

Full Length Research Paper

## Characterization of invertase from *Saccharomyces cerevisiae* MTCC 170

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Activity and stability of invertase obtained from *Saccharomyces cerevisiae* MTCC 170 were characterized with parameters like pH, temperature, metal ions, surfactants and chemical inhibitors. The pH stability of this enzyme was observed between pH 2 and 9 with an average of 63% retaining activity for 24 h. The crude enzyme showed optimum activity at pH 6 and 30°C. Enzyme activity was increased in the presence of 5 mM CaCl<sub>2</sub> (89.11%). Maximum invertase activity of 32.32% was recorded at polyethylene glycol (1%). Maximum invertase activity of 25.58% was recorded at EDTA for *S. cerevisiae* MTCC 170. The kinetic parameters (Km and Vmax) were determined at 30°C and pH 6 for *S. cerevisiae* MTCC 170 invertase for concentrations ranging from 0.5 to 5 mg/ml of sucrose as substrate. The Km and Vmax of *S. cerevisiae* MTCC 170 are 0.6894 mg/ml and 0.3201 µm/min/mg.

**Key words:** *Saccharomyces cerevisiae* MTCC 170, invertase, specific activity, relative activity, enzyme characterization.

### INTRODUCTION

Invertase [ $\beta$ -fructofuranosidases (EC.3.2.1.26)] is the yeast derived enzyme and a member of glycoside hydrolases, which include more than 370 enzymes of plant and microbial origin. Invertase from *Saccharomyces cerevisiae* is the high cost enzyme. Invertases are intracellular as well as extracellular (Ul-Haq and Ali, 2007). Invertase acts on non-reducing fructofuranoside terminal residues of  $\beta$ -fructofuranoside (Veana et al., 2011). The hydrolysis of sucrose which yields an equimolar mixture of glucose and fructose (invert syrup) is sweeter than sucrose due to high degree of sweetness of fructose. Consequently the sugar content can be increased considerably without crystallization of the material. Hence, one of the important applications of

invertase lies in the production of non-crystallizable sugar syrup from sucrose. Due to its hygroscopic nature, invert syrup is used as humectants in the manufacture of soft centered candies and fondants (Gehlawat, 2001).

Invertase is also used whenever sucrose containing substrates are subjected to fermentation viz. production of alcoholic beverages, lactic acid, glycerol, etc. Due to the associated inulinase activity, it is also used for the hydrolysis of inulin (polyfructose) to fructose. Other uses of the enzyme include, manufacture of artificial honey, plasticizing agents used in cosmetics, drug and paper industries and as enzyme electrodes for the detection of sucrose. Enzymatic hydrolysis of sucrose is preferable to acid hydrolysis as it does not result in the formation

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of undesirable flavoring agents as well as coloured impurities (Wiseman, 1975).

Many organisms produce invertase they include, *Neurospora crassa*, *Fusarium oxysporium*, *Phytophthora meganosperma*, *Bacillus macerans* (Kim et al., 2000), *Aspergillus niger*, *S. cerevisiae*, *Schizosaccharomyces pombe*, *Cladosporium cladosporioides* (Almeida et al., 2005), *Arthrobacter globiformis* (Win et al., 2004), *Kluyveromyces marxianus* (Workman and Day, 1983), *Saccharomyces fragilis*, *Aspergillus caespitosus*, *Candida guilliermondii*, *Penicillium chrysogenum*, *S. cerevisiae* (Mona and Mohamed, 2009), *Candida utilis* and *Pichia pastoris* (Ul-Haq and Ali, 2005).

## MATERIALS AND METHODS

### Invertase assay

The culture medium was centrifuged at 10000 rpm for 10 minutes at 4°C. The supernatant was used as crude enzyme source for invertase assay. Invertase activity was assayed as per the method of Sumner and Howells (1935) using 0.5 ml of sucrose as the substrate in 0.03 M acetate buffer (pH- 5.0) and incubated at 45°C for 30 min. The reaction was terminated by addition of 1 ml of DNS reagent and tubes were kept at boiling water bath for 5 min. After cooling the tubes at room temperature, 3 ml of distilled water was added in each tube. The intensity of the colour was read at 540 nm in UV-Vis spectrophotometer (Systronics, 119). Standard curve was performed with glucose solution. One unit of enzyme activity was defined as the amount of enzyme required to release 1 µ mol of glucose/ml/minute under assay condition. Enzyme activity was expressed in International units. Invertase activity was calculated using this formula:

$$\text{IU/ml} = \text{concentration of glucose} / 0.5 \times 30 \times 0.180$$

### Acetone precipitation

For protein precipitation, double the amount of acetone was added to the culture supernatant solution and the solution was left overnight at 4°C, the supernatant was removed and pellet of precipitated protein was kept and dried at laboratory temperature. The pellet which contained invertase was dissolved in 5 ml of double distilled H<sub>2</sub>O and it was dialyzed against double distilled H<sub>2</sub>O for 48 h at 4°C. This was further dialyzed against 50% (W/V) PEG in order to concentrate the protein sample (Mawadza et al., 2000).

### Effect of pH on activity and stability

The optimum pH of the crude invertase was determined by incubating the mixture of the crude invertase in the presence of the buffer (0.1 M citrate buffer) at pH 2 to 12. The pH effects on invertase activity were assayed at pH values ranging from 2 to 12 for 30 min. To determine pH stability, the enzyme was pre-incubated at 30°C for 24 h at pH 2 to 12 (Mase et al., 2008).

### Effect of temperature on invertase activity and stability

The effect of temperature on the activity of invertase was determined in the temperature range of 10-90°C in 0.1 M citrate buffer at pH 6 for 30 min. The invertase activity was determined under standard assay condition. To determine temperature stability,

the invertase was pre-incubated at 30°C for 24 h at pH 2 to 12 (Do et al., 2012).

### Effect of various metal ions on invertase activity

The crude invertase was mixed with 5 mM concentration of various salts such as CaCl<sub>2</sub>, CoCl<sub>2</sub>, MgCl<sub>2</sub>, ZnSO<sub>4</sub>, NiSO<sub>4</sub>, CuSO<sub>4</sub> and KCl for 30 min at 30°C pH 6 before adding the substrate and subsequently invertase activity was determined. To determine metal ions stability, the enzyme was pre-incubated at 30°C for 24 h at pH 6 (Shankar et al., 2010).

### Effect of various surfactants on activity

The effect of surfactants on the activity of crude invertase was determined by pre-incubating the enzyme in the presence of Triton X-100 (1%), Triton X-100 (2%), Triton X-100 (3%), Tween-20 (1%), Tween-20 (2%), Tween-20 (3%), SDS (0.1%), SDS (0.3%), SDS (0.5%), Poly ethylene glycol (0.1%), Poly ethylene glycol (0.3%), Poly ethylene glycol (0.5%) for 30, 60, and 90 min at 30°C before adding the substrate. Subsequently, relative invertase activities were measured at optimum temperature (Patil et al., 2012).

### Effect of different chemical inhibitors on invertase activity and stability

The effect of different chemical inhibitors on invertase activity and stability were determined individually for crude invertase of *S. cerevisiae* MTCC 170. The crude invertase was mixed with 0.1 mM concentration of different chemical inhibitors such as DMSO, EDTA, β-mercaptoethanol, H<sub>2</sub>SO<sub>4</sub> and H<sub>2</sub>O<sub>2</sub> for 30 min at 30°C, pH 6 before adding the substrate and subsequently invertase activity was determined. The relative activities were based on the ratio of the activity obtained at specific chemical inhibitors to the maximum activity obtained and expressed as percentage. To determine chemical inhibitors stability, the enzyme was pre-incubated at 30°C for 24 h at pH 6 (Aziz et al., 2011).

### Determination of kinetic parameters for crude invertase

The kinetic parameters (Michaelis-Menton constant) K<sub>m</sub> and maximal velocity V<sub>max</sub> of invertase activity of *S. cerevisiae* MK were determined individually from Lineweaver Burk plot optimal assay conditions, 45°C, pH 6 at 30 min for sucrose concentrations ranging from 0.5 to 5 mg/ml. The evaluation of these graph yielded the kinetic parameters for the invertase activity (Graph pad Prism 5.04 software) (Sivakumar et al., 2012).

## RESULTS

### Effect of pH on invertase activity and stability

The effect of pH on the activity of crude invertase was determined in the pH range of 2-12. Maximum invertase activity of 94.28% was recorded at pH 6. The enzyme activity was decreased to 15.14% at pH 12. The pH stability of enzyme was measured by the standard assay method with sucrose. An average 63% of retaining activity was observed between pH 4 and 8 (Figure 1).

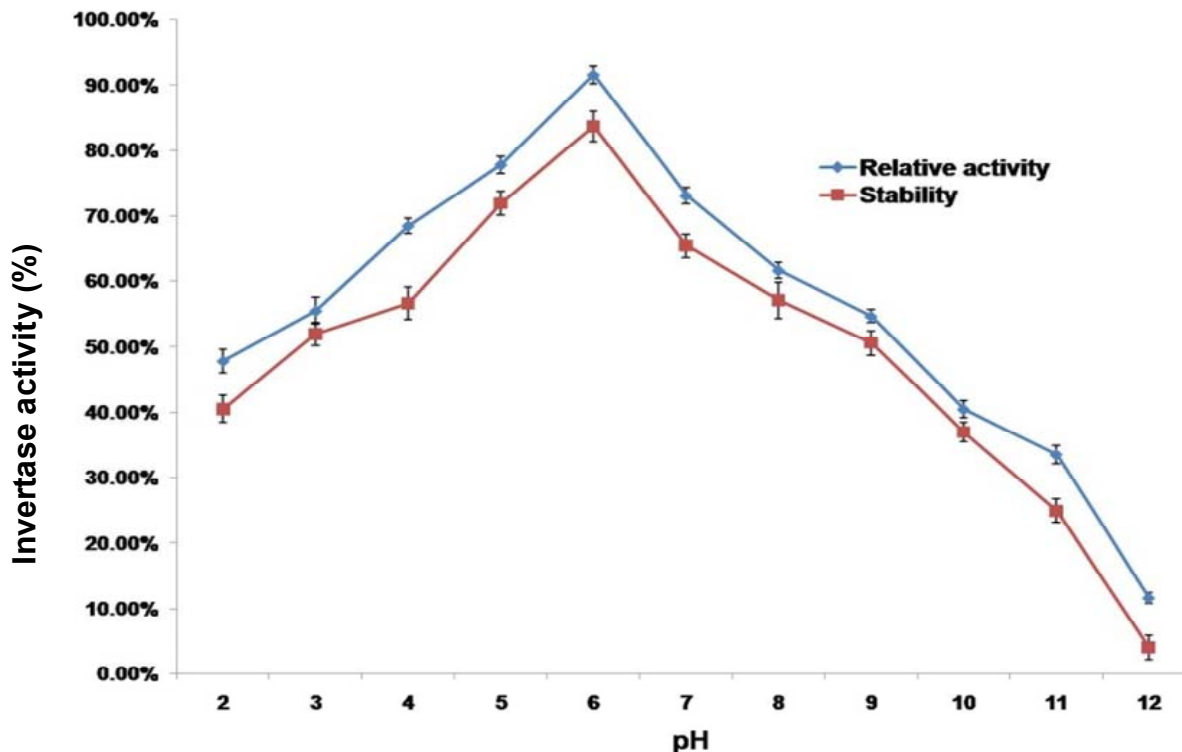


Figure 1. Effect of pH on invertase activity and stability by *S. cerevisiae* MTCC 170.

### Effect of temperature on invertase activity and stability

The effect of temperature on the activity of crude invertase was determined in the temperature range of 10-90°C. Maximum invertase activity of 95.54% was recorded at 30°C. Minimum invertase activity of 21.70% was recorded at 90°C. The original invertase activity was retained from 10 to 90°C approximately above 79% from 10-40°C (Figure 2).

### Effect of various metal ions on invertase activity

The crude invertase was pre-incubated at 30°C for 30 min at different concentration of the metal ions prior to standard invertase activity assay with sucrose. Maximum invertase activity of 89.11% was recorded at calcium chloride. Minimum invertase activity of 11.62% was recorded for potassium chloride. Partial inhibition of the crude invertase was in the order of  $KCl > MnSO_4 > ZnSO_4 > NiSO_4 > MgSO_4 > CoCl_2$  (Figure 3).

### Effect of different surfactants on invertase activity

The relative activity of invertase was decreased with increase in concentration of surfactants and also by time of exposure. At 1% surfactants concentration the relative

activity was high at the same time, at 5% surfactants concentration, it gradually reduced but not completely inhibited. Maximum invertase activity of 32.32% was recorded at polyethylene glycol (1%). Minimum invertase activity of 6.89% was recorded at triton X-100 (1%). The residual invertase activity for surfactants was given in Figure 4a and b.

### Effect of various chemical inhibitors on invertase activity and stability

The crude invertase was pre-incubated at 30°C for 30 min at different concentration of the chemical inhibitors prior to standard invertase activity assay with sucrose. Maximum invertase activity of 25.58% was recorded at EDTA. Minimum invertase activity of 9.30% was recorded at DMSO. The residual invertase activity for chemical inhibitors is given in Figure 5.

### Determination of kinetic parameters for *S. cerevisiae* MTCC 170

The kinetic parameters ( $K_m$  and  $V_{max}$ ) were determined at 30°C and pH 6 for *S. cerevisiae* MTCC 170 for concentrations ranging between 0.5 to 5 mg/ml of sucrose as substrate. The  $K_m$  and  $V_{max}$  of *S. cerevisiae* MTCC 170 are 0.6894 mg/ml and 0.3201  $\mu\text{m}/\text{min}/\text{mg}$

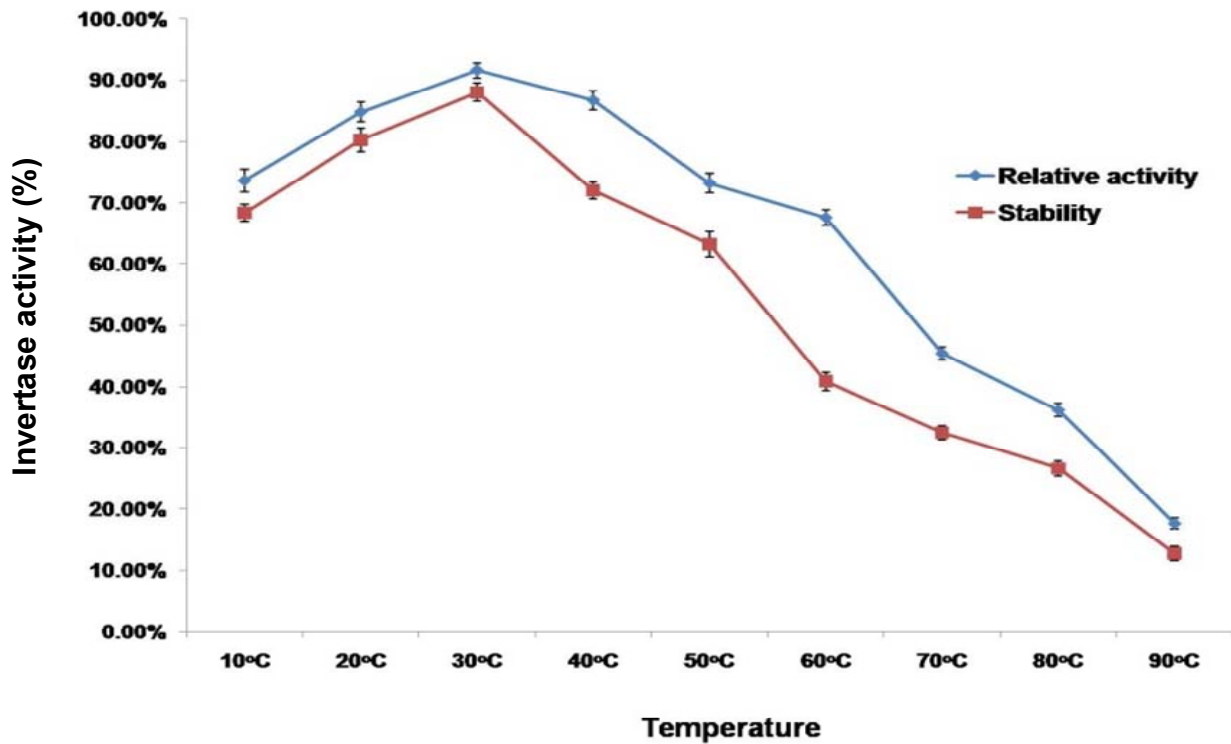


Figure 2. Effect of temperature on invertase activity and stability by *Saccharomyces cerevisiae* MTCC 170.

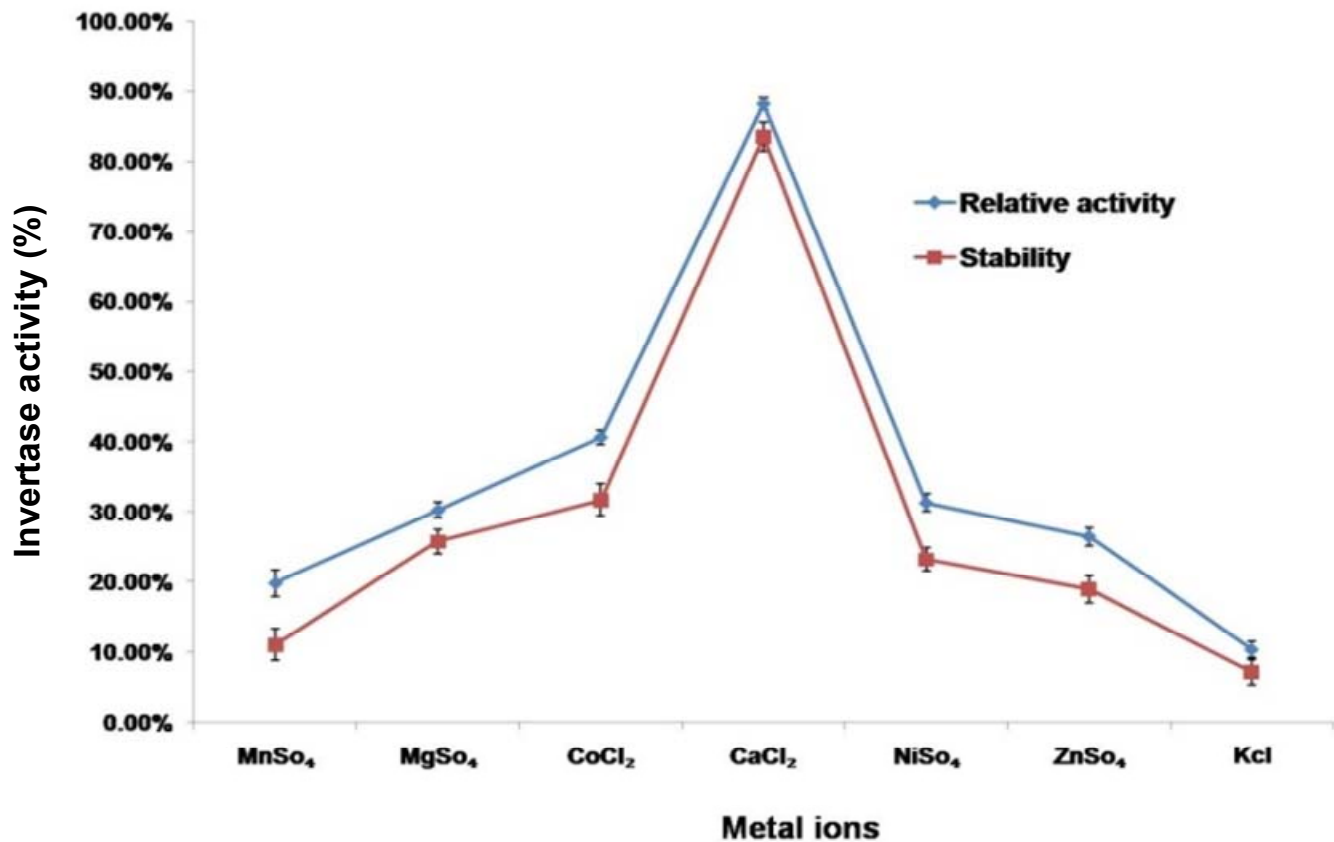


Figure 3. Effect of various metal ions on invertase activity and stability by *S. cerevisiae* MTCC 170.

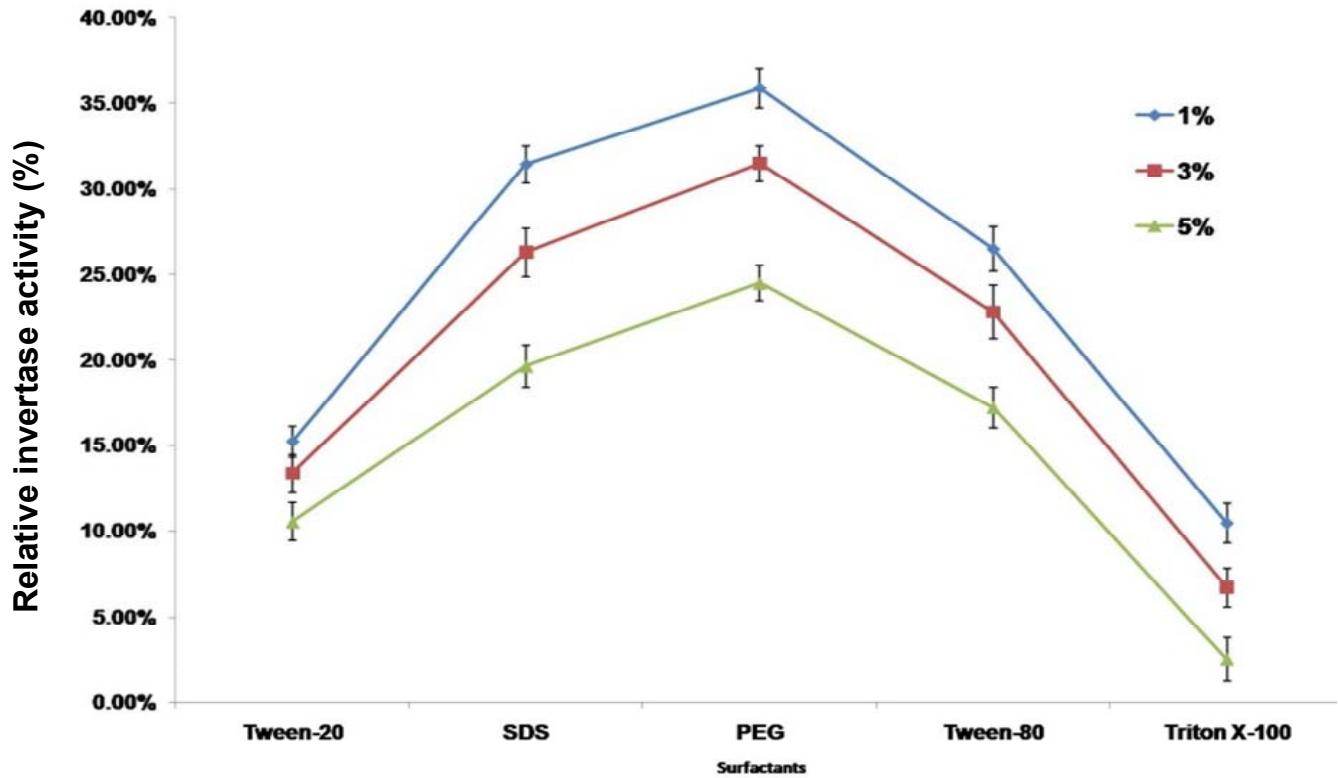


Figure 4a. Effect of different surfactants on invertase activity by *Saccharomyces cerevisiae* MTCC 170.

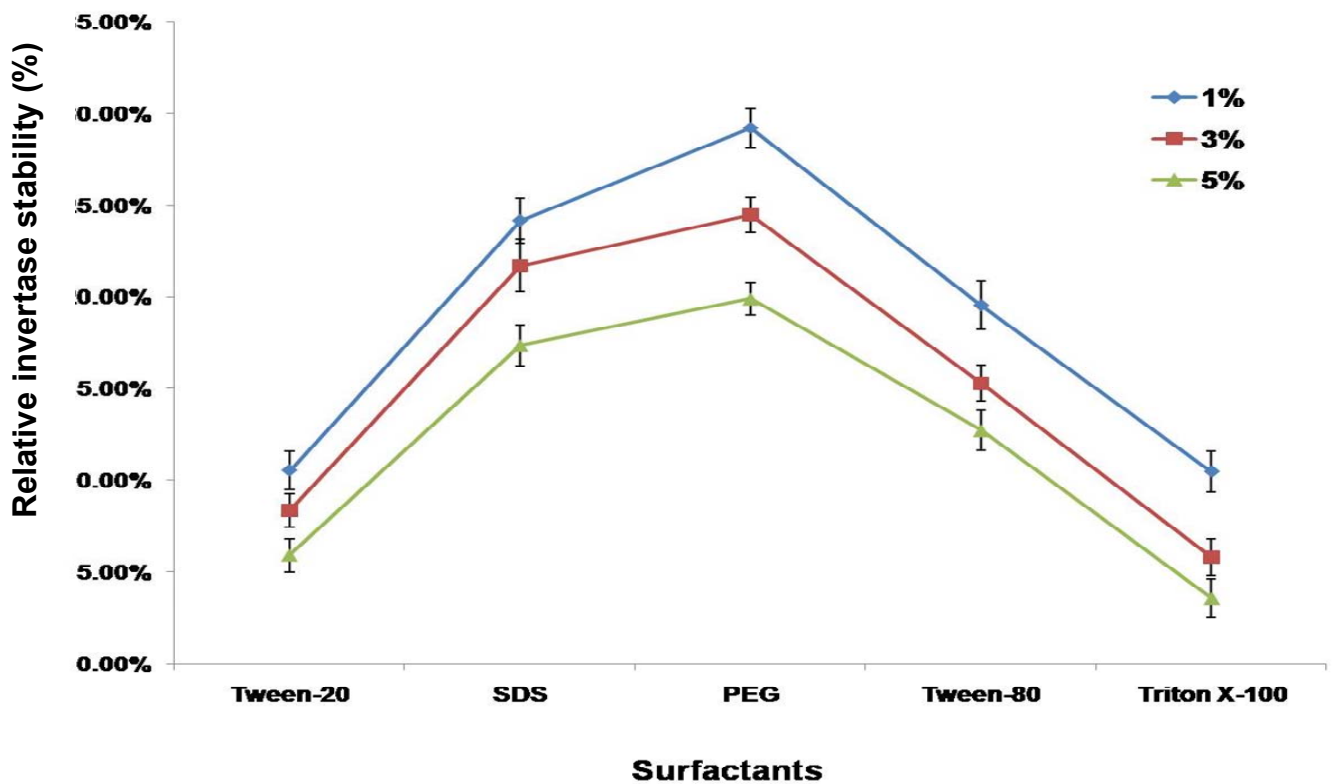
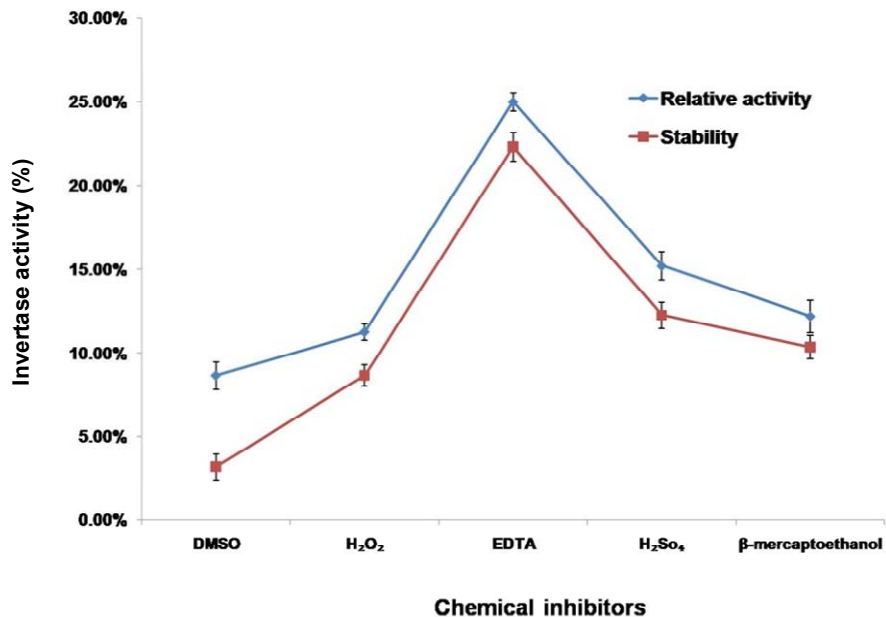


Figure 4b. Effect of different surfactants on invertase stability by *Saccharomyces cerevisiae* MTCC 170.



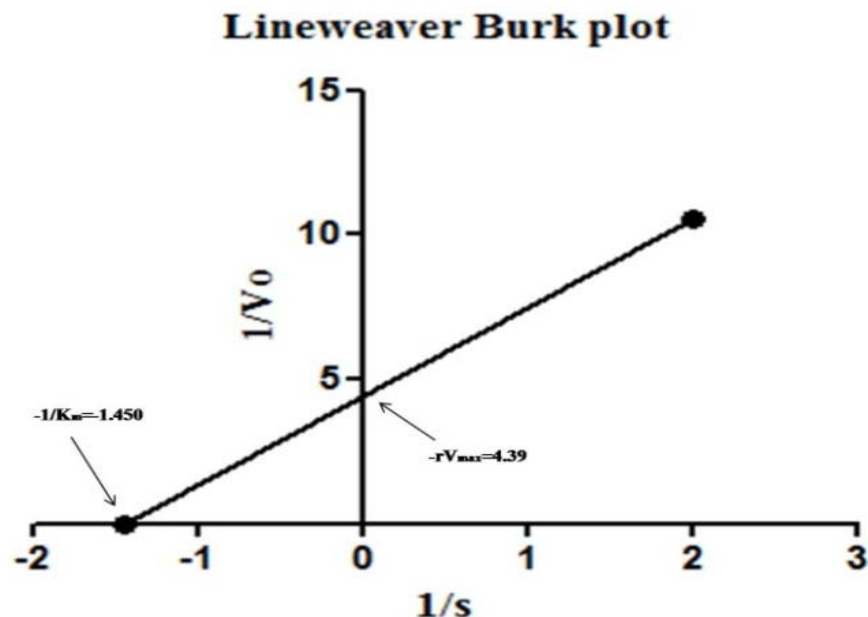
**Figure 5.** Effect of various chemical inhibitors on invertase activity and stability by *Saccharomyces cerevisiae* MTCC 170.

**Table 1.** Michaelis-Menton constant for *Saccharomyces cerevisiae* MTCC 170.

Michaelis-Menton	<i>Saccharomyces cerevisiae</i> MTCC 170
<b>Best-fit values</b>	
Vmax	0.3201
Km	0.6894
<b>Std. Error</b>	
Vmax	0.05047
Km	0.4680
<b>95% Confidence Intervals</b>	
Vmax	0.2037 to 0.4365
Km	0.0 to 1.769
<b>Goodness of Fit</b>	
Degrees of Freedom	8
R square	0.4760
Absolute Sum of Squares	0.02247
Sy.x	0.05300
<b>Constraints</b>	
Km	Km > 0.0
<b>Number of points</b>	
Analyzed	10

(Table 1). The kinetic parameters of enzymatic reaction were calculated by the Lineweaver-Burk linearization

using the Michaelis-Menton kinetic model, the  $-1/K_m$  value is -1.450 and the  $-r/V_{max}$  is 4.39 (Figure 6).



**Figure 6.** Lineweaver Burk plot for *Saccharomyces cerevisiae* MTCC 170.

## DISCUSSION

In the present work, the effect of pH for invertase activity by *S. cerevisiae* MTCC 170 was assessed. The effect of pH on the activity of crude invertase was determined in the pH range of 2-12. Maximum invertase activity of 91.55% was recorded at pH 6. The invertase activity was decreased to 11.64% at pH 12 in *S. cerevisiae* MTCC 170. Uma et al. (2010) stated that maximum invertase activity was recorded at pH 6.0 for invertase by *Aspergillus flavus*. Patil et al. (2012) evaluated the *Aspergillus* sp. invertase; it gave the good invertase activity for pH 6. Yamamoto et al. (1986) showed that maximum invertase activity was recorded at pH 6.8 for invertase from *Brevibacterium divaircatum*. Invertase exhibits marked stability towards temperature, pH, changes and denaturants. Invertase is used for the inversion of sucrose in the preparation of invert sugar and high fructose syrup (HFS) by Uma et al. (2012).

In the present study, the effect of temperature on invertase activity by *S. cerevisiae* MTCC 170 was investigated. The effect of temperature on the activity of crude invertase was determined in the temperature range of 10-90°C. Maximum invertase activity of 91.60% was recorded at 30°C. The invertase activity was decreased to 17.67% at 90°C in *S. cerevisiae* MTCC 170. Whereas, Kaur and Sharma (2005) reported that the 37°C gave good invertase activity for invertase by an actinomycete strain. Similarly, Maria and Rubio (1995) reported that 30°C gave good invertase activity for invertase by *Aspergillus niger*. Whereas, Gine et al. (2010) stated that the maximum invertase activity was recorded at 37°C for invertase by *Lactobacillus reuteri* CRL 100.

The effect of metal ions on invertase activity by *S. cerevisiae* MTCC 170 was investigated in the present work. The various metal ions such as  $\text{CaCl}_2$ ,  $\text{CoCl}_2$ ,  $\text{MgCl}_2$ ,  $\text{ZnSO}_4$ ,  $\text{NiSO}_4$ ,  $\text{CuSO}_4$  and  $\text{KCl}$  were tested. Maximum invertase activity of 88.30% was recorded in calcium chloride. The minimum invertase activity was recorded to be 10.34% at potassium chloride for *S. cerevisiae* MTCC 170 invertase. The effect of divalent metal ions  $\text{Cu}^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Co}^{2+}$  on the activity of the enzyme invertase showed that these ions affected the activity by a certain factor (Shankar et al., 2010). Similarly, Uma et al. (2010) evaluated that the maximum invertase activity was recorded at calcium chloride for invertase by *Aspergillus flavus*. In other hands, Guimaraes et al. (2007) stated that maximum invertase activity was recorded at magnesium chloride for invertase by *Aspergillus ochraceus*. Whereas, Uma et al. (2010) reported that the maximum invertase activity was recorded for sodium chloride and calcium chloride for invertase by *C. cladosporioides*. Moreno et al. (1979) showed that maximum invertase activity was recorded at cobaltous chloride for invertase by *S. cerevisiae* NRRL -Y 12623.

In the present work, the effect of surfactants on invertase activity by *S. cerevisiae* MTCC 170 was investigated. Various metal ions such as Triton X-100, Tween-20, SDS, polyethyleneglycol and Tween-80 were determined. Maximum invertase activity of 32.32% was recorded at poly ethylene glycol. The minimum invertase activity of 6.89% was recorded for Triton X-100 in *S. cerevisiae* MTCC 170 invertase.

The effect of chemical inhibitors on invertase activity by *S. cerevisiae* MTCC 170 was investigated in the present

study. The different chemical inhibitors such as DMSO, H<sub>2</sub>O<sub>2</sub>, EDTA, H<sub>2</sub>SO<sub>4</sub> and β-mercaptoethanol were tested. Maximum invertase activity of 25.58% was recorded in EDTA. The minimum invertase activity was recorded at 9.30% of DMSO in *S. cerevisiae* MTCC 170. Uma et al. (2012) reported that the maximum invertase inhibitory activity was recorded in toluidine for invertase by *C. cladosporioides*.

In the present investigation, the kinetic parameters (Km and Vmax) were determined at 30°C and pH 6 for *S. cerevisiae* MTCC 170 for concentrations ranging between 0.5 and 5 mg/ml of sucrose as substrate. The Km and Vmax of *S. cerevisiae* MTCC 170 are 0.6894 mg/ml and 0.3201 μmol/min/mg. Hocine et al. (2000) reported similar result for invertases from *A. niger*, Km and Vmax values for each enzyme were determined using the Lineweaver–Burk plots which were calculated to be 44.38 mM and 1030 mmol ml<sup>-1</sup> min<sup>-1</sup> for FTS and 35.67 mM and 398 mmol ml<sup>-1</sup> min<sup>-1</sup>. Ghazi et al. (2000) investigated the behaviour of fructosyltransferase from *A. aculeatus*, determined the kinetic constants (Km and Kcat) for both hydrolysis and transfer reactions. The reaction rates (μmol mg<sup>-1</sup> min<sup>-1</sup>) of sucrose concentrations up to 1.75 M were plotted.

Chang et al. (1994) reported that purified enzyme had an optimal pH (5-6), temperature (50°C) and a Km value of 0.53 M for catalyzing self transfer reaction from sucrose. Gine et al. (2010) reported that for invertase in *Lactobacillus reuteri* (CRL 1100), the Km (6.66 mM) and Vmax (0.028 μmol/min) values for sucrose were obtained. Workman and Day (1983) reported the Km value for sucrose was 13.6 mM in *Kluyveromyces fragilis*. Similar findings with the Bhatti et al. (2006) obtained Km value of 3.57 mM for sucrose in *Fusarium solani*. Hernalsteens and Maugeri (2008) reported the Km (13.4 g/l) and Vmax (21 μmol/ml/min) for sucrose by invertase in *Candida* sp. Buttner et al. (1990) determined Km value (71-83 mM) for sucrose in *Trichosporon adeninovorans* for two internal invertases. Whereas, Aziz et al. (2011) showed that the kinetic parameters of IM was about 1.74-fold more active (p=0.0002) than IW because their Vmax values were 564 and 325 U/mg of protein/min.

## Conclusion

The yeast (*S. cerevisiae* MTCC 170) capable of producing invertase was obtained from MTCC. The invertase activity and stability for the *S. cerevisiae* MTCC 170 was found to be at pH 6, 30°C, calcium chloride (metal ions), poly ethylene glycol (surfactants), EDTA (chemical inhibitors). The Km and Vmax of *S. cerevisiae* MTCC 170 are 0.6894 mg/ml and 0.3201 μmol/min/mg.

## Conflict of interests

The author(s) have not declared any conflict of interests.

## ACKNOWLEDGEMENT

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