6.3	5.2	3.9

Statistics

	ABCH	ABSS	BRSS	SHSS	ATF	MASS	TTD	ADSS	ATSS	GTSS
Std. Error of Mean	.58348	.60700	.48074	.37186	.37275	.36217	.45123	.32600	.32395	.48814
Std. Deviation	1.42922	1.48683	1.17757	.91086	.91305	.88713	1.10529	.79854	.79352	1.19569

samples *Aspergillus niger* ABCH and *Curvalaria sp* ADSS record same results while the samples *Penicillium notattum* BRSS and *Rhizopus stolineffer* ATF record same results also when the samples *Aspergillus niger* GDSS and *Aspergillus oryza* SHSS give a same results (Table 14) . When we use Ferric Sulphate there was exes in production compression with Barium Chloride while is the best concentration is a lower one . The higher production is 4.2 mg/l record by the local sample *Penicillium notattum* MASS at the lower concentration (1.0 gm/l) and 4.0 gm/l record by the local samples *Penicillium notattum* BRSS , *Rhizopus stolineffer* ATF and *Curvalaria sp* ADSS at the same concentration .when the local sample *Aspergillus oryza* ABSS record a lower product at same concentration and it is only 3,0 mg/l and the local sample *Fusarium sp* ATSS with 3.2 mg/l at same concentration . The lower production was recorded at a higher concentration (3.5 gm/l) when the samples *Rhizopus stolineffer* ATF and *Fusarium sp* ATSS record only 1.0 mg/l and the samples *Aspergillus oryza* SHSS and *Aspergillus niger* ABCH with 1.1 mg/l and 1.2 . The sample *Penicillium notattum* MASS witch record a higher production at the best concentration record only 2.0 mg/l at the higher concentration retain to record 2.0 mg/l at a higher concentration so that the product lowering to 50% and also the samples *Penicillium notattum* BRSS and *Curvalaria sp* ADSS which their record 1.9 and 1.8 . The sample *Rhizopus stolineffer* ATF lost 75% for her product at a higher concentration (3.5 gm/l) when it have record only 1.0 mg/l from 4.0 mg/l record by it at 1.0 gm/l concentration of Ferric Sulphate . The sample *Aspergillus niger* GDSS record 4.2 mg/l at 1.0 gm/l concentration of Ferric Sulphate and this result a good comparison with the other samples but this product bee lower to 1.1 mg/l at the higher concentration of salt (3.5 gm/l)(Table 15) . The using of Calcium Chloride($CaCl_2$) give a best results comparison with other upper ions , A higher product of

Table (14) Effect of $BaCl_2$ Concentration

Sample	Concentration of $BaCl_2$ (g/L)					
	1.0	1.5	2.0	2.5	3.0	3.5
ABCH	2.6	2.2	1.7	1.3	1.0	0.9
ABSS	2.2	2.0	1.9	1.3	1.1	0.8
BRSS	2.4	2.1	1.9	1.6	1.4	1.0
SHSS	2.0	1.8	1.4	1.0	0.9	0.6
ATF	2.4	2.1	1.8	1.4	1.1	0.7
MASS	2.8	2.4	2.0	1.6	1.0	0.6
ATD	2.0	1.8	1.5	1.1	0.9	0.5
ADSS	2.6	2.4	2.1	1.8	1.4	1.2
ATSS	1.9	1.6	1.2	0.9	0.6	0.3
GDSS	1.2	2.7	2.2	1.3	0.7	0.2

Statistics

	ABCH	ABSS	BRSS	SHSS	ATF	MASS	TTD	ADSS	ATSS	GTSS
Std. Error of Mean	.27739	.21858	.20602	.22274	.26002	.34124	.23238	.22571	.24687	.37896
Std. Deviation	.67946	.53541	.50465	.54559	.63692	.83586	.56921	.55287	.60470	.92826

Table(15) Effect of FeSO4 Concentration

Sample	Concentration of $FeSO_4$ (g/L)					
	1.0	1.5	2.0	2.5	3.0	3.5
ABCH	3.6	3.2	2.8	2.3	1.8	1.2
ABSS	3.0	2.9	2.6	2.1	19.	1.6
BRSS	4.0	3.7	3.2	2.7	2.1	1.9
SHSS	3.3	3.0	2.6	2.1	1.8	1.1
ATF	4.0	3.4	2.9	2.6	2.2	1.0
MASS	4.2	3.9	3.6	3.1	2.4	2.0
ATD	3.8	3.4	2.8	2.1	1.9	1.4
ADSS	4.0	3.4	2.8	2.4	2.1	1.8
ATSS	3.2	2.9	2.6	1.9	1.4	1.0
GDSS	4.2	3.9	3.1	2.5	1.9	1.1

Statistics

	ABCH	ABSS	BRSS	SHSS	ATF	MASS	TTD	ADSS	ATSS	GTSS
Std. Error of Mean	.47516	.67614	.44528	.23523	.41204	.30514	.57426	.56001	.64326	.57213
Std. Deviation	1.16390	1.65620	1.09072	.57619	1.00929	.74744	1.40665	1.37174	1.57565	1.40143

Glucoamylase enzyme record at a lower concentration of Calcium Chloride when the lower product recording at a higher concentration . The sample *Penicillium notattum* MASS record 5.1 mg/l as a higher product at this concentration , after that the samples *Penicillium notattum* BRSS and *Aspergillus oryza* ATD with 5.0 mg/l for etch while the sample *Aspergillus oryza* ABSS record 3.9 mg/l as a lower product at this best concentration . At the higher concentration of salt (3.5 gm/l) the higher product is 2.6 mg/l record by the sample *Penicillium notattum* MASS after it 2.4 mg/l at the sample *Penicillium notattum* BRSS and 2.3 mg/l at the sample *Aspergillus niger* ABCH , when the lower product is 1.2 mg/l at the sample ATF and 1.3 mg/l at the sample *Aspergillus oryza* SHSS . The sample MASS witch record a higher production at a lower concentration lost about 50% for production when it has record 2.6 mg/l at 3.5 gm/l concentration of Calcium Chloride . The samples *Aspergillus niger* ABCH , ATF and *Curvalaria sp* ADSSS record same results spicily at the best production at a lower concentration , while the samples *Penicillium notattum* BRSS ,*Penicillium notattum* MASS and *Aspergillus oryza* ATD record a same results . The sample *Aspergillus niger* GDSS record a higher production 3.7 mg/l at 1.0 gm/l concentration but this product retain to be 1.2 mg/l at a higher concentration (3.5 gm/l). The sample *Aspergillus oryza* SHSS lost more than 75% for her production when it had record 1.3 mg/l at 3.5 gm/l concentration and also the sample *Fusarium sp* ATSS witch it is record only 1.5 mg/l at same concentration of salt (Table 16) . Zinc Sulphate ($ZnSO_4$) one of some salts have too negative effecting on microbial cells activity so that the ion Zn is one of a heavy metals witch it is precipitate the proteins of cells an stopped the enzymes working. When we used this salt in production media , the quantum of enzyme has been a very few when the samples *Aspergillus niger* ABCH and *Curvalaria sp* ADSS record only 1.6 mg/l as a higher

Table(16) Effect of $CaCl_2$ Concentration

Sample	Concentration of $CaCl_2$ (g/L)					
	1.0	1.5	2.0	2.5	3.0	3.5
ABCH	4.7	4.3	3.7	3.1	2.9	2.3
ABSS	3.9	3.3	2.7	2.4	2.0	1.7
BRSS	5.0	4.7	4.0	3.3	2.9	2.4
SHSS	4.2	3.9	3.1	2.9	2.2	1.3
ATF	4.7	4.0	3.3	2.6	2.0	1.2
MASS	5.1	4.6	4.0	3.4	3.0	2.6
ATD	5.0	4.4	3.7	3.0	2.7	2.1
ADSS	4.7	4.2	3.6	3.1	2.8	2.2
ATSS	4.0	3.3	2.7	2.1	1.9	1.5
GDSS	3.7	3.1	2.6	2.1	1.4	1.2

Statistics

	ABCH	ABSS	BRSS	SHSS	ATF	MASS	TTD	ADSS	ATSS	GTSS
Std. Error of Mean	.36878	.33533	.41906	.43868	.52831	.39193	.44528	.37653	.38333	.39728
Std. Deviation	.90333	.82138	1.02648	1.07455	1.29409	.96003	1.09072	.92232	.93897	.97314

product at 1.0 gm/l concentration of salt , and the samples *Penicillium notattum* BRSS and *Penicillium notattum* MASS with 1.4 mg/l foe etch , while the lower production is only 0.9 mg/l recorded by the sample ATF . The lower production of enzyme record at 3.5 gm/l concentration of salt , at this concentration the samples *Aspergillus niger* ABCH and *Curvalaria sp* ADSS witch record a higher production at 1.0 gm/l concentration of Zink Sulphate retain to record 0.07 mg/l and 0.4 mg/l for them . The sample *Rhizopus stolineffer* ATF witch record a lower product at a lower concentration record only 0.01 mg/l as a lower product also at a higher concentration of salt (3.5 gm/l) and this is a lower production of all samples .The samples *Aspergillus niger* ABCH and *Curvalaria sp* ADSS record a same results when the samples BRSS and MASS record same results also when the samples *Aspergillus oryza* ABSS , *Aspergillus oryza* SHSS and *Aspergillus oryza* ATD record same results The sample *Aspergillus niger* GDSS record a higher product at 1.0 gm/l concentration and it is 1.3 mg/l when the lower product of it is 0.01 mg/l at 3.5 gm/l concentration of salt and this result same with the samples *Fusarium sp* ATSS and *Rhizopus stolineffer* ATF at same concentration (Table 17) . When we use Manganese Sulphate ($MnSO_4$) al the production was a lower also but a best than the Zinc Sulphate . At 1.0 gm/l concentration of this salt in the production media the higher product of enzyme is 1.4 mg/l record by the sample *Aspergillus oryza* SHSS , this sample record 0.5 mg/l at higher concentration (3.5 gm/l) and that means the production lost more than 50% at this concentration . The samples *Curvalaria sp* ADSS and BRSS come at second order if they record 1.2 mg/l for all at a lower concentration while they are record 0.3 mg/l and 1.0 mg/l respectively . The sample *Fusarium sp* ATSS record a lower production at stander concentration (1.0 gm/l) that is only 0.8 mg/l and the sample *Aspergillus oryza* ATD with 0.9 mg/l product and the sample *Aspergillus oryza*

Table (17) Effect of $ZnSO_4$ Concentration

Sample	Concentration of $ZnSO_4$ (g/L)					
	1.0	1.5	2.0	2.5	3.0	3.5
ABCH	1.6	1.2	0.8	0.4	0.1	0.07
ABSS	1.0	0.9	0.6	0.4	0.1	0.03
BRSS	1.4	1.2	1.0	0.98	0.7	0.3
SHSS	1.2	0.9	0.7	0.4	0.1	0.09
ATF	0.9	0.7	0.4	0.1	0.03	0.01
MASS	1.4	1.2	1.0	0.9	0.6	0.2
ATD	1.3	1.0	0.8	0.6	0.3	0.06
ADSS	1.6	1.4	1.1	0.9	0.6	0.4
ATSS	0.8	0.4	0.1	0.09	0.03	0.01
GDSS	1.3	0.9	0.5	0.2	0.02	0.01

Statistics

	ABCH	ABSS	BRSS	SHSS	ATF	MASS	TTD	ADSS	ATSS	GTSS
Std. Error of Mean	.25277	.16439	.15822	.18283	.15359	.17591	.18586	.18797	.12626	.21289
Std. Deviation	.61915	.40268	.38756	.44783	.37623	.43089	.45527	.46043	.30928	.52148

ABSS that is record 0.98 mg/l at the same concentration . At the higher concentration (3.5 gm/l) these samples record 0.08 mg/l , 0.1 mg/l 0.3 mg/l respectively . The samples *Aspergillus niger* ABCH , *Rhizopus stolineffer* ATF and *Penicillium notattum* MASS record a same results while the samples *Penicillium notattum* BRSS and *Curvalaria sp* ADSS record a same results also . The sample *Aspergillus niger* GDSS record a higher production of her at 1.0 gm/l and it is 1.4 mg/l while it had record 0.1 mg/l as a lower product at 3.5 gm/l concentration (Table 18) . The best production of Glucoamylase enzyme recorded when we use the mineral salts ions is a one s record at Magnesium Sulphate (Mg SO_4) especially at 1.0 gm/l concentration of this salt . at this concentration the sample *Aspergillus oryza* ATD record 8.9 mg/l as a higher production after that the sample *Aspergillus niger* ABCH with 8.7 mg/l while the lower production at this concentration is 6.8 mg/l recorded by the samples *Rhizopus stolineffer* ATF and *Fusarium sp* ATSS . At the higher concentration (3.5 gm/l) the higher product is 5.3 mg/l record by the sample *Aspergillus oryza* ATD witch it record a higher product at the lower concentration while the lower product is 3.1 mg/l record by the sample *Rhizopus stolineffer* ATF witch it also record a lower product at the lower concentration and that means the production has lost more than 50% . The samples *Aspergillus niger* ABCH , BRSS and *Aspergillus oryza* ATD record a same results while the samples *Rhizopus stolineffer* ATF and *Fusarium sp* ATSS record same results also . at same time the samples *Aspergillus oryza* SHSS and *Curvalaria sp* ADSS record same results and *Penicillium notattum* MASS and ABSS also record same results The sample *Aspergillus niger* GDSS record 7.7 mg/l as a higher product of it at 1.0 gm/l concentration but this product lost more than 50% at 3.5 gm/l concentration of salt while it record only 3.6 mg/l (Table 19)

Table(18) Effect of $MnSO_4$ Concentration

	Concentration of $MnSO_4$ (g/L)					
Sample	1.0	1.5	2.0	2.5	3.0	3.5
ABCH	1.0	0.98	0.77	0.71	0.62	0.4
ABSS	0.98	0.83	0.7	0.5	0.42	0.3
BRSS	1.2	1.0	0.99	0.87	0.55	0.1
SHSS	1.4	1.2	1.0	0.89	0.66	0.5
ATF	1.1	0.98	0.82	0.77	0.54	0.4
MASS	1.0	0.9	0.73	0.65	0.49	0.3
ATD	0.9	0.7	0.66	0.51	0.33	0.1
ADSS	1.2	1.0	0.97	0.87	0.56	0.3
ATSS	0.8	0.6	0.5	0.33	0.2	0.08
GDSS	1.4	1.3	1.0	0.9	0.3	0.1

Statistics

	ABCH	ABSS	BRSS	SHSS	ATF	MASS	TTD	ADSS	ATSS	GTSS
Std. Error of Mean	.09251	.10597	.16250	.13629	.10728	.10562	.11661	.13413	.10882	.21551
Std. Deviation	.22660	.25957	.39803	.33385	.26279	.25872	.28563	.32855	.26656	.52789

Table (19) Effect of $MgSO_4$ Concentration

Sample	Concentration of $MgSO_4$ (g/L)					
	1.0	1.5	2.0	2.5	3.0	3.5
ABCH	8.7	8.2	7.3	6.7	5.2	4.8
ABSS	7.4	7.0	6.5	5.4	4.9	3.8
BRSS	8.6	8.0	7.2	6.4	5.3	4.7
SHSS	8.2	7.3	6.7	6.0	5.2	3.9
ATF	6.8	5.7	4.9	4.0	3.6	3.1
MASS	7.9	7.2	6.6	5.4	4.2	3.8
ATD	8.9	8.1	7.7	6.9	6.0	5.3
ADSS	8.4	7.8	7.1	6.4	5.7	4.3
ATSS	6.8	6.2	5.7	4.9	4.0	3.2
GDSS	7.7	6.2	5.5	5.1	4.3	3.6

Statistics

	ABCH	ABSS	BRSS	SHSS	ATF	MASS	TTD	ADSS	ATSS	GTSS
Std. Error of Mean	.64261	.56135	.62183	.62685	.56887	.67614	.55000	.60741	.55718	.59104
Std. Deviation	1.57406	1.37502	1.52315	1.53547	1.39344	1.65620	1.34722	1.48784	1.36480	1.44776

4-2-4 Effect of pH :

pH is one of the important factors that determine the growth and morphology of microorganisms as they are sensitive to the concentration of hydrogen ions present in the medium. Earlier studies have revealed that fungi required slightly acidic pH and bacteria required neutral pH for optimum growth. pH is known to affect the synthesis and secretion of a-amylase just like its stability Fungi of *Aspergillus* sp. such as *A. oryzae, A. ficuum* and *A. niger* were found to give significant yields of a-amylase at pH=5.0–6.0 in SMF a-Amylase producing yeast strains such as *Saccharomyces cerevisiae* and *S. kluyveri* exhibited maximum enzyme production at pH=5.0 Bacterial cultures such as *B. subtilis, B. licheniformis,* and *B. amyloliquefaciens* requiredan initial pH of 7.0 *Rhodothermus marinus* was reported to yield good enzyme levels at initial pH range7.5 to 8. Hyper thermophilic archae such as *Pyrococcus furiosus, P. woesei* and *Thermococcus profundus* yielded optimum a-amylase at pH=5.0 Thermophilic anaerobic bacteria *Clostridium thermosulfurogenes* gave maximum titres of a-amylase at pH=7.0(Metin *et al*, 2010)

We know that the pH or the concentration of Hydrogen ions have a wide effecting on an enzymes activity at order on the life of (organism) . From different statics of pH ; 3.0 , 4.0 , 5.0 , 6.0 , 7.0 and 8.0 we founded that the pH 6.0 is a best one for production Glucoamylase enzyme . At this statics all the samples record higher product . The sample SHSS record10.8 mg/l as a higher production while the sample *Aspergillus oryza* ATD record 8.2 as a lower product at this statics of pH . the sample BRSS witch it record high product (10.6mg/l) at the pH 6.0 but it is retain to record 4.3 mg/l and 5.2 mg/l at PH 3.0 and pH 8.0 and that means the enzyme had a very sensitive for an alkaloid and acidify. All the sample had a same things but some sample like *Aspergillus oryza* ABSS , ATF and *Aspergillus oryza* ATD had a lower sensitive and it is a semi acidify . The samples *Aspergillus niger* ABCH ,*Penicillium notattum*

BRSS , *Aspergillus oryza* SHSS and *Curvalaria sp* ADSS record same results while the samples *Aspergillus oryza* ABSS , *Rhizopus stolineffer* ATF and *Fusarium sp* ATSS record same result (Table 20) .

4-2-5 Effect of time a(days) at enzyme production:

The samples was incubated at different periods between one day and seven days . The best production at general was recorded at fife days at same ecological condition . At fife days the sample *Aspergillus oryza* ATD record a higher product comparison with the other samples when it was record 14.8 mg/l and the sample *Aspergillus niger* ABCH witch it had record 14.6 mg/l , while the lower product at fife days was recorded by the sample *Penicillium notattum* MASS so that it was record 12.6 mg/l . The lower production of Glucoamylase enzyme at general was recorded at after one day from the incubation so that the sample *Aspergillus oryza* ATD witch it record a higher product at fifth day retard to record 11.7 mg/l as a higher product at one day , after it the sample *Curvalaria sp* ADSS witch it had record 11.2 mg/l while the lower product at one day was recorded by the sample *Penicillium notattum* MASS and it is only 8.0 mg/l . At the sixth and seventh day the production was allowed (Table 21) .

Table (20) Effect of pH on production of enzyme

Sample	p.H					
	3.0	4.0	5.0	6.0	7.0	8.0
ABCH	6.1	7.6	9.3	10.7	9.1	7.6
ABSS	6.0	7.3	8.4	9.8	8.6	6.4
BRSS	4.3	6.6	9.0	10.6	8.4	5.2
SHSS	5.2	6.8	8.1	10.8	8.1	5.0
ATF	6.4	7.0	7.7	9.8	7.6	4.6
MASS	4.2	5.8	9.2	10.4	8.4	6.2
ATD	6.6	6.8	7.3	8.2	6.2	4.1
ADSS	5.8	6.2	8.7	10.6	8.6	5.2
ATSS	4.6	5.7	6.8	9.0	6.2	4.2
GDSS	3.8	4.9	5.3	7.3	4.1	3.2

Statistics

	ABCH	ABSS	BRSS	SHSS	ATF	MASS	TTD	ADSS	ATSS	GTSS
Std. Error of Mean	.66232	.58977	.98107	.88456	.69781	.95696	.56194	.85884	.70542	.59311
Std. Deviation	1.62234	1.44465	2.40312	2.16672	1.70929	2.34407	1.37647	2.10373	1.72791	1.45281

Table (21) Effect of time (days) on production of enzyme

Sample	The Days					
	2	3	4	5	6	7
ABCH	8.7	9.3	12.0	14.6	13.0	12.4
ABSS	9.2	10.0	11.5	13.8	12.6	11.7
BRSS	9.7	10.6	12.2	14.2	13.7	12.6
SHSS	9.2	11.0	12.3	14.0	13.1	12.6
ATF	8.7	10.2	11.2	13.5	12.4	11.0
MASS	8.0	9.3	9.7	12.6	10.6	9.2
ATD	11.7	12.2	13.0	14.8	14.0	14.2
ADSS	11.2	12.1	12.9	14.1	13.0	11.6
ATSS	9.2	10.5	11.9	13.7	12.0	10.5
GDSS	8.7	9.8	9.9	12.3	11.7	10.8

Statistics

	ABCH	ABSS	BRSS	SHSS	ATF	MASS	TTD	ADSS	ATSS	GTSS
Std. Error of Mean	.92075	.68492	.71212	.69506	.68346	.63979	.49694	.43314	.64031	.54324
Std. Deviation	2.25536	1.67770	1.74432	1.70255	1.67412	1.56716	1.21724	1.06097	1.56844	1.33066

4-2 6 Effect of temperature on production of enzyme :

Results from table. (22) shows the effect of different incubation temperature on the production of Glucoamylase by Isolates., The maximum production of Glucoamylase was obtained at 55°C. The optimum temperature was observed for the production of "- Glucoamylase from *Aspergillus oryza* ATD sample was also35℃ as reported by(Krishna and Chandrasekaran,1996) Increase in incubation temperature, decreased the production of enzyme. The production of the enzyme was greatly inhibited at 35 and 60°C. It might be due to that at high temperature, the growth of the fungi was greatly inhibited and hence, enzyme formation was also prohibited . The higher product that is recording is 14.8mg/l when is the lower one at this sample is 10.2 mg/l at 35°C . At the best temperature the sample MASS record the lower product 12.6 mg/l and also the sample *Aspergillus niger* GDSS witch it have record 12.1 mg/l at same temperature while it has record 9.3 mg/l at 35°C similar at the ATSS and BRSS sample. From all results the lower one is only 8.7 witch it recording by the samples *Penicillium notattum* MASS and *Aspergillus niger* ABCH

4-3 Activity of enzyme :

The activity of Glucoamylase enzyme was measuring at differences effecting factors so as Time (mins) ,pH , Source of Nitrogen (Malt extract) . Source of Carbon (Starch) and Tempreture .

4-3-1 Effect of Time (mins) on activity of enzyme :

The glucoamylase activity was determined after every 10 mins of incubation in order to determine the optimum incubation period for maximum activity of extracellular glucoamylase. The enzyme activity howeve r, started after 10 mins of incubation and showed maximum

Table(22) Effect 0f Temperature (C˚) On Production of Enzyme

Sample	Temperature (C˚)					
	35	40	45	50	55	60
ABCH	8.7	9.4	10.0	12.6	14.6	13.5
ABSS	9.2	9.8	10.7	11.6	13.8	12.6
BRSS	9.4	10.6	11.7	12.8	14.2	12.4
SHSS	9.7	10.6	11.3	13.0	14.0	13.0
ATF	9.1	10.7	12.0	12.4	13.5	11.7
MASS	8.7	9.3	10.6	11.1	12.6	9.8
ATD	10.2	11.4	12.0	13.2	14.8	12.2
ADSS	10.0	11.2	12.6	13.0	14.1	11.6
ATSS	9.7	10.0	11.2	12.6	13.2	10.7
GDSS	9.3	9.9	10.6	11.9	12.1	8.7

Statistics

	ABCH	ABSS	BRSS	SHSS	ATF	MASS	TTD	ADSS	ATSS	GTSS
Std. Error of Mean	.98849	.70825	.69077	.65149	.61842	.57198	.64239	.59241	.57426	.56357
Std. Deviation	2.42129	1.73484	1.69204	1.59583	1.51482	1.40107	1.57353	1.45109	1.40665	1.38046

Activity at 30 mins of incubation so that the sample *Curvalaria sp* ADSS record a higher activity at this time and record 37.5 mg/l as a maximum activity of all fungi when it had record 28.3 mg/l as a lower activity of it at 10 mins . At same time the sample *Penicillium notattum* MASS record only 26.2 mg/l at the standard time (30 mins). *Aspergillus niger* ABCH record 36.7 mg/l at 30 mins as a second one (Table 23) show the result of effect of time on activity of Glucoamylase in ten isolate .

4-3-2Effect of p.H on activity of enzyme :

At the p.H 4.0 all the samples record a maximum activity when the sample BRSS record 41.1mg/l as a higher activity at this p.H and the sample GDSS record 31.0 as a lower activity at same p.H . At same time all of the sample record a lower activity at the very acidic and the higher alkaloid when the sample ABCH record 25.0mg/l at p.H 2.0 as a lower activity and also 29.2 mg/l at p.H 7.0 (table 24) .

4-3-3Effect of Malt extract concentration on activity of enzyme :

At different concentration of malt extract when found that the maximum activity of enzyme was record at 2.0gm/l concentration of malt extract when the sample ABCH record 46.7 mg/l at this concentration and 32.6 as a lower activity at 0.5gm/l concentration . At same time the sample ATSS record 43.9 mg/l as a lower activity at standard concentration (table 25) .

4-3-4Effect of Starch concentration on activity of enzyme :

The best concentration of starch is 0.4 gm/l at all sample . At this concentration the sample ATD record 54.3 mg/l as a higher activity and 42.3 mg/l at 0.1 gm/l concentration of starch while the sample MASS record only 47.6 mg/l as a lower activity at standard

Table (23) Effect of Time (mins) on Activity

Sample	The Time (Mins)					
	10	20	30	40	50	60
ABCH	20.2	23.0	36.7	30.7	29.5	27.1
ABSS	22.1	24.2	30.1	26.3	25.0	22.6
BRSS	25.5	31.0	35.2	33.6	29.0	26.6
SHSS	19.7	22.0	29.7	27.3	25.0	24.7
ATF	20.2	23.0	27.1	25.1	24.0	23.7
MASS	18.7	20.6	26.2	25.0	24.0	22.9
ATD	24.0	26.1	30.9	28.5	26.2	25.0
ADSS	28.3	32.5	37.5	35.2	33.0	32.7
ATSS	27.6	29.0	34.2	32.6	30.3	29.4
GDSS	18.5	22.8	29.8	27.2	20.9	15.5

Statistics

	ABCH	ABSS	BRSS	SHSS	ATF	MASS	TTD	ADSS	ATSS	GTSS
Std. Error of Mean	2.39286	1.19017	1.56711	1.46211	.93479	1.14659	1.02678	1.25539	1.00014	2.18338
Std. Deviation	5.86129	2.91530	3.83862	3.58143	2.28976	2.80856	2.51509	3.07506	2.44983	5.34818

Table(24) Effect of p.H on Activity

Sample	P.H					
	2.0	3.0	4.0	5.0	6.0	7.0
ABCH	25.0	30.2	40.6	39.5	38.4	29.2
ABSS	30.9	36.7	38.7	37.3	36.6	36.0
BRSS	36.8	37.2	41.1	30.5	27.3	25.6
SHSS	37.7	38.9	40.8	39.4	35.0	30.4
ATF	33.2	35.0	36.6	36.0	30.0	28.7
MASS	37.0	39.1	40.7	37.6	35.0	33.0
ATD	30.3	32.9	35.7	34.9	32.0	29.6
ADSS	33.6	37.2	39.6	36.0	34.1	30.9
ATSS	26.9	29.0	32.7	31.0	28.4	25.8
GDSS	20.5	25.1	28.5	24.9	22.6	15.8

Statistics

	ABCH	ABSS	BRSS	SHSS	ATF	MASS	TTD	ADSS	ATSS	GTSS
Std. Error of Mean	2.65486	1.09291	2.73505	1.54761	1.33060	1.13186	.99387	1.24168	1.04392	1.34104
Std. Deviation	6.50305	2.67706	6.69948	3.79087	3.25929	2.77248	2.43447	3.04149	2.55708	2.99867

Table (25) Effect of Malt Extract Concentration on Activity

Sample	Concentration of Malt Extract (g/L)					
	0.5	1.0	1.5	2.0	2.5	3.0
ABCH	32.6	34.6	38.7	46.7	45.2	42.3
ABSS	39.6	42.0	43.1	44.2	41.3	39.6
BRSS	39.7	42.0	43.9	45.1	43.7	40.7
SHSS	40.0	42.7	43.7	45.0	42.8	39.3
ATF	41.6	43.3	44.7	46.1	44.1	42.9
MASS	47.7	51.3	53.1	55.3	52.2	48.6
ATD	39.7	41.4	42.3	44.6	41.7	37.6
ADSS	40.6	42.3	44.1	46.0	44.1	41.9
ATSS	38.7	41.9	42.3	43.9	41.7	37.5
GDSS	30.1	33.9	38.2	40.6	37.3	26.9

Statistics

	ABCH	ABSS	BRSS	SHSS	ATF	MASS	TTD	ADSS	ATSS	GTSS
Std. Error of Mean	2.32972	.75836	.84633	.89322	.63478	1.15864	.97071	.78980	.98217	2.13088
Std. Deviation	5.70663	1.85760	2.07308	2.18792	1.55488	2.83807	2.37774	1.93460	2.40583	5.21958

concentration. The sample GDSS record 36.5 mg/l at a lower concentration 0.1gm/l 47.7 mg/l at the standard concentration 0.4gm/l (table 26) .

4-3-5Effect of Temperature on activity of enzyme :

The optimum temperature of the enzyme was 50°C for all isolates and 55-65°C . Temperature is the most important factors which markedly influence enzyme activity. Maximum Glucoamylase activity was recorded at 50ºCfor30 mins incubation (54.3mg/l of ATD and 53.0 mg/l of SHSS). Further increase in temperature resulted in decrease in the activity of Glucoamylase Table27).

4-4 Activity of enzyme at Purification

A good six samples then are using for purified the enzyme at differences conditions using ammonium sulphate methods and the given results was recording

4-4-1Effect of Time :

At the different time we find that the best activity of purification enzyme had record at 30 mins when the sample ADSS record 80.5 mg/l while the sample BRSS record a lower activity 73.0 mg/l at the same time . At the time 60 mins the samples record a lower activity when the sample ATF record only 38.1 mg/l as a lower activity at this time (table 28) .

4-4-2Effect of p.H :

The maximum activity of purification enzyme was recording at p.H 4.0 at the all sample while the sample ATF record 105.0mg/l as a

Table (26) Effect of Starch Concentration on Activity

Sample	Starch Concentration (g/L)					
	0.1	0.2	0.3	0.4	0.5	0.6
ABCH	33.6	39.7	42.3	50.6	50.0	47.1
ABSS	32.7	34.3	40.0	48.0	46.0	42.7
BRSS	40.0	43.0	47.6	52.3	49.7	48.1
SHSS	39.6	41.7	48.2	53.0	50.6	47.7
ATF	40.3	43.7	47.8	50.7	48.3	46.7
MASS	36.7	41.3	44.0	47.6	46.3	44.9
ATD	42.3	46.7	49.9	54.3	50.9	49.6
ADSS	41.0	46.7	48.1	52.6	52.0	50.8
ATSS	37.7	41.3	44.9	49.2	47.0	46.0
GDSS	20.3	27.9	36.4	45.4	40.1	30.5

Statistics

	ABCH	ABSS	BRSS	SHSS	ATF	MASS	TTD	ADSS	ATSS	GTSS
Std. Error of Mean	2.69956	2.52183	1.84091	2.10950	1.51124	1.61176	1.66368	1.77006	1.70387	3.68851
Std. Deviation	6.61254	6.17719	4.50929	5.16720	3.70176	3.94800	4.07517	4.33574	4.17361	9.03497

Table (27) Effect of Temperature(C ˚) on Activity

Sample	Temperature(C ˚)					
	20	30	40	50	60	70
ABCH	20.6	35.7	40.3	50.6	50.0	46.7
ABSS	20.0	26.8	32.0	48.0	47.1	44.8
BRSS	24.6	26.7	30.9	52.3	49.7	47.7
SHSS	21.7	29.8	36.9	53.0	51.0	48.3
ATF	26.0	30.4	36.7	50.7	48.6	46.8
MASS	20.7	26.7	33.9	47.6	46.0	44.3
ATD	24.3	33.6	41.6	54.3	51.6	49.0
ADSS	21.7	29.9	36.7	52.6	49.3	47.0
ATSS	20.1	27.8	34.3	49.2	47.3	44.0
GDSS	20.2	23.1	35.9	40.4	37.2	32.9

Statistics

	ABCH	ABSS	BRSS	SHSS	ATF	MASS	TTD	ADSS	ATSS	GTSS
Std. Error of Mean	4.65351	4.83072	5.13353	5.18764	4.21756	4.57068	4.74517	4.96902	4.76581	3.32259
Std. Deviation	11.39873	11.83279	12.57454	12.70707	10.33086	11.19583	11.62325	12.17155	11.67380	8.13865

Table (28) Effect of Time(mins) on Activity of purified Enzyme

Sample	Time(mins)					
	10	20	30	40	50	60
BRSS	55.6	60.3	73.0	70.0	63.0	40.9
ATF	57.7	65.0	73.5	68.3	60.1	38.1
MASS	40.9	50.1	75.0	70.5	60.0	60.0
ADSS	50.5	70.0	80.5	78.4	66.0	64.0
ATSS	55.5	66.4	74.0	70.0	61.3	39.4
GDSS	53.2	64.0	77.3	69.6	60.0	44.3

Statistics

	BRSS	ATF	MASS	ADSS	ATSS	GTSS
Std. Error of Mean	4.69380	5.03268	5.15114	4.45097	5.08593	4.79034
Std. Deviation	11.49742	12.32749	12.61767	10.90260	12.45793	11.73388

higher one, at the same time the sample GDSS record only 80.3mg/l as a lower one at this p.H (table 29)

4-4-3Effect of Malt extract concentration :

At different concentration of malt extract when found that the maximum activity of purification enzyme was record at 2.0gm/l concentration of malt extract when the sample MASS record 150.0 mg/l at this concentration and 110.0 as a lower activity at 0.5gm/l concentration . At same time the sample GDSS record 86.3 mg/l as a lower activity at standard concentration (table 30) .

4-4-4 5Effect of Temperature :

The optimum temperature of the purification enzyme was 50°C for all isolates .Maximum Glucoamylase activity was recorded at 50ºCfor30 mins incubation (160.0mg/l of ATF and 140.0 mg/l of BRSS). Fur the (table 31) .

4-4-5 Effect of Starch concentration :

The best concentration of starch is 0.4 gm/l at all sample . At this concentration the sample ATF record 160.0 mg/l as a higher activity and 70.6 mg/l at 0.1 gm/l concentration of starch while the sample BRSS record only 80.5 mg/l as a lower activity at standard concentration in local samples. The sample GDSS record 60.7 mg/l at a lower concentration 0.1gm/l and 86.4mg/l at 3.0 concentration of starch (table 32). .

Table (29) Effect of Ph on Activity of purified Enzyme

Sample	Ph					
	2.0	3.0	4.0	5.0	6.0	7.0
BRSS	40.6	75.3	98.7	87.7	74.3	60.0
ATF	70.3	90.4	105.0	100.0	95.0	60.1
MASS	40.0	49.0	90.3	80.0	73.0	61.7
ADSS	55.0	67.0	95.3	88.0	70.9	66.0
ATSS	60.6	70.0	85.0	80.4	71.0	45.0
GDSS	63.2	66.5	80.3	78.2	69.6	50.3

Statistics

	BRSS	ATF	MASS	ADSS	ATSS	GTSS
Std. Error of Mean	8.37057	7.23607	7.78527	11.53944	5.87893	4.45895
Std. Deviation	20.50363	17.72467	19.06994	28.26574	14.40037	10.92216

Table (30) Effect of Malt Extract Concentration on activity of purified enzyme

Sample	Concentration of Malt Extract (g/L)					
	0.5	1.0	1.5	2.0	2.5	3.0
BRSS	110.6	115.9	125.0	140.5	140.0	130.6
ATF	70,7	87.0	93.0	100.0	95.2	90.0
MASS	110.0	133.0	140.0	150.0	146.0	135.0
ADSS	112.8	120.0	124.0	130.0	128.0	119.3
ATSS	60.6	70.5	85.0	90.0	80.0	77.3
GDSS	60.3	73.3	80.7	86.3	77.7	70.8

Statistics

	BRSS	ATF	MASS	ADSS	ATSS	GTSS
Std. Error of Mean	5.03428	4.15716	5.76580	2.58908	4.29330	3.66949
Std. Deviation	12.33142	10.18293	14.12327	6.34192	10.51640	8.98838

Table(31) Effect of Temperature(C̊) on activity of purified enzyme

Sample	Temperature(C̊)					
	20	30	40	50	60	70
BRSS	50.0	65.0	96.6	140.0	130.0	97.6
ATF	60.0	77.0	90.0	160.0	140.0	40.0
MASS	46.2	60.5	80.0	110.0	95.1	30.6
ADSS	46.6	67.0	92.1	120.0	100.0	60.1
ATSS	50.9	68.3	95.0	130.6	120.3	70.1
GDSS	50.1	66.3	94.0	120.6	118.4	90.5

Statistics

	BRSS	ATF	MASS	ADSS	ATSS	GTSS
Std. Error of Mean	14.34133	19.01534	12.29919	11.28624	12.88839	11.42055
Std. Deviation	35.12894	46.57789	30.12673	27.64552	31.56999	27.97452

Table(32) Effect of Starch Concentration (gm/l) on activity of purified enzyme

Sample	Concentration of Starch (g/L)					
	0.1	0.2	0.3	0.4	0.5	0.6
BRSS	60.0	67.6	90.5	80.5	80.0	73.3
ATF	70.6	95.0	120.0	160.0	140.0	100.5
MASS	60.7	77.0	87.1	110.0	90.0	56.4
ADSS	77.7	83.6	110.0	90.6	70.0	66.0
ATSS	66.3	78.6	90.3	87.0	77.0	75.0
GDSS	60.7	80.8	86.4	80.0	75.4	70.0

Statistics

	BRSS	ATF	MASS	ADSS	ATSS	GTSS
Std. Error of Mean	4.38767	13.23389	8.14170	6.51462	3.52672	3.72574
Std. Deviation	10.74754	32.41628	19.94302	15.95750	8.63867	9.12617

Chapter five

5-Discussions

The ten fungal species *Aspergillus niger*, *Rhizopus stolineffer*, *Fusarium oxysporum*, *Penicillium notattum* , *Curvalaria sp* and *Aspergillus oryza*e investigated in this study were all able to produce glucoamylase on the substrates starch at concentration 4.0gm/L at well than other substrates .and other concentration. The amounts of glucoamylase produced by the fungal species varied on the different substrates. *Rhizopus stolineffer* produced higher amounts of glucoamylase on each of the substrates when compared to the other fungal species tested in this study. *Rhizopus stolineffer* has been shown to be an active producer of glucoamylase and is being exploited or used on large scale for industrial production of the enzyme. In an investigation similar to what is being reported on, Varalakshmi *et al*., (2009) and (Suganthi *et al*., 2011) reported that *Penicillium notattum* produced glucoamylase more actively than several fungal species they investigated on different substrates.(Imai *et al*., 1994) also reported that *Aspergillus oryza* is a very useful fungus in the industrial production of glucoamylase. Imai and colleagues in the same study also found *Rhizopus stolineffer* to be very useful for industrial production of glucoamylase. *Penicillium notattum* investigated in this study was next to *Rhizopus stolineffer* when its glucoamylase production capacity was compared with the other fungal species. With respect to the substrates used, Glucose and Sucrose are un stable for use it as substrates. The *Penicillium notattum* and *Rhizopus stolineffer* investigated in this study could be depended on for the production of glucoamylase for industrial purposes. In this study, the best activities achieved with *Rhizopus stolineffer* and *Penicillium notattum* on starch were respectively 160mg/l and 140mg/l. In similar studies,(Ellaiah *et al*., 2002) and (Sun *et al*., 2009) respectively reported glucoamylase activities of about 125.5 and 136.22 mg/l .

Temperature, pH, Nitrogen concentration and incubation period of culture media influenced glucoamylase production and Activity in this study. In this study, increasing temperature from 20ºC to70ºC led to an increase in glucoamylase activity. There was a decline in glucoamylase activity when the temperature of the culture mixture was increased to 50ºC for all fungal species studied. (Deshmukh *et al*., 2011) also demonstrated that temperature influenced the production of glucoamylase. In their study, it was found that increasing the temperature of the culture mixtures from 30ºC to 40ºC increased glucoamylase activities significantly. Further increase in temperature to 55ºC decreased glucoamylase activities.

In this study, highest glucoamylase activities were recorded at pH 4.0 for *Penicillium notattum* and also for *Rhizopus stolineffer*.(Ellaiah *et al*., 2002) demonstrated that pH influenced glucoamylase activity. In their study, it was reported that the highest glucoamylase activity was achieved at pH 5 for *Aspergillus niger*. Work carried out by(Suntornsuk and Hang, 1997) on effect of pH on glucoamylase activity showed that highest glucoamylase activities were achieved at pH between 4 and 5 for *Rhizopus sp*ecies. Optimum Nitrogen concentration recorded in this study was 2.0 g/l (Barton *et al*., 1969) produced highest glucoamylase activities at nitrogen concentration of 5g/l. In this study, highest glucoamylase activities were achieved with *Rhizopus stolineffer* and *Penicillium notattum* on starch at 30 mins of incubation period . (Deshmukh *et al*., 2011) demonstrated that incubation period influenced glucoamylase activity. In their work, highest glucoamylase activity was achieved at 40mins of incubation period. By comparing the best glucoamylase activities achieved in this study with what have been reported by other workers, activities in this study are low. For industrial usage and profitability, the local fungal isolates identified in this study (i.e. *Penicillium notattum* and *Rhizopus* species) would have to be improved genetically or otherwise to make them better producers of glucoamylase. Improving other parameters such as substrates, temperature, pH,

Nitrogen, incubation period and composition of substrates to achieve higher glucoamylase activity may be the best option to consider if the local fungal isolates are to be used for enzyme production. It is recommended that further investigation to define optimum carbon requirements, temperature, pH, incubation period and Nitrogen concentrations among others are carried out aimed at increasing the levels of glucoamylase activities produced by the local isolates of fungi used in this study. (Nagwa *et a*l, 2000) Isolate the enzyme from *A. flavus* and record maximum production after 72 hours of incubator. And also (Ayansina and Owoseni ,2010). (Meshra and Behara ,2008) fined the maximum activity of amylase in *Bacillus sp* is 23.6 mg/l at 35C as a good temperature of bacteria for production this enzyme . A crossing of the time there are more researcher have study the production and activity of Glucoamylase enzyme at difference condition ,So that (Figueira and Hiooka; 2000 ,Shafeeque *et al* ,2009 ; Adeniran and Abiose,2009 ;Stevenson *et al* ,1984) . Some of these record the best of enzyme can be isolate from *Aspergillus sp* for industrial application (Sevenson *et al* , 1982;Fabiana *et al* ,2008 ,Sasi, *et al* ,2010; Mervak and El Gendi ,2012 ,Boel *et al* , 1984, Johnson *et al* , 1968, Okolo, *et al* 1995 ; and Lineback *et al*, 1972) . Other one record the best enzyme production may be from *Penicillium sp* (Metin *et al*, 2010) And other report the good production at *Candida sp* and yeast (Bertrand *et al*, 2005; Stevenson *et al*, 1984 , Lagzouli *et al*, 2007) . (Haq *et a*l, 2005) report the maximum production of glucoamylase at *Fusarium sp* is 32.5 mg/l at 40C . (Takahashi *et al*,
1994) record that the genus *Rhizopus sp* is a good source of Glucoamylase and also (Chrisstiane *et al*, 2011; Nagwa *et al*, 2010 ; Tahar *et al*, 2010 , Nahar *et al*,2008 ,Ominyi *et al*, 2013and Pacheco *et al*,2004). (Yamasaki and Suzyki,1977) isolated enzyme from *Mucor sp* at difference condition an he found that the best temperature is 40C at 3 days incubation . Measured K max and Vmax values for glucoamylase produced from *Penicillium notattum* in this study are 0.0009548 g/l and 2.387 g/l. min respectively. For *Rhizopus* species,

the measured K max and V max values are 0.0007443 g/l and 2.481 g/l. min respectively. The higher value of K max for glucoamylase of *Penicillium notattum* means glucoamylase produced by *Penicillium notattum* a lower binding affinity for starch than that produced by *Rhizopus* species. Therefore, at maximum velocity of the glucoamylase-starch reaction, glucoamylase of *Penicillium notattum* would contain more starch molecules than that of *Rhizopus* species. Hence Kmax or turnover number or maximum number of starch molecules converted to glucose per active site of glucoamylase enzyme per unit time when the glucoamylase enzyme of *Aspergillus niger* is saturated is expected to be higher than that of *Rhizopus* species. Therefore, glucoamylase activity of Aspergillus niger is expected to be higher. Some reported K max values for *Penicillium notattum* and *Rhizopus* species are 3.5 g/l (Selvakumar *et al*., 1996) and 12.2 g/l (Suntornsuk and Hang, 1997) respectively.

Results in Table (29-33) indicated that as Glucoamylase concentration increased the Glucoamylase activity increased. This behavior is in accordance with the observations of West, *et al.* (1967) who stated that within fairly wide limits the speed of enzyme concentration is directly proportional to the enzyme concentration. Abd El-Rahman, (1990) and El-Safey, (1994) previously reported the same observation.

The Glucoamylase activity reached the maximum with an optimum substrate (starch) concentration of 4.0gm/l with enzyme activity 160mg/ll. Any increase or decrease of substrate concentration gave a corresponding decrease in Glucoamylase activity (Table33).(Kuiper, *etal*,1978) reported that the maximum activity of Glucoamylase enzyme was obtained at 1.67 % of substrate (starch) concentrations. In addition to that,(Abd El-Rahman, 1990) concluded that, the optimal concentration of starch for maximum Glucoamylase activity was between 2–3%. Moreover,(El-Safey, 1994) reported that, the optimal substrate (starch) concentration in reaction mixture of the MM-Glucoamylase enzyme was found to be 0.1 % (w/v) corresponding to 2% (w/v) for RH-

Glucoamylase enzyme. The effect of temperature on the activity of the purified amylase is shown in (Table32). The optimum incubation temperature for purified Glucoamylase enzyme was 50ºC. the purified Glucoamylase activity reached up to 160mg/l. While the temperature below or above 50 ºC exhibited lower activities of Glucoamylase. Other investigators were reported that, the optimum temperature for maximum purified Glucoamylase activity was 30ºC (Strumeyer and Fisher, 1982). The same finding was reported by(El-Safey, 1994) who indicated that, the purified MM-Glucoamylase displayed maximal activity at 30ºC corresponding to 50ºC for purified RH-Glucoamylase. On the other hand, (Abd El-Rahman1990) and(Lin *et al*, 1998), concluded that, the optimum temperature for purified Glucoamylase ranging from 30 to 85 ºC.(Moreover, Khoo *et al*, 1994) reported that, the optimum temperature for purified Glucoamylase was 55ºC.(Chakarabarty *et al*, 2000) found that the maximum activity of a Thermostable purified Glucoamylase was observed at 50ºC. Moreover, (Odibo and Ulbrich-Hofmann, 2001) concluded that the optimum temperature for the enzymes were 60 ºC for α-amylase and 70ºC for glucoamylase, respectively.

The enzyme activity of the Glucoamylase was determined at different pH values. As shown in (Table 30) the pH for maximal activity is 4.0with 105.5Glucoamylase produced by several fungi and bacteria are active at pH ranging from 5.5 to 8.5 (Uchino and Katano, 1981). (, El-Safey, 1994) concluded that the optimum pH values for Glucoamylase enzyme was ranged between 5.8 and 6.4 for RH-α-amylase. In addition,(Amirul *et al*, 1996) found that the optimal activities of the purified enzymes were found to have pH optimum of 4.2 and 4.5 for GA1 and GA2.(Khoo *et al*, 1994) reported that the Glucoamylase enzyme was found to have maximum activity at pH 6.0. (Abou-Zeid 1997) found that the optimal activity of the purified enzyme was achieved at pH 7.0. (Moreover, Chessa *et al*, 1999) reported that the optimum pH value for maximum Glucoamylase activity was at found pH 7.5. (Chakarabarty *et al*,

2000) concluded that the purified Glucoamylase showed a wide range of pH tolerance and maximum activity was observed at 7.0. (Moreover, Malhotra *et al*, 2002) concluded that the purified Glucoamylase showed a maximum activity at the optimum pH value of 8.0. In addition, (Lin *et al*, 1998) recorded that, the optimal pH for alpha-amylase enzyme activity was 9.0. Nevertheless,(Odibo, and Ulbrich-Hofmann, 2001) reported that the optimum pHs for enzymes were found to be 5.0 for α-amylase and 6.0 for glucoamylase. The results (Table 29) indicated that, as time increased the enzyme activity increased. The optimum incubation period for Glucoamylase activity was obtained at 30mins. (80.5 mg/l) beyond which the enzyme activity get affected due to the length of incubation period.

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APPENDEX

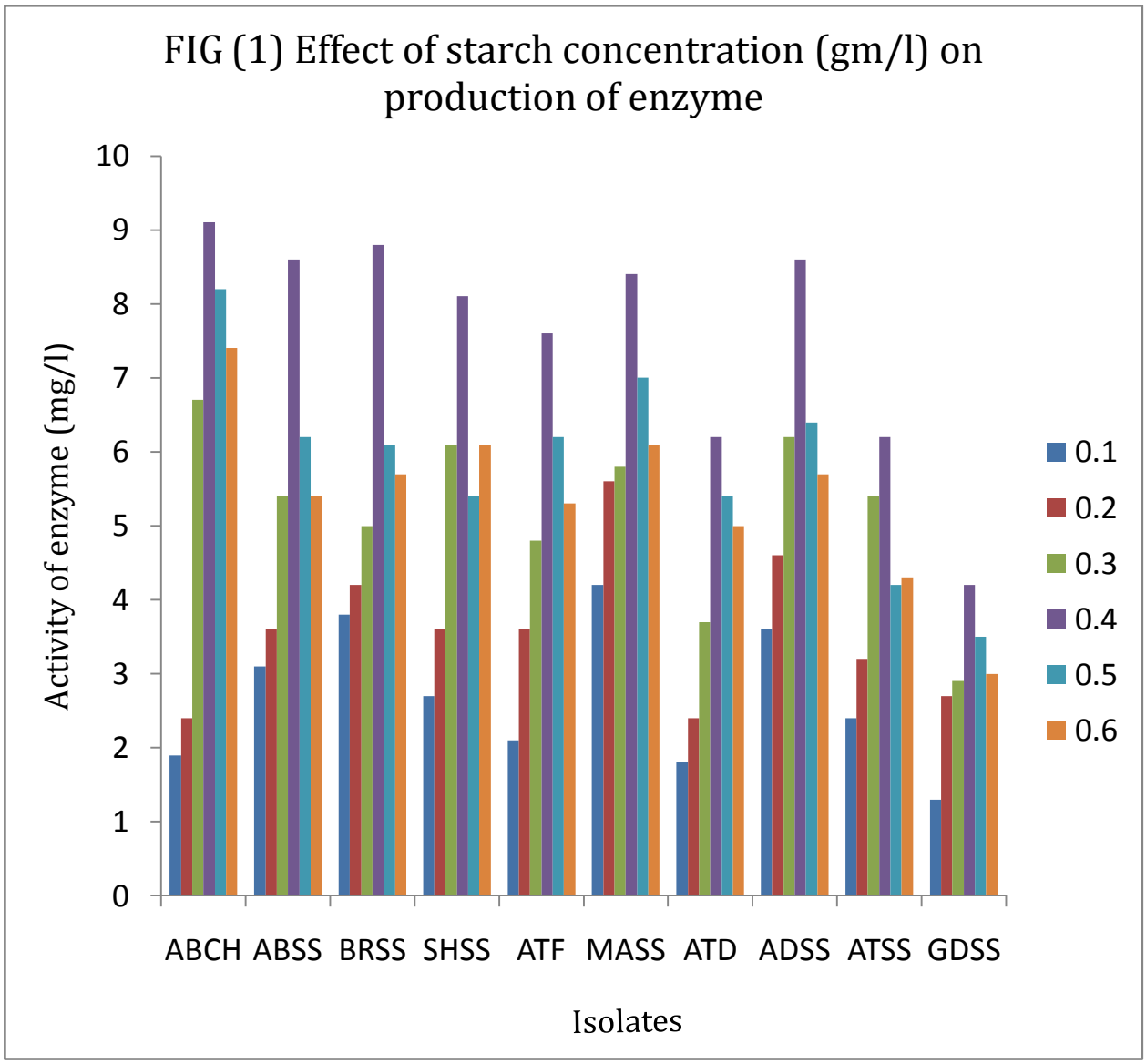


FIG (1) Effect of starch concentration (gm/l) on production of enzyme
Activity of enzyme (mg/l)
10
9
8
7
6
5
4
3
2
1
0
ABCH
ABSS
BRSS
SHSS
ATF
MASS
ATD
ADSS
ATSS
GDSS
Isolates
0.1
0.2
0.3
0.4
0.5
0.6

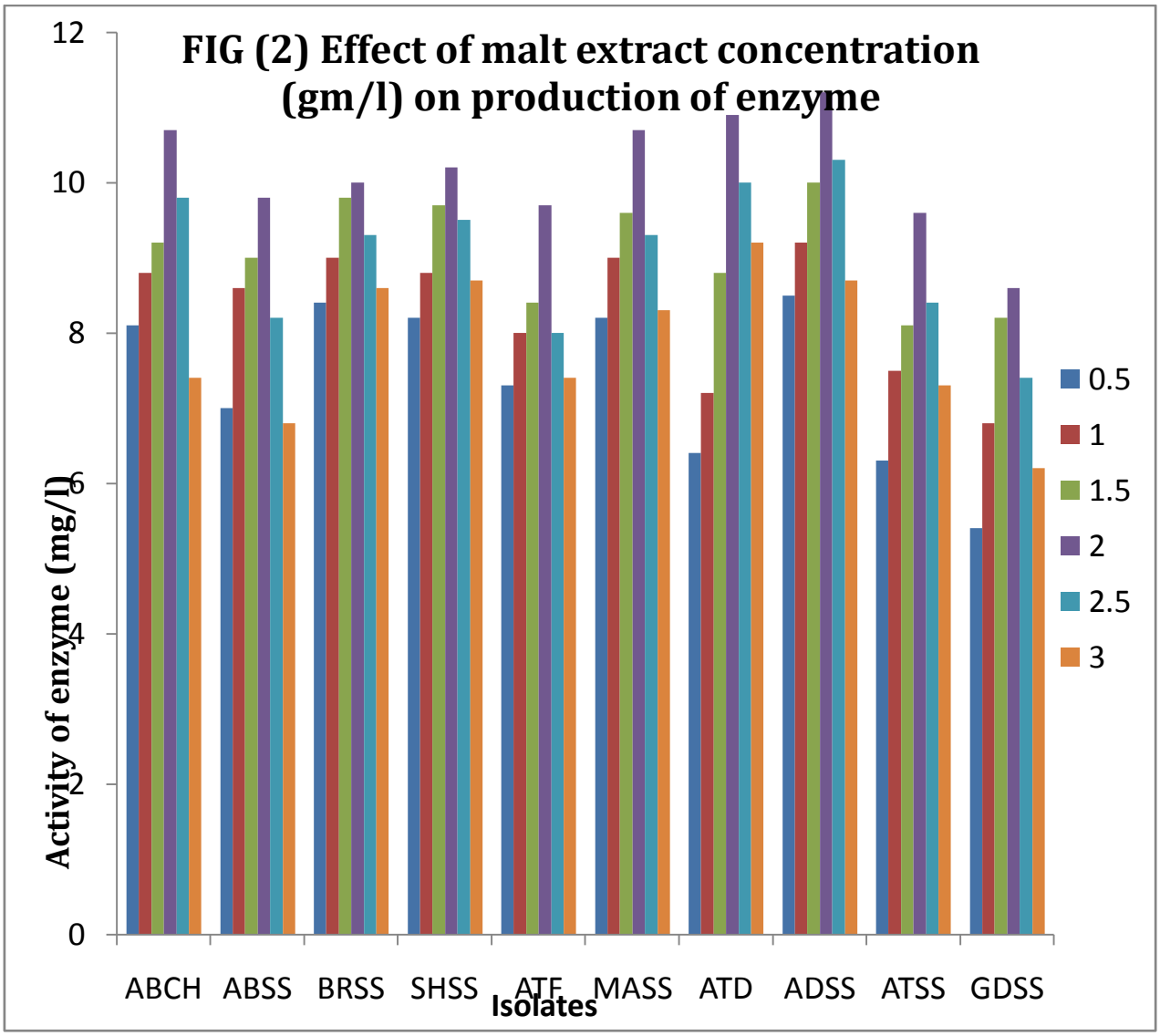
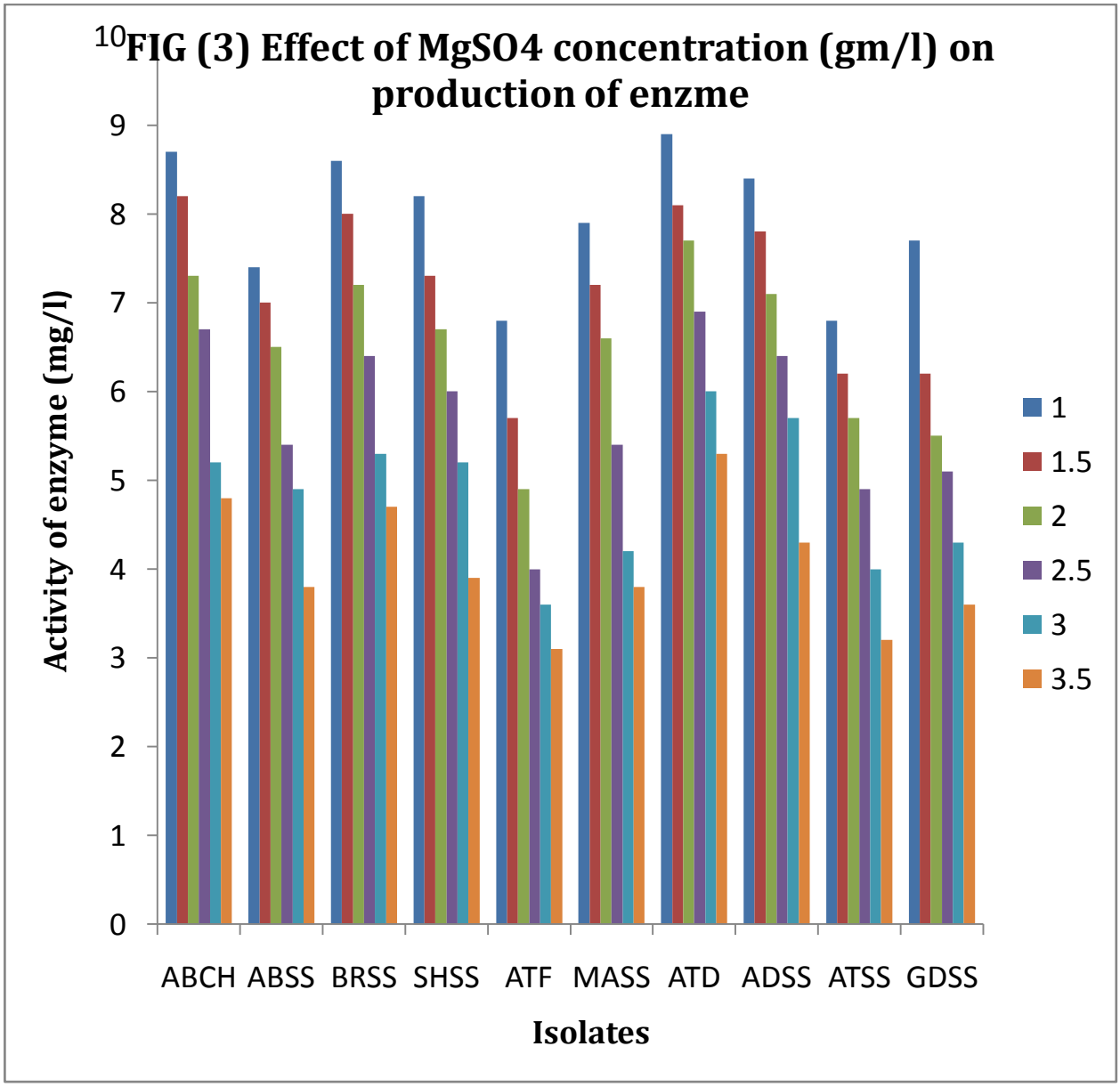
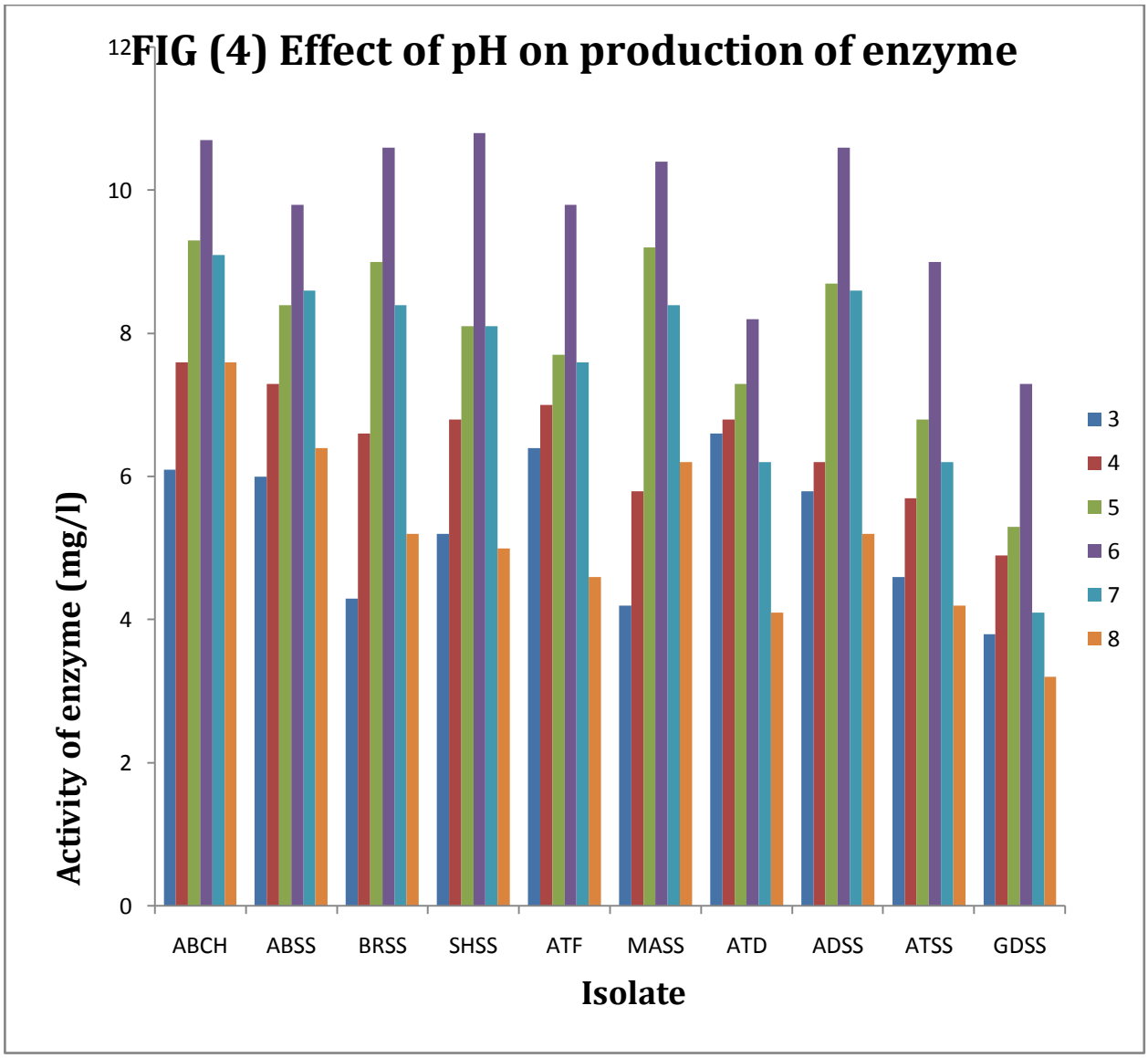


FIG (2) Effect of malt extract concentration (gm/l) on production of enzyme
Activity of enzyme (mg/l)
12
10
8
6
4
2
0
ABCH
ABSS
BRSS
SHSS
ATF
MASS
ATD
ADSS
ATSS
GDSS
Isolates
0.5
1
1.5
2
2.5
3


10FIG (3) Effect of MgSO4 concentration (gm/l) on production of enzme
Activity of enzyme (mg/l)
0
1
2
3
4
5
6
7
8
9
ABCH
ABSS
BRSS
SHSS
ATF
MASS
ATD
ADSS
ATSS
GDSS
Isolates
1
1.5
2
2.5
3
3.5


12FIG (4) Effect of pH on production of enzyme
Activity of enzyme (mg/l)
0
2
4
6
8
10
ABCH
ABSS
BRSS
SHSS
ATF
MASS
ATD
ADSS
ATSS
GDSS
Isolate
3
4
5
6
7
8

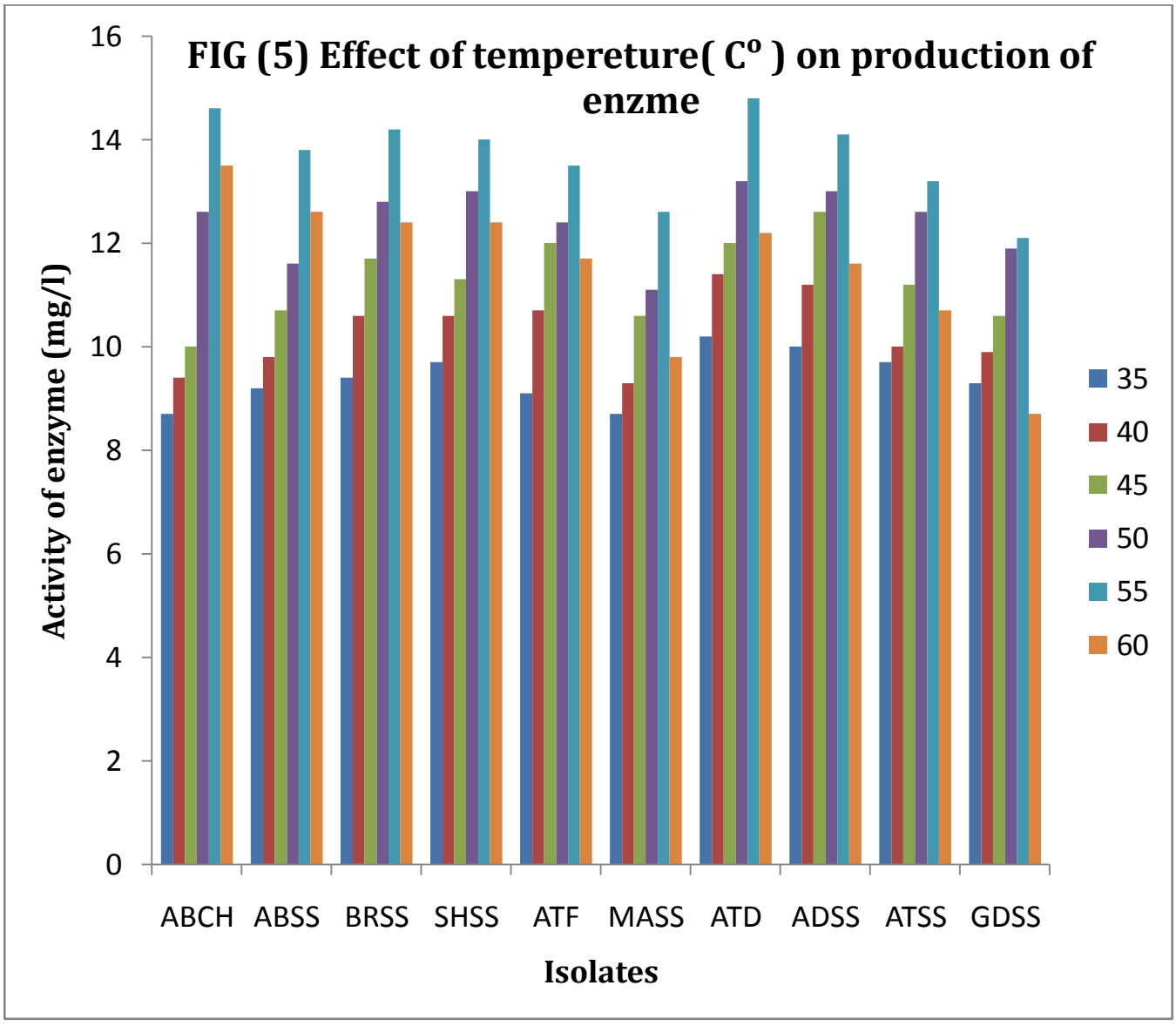


FIG (5) Effect of tempereture(Cº) on production of enzme
Activity of enzyme (mg/l)
16
14
12
10
8
6
4
2
0
ABCH
ABSS
BRSS
SHSS
ATF
MASS
ATD
ADSS
ATSS
GDSS
Isolates
35
40
45
50
55
60

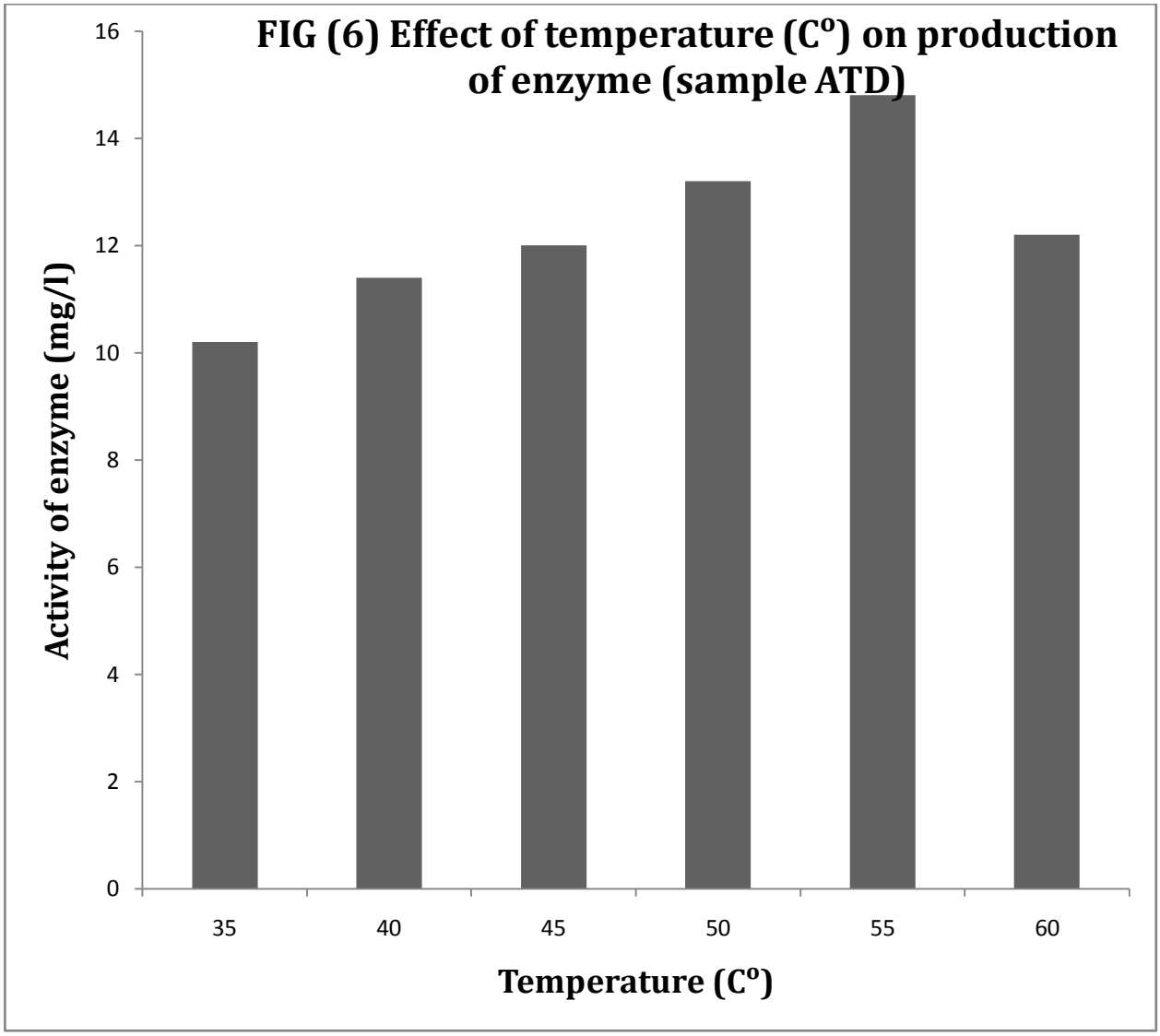


FIG (6) Effect of temperature (C°) on production of enzyme (sample ATD)
Activity of enzyme (mg/l)
16
14
12
10
8
6
4
2
0
35
40
45
50
55
60
Temperature (C°)

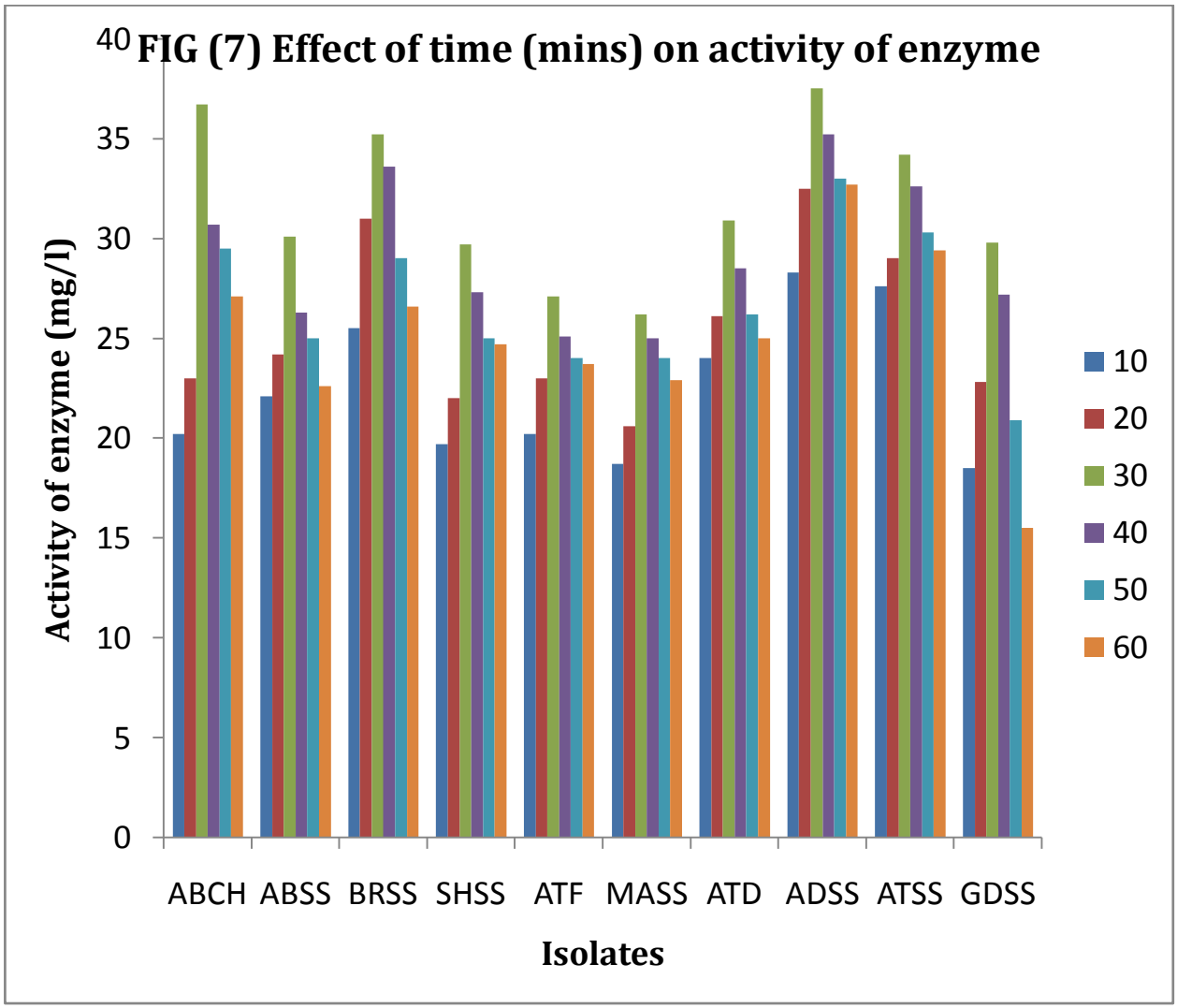


FIG (7) Effect of time (mins) on activity of enzyme
Activity of enzyme (mg/l)
40
35
30
25
20
15
10
5
0
ABCH
ABSS
BRSS
SHSS
ATF
MASS
ATD
ADSS
ATSS
GDSS
Isolates
10
20
30
40
50
60

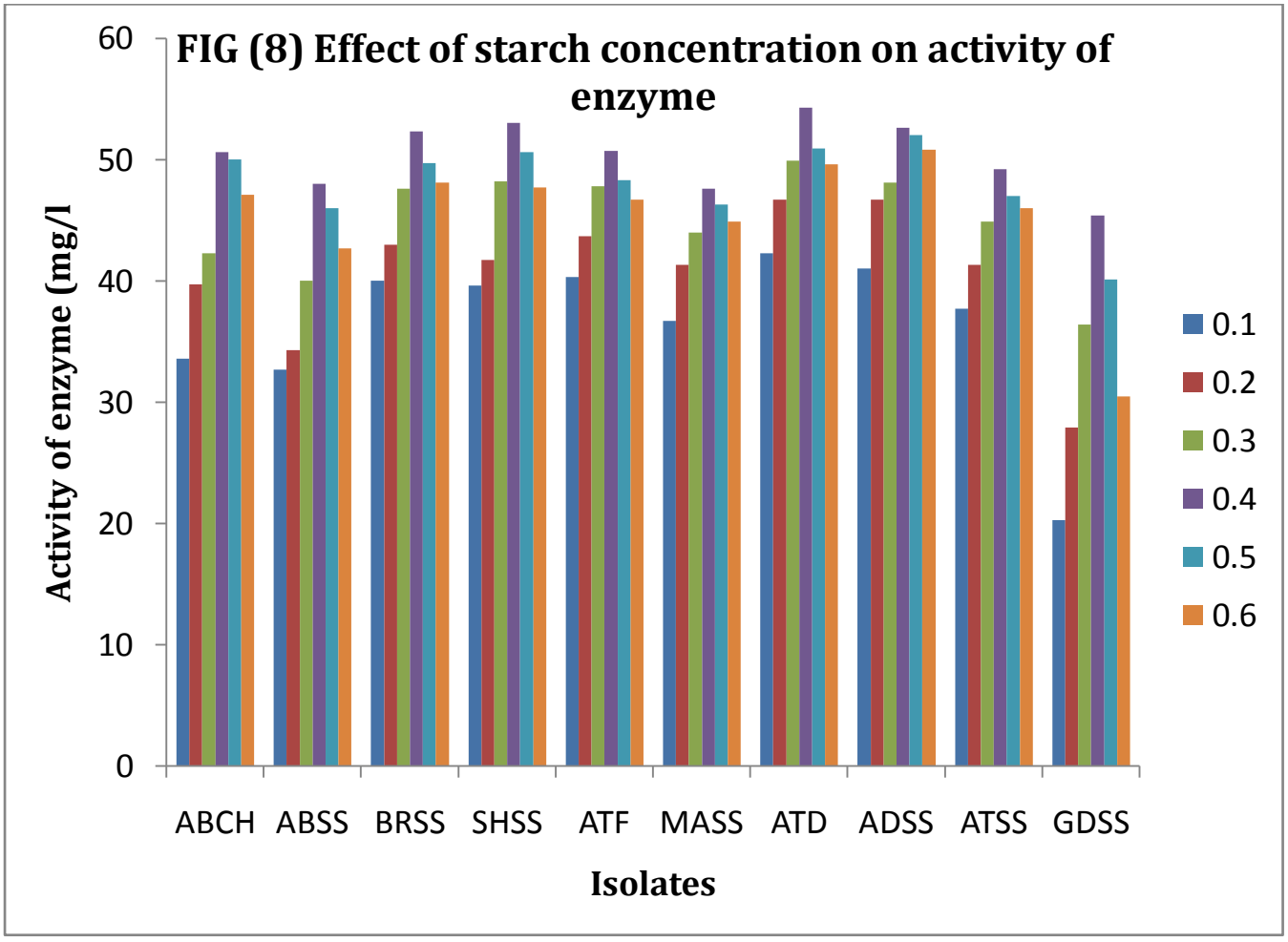


FIG (8) Effect of starch concentration on activity of enzyme
Activity of enzyme (mg/l
60
50
40
30
20
10
0
ABCH
ABSS
BRSS
SHSS
ATF
MASS
ATD
ADSS
ATSS
GDSS
Isolates
0.1
0.2
0.3
0.4
0.5
0.6

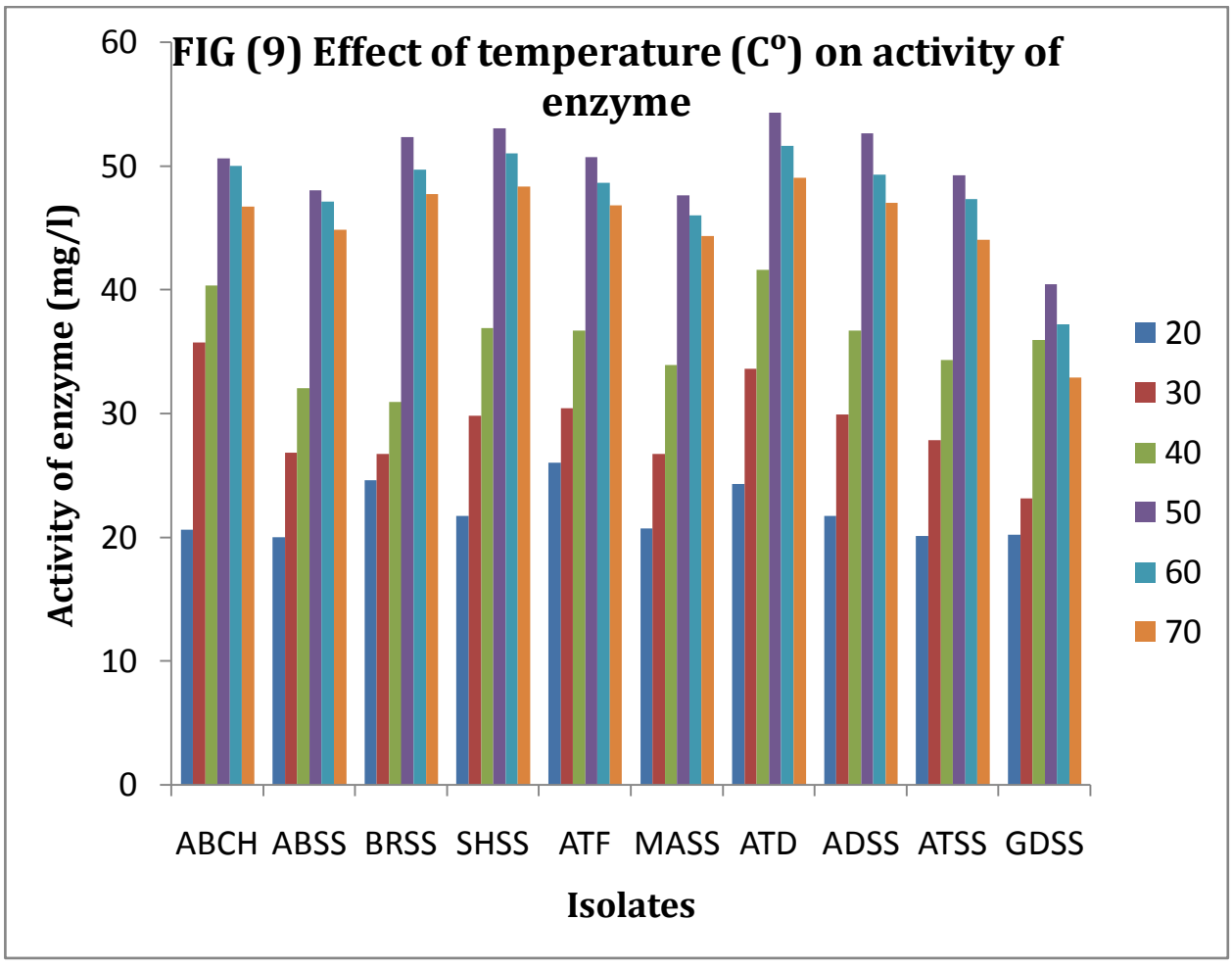


FIG (9) Effect of temperature (C°) on activity of enzyme
Activity of enzyme (mg/l)
60
50
40
30
20
10
0
ABCH
ABSS
BRSS
SHSS
ATF
MASS
ATD
ADSS
ATSS
GDSS
Isolates
20
30
40
50
60
70

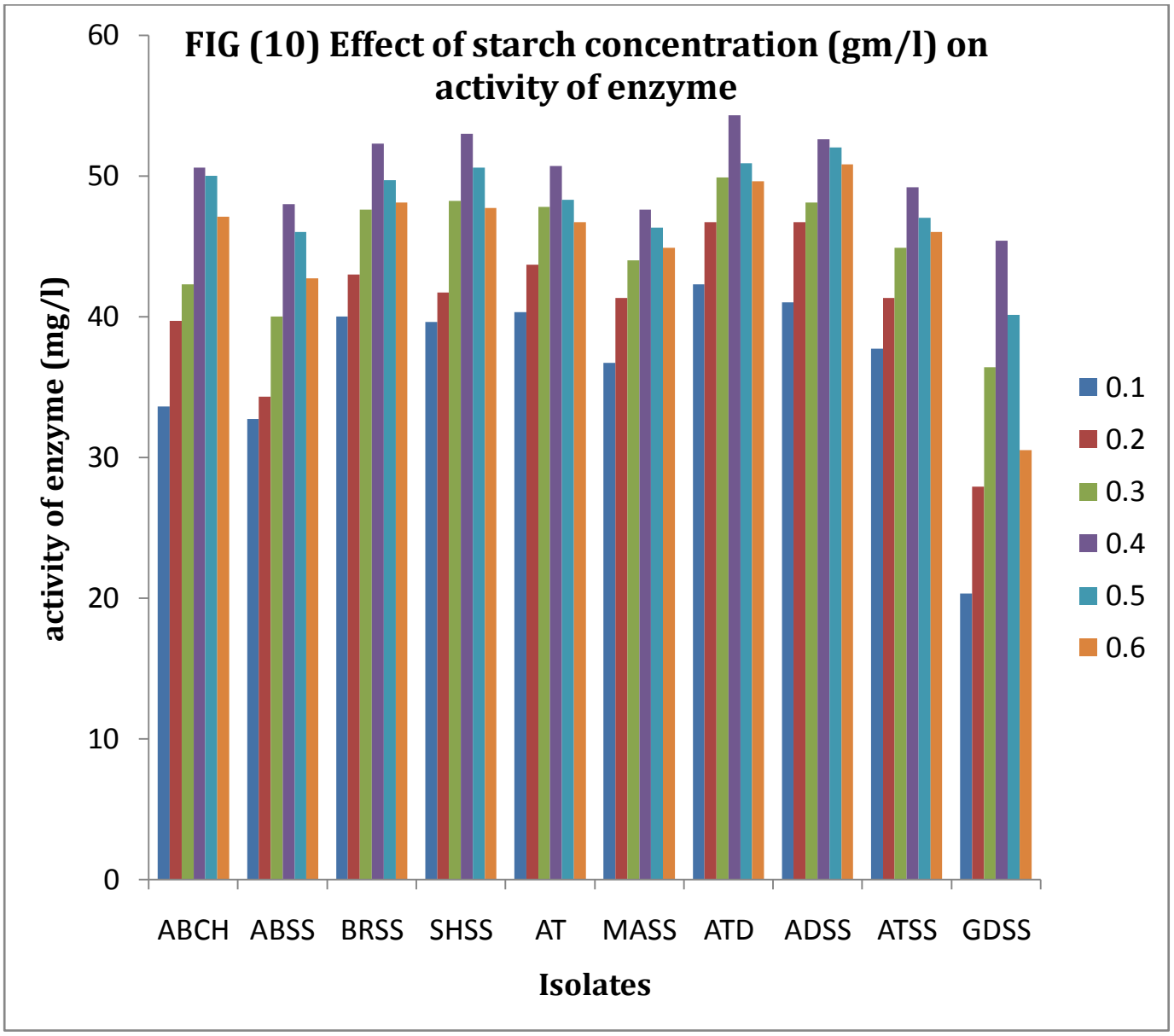
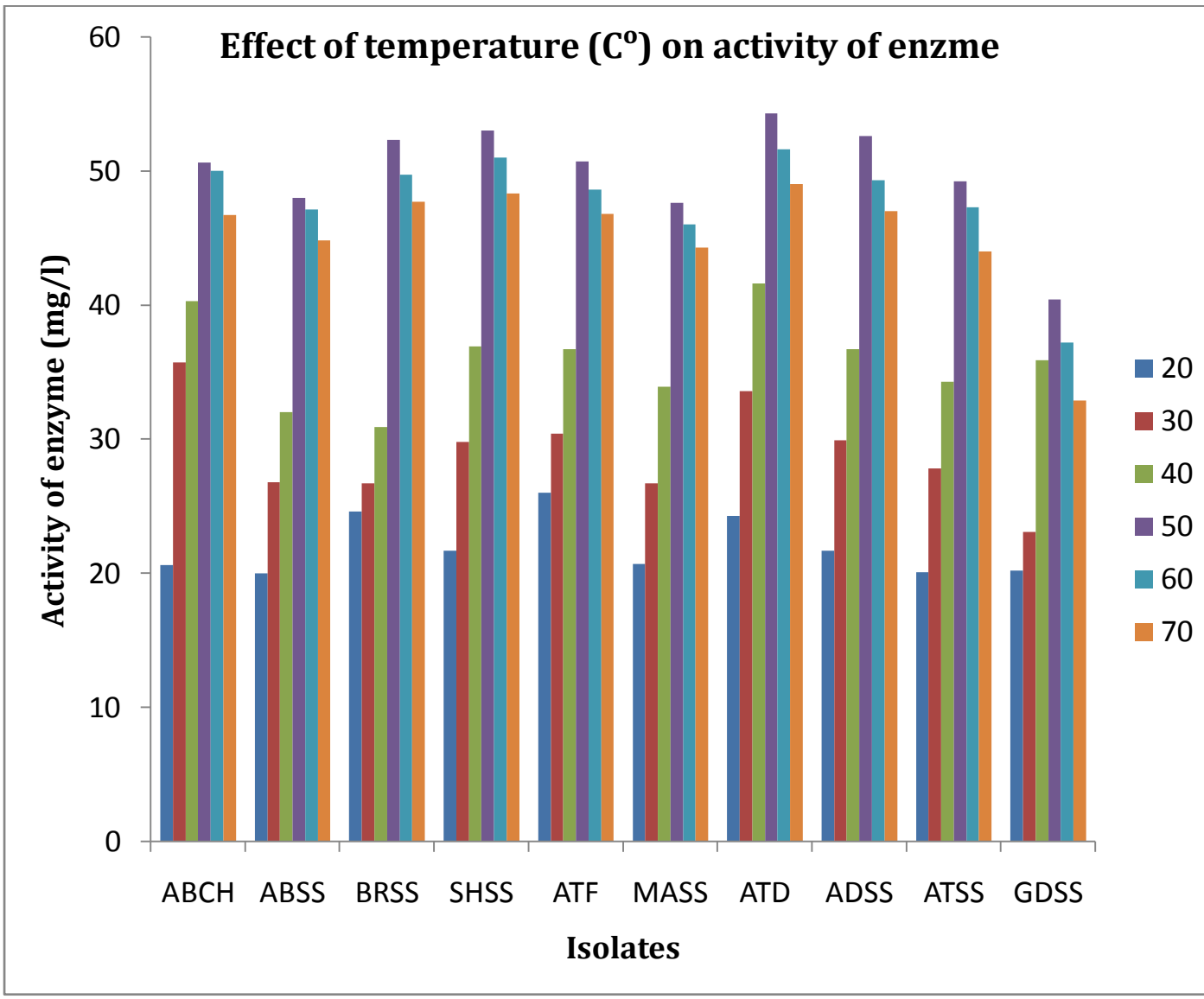
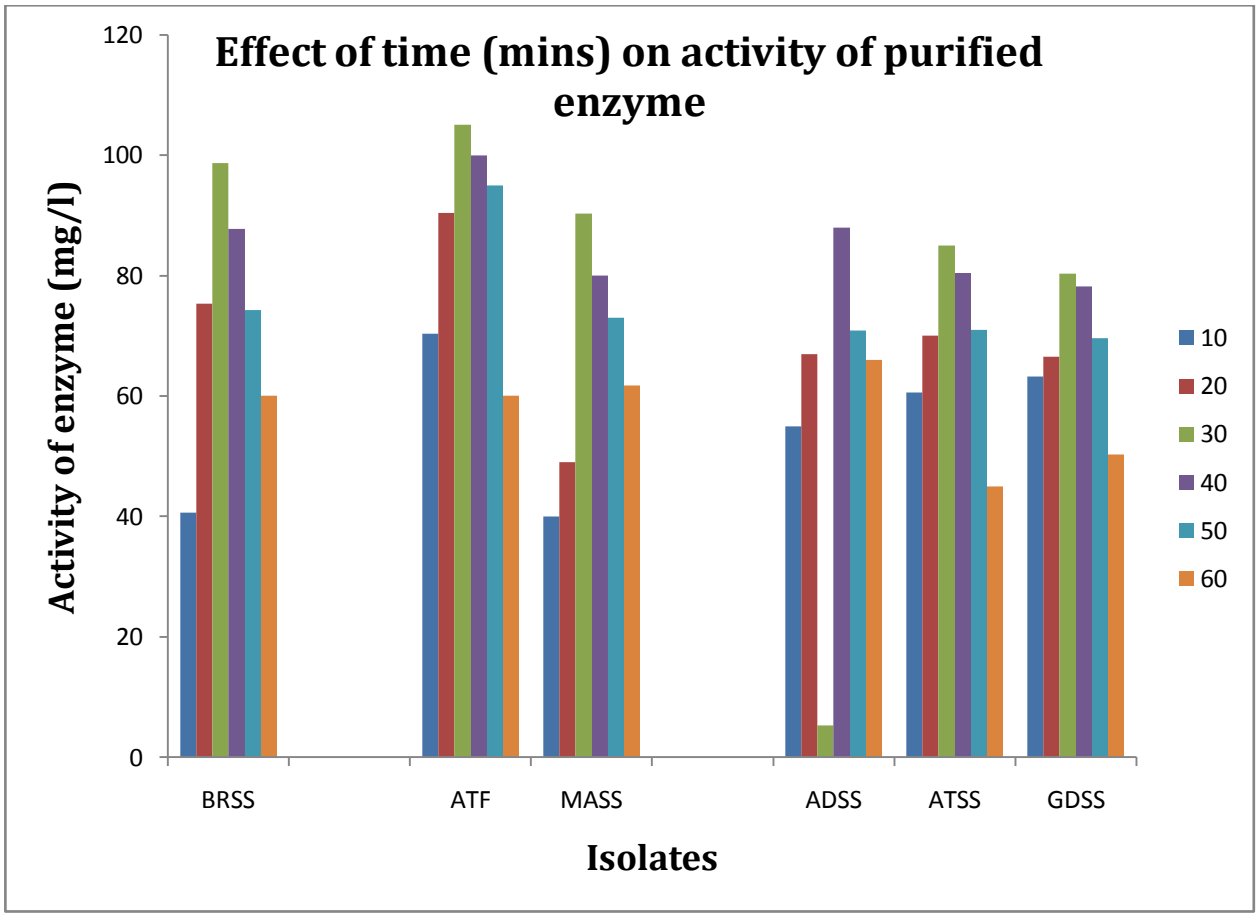


FIG (10) Effect of starch concentration (gm/l) on activity of enzyme
activity of enzyme (mg/l)
60
50
40
30
20
10
0
ABCH
ABSS
BRSS
SHSS
AT
MASS
ATD
ADSS
ATSS
GDSS
Isolates
0.1
0.2
0.3
0.4
0.5
0.6


Effect of temperature (Cº) on activity of enzme
Activity of enzyme (mg/l)
60
50
40
30
20
10
0
ABCH
ABSS
BRSS
SHSS
ATF
MASS
ATD
ADSS
ATSS
GDSS
Isolates
20
30
40
50
60
70


Effect of time (mins) on activity of purified enzyme
Activity of enzyme (mg/l)
120
100
80
60
40
20
0
BRSS
ATF
MASS
ADSS
ATSS
GDSS
Isolates
10
20
30
40
50
60

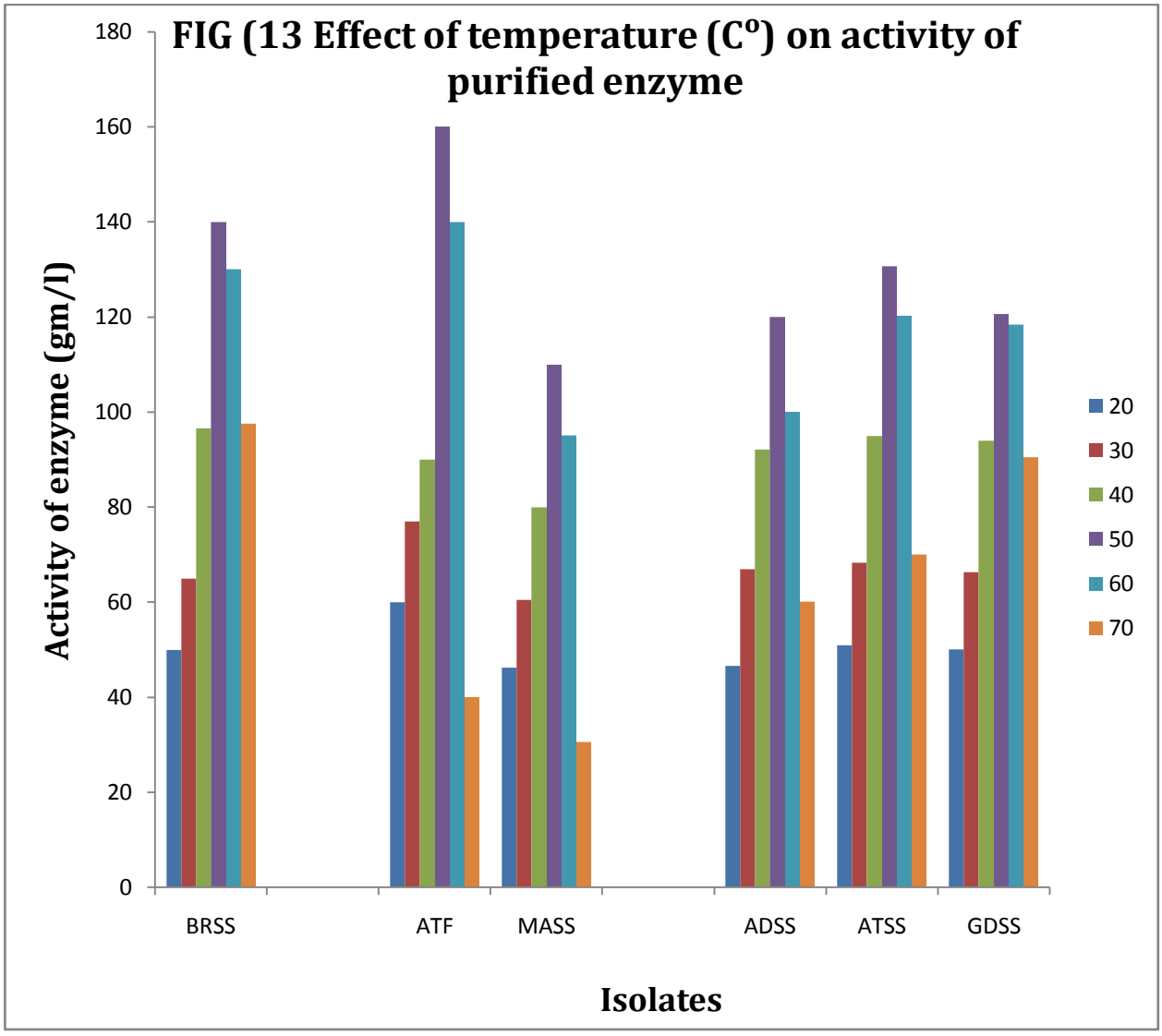
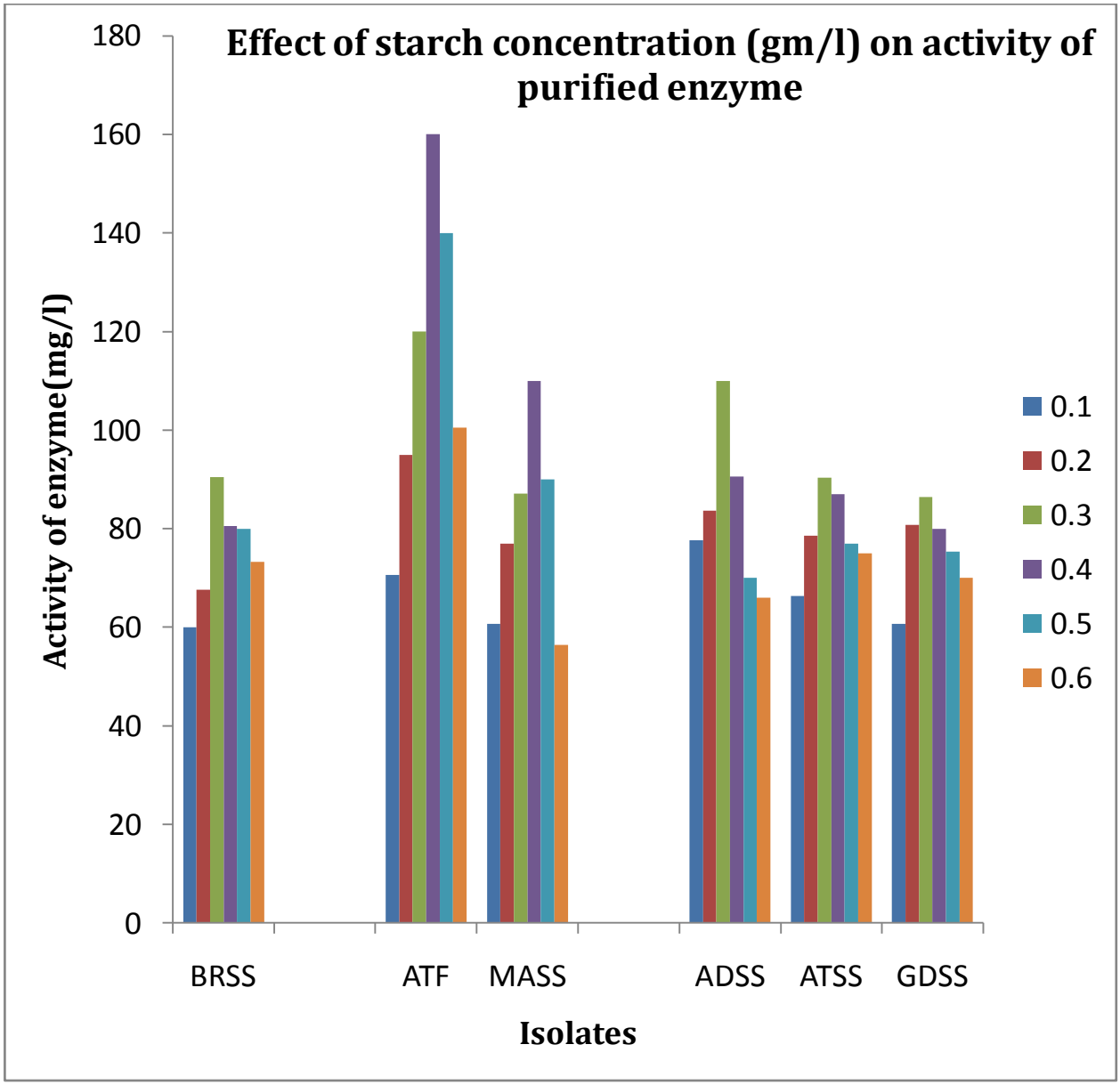


FIG (13 Effect of temperature (C°) on activity of purified enzyme
Activity of enzyme (gm/l)
180
160
140
120
100
80
60
40
20
0
BRSS
ATF
MASS
ADSS
ATSS
GDSS
Isolates
20
30
40
50
60
70


Effect of starch concentration (gm/l) on activity of purified enzyme
Activity of enzyme(mg/l)
180
160
140
120
100
80
60
40
20
0
BRSS
ATF
MASS
ADSS
ATSS
GDSS
Isolates
0.1
0.2
0.3
0.4
0.5
0.6