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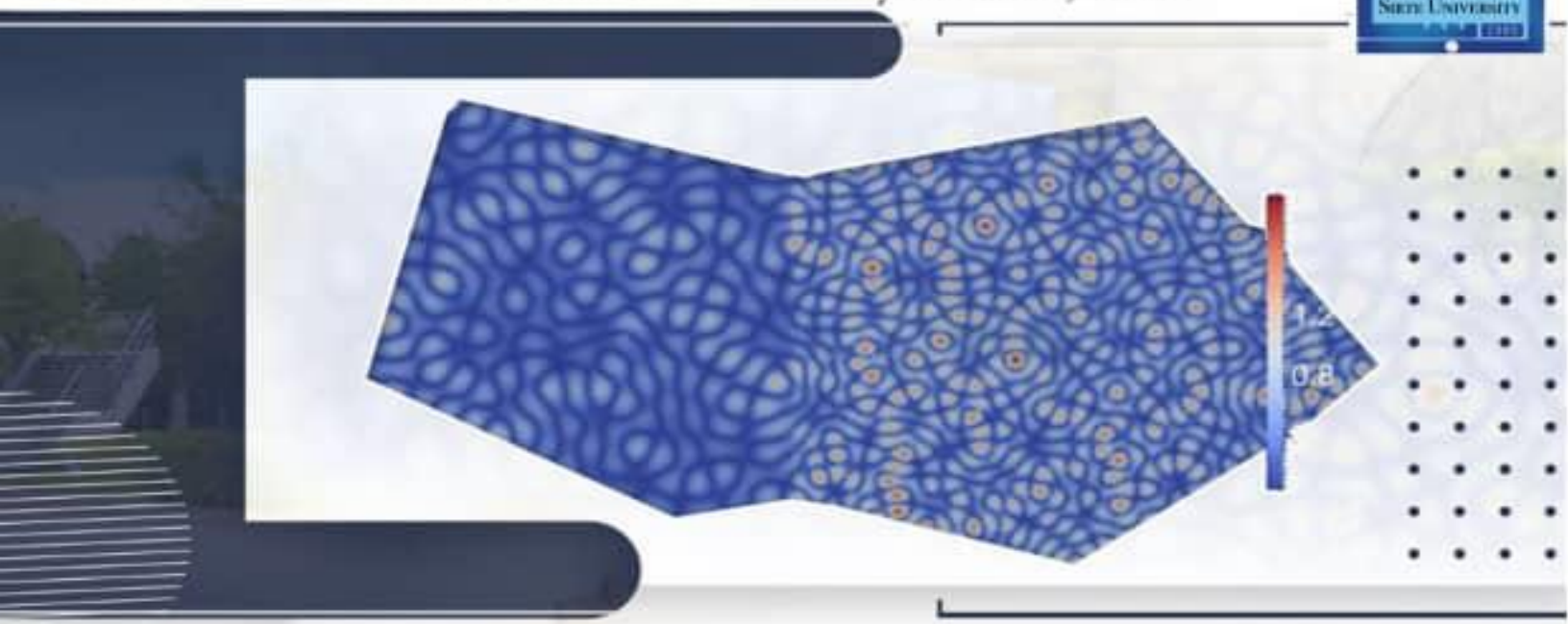

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Biochemical Study on the Kinetic Properties of the Invertase Produced by *Saccharomyces Cerevisiae*

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A B S T R A C T

Invertases are enzymes that hydrolyze sucrose to produce an equimolar mixture of glucose and fructose, which is of interest for various industrial applications. The present study aimed to produce invertase by *Saccharomyces cerevisiae* isolated from Baker's yeast and grape samples using the standardize technique. The enzyme activity was characterized with some parameters like pH, temperature, metal ions, kinetic parameters and inhibitors (fructose, glucose and copper (II) sulfate). Spectrophotometric methods were used to study enzyme kinetics and to determine the factors affecting enzyme activity. The optimum activity was recorded at 55˚C for both invertases. The optimum activity was at pH 6.0 for Baker's yeast invertase and at pH 10 for grape invertase. From Lineweaver-Burk Plot, V_{max} was found to be 24.39 ± 2.44 nmol/min/mg protein and the K_m approximately 0.860 ± 0.04 mM for Baker's yeast invertase but in case of grape invertase, V_{max} was 23.25 ± 3.14 nmol/min/mg protein and the K_m approximately1.243 ± 0.07 mM. Enzyme activity was increased in the presence of 5 mM Ca^{+2} ions for Baker's yeast, whereas showed the maximum activity at 5 mM Mg^{+2} ions in case of grape fruit invertase. Using sucrose as substrate, the K_{cat}, K_{cat}/K_m and Ks values were 0.28 ± 0.02 min^{-1}, 0.325 ± 0.03 mM^{-1} min^{-1} and 27.03 ± 5.24 ml/min/mg protein for Baker's yeast invertase activity, whereas were 0.56 ± 0.008 min^{-1}, 0.045 ± 0.003 mM^{-1} min^{-1} and 24.39 ± 7.11 ml/min/mg protein for grape invertase values. In conclusion, the *S. cerevisiae* isolated from grape fruits was more potent for invertase production in comparable with that isolated from Baker's yeast.

1 Introduction

Enzymes are proteins that produced by all living cells as catalysts for specific chemical reactions. In enzymatic reactions, the molecule at beginning is called substrates, and then they convert into different molecules called products. Almost all biological cells need enzymes to increase the rate of chemical reactions in order to occur at rates sufficient for life. Enzymes are very important part in some food processes, such as making of cheese, bread, wine and beer, for thousands of years (Dewdney, 1973). Invertase (EC 3.2.1.26) is one of the most widely used enzymes in confectionery industry for preparation of jams and candies (Klein *et al*., 1989). Invertase, also known as -fructofuranosidase, catalyzes the conversion of sucrose, a non-reducing disaccharide, to fructose and glucose, which are reducing monosaccharides (Arise *et al*., 2020). Inverted sugar syrup is the result of combining

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glucose and fructose (Mobini-Dehkordi *et al*., 2008). Invertases are found in nature in various isoforms that are distinguished by their subcellular locations. For example, in yeast cells, it can be found in two forms: extracellular or intracellular invertase, while in plants, there are three isoforms that differ in biochemical properties and subcellular locations (Acosta *et al*., 2000). Invert sugar is a sugar composed of an equimolar mixture of fructose and glucose that is sweeter and has less crystallinity than sucrose (Goosen *et al*., 2007). Invertases are known to be used in a variety of industrial food applications, including the production of non-crystallizing creams, artificial honey, lactic acid, ethanol, confectionary (food), digestive aid tablets, powder milk for infants, and other infant foods (Phadtare *et al*., 2004; Sikander, 2007). Despite invertase wide range of applications in numerous industries, commercially available invertase is quite expensive, restricting the enzyme's usefulness. Microorganisms are primarily used in the synthesis of invertase, which requires very precise control of production conditions and a high level of purification for taste and health reasons, making the enzyme pricey (Laluce, 1991). It occurs widely in microorganisms and was first isolated in 1860 by Berthelot using alcohol precipitation (Neumann, & Lampen, 1967).

Saccharomyces cerevisiae is particularly interesting microorganism, it synthesizes two invertase is biosynthesized by yeast strains, Aureobasidium sp. Rhodotorulaglutinis, Saccharomyces cerevisiae (Mona, & Mohamed, 2009), Saccharomyces carlsbergenesis (Shankar *et al*., 2010). Saccharomyces cerevisiae is the organism of choice for invertase production because of its characteristic high sucrose ferment ability. The aim of this study was carried out to produce extracellular invertase enzyme of Saccharomyces cerevisiae newly isolated and partial purification from Baker’s yeast and grape samples to determine the kinetic factors and conditions that will maximize the activity of the enzyme which will help boost its suitability for industrial prospects.

2 Materials and Methods

Yeast, sucrose, glacial acetic acid, sodium acetate, sodium potassium tartrate, sodium hydroxide, silver nitrate, glucose, fructose, Bovine serum albumin (BSA), Coomassie brilliant blue (G-250) and 3,5-dinitrobenzoic Acid (DNS) were obtained from Sigma/Aldrich Chemical Company Ltd, Poole, UK.

2.1 Isolation of Microorganisms

In this study, *Saccharomyces cerevisiae* strains were isolated from Baker's yeast and fruit samples such as grape and used to produce invertase. Grape samples were gathered from El-beida city's local market and utilized to isolate invertase-producing yeast. Each cotton wool plugged Erlenmeyer flask was filled with YPS agar medium containing: (sucrose 30.0 g/l, peptone 5.0 g/l, and yeast extract 3.0 g/l). The flasks were sterilized in an autoclave for 15 minutes at 15 lbs pressure (121°C) and then cooled to room temperature (Dworschock & Wickerham, 1961). For 2-3 days, the Petri plates were incubated at 30°C in an incubator (Model: MIR-153, Sanyo Japan). YPS agar slants were used to transfer the colonies. Cultural and physical traits were used to identify the isolates (Barnett *et al*., 1979).

2.1.1 Isolation of Efficient Invertase Producer

By inoculating a culture into sucrose broth, an efficient invertase producer (*Saccharomyces cerevisiae*) was isolated. The broth was tested for invertase activity after three days of incubation by boiling it with Benedict's reagent (green to brick red color indicates positive result) (Arumugam *et al*., 2014).

2.1.2 Identification of *Saccharomyces Cerevisiae* Gram’s Staining

Gram's staining was applied to a loopful broth culture and viewed microscopically under an oil immersion microscope. Budding yeast cells that were Grams positive were observed (Arumugam *et al*., 2014).

2.1.3 Lacto Phenol Cotton Blue Staining

Lacto phenol Cotton Blue Staining producer was used to stain a loop of broth culture, which was observed under high power objectives. The presence of budding yeast cells was observed (Arumugam *et al*., 2014).

2.1.4 Extracellular Invertase Production

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.

2.2 Measurement of Invertase Activity

The activity of the enzyme was determined using sucrose as substrate. A suitable amount (100µl) of the enzyme solution was mixed with buffered (acetate buffer pH 6.0 for invertase from Baker’s yeast and sodium phosphate buffer pH 10.0 for invertase from grape sample) 30 mM aqueous sucrose solution. The reaction was carried out at

55°C for 30 min and the volume was made up to 3 ml by adding distilled water. To stop the reaction, 3 ml of dinitrosalicylic acid reagent (DNS) was added and the mixture was heated for five minutes in a boiling water bath. After cooling, the intensity of the color was read at 540 nm in UV-Vis spectrophotometers (Miller, 1959).

The invertase units in each sample were determined by using the following formula:

Units of invertase activity in each assay

$$\text{Tube} = \frac{\Delta ABS/30.0\text{min} * \text{Final volume}}{a * 1.0\text{cm}}$$

Where, ΔABS = absorbance change

a = mill-molar absorptivity constant

Total units = Number of units / Final volume * Final volume / ml of dilute enzyme added * total ml of dilute enzyme / ml of stock added * ml of stock fraction/ 1. Protein concentration was estimated according to the method described by Bradford depending on bovine serum albumin (BSA) standard curve (Bradford, 1976).

The specific activity was determination by using following equation:

$$= \frac{\text{U}}{\text{mg}} = \frac{\text{Enzyme activity } \frac{\text{U}}{\text{ml}}}{\text{Protein concentration } \frac{\text{mg}}{\text{ml}}}$$

2.3 Protein Estimation

The protein concentration of enzyme solutions was measured by Bradford method (Bradford ,1976). Bradford reagent (5ml) was added to a test-tube containing 0.1 ml of the diluted enzyme. A blank was run parallel. The tubes were vortexed. The absorbance was noted at 595 nm on a spectrophotometer. The bovine serum albumin (BSA) standard curve was used to calculate the amount of protein in each sample.

2.4 Effect of pH and Temperature on Invertase Activity

The effect of pH on invertase activity was determined by assaying the enzyme as mentioned before with the difference that the activity was determined at different pH ranging from 3-13 (50mM acetate and 50mM sodium phosphate buffer) at 55°C as described earlier (Amin *et al*., 2010). The optimal temperature for invertase activity was determined by measuring the activity at various temperatures (25-70°C), as described by Amin *et al* (2010).

2.5 Effect of Various Metal Ions on Invertase Activity

The crude invertase was mixed with 5 mM concentration of various salts such as $CaCl_2$, $CuCl_2$, $MgCl_2$, $SnCl_2$, $NiCl_2$, $CuCl_2$ and NaCl for 30min at 55°C pH 6 and 10 for invertase from Baker's yeast and grape sample respectively before adding the substrate and subsequently invertase activity was determined (Shankar *et al*., 2010).

2.6 Determination of The Mode of Inhibition and Inhibitor Constant (Ki)

For crude invertase of *S. cerevisiae*, the effect of different chemical inhibitors on invertase activity was studied individually. Before adding the substrate, the crude invertase was mixed with 0.5, 1, 2, and 5mM concentrations of different chemical inhibitors such as fructose, $CuSO_4$, and glucose for 30 minutes at 55°C, pH 6.0 and 10 for invertase from Baker's yeast and grape sample, respectively (Aziz *et al*., 2011).

2.7 Determination of Kinetic Parameters for Crude Invertase

The kinetic parameters (Michaelis-Menton constant) K_m and maximal velocity V_{max} of invertase activity of *S. cerevisiae* were determined individually from Lineweaver Burk plot optimal assay conditions, 55°C, pH 6.0 and 10 for invertase from Baker's yeast and grape sample respectively at 30 min for sucrose concentrations ranging from 0.25, 0.5, 1, 2, 5, 10 and 20 mM. The evaluation of these graph yielded the kinetic parameters for the invertase activity (Sivakumar *et al*., 2012).

2.8 Statistical Analysis

Results were expressed as means ± standard division of the mean (n = 6). Statistical significance was set at $P \leq 0.05$ by using a t-test. Microsoft Excel has calculated kinetic parameters for invertase activity.

3 Results

3.1 Isolation and Identification of Yeast Cultures

Different strains of *Saccharomyces cerevisiae* were isolated from Baker's yeast and grape fruit samples. On the basis of morphology and sporulation, they were identified using conventional yeast identification procedures.

3.2 Effect of pH on *S. cerevisiae* Invertase Activity

In the present study, the effect of pH for invertase activity by S. cerevisiae of Baker's yeast and grape fruit were

assessed. The effect of pH on the activity of crude invertase was determined in the pH range of 3.0-13. Maximum invertase activity of was recorded at pH 6.0 and pH 10 for both extracellular invertases (*S. cerevisiae*) were isolated from Baker's yeast and grape fruit respectively. The enzyme activity was decreased at pH 13 for both enzymes. The pH stability of enzyme was measured by the standard assay method with sucrose. An average of retaining activity was observed between pH 5.0 and 8.0 for Baker's yeast invertase and between pH 8.0 and 11 for invertase from grape sample (Fig. 1 and 2).

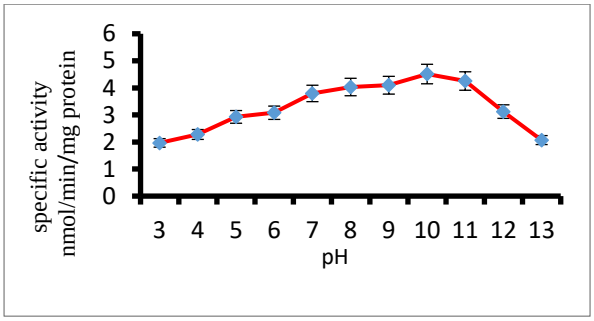

Figure (1). Effect of pH on invertase activity by *S. cerevisiae* (grape fruit).

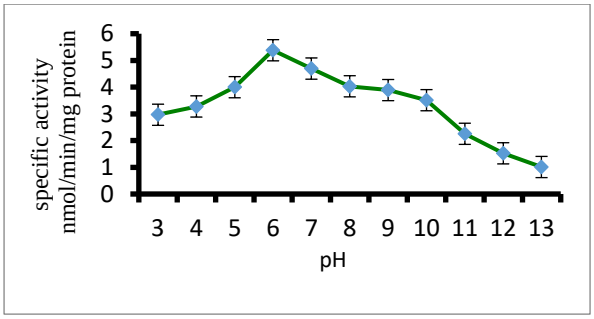

Figure (2). Effect of pH on invertase activity by *S. cerevisiae* (Baker's yeast).

3.3 Effect of Temperature on *S. cerevisiae* Invertase Activity

In the study of effect of temperature on invertase, it was observed that invertase was sensitive to temperature; the higher the temperature, the higher the rate of reaction. The increase in the rate of reaction is due to an increase in the number of molecules that have sufficient energy to enter into the transition state. From (Fig. 3), the reaction rate of invertase for both, extracellular invertases increased from 35°C to 55°C.

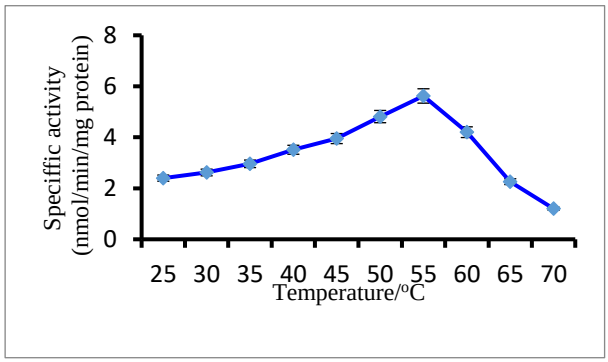

Figure (3). Effect of temperature on invertase activity by *S. cerevisiae* (Baker's yeast and grape fruit).

3.4 Effect of Various Metal ions on *S. cerevisiae* Invertase Activity

The effect of different metals ions ($CaCl_2$, $CuCl_2$, $MgCl_2$, $SnCl_2$, $NiCl_2$, $CuCl_2$ and NaCl) on invertase activity for both, extracellular invertases of *S. cerevisiae* was examined by incubating various metal ions with extract at 55°C for 30 minutes (Fig. 4 and 5). Maximum amount of invertase activity of 2.69 ± 0.03 nmol/min/mg protein was recorded in calcium chloride and minimum invertase production of 0.34 ± 0.005 nmol/min/mg protein was recorded in tin (II) chloride by *S. cerevisiae* of Baker's yeast (Fig. 4). Among the tested metal ions, the maximum amount of extracellular invertases for grape fruit was recorded in magnesium chloride (1.25 ± 0.02 nmol/min/mg protein), whereas the minimum amount of invertase production was observed in sodium chloride (0.09 ± 0.001 nmol/min/mg protein) (Fig. 5).

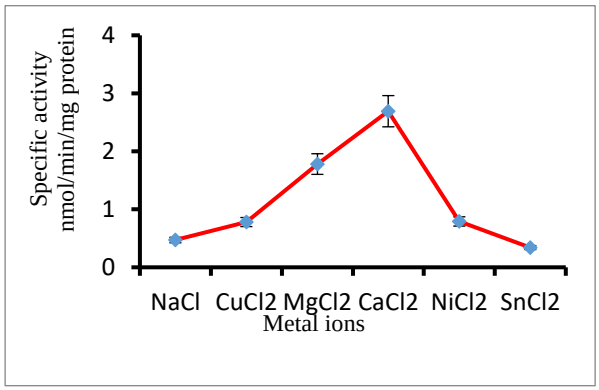

Figure (4). Effect of metal ions on invertase activity by *S. cerevisiae* from Baker's yeast.

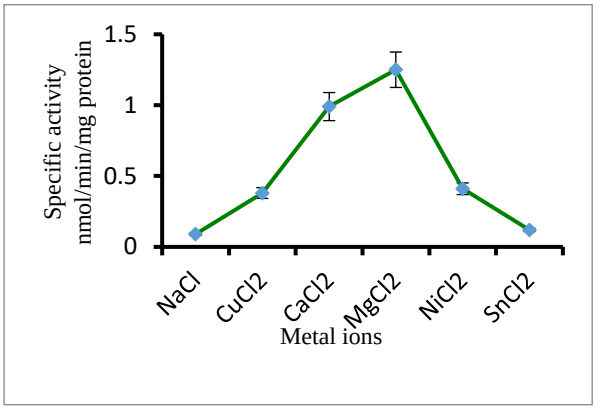

Figure (5). Effect of metal ions on invertase activity by *S. cerevisiae* from grape sample.

3.5 Determination of Kinetic Parameters for *S. cerevisiae* Invertase

The kinetic parameters of enzymatic reaction were calculated by the Lineweaver-Burk linearization using the Michaelis-Menton kinetic model. The kinetic parameters (K_m and V_{max}) were determined at 55°C and pH 6 and 10 for both, extracellular invertases (*S. cerevisiae*) for concentrations ranging between 0.25 to 5 mM of sucrose as substrate. The K_m and V_{max} of *S. cerevisiae* for Baker's yeast is 0.860 ± 0.04 mM and 24.39 ± 2.44 nmol/min/mg protein (Fig. 6). The K_m and V_{max} of S. cerevisiae for grape fruit is 1.243 ± 0.07 mM and 23.25 ± 3.14 nmol/min/mg protein (Fig. 7). The results of this study indicated that sucrose is a better substrate of extracellular invertases (Baker's yeast) than extracellular invertases (grape fruit) with a K_m value 1.5-fold higher (1.243 ± 0.07 mM) and the lower V_{max} (23.25 ± 3.14 nmol/min/mg protein).

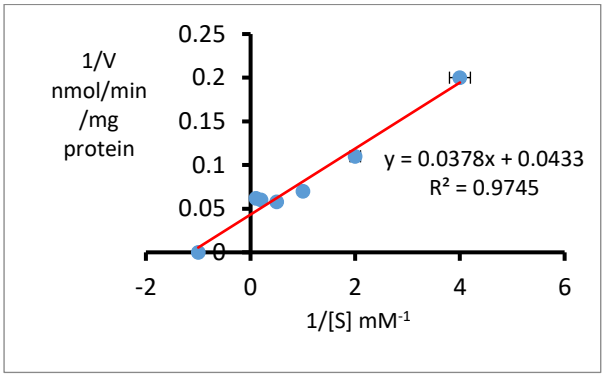

Figure (6). Determination of K_m and V_{max} of *S. cerevisiae* of Baker's yeast invertase (Lineweaver Burk plot; 50 mM citrate pH 6, 55°C, 30 min, substrate concentration ranging from 0.25-5 mM; Mean and standard deviation were determined from three replicates). The intercept on the y-axis corresponding to $1/V_{max}$ = 0.0433, slope corresponding to K_m/V_{max} = 0.0378.

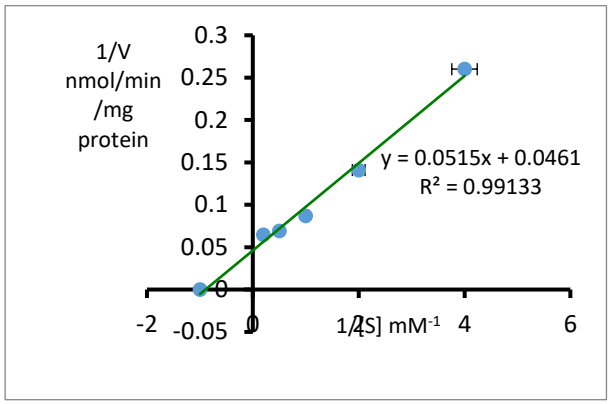

Figure (7). Determination of K_m and V_{max} of *S. cerevisiae* of grape fruit invertase (Lineweaver Burk plot; 50 mM sodium phosphate pH 10, 55°C, 30 min, substrate concentration ranging from 0.25-5 mM; Mean and standard deviation were determined from three replicates). The intercept on the y-axis corresponding to $1/V_{max}$ = 0.0461, slope corresponding to K_m/V_{max} = 0.0515.

3.6 Kinetics of Sucrose Hydrolysis by *S. cerevisiae* Invertases

Extracellular invertases (*S. cerevisiae*) for Baker's yeast and grape fruit were assayed at 55°C in the reaction mixtures (pH = 6.0 and 10, respectively) containing different amounts of sucrose (0.25-5.0 mM). The data were plotted according to the Lineweaver-Burk plot to determine the values of kinetic constants (V_{max} and K_m, K_{cat}, and K_{cat}/K_m, Fig. 6 and 7) as described previously. A Lineweaver-Burk plot was applied to determine the kinetic constants (K_m and V_{max}). The catalytic center activity for both, extracellular invertases (*S. cerevisiae*) (K_{cat}) and specificity constant (K_{cat}/K_m) of sucrose was determined (Tab. 1).

Table (1). The catalytic properties of invertase from *S. cerevisiae.*

Invertases types		
Catalytic properties	**Baker's yeast**	**Grape fruit**
K_m (mM)	0.860 ± 0.04	1.243 ± 0.07
V_{max} (nmol/min/mg protein	24.39 ± 2.44	23.25 ± 3.14
[a]K_{cat} (min^{-1})	0.28 ± 0.02	0.56 ± 0.008
K_{cat}/K_m (mM^{-1} min^{-1})	0.325 ± 0.03	0.045 ± 0.003
Ks (V_{max}/K_m) (ml/min/mg protein)	27.03 ± 5.24	24.39 ± 7.11

The substrate efficiency ($Ks = V_{max}/K_m$).
The turnover number (K_{cat}) = $V_{max}/[E]$.
[a] Calculated estimating a molecular mass of 270 kDa for the active enzyme.
The kinetic constants (K_m & V_{max}).
The specificity constant (K_{cat}/K_m).

3.7 Determination of Inhibition constants (Ki) for *S. cerevisiae* Invertases

In order to determine the Ki for *S. cerevisiae* invertases inhibitors with a series of experiments were conducted with different chemical inhibitors. The crude invertase was mixed with 0.5, 1, 2 and 5 mM concentration of different chemical inhibitors such as fructose, $CuSO_4$ and glucose for 30 min at 55°C, pH 6.0 for invertase from Baker's yeast before adding the sucrose as substrate and subsequently invertase activity was determined. Lineweaver-Burk plots from this data were used to determine the inhibitor constant (Ki). (Fig. 8 to 13) show the effect of these inhibitors on extracellular invertase. (Tab. 2) summarizes the Ki and mode of inhibition of different inhibitors with invertase activity.

Table (2). Summary of inhibition constant (Ki) and mode of inhibition of different inhibitors with Baker's yeast extracellular invertase

Invertase inhibitor	Ki (mM)	Mode of inhibition
Fructose	0.45 ± 0.09	Competitive
$CuSO_4$	0.066 ± 0.003	Uncompetitive
Glucose	0.014 ± 0.001	Non-competitive

The values shown are the (Mean ± S.D), obtained from three separate the crude invertase for Baker's yeast.

It was found that all extracellular invertase inhibitors had a Ki values between 0.01 mM to 0.4 mM with the lowest Ki value with glucose. The weakest extracellular invertase inhibitors found was fructose. Lineweaver-Burk plot analysis revealed that the inhibitors had different modes of inhibition. Glucose was a non-competitive inhibitor (Fig. 8 and 9), while $CuSO_4$ was uncompetitive inhibitors (Fig. 10 and 11). Fructose was found to be a competitive inhibitor (Fig. 12 and 13).

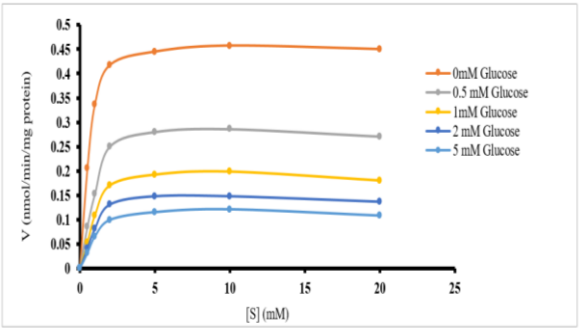

Figure (8). Saturation kinetics plot for glucose inhibition with sucrose as an invertase substrate in extracellular invertase for Baker's yeast.

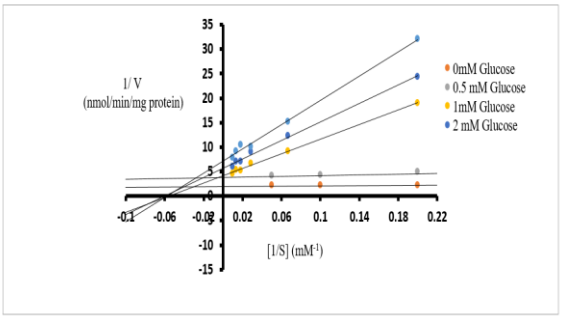

Figure (9). Lineweaver-Burk plot for glucose inhibition (non-competitive) with sucrose as an invertase substrate in extracellular invertase for Baker's yeast.

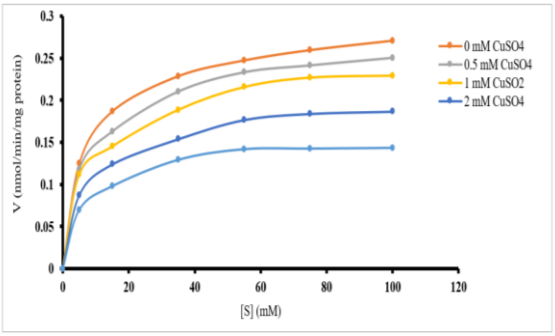

Figure (10). Saturation kinetics plot for $CuSO_4$ inhibition with sucrose as an invertase substrate in extracellular invertase for Baker's yeast.

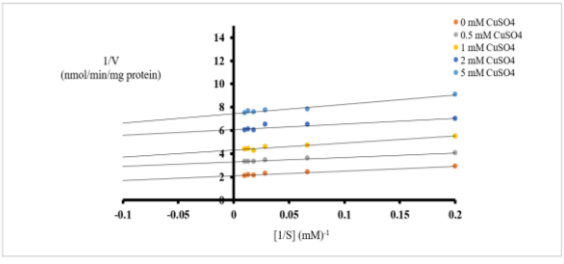

Figure (11). Lineweaver-Burk plot for $CuSO_4$ inhibition (uncompetitive) with sucrose as an invertase substrate in extracellular invertase for Baker's yeast.

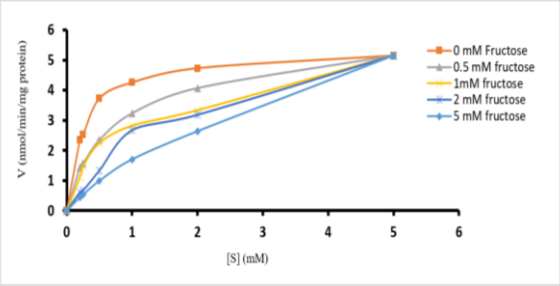

Figure (12). Saturation kinetics plot for fructose inhibition with sucrose as an invertase substrate in extracellular invertase for Baker's yeast.

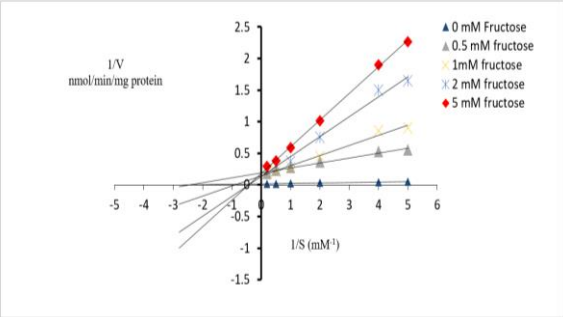

Figure (13). Lineweaver-Burk plot for fructose inhibition (competitive) with sucrose as an invertase substrate in extracellular invertase for Baker's yeast.

4 Discussion

The identification of *S. cerevisiae* was carried out (Barnett *et al*., 1979; Gustavo *et al*., 2022). Colonies were flat, smooth, moist, glistening, and creamy in color. They appeared on YPS agar plates after 24 h, rapidly grew and fully matured within 3 days. Microscopic studies showed that *S. cerevisiae* is one of the budding yeast. Cells in the sediment of YPS broth were unicellular and globose. The bud raised on different parts of the cell surface on a narrow base as said to be multilateral (multipolar) budding was typical. The spores formed by *S. cerevisiae* were 1-4 spores per ascus. They were rounded and slightly oval in shape. Screening of *S. cerevisiae* isolates was carried out in shake flask by submerged fermentation for invertase production. The effect of pH on the activity of crude invertase was determined in the pH range of 3.0-13. A change in pH affects the ionization of essential active site amino acid residues that are involved in substrate binding and catalysis. The ionization of these residues may cause distortion of the active site cleft and hence may indirectly affect enzyme activity. The effect of pH is related to the growth and metabolic activities of the organism (Bodade *et al*., 2010). This result was supported by the finding of Shankar *et al*. (2014) who showed that the maximum invertase activity was at pH 6.0 by *Saccharomyces cerevisiae* MK. Uma *et al*. (2010) reported that the maximum invertase activity was found at pH 6 by *Aspergillus flavus*. Patil *et al*. (2012) evaluated the *Aspergillus* sp. invertase; it gave the good invertase activity for pH 6. Yamamota *et al*. (1986) showed that maximum invertase activity was recorded at pH 6.8 for invertase from *Brevibacterium divaircatum*. Invertase exhibits marked stability towards pH changes and denaturants. Unlike other enzymes, invertase exhibits relatively high activity over a broad range of pH (3.5-5.5), with the optimum near pH of 4.5. (Essel & Osei, 2014). Invertases from *Saccharomyces cerevisiae* are usually active in slightly acidic to neutral pH (3.0-7.0), to close to neutral pH (6.5-7.0) (Belcarz *et al*., 2000). However, optimum activity was highly active at pH 3.6 to 5.0 with optimum pH 4.5. Nguyen *et al*., (2000) found that *Aspergillus niger* invertase was stable at pH range from 5.0 to 6.5. However, Benattouche *et al*. (2016) was noted that the invertase by *Streptococcus sp*. isolated from the date increased in pH from pH 7.0 to pH 8.0. Result also, showed that the invertase activity maxima observed at pH 10 (Fig. 1) by *S. cerevisiae* (grape fruit). These results are in line with Lee & Strum, (1996) results who reported that the maximum invertase increased above pH 9.0 by alkaline invertase isolated from carrot. On the other hand, the pH of a soluble invertase present in Semillon grape berry juice has been determined to be acidic not alkaline (Nakanishi *et al*., 1991). Thus, further basic research is still needed. For instance, it remains unclear whether or not differences exist in gene expression patterns and regulation of sugar metabolism between grape species (Pan *et al*., 2009). The activity of the invertase declined from 60°C to 70°C. This implies that the enzyme was denatured which led to the loss of activity of the invertase. Since enzymes, like invertase, were proteins with tertiary structure, they can be denatured when exposed to high temperatures. Denatured proteins do not react as much as the newly extracted proteins, thus resulting into a drastic drop of the rate of reaction (Campbell & Farrell, 2012). The temperature optimum was found 55°C in the present study (Fig. 3). These results agree with the findings by L'Hocine *et al*. (2000) who reported that the 55°C gave good invertase activity for invertase from *Aspergillus Niger* strain. On the other hand, Shankar *et al*. (2014) reported that the 30°C gave good invertase activity for invertase by an *S. cerevisiae* strain. In addition, Gine *et al*. (2010) found that 37°C gave good invertase by *Lactobacillus reuteri* CRL 100. Moreover, Benattouche *et al*. (2016) also

reported that the maximum invertase activity was recorded at 50°C for invertase by *Sterptococcus sp*. The similar results, Amin *et al*. (2010) reported that a slight increase in the activity of acid invertase was observed with Ca^{+2}, Mn^{+2} and Mg^{+2} ions. Shankar *et al*. (2014) showed that maximum invertase of 89.11% was recorded at $CaCl_2$. However, 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 invertases 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, 2004). (Dahot & Noomrio 1996) reported increase in invertase activities in the presence of $MgCl_2$ and $CaCl_2$ ions. They suggested that thiol groups at the catalytic site are important for the invertase activity. Metal ions play important roles in the biological function of many enzymes. The various modes of metal-protein interaction include metal-, ligand-, and enzyme-bridge complexes. Metals can serve as electron donors or acceptors. Chang *et al*. (1994) reported that purified enzyme had an optimal pH (5-6), temperature (50°C) and a K_m value of 0.53 mM for catalyzing self-transfer reaction from sucrose. Gine *et al*. (2010) reported that for invertase in *Lactobacillus reuteri* (CRL 1100), the K_m (6.66 mM) and V_{max} (0.028 µmol/min) values for sucrose were obtained. Uma *et al*. (2010) reported the result for invertase by *Aspergillus flavus* in Lineweaver-Burk plot of the enzyme affinity for sucrose gave a straight line plot from which the K_m as 0.23 mg/ml and V_{max} was 15.8 U/mg. Ribeiro and Vitolo, (2005) stated the result for conventional Lineweaver-Burk plot, the kinetic constants for soluble invertase (K_m = 18.3 mM and V_{max} = 0.084 U/mgE) and insoluble invertase (K_m = 29.1 mM and V_{max} = 0.075 U/mgE) invertase were calculated. Almeida *et al*. (2005) investigated the kinetic parameters of enzymatic reaction were calculated by the Lineweaver-Burk linearization using the Michaelis-Menton kinetic model. For the auto-immobilized enzyme it was obtained a K_m of 447 mM and V_{max} of 2,805 mmol/min. Uma *et al*. (2012) also stated the Michaelis-Menton kinetics for and constants were determined from a Lineweaver-Burk plot. The kinetic constant of invertase was V_{max} 28.57U/mg and K_m of 0.26 mg/ml. Whereas, Mona *et al*. (2009) evaluated the invertase from *Saccharomyces cerevisiae* NRRL Y-12632 in K_m value of the pure enzyme was found to be 60 mM while its V_{max} was 35.5 min mg protein as calculated by Hanes-Woolf plot. The rate of sucrose hydrolysis; decreased by increasing substrate concentration, which may be due to substrate inhibition. However, Talekar *et al*. (2010) reported the kinetic constants for soluble invertase (K_m = 10.80 mM and V_{max} = 21.59 µmol/minutes) were calculated from nonlinear regression. Whereas, Guimaraes *et al*. (2007) stated that invertase from *Aspergillus niveus* had K_m value of 5.78 mM and V_{max} of 28.46 U per mg of protein per min and Chavez *et al*. (1997) investigated that invertase from *Candida utilis* and *Saccharomyces cerevisiae* exhibited K_m values of 11 and 25 mM, respectively. In a summary, a kinetic constant of invertase isolated from many organisms is affected by many factors such as enzyme sources, optimum pH, optimum temperature, thermal stability and molecular mass. This means that the good affinity of the extracellular invertases (Baker's yeast) for sucrose substrate. This was due to the lower accessibility of the substrate to the active site of the invertase enzyme (Bayramolu *et al*., 2003). The kinetic parameters of both, extracellular invertase shown in Table 1 shows the most dramatic change, with about a 22.5% reduction in Kcat for grape invertase. There was big change in the $Kcat/K_m$ of Baker's yeast invertase. The $Kcat/K_m$ value of Baker's yeast invertase was about ≈ 8-fold increase in the catalytic process. The key advantages revealed here are the low K_m (0.860 ± 0.04 mM), the high V_{max}, (23.25 ± 3.14 nmol/min/mg protein) and the high $Kcat/K_m$ (0.28 mM^{-1} min^{-1}). This could be due to the ionized state of the Baker's yeast invertase whose charges have been reserved and that the sucrose binds more strongly to the active site than transition state of the substrate substantiating with the study of Myers *et al*., (1997). This indicated that the Baker's yeast had higher affinity towards binding sucrose to the active site. The turnover number (K_{cat}) and specificity constant (K_{cat}/K_m) values of both enzymes (Tab. 1) showed that the activity of the Baker's yeast was better compared to grape invertase. Furthermore, specificity constant Ks (27.03 ml/min/mg protein) again confirmed that the Baker's yeast invertase was higher and more specific for sucrose as compared to grape invertase Ks (24.39 ml/min/mg protein) (Tab. 1). There are a few reports investigating the kinetic parameters of *S. cerevisiae* invertases. Partially purified invertases from *S. cerevisiae* K_{cat} and K_{cat}/K_m for sucrose substrate was 5.66 min^{-1} and 2.16 x 10^7 $M^{-1}Min^{-1}$ (Reddy & Maley, 1996). Invertase production by *Saccharomyces cerevisiae* on from the wild-culture had K_{cat} and K_{cat}/K_m for sucrose equal to 122 min^{-1} and 19.51 $M^{-1}min^{-1}$ (Ul-Haq & Ali, 2005). In this study preliminary experiments were performed with three well-documented inhibitors of

extracellular invertase for Baker's yeast. Lee and Sturm (1996) support this finding who reported that invertase enzyme of carrot root was inhibited by their reaction products glucose and fructose. Fructose was a competitive inhibitor of neutral (Ki, 13.4 mM) and alkaline (Ki 16.3 mM) invertase. In contrast, glucose inhibited neutral (Ki, 28 mM) and alkaline (Ki, 33 mM) invertase noncompetitively. Fructose and glucose were competitive and noncompetitive inhibitors, with Ki values of 38 mM and 72 mM, respectively of rice alkaline invertase (Lin *et al*., 1999). Fructose acts as a competitive inhibitor for the invertases, which implies that there is tighter binding of fructose to the active site in these enzymes (although the binding is still weaker than that of glucose) (Wu & Birch, 2005). These results indicated that the enzyme activity could be modulated by its end products.

5 Conclusions

The current study established the presence of invertase activity in *S. cerevisiae* which was isolated from both Baker's yeast and grape samples using the standardize techniques. Invertase isolated from *S. cerevisiae* was found to have maximum activities at pH 6 and 10 and maximum temperature of 50°C. The activity of the enzyme was affected by the presence of metal ions in various degrees. A comparison of a number of kinetic parameters of Baker's yeast and grape fruit invertase showed marked differences. The inhibition of hydrolysis of sucrose by invertase was characterized for three different inhibitor compounds. Inhibition studies showed that fructose, glucose and $CuSO_4$ inhibited invertase competitively, non-competitively and un-competitively respectively. Hence, *S. cerevisiae* may be recommended as a local source for the production of invertase enzyme, thus reducing the cost of production significantly.

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