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Biosynthesis, partial purification and characterization of invertase through carrot (*Daucus carota* l.) peels

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Abstract

The present study was investigated on the production of Invertase under solid-state fermentation (SSF) through Aspergillus species, by using Carrot peels (*Daucus carota L.*) as a substrate. The highest productivity of Invertase (7.95±0.1 U/ml) was achieved by using *Aspergillus niger* at 90% initial moisture content with 1×10^6 spores/ml after 72 hours of incubation period. The enzyme was purified about 1.42-fold by ammonium sulphate precipitation. It showed thermal stability from 20-70^oC over a pH range (5.5 to 6.5) with maximum activity at pH 5.5 and 50°C. The enzyme was highly active towards sucrose at both concentrations viz: 0.1 M and 0.5 M, but it showed less activity towards glycerol. It was completely inhibited by Hg^{2+} (1mM) and slightly stimulated by Co^{2+} and Na^{+} at the same concentration.

Key words: Food processing wastes, Carrot peels, Invertase, Purification, *Aspergillus niger*, solid-sate Fermentation.

Introduction

Agro-industrial wastes and by-products are renewable form of resources generates round the year all over the world. Wheat and rice bran, sugar cane bagasse, corn cobs, citrus and mango peel etc. are one of important wastes of food industries. These materials are accumulated in enormous amounts in Pakistan alone and serve as source of environmental pollution (GOP 2009). Bioconversion of carbohydrate wastes is receiving increased attention in view of the fact that these wastes can act as substrates for the production of useful biomaterials and chemical intermediates with rapid industrial development, there is a need for environmentally sustainable processes, and there is a general agreement that sustainable environmental protection can only be achieved by integrating a general environmental awareness. Recently, conversion of renewable raw materials into chemicals has become a major subject of research and process development around the world (Jin et al. 2005).

Increasing concern about pollution that occurs from agricultural and industrial wastes has stimulated interest in converting waste materials into commercially valuable products. The food industry produces large volumes of wastes, both solids and liquids resulting from the production, preparation and consumption of food. Beside their pollution and hazardous aspects, in many cases, food processing wastes might have potential for recycling raw materials or for conversion into useful product of higher value (Sangeetha et al. 2004; Mamma et al. 2008; Rashad & Nooman, 2008; Guimaraes et al. 2009).

Carrots (*Daucus carota* L.) are rather inexpensive and highly nutritious as it contains appreciable amount of vitamins and minerals. They are rich source of energy because it contains a lot of sucrose (Manjunatha et al. 2003). After processing, carrot residues, e.g. peels, pomace, are usually discarded or used as animal feed. However, carrot by-products still contain high contents of beneficial substances, especially bioactive compounds with antioxidant activities (Zhang & Hamauzu 2004).

Advent of biotechnology helped to unlock novel food ingredients through the use of biotechnologically-derived industrial enzymes. Microbes have played pivotal role in this progress and currently the fermented products contribute adequate share. Their role in bioconversion of waste commodities into value added products has been highlighted in the recent decades (Pandey et al. 2000; Ahuja et al. 2004). The utilization of microbial enzymes has found broad technological application in different industrial processes. Among the various enzymes commercialized many are products of fermentation of filamentous fungi (Piccoli-valle et al. 2001). The increase use of enzymes to replace traditional chemical transformation processes is obsessed by an aspiration for better production economics, new product functionalities, improved

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safety, and an increasing desire to reduce the environmental pollution. To meet this increasing demand for enzymes, most new enzymes are produced from fungal or bacterial kingdom grown in large-scale fermenters using agro-industrial waste products (Cherry et al. 2001).

Invertase (β-D-fructofuranosidases fructohydrolases, EC. 3.2.1.26) is used for the inversion of sucrose in the preparation of invert sugar and high fructose syrup (HFS). It is one of the most widely used enzymes in food industry where fructose is preferred than especially in the preparation of jams and candies because it is sweeter and does not crystallize easily D-glucose and D-fructose at concentrations lower than 10% sucrose, thus making these enzymes suitable for biotechnological applications (Uma et al. 2010). Biologically active enzymes may be extracted from any living organisms like plants, animals and micro organisms. Microbes are preferred to plants and animals as sources of enzymes because of less harmful materials than plant and animal tissues. The majority of enzymes used in industrial/biotechnological applications are derived from particular fungi and bacteria (Hussain et al. 2009). Several filamentous fungi Invertases have also been characterized such as those from *Aspergillus* sp. (Ashokkumar et al. 2001; Nguyen et al. 2005; Guimaraes et al. 2007, 2009).

Extensive studies have been done by using synthetic medium for preparation of Invertase while a little attention has been paid on its production from un-convenentional inexpensive sources (Vitolo et al. 1995; Ashokkumar et al., 2001; Rashad et al., 2006; Guimaraes et al. 2007, 2009).
The present study was conducted to produce Invertase enzyme by utilizing agro waste i.e. carrot peels through *A.niger* under solid state fermentation when grown in shaken cultures. Further enzymes was purified and characterized to get pure enzyme.

Material and methods

Microorganism

Five pure culture of the *Aspergillus* spp. i.e. *A.niger* L**.** FCBP-840, *A.fumigatus* L**.** FCBP-971*, A.flavus* L**.** FCBP-1002*, A.terreus* L**.** FCBP-1011 *and A.candidus* L**.** FCBP-844 were obtained from the First Fungal Culture Bank of Pakistan, Institute of Agricultural Sciences, University of the Punjab, Lahore. They were propagated on malt extract agar (MEA) slants.

Food processing waste

Carrot peels (*Dacus carota* L.) were used as a substrate for Invertase production. The peels obtained from local juice shops and house hold wastes, were washed with tap and distilled water, excess water was removed. After fine chopping, peels were stored in plastic bags at 4^{o}C for subsequent use as substrate for fermentation medium (Chantaro et al. 2008).

Chemical analysis of carrot peels

Proximate analysis of carrot peels were analyzed for moisture content, ash, crude protein, crude fat, crude fiber, total carbohydrates and pH (AACC 2000).

Fermentation media and growth conditions

Carrot peels were used as basic substrate for solid state cultivation and fermentation was carried out in 250-mL conical flasks containing 10 g of each substrate, sterilized at 121±1°C for 20 minutes. For inoculums development, 2mL of sterilized water was then added in a slant containing pure culture. Fungal mass was scratched gently using a glass rod and a spore suspension was poured in sterilized substrates. All was done under sterilized conditions to avoid any contamination. The number of spores per mL was measured using heamatocytometer. Then cotton plugged flasks were incubated in static incubator at 30±2^{o}C for 72 hours (Rashad and Nooman 2009).

Enzyme extraction

At the end of the specified fermentation period, 50mL of distilled water were added to each flask. The extraction of enzyme was carried out on rotary shaker at 250 rpm for 90 min at 30±2^{o}C. The substrate extract was filtered by using filter paper (Whatman No.1) and the filtrate was used as crude extracellular enzyme for measuring activity (Sangeetha et al. 2004).

Enzyme assay

Invertase activity in the culture filtrate was measured by estimating the liberated reducing sugar released by the hydrolysis of sucrose as described by Sumner and Howell method (Sumner and Howell 1935) using glucose as a standard. One Invertase unit is defined as the amount of enzyme which releases 1 mg of inverted sugar in 5 minutes at 20°C, at pH 4.5.

Protein determination

Protein was measured according to Lowry et al. (1951) method using bovine serum albumin (BSA) as a standard.

Dry cell mass evaluation

The dry weight of fungal mycelium was determined after required fermentation period, which was harvested by filtration using a Whatman no.1 filter paper. Dry weight was estimated through oven drying of mycelium at 105^{o}C for six hour (Myers et al., 1997). Final weight was taken to obtain dry cell mass.

Optimization of cultural conditions for Invertase production

Moisture content

For the best production of Invertase, fermentation was carried out by growing the organism on their particular substrate with varying levels of moisture i.e. 10, 30, 50, 70 and 90% at 30±2°C for 72 hours.

Incubation period

Invertase production varies with the increase in incubation period. For this purpose, different incubation times at 36, 48, 60, 72, 84 and 96 hours were used under optimal fermentation conditions at 30±2°C. The activity of enzyme was estimated after 12 hours under pre-optimized conditions.

Inoculum's size

For the best production of Invertase five different Inoculum's size (1×10^{1}, 1×10^{2}, 1×10^{3}, 1×10^{4}, 1×10^{5}, 1×10^{6}, spores/mL) were evaluated for the maximum enzyme production under optimal fermentation conditions at 30±2°C.

Partial purification of invertase

Ammonium sulphate precipitation

The method of Madhan et al. (2009) was followed for purification of Invertase enzyme. Different levels of ammonium sulphate (40, 50, 60, 70 and 80%) in acetate buffer (pH 4.5) were used for the enzyme precipitation.

Invertase was purified from the crude culture filtrate, which was initially subjected to 40% precipitation with ammonium sulfate and pelleted at 10,000 rpm for 20 min at 4°C. The pellet obtained was dissolved in 1mL of 0.2 M acetate buffer. The supernatant was further saturated to 80% concentration of ammonium sulfate and pelleted as before. The pellet was dissolved in 1mL of the acetate buffer and the activity was estimated infractions.

Characterization of enzyme

Effect of pH on activity and stability of enzyme

Small aliquots of enzyme were assayed with four buffering agents, namely citrate (pH 3.0-4.0), acetate (pH 4.5-5.5), phosphate (pH 6.0-7.0) and Tris-HCl (pH 7.5-9.0) at 0.1 M for each one, for recording pH profile under the standard assay conditions.

To study the effect of pH on the stability, enzyme was pre-incubated for 30 min at 30°C with the four previous buffering systems before testing the enzymatic activities at the standard assay conditions, and then relative activity was calculated.

Effect of temperature on activity and stability of invertase

The maximum activity of the enzyme was determined at different incubation temperatures ranged from 20-70°C. Thermal stability was studied by incubating the purified enzyme at various temperatures (20-70°C) for 1 hour and the remaining enzyme activity was then assayed using the standard assay condition.

Determination of substrate specificity

Influence of metal ions on enzyme activity

1mM solutions of various metals were prepared.1mL of these solutions was pre-incubated at 30±2°C for 30 min with 1mL of enzyme solution. Then Invertase activity was measured and % reduction in Invertase activity was estimated. Blank was taken before adding the metals. The chemicals which were used namely, Nickel chloride (NiCl), Zinc sulphate ($ZnSO_4$), Cobalt chloride ($CoCl_2$), Sodium chloride (NaCl), Ferrous sulphate ($FeSO_4$), Copper sulphate ($CuSO_4$), Calcium chloride ($CaCl_2$), Mercuric chloride ($HgCl_2$), Magnesium chloride ($MgCl_2$).

The activity of the Invertase on several substrates such as sucrose, maltose, fructose, and glycerol was tested. All substrates were used in concentration of 0.1 and 0.5 M and determined with reducing sugar assay with glucose as a reference.

Statistical analysis

Treatments means, standard error and Duncan s multiple range test (Steel and Torrie 1980) were calculated from the data obtained from various parameters during present studied by using software package Co-stat Version 3.03.

Results and Discussion

All of the experiments and analyses were run in triplicate, and data values have been given as means (standard errors (SE). The (SE values have been presented as error bars in the figures determined by (DMR) Duncan's multiple range test. In this work, five strains of *Aspergillus spp.* were screened for the production of Invertase. The cultivation was carried out on carrot peels wastes without any additive nutrients by using solid state fermentation (SSF). The data showed a wide variation in the yield of enzyme on carrot peels (4.49±0.08-7.95±0.1 U/ml) The maximum production of Invertase was observed by using *Aspergillus niger* in case of carrot peels as a substrate, followed by *A .candidus, A. terreus and A. flavus* (7.71±0.06, 6.86±0.1 and 6.64±0.07 U/ml) respectively, which showed considerable amount of Invertase while negligible amounts of the enzyme was detected in *Aspergillus fumigatus* (Fig 1). During higher production of Invertase by *A. niger* dry cell mass and sugar consumption were 8.96±0.15 and 20.6±0.51 mg/ml, respectively and 1.01±0.05 mg/ml protein activity was recorded. Santos et al. (2004) reported that fungi have been considered to be the organisms most adapted to SSF because their hyphae can grow on particle surfaces and penetrate into the inter particle spaces and thereby colonizing solid substrates. Viniegra-Gonzalez et al. (2003) found that *A. niger* grew more efficiently and produced more Invertase in SSF culture than in submerged culture when sucrose levels were high. His studies suggested that higher sugar levels prevented denaturizing of Invertase in SSF. Mamma et al. (2008) found that the highest Invertase activity produced by *A.niger* cultivated on dry orange peel was 72.5 U/g dry substrate at initial pH 5 and 90% moisture content, while the highest activity which was produced by *Neurospora crassa* was 74.0 U/ g dry substrate under the same conditions. These values are much higher than those reported in the present study, by the same microorganism grown on different carbon source. Madhan et al. (2009) investigated the efficiency of *A. niger* to produce Invertase by using powdered stem of Lemon grass as sole substrate and sole carbon source for the microorganism.

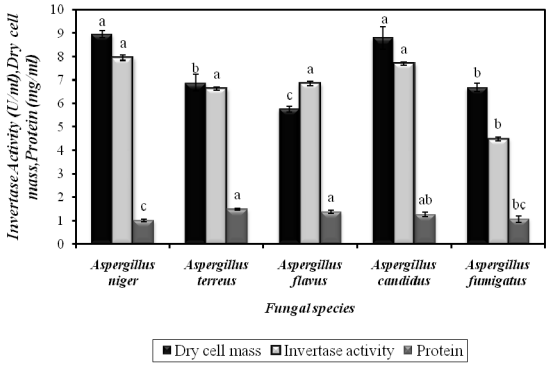

Figure 1: Screening of Aspergillus species on carrot peels used as a substrate for Invertase production at standard conditions.

The economic feasibility of the microbial enzymes production application generally depends on the cost of its production processes. In order to obtain high and commercially viable yields of Invertase enzymes, it was essential to optimize the fermentation medium used for fungal growth and enzymes production. In SSF, the existence of an optimum moisture content of the medium has been stressed as it has profound effects on growth kinetics, and on the physicochemical

properties of solids, which in turn affects productivities (Lonsane et al. 1992).It was noted that Invertase production was increased by increasing the moisture content from 30 % to 90 % as depicted in (Fig 2). *A.niger* on carrot peels showed maximum Invertase (7.95±0.13) production during 90% of moisture. While dry cell mass, sugar consumption and protein were 8.04±0.35, 19.3±0.83 and 1.02±0.11 mg/ml, respectively. Krishna (2005) found that lower moisture tends to reduce nutrient diffusion, microbial growth, enzyme stability and substrate swelling. Similar results were reported by Mamma et al. (2008) who found that the optimum moisture content for the production of Invertase from different fungal strains by SSF using orange peels was 90%.

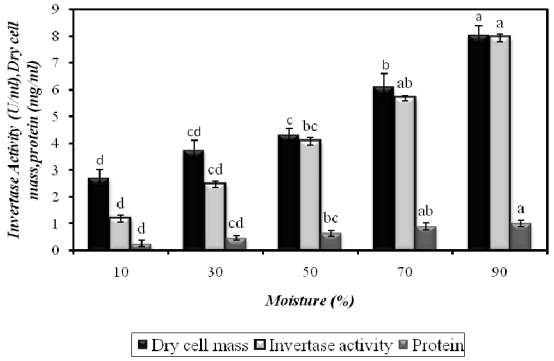

Figure 2: Effect of different moisture content (10-90%) on Invertase production from *Aspergillus niger*.

Incubation period had an obvious effect on invertase production by *Aspergillus niger*, it seems from the results that a lag phase was observed during the first 24 hours of spore germination with practically less enzyme synthesis. The effect of incubation periods on the production of Invertase is demonstrated in (Fig 3).Maximal Invertase productivity was observed at the end of 72 hours in *Aspergillus niger* (7.95±0.05 U/ml) after which a decline in enzyme activity was observed. The dry cell mass, sugar consumption and protein were 8.04±0.35, 19.3±0.83 and 1.02±0.11mg/ml, respectively. Similar results were concluded by Rashad et al. (2005)

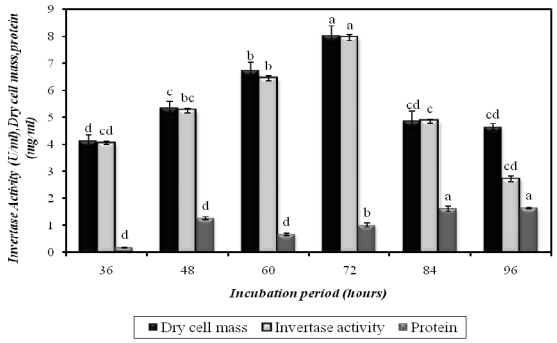

Figure 3: Effect of different incubation periods (36-96 hours) on Invertase production by *Aspergillus niger*.

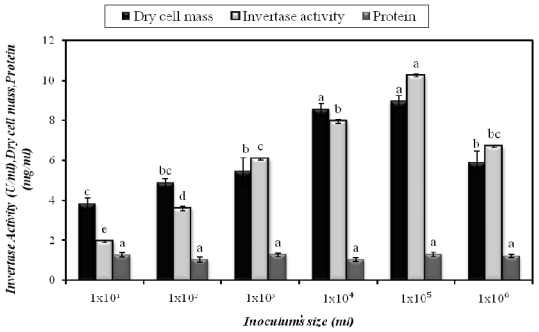

Figure 4: Effect of different Inoculum's size ($1x10^1$-$1x10^6$spores/mL) on Invertase production from *Aspergillus niger*.

from *Saccharomyses cerevisiae*. Sirisansaneeyakul et al. (2000), reported incubation period of 72 hours for both intra and extracellular Invertase from *A.niger* which was similar to present results. It was very essential parameter that has to be controlled because it varies from organism to organism. Parks & Pastores (2006) observed that extracellular Invertase from *A.niger* reached at maximum activity after 4 days of incubation followed by a decline in the enzyme activity till the 6th day of incubation.

Concerning the effect of inoculums's size, the obtained results revealed that, the optimal inoculum's size needed to produce the highest yield of enzyme. The effect of inoculums size on the production of Invertase is demonstrated in (Fig 4). Maximum enzyme production (10.24±0.06 U/ml) from *A. niger* on carrot peels was achieved at Inoculum's size $1x10^6$ (spores/ml). While dry cell mass, sugar consumption and protein were estimated to 8.94±0.3, 19.5±0.63 and 1.21±0.1 mg/ml, respectively. However, after certain limit the competition for the nutrients resulted in a decrease of the metabolic activity of the organism. With optimum inoculum's size, there was a balance between biomass synthesis and availability of nutrients that supports production of enzyme (Nampoothiri et al. 2004).

The proximate composition of the fermented and non-fermented carrot peels in (Table 1) reveals that fermentation of the peels increases moisture content of carrot peels (26.7±0.29 %) as compared to the non-fermented dry sample (7.46±0.35%). There is slight increase in protein of fermented carrot in contrast to non-fermented sample. There was significant decrease in the fiber, fat and ash content of the fermented sample. This affected the carbohydrate content (calculated by difference) which was high in non-fermented sample. However pH of the fermented carrot peel also show decline as compare to non-fermented one**.** Chemical analysis of substrate revealed different composition of nutrients which suggested that it can be utilized for microbial growth. Krishna

Table 1. Proximate chemical compositions of fermented and non-fermented carrot peels

Chemical analysis	Moisture (%)	Ash (%)	Fiber (%)	Fat (%)	Protein (%)	Carbohydrate (%)	pH
non-fermented	7.46±0.35	11.2±0.70	17±0.59	3.84±0.004	4.47±0.14	56.0±0.60	5.75±0.037
fermented	26.7±0.29	7.8±0.10	12.75±0.46	1.54±0.004	7.81±0.23	43.4±0.037	4.65±0.075

Each value is an average of three replicates; ± represents Standard Deviation among replicates at 0.5 % level of significance ($p \leq 0.05$).

(2005), reported that numerous nutrients can regulate sporulation through metabolic effects in SSF. These nutrients include carbon and nitrogen sources, minerals, and vitamins or cofactors. Moisture content of fermented peels was high as compare to non-fermented, so it can serve as substrate for the production of enzymes using SSF technique. There is slight increase in protein content. The increase in protein of the fermented peels samples may be due to the fact that the microorganisms identified which degrades the sample readily may have secreted extracellular enzymes in the peels which subsequently increases the protein content of the fermented sample as well as microbial biomass (Odetokun 2000). There was no considerable difference in the fat, while there was a decrease in fiber content of the fermented sample. This affected the carbohydrate content (calculated by difference) in which there was no considerable difference. (Aykroyd & Doughy 1982; Brough & Azam-Ali 1992; Odetokun 2000) reported that increase in carbohydrate content during fermentation may be due to a reduction in the fiber content and increase in both reducing sugars and total soluble sugars. Suutarinen et al. (2003) reported proximate composition of carrot and to determine the enzymatic potential of various vegetable peels. (Rashad & Nooman, 2009) reported chemical analysis of red Egyptian carrot for the production of extracellular Invertase from *Saccharomyses Cerevisiae*. Various authors reported this analysis on the basis of their substrate conditions. Change in pH in fermenting carrot was also observed and it was noted that there was decrease in pH throughout the fermentation. (Raimbault & Tewe 2001) indicated that the pH of a culture may change in response to metabolic activities. The most obvious reason being the secretion of organic acids as citric, acetic or lactic this causes pH to decrease.

The crude enzyme preparation was obtained by conducting fermentation processes under the optimal culture conditions tested in the present study. The whole optimum culture media obtained from several batches were collected and the culture filtrate was concentrated to be used in the trials of getting the enzyme in a pure form. The crude culture filtrate containing Invertase was subjected to ammonium sulfate precipitation. After conducting some preliminary trials, 80% concentration was selected for the enzyme precipitation. The results indicate specific activity of 8.09 U/mg, purification procedure was low; nevertheless, our goal was to purify and characterize the isolated enzyme.

The enzyme was characterized to find the conditions at which it showed best performance. The activity and stability of purified enzyme were investigated at different temperatures and pH in (Fig.5 & 6). The enzyme showed maximum activity

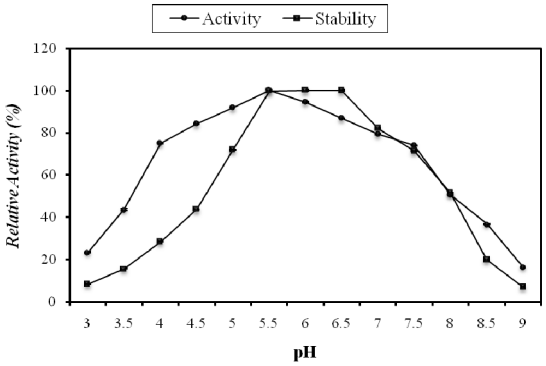

Figure 5: Effect of pH on Activity (●) and Stability (■) of Invertase by *Aspergillus niger*.

at 50°C. This result was similar to that, reported by Nguyen et al. 2005 who found that the optimum temperature for Invertase production by *A. niger* was 50°C. Akardere et al. (2010) found Invertase activity at 60°C. Higher values of optimum Invertase temperatures were reported by many authors (Rubio et al. 2002 ; Guimaraes et al. 2007 ; Hussain et al. 2009), while lower value (30°C) was reported by Rashad et al. (2006). The thermal stability of the Invertase was found from 20-40°C. The enzyme stability in *A.niger* was in the same range as illustrated by (Quiroga et al. 1995; Rashad & Nooman, 2009). However Madhan et al. (2009) found that Invertase from *A. niger* was found to be stable till a temperature of 55°C. The enzyme showed maximum activity at pH 5.5.The similar results were described by Nguyen et al. (2005) for *A. niger*. The optimum pH value was higher than those reported by many authors

Table 2. Purification steps of Invertase from *Aspergillus niger*

Step	Activity (IU)	Protein (mg)	Specific activity (IU/mg)	Recovery (%)	Purification (fold)
Crude extract	512	63.3	8.09	100	1
40-80% $(NH_4)_2SO_4$	336	29	11.5	65.6	1.42

respectively. However in partially purified form specific activity was 11.5 with 1.42 purification fold (Table 2) Hocine et al. (2000) reported purification of extracellular β-d-fructofuranosidase from *A.ochraceus*. The results of ammonium sulfate fractionation showed that 80% of total fructosyltransferase and Invertase activities could be recovered in the fraction of 30–100%ammonium sulfate saturation with a purification of 5.3 and 5.1 fold, respectively.
Bhatti et al. (2006) purified the Invertase from *Fusarium solani* with 3.1 purification fold. Akardere et al. (2010) used three-phase partitioning (TPP) to purify Invertase from Baker's yeast (*Saccharomyces cerevisiae*) in a single step. They concluded that under optimized conditions the Invertase was purified to 15-fold with 363% activity recovery. The efficiency of the salting out of proteins will depend on sulfate concentration as well as, on the net charge of the proteins. Therefore, ammonium sulfate saturation is of critical importance and must be optimized (Dennison & Lovrein 1997; Sharma et al. 2000). Invertase yield obtained by this (Quiroga et al. 1995; Rubio et al. 2002; Rashad et al. 2006; Guimaraes et al. 2007, 2009), while it was lower than that reported by (Vorster & Botha, 1998) who found optimum pH of neutral Invertase to 7.2. While in present study Invertase was found to be stable from pH 5.5 to 6.5, respectively. Nguyen et al. (2005) found that *A.niger* Invertase was stable in the pH range from 5.0 to 6.5. Different pH-stability profiles of Invertases have been obtained depending on the enzyme source and also assay method applied (Bhatti et al. 2006; Nguyen et al. 2005; Belcarz et al. 2002).

The purified extracellular Invertase was tested for substrate specificity. Based on (Fig 7) Invertase was highly active towards sucrose at both concentrations viz: 0.1 M and 0.5 M, but it showed less activity towards glycerol. Invertase was highly active towards sucrose (100%) and slightly active towards raffinose (14.7%) but it nearly did not show any activity towards maltose and glycerol (Rashad & Nooman,

2009) Similar behavior of extracellular Invertases towards sucrose and raffinose has been detected by many workers (Rubio et al. 2002; Rashad et al. 2006 and Guimaraes et al. 2009) using different microorganisms (*R. glutinis, S. cerevisiae* and *A. niveus*

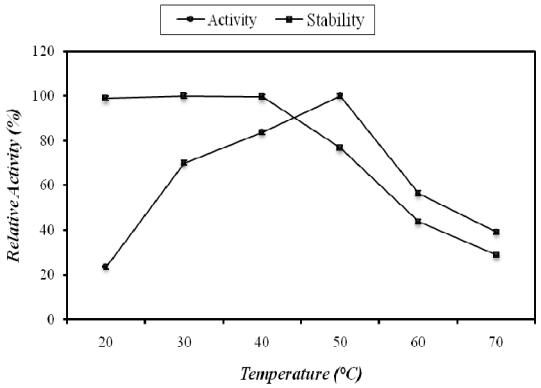

Figure 6: Effect of temperature on Activity (●) and stability (■) of Invertase by *Aspergillus niger*.

respectively). These results suggest that the enzyme is a β-D-fructofuranoside fructohydrolase, able to attack the β-D-fructofuranosides from the fructose end.

The effects of metal ions on the activity of the Invertase are shown in (Fig 8). Different metals were assessed in present work and results showed that Hg^{2+} inhibited the enzyme activity. While slight inhibition was noted by other metals however increase in Invertase activity was observed in case of Co^{2+}. (Rashad & Nooman, 2009) reported similar results by detecting complete inhibition by Hg^{2+}, as well as noticed slight increase in the enzyme by using Co^{2+} at low concentration (1mM).The inhibition of Invertase by Hg^{2+} was reported by many authors (Ghosh et al. 2001; Rashad et al. 2006; Guimaraes et al. 2009) and they suggested that thiol groups at the catalytic site are important for the Invertase activity. Stimulation of Invertase activity by Co^{2+} was also reported by Rubio et al. (2002) and Rashad et al. (2006), while Nguyen et al. (2005) found that the enzyme was slightly inhibited by addition of 1mM Co^{2+}. Guimaraes et al. (2007) also reported that Cu^{2+} inhibited the extracellular β-fructofuranosidase activity of *A. ochraceus*. Salt addition may affect amino acid residues both at the active site and the exterior surface of enzymes, possibly inducing charge alterations of the catalytic amino acids and/or structure distortions (Salis et al. 2007).

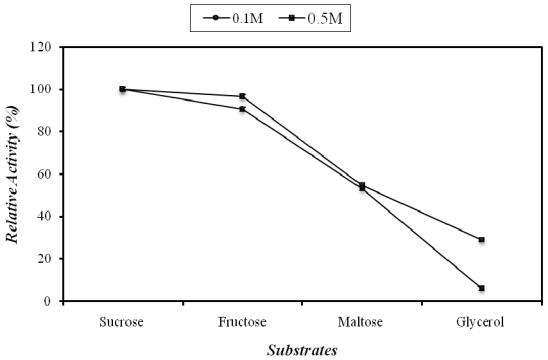

Figure 7: Effect of different substrates concentrations 0.1M (●) and 0.5M (■) on activity of Invertase from *Aspergillus niger*.

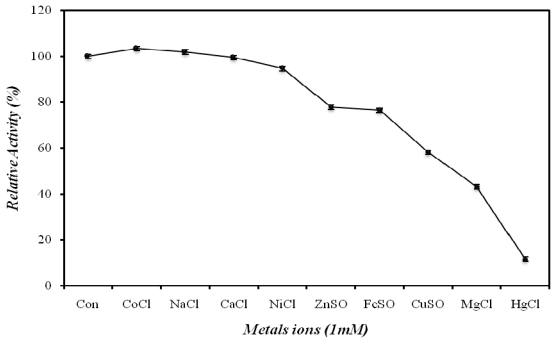

Figure 8: Effect of various metal ions on the activity of the Invertase from *Aspergillus niger*.

Nakanishi et al. (1991) reported that effect of 0.01 M of KCl, NaCl, $MgCl_2$ and $CaCl_2$ on the activity of both wine and grape juice invertase were negligible. Voster & Botha (1998) reported similar effects of Hg^{2+} on the activity of sugarcane neutral invertase. It was also reported that 0.005 M $FeCl_2$, $CuCl_2$, $ZnCl_2$, $CdCl_2$ and $AlCl_3$ reduced the activity of Invertase approximately 80, 73, 32, 45 and 22% respectively (Mahbubur-Rahman et al. 2004).

Conclusion

This study indicates the potential for the use of food processing wastes such as red carrot peels for the efficient production of Invertase by *A.niger* in SSF, thereby resulting in recycling waste materials for conversion into useful product of higher value as a by-product.

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