# A COMPARISON STUDY FOR CYTOSINE DEAMINASE PRODUCTION FROM *SACCHAROMYCES CEREVISIAE* OF DIFFERENT READY-MADE BREAD YEAST TYPES PURCHASED FROM LOCAL MARKET

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## ABSTRACT

**Background**: In the current study, the enzyme cytosine deaminase was extracted from ready-made bread *Saccharomyces cerevisiae* yeast, which represented different types from the local market. **Methodology**: About 100 grams were subjected to 50 mL of toluene with the aid of gentle heating at 45°C. Then, 100 mL of cold water was added, and incubated at 4°C for 18 hours. The water layer was then centrifuged and subjected to various purification steps for the enzyme, including salt precipitation, ion exchange, and gel filtration. **Results**: The local Iraqi type showed the best specific activity of 12.267 U/mg, while the specific activity of the enzyme after DEAE-cellulose purification was 216.66 U/mg. The specific activity reached 571,428 IU/mg after the Sephadex G-200 gel exclusion step. **Conclusion**: The choice of bread yeast for the extraction of the cytosine deaminase enzyme is due to its ready availability, fast growth and reproduction, dense growth, and lack of any negative impact on human health. Among the tested yeast strains, the local strain Iq.3 demonstrated superior efficiency in enzyme production when compared to others. Given the characteristics of the local yeast, it is crucial to investigate and utilize its potential for medical applications.

**Keywords:** Cytosine deaminase, DEAE-cellulose, Gel filtration, Yeast *Saccharomyces cerevisiae*.

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## INTRODUCTION

The Yeast *Saccharomyces cerevisiae* is considered an important fungus for economic and medical applications. Its importance has progressed nowadays as it is widely entered into the field of industrial fermentations and vitamins, fats, proteins, biological control agents, and other food materials production (1, 2, 3) Besides; Yeast has been used in combating soil fungi, and combating some fungal diseases, it is regarded to get a promise rule that received great attention in combating and treating some diseases especially in cancer (4,5). The enzyme cytosine deaminase has been detected in many living organisms, including bacteria, yeast, fungi, and prokaryotic cells, whereas higher eukaryotic organisms, such as plants and mammals, lack the presence of this enzyme (6). This enzyme, extracted from baker's yeast *S. cerevisiae*, removes the amine group from the base 5-fluorocytosine (5-FC) and converts it to 5-fluorouracil. The latter is one of the most famous chemotherapeutic agents that possesses a role in cancer treatment (7,8). The compound 5-fluorouracil is one of the fluorinated pyrimidines, which are analogs of the natural nitrogenous base uracil. It has been used for more than 30 years as an anti-cancer agent to treat cancerous tumors such as skin, breast, and colon cancer (9,10). Therefore, many researchers have suggested giving (5-FC) orally and implanting the extracted enzyme capsule at the treatment site, near or in the tissue affected by the cancerous tumor (11,12). Therefore, the current study focuses on the importance of this enzyme and aims to extract and purify the enzyme cytosine deaminase from different sources of commercial bread yeast (*S. cerevisiae*) purchased from Iraqi markets, and to compare it with the locally handmade bread yeast culture regarding the enzyme yields and its specific activity.

## METHODOLOGY

**Extraction and production of the enzyme cytosine deaminase from bread yeast *S. cerevisiae*:**

Depending on the method described by the researchers Ipata and Cerignani (1978), with some modifications in the proportions of extracted solvents (13).

**Sample collection**

From the Iraqi market, four beaker yeast samples as dry yeast granules of *S. cerevisiae* were purchased, representing different origins of well-known brands supplied by foreign companies found at Baghdad local markets in July 2022. In addition, an isolate of *S. cerevisiae* was obtained from dough and employed in the current study. The symbols in Table (1) represent the four foreign companies' yeast sources and the local one, with three replicates for each.

Table (1): The four kinds of bread yeast *S. cerevisiae* with their origin and symbols

| Sample Kind | Trade mark | Origin | Symbol |
|---|---|---|---|
| **Turkish yeast1** | Saf-instant | Türkiye | T1 |
| **Turkish yeast2** | Saf-instant | Türkiye | T2 |
| **Turkish yeast3** | Saf-instant | Türkiye | T3 |
| **Iranian yeast1** | Klar Maya | Iran | Ir 1 |
| **Iranian yeast2** | Klar Maya | Iran | Ir 2 |
| **Iranian yeast3** | Klar Maya | Iran | Ir 3 |
| **Russian yeast1** | GLORIPAN | Russia | R 1 |
| **Russian yeast2** | GLORIPAN | Russia | R 2 |
| **Russian yeast3** | GLORIPAN | Russia | R 3 |
| **Iraqi yeast1** | Local production | Iraq (handmade) | Iq 1 |
| **Iraqi yeast2** | Local production | Iraq (handmade) | Iq 2 |
| **Iraqi yeast 3** | Local production | Iraq (handmade) | Iq 3 |

**Extraction Cytosine Deaminase Enzyme:**

About 100 grams of ready-made bread yeast from each type mentioned in Table (1) were suspended with 50 mL of toluene to extract the enzyme cytosine deaminase from the yeast. Organic toluene will cause cell wall rupturing with the aid of a water bath at 45°C for one hour, with three replicates. The mixture was left at room temperature for 2-3 hours, and then transferred to a separator funnel. 100 mL of cold water was added to the mixture, with gentle shaking for half an hour. The mixture was incubated at 4°C for 18 hours. The separated aqueous layer had been subjected to a cool centrifuge at 10000 rpm for 20 minutes at 4°C. The supernatant represented the crude extract for the enzyme, which had been subjected to different steps. Provide a concise and precise description of the experimental results, their interpretation, as well as the experimental conclusions that can be drawn from enzyme purification employing the measurement of specific enzyme activity and protein content in each step.

**Measuring the Activity of the Enzyme Cytosine Deaminase:**

According to the Ipata method (13), the enzyme activity was measured by taking 0.2 mL from the crude extract mixed with 0.3 mL of 0.1M Tris-base buffer, pH 7and an adequate amount of 0.5 mL of the enzyme-substrate 0.001M cytosine in a glass test tube to get the total volume of 1 mL. After 30 min of incubation at 37°C, the mixture was diluted with 4 mL of 0.1 N HCI to stop the reaction and measure the conversion of the substrate cytosine to the product uracil with a spectrophotometer at 286nm. The blank was prepared in the same manner by adding 0.5 mL $H_2O$ instead of the substrate. Enzyme activity, enzyme-specific activity, and the enzymatic yield were calculated by applying the following equations (14)

*Enzymatic activity (U/mL) =Total volume of reaction medium × absorbance / Volume of enzyme added to the active agent x time x enzymatic factor

* Specific activity = enzymatic activity/protein concentration

* Enzymatic yield = total activity of the step / total activity of the purification step of the extract x 100.

*International unit (U) = a unit of an enzyme's catalytic activity. U (µmol/min) is defined as the amount of the enzyme that catalyzes the conversion of one micromole of substrate per minute under the specified conditions of the assay method.

**Estimation of Total Protein Concentration:**

The Biuret method (15) was used to estimate the amount of protein in the crude extract of bread yeast *S. cerevisiae* for all types as well as in all stages of extraction, purification, and characterization. This method depends on the reaction of the basic copper solution (basic copper sulfate) present within the contents of the reagent solution with Nitrogen atoms present in the peptide bonds of the protein, forming a complex with a purple color. A standard curve for the protein of known concentrations of bovine serum was employed, and a straight-line equation was applied for the calculation of protein concentration in each step. The assay involved a reaction of 1 mL of the extracted enzyme with 0.5 mL of the Biuret reagent; the change in the color was read at 540 nm.

**Steps for Purification of the Enzyme Cytosine Deaminase:**

**A- Precipitation of the enzyme with ammonium sulfate salts**

The volume of the crude extract solution for all types, with their replicates, had been measured. According to that, specific amounts of ammonium sulfate $(NH_4)_2SO_4$ were added to each crude extract of the enzyme to get saturation percentages that ranged between (60-70) % (13). The precipitated proteins were separated using a Cooling Centrifuge at a speed of 10,000 rpm at 4°C for 20 minutes. This process was repeated by taking the filtrate and adding ammonium sulfate crystals to it until the saturation rate reached 70% under the same conditions. The filtrate was discarded, and the precipitate was dissolved in the smallest volume of 0.1 M Tris-base buffer at pH 7. The enzymatic activity, protein concentration, and specific activity were measured for all solutions.

**B- Purification of the enzyme with membrane sorting (Dialysis)**:

The dialysis process was carried out on the enzyme solution obtained from the ammonium sulfate precipitation step to remove the salt residue. The enzyme's solution was placed in a dialysis bag supplied by the USA/Spectrum Company, which allows the passage of substances less than 120 kDa. The bag was placed in a container containing 0.1M Tris-base buffer solution, pH 7, for 24 hours. The dialysis bag was placed in a container containing fine sucrose particles for the enzyme solution's concentration. Enzymatic activity, enzyme-specific activity, and protein concentration were estimated in the selected yeast samples. The semi-purified enzyme was stored at -20°C until other purification steps were performed (16).

A comparison of specific activity and protein concentration among the four types in this step was made, so that the most efficient and effective enzyme activity for the yeast type is selected to complete the subsequent purification steps to obtain a pure enzyme that possesses biological applications.

**C-Purification of the enzyme with Ion-exchange chromatography**

The diethylaminoethyl cellulose exchanger DEAE-Cellulose was prepared according to the method described by (17). The DEAE-Cellulose ion exchanger column had dimensions of (3 x 12) cm, with the total volume of the column being 85 mL. The flow speed was adjusted to 30 mL/hour, with pH of 7.2 using buffer solution of 0.05 M. After performing the dialysis process, 10 mL of the concentrated protein solution resulting from the ammonium sulfate precipitation step was added to the walls of the DEAE-Cellulose ion exchanger column slowly using a dropper. The column was washed with a Tris-base buffer solution at a concentration of 0.05 M, pH 7.2, to remove unbound proteins at a flow speed of 30 mL/hour. Each 5 mL eluate was collected from the column into separate tubes, and an absorbance reading was taken for each portion at 280 nm using a spectrophotometer. The proteins bound to the column were recovered with a 0.1-0.5 salt gradient using a 0.05 M base buffer solution with a pH of 7.2 containing 0.5 M potassium chloride with a flow rate of 30 mL/hour and collecting the eluted portions in parts of 5 mL/part, followed by measuring the absorbance in each recovered part at a wavelength of 280 nm.

**D- Purification of the enzyme with Gel filtration chromatography**

The gel filtration chromatography technique was employed for further enzyme purification. Using a Sephadex G-200 column prepared according to the manufacturer's instructions (Pharmacia Fine Chemicals), the gel was packed into a glass column with dimensions (1.5 x 80) cm, and the material was left to settle. The column was calibrated using a 0.25 M Tris-base buffer solution at pH 7.2 and a flow rate of 30 mL/hour to collect an elution of 5 mL in separate tubes. An Aliquot of 5 mL from the concentrated semi-purified enzyme in the ion exchange step was placed gradually on the internal walls of the gel filtration column. The collected 5 mL eluate was followed by an absorption reading at 280 nm. Enzymatic activity, enzyme-specific activity, and protein concentration were estimated for the

portions that gave positive results in the disc method for enzyme detection (18).

**Detection of the enzyme by the disc method:**

The semi-purified enzyme was detected using the disc method after collecting the portions with the best peak readings, in all purification steps, to improve their enzymatic activity. The cytosine substrate was prepared with a concentration of 0.05 M, and 0.01% phenol red dye was added to agar at 1.5% to prepare 100 mL using a phosphate buffer solution of 0.1 M at pH 5.9. The mixture was sterilized through a 0.22 µm Millipore filter and poured into a sterile petri dish to form a 2 mL layer. A sterile disc of Whatman No.1 filter paper, 6mm in diameter, saturated with enzyme mixture, was placed on the surface of the agar and incubated in the Petri dishes at 37 °C for 8-12 hours. A control dish was used by submerging the paper disc in a phosphate buffer rather than the enzyme mixture. The positive result will indicate the appearance of a red area around the disc. Enzymatic activity, enzyme-specific activity, and protein concentration for the positive-elution fractions were estimated. These parts were stored at -20°C for subsequent purification steps (19).

## RESULTS

**Extraction and Purification of Cytosine Deaminase Enzyme from Four Types of Bread Yeast *S. cerevisiae***

**A-Crud extraction of the Enzyme from the yeast**

The cytosine deaminase enzyme was extracted from *S. cerevisiae* bread yeast using toluene for cell wall rupturing according to the method described by Ipata (13). The specific activity of the crude extract was estimated for replicates of all yeast types included in the current study, as shown in Figure (1).

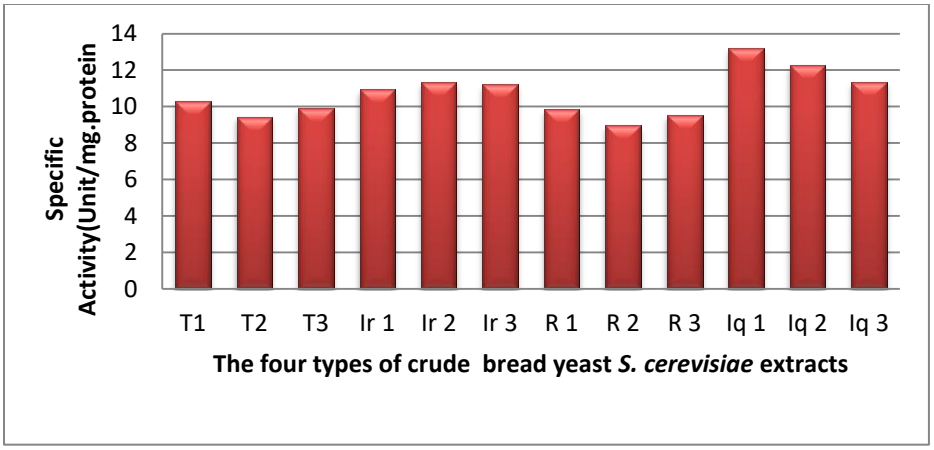


**Figure (1): The Specific Activity of the cytosine deaminase enzyme for the four types of crude bread yeast *Saccharomyces cerevisiae* extracts with their replicates.**

The average specific activity of the cytosine deaminase enzyme extracted from different types of bread yeast in triplicate is as follows: Turkish type (9.891) U/mg protein, the Iranian type (11.15) U/mg of protein, the Russian type (9.44) U/mg of protein, while for the local type, the specific activity was (12.2683) U/mg as the best result. The cytosine deaminase extraction procedure was similar to a study by (20) for manufactured bread yeast, as the specific activity of the crude extract reached 9.6 U/mg protein.

**B- Precipitation of the enzyme with ammonium sulfate salts:**

The enzyme precipitation procedure for the four types of bread yeast extracts (Turkish, Iranian, Russian, and the local) by ammonium sulfate in saturation percentages of 60-70% showed enzymatic activity and specific activity after performing the dialysis process, as appeared in Tables (2, 3, 4, and 5), respectively.

**TABLE (2).THE CRUD AND SALT PRECIPITATION CYTOSINE DEAMINASE ENZYME SPECIFICATION FOR TURKISH BREAD YEAST**

| The Step | Yeast Type | Volume (ml) | Enzyme Activity (U/ml) | Protein Concentration (mg/ml) | Specific activity (U/mg) | Total Activity (U) | No. of Purification | % Enzyme Yield |
|---|---|---|---|---|---|---|---|---|
| Crud Extract | T1 | 88 | 7.75 | 0.78 | 9.93 | 682 | 1 | 100 |
| | T2 | 85 | 7.266 | 0.77 | 9.43 | 617.6 | 1 | 100 |
| | T3 | 90 | 8.716 | 0.84 | 10.37 | 784.4 | 1 | 100 |
| Precipitation with Ammonium Sulphate (70% saturation) | T1 | 15 | 5.016 | 0.39 | 12.86 | 75.24 | 1.29 | 11.03 |
| | T2 | 14 | 4.75 | 0.38 | 12.5 | 66.5 | 1.32 | 10.76 |
| | T3 | 16 | 5.983 | 0.45 | 13.29 | 95.77 | 1.28 | 12.20 |
| LSD value | | 11.02 * | 1.217 * | 0.381 * | 2.074 * | 52.95 * | 0.328 NS | 8.63 * |
| | | | | (*) P≤0.05 | | | | |

**TABLE (3).THE CRUD AND SALT PRECIPITATION CYTOSINE DEAMINASE ENZYME SPECIFICATION FOR IRANIAN-TYPE BREAD YEAST**

| The Step | Yeast Type | Volume(ml) | Enzyme Activity (unit/ml) | Protein Concentration (mg/ml) | Specific activity (unit/mg) | Total Activity (unit) | No. of Purification | % Enzyme Yield |
|---|---|---|---|---|---|---|---|---|
| Crud Extract | Ir1 | 88 | 9.867 | 0.88 | 11.2 | 888.03 | 1 | 100 |
| | Ir2 | 85 | 10.08 | 0.82 | 12.29 | 887.04 | 1 | 100 |
| | Ir3 | 90 | 9.516 | 0.87 | 10.93 | 789.82 | 1 | 100 |
| Precipitation with Ammonium Sulphate (70% saturation) | Ir1 | 15 | 6.083 | 0.45 | 13.5 | 109.49 | 1.20 | 12.32 |
| | Ir2 | 14 | 7.066 | 0.52 | 13.58 | 105.99 | 1.1 | 11.94 |
| | Ir3 | 16 | 5.983 | 0.48 | 12.46 | 71.796 | 1.13 | 9.09 |
| LSD value | | 11.02 * | 2.156 * | 0.391 * | 2.063 * | 72.37 * | 0.302 NS | 9.162 * |
| | | | | (*) P≤0.05 | | | | |

**TABLE (4).THE CRUD AND SALT PRECIPITATION CYTOSINE DEAMINASE ENZYME SPECIFICATION FOR THE RUSSIAN TYPE BREAD YEAST**

| The Step | Yeast Type | Volume(ml) | Enzyme Activity (unit/ml) | Protein Concentration (mg/ml) | Specific activity (unit/mg) | Total Activity (unit) | No. of Purification | % Enzyme Yield |
|---|---|---|---|---|---|---|---|---|
| Crud Extract | R1 | 82 | 6.183 | 0.65 | 9.5 | 507 | 1 | 100 |
| | R2 | 89 | 5.116 | 0.57 | 8.97 | 455.3 | 1 | 100 |
| | R3 | 80 | 6.2 | 0.63 | 9.84 | 496 | 1 | 100 |
| Precipitation with Ammonium Sulphate (70% saturation) | R1 | 13 | 4.466 | 0.42 | 10.63 | 58.05 | 1.11 | 11.45 |
| | R2 | 15 | 3.266 | 0.32 | 10.2 | 48.99 | 1.13 | 10.75 |
| | R3 | 12 | 4.916 | 0.43 | 11.43 | 58.99 | 1.16 | 11.89 |
| LSD value | | 10.46 * | 2.075 * | 0.309 * | 2.192 * | 61.28 * | 0.297 NS | 10.02 * |
| | | | | (*) P≤0.05 | | | | |

**TABLE (5).THE CRUD AND SALT PRECIPITATION CYTOSINE DEAMINASE ENZYME SPECIFICATION FOR THE LOCAL IRAQI-TYPE BREAD YEAST**

| The Step | Yeast Type | Volume(ml) | Enzyme Activity (U/ml) | Protein Concentration (mg/ml) | Specific activity (U/mg) | Total Activity (U) | No. of Purification | % Enzyme Yield |
|---|---|---|---|---|---|---|---|---|
| Crud Extract | Iq1 | 90 | 9.53 | 0.84 | 11.34 | 857.9 | 1 | 100 |
| | Iq2 | 95 | 10.18 | 0.83 | 12.26 | 967.1 | 1 | 100 |
| | Iq3 | 93 | 10.3 | 0.78 | 13.205 | 957.9 | 1 | 100 |
| Precipitation with Ammonium Sulphate (70% saturation) | Iq1 | 15 | 6.65 | 0.52 | 12.78 | 99.75 | 1.126 | 11.62 |
| | Iq2 | 16 | 8.283 | 0.59 | 14.03 | 132.5 | 1.144 | 13.70 |
| | Iq3 | 14 | 7.78 | 0.54 | 14.40 | 108.9 | 1.09 | 1136 |
| LSD value | | 10.61 * | 2.188 * | 0.309 * | 2.762 * | 62.36 * | 0.241 NS | 8.934 * |
| | | | | (*) P≤0.05 | | | | |

As shown in the previous tables for the foreign types, results for specific activity were arranged as follows: Iranian specific activity (13.18U/mg), Table (2), the Turkish yeast specific activity (12.883 U/mg), Table (3), and finally, Russian specific activity (10.7533 U/mg), Table (4). The study results for the precipitation process showed the highest specific activity, reaching (13.8566 U/mg). Several purification times 1, 09 times, and an enzymatic yield of 11,47 %, as in Table (5), for a saturation rate of 60%, thus purification steps for the cytosine deaminase enzyme extracted from the local yeast type had been chosen in the remaining steps.

**Choosing the most efficient type of yeast in producing the enzyme cytosine deaminase:**

The choice of bread yeast in the production of the cytosine deaminase enzyme is due to its ease of obtaining, rapid growth and reproduction, dense growth, and no pathological effect on human life. To determine the most efficient type, the enzyme was extracted from the four models (Turkish, Iranian, Russian, and local). There were three repetitions of the extraction and purification process by precipitation. During the extraction process, local yeast **Iq.3** was identified as the best and most efficient for producing the enzyme, with a specific activity of 13.2 U/mg of protein, as shown in Figure (1). Based on these results, local yeast Iq. 3 were selected for enzyme production, and the subsequent purification processes were carried out. The enzyme was characterized, and the optimal conditions for it were studied.

**C-Ion exchange chromatography:**

The ion exchange results showed that only one protein peak appeared in the washing step, devoid of enzymatic activity in its constituent parts. In contrast, two protein peaks appeared in the recovery process using potassium chloride, as shown in Figure (2), and when enzymatic activity was detected in these peaks using the detection disc method: Parts (36 - 41) in the recovery stage showed enzymatic activity, and their absence in the other protein peak parts.

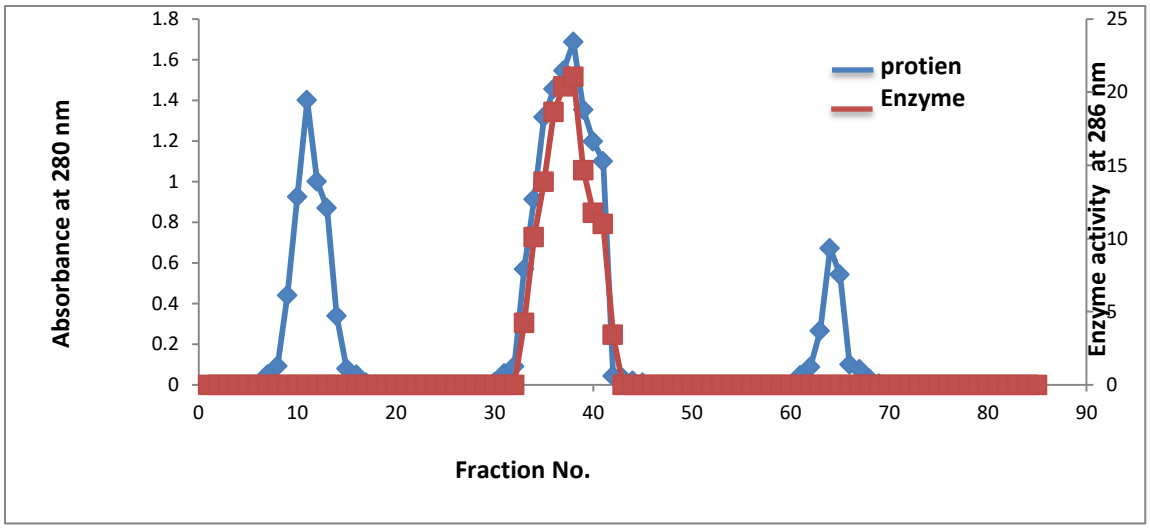


Figure (2): Ion exchange chromatography using the DEAE-Cellulose ion exchanger with dimensions of (2 x 21) cm to purify the enzyme cytosine deaminase produced from bread yeast *S. cerevisiae*, the local type with a Tris-base buffer solution, concentration of 0.05 mol.

In this step, it is possible to increase the enzyme's specific activity by concentrating the protein peak component parts that give enzymatic activity. The specific activity was raised to 216.66 U/mg, with a number of purifications of 16,407 times and an enzyme yield of 1.475% as shown in Table (6).

**D-Gel filtration using Sephadex G-200 gel:**

The enzymatic solution obtained from the ion-exchange process was concentrated and added to a gel filtration column (Sephadex G-200). It was observed that a single protein peak appeared when measuring fractions at 280 nm Figure (3) and upon detecting enzymatic activity in the constituent fractions. For these peaks, using the disc method,

the parts that make up the protein peak (6-19) showed enzymatic activity, whereas it was absent in the parts that make up the other protein peaks. Then, the collected parts of the protein peak that gave activity were concentrated, and the specific activity was measured. It was 571,428 U/mg, with a number of purifications of 43,273 times, and an enzymatic yield of 0.647%, as shown in Table (6).

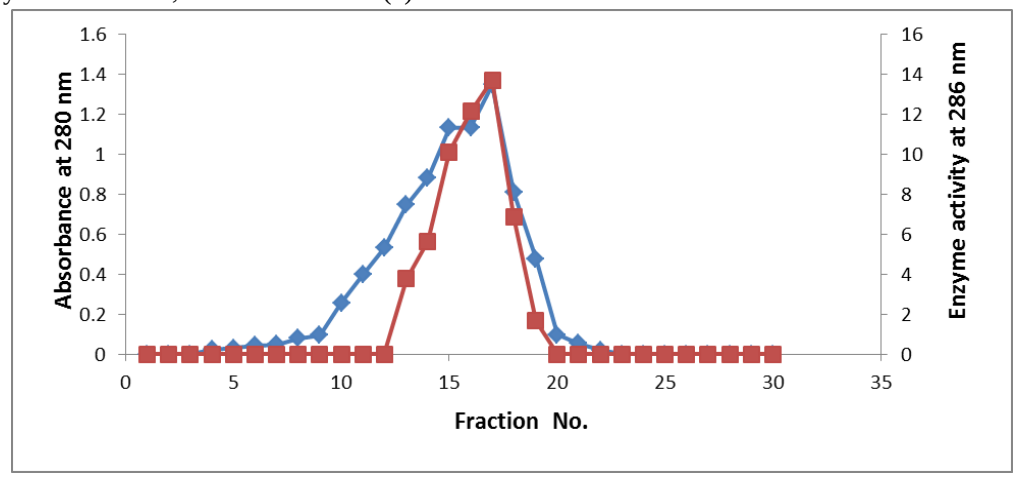


**Figure (3): Gel filtration to purify the enzyme cytosine deaminase produced from yeast using a Sephadex G-base column (1.5 x 70 cm) at a flow speed of 30 ml/hour with 0.25 M Tris-base buffer, pH 7.2, the ratio of collection 5 ml/part.**

**Table (6): Stages of purification of the cytosine deaminase enzyme extracted from local type (Iq3) *S.cerevisiae* bread yeast**

| The Step | Yeast Type | Volume (ml) | Enzyme Activity (unit/ml) | Protein Concentration (mg/ml) | Specific activity (unit/mg) | Total Activity (unit) | No. of Purification | % Enzyme Yield |
|---|---|---|---|---|---|---|---|---|
| **Crud Extract** | Iq3 | 150 | 10.3 | 0.78 | 13.205 | 1545 | 1 | 100 |
| **Precipitation with Ammonium Sulphate (70% saturation)** | Iq3 | 15 | 7.78 | 0.44 | 17.681 | 116.7 | 1.338 | 7.553 |
| **Ion exchange chromatography purification with DEAE-Cellulose ion exchanger** | Iq3 | 10 | 4.55 | 0.021 | 216.66 | 45.5 | 16.407 | 2.944 |
| **Gel filtration using Sephadex G-200 gel Purification** | Iq3 | 5 | 4 | 0.007 | 571.428 | 20 | 43.273 | 1.294 |

**Detection of the enzyme activity by the disc method:**

The purified enzyme from the ion exchange and gel filtration purification steps was detected using the disc method after collecting the portions with the best peak readings separately, to improve the presence of this specific enzyme and its enzymatic activity. From Figure 4, it was observed that a red halo formed around the tablet due to the enzyme's activity in removing the amino group from cytosine, converting it to uracil and forming ammonia, thereby altering the medium's pH and color from yellow to red.

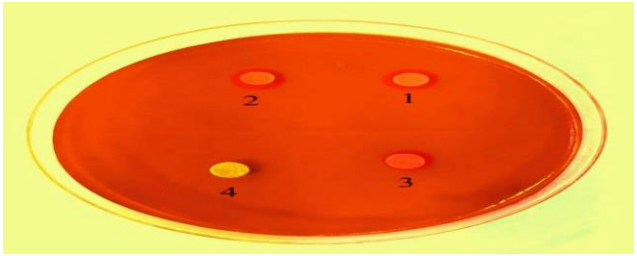


**Figure (4): Enzyme detection test by the disc method (1. Ion exchange., 2. Gel filtration, 3. Crude extract, 4. Control disc)**

## DISCUSSION

In some studies, the cytosine deaminase enzyme was extracted from bread yeast using ethyl acetate rather than toluene, and the specific activity reached (0.037) U/mg protein (21,22). Other studies have reported the use of several methods for extracting enzymes from various microorganisms. The enzyme was extracted from the bacteria *Escherichia coli* after the disruption of the bacterial cell wall with an ultrasonic oscillation device, and the specific activity reached (5.28) U/mg protein (23). The Cytosine deaminase enzyme was extracted from the parasite *Trichomonas vaginalis*, with a specific activity of 3.18 U/mg protein (24), whereas in another study, the enzyme was extracted from Turkish-type bread yeast by ammonium sulfate precipitation, the specific activity reach the best value 12.5 U/mg at a saturation rate of 60% ammonium sulphate with several purification times of 1.302 times, and an enzymatic yield of 4.82% (20). While in *Ipata's* study 1978 (13) saturation rates (60-70) % of ammonium sulfate were used to concentrate the enzyme from bread yeast, the specific activity reached 14.3 U/mg of protein and an enzymatic yield of 53%, with a number of purification times of 1.5 times. Another study used (30-55%) of ammonium sulphate to concentrate the enzyme produced by *E.coli* bacteria, with a specific activity reached 10,817 U/mg of protein, 2,048 times of purification, with an enzymatic yield of 5,589% (21). An additive purification step was used by Abbas, 2000 (2) ,who purified the enzyme from manufactured bread yeast . The ion exchanger was used after concentrating the portions of the protein's peak that showed enzymatic activity; their specific activity was measured, reaching 358 unit/mg protein, with 37.29 times purified, with an enzymatic yield of 2.55%. Another study used an ion exchanger as a third step to purify the enzyme from bread yeast after gel filtration chromatography; the specific activity reached 1973 IU/mg, with a number of purification times of 214.4 times and an enzyme yield of 14% (13). In a previous study, the enzyme cytosine deaminase ,which purified from *E.coli* bacteria using an ion exchanger as a second stage for purifying the enzyme, the specific activity reached 189.52 units/mg with a number of purifications of 35.89 times, and enzyme yield of 5.025% (23). The enzyme acting to bind to the ion exchanger could indicate that the cytosine enzyme carries a negative net charge at pH 7.2, as an opposite charge to the ion exchanger, which is the result of the enzyme's binding to the positive groups possessed by the ion exchanger.

A study by (20) used a separation column (Sephadex G-200) after the ion exchange step in purifying the cytosine deaminase enzyme, indicating that the specific activity of this enzyme extracted from manufactured bread yeast reached 400 units/mg protein with 41.6 purification times, with an enzymatic yield of 1.19%. In another study, the separation column was used as a second step , in purifying the enzyme from bread yeast , following the precipitation process with ammonium sulfate .The specific activity was 494 U/mg protein, the number of purification times was 53.69 times, and the enzymatic yield was 19% (13). For the researcher (Katsuragi et al, 1989 (1), who used Sepharose CL-4B gel filtration as a third step in purifying the enzyme cytosine deaminase from bread yeast, the specific activity reached 42 U/mg protein, with 1,100 puification times, with an enzymatic yield of 5.7%. whereas Al-Baer *et al*.,2017 used a Sepharose 6B gel filtration column as a third step in purifying the enzyme from *E. coli*, where the specific activity reached 302.272 U/mg of protein and the number of purification times was 57.248 times, with an enzymatic yield of 2.099% .(23)

The Yeast *S. cerevisiae* is considered an important fungus for economic and medical applications, and due to its importance, recent studies have focused on its molecular and genetic levels (25). The benefits of this yeast were not limited to being used in the manufacture of bread and pastries as a main food ingredient in many countries, especially developing ones (26), also this yeast was also included in systems and strategies for improving and developing food sources, both commercially and industrially, in addition to important modern studies that focus on the production of important food and therapeutic materials (27,28,29,30,31). Including the enzyme under study. All of this has led scientists and researchers to widely understand the structure of this yeast and to identify its genetic components (12), enabling them to use it in genetic modification and cloning studies to produce many food, agricultural, and medical materials.

## CONCLUSION

The choice of bread yeast for the extraction of the cytosine deaminase enzyme is due to its ready availability, fast growth and reproduction, dense growth, and lack of any negative impact on human health. Among the tested yeast strains, the local yeast strain Iq.3 demonstrated superior enzyme production efficiency when compared to others. Given the characteristics of the local yeast, it is crucial to investigate and utilize its potential for medical applications.

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# دراسة مقارنة إنتاج إنزيم السايتوسين دي أمنيز من مختلف أنواع خميرة الخبز الجاهزة في السوق المحلي

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**الخلاصة**

**خلفية عن الموضوع**: في الدراسة الحالية تم استخلاص انزيم السايتوسين دي امنيز من عدة انواع من خميرة الخبز المتوفرة في السوق المحلية. **المواد وطرق العمل**: تم تعريض حوالي 100 غرام من كل من الانواع قيد الدراسة الى50 مل تولوين كمذيب عضوي لتحطيم جدارخلية الخميرة، مع تسخين في حمام مائي عند درجة حرارة 45 درجة مئوية وأضيف 100 مل من الماء البارد إلى الخليط البارد وحضنت عند درجة حرارة 4 درجة مئوية لمدة 18 ساعة، لاستخراج الإنزيم ثم تم طرد طبقة الماء مركزياً مع إخضاعها لخطوات تنقية مختلفة للإنزيم بما في ذلك الترسيب بالملح والتبادل الأيوني وتنقية الترشيح الهلامي . **النتائج**: وكانت النتيجة أن متوسط النشاط النوعي للمستخلص الخام النوع العراقي المحلي قدره (12,267) وحدة دولية/ ملغم كأفضل نتيجة تم اختيارها لخطوات التنقية الإضافية المتضمنة تطبيق عمود DEAE- السليلوز الذي أعطى نشاط نوعي قدره 216,66 وحدة دولية/ ملغم. وصل النشاط النوعي إلى 571,428 وحدة دولية/ملغم بعد خطوة الاستخلاص الجل Sephadex G-200. **الاستنتاجات**: يعود اختيار خميرة الخبز لاستخلاص إنزيم السيتوزين ديامينياز إلى سهولة توفرها وسرعة نموها وتكاثرها وكثافة نموها وعدم وجود أي تأثير سلبي على صحة الإنسان. من بين سلالات الخميرة التي تم اختبارها، أظهرت سلالة الخميرة المحلية Iq.3 كفاءة فائقة في إنتاج الإنزيم بالمقارنة مع غيرها. ونظرا لخصائص الخميرة المحلية، فمن الأهمية بمكان التحقيق والاستفادة من إمكاناتها للتطبيقات الطبية.

**الكلمات المفتاحية:** إنزيم نازعة أمين السيتوزين، عمود السليلوز DEAE، الترشيح الهلامي، خميرة الخبز.