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A Cost-effective Medium for Enhanced Production of Extracellular α-galactosidase in Solid Substrate Cultures of *Aspergillus awamori* and *A. carbonarius*

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Abstract: α-Galactosidase (α-Gal) produced by *Aspergillus awamori*, *A. carbonarius*, in solid substrate cultures was investigated. These fungi were screened for α-Gal production on agricultural by-products (cane baggase, corn bran, soft wood saw dust and soya flour).The results showed that corn bran added to fermentation medium in the ratio of (1:1,w/v, the initial moisture content [MC], 60.90%) was the best substrate for α-Gal production by *A. awamori* and *A. carbonarius* . The inoculum density of 5.0×10^5 CFU/ml and 5.7×10^5 CFU/ml were the best for *A. awamori* and *A .carbonarius*, respectively. Six days was the best incubation period for maximal production of α-Gal by the two experimental fungi. Using of yeast extract, malt extract, beef extract, corn steep liquor, cane molass, beet molass and whey increase the production of α-Gal by both *A. awamori* and *A. carbonarius*. Maximum α-Gal production was obtained when the two experimental fungi were grown on corn bran fortified with 0.05 % malt extract. The basal growth solution was treated beet molass and crude whey, in case of *A. awamori* and *A. carbonarius* respectively. The crude enzyme was precipitated by ammonium sulphate (60%) for both *A. awamori* and *A. carbonarius*. The enzyme was partially purified by ion exchange chromatography on bentonite (2.5%) the activity of the enzyme was 6.38 u/ml and 5.62 u/ ml for *A. awamori* and *A. carbonarius* respectively. The temperature and pH optima of partially purified α-Gal of both *A. awamori* and *A .carbonarius* were 55 ºC and 4.8, respectively. Thermostability over a wide range of temperature (30-60 ºC) and pH stability over a wide range of pH (3.6-4.8). Partially purified (PP) α-Gal of *A. awamori* was inhibited with Na^+, Li^+, Ag^{2+}, Hg^{2+}, Zn^{2+}, Mo^{2+}, Cd^{2+}, Pb^{2+} and Fe^{2+} at 1mM & 5mM. Mg^{2+}, Co^{2+}, Mn^{2+} and Fe^{3+} slightly enhanced the enzyme activity at 1mM while at 5mM they caused inhibition. Ca^{2+} slightly enhanced the enzyme activity at 5mM. On the other hand, PP α-Gal of *A. carbonarius* was inhibited with Na^+, Li^+, Ca^{2+}, Ag^{2+}, Hg^{2+}, Zn^{2+}, Mo^{2+}, Cd^{2+}, Pb^{2+} and Fe^{2+} at 1mM & 5mM. Co^{2+} and Fe^{3+} slightly enhanced the enzyme activity at 1 mM while at 5 mM caused inhibition. Mg^{2+} and Mn^{2+} slightly enhanced the enzyme activity at 1mM & 5mM. The enzyme was stored at 0, 4, 30, 45 ºC and the activity was assayed after 5, 10, 15, 20, 25, 30 days. The enzyme was stable when stored at 4 ºC for 20 days for the two experimental fungi.

Key words: *A. awamori, A. carbonarius*, α-Gal; Production, Purification, properties

INTRODUCTION

α-Galactosidase (α-Galactoside galactohydrolase, EC 3.2.1.22) catalyzes the hydrolysis of α-1,6 linked α-galactoside residues from simple and complex oligosaccharides containing terminal α-D-galactosyl groups (Ulezlo and Zaprometova, 1982) such as melibiose, raffinose, stachyose, verbacose and from xylan and galacto(gluco)mannans, galactolipides and other polymeric substrates liberating galactose (Dey and Pridham, 1972). α-Galactosidase (α-Gal) activity has been reported from animals, plants, and microbes (Chinen *et al*., 1981 and Rezessy–Szabo *et al*., 2000), but human and monogastric animals lack α-Gal in their digestive tract. (Viana *et al*., 2007). There are extensive applications of α-Gal in food, feed, and pharmaceutical industries (Cruz & Park, 1982; Dey *et al*., 1993 and Gdala *et al*., 1997). α-Gal is used for hydrolyzing the α-D-galactosyl linkage present in the simple raffinose family oligosaccharides as well as in more complex polysaccharides, which accumulate in the small intestine without digestion, then intestine and undergo anaerobic fermentation producing gases resulting in gastrointestinal disorders (Zaprometova, 1982). Flatulence, which is often accompanied by headache, dizziness, dyspepsia is suggested to be the most important factor that limits the wider utilization of beans in diets (Castillo *et al*., 1990).

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α-Gal has also various industrial applications as in the pulp and paper industry (Adya & Elbein, 1977) biobleaching can be also improved by adding α-Gal in combination with xylanase (Ratto *et al*., 1993). It may also be involved in modification of wood derived materials (Clarke *et al*., 2000). In the processing of beet sugar, α-Gal used in sugar refining because it reduces the raffinose content of sugar beet syrups, where raffinose inhibits sucrose crystallization from syrups there by reducing the yield of the process (Ulezlo & Zaprometova, 1982). A partial or total deficiency of α-Gal may cause a disease called Fabryı s disease, which results from the progressive accumulation of neutral glycosphingolipids in most fluids and tissues of the body (David *et al*., 2007). The Fabryı s disease can be treated by the enzyme replacement therapy (Fuller *et al*., 2004). In addition, it can be used for blood type conversion (Olsson *et al*., 2004), where it cleaves the terminal α-galactose residues from oligosaccharides chain on the surface of blood type B red cells, thus generating type O cells (Zhu *et al*., 1995).

Production of α-Gal has been reported by many microorganisms, such as bacteria (Gote *et al*., 2007; Garro *et al*., 1996; Duffaud *et al*., 1997; Ledar *et al*., 1999; Tzortzis *et al*., 2003 and Athanasios *et al*., 2007). *Streptomyces griseoloalbus* (Anisha and Prema, 2008). Yeasts (Durance and Skura, 1985; Naumova *et al*., 1996), Giuseppin *et al*., 1993), Oda and Tonomura, 1996; Chen *et al*., 2000 and Garcia *et al* ., 2001). α-Gal from fungi was also reported (Arnaud *et al*., 1976; El-Gindy, 2002 and Borzova & Verbanets, 2003; Saad, 2005; Szendefy *et al.,* 2006 and Shankar & Mulimani, 2007; Filho *et al.,* 2008 and Pessela *et al.,* 2008). Solid substrate fermentation (SSF) has gained importance for the production of microbial enzymes due to economical advantages over conventional submerged fermentation (Pandey *et al*., 1999 and Holker *et al*., 2004) and due to the possibility of using cheap and abundant agro-industrial wastes as substrate. It can be of special interest in those processes where the crude fermented product may be used directly as the enzyme source (Pandey *et al*., 1999; Holker & Lenz 2005; and Krishna, 2005). There has been considerable interest to produce α-Gal in SSF processes among various groups of microorganisms (Zeilinger *et al*. 1993; Wang *et al*. 2004; Krishna, 2005 ; Saad, 2005 andShankar and Mulimani , 2007).

This investigation deals with the production of α-galactosidase by *Aspergillus awamori*, *A. carbonarius* in solid substrate cultures using some cheap industrial and natural by-products

MATERIALS AND METHODS

Fungi:

Two selected fungi i.e., *Aspergillus awamori* Nakazawa and *Aspergillus carbonarius* (Bainier) Thom. were isolated from a composite garden soil samples collected from the botanical garden, Biological Sciences Department, Faculty of Education, Cairo, Egypt. They were isolated by direct soil plate method (Warcup, 1950) and identified according to Moubasher (1993). Pure cultures were maintained on Czapek's agar slants.

Culture Medium and Conditions:

A basal solution consisted of 1 g of KH_2PO_4, 0.5 g of KCl, 0.5 g of $MgSO_4.7H_2O$ and 5 g of yeast extract in tap water to a volume of 1000 ml. L-asparagine (4.0 g) is used as a nitrogen source for *A. awamori* while $NaNO_3$ (4.0 g) is used as a nitrogen source for *A. carbonarius* (Ali., *et al* 2007) For each particular treatment a duplicate set of (250 ml) Erlenmeyer conical flasks were prepared containing the basal or optimized media, sterilized at 121 ºC for 20 min. under 1.5 atmospheric pressure and cooled to room temperature. On cooling they were inoculated by evenly prepared spore suspension (10^5 spores ml^{-1}, if otherwise not stated) and incubated at 30 ºC for 10 days. The content of each flask was gathered up and thoroughly mixed with 20 ml cooled sterilized distilled water, squeezed through muslin, filtered and completed volume (20 ml) with cooled sterilized distilled water, then α-Gal activity and protein content of the culture filtrate were determined.

Carbon Substrates:

Powder of cane bagasse, corn bran, soft wood saw dust and soybean, were used in the screening experiment to choose the most potent isolates for α-Gal production.

Industrial By-products:

By-products from different industries were tried to enhance α-Gal production by investigated fungi. These are whey (dairy industry), cane and beet molasses (sugar industry) and corn steep liquor (corn industry). Whey was collected from the rural area of Kaluobia. Cane and beet molasses were obtained from Hawamdiah and Kafr-El-Sheikh sugar factories, respectively.

Treatment of Cane and Beet molasses (CM&BM):

200 ml of either cane or beet molasses was completed to 1liter by tap water, autoclaved at 121 ºC for 20 min. under 1.5 atmospheric pressure and cooled to room temperature, then the molasses were filtered to get rid of the precipitated mud. The filtrate was stored in dark bottles at refrigerator till use.

Treatment of Whey:

Whey used as additive in two forms; crude whey (CW) and whey permeate (WP). The last was prepared as follows:

Crude whey autoclaved at 121 ºC for 20 min. under 1.5 atmospheric pressure cooled to room temperature and then filtered. The filtrate (WP) was stored in dark bottles at refrigerator till use.

The previous by-products (molasses and whey) were kindly analyzed for nitrogen content (NC); carbohydrates content (CC) and mineral content (MC) by the authorities of Desert Research Center, Cairo, Egypt; Results are shown in Table (I).

Table 1: Nitrogen content (NC); carbohydrates content (CC) and minerals content (MC) of molasses and whey.

By-product	NC (mg %)	CC (mg %)	MC (mg %)
Cane molass (CM)	10.9	17.982	Na (61.0), K (650.0), Ca (248.35), Mg (57.35), Fe (6.45), Mn (0.503) and Zn (0.333).
Beet molass (BM)	22.7	12.873	Na (328.5), K (700.0), Ca (17.76), Mg (3.734), Fe (1.099), Mn (0.065) and Zn (0.248).
Crude whey (CW)	1.5	2.104	Na (41.0), K (70.0), Ca (133.70), Mg (16.47), Fe (0.50), Mn (0.015) and Zn (0.428).
Whey permeate (WP)	0.500	0.701	Na (41.0), K (95.0), Ca (85.05), Mg (15.04), Fe (0.18), Mn (0.008) and Zn (0.118)

α-gal Assay:

α-Gal assay was determined using the method adopted by (Rezessy–Szabo *et al.*, 2003).A reaction mixture containing 0.5 ml of 15 mM r-nitrophenyl-a-D-galactopyranoside (PNP-a-gal, Sigma Chemical Co., USA), 0.3 ml of 100 mM acetate buffer (pH 5.0) and 0.2 ml of enzyme solution was incubated at 30°C for 5 min. The reaction was terminated by adding 5 ml of 0.1 M Na_2CO_3 and the released r-nitrophenol was determined by measuring the absorbance at 405 nm (Rezessy-Szabo, 2003). All assays were carried out at pH 5.0. One α -galactosidase Unit (α-gal U) is that amount of enzyme which liberates 1µmole of galactose in one min. under the assay conditions.

Protein Determination:

The method used was described by Lowry *et al.* (1951), using bovine serum albumin as a standard.

Purification of α-Galactosidase:

Purification of α-Gal was carried out through sequential steps. The steps of enzyme purification can be summarized as follows: Cell Free Filtrate (CFF) giving the highest enzyme activity was used in α-Gal purification by growing the experimental fungi on SSC optimized media. Cooled CFF was dialyzed in Sigma dialyzing bags against sterilized distilled water for 24 h at 4 ºC. The Obtained cell-free dialysate (CFD) was precipitated by ammonium sulphate, (60%). The most active fractions were subjected to bentonite (2.5%) as cationic exchanger and eluted with 10 ml of 0.1 M NaCl. The filtrate was represented the partially purified α-Galactosidase. Some properties of the enzyme were elucidated.

RESULTS AND DISCUSSION

Results:

Effect of Different Substrates at Different Initial Moisture Content (MC%) on the α-Gal production:

The potentiality of the two experimental fungi; *A. awamori* and *A. carbonarius* to produce α-Gal in solid substrate cultures was investigated on four different substrates; namely cane bagasse, corn bran, soft wood saw dust and soya flour. Each one of the different substrate was mixed with optimized basal solution in the ratio of (1:1, 1:2, 1:3, 1:4 and 1:5, w/v). One set was used for *A. awamori* and the other for *A. carbonarius*. The moisture content for each substrate was determined.

A. awamori produced highest α-Gal activity (1.218, 0.240 and 1.132 U/g) when grown on corn bran, soft wood saw dust and soya flour medium in the ratio of (1:1, w/v) and moisture content (60.90, 56.36, 55.45%), respectively as carbon substrates (Table 2). *A. carbonarius* showed highest α-Gal activity (1.284, 0.306 and 1.048 U/g) when grown the same substrates in the same ratios and moisture content. (Table 3).

Table 2: Effect of initial moisture content (MC) and carbon substrate/basal solution ratio on the production of α-Galactosidase by *Aspergillus awamori*.

Substrate	Weight of solid substrate : Volume of basal solution (W/V)*														
	1:1			1:2			1:3			1:4			1:5		
	**MC (%)	Specific activity U/mg protein	α-Gal activity U/g Sub-strate	**MC (%)	Specific activity U/mg Protein	α-Gal activity U/g Sub-strate	**MC (%)	Specific activity U/mg Protein	α-Gal activity U/g Sub-strate	**MC (%)	Specific activity U/mg Protein	α-Gal activity U/g Sub-strate	**MC (%)	Specific activity U/mg Protein	α-Gal activity U/g Sub-strate
Cane bagasse	59.09	0.004	0.004	71.88	0.044	0.068	78.57	0.022	0.038	82.69	0.021	0.062	85.48	0.160	0.176
Corn bran	60.90	0.353	1.218	73.13	0.179	0.982	79.52	0.184	1.032	83.46	0.204	1.002	86.13	0.166	0.926
Soft wood saw dust	56.36	0.167	0.240	70.00	0.056	0.030	77.14	0.021	0.030	81.54	0.139	0.272	84.52	0.396	0.864
Soya flour	55.45	0.124	1.132	69.37	0.118	0.930	76.66	0.094	1.006	81.15	0.058	0.860	84.19	0.045	0.838

*(W/V): ratio of solid substrate weight: volume of basal solution.
** (MC): Moisture Content.

Table 3: Effect of initial moisture content (MC) and carbon substrate/basal solution ratio on the production of α-Galactosidase by *Aspergillus carbonarius*.

Substrate	Weight of solid substrate : Volume of basal solution (W/V)*														
	1:1			1:2			1:3			1:4			1:5		
	**MC (%)	Specific activity U/mg protein	α-Gal activity U/g Sub-strate	**MC (%)	Specific activity U/mg Protein	α-Gal activity U/g Sub-strate	**MC (%)	Specific activity U/mg Protein	α-Gal activity U/g Sub-strate	**MC (%)	Specific activity U/mg Protein	α-Gal activity U/g Sub-strate	**MC (%)	Specific activity U/mg Protein	α-Gal activity U/g Sub-strate
Cane bagasse	59.09	0.014	0.014	71.88	0.026	0.046	78.57	0.012	0.22	82.69	0.055	0.126	85.48	0.163	0.314
Corn bran	60.90	0.361	1.284	73.13	0.325	1.138	79.52	0.136	1.002	83.46	0.218	1.148	86.13	0.348	1.212
Soft wood saw dust	56.36	0.449	0.306	70.00	0.007	0.012	77.14	0.384	1.044	81.54	0.306	1.162	84.52	0.332	1.296
Soya flour	55.45	0.100	1.048	69.37	0.124	1.058	76.66	0.137	0.964	81.15	0.156	0.878	84.19	0.126	0.822

*(W/V): ratio of solid substrate weight: volume of basal solution.
** (MC): Moisture Content.

Effect of Corn Bran Loadage on the α-Gal production:

The α-Gal production by the experimental fungi as affected by corn bran loadage (5, 10, 15, 20, 25g/250ml Erlenmeyer conical flasks) was studied. In this experiment; corn bran was added to basal medium in different volumes in the ratio of (1:1, w/v) for cultivation of *A. awamori* and *A. carbonarius*. Incubation period lasted for 10 days at 30 ºC. In each treatment α-Gal activity was assayed. Results (Fig. 1) show that 5g corn bran per flask was the best weight for the α-Gal production where the activity was (1.316 U/g &1.285 U/g) for *A. awamori* and *A. carbonarius* and gradually decreased with the increase of corn bran weight.

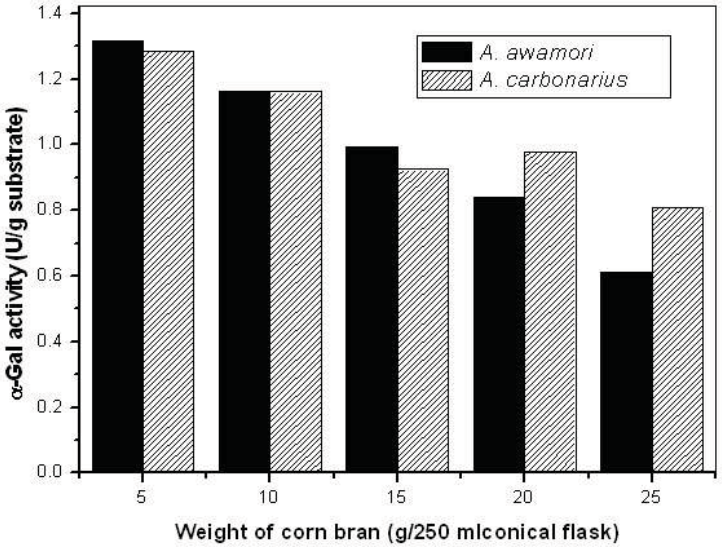

Fig. 1: Effect of corn bran loadage on the on the production of α-Galactosidase by *A. awamori* and *A. carbonarius.*

Effect of Inoculum Density (CFU/ml) on the α-Gal production:

Optimized cultivation medium was inoculated with (2.5×10^5, 5.0×10^5, 7.5×10^5, 1.0×10^6 and 1.25×10^6 CFU /ml spore suspension for *A. awamori* and *A. carbonarius*. Incubation period lasted for 10 days at 30 ºC after which α-Gal was assayed. Results Fig. 2 show that in case of *A. awamori* 5.0×10^5 CFU was the best

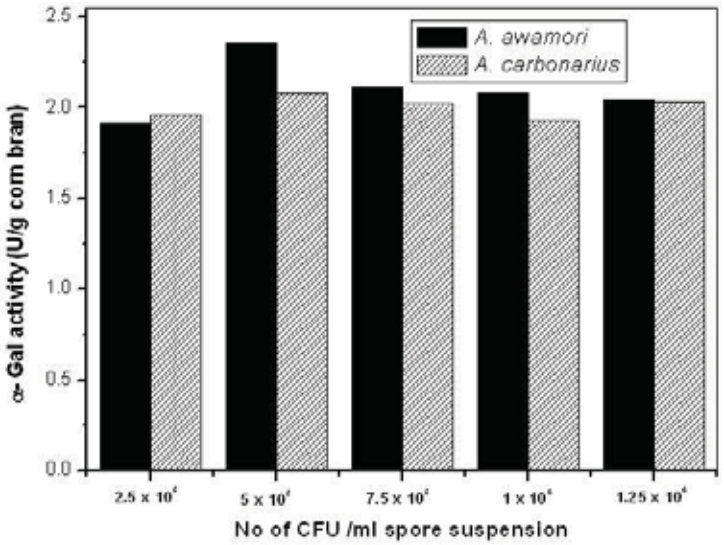

Fig. 2: Effect of inoculums density (CFU / ml) on the production of α-Galactosidase by *A. awamori* and *A. carbonarius.*

inoculum for the α-Gal production where the activity reached (2.356 U/g). While 5.7 $\times 10^5$ CFU was the best inoculum for the α-Gal production for *A. carbonarius* and resulted in activity of (2.080 U/g).

Effect of Incubation Period on the α-Gal production:

α-Gal activity was determined in the culture filtrate after 3, 4, 5, 6, 7, 8, 9 and 10 days incubation temperature was 30 ºC. Results (Fig. 3) show that 6 day incubation was the best for the experimental fungi where *A. awamori* produce a maximum α-Gal production (2.172 U/g) at incubation period of 6 days. While *A. carbonarius* reached a maximum α-Gal production at incubation period of 6 days (2.280 U/g).

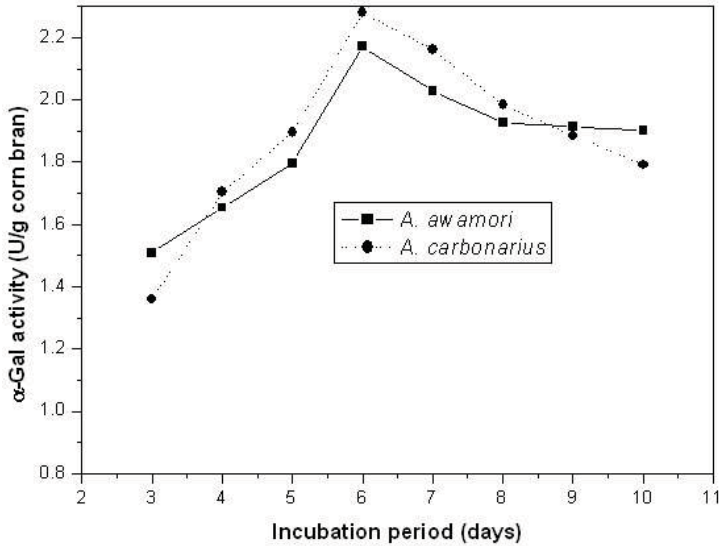

Fig. 3: Effect of incubation period on the production of α-Galactosidase by *A. awamori* and *A. carbonarius.*

Effect of Some Agro-industrial and Additives on the α-Gal production:

Cheap agro-industrial by-products (Corn steep liquor, molasses and whey) and some additives (yeast, malt and beef extracts) were used to motivate the α-Gal production by the experimental fungi in solid substrate

culture. These by-products are renewable and produced in great quantities and often disposed to the environment causing pollution. Due to governmental environmental pressures, it is suitable from the economic point of view to recycle such cheap by-products in other industries.

Effect of Yeast Extract on the α-Gal production:

Optimized medium was fortified different concentrations of yeast extract (0.05, 0.10, 0.15, 0.20, 0.25%). Results Fig. 4 show that α-Gal production was higher when optimized medium was fortified by (0.20%) yeast extract for *A.awamori*, the activity was (2.946 U / g). While addition of yeast extract (0.10%) to optimized medium increased the α-Gal production by *A. carbonarius* (3.123 U/g).

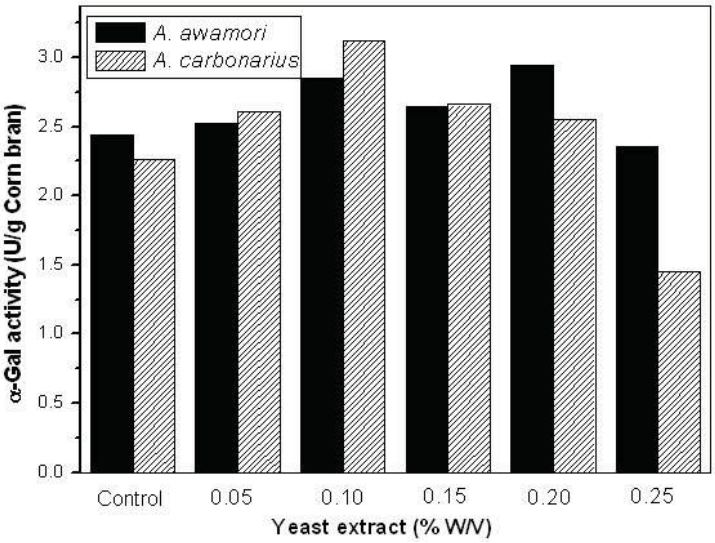

Fig. 4: Effect of yeast extract on the production of α-Galactosidase by *A. awamori* and *A. carbonarius.*

Effect of Malt Extract on the α-Gal production:

Optimized medium was fortified by different concentrations (0.05, 0.1, 0.15, 0.20 and 0.25%) of malt extract. The results (Fig.5) and illustrated in show that addition of malt extract (0.05%) to optimized medium increased the production of α-Gal by *A. awamori* and *A. carbonarius*, where the activity was (3.560 and 3.277 U/g, respectively).

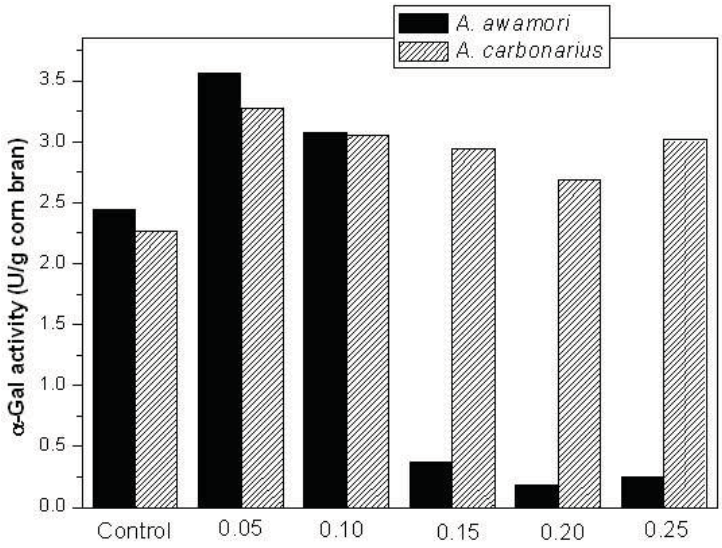

Fig. 5: Effect of malt extract on the production of α-Galactosidase by *A. awamori* and *A. carbonarius.*

Effect of Beef Extract on the α-Gal production:

Optimized medium was fortified by different oncentrations (0.1, 0.2, 0.3, 0.4 and 0.5%) of beef extract. The (Fig. 6) showed that addition of beef extract to optimized medium motivated the production of more α-Gal enzyme by the experimental fungi. In case of *A. awamori* at (0.4%) resulted in α-Gal activity (3.212 U/g). While *A. carbonarius* addition of beef extract (0.3%) increased α-Gal activity to (3.213 U/g).

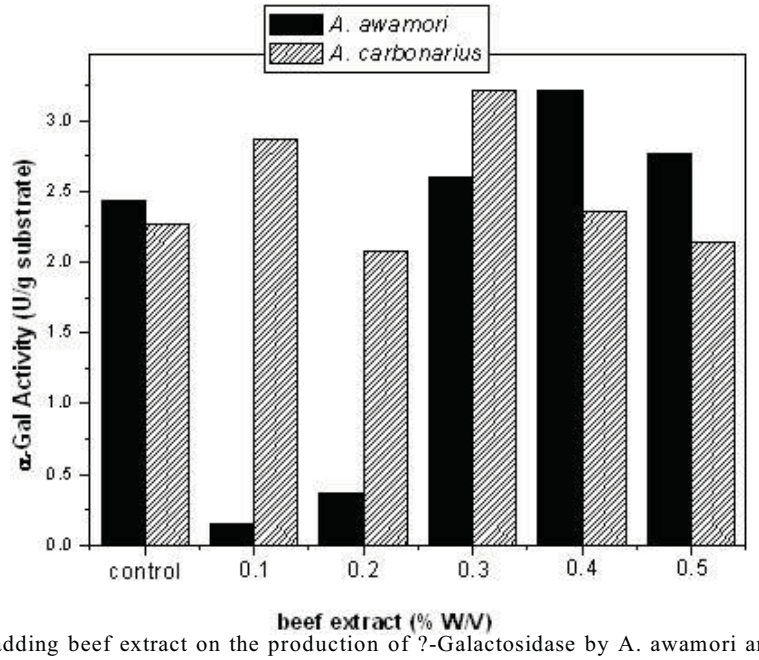

Fig. 6: Effect of adding beef extract on the production of ?-Galactosidase by A. awamori and A. carbonarius.

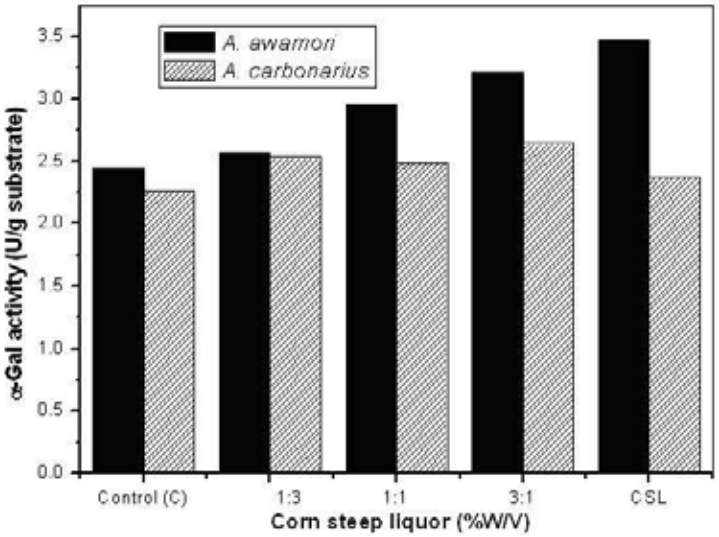

Fig. 7: Effect of addition of corn steep liquor (CSL) on the production of α-Galactosidase by *A. awamori* and *A. carbonarius.*

Effect of Corn Steep Liquor (CSL) on the α-Gal production:

The effect of corn steep liquor (CSL) as a cheap by-product of corn industry on the α-Gal production by the experimental fungi was studied. CSL was added to optimized medium in the ratio of (1:3, 1:1, 3:1 V/V), respectively. Also corn steep liquor was used as the basal solution with corn bran for *A. awamori* and *A. carbonarius*. From the results obtained ; it is very clear that the increase of corn steep liquor to optimized

medium caused an increase in the α-Gal production. Results (Fig. 7) show that in the case of *A. awamori* the increase in corn steep liquor up to (100%) resulted in an increase of α-Gal production when CSL used as the basal solution, where the activity reached (3.467 U/g). On the other hand *A. carbonarius* CSL supported production of α-Gal, where the increase in (CSL) concentration to optimized medium by the ratio (3:1, V/V) caused an increase in α-Gal production, where the activity was (2.643 U/g).

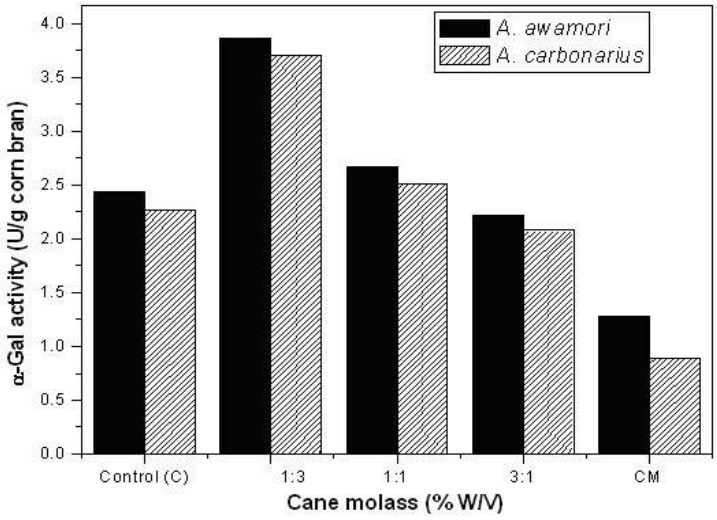

Fig. 8: Effect of addition of Cane molass (CM) on the production of α-Galactosidase by *A. awamori* and *A. carbonarius*

Effect of Cane Molass (CM) on the α-Gal production:

The effect of treated cane molass (CM) as a cheap by-product of cane sugar industry on the production of α-Gal by the experimental fungi was studied. In this experiment; cane molass (CM) was added to optimized medium in the ratio of (1:3, 1:1, 3:1, V/V), respectively. Also cane molass was used as the basal solution with the carbon source (corn bran) for *A. awamori* and *A. carbonarius*. Results (Fig. 8) reveal that addition of cane molass to optimized medium by the ratio of (1:3, V/V) caused high increase in α-Gal production for both of the experimental fungi , the activity was (3.861 U/g and3.707 U/g) for *A.awamori* and *A. carbonarius* respectively and then gradually decreased with the increase in cane molass, the α-Gal activity reached (1.282 U/g and 0.895 U/g) when cane molass was used as the basal solution for *A.awamori* and *A. carbonarius* respectively.

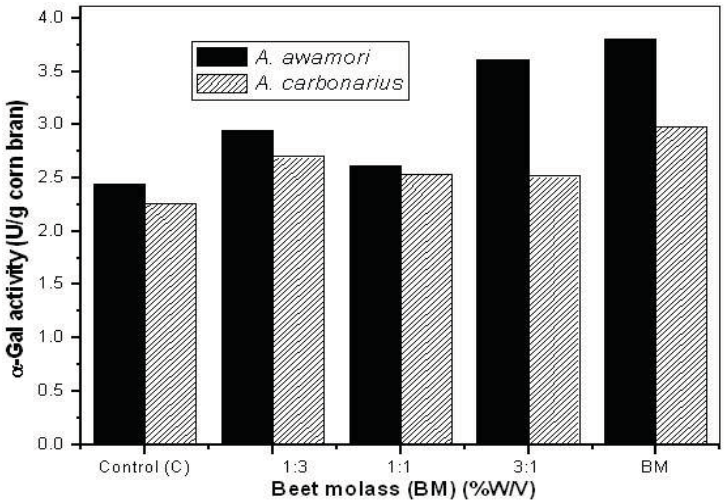

Fig. 9: Effect of addition of Beet molass (BM) on the production of α-Galactosidase by *A.awamori* and *A. carbonarius*.

Effect of Beet Molass (BM) on the α-Gal production:

The effect of treated beet molass (BM) as a cheap by-product of beet sugar industry on the production of α-Gal by the experimental fungi was investigated. In this experiment; beet molass (BM) was added to optimized medium in the ratio of (1:3, 1:1, 3:1, V/V), respectively. Also beet molass was used as the basal solution with corn bran for *A. awamori* and *A. carbonarius*. The results ((Fig. 9) show that the increase in beet molass up to (100%) resulted in an increase of α-Gal production by *A. awamori* and *A. carbonarius* ,when beet molass used as the basal solution, for *A. awamori* the α-Gal activity was (3.804 U/g), while when used as as the basal solution for *A. carbonarius* the α-Gal activity was (2.982 U/g).

Effect of Crude Whey (CW) on the α-Gal production:

Production of α-Gal by using natural by-product from cheese industry was investigated. In this experiment; crude whey (CW) was added to optimized medium in the ratio of (1:3, 1:1, 3:1, V/V), respectively. Also crude whey was used as the basal solution with the corn bran for *A. awamori* and *A. carbonarius*. Results (Fig. 10) reveal that α-Gal for *A. awamori* was much higher when crude whey was added to optimized medium in the ratio of (1:3, V/V), where the α-Gal activity was (3.718 U/g) and gradually decreased with the increase of crude whey. α-Gal activity was (3.092 U/g) when crude whey was used as the basal solution.In the case of *A. carbonarius* α-Gal production gradually increased with the increase in crude whey, where the activity reached (3.781 U/g) when crude whey used as the basal solution.

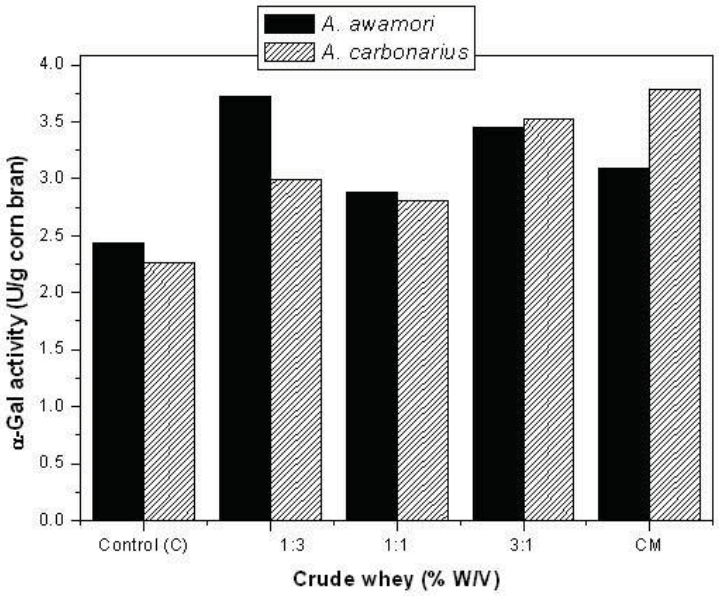

Fig. 10: Effect of addition of Crude whey (CW) on the production of α-Galactosidase by *A. awamori* and *A. carbonarius.*

Effect of Whey Permeate (WP) on the α-Gal production:

The effect of whey permeate (WP) as a natural by-product from cheese industry on the α-Gal production by the experimental fungi was studied. In this experiment; whey permeate (WP) was added to optimized medium in the ratio of (1:3, 1:1, 3:1, V/V), respectively. Also whey permeate was used as the basal solution with corn bran for *A. awamori* and *A. carbonarius*. The results (Fig. 11) show that addition of whey permeate to optimized medium by the ratio of (1:3, V/V) increased in α-Gal production by *A. awamori*, where the activity was (3.357 U/g) and gradually decreased with the increase of whey permeate where the activity reached (3.021 U/g) when whey permeate was used as the basal solution.In case of *A. carbonarius* the results show that 1:3 (V/V) of whey permeate to optimized medium resulted in high production of α-Gal activity (3.850 U/g) and gradually decreased with the increase of whey permeate and reached to (3.550 U/g) when permeate whey was used as the basal solution.

Purification of α-Gal Produced by the Experimental Fungi:

A.awamori was grown on a cheap natural medium consists of 5.0g corn bran as carbon substrate moistened with 5 ml treated beet molass, fortified with 0.05% malt extract, 5.0×10^5 CFU/ml. The pH of optimized

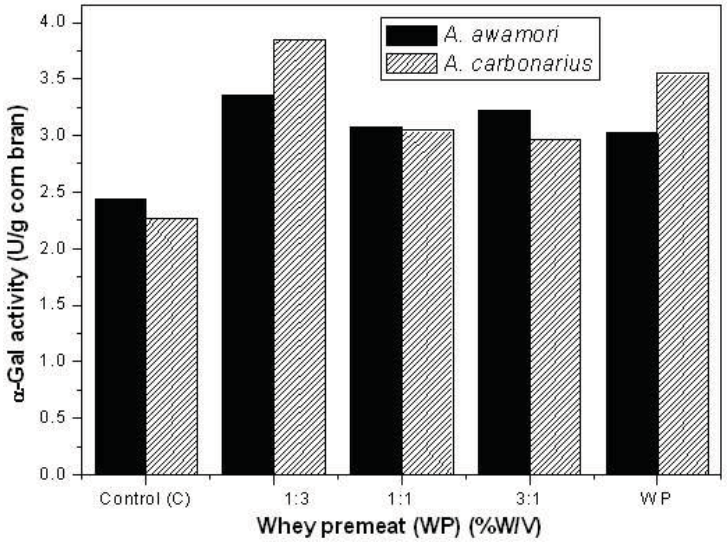

Fig. 11: Effect of addition of whey premeat (WP) on the production of α-Galactosidase by *A.awamori* and *A. carbonarius.*

medium was 6.1. Incubation temperature was 30 ºC for 6 days; giving α-Gal activity (3.844 U/g substrate). *A. carbonarius* was grown on a cheap natural medium consists of 5.0 g corn bran as carbon substrate moistened with 5 ml crude whey, fortified with 0.05% malt extract, 5.7 $\times 10^5$ CFU/ml. The pH of optimized medium was 3.9. Incubation temperature was 30 ºC for 6 days; giving α-Gal activity (3.912 U/g substrate). The pH of culture medium for both *A. awamori* and *A. carbonarius* was adjusted to pH 5.2. Cell free filtrate (CFF) was used as a source of crude α-Gal enzyme.

Precipitation of α-Gal Enzyme Produced by the Experimental Fungi:

In this experiments; the cell free filtrate (CFF) of the two experimental fungi were dialysed for 24 h. using dialysis bags (Sigma, USA) at 4 ºC against distilled water and precipitated by salting out with ammonium sulphate. The results show that the best α-Gal activity was obtained at 60 % ammonium sulphate (0.809 U/ml), specific activity (0.889 U/mg), recovery (85.79%) and purification fold (3.87) for *A. awamori*. The results for *A. carbonarius* show that α-Gal was most active at (60%) with activity of (0.850 U/ml), specific activity (0.877 U/mg), recovery (87.63%) and purification fold (3.93).

Partial Purification of α-Gal of A. Awamori on Ion Exchange Chromatography on Bentonite:

The resultant precipitate resulted from (60%) ammonium sulphate treatment of cell free dialysate (CFD) preparation of *A. awamori* was treated by bentonite. The results show that the treatment of ammonium sulphate dialysate (ASD) by (2.5%) bentonite revealed best α-Gal activity (0.116 U/ml), specific activity (1.289 U/mg), recovery (12.30%) and purification fold (5.60). A summary of treatments used for partial purification of α-Gal of *A. awamori* is shown in Table (4).

Partial Purification of α-Gal of A. carbonarius on Ion Exchange Chromatography on Bentonite:

The resultant precipitate resulted from (60%) ammonium sulphate treatment of cell free dialysate (CFD) preparation of *A. carbonarius* was treated by bentonite. The results show that the treatment of ammonium sulphate dialysate (ASD) by (2.5%) bentonite revealed best α-Gal activity (0.108 U/ml), specific activity (1.213 U/mg), recovery (11.13%) and purification fold (5.44). In (Table 5) Partial purification steps of α-Gal of *A. carbonarius* are summarized.

Some Properties of α-Gal Enzyme Produced by the Experimental Fungi:

The effect of temperature, pH, addition of modulators, and effect of prolonged storage storage on the activity of partially purified α-Gal of *A. awamori* and *A. carbonarius* 1.2 U/mg protein were studied.

Table 4: Summary of partial purification steps of α-Galactosidase from *Aspergillus awamori.*

Purification step	Volume (ml)	Total Protein (mg)	Total activity (U)	Specific activity (U/mg protein)	Recovery (%)	Purification fold
CFF	200	818.0	188.60	0.230	100.0	1.00
CFD	152	560.88	142.88	0.254	75.76	1.10
$(NH_4)_2SO_4$ (60%)	60	93.60	54.36	0.580	28.82	2.52
ASD	64	58.24	51.78	0.889	27.45	3.87
Bentonite (2.5%)	55	4.95	6.38	1.289	3.38	5.60

Table 5: Summary of partial purification steps of α-Galactosidase from *Aspergillus carbonarius.*

Purification step	Volume (ml)	Total Protein (mg)	Total activity (U)	Specific activity (U/mg protein)	Recovery (%)	Purification fold
CFF	200	872.0	194.00	0.223	100.0	1.00
CFD	152	601.92	146.07	0.243	75.29	1.09
$(NH_4)_2SO_4$ (60%)	60	130.80	54.06	0.413	27.87	1.85
ASD	64	62.08	54.40	0.877	28.04	3.93
Bentonite (2.5%)	52	4.63	5.62	1.213	2.90	5.44

Table 6: Effect of some metal ions on the activity of partially purified α-Gal of *A. awamori* and *A. carbonarius.*

Metal ion	Conc. (mM)	Relative activity (%)*	
		A. awamori	*A. carbonarius*
Control	0	100.0	100.0
Na^+	1	94.49	98.33
	5	84.62	90.71
Li^+	1	97.98	79.76
	5	71.99	70.83
Ca^{2+}	1	87.83	85.83
	5	111.94	70.59
Mg^{2+}	1	106.31	108.21
	5	68.77	106.90
Co^{2+}	1	105.40	102.62
	5	91.85	94.40
Ag^{2+}	1	47.07	66.07
	5	39.49	56.07
Hg^{2+}	1	65.56	62.38
	5	59.59	55.95
Mn^{2+}	1	109.53	113.57
	5	99.89	106.31
Zn^{2+}	1	92.08	76.90
	5	71.76	59.76
Mo^{2+}	1	75.09	79.76
	5	66.70	75.48
Cd^{2+}	1	66.89	65.87
	5	33.66	58.66
Pb^{2+}	1	53.73	67.86
	5	45.46	33.33
Fe^{2+}	1	98.46	99.95
	5	29.82	75.70
Fe^{3+}	1	102.41	101.26
	5	75.11	61.61

*Time of pre-incubation: 5 min.
Molarity and type of buffer: (0.2M) citrate-phosphate buffer.
Temperature of incubation: 55 ºC

Effect of Reaction Temperature on the α-Gal activity:

This was studied by incubation of the partially purified enzyme with the standard reaction mixture in water bath set at different temperatures ranging from 30 to 60 ºC for 5 minutes, then the α-Gal activity was determined as previously described. The results (Fig. 12) show that the activity of partially purified α-Gal of *A. awamori* and *A. carbonarius* increased with temperature increase and reached its maximum activity at 55 ºC, where the activity was 0.812 and 0.932 U/ml, respectively followed by gradual decrease with increase of temperature.

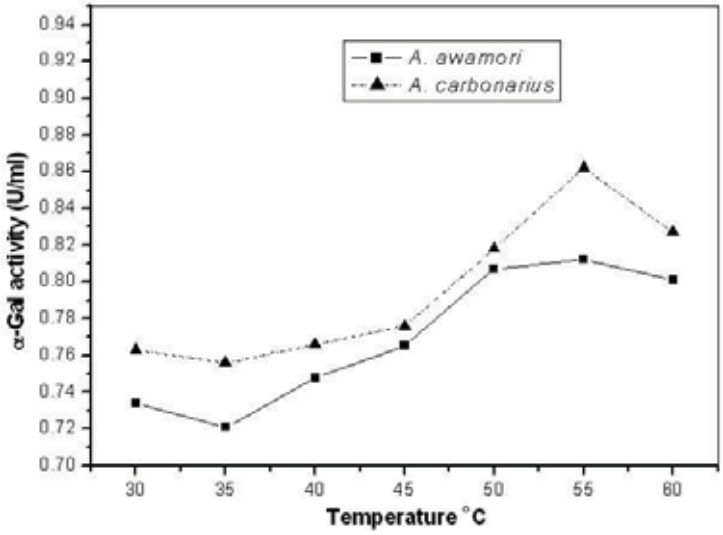

Fig. 12: Effect of temperature on the activity of partially purified α-Galactosidase of *A. awamori* and *A. carbonarius.*

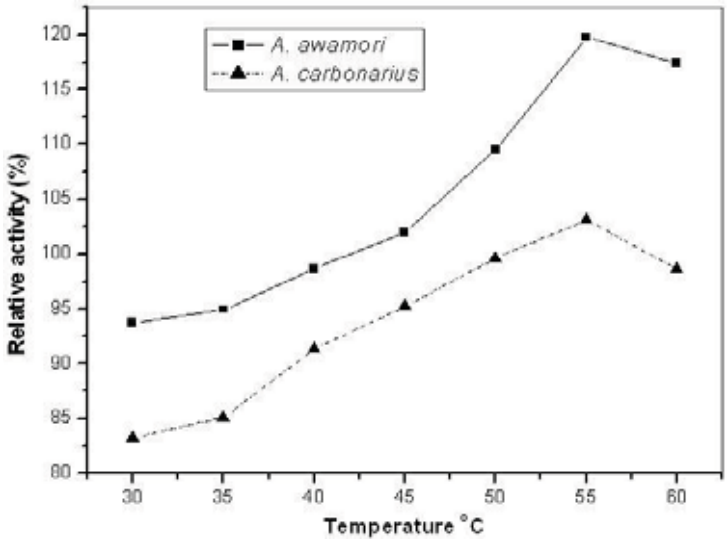

Fig. 13: Effect of thermal stability on the activity of partially purified α-Galactosidase of *A. awamori* and *A. carbonarius.*

Thermal Stability of the Partially Purified α-Gal:

In this experiment; enzyme solution was pre-incubated at different temperatures ranging from 30 to 60 ºC for 30 minutes, after which, the relative activity assayed at 55 ºC.

The results (Fig. 13) show that the partially purified α-Gal of *A. awamori* and *A. carbonarius* was stable at 55 ºC for 30 minutes and the relative activity reached 117.44% and 98.62%, respectively after 30 minutes at 60 ºC.

Effect of pH Value on the Activity of Partially Purified α-Gal:

In this experiment, citrate-phosphate buffer (0.2 M) with different pH values ranging between 3.6 and 5.6 was used at standard conditions of enzyme assay. The results (Fig. 14) show that the best pH value was 4.8, where the activity was 0.859 and 0.865 U/ml for *A. awamori* and *A. carbonarius*, respectively followed by gradual decrease with increase of pH value.

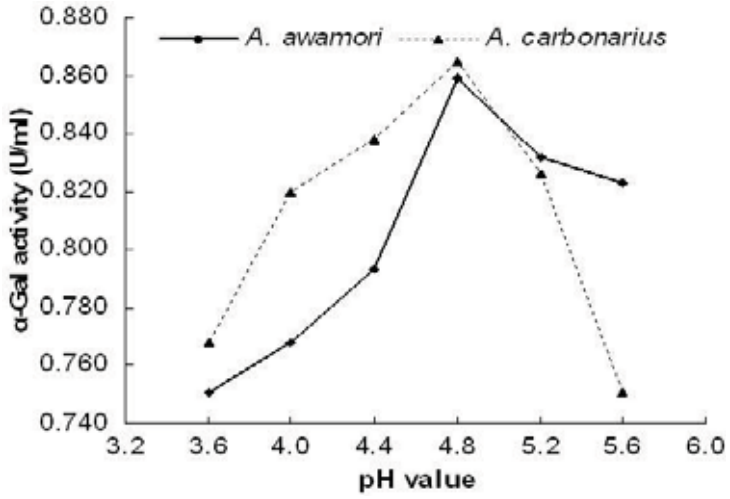

Fig. 14: Effect of pH value on the activity of partially purified α-Galactosidase of *A. awamori* and *A. carbonarius.*

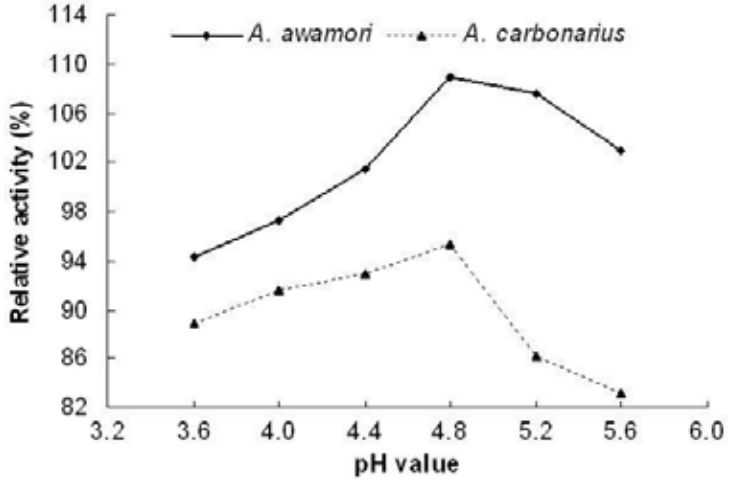

Fig. 15: Effect of pH value on the stability of partially purified α-Galactosidase of *A. awamori* and *A. carbonarius.*

Effect of pH value on the stability of partially purified α-Gal:

The effect of pH value on the enzyme stability could be distinguished experimentally by pre-incubating the enzyme at different pH values (3.6-5.6) for 24 h , after which the enzyme substrate was added and incubation for 5 minutes, the other steps for determination of α-Gal activity completed and the relative activity after that was estimated. The results (Fig. 15) show that the partially purified α-Gal of *A. awamori* and *A. carbonarius* was stable at pH 4.8 for 24 h.

Effect of Some Metal Ions on the Activity of Partially Purified α-Gal:

The influence of fourteen different metal ions was individually investigated upon the partially purified α-Gal of *A. awamori* and *A. carbonarius*. The enzyme was incubated for 5 minutes with various modulators at final concentrations 1.0 & 5.0 mM, and then assayed for enzyme activity.The results (Table 6) show that the activity of partially purified α-Gal of *A. awamori* was inhibited by Na^{+}, Li^{+}, Ag^{2+}, Hg^{2+}, Zn^{2+}, Mo^{2+}, Cd^{2+}, Pb^{2+} and Fe^{2+} at the two investigated levels. Mg^{2+}, Co^{2+}, Mn^{2+} and Fe^{3+} slightly enhanced the enzyme activity at low concentration while at the high concentration caused inhibition. High concentration of Ca^{2+} slightly enhanced the enzyme activity and low concentration decreased it.In case of *A. carbonarius*; the activity of partially purified α-Gal was inhibited by Na^{+}, Li^{+}, Ca^{2+}, Ag^{2+}, Hg^{2+}, Zn^{2+}, Mo^{2+}, Cd^{2+}, Pb^{2+} and Fe^{2+} at the

Table 7: Effect of some enzyme inhibitors on the activity of partially purified α-Galactosidase of *A. awamori* and *A. carbonarius*

Inhibitor	Conc. (mM)	Relative activity (%)	
		A. awamori	*A. carbonarius*
Control	0	100.0	100.0
Sodium arsenate	1	84.04	74.88
	5	78.19	69.29
EDTA *	1	52.01	55.12
	5	23.31	45.24
PMSF **	1	14.58	13.69
	5	9.53	11.55
DCPAB ***	1	30.54	29.17
	5	14.24	19.40

EDTA * = Ethylenediaminotetraacetic acid.
PMSF** = Phenylmethylsulfonylfluoride.
DCPAB*** = Dichloroparaaminobenzoic acid.

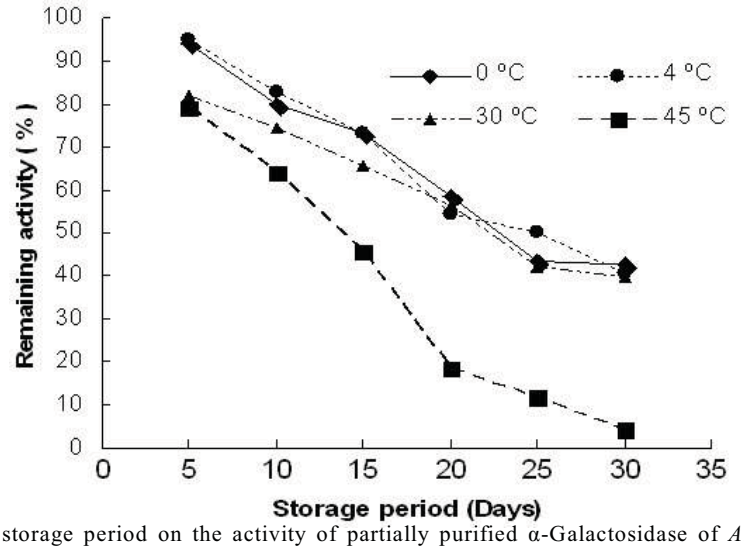

Fig. 16: Effect of storage period on the activity of partially purified α-Galactosidase of *A. awamori.*

two investigated levels. Co^{2+} and Fe^{3+} slightly enhanced the enzyme activity at low concentration while at the high concentration caused inhibition. Mg^{2+} and Mn^{2+} slightly enhanced the enzyme activity at the two investigated levels.

Effect of Some Enzyme Inhibitors on the Activity of Partially Purified α-Gal:

Four different enzyme inhibitors were separately added to the reaction mixtures to test their possible influence on the partially purified α-Gal of *A. awamori* and *A. carbonarius* as described in the previous experiment. The results (Table 7) show clearly that all the tested inhibitors produce an inhibiting effect on partially purified α-Gal. The inhibition caused by enzyme inhibitors ranged between 9.53 and 84.04% for *A. awamori* while ranged between 11.55 and 74.88% for *A. carbonarius*. (PMSF) and (DCPAB) the most effective inhibitors.

Effect of Prolonged Storage on the Activity of Partially Purified α-Gal:

In this experiment; the partially purified α-Gal was stored at different conditions; were it was stored at 0, 4, 30, 45 ºC. The α-Gal activity was assayed after 5, 10, 15, 20, 25, 30 days. The results (Figs. 16 &17) show that ; in case of *A. awamori* ; the enzyme retained about 60% of its activity after prolonged storage for 20 days at 0 ºC and retained 50% of its activity after 25 and 20 days at 4 and 30 ºC, respectively. The enzyme retained 79% of its activity after storage for 5 days at 45 ºC. The partially purified α-Gal of *A. carbonarius* retained about 60% of its activity after storage for 15 days at 0 ºC and retained 50% of its activity after 20 and 15 days at 4 and 30 ºC, respectively. The enzyme retained 72% of its activity after storage for 5 days at 45 ºC. Generally the partially purified α-Gal was stable when stored at 4 ºC for 20 days.

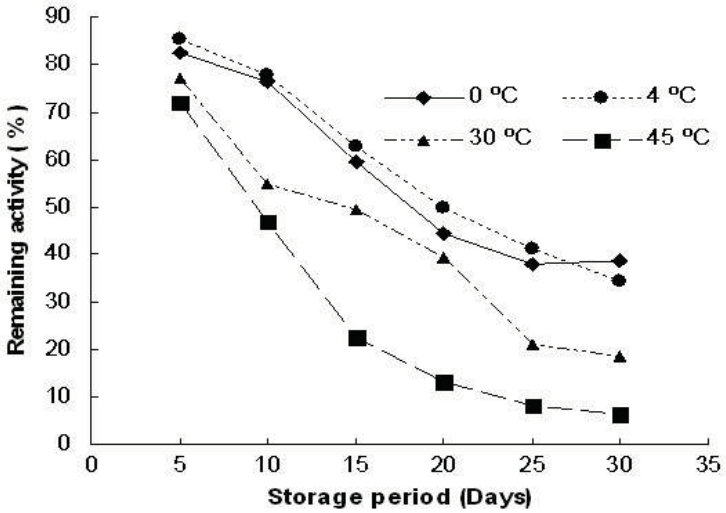

Fig. 17: Effect of storage period on the activity of partially purified α-Galactosidase of *A. carbonarius.*

Discussion:

α-Galactosidases (α-Galactoside galactohydrolase, EC 3.2.1.22) hydrolyze the α-1,6-linked α-galactoside residues from α-galactosides including galacto-oligosaccharides, galactomannans and galactolipides (Dey & Pridham, 1972, Ulezlo & Zaprometova, 1982 and Naumoff, 2004). Transgalactosidases activity was also demonstrated in case of some α-Galactosidases (Adya & Elbein, 1977). The α-Galactosidases have an increasingly practical potential in food, feed, and pharmaceutical industries (Cruz & Park, 1982; Dey *et al*., 1993 and Gdala *et al*., 1997). Formerly the α-Galactosidase enzymes were mainly extracted from plants (Chinen *et al*., 1981) with small efficiency and high cost. Applying fermentation technology with relevant microorganisms makes this process more efficient (Pandey *et al*., 1999 and Holker *et al*., 2004). One approach has been to obtain a potent α-Galactosidase producer through gene manipulations and cloning which is very expensive. A second attractive option is to develop natively occurring α-Galactosidase producing fungi. In recent years, there has been growing interest in α-Galactosidase production by fungi (e.g. Puchart *et al*., 2000; Rezessy–Szabo *et al*., 2000, 2002, 2003 and 2007; Ademark *et al*., 2001; El-Gindy, 2002 and Borzova & Verbanets, 2003; Wang *et al*., 2004; Sonia *et al*., 2005; Saad, 2005; Szendefy *et al.,* 2006; Shijun *et al*., 2007; Cao *et al.,* 2007 and Pessela *et al.,* 2008).

α-Gal production by the two promising fungi i.e. *A. awamori* and *A. carbonarius* was optimized under Solid Substrate Culture (SSC) conditions. There has been considerable interest to produce α-Gal in Solid Substrate Fermentation (SSF) processes. The filamentous fungi are most exploited because their ability to grow on SSF and to produce a wide range of extracellular enzymes (Krishna, 2005). Solid Substrate Fermentation (SSF) is an efficient enzyme production method, mimicking nature, producing concentrated enzyme solution, employing cheap and easily available substrates and reduces the cost of the enzyme production. Corn bran (CB) was the best solid substrate enhancing α-Gal production by the two experimental fungi. To the best our knowledge, this is the first report about α-Gal production on corn bran (CB). Wheat bran is a popular carbon source in SSF for production of α-Gal by fungi (Cruz & Park, 1982; Annunziato *et al.,* 1986; Kotwal *et al.,* 1998; Pandey *et al*., 1999 and Wang *et al*., 2004). Soybean meal and sugar beet pulp are also repeatedly used in α-Gal biosynthesis (Brumer *et al*., 1999 and Saad, 2005). El-Gindy (2002) used soft wood pulp as substrate in the production of α-Gal**.** When moisture content (MC %) of the medium was (60.90%) α-Gal activity reached its peak value (1.218 and 1.284 U/g substrate) in case of *A. awamori* and *A. carbonarius*, respectively. Similar results were obtained (Annunziato *et al.,* 1986; Wang *et al*., 2004 and Shankar & Mulimani, 2007). The moisture content is known to affect the hydrolytic enzyme production in SSF significantly (Battalino *et al.,* 1991). Lower moisture levels leads to suboptimal growth, a lower degree of substrate swelling and high surface tension, whereas higher moisture level decrease porosity, changes corn bran particle structure and promotes the development of stickiness and agglomeration which would cause lower oxygen transfer and heat dissipation and enhanced formation of surface mycelium (Lonsane *et al.,* 1985). *A. awamori* and *A. carbonarius* are aerobic fungi, and both free water and oxygen are essential, so good substrate porosity is quite critical for their metabolism.

The effect of medium loadage play an important role in fungal growth and enzyme production. 5g CB/250 ml conical flask inoculated with 5.0×10^5 and 5.7×10^5 CFU/ml from seven days old culture of both *A. awamori* and *A. carbonarius*, respectively enhanced α-Gal production. Adequate inoculum and an appropriate loadage can initiate fast mycelium growth and enzyme production (Wang *et al*., 2004). The pH of SSF medium was 5.1 and 4.9 in case of *A. awamori* and *A. carbonarius*, respectively. It is well known that solid substrate contributes to better efficiency and filamentous fungi have reasonably good growth over a broad range of pH 2-9, with and optimal range of 3.8-6.0 (Krishna, 2005).

The effect of certain additives such as yeast extract (0.05, 0.1, 0.15, 0.20 and 0.25%), malt extract (0.05, 0.1, 0.15, 0.20 and 0.25%) and beef extract (0.1, 0.2, 0.3, 0.4 and 0.5%), in addition to some agro-industrial and dairy by-products such as corn steep liquor, cane molass, beet molass, crude whey and permeate whey at different ratios: optimized basal solution (OBS), (1:3, 1:1, 3:1 and 100%) on the enzyme production was investigated. Diluted treated molasses were used. An obvious increase α-Gal by the two experimental fungi was noticed.

Yeast-, malt- and beef-extracts are routinely used in formulation of different culture media for growth and enzyme production of fungi. The results of our study reveals that addition of these extracts enhanced α-Gal production by the experimental fungi. This may be attributed to the presence of amino acids, vitamins and minerals in these extracts. Addition of malt extract (0.05%) resulted in production of 3.560 and 3.277 U/g substrate from *A. awamori* and *A. carbonarius*, respectively. Beet molass (100%) and crude whey (100%) supported fungal growth and α-Gal production in case of *A. awamori* (3.804 U/g CB) and *A. carbonarius* (3.781 U/g CB), respectively.

Beet molass, a by-product of beet sugar industry, is one of the cheapest sources of carbohydrates (12.873 mg %), nitrogenous substances (22.7 mg %) and minerals (mg %) Na, 328.5; K, 700.0; Ca 17.76; Mg, 3.734; Fe, 1.099; Mn 0.065 and Zn, 0.248. In addition, large amounts of vitamins such as thiamine, riboflavin, pyridoxine, folic acids, biotin and pantothenic acid were reported (Crueger and Crueger, 2000).

Whey can be considered as an important substrate for fungal growth; which accumulates primary as a by-product from cheese manufacture or whey processing industries. Whey is rich in carbohydrates (10.5 mg %), nitrogenous substances (2.104 mg %) and minerals (mg %) Na, 41.0; K, 70.0; Ca 133.70; Mg, 16.47; Fe, 0.50; Mn 0.015 and Zn, 0.428. Lactose is the major carbohydrate while casein in an important protein in crude whey. Today whey (lactose) is an important carbon source for glycosidase fermentations that can be used economically (Weignerova *et al.,* 1999 and Seiboth *et al.,* 2007). Inclusion of 4.0% guar gum and 1.0% lactose in a basal wheat bran medium in SSF increased the production of α-Gal from *A. niger* by 32.62% (Srinivas *et al.,* 1993). On a technical scale; lactose is an important carbon source for hemicellulolytic enzyme (including α-Gal) fermentations with the ascomycete *Hypocrea jecorina* (*Trichoderma reesei*) (Cherry and Fidantsef, 2003). Alpha linked galactooligosaccharides was produced from lactose *via* reverse hydrolysis catalysed by the alpha-galactosidase of *Candida guilliermondii* (Hashimoto *et al.,* 1994). Formation of α-galactooligosaccharides *via* reverse hydrolysis of lactose present in whey induces the production of α-Gal by *A. awamori* and *A. carbonarius*.

Optimized Solid Substrate Fermentation (OSSF) for both *A. awamori* and *A. carbonarius* affords cheap renewable substrates (corn bran, beet molass and whey) cost effective for α-Gal production in titres comparable with other fungi. The process of α-Gal production in laboratory scale may have the potential to scale-up.

There is no universal prescription for the order in which the various techniques should be applied to the purification of a particular protein (Wilson and Walker, 1994). A simple and inexpensive method was used for partial purification of α-Gal from culture filtrate of both A. *awamori* and *A. carbonarius*. Salting out with ammonium sulphate (60%) gave 28.82 and 27.87% yield with purification fold 2.52 and 1.85 for the two experimental fungi, respectively. A yield of 3.38 and 2.90% with purification fold 5.60 and 5.44 was obtained after bentonite (2.5%) treatment in case of the two experimental fungi, respectively. Bentonite is nontoxic adsorbent behaves essentially as cationic exchanger. Bentonite has been used for purification of a number of enzymes such as pectinlyase, polygalacturonase, β-glucosidase, α-L-arabinofuranosidase and α-Galactosidase (Bailey and Ojamo, 1990; Spagna and Pifferi 1994; Spagna *et al.,* 1998; El-Gindy 2002 and Saad 2005).

Some properties of the partially purified enzyme were investigated. The optimum temperature was 55 ºC and the enzyme was stable at a wide range of temperature (30-60 ºC) for both fungi. *A. carbonarius* retained less activity.

The optimum pH was 4.8 and the enzyme was stable at a wide range of pH (3.6-4.8) for both the experimental fungi, after which the enzyme stability declined. An α-Gal with an optimal pH 4.0 was isolated from *A. niger* (Bahl and Agrawal, 1969). Strain of *A. niger* produced α-Gal with pH 4-4.5 (Adya and Elbein, 1977). Somiari and Balogh (1995) isolated α-Gal from *A. niger* with optimal activity pH 5.0 and

50 ºC. Manzanares *et al.,* (1998) isolated α-Gal from *A. niger* strain optimally active at pH 4.5 and 50-55 ºC. El-Gindy (2002) purified α-Gal from a local *A. niger* isolate. The enzyme was optimally active at pH 5.0 and 50 ºC and stable over a wide range of pH (4.0-5.0) and temperature (37-70 ºC). The properties of our α-Gal introduce it as a potential candidate for application processes.

Partially purified (PP) α-Gal of *A. awamori* was inhibited with Na^{+}, Li^{+}, Ag^{2+}, Hg^{2+}, Zn^{2+}, Mo^{2+}, Cd^{2+}, Pb^{2+} and Fe^{2+} at 1mM & 5mM. Mg^{2+}, Co^{2+}, Mn^{2+} and Fe^{3+} slightly enhanced the enzyme activity at 1mM while at 5mM caused inhibition. Ca^{2+} slightly enhanced the enzyme activity at 5mM. On the other hand, PP α-Gal of *A. carbonarius* was inhibited with Na^{+}, Li^{+}, Ca^{2+}, Ag^{2+}, Hg^{2+}, Zn^{2+}, Mo^{2+}, Cd^{2+}, Pb^{2+} and Fe^{2+} at 1mM & 5mM. Co^{2+} and Fe^{3+} slightly enhanced the enzyme activity at 1 mM while at 5 mM caused inhibition. Mg^{2+} and Mn^{2+} slightly enhanced the enzyme activity at 1mM & 5mM. The inhibition of α-Gal by heavy metals has been previously reported (Zapater *et al.,* 1990; Kotwal *et al.,* 1999 and Rezessy-Szabo *et al.,* 2007). This may be attributed to the presence of sulfhydryl (-SH) group at the active site of α-Gal.

Heavy metals interfere with substrate in the catalytic pocket and inhibit the activity of α-Gal. SH groups play an important role in supporting the active conformation of the protein molecule (Malanchuk *et al.,* 2000).

The enzyme was stable when stored at 4 ºC for 20 days; In case of *A. awamori* ; the enzyme retained about 60% of its activity after prolonged storage for 20 days at 0 ºC and retained 50% of its activity after 25 and 20 days at 4 and 30 ºC, respectively. The enzyme retained 79% of its activity after storage for 5 days at 45 ºC. PP α-Gal of *A. carbonarius* retained about 60% of its activity after storage for 15 days at 0 ºC and retained 50% of its activity after 20 and 15 days at 4 and 30 ºC, respectively. The enzyme retained 72% of its activity after storage for 5 days at 45 ºC. Possibility of prolonged storage is an important character in enzyme technology. This property is very useful for their technical application.

Conclusions:

Alpha-Galactosidases from *A. awamori* and *A. carbonarius* are promising for application. The enzyme can be produced by developing an inexpensive process of SSF on corn bran, thus holding a promise for developing countries. This affords a cheap alternative for mass production of α-Gal and application in food, feed, pharmaceutical industries.

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