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Production of α-Amylase by Aspergillus oryzae, Penicillium chrysogenum and Rhizopus stolonifer causing spoilage of slice breads

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Abstract: *Amylases are a group of hydrolases that can specifically cleave the α-glycosidic linkage in starch. α-amylases (endo-1, 4-α-D-glucan glucohydrolase, EC 3.2.1.1) are extra cellular enzymes that randomly cleave the 1,4-α-D-glycosidic bonds between adjacent glucose units in the linear amylase chain and are classified according to their action and properties.*
In the present investigation production of extracellular α-amylase by Aspergillus oryzae, Penicillium chrysogenum and Rhizopus stolonifer isolated from slice breads was studied at different pH, temperature, incubation periods, carbon and nitrogen sources. The results revealed that maximum production of α-amylase was achieved at 7 days of incubation, 30^{0}C temperature and at pH 7.0. Glucose was the best carbon source which induced maximum production of α-amylase followed by fructose and sucrose. Ammonium sulphate and peptone were the best nitrogen sources for the production of α-amylase.
Key Words: *Slice breads, α-amylase, Aspergillus oryzae, Penicillium chrysogenum, Rhizopus stolonifer*

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I. Introduction

Bread is a stable food prepared by cooking a dough of flour and water and some additional ingredients. Salt, fat and leave ling agents such as Yeast *(Saccharomyces cerevisiae*) and baking soda are common ingredients. Bread may contain milk, egg, sugar, spice, fruit, vegetables, nuts or seeds.

Fresh bread is prized for its tastes, aroma, quality, appearance and texture. There are several different types of bread prepared around the world, viz., white bread, brown bread, whole meal bread, wheat germ bread, whole grain bread, Roti or Chapatti, Granary bread, Rye bread, unleavened bread or matzo, sourdough bread, flat bread, hemp bread, crisp bread etc.

In breads the amount of flour is always stated as 100% and the amounts of the rest of the ingredients are expressed as a percent of that amount by weight. The grains used in flour making provides starch and proteins needed to form bread. The protein content of the flour is the best indicator of the quality of bread. In addition to starch, the wheat flour contains three water soluble proteins viz., albumin, globulin and proteases, and two water insoluble protein, glutenin and gliadin. When flour is mixed with water, the water soluble proteins dissolve, leaving the glutenin and gliadin to form the structure of the resulting bread. Ascorbic acid, hydrochloride, sodium metabisulphate, ammonium chloride, various phosphates, amylase and protease are commonly used as ingredients to improve the quality of bread. In addition to these, three natural phenolic glucosides viz., secoisolariciresinol, p-coumaric acid glucoside and Ferulic acid glucoside are also found in commercial breads.

The composition of a typical white and wheat slice bread can be summarized as follows:

Nutritional value per 100gm(3.5oz)	White bread (typical bread)	Brown bread (Whole wheat bread)
Energy	1,113 KJ(266k.cal)	1,034KJ(247kcal)
Carbohydrates	51g	41g
Dietary fiber	2.4g	7g
Fat	3g	3g
Protein	8g	13g
Thiamine(Vit.B1)	0.5mg(43%)	0.4mg(35%)
Riboflavin(Vit.B2)	0.3mg(25%)	0.2mg(17%)
Niacin(Vit.B3)	4mg(27%)	4.7mg(31%)

Folate(Vit.B9)	111µg(28%)	50µg(13%)
Choline	14.6mg(3%)	26.5(5%)
Vitamin K	3.1µg(3%)	7.8µg(7%)
Calcium	151mg(15%)	107mg(11%)
Iron	3.74mg(29%)	2.43mg(19%)
Magnesium	23mg(6%)	82mg(23%)
Potassium	100mg(2%)	248mg(5%)
Sodium	681mg(45%)	472mg(31%)

Percentages are relative to US recommendation for adult

Slice bread is subjected to microbial attack that causes spoilage of nutrients by way of decomposition. Carbohydrates (starch), fats and proteins are the basic constituents of slice bread. Hence slice bread is susceptible to degradation by a great many species of fungi, yeasts and bacteria. The microorganisms causing breakdown of carbohydrates, fats and proteins vary with the environment. Under aerobic conditions a wide range of saprophytic fungi such as *Mucor, Rhizopus, Eurotium, penicillium, Aspergillus, Cladosporium, Auriobasidium, Thermoascus, Monila,* etc. colonize the bread. Growth of amylolytic, lipolytic and proteolytic fungi helps in the decomposition and spoilage of bread. Among different groups of microbes, fungi have an edge over others in initiating the breakdown of solid substrata, partly because of their superior enzymatic equipment and partly because of their filamentous growth.

Slice bread provides a suitable substratum for fungi. The fungi cause complete spoilage and destruction of bread by their amylolytic, lipolytic and proteolytic activity and so it has been decided to study the role played by some dominant fungal flora in the spoilage of slice bread. The quality of bread, the physical factors of the environment helping spoilage of bread and the chemical factors greatly affect the growth and sporulation of fungi colonizing the bread.

Amylases are a group of hydrolases that can specifically cleave the α-glycosidic linkage in starch. Two important groups of amylases are glucoamylase and α-amylase. Glucoamylase (exo- 1,4-α-D-glucan glucanohydrolase) produces single glucose units from nonreducing ends of amylose and amylopectin (Anto *et al*., (2006) [1]. Whereas, α-amylases (endo-1,4-α-D-glucan glucohydrolase) are extracellular enzymes that randomly cleave the 1,4-α-D-glucosidic linkage between adjacent glucose units inside the linear amylose chain (Pandey *et al*., (2005); Castro *et al*., (2010) [2, 3]. α-amylases are widely distributed in nature and can be derived from various sources such as plants, animals and microorganisms (Pandey *et al*., (2005); Omemu *et al*., (2005) [2, 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.

Nowadays, the new potential of using microorganisms as biotechnological sources of industrially relevant enzymes has stimulated renewed interest in the exploration of extracellular enzymatic activity in several microorganisms (Akpan *et al*., 1999; Bizzini and Martini (2002); Gupta *et al*., 2008) [5, 6, 7]. Many species of *Aspergillus* and *Rhizopus* are used as a source of fungal α-amylase (Pandey *et al*., 2005; Gupta *et al*., (2008; Khan and Yadav, 2011; Kim *et al*., (2011); Irfan and Nadeem, 2012) [2, 7, 8, 9, 10]. Spectrum of applications of α-amylase is also extending in many other areas such as analytical chemistry, clinical and medicinal diagnosis (Murlikrishna and Nirmala, 2005; Chimata *et al*., 2010; Nimkar *et al*., 2010) [11, 12, 13].

There are various types of amylases, namely α, β, and glucoamylases. α-amylases (endo-1, 4-α-D-glucan glucohydrolase, EC 3.2.1.1) are extra cellular enzymes that randomly cleave the 1,4-α-D-glycosidic bonds between adjacent glucose units in the linear amylase chain and are classified according to their action and properties. β-amylases (β-1, 4-glucan maltohydrolase, EC 3.2.1.2) are usually of plant origin, but a few microbial strains are also known to produce them. It is an exoacting enzyme that cleaves nonreducing ends of amylose, amylopectin, and glycogenmolecule. Glucoamylase (amyloglucosidase, glucanogenic enzymes, starch glucogenase, and exo-1, 4-α-D-glucan glucanohydrolase (EC 3.1.2.3)) hydrolyses single glucose units from the nonreducing ends of amylose and amylopectin in stepwise manner. The fungal amylases are preferred over other microbial sources because of their more acceptable and regarded as safe.

The present investigation is aimed to study the extracellular production of α-amylase by *Aspergillus oryzae, Penicillium chrysogenum* and *Rhizopus stolonifer* causing spoilage of slice bread.

II. Materials and Methods

Slice breads were collected from local market of Patna. They were kept in laboratory in a sterilized polythene bag for laboratory analysis of fungi.

Isolation and Identification of fungi: Bread mycoflora were isolated from spoiled slice breads by standard blotter method (baiting) (Baki and Anderson, 1973) [14] on potato dextrose agar medium consisted of potato 200 g, dextrose 20 g, agar 20 g, and distilled water 1 L. 0.5 mg/ml of amoxicillin was added to PDA medium as anibacterial agent. Five slice breads were kept separately in moist Petri dishes. Experiment was conducted in replicates of five. All Petri dishes were incubated at 28°C for 6 days. Spoiled breads were assayed for their

fungal contents. Fungi were identified on the basis of macro- and microscopic characteristics. All fungal cultures were confirmed by studying the morphology of colonies, microscopic examination and characterization and spore shape and colour following standard keys (Ainsworth and Bisby, 1971; Domsch and Gams, 1972; Domsch *et al*., 1980; Ellis, 1976; Lesli and Summerell, 2006; Pitt, 1985) [15, 16, 17, 18, 19, 20].

Screening of fungi for α-amylase production: Eleven fungal floras were isolated from spoiled slice breads viz. *Penicillium chrysogenum*, *Mucor* sp. *Rhizopus stolonifer*, *Fusarium oxysporum*, *Alternaria* sp. *Phoma* sp. *Curvularia lunata*, *Cladosporium herbarum*, *Eurotium* sp. and one yeast *Pitchia burtonii*. Out of which only three isolates viz. *Aspergillus oryzae, Penicillium chrysogenum* and *Rhizopus stilonifer* were screened for their ability to produce extracellular α-amylase production under different cultural conditions.
Screening of fungal α-amylase activity: *Aspergillus oryzae, Penicillium chrysogenum* and *Rhizopus stolonifer* isolated from spoiled slice breads were assayed for their activity to produce extracellular α-amylase. These three fungal isolates were cultured on solid starch yeast extract agar (SYE) medium consisted of soluble starch, 5.0; yeast extract, 2.0; KH2PO4, 1.0; MgSO4.7H2O, 0.5 and agar, 15g and distilled water 1L (Barnett (1971) [21]. Fungal isolates were tested for amylase production by starch hydrolysis. Starch agar medium consisted of peptone, 0.5; beef extract, 0.15; yeast extract, 0.15; sodium chloride, 0.5; starch, 1; agar, 2 g; distilled water, 100 ml was inoculated with fungal isolates separately and incubated at 30°C, then flooded with iodine solution (Iodine, 0.2; potassium iodide, 0.4; distilled water, 100 ml). The clear zone around fungal growth indicated the production of amylase (Suganthi *et al*., (2011) [22]. On the basis of the clear area, *Aspergillus oryzae*, *Penicillium chrysogenum* and *Rhizopus stolonifer* were selected for further assays on amylase production.

Amylase activity was estimated by analyzing the reducing sugar released during hydrolysis of 1% (w/v) starch in 0.1 M phosphate buffer, pH 6.5, at 25ºC for 20 min by the Dinitrosalicylic acid method (Miller, 1959). One unit of amylase activity was defined as the amount of enzyme that releases 1 µmol of reducing sugar as glucose per min under the assay conditions. Enzyme activity is expressed as specific activity, which is represented as U/mg of protein. The protein concentration was determined by the Lowry's method (Lowry et al., 1951) using bovine serum albumin as the standard. The amount of reducing sugars released was estimated by determining the optical density i.e. absorbance at 700 nm wave length using the spectrophotometer.

Optimization of Cultural conditions for production of α-amylase by fungal isolates:
Effect of Incubation period on the production of α-amylase: The fungal isolates viz. *Aspergillus oryzae, Penicillium chrysogenum* and *Rhizopus stolonifer* were cultured separately for six different incubation periods viz. 4, 5, 6, 7, 8 and 9 days for their production of α-amylase. Fifty ml of SYE liquid medium (pH 6.0) were dispensed into 250 ml Erlenmeyer flasks and then autoclaved for 15 minutes at 1.5 atm. Each flask was inoculated with two agar mycelial discs (10 mm diameter) obtained from 7 days old cultures. Inoculated flasks were incubated at 28°C. After 2 days intervals, cultures were filtered. Clear supernatants obtained after centrifugation of filtrates were used for assaying amylase activity.

Effect of temperature on the production of α-amylase: The influence of six different temperatures viz. 15, 20, 25, 30, 35 and 40°C on the activity of amylase was investigated by incubation of *A. oryzae, Penicillium chrysogenum* and *R. stolonifer* cultures in liquid medium for 7days. After the incubation period, the cultures were filtered. The filtrates were centrifuged and the clear supernatants were used for assaying amylase activity.

Effect of pH values on the production of α-amylase: The influence of six different pH viz. 4, 5, 6, 7, 8, 9 on amylase production was studied by incubating *A. oryzae, P. chrysogenum* and *R. stolonifer* cultures at 30°C in liquid medium previously adjusted to different pH values for 7 days. After the incubation period, the filtrates were centrifuged and the clear supernatants were used for assaying amylase activity.

Effect of different carbon sources on the production of α-amylase: The effect of seven different carbon sources viz. Glucose, Fructose, Maltose, Lactose, Sucrose, Cellulose and Starch on the production of α-amylase by the three fungal isolates viz, *A. oryzae, P. chrysogenum* and *R. stolonifer* was investigated. The fungal isolates were grown in Erlenmeyer flasks (250 ml) containing 50 ml liquid medium. Seven different carbon sources were added individually to the basal medium by 1% ratio. The flasks were sterilized at 121°C for 20 minutes, inoculated with two mycelial discs (10 mm) cut out from 7 days fungal cultures grown on potato dextrose agar medium. Inoculated flasks were incubated at 30°C for 7 days. At the end of the incubation period, amylase activity was determined.

Effect of different nitrogen sources on the production of α-amylase: Similarly the effect of different nitrogen sources viz. ammonium chloride, ammonium sulphate, ammonium nitrate, peptone, potassium nitrate, calcium nitrate and sodium nitrate on the production of α-amylase was investigated.

A. oryzae, *Penicillium chrysogenum* and *R. stolonifer* were grown in Erlenmeyer flasks (250 ml) containing 50 ml of liquid medium. Seven nitrogen sources viz. ammonium chloride, ammonium sulphate, ammonium nitrate, peptone, potassium nitrate, calcium nitrate and sodium nitrate) were added individually to the basal medium by 0.3% ratio. The flasks were sterilized at 121°C for 20 minutes, inoculated with two mycelial discs (10 mm) cut out from 7 days fungal cultures grown on potato dextrose agar medium. The inoculated flasks were incubated at 30°C for 7 days. At the end of the incubation period, amylase activity was determined.

All experiments were carried out in triplicates, and repeated three times. The samples collected from each replicate were tested for amylase production and activity. The data were analyzed by measuring SD and SE at 5% level of significance.

III. Results and Discussion

Fungal isolates were tested for amylase production by starch hydrolysis on starch agar medium. The clear zone around fungal growth indicated the production of amylase. The results revealed that *Aspergillus oryzae* and *Rhizopus stolonifer* were more active in producing α-amylase in comparision to *Penicillium chrysogenum* (Figure-1and 2). The present findings gain support from the work of Salem and Ebrahim (2013) [25] who have studied the production of α-amylase by *Aspergillus niger* and *Rhizopus stolonifer* and found highest activity of extracellular α-amylase by these fungi.

Varalakshmi *et al*., (2009) [26] reported that *Aspergillus niger* JG124 was the best amylase producer. Mishra and Dadhich (2010) [27] examined fifteen isolates of filamentous fungi obtained from soil samples for their ability to produce amylase. *Aspergillus niger* RJ1 produced the highest level of extracellular amylase. Recently, Tripathy *et al*., (2011) [28] investigated 66 fungal isolates for amylase production. Major number of isolates showed presence of amylolytic activity. 9% of total culture isolates yielding high production of amylase.

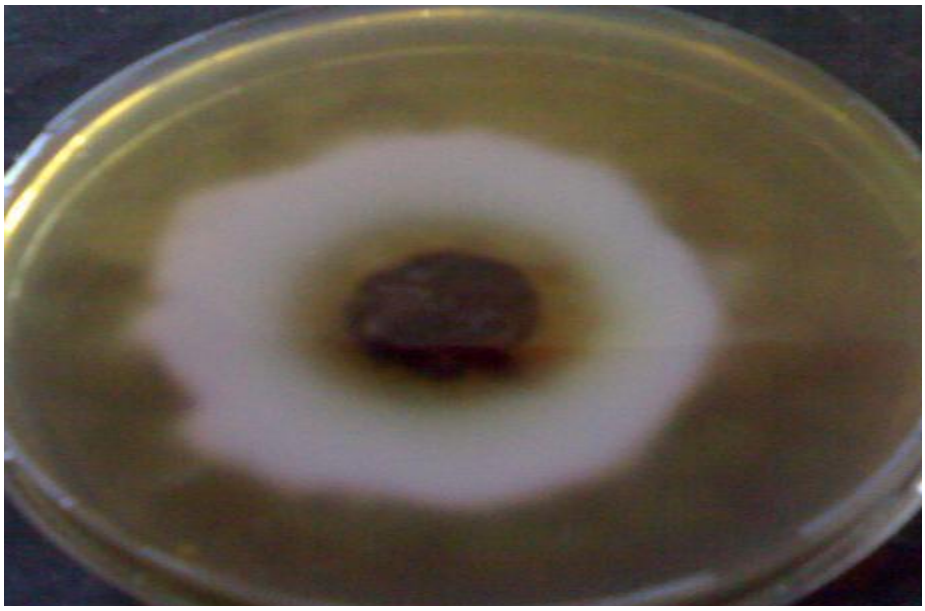

Figure-1: α-amylase activity by *Aspergillus oryzae*

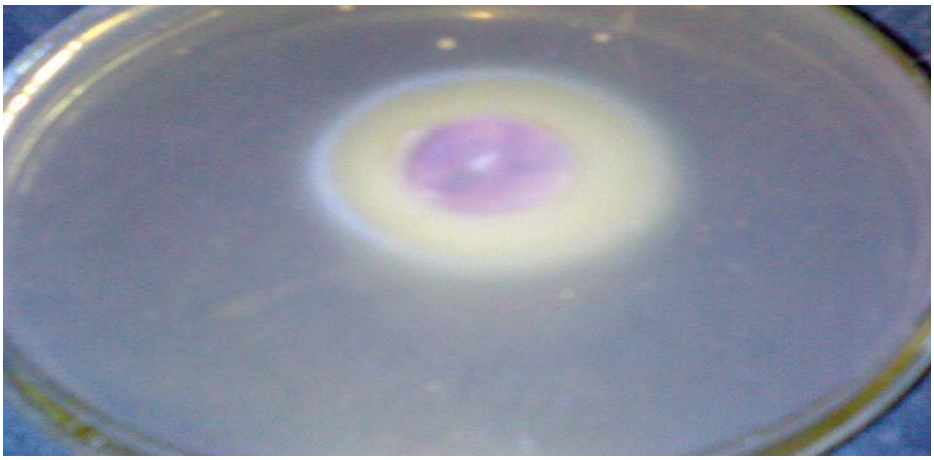

Figure-2: α-amylase activity of *Rhizopus stolonifer*

Effect of Incubation Period on the production of α-amylase: Amylase enzyme produced by *A. oryzae*, *Penicillium chrysogenum* and *R. stolonifer* increased with the increased incubation period showing its maximum activity after 7 days. After 7 days of incubation the α-amylase activity decreased (Table-1; Fig-3). *Aspergillus oryzae* and *Rhizopus stolonifer showed* maximul α-amylase activity in comparison to *Penicillium chrysogenum.* At 7 days of incubation *A. oryzae* and *R. stolonifer* showed maximal enzyme activity (7.8 U/mg and 8.0 U/mg of protein respectively (Table-1; Figure-3). Uguru *et al.*, (2011) [29] and Gupta *et al.*, (2008) [7] reported that the maximum production of α-amylase by *A. niger* which was achieved after 6 and 5 days of incubation, respectively. Singh *et al.*, (2009) [30] studied α-amylase production by *Humicola lanuginosa*. Maximum amylase production was observed after 144 hours of incubation. Recently, Chimata *et al.*, (2010) [12] reported that the best α-amylase production by *Aspergillus* MK07 were after 120 hours of incubation. Erdal and Taskin (2010) [31] revealed that maximum production of amylase by *Penicillium expansum* was achieved after 6 days of incubation. Alva *et al.*, (2007) [32] have also studied the maximal α-amylase activity of *Aspergillus* sp. after 6 days of incubation.

Table-1: Effect of Incubation period on α-amylase activity in U/mg of protein of three fungal isolates

Fungal isolates	Days of Incubation					
	4	5	6	7	8	9
Aspergillus oryzae	0.8	1.8	6.7	7.8	6.9	0.5
Penicillium chrysogenum	0.5	1.0	4.5	5.7	5.0	0.2
Rhizopus stolonifer	0.8	1.9	7.0	8.0	6.5	0.4

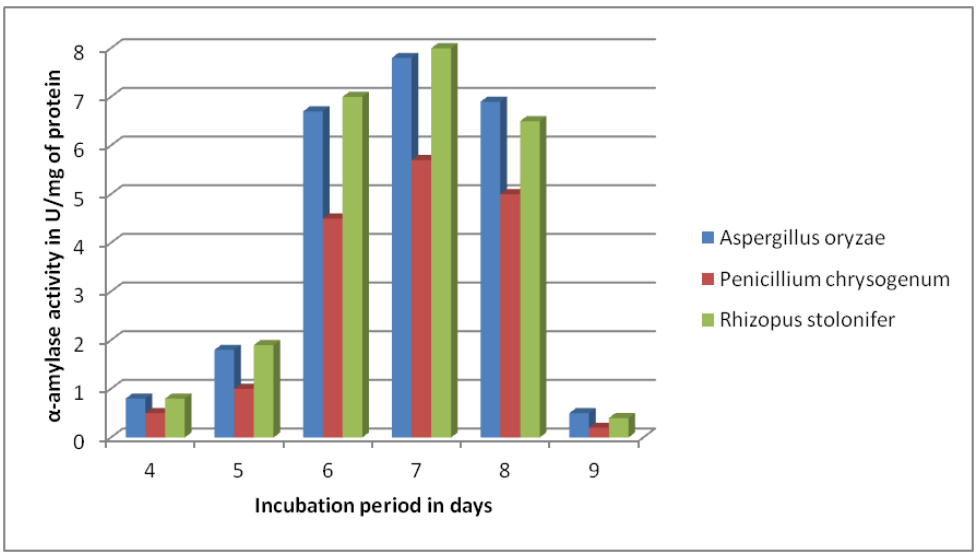

Figure-3: α-amylase activity in U/mg of protein by three fungal isolates

Effect of temperature on α-amylase activity in U/mg of protein of three fungal isolates

From the results it is evident that maximum production of α-amylase was achieved at 30^0C by all the three fungal isolates viz. *Aspergillus oryzae, Penicillium chrysogenum* and *Rhizopus stolonifer*. Production of α-amylase by A.oryzae and R. stolonifer was maximum (12.5 U/mg of protein and 12.7 U/mg of protein respectively. However, α-amylase production by *Penicillium chrysogenum* was comparatively low (8.5 U/mg of protein) at this temperature (Table-2; Figure-4). At 15, 20, 25 and 40°C α-amylase was also synthesized by the present fungal isolates, but its amounts were generally low (Table-2; Figure-4). Saleem and Ebrahim (2013) [25] have also observed maximum amylase production by *Aspergillus niger* and *Rhizopus stolonifer* at 30^0C. Haq *et al.,* (2002) [33] and Gupta *et al.,* (2008) [7] found that the optimum temperature for production of amylase by *A. niger* was 30°C. However, Khan and Yadav (2011) [8] reported that amylase production by *A. niger* was optimum at 28°C. Alva *et al.,* (2007) [53] and Chimata *et al.,*(2010) [12 reported that 30°C was the optimum temperature for amylase production by *Aspergillus.* Erdal and Taskin (2010) [35] revealed that maximum production of amylase by *Penicillium expansum* was achieved at 30**°**C. Irfan *et al.,* (2012). [10] have also reported that maximum α-amylase production by *A.niger*-ML-17 and *R. oligosporus* –ML-10 was recorded at 30 and 35°C, respectively.

Table-2: Production of α-amylase by three fungal isolates at different temperature (α-amylase in U/mg of protein)

Fungal isolates	Temperature in degree Celsius (^{0}C)					
	15	20	25	30	35	40
Aspergillus. oryzae	6.2 ±0.11	6.7 ±0.12	7.2 ±0.17	12.5 ±0.12	9.5 ±0.12	6.5 ±0.11
Penicillium chrysogenum	5.5 ±0.11	6.0 ±0.10	6.5 ±0.11	8.5 ±0.15	8.0 ±0.13	6.5 ±0.13
Rhizopus stolonifer	6.0 ±0.13	6.6 ±0.14	7.0 ±0.14	12.7 ±0.11	9.2 ±0.14	6.0 ±0.16

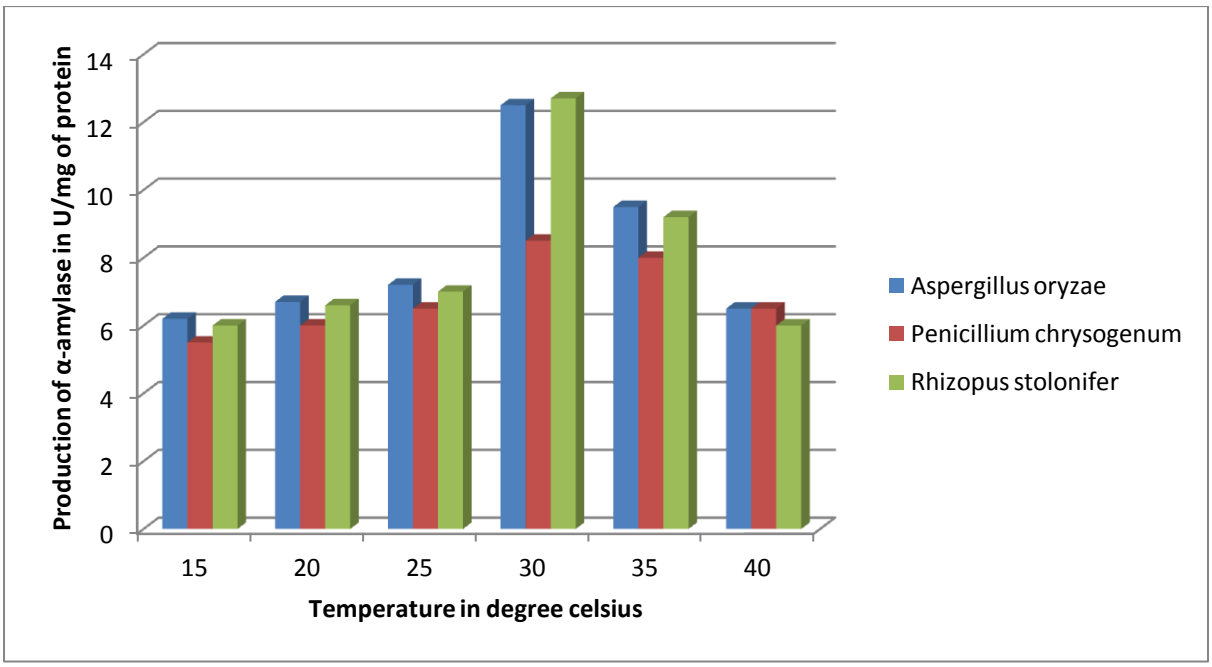

Figure-4: Production of α-amylase (U/mg of protein) by three fungal isolates at different temperature

Effect of pH on production of α-amylase

The optimum pH value for α-amylase production by *Aspergillus oryzae, Penicillium chrysogenum* and *Rhizopus stolonifer* was 7.0. At this pH *A. oryzae, P. chrysogenum* and *R. stolonifer* produced 12.5, 9.5 and 12.4 U/mg of protein respectively (Table-3; Figure-5). These three fungal isolates also produced considerable amounts of the enzyme at pH 5, 7 and 8, while low amounts of the enzyme were observed in more acidity or alkalinity cultures.

Uguru *et al., (*2011) [29] and Khan and Yadav (2011) [8] showed that maximum production of α-amylase by *A. niger* was achieved in the medium initially adjusted to pH 6 and 6.2, respectively. Alva *et al.,* (2007) [34] found that pH 5.8 was the best for production of *Aspergillus* sp. JGI 12 amylase. Singh *et al.,* (2009) [30] studied α-amylase production by *Humicola lanuginosa*. Maximum amylase production was observed at pH

medium initially adjusted to 6.0 Ayansina and Owoseni (2010) [36] studied amylolytic activity of *A. flavus* isolated from mouldy bread. The optimum pH medium for amylase activity was 7.0. Erdal and Taskin (2010) [35] revealed that maximum production of amylase by *Penicillium expansum* was achieved entire the medium initially adjusted to pH 6. Irfan *et al., (*2012) [10] have also reported that the optimum pH value for α-amylase production by *A. niger*-ML-17 and *R. oligosporus*–ML-10 were 5 and 6, respectively.

Table-3: Production of α-amylase (U/mg of protein) by three fungal isolates at different pH

Fungal isolates	pH					
	4.0	5.0	6.0	7.0	8.0	9.0
Aspergillus. oryzae	1.5 ±0.07	1.7 ±0.11	2.0 ±0.13	12.5 ±0.15	7.5 ±0.12	6.0 ±0.13
Penicillium chrysogenum	0.5 ±0.05	1.0 ±0.10	1.7 ±0.11	9.5 ±0.16	5.5 ±0.14	3.3 ±0.12
Rhizopus stolonifer	1.4 ±0.11	1.8 ±0.12	2.6 ±0.15	12.4 ±0.11	7.0 ±0.13	6.2 ±0.15

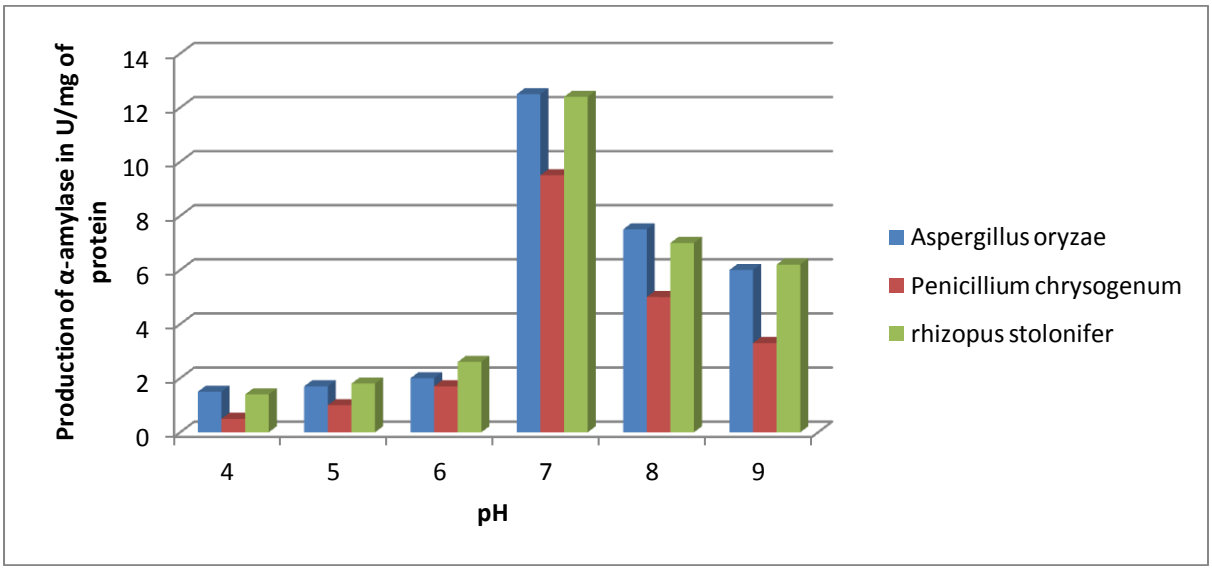

Figure-5: Production of α-amylase (U/mg of protein) by three fungal isolates at different pH

Efeect of Carbon sources on production of α-amylase

Among seven carbon different sources incorporated separately in culture medium, glucose yielded maximum amylase production by *A. oryzae*, *Penicillium chrysogenum* and *R. stolonifer* followed by starch, fructose and sucrose, lactose and maltose. Maltose was the least inducible carbon sources for amylase production by the present fungal isolates (Table-4; Fig-6). Balkan and Ertan (2007) [37] revealed that maximum production of amylase by *Penicillium chrysogenum* was achieved with the incorporation of starch as carbon source. Gupta *et al.*, (2008) [7] recorded that starch was the best carbon source for α-amylase production by *A. niger*. Chimata *et al.,(*2010) [12] found that starch was the optimum carbon source for α-amylase production by *Aspergillus* MK07. Erdal and Taskin (2010) [35] revealed that maximum production of amylase by *Penicillium expansum* was achieved with the incorporation of starch as a carbon source.

Table-4: Effect of different carbon sources on the production of α-amylase (U/mg of protein) by three fungal isolates

Fungal isolates	Carbon sources						
	Glucose	Fructose	Maltose	Lactose	Sucrose	Cellulose	Starch
Aspergillus. oryzae	12.7 ±0.17	9.7 ±0.11	3.5 ±0.14	6.5 ±0.15	9.5 ±0.12	7.5 ±0.13	8.5 ±0.12
Penicillium chrysogenum	6.5 ±0.15	5.5 ±0.11	3.4 ±0.11	3.0 ±0.16	5.7±0.13	6.0 ±0.12	6.4 ±0.16
Rhizopus stolonifer	12.4 ±0.11	9.5 ±0.12	3.0 ±0.15	6.1 ±0.16	9.0 ±0.13	7.2 ±0.15	8.2 ±0.17

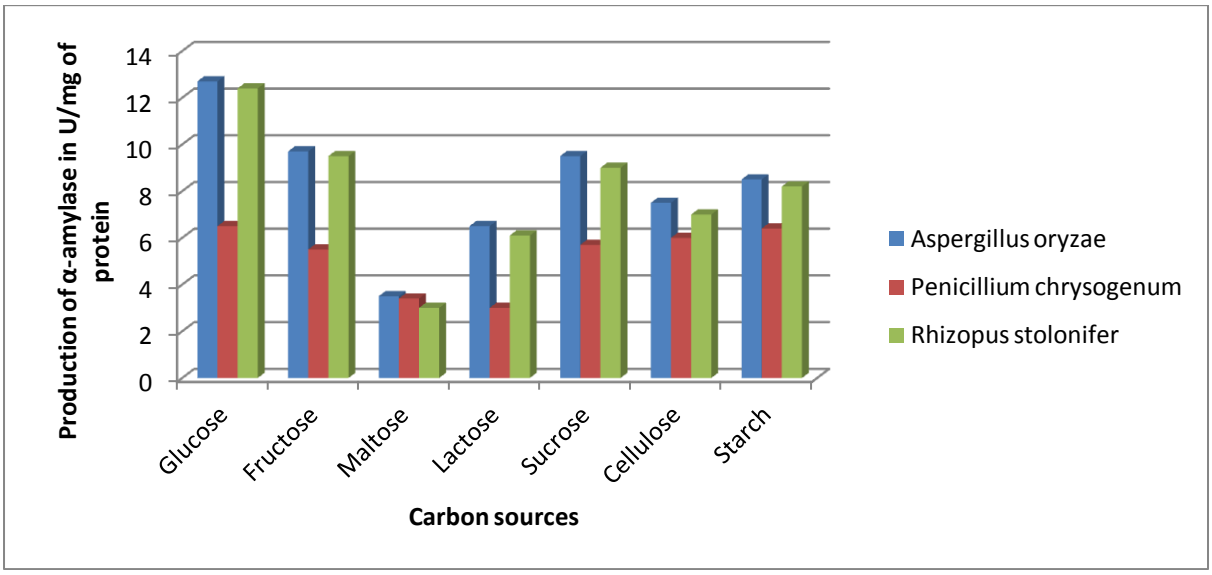

Figure-6: Effect of different carbon sources on the production of α-amylase (U/mg of protein) by three fungal isolates

Effect of different nitrogen sources on production of α-amylase

The highest yields of α-amylase by *A. oryzae, Penicillium chrysogenum* and *R. stolonifer* was achieved when the culture medium was supplemented with ammonium sulphate as nitrogen source followed by peptone (Table-5; Figure-7)). Potassium nitrate, Calcium nitrate, Ammonium nitrate, Sodium nitrate and Ammonium chloride exhibited more or less equal nitrogen sources for the production of α-amylase by the three present fungal isolates. Balkan and Ertan (2007) [37] revealed that maximum production of amylase by *Penicillium chrysogenum* was achieved with the incorporation of sodium nitrate as nitrogen source. Gupta *et al.*, (2008) [7] and Chimata *et al.*, (2010) [12] found that the optimum α-amylase production by *A. niger* and *Aspergillus* MK07 was achieved with the supplementation of peptone as nitrogen sources. Erdal and Taskin (2010) [35] revealed that maximum production of amylase by *Penicillium expansum* was achieved with the incorporation of peptone as nitrogen source.

Table-5: Effect of different nitrogen sources on the production of α-amylase (U/mg of protein) by three fungal isolates

Fungal isolates	Carbon sources						
	$(NH_4)SO_4$	Peptone	KNO_3	$Ca(NO_3)_2$	NH_4NO_3	$NaNO_3$	NH_4Cl
Aspergillus. oryzae	12.5 ±0.18	10.5 ±0.12	6.5 ±0.16	6.7 ±0.15	6.4 ±0.18	7.5 ±0.15	6.5 ±0.15
Penicillium chrysogenum	8.5 ±0.14	7.3 ±0.13	4.5 ±0.13	4.7 ±0.17	4.0±0.13	5.0 ±0.11	3.5 ±0.14
Rhizopus stolonifer	12.3 ±0.15	10.2 ±0.14	6.2 ±0.15	6.5 ±0.16	6.0 ±0.17	7.6 ±0.16	6.0 ±0.17

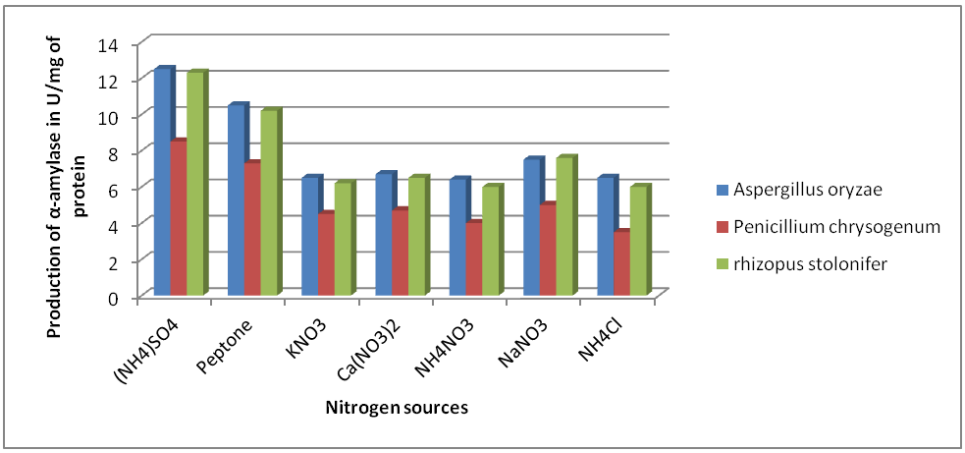

Figure-7: Effect of different nitrogen sources on the production of α-amylase (U/mg of protein) by three fungal isolates

IV. Conclusions

α-amylase is one of the most widely used enzymes required for the production of fermented foods and starch industries. The demand of amylase is increasing continuously with increase in its application spectrum. In the present investigation three fungal isolates from the slice breads were investigated for their abilities to produce α-amylase enzyme. *Aspergillus oryzae* and *Rhizopus stolonifer* were the best local isolates for α-amylase enzyme production. The improvement of environmental and nutritional conditions increased the production of fungal amylase. Employment of the most active fungi for production of α-amylase would contribute in production of the enzyme in large scale and development of some food and starch industries.

It can be concluded that *Aspergillus* oryzae and Rhizopus stolonifer can be exploited for the synthesis of α-amylase at industrial level and strain improvement studies can be carried out to enhance enzyme production.

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