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THE USE OF LOW QUALITY SWEET POTATOES ROOTS *(Ipomoea batatas)* FOR ALPHA-AMYLASE PRODUCTION BY *Aspergillus oryzae*

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ABSTRACT

Low quality sweet potatoes roots were used as a carbon source to produce extracellular alpha–amylase by *Asperigillus oryzae* in shake flask cultures. Peak alpha – amylase production was obtained by local strain of *Aspergillus oryzae* on sweet potatoes filtrate medium amended with salts solutions and sodium nitrate, pH 6.0 after incubation at 30°C for 5 days. The optimum temperature and pH of the enzyme activity was 50°C and 5.6, respectively. The optimum time for enzyme activity, using starch buffered 0.5%, was 1h at optimum pH 5.6 and optimum temperature 50°C. The produced enzyme is stable at temperatures below 50°C, while the enzyme lost 13.1, 24.5, 46.8 and 57.2% of its original activity after exposure at 55, 60, 65 and 75°C for 1h., respectively.

Key words : alpha – amylase – Low quality sweet potatoes – *Aspergillus oryzae*

INTRODUCTION

A large number of industerial enzymes are derived from filamentous fungi belonging to the genus Aspergillus. It is a characteristic of these fungi that they are common and mostly harmless, and that they secrete a number of extracellular enzymes in relatively large quantities.

Alpha – amylase has a vital importance to several industeries among these are textile, paper industeries, sugar refining industry. The enzyme is also used widely for starch digestion to produce ethanol or yeast biomass (De-Moraes *et al* 1995).

Fungal alpha – amylase was produced by many fungi under different fermentation systems such as *Aspergillus niger* (Guo *et al.*, 1991) *A. oryzae* (Carlsen *et al.*, 1996 and Moerkeberg *et al.*, 1995) and *Penicillium expansum* (Dahot *et al.*, 1988).

The alpha – amylase produced by the fungus *Aspergillus oryzae* is widely used in starch saccharification for the food industry, playing an important role in enzymatic supplementaion of flour for the baking process (Fogarty and Kelly, 1980 and Terebizinik *et al.*, 1996).

Sweet potatoes are very popular , the fleshy edible tubers root are highly nutritious and contain starch (18%) ,protein (2%) , other carbohydrates (8%) , minerals (1%), water (70%), besides iron, calcium,vitamines and 1180 IU of carotene per 100 gram (Pandey and Chadha, 1996). According to reports of Central Administration for Agricultural Economics and Statistics, Ministry of Agriculture , Egypt (2000) ,the area under cultivation is about 18271 feddan with an yield of 188862 tons. About 1-2 % of the yield is considered low quality.

production of alpha – amylase using locally selected strain of *Aspergillus oryzae*. Optimization of growth conditions for producing high level of the enzyme as well as some factors affecting enzyme activities were studied.

MATERIALS AND METHODS

Microorganism:

The organism, *Aspergillus oryzae*, used in this study was kindly obtained from Plant Pathology Dept., Fac. of Agric., Mansoura Univ. The stock cultures were maintained on slopes of potato dextrose agar (PDA) medium (Demain and Solomon, 1986) at 5°C and subcultured monthly.

Propagation :

Spore suspension of 7-days old cultures, containing $7x10^6$ spores/ml in 0.8% NaCl sterilized solution, was used as inoculum.

Media used :

Sweet potatoes filtrate (20g/l) amended with saline solution as recommended by Uguru *et al.* (1997) was used for alpha - amylase production. The saline solution consists of (g/l) : K_2HPO_4, 1.0; $MgSO_4.7H_2O$, 0.5; $NaNO_3$, 2.0; $FeSO_4.7H_2O$, 0.001 and KCl, 0.5. The medium's pH was adjusted to 7.0 before sterilization at 121°C for 15 min.

Preparation of sweet potatoes filtrate :

Low quality roots of sweet potatoes were collected from the market. The potato roots were cleaned by washing throughly with water and then cut up into small pieces. The small pieces were washed, dried in the oven at 60°C to constant weight and ground into fine powder in a laboratory mill. The powder was sieved and kept in a desiccator until used. Twenty gram of the powder was dissolved in water at 80°C using water path with gentle shaking for 1 hour in order to extract the most of the soluble carbohydrates. The resulting filtrate strained through two layers of muslin cloth and amended with the saline solution (Uguru *et al.* 1997).

Culture conditions and optimization studies :

A series of 500 ml Erlenmyer flasks, each containing 100 ml of potato filtrate amended with the saline solution, pH 7.0, were sterilized at 121°C for 15 min and inoculated with 1 ml of spore suspension. Cultivation was performed on a rotary shaker (180 rev/min). After incubation period the cultures were harvested by filtration through glass wool filter and then centrifuged. The clear supernatants were used for enzyme assay. Some factors affecting alpha – amylase production by *A. oryzae* such as time course, incubation temperature, initial pH and nitrogen sources were studied.

Factors affecting enzyme activity :

Optimal pH :

The optimal pH was determined using soluble starch in 0.05 M citrate buffer (pH 4.0 – 6.6), 0.05 M sodium phosphate buffer (pH 7.0 – 8.0), under standard conditions.

Optimal temperature :

It was determined with soluble starch buffered at temperatures ranging from 30 to 70 °C under standard conditions.

Optimal time of enzymatic reaction:

It was done by incubating the reactin mixture for 10 to 75 minutes under standard conditions.

Thermal stability :

It was determined after incubating samples of enzyme at different temperatures (30-70°C) for 1 h. Afterwards the enzyme was immersed in an ice bath and then the activity was tested under standard conditions.

Enzyme assay :

Alpha – amylase activity was measured by iodine method as described by Hernandez and Pirt (1975), as follow :2.5 ml of 0.5% soluble starch in citrate buffer (pH 6.0) was mixed with 0.5 ml of culture filtrate, incubated for 15 min at 40°C. The reaction was stopped by adding 1.0 ml of 0.1 N HCl. From each reaction tube 0.5 ml was obtained and mixed with 1.0 ml of a (0.2% iodine – 0.4% KI) solution and 2.0 ml distilled water, allowed to stand for 15 min at room temperature. The color intensities were measured at 620 nm using spekol 111. One unit of alpha- amylase activity was defined as the amount of enzyme that hydrolyze 0.1 mg of starch in 15 min at optimal assay conditions.

Starch disappearance :

It was determined as described by Uguru *et al* (1997). To 0.5 ml of culture filtrate (contains residual starch), 2 ml of 0.1 M H_2SO_4 was added. Afterwards, 5ml of Iodine solution (0.3% I_2 and 3% KI) was added and the absorbance measured at 600 nm against water / iodine blank.

RESULTS AND DISCUSSION

Some factors affecting alpha- amylase production by *A. oryzae* :

Time course :

The effect of time course on alpha- amylase production by *A. oryzae* is shown in Table (1). The organism showed activity in mineral salts containing either 2% (w/v) soluble starch or sweet potatoes as the sole carbon source. Among nine days of incubation the 5th day was the proper time for enzyme production in both media. The corresponding figure for final

pH's values were 6.5 and 5.8, respectively. Results are in harmony with those obtained by Carlsen *et at.* (1996). They reported that the optimum production time of alpha- amylase by *A. oryzae* was between 72 – 120 hr. On the other hand. Uguru *et al.* (1997) found that the higher amylase activity in *A. niger* grown in yam peet was obtained, during the early stationary phase, at the end of the 4th day of incubation.

Table (1) : Effect of time course on alpha – amylase production by *A. oryzae* grown on sweet potatoes filtrate or starch supplemented with mineral salts, pH 7.0 at 30 °C

Time / day	Sweet potatoes				Soluble starch			
	final pH	α –amylase u/ml	% residual starch	Exhausted starch %	final pH	α –amylase u/ml	% residual starch	exhausted starch %
2	6.9	5.869	71.73	28.27	6.8	1.87	4.3	95.70
3	6.6	16.218	66.39	33.61	6.8	4.78	15.84	84.16
5	5.8	21.85	27.13	72.87	6.5	5.95	34.91	65.09
7	4.5	19.744	19.42	80.58	6.0	4.9	52.85	47.15
9	6.1	17.367	11.91	88.09	6.2	2.85	70.62	29.38

*** Enzyme activity was done at pH 5.0, 40 °C. for 15 min.**

Also, data showed that, alpha- amylase activity level was higher when the organism was grown on sweet potatoes than on soluble starch medium. In the mineral salts medium contaning soluble starch, the highest amylase activity was 5.95 µ/ml while the corresponding value was 21.85 in mineral salts medium containing sweet potatoes. The stimulatory effect of sweet potatoes in the medium may be due to the presence of monostarch carbohydrates, proteins, amino acids, etc. (Pandey and Chadha, 1996) in contrasting to the defined medium containing soluble starch. Similar results were obtained by Uguru *et al.* (1997). Data revealed that, at the maximum alpha- amylase activity, about 72.87 % of the sweet potatoes starch was utilized while 34.91 % of the soluble starch was used. This an indication of easy degradation of the sweet potatoes starch by *A. oryzae* strain.

Incubation Temperature :

Optimal temperature for alpha- amylase production was determined at temperaturers ranging from 24 to 36°C. Data in Table (2) show that both of alpha- amylase production and exhausted starch were greatly affected by incubation temperature. The optimum temperature for alpha- amylase production by *A. oryzae* was 30 °C. Domingues and Peralta (1993) found that the optimum temperature for alpha- amylase production from *A. fumigatus Fresenius* was 30 °C.

Table (2): Effect of incubation temperature on alpha – amylase production by *A. oryzae* grown on sweet potatoes filtrate amended with salts solution, pH 7.0 for 5 days.

Temp. (C)	final pH	alpha – amylase µ/ml	residual starch %	exhausted starch %
24	6.3	16.87	37.7	62.3
27	6.2	19.13	29.09	70.91
30	5.9	21.85	27.13	72.87
33	6.4	13.19	42.91	57.09
36	6.5	8.21	57.86	42.14

* Enzyme activity was done at pH 5.0, 40 °C. for 15 min

Effect of initial pH on alpha- amylase production :

Data in Table (3) clearly show that the initial pH value of the fermenting medium affected the formation of alpha- amylase produced by the fungal strain. The enzyme was increased by increasing the initial pH value up to pH 6.0 then decreased. Fadel (2000) found that the optimum pH of alpha-amylase production by *A. niger* was 6.5.

Table (3) : Effect of initial pH on alpha – amylase production by *A. oryzae* grown in sweet potatoes filtrate amended with salts solution for 5 days at 30 °C.

Initial pH	final pH	Alpha – amylase µ/ml	residual starch %	exhausted starch %
3	3.3	1.750	93.84	6.16
4	5.4	19.498	50.827	49.173
5	5.5	24.348	27.13	72.87
6	5.6	26.290	25.77	74.23
7	5.8	21.85	28.33	71.67
8	6.5	18.348	30.41	69.59

*Enzyme activity was done at pH 5.0, 40 °C. for 15 min

Effect of nitrogen sources on alpha- amylase production :

To investigate the effect of nitrogen sources on alpha- amylase production by the organism, seven nitrogen sources in addition to the control (sodium nitrate) were added, in equivalent nitrogen amount, to salt solution amended with sweet potatoes (20g/l). Table (4) reveals that sodium nitrate came on the front of nitrogen sources suitable for high level followed by yeast extract, peptone, potassium nitrate, ammonium nitrate, beef extract and ammonium sulphate. A reduction in enzyme production was detected by using ammonium chloride and this may be due to the effect of the salt in lowering pH culture. Semilar results were obtained by Raimbault and Alazard (1980) and Fadel (2000). The disappearance of the positive role of yeast extract, peptone and beef extract on alpha – amylase production may due to the presence of proteins and amino acids in sweet potatoes filtrate.

Table (4) : Effect of nitrogen source on alpha – amylase production by *A. oryzae* grown on sweet potatoes filtrate amended with salts solution under different N. sources, pH 6, at 30 °C for 5 days.

Nitrogen source	Final pH	alpha – amylase µ/ml	Residual starch %	exhausted starch %
Sodium nitrate (control)	5.6	26.29	26.77	73.23
Potassium nitrate	5.5	20.14	37.87	62.13
Ammonium sulphate	4.9	17.41	44.07	55.93
Ammonium nitrate	4.8	19.54	40.28	59.72
Ammonium chloride	4.0	13.62	48.81	51.19
Peptone	5.5	20.98	36.3	63.7
Yeast extract	5.8	24.68	28.1	71.9
Beef extract	5.7	19.08	32.5	67.5

*** Enzyme activity was done at pH 5.0, 40°C. for 15 min**

Some factors affecting enzyme activity :

Culture filtrate of *Aspergillus oryzae* grown on sweet potatoes filtrate medium containing mineral salts and sodium nitrate as nitrogen source, pH 6.0 for 5 days at 30°C, was used as alpha- amylase source. Some factors affecting enzyme activity were studied.

Effect of pH :

The optimal pH was determined using soluble starch as substrate in 0.05 M citrate buffer (pH 4.0 - 6.6) and 0.05M sodium phosphate buffer (pH 7.0 – 8.0) under standard conditions. As shown in Fig (1) the optimal pH for alpha- amylase activity was 5.6 and the enzyme had a wide range of pH (4.6 – 7.5). Similar results were found by Uguru *et al.* (1997), they found that the optimum pH for amylases of *Aspergillus niger* was 5.5, but Domingues and Peralta (1993) found that the optimum pH for amylases obtained from *Aspergillus fumigatus Fresenius* was 6.0.

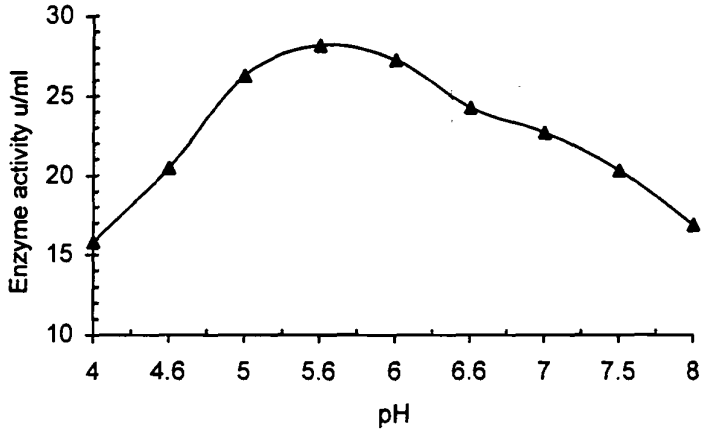

Fig (1) : Effect of reaction mixture pH values on alpha – amylase activity. (reaction was done at 40 °C for 15 min)

Effect of Temperature :

The effect of temperature is presented in Fig (2). It is obvious from data illustrated by Fig. (2) that the optimal temperature for alpha- amylase activity was 50°C. These results are in agreement with those obtained by Morita and Wadano (1971); Bhella and Altosaar (1984); Selim *et al.* (1992) and Domingues and Peralta (1993) who stated that the optimum temperature for alpha-amylase activity produced either by *Aspergillus niger, A. fumigatus* or *A. oryzae* was 50°C.

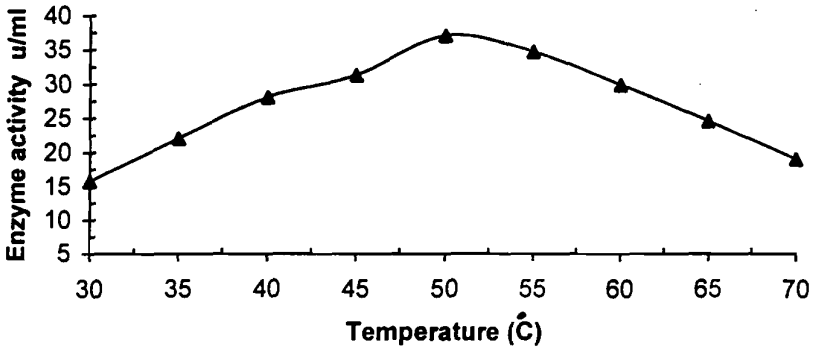

Fig (2): Effect of reaction mixture temperature on alpha – amylase activity (reaction was done at pH 5.6 for 15 min).

Optimum time of reaction and thermal stability :

Fig (3) showed that the time of reaction is greatly affected alpha-amylase activity since it increased with reaction time proceeded up to 60 min.

Concerning thermal activity, it is clearly shown from Fig. (4), , that the alpha – amylase activity was stable at temperatures below 50°C after exposure for 1 hour at each treatment. The illustrated data revealed that the enzyme lost 13.1, 24.5, 46.8 and 57.2% of its original activity after exposure at 55, 60, 65, and 70 for 1 h., respectively.

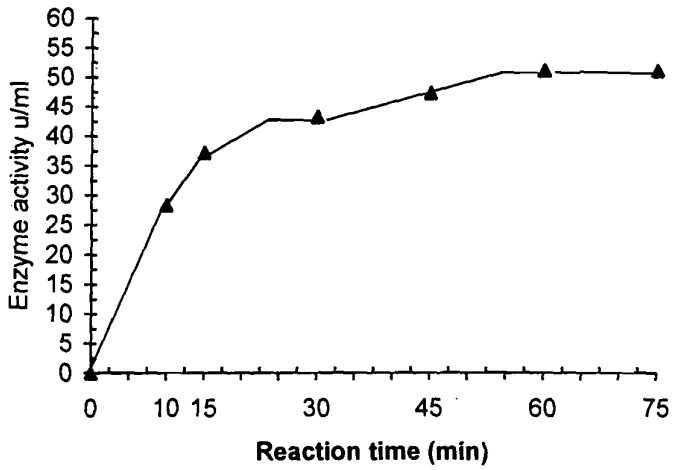

Fig (3): Effect of incubation time on alpha – amylase activity (the reaction was done at 50 °C, pH 5.6)

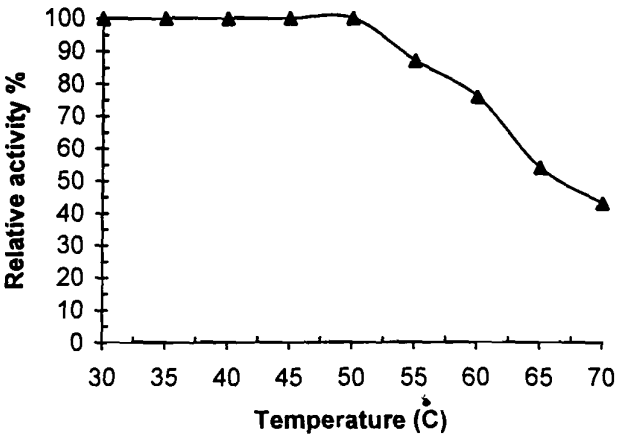

Fig (4) Effect of enzyme exposure at different temperatures for 1h at its original activity. (reaction was done at 50 °C, pH 5.6 for 1h.)

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استخدام جذور البطاطا الحلوة منخفضة القيمة في إنتاج إنزيم الألفا أميليز من فطر اسبرجلس أوريزا

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تم في هذا البحث استخدام مسحوق جذور البطاطا الحلوة منخفضة القيمة كمصدر كربوني لإنتاج إنزيم الألفا أميليز بواسطة ميكروب أسبرجلس أوريزا. ودرست خلال البحث بعض العوامل المؤثرة في إنتاج الإنزيم وبعض العوامل المؤثرة على نشاط الإنزيم وتم التوصل إلى النتائج التالية :

- كانت أعلى كمية لإنتاج الإنزيم بعد خمسة أيام وذلك خلال فترة تحضين مقدارها ٩ أيام
- كانت أفضل درجة حرارة لإنتاج الإنزيم هي ٣٠ درجة وأفضل درجة pH هي ٦
- عندما استخدمت مصادر مختلفة كمصدر نيتروجيني أضيفت للبيئة كان أنسب مصدر نيتروجيني هو نترات الصوديوم
- عند دراسة خصائص الإنزيم تم الحصول على النتائج التالية :
- أنسب درجة حرارة لنشاط الإنزيم كانت ٥٠ درجة مئوية
- أنسب درجة pH لنشاط الإنزيم كانت ٥,٦
- عند دراسة أنسب وقت لتحضين مخلوط الإنزيم ومادة التفاعل (نشا ٠,٥ % في المحلول المنظم) اتضح أن أنسب وقت للتحضين هو ساعة
- عند تعريض الإنزيم على درجات حرارة مختلفة لمدة ساعة ثم قياس النشاط الإنزيمي اتضح ما يلي :
- لم يتأثر الإنزيم بالتعرض لدرجات الحرارة ما دون الخمسين درجة مئوية
- بتعرض الإنزيم لدرجات ٥٥ ، ٦٠ ، ٦٥ ، ٧٠ م لمدة ساعة فقد ١٣,١ ، ٢٤,٥ ، ٤٦,٨ ، ٥٧,٢ % من نشاطه ، على الترتيب .

-وتتضح أهمية البحث التطبيقية في إنتاج انزيم الألفا أميليز من جذور البطاطا الحلوة المنخفضة القيمة والتي تمثل ٢:١% من قيمة المحصول الكلى المنتج فى مصر والمقدر قيمته بحوالي ١٨٨٨٦٢ طن من العروات المختلفة