

## Utilization of pomegranate peel waste for the production and characterization of invertase using *Cladosporium sp.*

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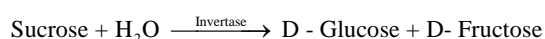
### ABSTRACT

Invertase production by *Cladosporium sp.*, at varying pH, temperature, period of incubation was studied by using Czapek Dox as basal medium. Sucrose in the medium was substituted with pomegranate peel waste. The effect of metal ions of invertase was also carried out. The results of the study revealed that the production of invertase was maximum on the 5<sup>th</sup> day of incubation at an optimum pH of 6.0 and optimum temperature of 30°C. Purification of invertase was carried out using ammonium sulfate, dialysis followed by acetone precipitation. Protein content and thermal stability of the purified invertase was determined.

**Key words:** Invertase, Pomegranate peel, *Cladosporium sp.*,

### INTRODUCTION

The enzyme invertase (B-D-fructofuranoside fructohydrolase, E.C.No:3.2.1.26) catalyses the hydrolysis of any compound with an unsubstituted β -D-fructofuranosyl residue and the prime substrate is sucrose.(George Boguslawski, 1983).



A wide range of microorganisms produce invertase and can thus, utilize sucrose as a nutrient. They hydrolyse sucrose to produce fructose and glucose in an equimolar mixture named inverted sugar. The inverted sugar is incorporated more easily in industrial preparations and has more added value than sucrose. (Chou and Jasovsky ,1993).

The high cost of invertase production process, together with the low process extraction yield and purification have been the limiting factors for the use of the enzymatic hydrolysis in the industrial process. Reduction in the production cost could make it a more efficient and reliable technique

to be used industrially for the commercial production (Rodrigues *et al.*, 2000).

Invertase is basically a yeast derived enzyme. But many microorganisms including fungi and bacteria, produce this enzyme. Invertase production by microorganisms such as *Aureobasidium pullulans* KFCC10525 (Kurkaka *et al.*, 1996), *Zymomonas mobilis* (Park *et al.*, 1991) and *Clavibacter michiganensis* (Baer *et al.*, 1998) are known. (A.B.Dhake and M.B patil , 2005).

Invertase has gained importance in recent times due to its various biotechnological applications. Major applications of invertase includes confectionary, beverage, bakery and other pharmaceutical formulations for the preparation of invert sugar and high fructose syrup(HFS) from sugar (Gehlawat, 2001). It is one of the most widely used enzymes in food industry where fructose is preferred than sucrose especially in the preparation of jams and candies, because it is sweeter and does not crystallize easily (Cuitlahuac Aranda *et al.*, 2006).

## MATERIAL AND METHODS

### Isolation of pure culture

The fungi isolated from the soil and single germinating spores were picked from the mixed culture. A pure culture was produced by repeated subculturing which was transferred to agar slant and subcultured fortnightly which was then stored at 4°C until use (Anjali Hans and Arun Dev Sharma, 2005).

### Selection of organism based on the utilization of sucrose

The organism present in the pure culture were identified using "Lactophenol cotton blue mounting method", which was especially used for the identification of fungi. The organism used through out the experiment was *Cladosporium* species was selected and identified commonly using 2% glucose medium (Srinivasan *et al.*, 1991 Nakamura *et al.*, 1997 and Domsch KM *et al.*, 1980).

### Growth media and cell suspensions

*Cladosporium* species was maintained in the Czapek Dox medium as basal medium and sucrose was substituted with pomegranate peel waste in the fermentation media as the carbon source. (Marcin Skowronek *et al.*, 2003).

### Culture condition

The production medium was inoculated with a loop of culture from 24 hours old stock culture slants (Gogoi *et al.*, 1998) and incubated at 30°C. After 3 days of incubation, invertase, biomass, pH and reducing sugar content of the medium were determined for 7 days with 24 hours interval. (Anjali Hans and Arun Dev sharma., 2005).

### Cell free extract preparation

The mycelial mass was collected by filtration after 3 days of incubation. The filtrate was centrifuged at 10,000 rpm for 20 minutes at 4°C. The supernatant was used as the source of invertase enzyme mainly contains extracellular enzyme. The mycelial mass was washed several times with distilled water and used for dry weight determination. The cell growth (biomass) in the liquid culture was monitored by dry weight that was expressed as  $g\ l^{-1}$  of the microbial culture. (Dhake

and Patil, 2005; Anjali Hans and Arun Dev Sharma 2005 )

### Optimization of the culture condition

The enzyme activity was measured by altering the individual the parameter such as pH, temperature and substrate concentration of the medium keeping the remaining parameter unaltered. (Peter. F. Stanbury *et al.*, 1997).

### Assay of enzyme activity

The determination of invertase activity was carried out by DNSA method.

The crude enzyme was purified using ammonium sulfate, dialysis and acetone. The thermal stability of the purified invertase was performed by incubating the purified sample in 0.01M acetate buffer of pH 6.0 at 50°C respectively.

### Enzyme units

One unit of invertase (IU) was defined as the amount of enzyme which liberated / mg of product / minute /ml under the assay condition. (Anjali Hans and Arun Dev Sharma ,2005)

## RESULTS AND DISCUSSION

Invertase production was reported in *Cladosporium sp.*, (Dhake and Patil, 2005). The cultural parameters have a significant impact on both enzyme production and the activity. The effect of some cultural parameters like incubation time, pH, temperature and substrate feeding (Pomegranate peel waste) has been studied on invertase production by *Cladosporium*.

The organism was grown for a period of 7 days. The enzyme activity was measured at regular intervals of 24hrs at pH 4-7. The maximum invertase was produced after 5 days of inoculation at pH 6 Table 1. Similar observation was obtained by Anaclaudia *etal.*, 2005 using *Cladosporium cladosporioides* cells and by B.k.Gogoi *etal.*, 1998 using *Saccharomycopsis fibuligera*.

Optimum temperature for invertase production by *Cladosporium* was found to be 30°C Table 2, on the same 5<sup>th</sup> day. Kazhurio Fukui *et al.*,

1974 also reported the temperature of 40°C as optimum temperature for enzyme production by streptococcus mutants. This observation was also supported by Shaik Yazdani Basha *et al.*, 1994 using *Thermomyces lagnuginosus*.

The enzyme production at different substrate concentration (Pomegranate peel waste) 2-8 gm was also done. The maximum enzyme production was found to be at substrate

concentration of 4gm at 5<sup>th</sup> day of incubation in optimum culture medium (Table 3). the Km value of the enzyme obtained after 5 days of incubation is 1.4 (Fig. 1) which was supported by Gogoi *et al.*, 1998.

Purification procedure resulted in a 2-fold increase in specific activity of the enzyme (Table 4). The thermal stability of partial purified invertase on exposure to different temperatures. The

**Table 1: Effect of pH in Invertase production by *Cladosporium Sp.*, (Pomegranate Peel Waste), Invertase activity IU/ml**

pH	Incubation Period (Days)				
	III	IV	V	VI	VII
4	1.323(0.91)	1.344(1.08)	2.646(2.13)	1.890(1.49)	1.806(1.30)
5	1.932(1.86)	2.436(2.06)	3.255(3.99)	3.150(3.63)	2.499(2.09)
6	2.877(2.57)	3.108(2.89)	4.494(6.31)	4.284(5.20)	2.835(2.45)
7	1.785(1.32)	2.394(1.99)	3.192(3.81)	2.840(2.84)	2.289(1.89)

The data in parenthesis indicates mycelial biomass

**Table 2: Effect of temperature in invertase production by *Cladosporium Sp.*, (Pomegranate Peel Waste), Invertase activity IU/ml**

Temperature (°C)	Incubation Period (Days)				
	III	IV	V	VI	VII
30	4.6(3.4)	7.7(5.6)	9(6.8)	6(4.8)	5.9(4.3)
40	12.3(9.7)	14.1(10.6)	17.5(13.6)	12(9.4)	10.5(7.9)
50	4.1(2.9)	6.8(5.1)	8(5.8)	5.5(3.8)	4(3.0)
60	2.2(1.8)	6.3(5.2)	7(5.3)	3(2.3)	2(1.8)

The data in parenthesis indicates mycelial biomass

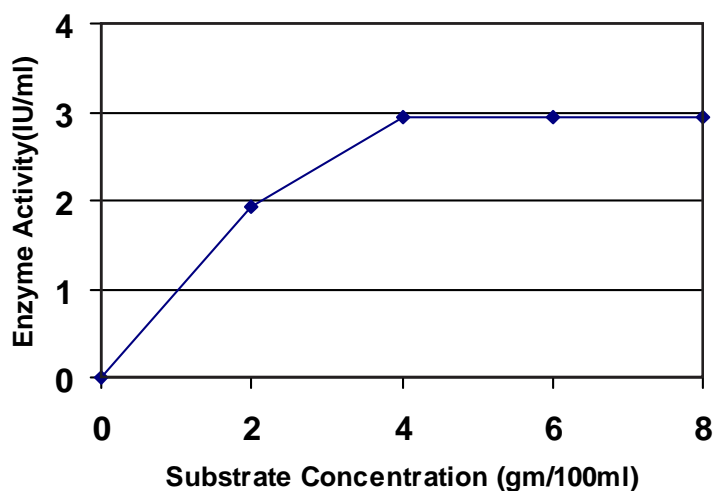
**Table 3: Effect of Substrate Concentration in Invertase production by *Cladosporium Sp.*, (Pomegranate Peel Waste), Invertase activity IU/ml**

Concentration gm/100ml	Incubation Period (Days)				
	III	IV	V	VI	VII
2	1.449(1.42)	1.596(1.80)	1.932(1.33)	1.457(1.62)	1.171(1.51)
4	2.457(1.36)	2.604(2.62)	2.940(2.98)	2.465(2.57)	2.179(2.19)
6	2.457(1.53)	2.604(2.86)	2.940(3.67)	2.465(3.47)	2.179(3.28)
8	2.456(1.21)	2.603(1.10)	2.940(1.58)	2.464(1.31)	2.177(1.49)

The data in parenthesis indicates mycelial biomass

**Table 4: Partial purification of invertase**

Description	Invertase activity IU/ml	Total protein mg/ml	Specific activity
Crude extract	0.924	0.421	2.196
30%Ammonium sulphate saturation	1.639	0.299	5.4
Dialysed	2.152	0.190	11.326
50%Acetone	2.659	0.156	17.04



**Fig. 1: Effect of substrate concentration in invertase production by *Cladosporium sp.*, (pomegranate peel waste)**

full activity was retained when the enzyme was exposed to 10 minutes at 50°C (Fig. 2). The result obtained was supported by Skowronek et al., 2003 by using the strain *Cladosporium herbarum*.

The effect of metal ions on the activity of the enzyme was examined by incubated with various metal ions of concentration 0.01M at pH 6 and

temperature 30°C for 10 minutes. The cations tested had a significant inhibitory effect on the hydrolysis by invertase. Zn was found to be a potent inhibitor of invertase (Fig. 3). The result obtained was supported by Gogoi et al., 1998 by using the strain *Saccharomycopsis fibuliger* and concluded that no metal ions can activate the enzyme.

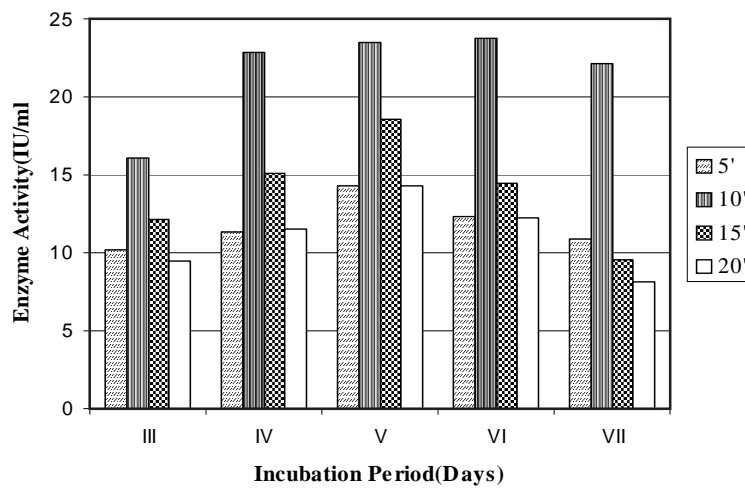


Fig. 2: Thermal stability of purified invertase

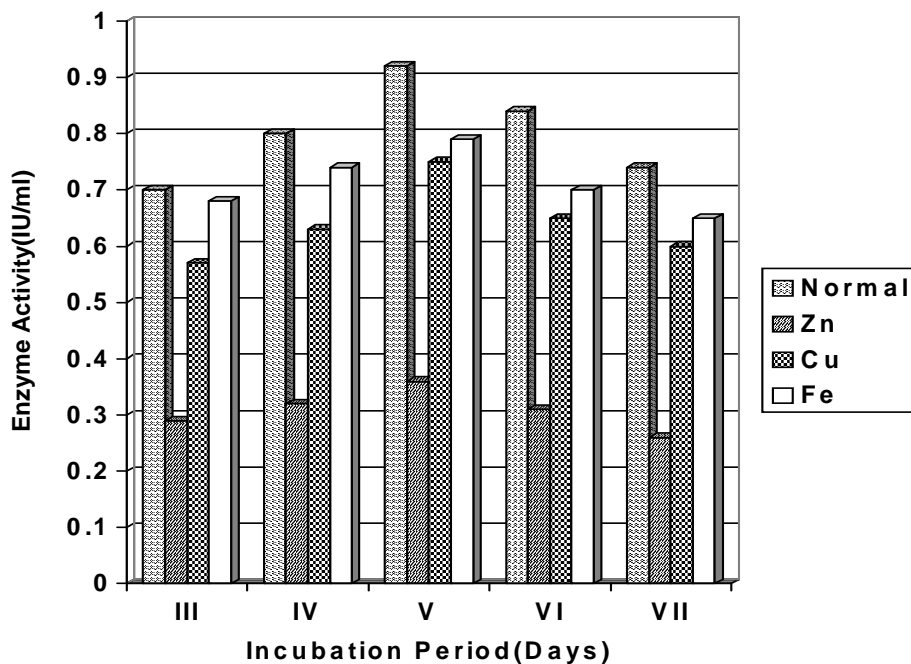


Fig. 3: Effect of metal ions in invertase activity

The present study concludes that the pomegranate peel waste can be more effectively used as a carbon source for the production of invertase using *Cladosporium sp.*, under the optimized culture condition. Hence it can be substituted as a carbon source for the commercial production of invertase.

#### ACKNOWLEDGEMENTS

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