Iraqi Journal of Biotechnology, 2025, Vol. 24, Special Issue 2, 166-172

Production of Invertase from Baker's Yeast (*Saccharomyces cerevisiae*) using Agricultural Residues

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Received: February 20, 2025 / Accepted: July 16, 2025 / Published: November 16, 2025

Abstract. Invertase (*β-fructofuranosidase*) is a group of important enzymes that catalyze the breakdown of sucrose into fructose and glucose. It plays a major role in the food, cosmetics, and pharmaceutical industries. In this study, the invertase enzyme was produced using local baker's yeast (*Saccharomyces cerevisiae*). The study involves the production of invertase in submerged fermentations. The effect of carbon source (using glucose, lactose, maltose, fructose, and galactose), nitrogen source (yeast extract, peptone, urea, ammonium sulfate and meat extract), pH (3, 4, 5, 6, 7) and inoculum size (2, 4, 6, 8, 10%v/v) were optimized for invertase production. The second objective of the present study was the production of invertase enzyme from Baker's yeast using Orange, Banana, and Potato peel wastes as a carbon source for invertase production.

Keyword: Invertase, *Saccharomyces cerevisiae*, Baker's yeast, Submerged fermentation, Agricultural residues.

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Introduction

Enzymes are biomolecules produced by living organisms that accelerate chemical reactions, making them known as biocatalysts. Consequently, enzymes have numerous applications in various fields, including medicine, the environment, and industry. Etc. Among these, invertase *(D-fructofuranosidfructohydrolase*, EC 3.2.1.26) which are important in the industrial field, specifically in the food industry, because it belongs to the family of hydrolytic enzymes that break down sucrose into D-fructose and D-glucose, producing invert sugar, as depicted in Figure 1. This sugar is widely used as a sweetener and humectant in the food industry. (1)

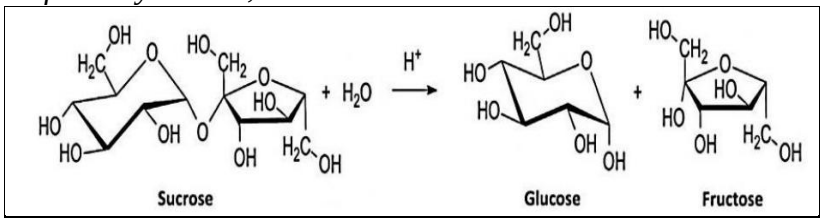

Figure (1): Sucrose hydrolysis by invertase (1).

Invertase is widely used for commercial purposes in several industries, including food (2), biofuels (3), beverages, pharmaceuticals, and biosensors (4). Invertase can be synthesized by yeasts (5,6,7), bacteria (8,9), and also by some filamentous fungi (10). One of the most common sources of invertase is Baker's yeast, a strain of *Saccharomyces cerevisiae*. This yeast is widely used in the food industry and is known for its ability to produce substantial amounts of invertase during fermentation. It is an excellent source of invertase due to its wide availability, and it is also considered a natural product of the enzyme during fermentation, and fact that its yields are excellently high, and also the efficiency of yeast in the submerged fermentation process, its complete compatibility with food processes, and the safety of its consumption play an effective role in its emergence in the production of invertase for various purposes. Industries.

In addition, the progress made in genetic engineering allows the allocation of new yeast strains to enhance invertase production, which enhances its position as a good source of this important and versatile enzyme. Currently, most invertase production processes are performed via submerged state fermentation (SmF), followed by solid-state fermentation (SSF) (11).

Materials and Methods

Media and Chemicals

Baker's yeast (*Saccharomyces cerevisiae*) was obtained from the local market in Baghdad /Iraq. Yeast extract broth media, DNS (Dinitrosalicylic acid), and other chemicals were supplied by Himedia / India.

Yeast Activation

The yeast activation is achieved by dissolving 1.5g of yeast in 100 ml of yeast extract broth media and incubating in a rotary shaking incubator at 30 °C for 24hrs at 150 rpm.

Production Media and Conditions

After yeast activation, 5 mL of the yeast culture was transferred to 250 mL of production media containing (Sucrose 30g, K_2HPO_4 1g, $MgSO_4$ 0.3g, $FeSO_4$ 0.3g, $ZnSO_4$ 0.3g, Yeast Extract 5g) /L, pH adjusted to 5. The medium was incubated in a rotary shaking incubator at 30^o for 72hrs (The agitation rate was kept at 150 rpm). After 72hrs, the production broth culture was filtered by using a cooling centrifuge at 10000 rpm and 4°C for 10 min to obtain supernatant. This supernatant was used as Invertase crude. The Invertase activity in the supernatant was determined spectrophotometrically. All media used in the study were sterilized by using an autoclave at 121°C for 20 min.

Invertase Assay

The enzyme activity measurement was performed by using the DNS method for the assay procedure (12). 0.2 mL of the enzyme solution was added to 0.8 mL of a 2% sucrose solution (sucrose used as the substrate) and incubated at 37°C for 30 min. Then, 1 mL of DNS reagent was added and placed in a boiling water bath for 5 minutes. After that, allow the tubes to cool to room temperature. The absorbance was measured for each reaction mixture using a spectrophotometer at 540 nm. Invertase activity was determined by using the following equation:

$$\text{Invertase activity (U/ml)} = \frac{A \times V \times 106}{€ \times t \times v}$$

Where: A: Absorbance at 540 nm, V: Total volume of reaction mixture in (2 ml), €: molar extinction coefficient, t: incubation time (30min), and v: volume of enzyme used in (0.2ml). invertase activity (one unit) was defined as the

crude enzyme amount required for releasing 1 µg of reducing sugar under assay conditions.

Submerged Fermentation Optimization Studies

Effect of Carbon Sources

To determine the effect of carbon sources on invertase production, different sugar types used as carbon sources, such as maltose, fructose, glucose, lactose, and galactose, were evaluated.

Effect of Nitrogen Sources

To determine the effect of nitrogen sources on invertase production was evaluated by using different nitrogen sources such as meat extract, urea, and ammonium sulfate.

Effect of pH

A range of pH (3-7) was tested for invertase production (1.0 M H_2SO_4/1.0M NaOH used to change pH).

Effect of Inoculum Size

To determine the effect of inoculum size on invertase production, different yeast concentrations were used (2,4,6,8, and 10%).

Effect of Agricultural Residues on Invertase Production

Locally available fruit substrates peels were collected such as potato, orange, and banana, washed, shade dried, ground, and stored in polyethylene bags at room temperature. The media and methods of invertase production are the same as those used in SMF, and agricultural waste is added at 30g/L as a replacement for the sucrose.

Results and Discussion

Effect of Carbon Sources

The results of the study effect of carbon sources (by using glucose, lactose, maltose, fructose, and galactose (30g/L)) on invertase production by *Saccharomyces cerevisiae* after 72 hrs. incubation period at 30°C and pH 5, with 2% inoculum, are given in Figure 2.

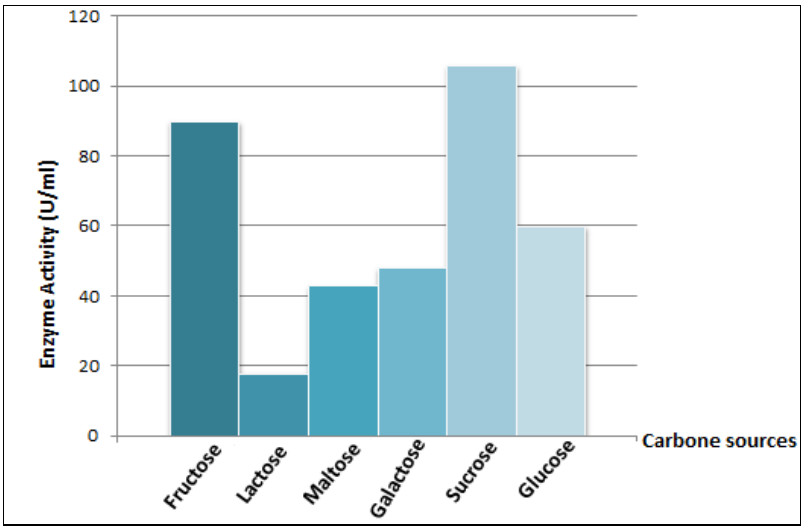

Figure (2): Different carbon sources effect on invertase production.

The highest enzyme activity was when using sucrose (with enzyme activity of 105 U/ml) as a carbon source, then fructose with enzyme activity of 90 U/ml, followed by glucose, galactose, and maltose with enzyme activity of 59 U/ml, 48 U/ml and 42 U/ml, receptivity, while less enzyme activity was recorded when using lactose with enzyme activity 18 U/ml.

Sucrose is a disaccharide composed of two monosaccharides, glucose and fructose, which are rapidly absorbed by yeast when used as a carbon source, so the yeast has high enzyme activity when using sucrose as substrate. Also, there is invertase enzyme activity when glucose and fructose when used as substrates, because they are monosaccharides, so they are absorbed rapidly by yeast. When lactose is used as a carbon source, there is no enzyme activity, as yeast lacks the enzymes that break down lactose (β-galactosidase) and therefore cannot consume it. However, in some cases, the invertase may be active when lactose is used. This occurs when the yeast needs an energy source, and only lactose is available; lactose can stimulate enzyme production through a phenomenon called catabolite repression.

These results were similar to the results of Shankar *et al.* (13), who optimized invertase production from *Saccharomyces cerevisiae MK* by manipulating various cultural conditions; they found that maximum invertase production was recorded in sucrose (0.36 U/ml) supplemented medium. The minimum invertase production was recorded in lactose (0.01 U/ml).

Different carbon sources (sucrose, maltose, galactose, and glucose) effect were studied by Qureshi *et al.* (14) on enzyme production and yeast growth from *S. cerevisiae* and found that the enzyme activites were (109 U/ml) for glucose and (124 U/ml) for sucrose.

Effect of Nitrogen Sources

Effect of nitrogen sources (yeast extract, peptone, urea, meat extract, and ammonium sulphate with concentraction of (5g/L)) on enzyme production by *S. cerevisiae* were tested in period of 72 hrs. of incubation at 30°C and pH 5, with 2% inoculum, the result is shown in (Figure 3).

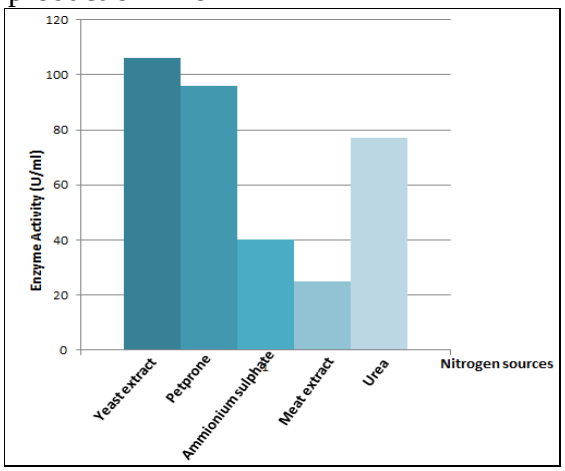

Figure (3) : The effect of nitrogen sources (5 g/L) on invertase production.

The highest invertase activity was when using yeast extract (105 U/ml), then peptone (95 U/ml), urea (78 U/ml), ammonium sulfate (40 U/ml), and finally meat extract (23 U/ml). Both yeast extract and peptone, a mixture of proteins, peptides, amino acids, vitamins, and minerals, are important source for providing yeast with the nitrogen that is necessary for enzyme production. They have a role in enhancing enzyme production and activity, biomass production, and increasing yeast performance during the fermentation process to produce the enzyme Urea and ammonium sulfate are considered simple inorganic sources of nitrogen, but they are good sources of

nitrogen to increase enzyme productivity because they are direct sources and quickly used by the yeast, helps it in the synthesis of proteins, including invertase.

The effect of nitrogen sources such as yeast extract, meat extract, urea ,peptone and ammonium sulphate was studied by Qureshi *et al.* (14),. They found that the highest invertase yield obtained at 155 U/ml of yeast extract. In contrast, other researchers found that using urea as a nitrogen source gave a maximal invertase yield, Ali *et al.* (15). Others reported that the yeast extract and peptone in invertase production were significant by *Saccharomyces cerevisiae 3090* Suresh *et al.* (16). The urea and yeast extract were best for invertase production for *Saccharomyces cerevisiae* Pandey and Soccol (17), Alegre *et al.* (18).

Effect of pH

Figure 4 illustrate the Impact of initial pH (3,4,5,6, and 7) on the enzyme production. The highest invertase production was noted at pH 5.5. The production process from yeast sharply decreased at pH 7. Although, no significant difference in the enzyme production was found at the pH values of 5 and 6.

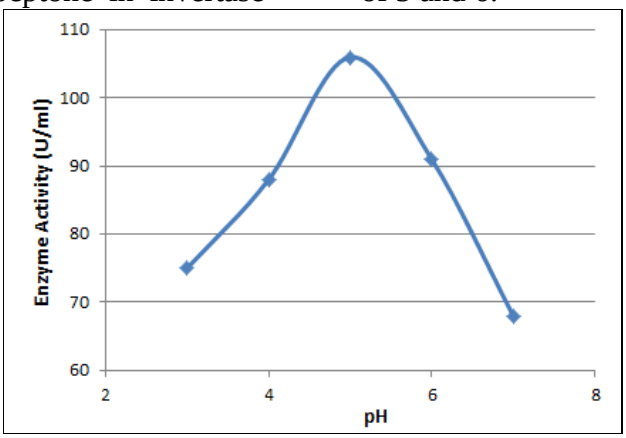

Figure (4): The effect of pH on invertase production.

Qureshi *et al.* (14) found the highest invertase was noted at 5.5. Enzyme secretion sharply decreased at pH 6.5. Our results are also similar to the results of Uma *et al.* (19) for invertase production.

Effect of Inoculum Size

Different inoculum sizes, such as (2,4,6,8 and 10) %, were investigated for the ability to stimulzte invertase production in the production medium. The results showed in Figure 6, the maximum activity was observed at the 6% (115 U/ml) of inoculum level.

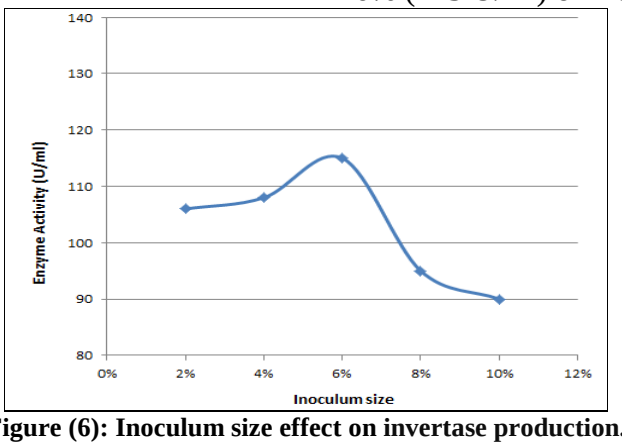

Figure (6): Inoculum size effect on invertase production.

When the inoculum size increases, invertase activity increases significantly, reaching the optimum level, where the maximum enzyme activity occurs. As the inoculum size continues to increase, enzyme activity declines. This is due to the rapid depletion of nutrients and the accumulation of wastes and byproducts during the growth phase (20).

Effect of Agricultural Residues as Carbon Source

Orange, banana, and potato peels were used as a substrate for invertase production. Data recorded in Figure 7 revealed that maximum invertase activity was obtained in the fermentation media inoculated with baker's yeast containing orange peel waste as substrate, which produced 126 U/ml, followed by banana peels as substrate, which produced 100 U/ml, and then by potato peel, which produced 96 U/ml.

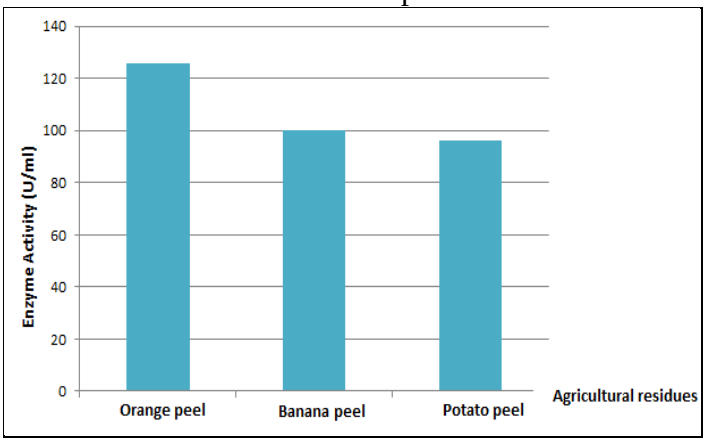

Figure (7): The effect of orange, banana & potato peels on invertase production.

Orange and banana peels, as well as potatoes, are complex carbohydrates, consisting of cellulose, pectin, and starches. The main substrate on which the invertase enzyme works is sucrose, so there is a need to convert the complex sugars that are present in the peels into a simpler form. However, it has proven efficient when used as a substrate instead of sucrose, which is used in pure form for the invertase production. This may be because yeast, in the presence of complex sugars (peels), stimulates the production of hydrolysis enzymes and thus increases the production of invertase.

Conclusion

Different parameters (Carbon source, nitrogen source, inoculum size, and pH) were optimized for invertase production from Baker's yeast. It found that the best carbon sources are sucrose and yeast extract when used as a nitrogen source. The optimum pH for invertase production was 5.5, and at a 6% inoculum size. The agricultural waste was successfully utilized as a carbon source for invertase production from baker's yeast, using orange, banana, and potato peels.

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