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Utilization of Agricultural Wastes for Production of Pharmaceutical Glucose by Microbial Amylolytic Enzymes

Yasser Fathy Abdelaleim[1], Alaa Ropy Mahmod[2*], Mohamed H.H. Roby[3]
1. Agricultural Microbiology Department, Faculty of Agriculture, Fayoum University, 63514 Fayoum, Egypt.
2. Chemistry Department, Faculty of Science, Fayoum University, 63514 Fayoum, Egypt.
3. Food Science and Technology Department, Faculty of Agriculture, Fayoum University, 63514 Fayoum, Egypt.
[*]**Correspondence author:** Yasser F. Abdelaleim, Email: yfa00@fayoum.edu.eg

Abstract

The amylolytic enzymes are a group of hydrolytic enzymes of wide application in many industries such as food, textile, paper and digestive pharmaceutical preparations. The aim of the present study was to find the best nutritional conditions for the production of the two amylolytic enzymes namely; α-amylase and glucoamylase, using cheap and locally available wastes. For this reason, strains of *Bacillus subtilis* and *Aspergillus foetidus* were used for the production of α- amylase and glucoamylase, respectively. Several media were tested for the production both enzymes on laboratory scale using agricultural wastes. The best medium for α-amylase production (230 U/ml) consists of 6% of both corn starch and dried yeast, 0.1% K_2HPO_4, 0.02% $MgSO_4.7H_2O$, 0.001% $MnSO_4$ and 0.001% $Fe_2(SO_4)_3$, and NaCl. The highest activity of glucoamylase (46 U/ml) was obtained on a medium containing 6% wheat bran, 4% dried yeast, 0.1% K_2HPO_4, 0.05%$MgSO_4$, 0.05% KCl and 0.001% $FeSO_4$. The using of these two enzymes for production of glucose from corn starch was remarkable with 88-90% conversion efficiency. The IR spectroscopic analysis of the produced dextrose powder showed that it meets the pharmaceutical grade.

Keywords: α-amylase, glucoamylase, starch saccharification, *Bacillus subtilis* and *Aspergillus foetidus*.

Introduction

Amylases naturally secreted by microorganisms, plants and animals, from which the microbial source is considered very suitable and great importance to industry due to its thermal stability and higher efficiency **(Gupta *et al.*, 2003)**. Also, microbial amylases have a wide range of thermal and pH stability, and can be produced in high amounts easily in short time **(Dey *et al.*, 2016; Hiteshi and Gupta 2014; Kelly *et al.*, 2009; Mehta and Satyanarayana 2016 and Sindhu *et al.*, 2017)**. Microbial amylases are used as biological catalysts in many industrial applications such as food, textile, paper, brewing, and digestive pharmaceutical preparations **(Hostinová and Gašperík 2010; Mehta and Satyanarayana 2016 and Souza D. and Oliveira D. 2010)** .

Starch is a naturally abundant storage carbohydrate in many plant tissues and parts; cereals, tubers, roots, stems, leaves and unripe fruits, and so it is common in diets and many food products. Concerning the chemical structure, starch is a polysaccharide of glucose units which are linked together either by α-1,4-glycosidic bonds only in amylose molecules, or by both α-1,4- and α-1,6-glycosidic bonds in amylopectin molecules **(Bello-Perez *et al.*, 2018 and Magallanes-Cruz *et al.*, 2017)**. To get advantage from this vast carbohydrate stock in food industry, starch can be isolated from the above botanical origins and enzymatically processed to produce more useful products with better characteristics like dextrose and biofuel **(Kelly *et al.*, 2009; Lindeboom *et al.*, 2014; Linko and Javanainen 1996 and Magallanes-Cruz *et al.*, 2017).**

Both of α-amylase and glucoamylase are hydrolytic enzymes that act on polysaccharides like starch in a different manner. Where, α-amylase (EC 3.2.1.1) is an endoamylase that randomly hydrolyses α-1,4-glycosidic linkages in starch polysaccharide chains converting these long chains into oligosaccharides of variable lengths, maltotriose and maltose **(Maarel van der *et al.*, 2002; Mehta and Satyanarayana 2016; Souza D. and Oliveira D. 2010 and Zhang, *et al.*, 2017)**. Conversely, glucoamylase (EC 3.2.1.3) is an exoamylase with exo-1,4-α-glucosidase activity releasing glucose units successively from the nonreducing ends of starch chains. Glucoamylase can completely convert starch polymer into glucose units by hydrolysis of α-1,4-glycosidic linkages with high rate, and α-1,6-glycosidic linkages with slow rate **(Marín-Navarro and Polaina 2011 and Norouzian *et al.*, 2006)**. It was reported that using different amylase activities like α-amylase and glucoamylase was efficiently useful for starch liquefaction and saccharification **(Chu-Ky *et al.*, 2016; Li, *et al.*, 2018; Linko and Javanainen 1996; Soni *et al.*, 2003, and Soni *et al.*, 2012).** It was well reported that composition of the growth medium affects enzyme production; so that, optimization of carbon source and nitrogen source is very important for amylase production and its dependent industrial applications **(Divakaran *et al.*, 2011; Mehta and Satyanarayana 2016; Özdemir *et al.*, 2011 and Roy,**

***et al.*, 2012)**. Agricultural wastes can be utilized for production of α-amylase and some other enzymes because of their economic benefits having low cost production and positive environmental impact through recycling of agro-industrial wastes into useful products **(Attia and Ali 1977; Hassan and Abd Karim 2012 and Rajagopalan and Krishnan 2008, 2009)**.

Alpha amylase was produced from the Gram-positive bacterium *Bacillus subtilis* which is popular in biotechnological enzyme production because it was well studied and had high protein yield without toxic by-products **(Dijl V. *et al.*, 2013; Lyubenova *et al.*, 2011; ÿztürk *et al.*, 2016)**. Also, *Aspergillus* sp. was used for industrial production of enzymes as it is characterized by high enzyme yield with broad substrate spectrum and acid tolerance **(Knuf and Nielsen 2012; de Vries and Ronald 2017).**

In this study, the authors aimed to optimize conditions for α-amylase and glucoamylase production using agricultural wastes from *Bacillus subtilis* and *Aspergillus foetidus*, respectively. Additionally, both enzymes were used for starch liquefaction, saccharification, and production of dextrose.

Materials and Methods

Microorganisms

Bacillus subtilis S. 3217 and *Aspergillus foetidus* (ATCC 14916), were used for production of α-amylase and glucoamylase, respectively. Strains were obtained from the Agricultural Microbiology Department, Faculty of Agricultural, Fayoum University, Egypt. The cultures were maintained on Nutrient agar and Potato dextrose agar slant for *Bacillus subtilis* and *Aspergillus foetidus* respectively, stored at 5°C and sub culturing were mad every two weeks.

Materials:

a. Corn steep liquor, Corn starch and Protelan were obtained from the starch and glucose company at Cairo, Egypt.
b. Corn starch, Rice starch and potato starch were obtained from the big markets in Cairo, Egypt.

Media:

a. A basal medium containing the following mineral salts: 0.1% K_2HPO_4, 0.02% $MgSO_4.7H_2O$ and 0.001% of each of $MnSO_4$, $Fe_2(SO_4)_3$ and NaCl. And adjusted Ph at 7.0 with NaOH and this basal medium was sterilized by autoclaving at 121 °C for 15 minutes. for *Bacillus subtilis* and for *Aspergillus foetidus* using a basal Czapek´s medium.
b. Fermentation medium using a basal medium modifies are listed in Table 1 for *Bacillus subtilis* and a basal Czapek´s modified medium in Table 5 for *Aspergillus foetidus.*

Fermentation experiments:

a. Preparation of inoculum for α-amylase production.

After incubation period of 24 hrs, culture of the tested strain obtained on nutrient agar slants were suspended in sterilized distilled water. A 1.0 ml of the culture was aseptically trans pored to 500 ml flask containing 100 ml of sterile nutrient broth medium. Flaskes were incubated on a rotary shaker at 100 rpm and 32°C for 48h.

b. Fermentation conditions for α-amylase production

Laboratory experiments were carried out under sterile conditions using a glass conical flask of 500 ml containing 100 ml media inoculated with 2 ml of 24 h culture of *Bacillus subtilis* S. 3217. The inoculated media were incubated for 48 h. old at 32 °C and 100 rpm for α-amylase production. Several media (1-14) were tested in comparison with the medium used for commercial production which consists of 4% protelan, corn steep liquor (CSL) and inorganic salts, medium 15 (table 1).

For the preparation of clear cell free extract (CFE) containing crude α-amylase from *Bacillus subtilis*, 50 ml of the culture was mixed with 5 ml phosphate buffer (0.1 M, pH 6.0) and 1.25 ml of $CaCl_2.2H_2O$ (20%) solution **(Beckord *et al.*, 1946)**. After shaking for 20 min at room temperature, CFE was obtained by centrifugation at 8000 rpm for 15 min at 4°C.

c. Preparation of inoculum for spore suspension for glucoamylase production.

Fungal culture was grown on PDA slants at 28°C until well sporulated. Spores were harvested by adding sterialized Tween 80 solution 01% (v/v), filtered through several layers of sterilized cheese cloth, centrifuged, washed three times with sterilized distilled water, suspended in Sterilized Tween 80 solution 0.01% (v/v). The number of spore suspension was adjusted to contain approximately 10^6 spores/ml.

Fermentation conditions for glucoamylase production

Experiments were carried out in a flask of 500 ml containing 100 ml media, described below, inoculated with 2 ml of 48 h. old *Aspergillus foetidus* culture and incubated for 72 h at 30 °C and 100 rpm for glucoamylase production. These experiments were carried out for detection of the best nutritional conditions for fungal glucoamylase production using a basal Czapek´s medium containing 1.2% total carbon content and 0.05% nitrogen content. The effect of different carbon sources like sucrose, glucose, fructose, maltose, dextrin, corn starch, rice starch, potato starch, wheat bran, rice bran and portelan on enzyme production at a constant carbon content of

1.2% was tested (Table 2). Also, tested the effect of was tested increasing concentrations (0.02-0.2%) of $NaNO_3$, $(NH_4)_2$ HPO_4, $(NH_4)_2SO_4$ and NH_4NO_3 as inorganic nitrogen sources on the enzyme production (Table 3). Likely, the effect of organic nitrogen sources like CSL, dried yeast, gluten and soya bean with concentration ranging from 0.5% to 5.0% was studied (Table 4). Additionally, the effect of seven media containing mixture of nitrogen and carbon sources on glucoamylase production was studied (Table 5). These seven media contained 0.1% K_2HPO_4, 0.05% $MgSO_4$, 0.05% KCl, and 0.001% Fe_2SO_4.

Also, the culture fluid of *Aspergillus foetidus* was filtered through Seitz filter with bacterial asbestos filter discs, and centrifuged as mentioned above. The clear filtrate containing crude enzyme was used for the determination of glucoamylase activity starch liquefaction and saccharification.

Enzyme assay:

a. Alpha amylase assay

The method was according to a previous study **(Swain and Ray 2007)** where the assay reaction contained 0.5 ml of enzyme solution (*B. subtilis* CFE) to 1 ml of 0.5% starch dissolved in phosphate buffer (0.1 M, pH 6.0). The mixture was placed in a water bath at 40 °C for 10 min. Then, 0.5 ml of (0.1 N) HCl was added and followed by 0.5 ml of iodine solution (2% KI, 0.2%I). The optical density of the resulting blue colour was measured at 690 nm. Where, one α-amylase unit corresponds to the enzyme amount that reduces the intensity of the blue colour by 0.01% at 40 °C in minute per ml of enzyme solution.

b. Glucoamylase assay

In this assay, 0.5 ml of enzyme solution (*A. foetidus* CFE) was mixed with 0.5 ml of 1% starch dissolved in sodium acetate buffer (0.1 M, pH 4.2), and incubated at 60 °C for 10 min. After that, 1 ml of 3,5-dinitrosalicyclic acid (DNS) reagent was added, and the final mixture was incubated in boiling water bath for 5 min, then cooled and diluted to 10 ml with distilled water. The optical density of the coloured product was measured at 540 nm, and the enzyme activity was calculated from glucose-DNS standard curve. One glucoamylase unit is expressed as one µmole glucose liberated per ml per minute at assay conditions **(Pasin *et al*., 2017 and Wang *et al*., 2008).**

Starch liquefaction and saccharification

Corn starch (pH 6.0) was gelatinized at 110 °C for 5 min and then liquefied with *B. subtilis* α-amylase (28 U/g corn starch) at 90 °C for 3 h. Thereafter, the starch hydrosylate was cooled to 55-60 °C and the pH was adjusted to 4.5-5.0. Then, saccharification was achieved by incubation with glucoamylase (13 U/g corn starch) with gentle stirring for 72 h **(Vieille and Zeikus 2001).** Periodic samples were analysed for reducing sugars. The high glucose syrup was treated with 0.5-%1 w/v activated charcoal at 60 °C with efficient stirring for 20-30 min. The mixture was centrifuged at 400 rpm to obtain clear saccharified liquor, which was then passed through ion exchange resin columns. The clear solution was concentrated under vacuum at 40-45 °C until total solids reached 70-75% **(Maarel van der *et al*., 2002)**. Seed crystals were added (anhydrous glucose) with continuous gentile stirring. The temperature of the mixture was decreased to 20 °C, over a period of two days, about 60-65% of dextrose was crystalized as glucose monohydrate and easily separated and dried under vacuum. The mother liquor was concentrated and crystalized to recover another yield of crystals. The dextrose powder produced was analysed by Beckman IR-20 infrared spectrophotometer, and compared to a pharmaceutical grade of standard dextrose monohydrate **(Figures 1 and 2).**

Results and Discussion

Production of bacterial α-amylase

Results showed that increasing the concentration of carbon source from 3% to 4% as corn starch with a fixed concentration of nitrogen source in the medium increased the α-amylase production with corresponding activities of 195 U/ml and 200 U/ml, respectively (table 1). At a higher concentration of corn starch (5%), the activity was decreased markedly to 140 U/ml. This may be due to lower nitrogen content in respect to that of carbon concentration, and indicates that the optimum C/N ratio is an important factor for the production of α-amylase. Similarly, the same effect was found with increasing starch concentration up to a certain value, beyond which the α-amylase activity decreased **(Kalpana, *et al*., 2014 and Santos and Martins 2003)**. This decrease in α-amylase production was explained by the inhibitory effect of reduced free oxygen transfer necessary to bacterial growth due to higher viscosity resulted from high starch concentrations **(Kalpana *et al*., 2014)**.

Also, it was reported that using starch as a carbon source resulted in the highest α-amylase activity in *Bacillus* sp. **(Irfan *et al*., 2017; Kalpana *et al*., 2014; Nwokoro and Anthonia 2015 and Santos and Martins 2003)**. On the other hand, using galactose gave maximum α-amylase production compared to starch in *Bacillus* sp. strain **(Özdemir *et al*., 2011)**. Additionally, corn flour produced the highest amylase activity in *Bacillus amyloliquefaciens* P-001 **(Deb *et al*., 2013)**.

Present results show that corn steep liquor (CSL) is a promising nitrogen source for enzyme production where the maximum activity obtained was at 2% CSL concentration. Conversely, the enzyme production was sharply dropped to 50% when CSL was completely replaced with the same concentration of corn steep precipitate (CSP). This may be due to poor or lack of growth factors in CSP, which is known to be present in CSL. Similarly, it was mentioned that addition of 0.3% CSL and 0.1% wheat protein

concentrate to a basal medium containing 0.25% starch resulted in maximum α-amylase production in *Bacillus* sp. SMIA-2 compared to the basal medium **(Corrêa *et al.*, 2011)**.

Locally produced dried yeast as a by-product of alcohol and beer industries was found to be a good nitrogen source. The yeast cells is known to contain growth factors and essential amino acids in assimilable form. By using different concentrations of corn starch and dried yeast ranging from 3% to 7% in media 8-14, (Table 5) it was found that α-amylase production increased with increasing the concentrations up to a certain limit. Where, medium 10 containing 3% of both of corn starch and dried yeast, and medium 13 containing 6% from both of them produced activities of 150 U/ml and 230 U/ml, respectively. Using medium 14 containing 6% corn starch and 7% dried yeast slightly decreased the enzyme activity to 225 U/ml. Similarly, it was reported that α-amylase activity increased with increasing the concentration of yeast extract up to 5 g/L, then, the activity was decreased by the increase in yeast extract concentration in *Bacillus* sp. **(Santos and Martins 2003)**. Also, it was reported that α-amylase production in *Bacillus subtilis* KCC103 increased with increasing the concentration of nitrogen sources like beef extract and yeast extract, however, it decreased at high concentrations **(Rajagopalan and Krishnan 2009)**.

Comparing the effect of organic and inorganic nitrogen sources, results showed that organic nitrogen was a great importance for enzyme production. When a part of dry yeast was substituted with $(NH_4)_2HPO_4$, the enzyme production was decreased.

As mentioned above, the gradual balanced increasing of both carbon and nitrogen sources in the medium was accompanied by increasing production of the enzyme. The highest enzymatic activity was obtained when using media No. 12, 13 and 14. Table(1) where, the maximum activity obtained in this case was 230 U/ml by using a medium containing 6% of both corn starch and dry yeast. While, the comparative protelan medium gave only 100 U/ml corresponding to about 50% of the activity of the tested media. The increase of dry yeast to 7% showed no effect on enzyme productivity, that it may be excess than the organism requirements.

From previous results and published data mentioned above it could be said that the increase in both carbon and nitrogen sources enhance the production of α-amylase to a certain limit. The increase in nitrogen concentration over a certain limit was no effect, while in case of increasing carbon concentration, the enzyme production was decreased.

Table 1. Effect of nutritional conditions on the production of bacterial α-amyase by *Bacillus subtilis*.

No.	Medium condition*
1	3% Corn starch, 4% CSL, 0.5% $(NH_4)_2HPO_4$
2	4% Corn starch, 4% CSL, 0.5% $(NH_4)_2HPO_4$
3	5% Corn starch, 4% CSL, 0.5% $(NH_4)_2HPO_4$,
4	4% Corn starch, 3% CSL, 1% CSPP, 0.5% $(NH_4)_2HPO_4$
5	4% Corn starch, 2% CSL, 2% CSPP, 0.5% $(NH_4)_2HPO_4$
6	4% Corn starch, 4% CSPP, 0.5% $(NH_4)_2HPO_4$
7	4% Corn starch, 3% CSPP, 0.5% $(NH_4)_2HPO_4$
8	4% Corn starch, 3% dried yeast, 0.5% $(NH_4)_2HPO_4$
9	4% Corn starch, 3% dried yeast
10	3% Corn starch, 3% dried yeast
11	4% Corn starch, 4% dried yeast
12	5% Corn starch, 5% dried yeast
13	6% Corn starch, 6% dried yeast
14	6% Corn starch, 7% dried yeast
15	Control (Protelan medium): 4% protelan, 1.5% CSL

*Each medium containing the following mineral salts: 0.1% K_2HPO_4, 0.02% $MgSO_4.7H_2O$ and 0.001% of each of $MnSO_4$, $Fe_2(SO_4)_3$ and NaCl. CSL: corn steep liquor; CSPP: corn steep precipitate.

Likely, it was reported that using yeast extract produced the maximum α-amylase production when compared to other organic nitrogen sources in *Bacillus* sp. **(Hassan and Abd Karim 2012 and Sreekanth *et al.*, 2013).** Additionally**, Kalpana *et al.*, (2014)** found that using yeast extract in combination with other organic sources like 1% tryptone produced the maximum amylase activity in *B. subtilis* S8-18. On the other hand, it was found that using peptone as a nitrogen source was more effective than yeast extract and produced the maximum alkaline α-amylase activity in *B. subtilis* **(Al-Johani *et al.*, 2017 and Irfan *et al.*, 2017)**. Compared to other organic nitrogen sources, 0.2% tryptone displayed higher enzyme activity, while the inorganic source presented as ammonium nitrate produced the maximum α-amylase activity in *Bacillus amyloliquefaciens* P-001 **(Deb *et al.*, 2013)**. Moreover, 5% beef extract produced the maximum α-amylase activity in *Bacillus subtilis* MTCC 6537, followed by yeast extract, peptone and tryptone where high concentration of nitrogen source seemed to be inhibitory for enzyme

production **(Pavithra *et al*., 2014)**. These variations in enzyme production may be affected by the type of bacterial and fungal strains, composition of the growth medium and growth conditions **(Sun *et al*., 2010)**.

Production of glucoamylase

It is clear from the obtained results that the agricultural wastes as a source of carbon were more suitable for the production of glucoamylase than the other tested carbon sources, i.e. sugars and different types of starch (table 2), which is in agreement with a previous study **(Attia and Ali 1977)**. Among these agricultural wastes, wheat bran was the best one (34 U/ml), followed by rice bran (29 U/ml). The relative higher productivity given by such carbon sources may be due to their high content of growth promoting substances like minerals, proteins and starch **(Sreekanth *et al*., 2013)**. Additionally, it was reported that rice bran followed by corn bran and wheat bran produced the highest glucoamylase activity in *Aspergillus awamori* among the tested agricultural by-products **(Attia and Ali 1977)**. It was found that dextrin and corn starch produced glucoamylase activities of 24 and 23 U/ml, respectively. However, lower enzyme activities were obtained when using rice starch, potato starch, maltose, sucrose, glucose and fructose (table 2). It was found that soluble starch followed by corn starch, potato starch, Dextrin and sucrose produced the best glucoamylase activities in *Aspergillus flavus* **(Ayodeji *et al*., 2017)**. Moreover, it was reported that the maximum glucoamylase production from *A. japonicus* was achieved using amylopectin followed by rice starch, potato starch, glycogen, maltose and corn starch **(Pasin *et al*., 2017)**.

With regard to the nitrogen source, different organic and inorganic sources were tested. From the obtained results, it is clear that the organic nitrogen sources (table 4) were more suitable for glucoamylase production than the inorganic sources (tables 3). It was found that glucoamylase activity was increased with the increasing of nitrogen source concentration till a maximum limit for each source, then decreased. For example, using concentrations of 0.5%, 1.0%, 2.0%, 3.0%, 4.0% and 5.0% of dried yeast produced enzyme activities being 2.0, 3.2, 15.0, 15.6, 20.0 and 17, respectively (table 4). Results show that the limiting factor in glucoamylase production was not only the nitrogen concentration but also its bioavailability which plays an important role in this respect. This conclusion was reached when comparing enzyme activities using two media containing 4% dry yeast and 3% soya bean which displayed 20.0 U/ml and 11.5 U/ml, respectively. Also, 4% gluten produced glucoamylase activity of 20 U/ml, while, 5% CSL gave 9.8 U/ml (table 4). These results indicate that 4% of dried yeast and gluten were the best for the enzyme production. In general, dried yeast was the most favourable raw material as nitrogen source for its high productivity and availability.

The effect of media containing different combinations of the best carbon and nitrogen sources on glucoamylase production is presented in **Table (5).** By comparing productivity of media (No. 1, 5 and 6) containing different concentrations of corn starch in combination with dried yeast, data showed that medium No. 6 was the best for producing enzyme activity of 45 U/ml. Also, medium No. 1 containing 4% dried yeast produced activity of 40 U/ml, compared to 20 U/ml from medium No. 2 containing 4% gluten. Moreover, medium containing 7% dextrin, 3% dried yeast displayed activity of 46 U/ml. The best productivity attained using medium No. 4 containing 6% wheat bran and 4% dried yeast with 51 U/ml glucoamylase activity.

It was reported that rice bran followed by corn bran and wheat bran produced the highest glucoamylase activity for *Aspergillus awamori* among the tested agricultural wastes **(Attia and Ali 1977)**. They also, found that using different carbon sources like maltose followed by potato starch, dextrin and glucose gave high amounts of glucoamylase production. It was stated that agro-industrial waste products like crushed yellow maize resulted in the maximum glucoamylase production higher than wheat bran and rice bran using *A. foetidus* **(Makhmud *et al*., 1978)**. On the other hand, it was stated that KH_2PO_4 was more effective than $(NH_4)_2SO_4$ and yeast extract for glucoamylase production of *A. niger* **(Wang *et al*., 2008)**.

Similar to present findings, it was reported that yeast extract gave the maximum glucoamylase production compared to other organic and inorganic nitrogen sources of *Aspergillus* spp **(Jain and Katyal 2018)**. Also, it was found that soluble starch followed by corn starch, potato starch, Dextrin and sucrose produced the best glucoamylase activities of *Aspergillus flavus* **(Ayodeji *et al*., 2017)**. Additionally, it was reported that the maximum glucoamylase production from *A. japonicas* was achieved using amylopectin followed by rice starch, potato starch, glycogen, maltose and corn starch **(Pasin *et al*., 2017)**.

Starch saccharification and production of dextrose

Using 12 U of α-amylase and 1.5 U of glucoamylase per gram of corn starch was successful for starch gelatinization and saccharification, respectively, and also the production of dextrose. These figures of the locally produced enzyme were found promising for the conversion of starch to glucose with overall efficiency of 88-90 percent. The dextrose powder produced was analysed spectroscopically in comparison with pharmaceutical grade of dextrose monohydrate as standard. It was clear from the IR analysis that the produced dextrose meets the pharmaceutical requirements (Figures 1 and 2). Gelatinization solubilizes starch and makes its polymeric chains ready for liquefaction by the action of α-amylase to produce short-chain dextrins which can be saccharified by glucoamylase into glucose **(**

Maarel van der *et al*., 2002 and Vieille and Zeikus 2001)

Table 2. Effect of different carbon sources on glucoamylase production by *Aspergillus foetidus.*

Carbon source*	**Enzymatic Activity (U/ml)**
Sucrose	4.0
Glucose	8.4
Fructose	8.0
Maltose	10.0
Dextrin	24.0
Corn starch	23.0
Rice starch	15.2
Potato starch	10.0
Wheat bran	34.0
Rice bran	29.0
Protelan	**17.8**

* calculated as 1.2%C.

Table (3). Effect of various concentration of some inorganic sources on production of glucoamylase by *Aspergillus foetidus.*

Inorganic Nitrogen Source	Concentration%							
	0.02	0.04	0.06	0.08	0.10	0.13	0.15	0.20
	Enzyme activity (U/ml)							
$NaNO_3$	1.7	5.1	5.9	6.6	7.5	7.0	5.8	5.0
$(NH_4)_2 HPO_4$	1.8	4.4	3.8	3.2	3.0	3.0	2.5	2.2
$(NH_4)_2 SO_4$	1.0	2.5	1.9	1.2	0.7	0.7	0.3	0.0
$NH_4 NO_3$	3.0	1.6	1.1	0.5	0.5	0.0	0.0	0.0

Table 4. Effect of various concentration of some organic nitrogen sources on production of glucoamylase by *Aspergillus foetidus.*

Organic Nitrogen Source *	Concentration%					
	0.5	1.0	2.0	3.0	4.0	5.0
	Enzyme activity (U/ml)					
CSL	0.9	2.5	3.6	5.0	7.3	9.8
Dried yeast	2.0	3.2	15.0	15.6	20.0	17.0
Gluten	3.0	6.3	12.0	13.2	20.0	18.0
Soyabean	3.6	5.7	7.5	11.5	9.5	0.0

Table 5. Effect of combinatory supplementation of carbon and nitrogen sources on glucoamylase production.

No.	Media*	Enzyme Activity (U/ml)
1	3.3% Corn starch, 4% dried yeast, salts	40
2	3.3% Corn starch, 4% gluten, salts	25
3	6% Wheat bran, 3% dried yeast, salts	33
4	6% Wheat bran, 4% dried yeast, salts	51
5	6% Corn starch, 3% dried yeast, salts	39
6	7% Corn starch, 3% dried yeast, salts	45
7	7% Dextrin, 3% dried yeast, salts	46

*Salts: 0.1% K_2HPO_4, 0.05% $MgSO_4$, 0.05% KCl, 0.001% Fe_2SO_4.

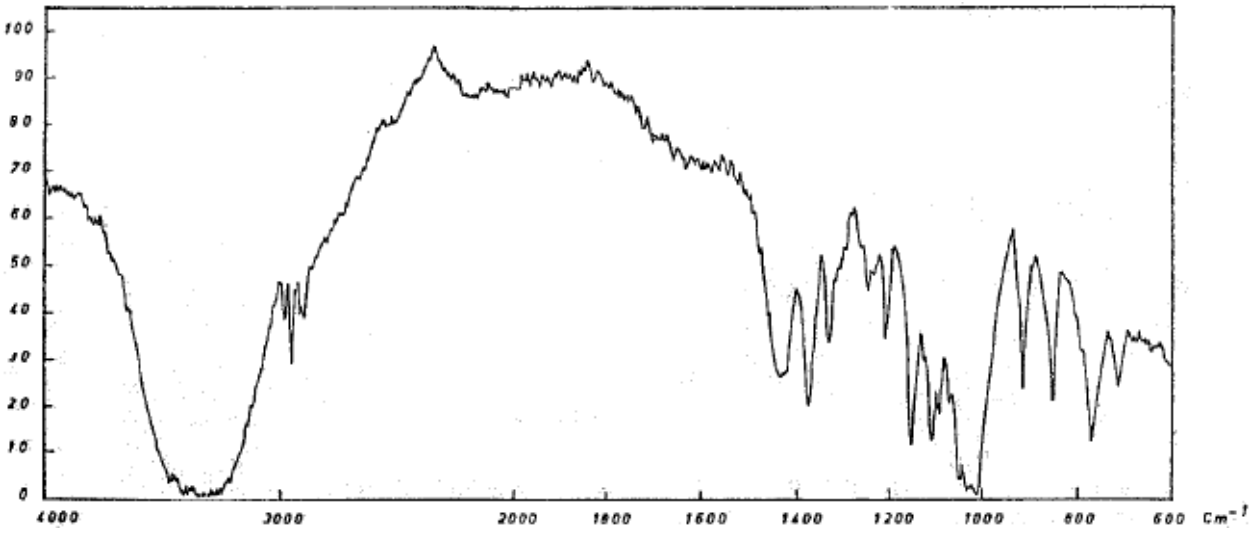

Fig.1: IR analysis spectrum of standard glucose.

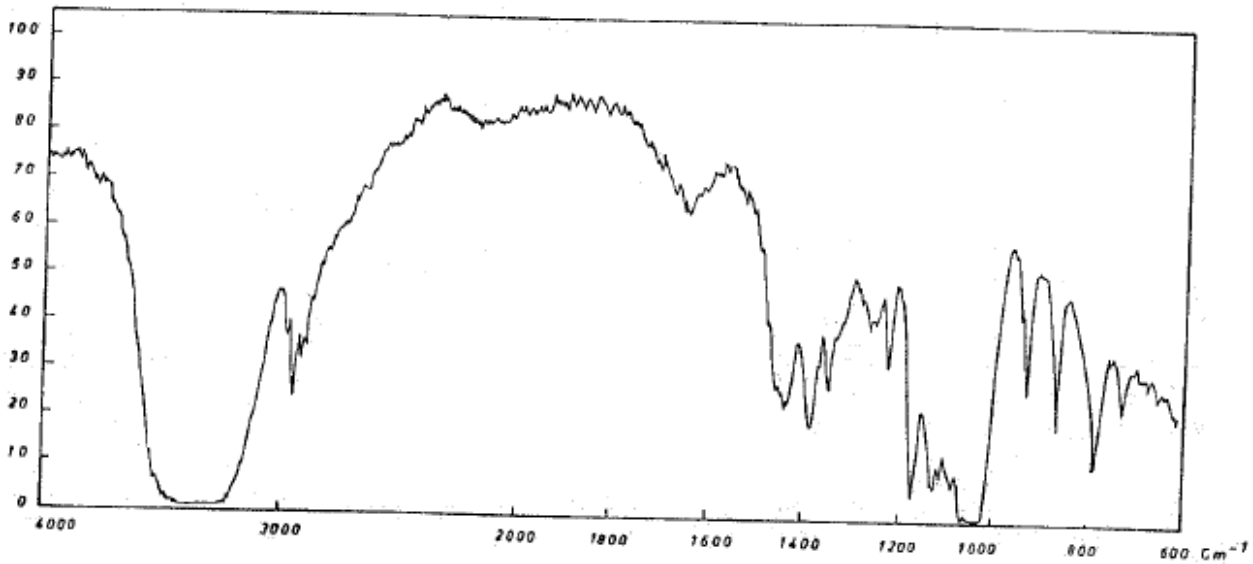

Fig.2: IR analysis spectrum of glucose produced from starch saccharification by the action of α-amylase and glucoamylase.

Conclusion

From the above mentioned results, it could be concluded that the best medium, for the production of α-amylase by *B. subtilis* consists of 6% corn starch, 6% dried yeast, 0.1% K_2HPO_4, 0.02% $MgSO_4$. $7H_2O$ and 0.001% of each $MnSO_4$, $Fe_2(SO_4)_3$ and NaCl. While the best medium for glucoamylase production consists of 6% wheat bran, 4% dried yeast, 0.1% K_2HPO_4, 0.05% $MgSO_4$, 0.05% KCl and 0.001% $FeSO_4$. These media are preferred to the commercial portelan medium due to high enzyme production and extraction. Both amylolytic enzymes obtained in this work were successfully used for the production of pharmaceutical grade of glucose powder from corn starch.

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الأستفادة من المخلفات الزراعية في إنتاج الجلوكوز الصيدلاني بواسطة إنزيمات الاميليز الميكروبية

ياسر فتحي عبد العليم[1]، علاء روبي محمود [2] ، محمد حسين حمدي[3]

[1] قسم الميكروبيولوجيا الزراعية– كلية الزراعة – جامعة الفيوم– الفيوم – مصر.

[2] قسم الكيمياء – كلية العلوم – جامعة الفيوم– الفيوم– مصر.

[3] قسم علوم وتكنولوجيا الأغذية– كلية الزراعة – جامعة الفيوم– الفيوم – مصر.

إنزيمات الأميليز هي مجموعة من إنزيمات التحلل المائي ذات التطبيق الواسع في العديد من الصناعات مثل المواد الغذائية والمنسوجات والورق والمستحضرات الصيدلانية المساعدة على الهضم. كان الهدف من هذه الدراسة هو تحديد أفضل الظروف الغذائية لإنتاج نوعين من إنزيمات الأميليز وهما ؛ الألفا أميليز والجلوكوأميليز باستخدام مخلفات رخيصة ومتوفرة محليًا. لهذا السبب، تم استخدام سلالات *Bacillus subtilis*، *Aspergillus foetidus* لإنتاج الألفا أميليز والجلوكوأميليز على التوالي. تم اختبار العديد من البيئات لإنتاج هذه الإنزيمات على النطاق المعملي باستخدام المخلفات الزراعية. ولقد وجد أن أفضل وسط لإنتاج ألفا أميليز (230 وحدة / مل) يتكون من 6٪ من كل من نشا الذرة والخميرة الجافة و 0.1٪ K_2HPO_4 و 0.02٪ $MgSO_4.7H_2O$ و 0.001٪ $MnSO_4$ و 0.001٪ $Fe_2(SO_4)_3$ و NaCl بينما تم الحصول على أعلى نشاط للجلوكواميليز (46 وحدة/مل) من وسط يحتوي على 6٪ نخالة قمح،4٪ خميرة مجففة، 0.1٪ K_2HPO_4، 0.05٪ $MgSO_4$ ، 0.05٪ KCl و 0.001٪ $FeSO_4$. ولقد أظهر استخدام هذين الإنزيمين لإنتاج الجلوكوز من نشا الذرة كفاءة تحويل ملحوظة بنسبة 88–90 ٪. وأظهر التحليل الطيفي للأشعة تحت الحمراء لمسحوق الدكستروز المنتج أنه يفي بالدرجة الصيدلانية.