

Original Article

Extraction of Protease from *Cicer Arietinum*

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Abstract:

Proteases are essential enzymes with wide-ranging applications in food processing, pharmaceuticals, detergents, and biotechnology. This study focuses on the crude extraction of protease from chickpea (*Cicer arietinum*) seeds followed by a determination and estimation of enzyme activity under different conditions using the Biuret method. The objective was to compare the efficiency, stability, and inhibition susceptibility of enzyme extracted by analyzing the effects of temperature, substrate concentration, and activity of enzyme in presence of inhibitors (CuSO_4). The results showed that proteases extracted from *Cicer arietinum* showed optimum activity at 55°C. An increase in substrate concentration resulted in higher enzyme activity. It was observed that in presence of 0.5% CuSO_4 , the enzyme activity decreased.

Keywords: Protease, *Cicer arietinum*, Biurette method, substrate concentration, inhibitors, optimum temperature.

Introduction: Pulse consumption has been associated with positive health outcomes across multiple populations (Viguiouk et al., 2017). Consumption of Chickpeas (*Cicer arietinum*) has been proposed to be beneficial in the management of multiple chronic diseases such as cardiovascular diseases and obesity (Gupta et al., 2017; Padhi and Ramdath, 2017). It is also an important source of carbohydrates, B-group vitamins, and certain minerals. During germination some hydrolytic enzymes such as amylase, invertase, protease and lipase are activated. Proteases are a group of enzymes that catalyze hydrolysis of bonds in polypeptide chains and split them into smaller polypeptides or else free amino acids. Proteases are the enzymes that perform proteolysis i.e. digestion of proteins. Proteases, enzymes that break down proteins, have wide-ranging industrial applications, particularly in detergents, food processing, leather tanning, and waste management (Neha Thakur, et al., 2018). Proteases are widely used in the food industry for various applications, including meat tenderization, cheese production, baking, and improving the nutritional value of foods. They can also be used to modify proteins for better flavor, solubility, and digestibility, as well as in waste management and brewing (Sindhu Raveendran, 2018). The current project focuses on the crude extraction of the protease enzyme from *Cicer arietinum* and comparing the enzyme activity of the protease extracted from bacterial and plant sources in order to have a good efficient and stable yield of the enzyme for commercial use. **Materials And Methods:**

1. Extraction of Enzyme from *Cicer arietinum*

- Soak chickpea seeds in water for 8–12 hours.
- Germinate for 24–48 hours in a moist environment. This enhances protease activity.

To check for protease production in chickpea before Extraction: Seed Sterilization :- Wash on soaked *Cicer arietinum* seed by submerging in tap water for 2 mins then wash it with a few drops of mild detergent for 1 min. Decant the detergent and wash it with distilled water for 2mins.



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- Cover the Chickpea seed with 70% Ethanol and Shake it for 5mins. Add 0.2% HgCl_2 and Wash it again with Sterile Distilled Water.
- **Plating On Sterile Casein Agar Plate:** - Now, transfer the Sterile *Cicer arietinum* aseptically on a Sterile Casein agar plate and incubate the plate at room temperature for 24-48 hours and observe for zone of inhibition.
- Weigh about 50 g of *Cicer arietinum* seeds (germinated or raw).
- Grind them in a blender or mortar & pestle with a cold phosphate buffer (pH 7.0, 50 mM) in a 1:5 ratio (1 part seed, 5 parts buffer). Maintain the mixture on ice to prevent enzyme denaturation.
- Filter the homogenate using cheesecloth or muslin cloth to remove solid debris.
- Centrifuge the filtrate at 10,000–12,000 rpm for 15–20 minutes at 4°C.
- Collect the clear supernatant – Crude protease enzyme.

Determination of effect of different parameters on the enzyme: The Biuret method was used to estimate protein concentration and enzyme activity considering different parameters viz. Optimum temperature, substrate concentration, effect of inhibitors. The absorbance of the system was determined spectrophotometrically at 550 nm.

A. Effect Of Temperature:

- Take 5 tubes with 2 ml 1% Casein substrate in each of the tube
- Add 2 ml phosphate buffer in the above tubes.
- 1 ml of enzyme extract was added to all tubes.
- Incubate the above tubes at respective temperature (4°, 28°, 37°, 55°, 100°) for 30 mins
- Centrifuge the tubes at 4°C at 10,000 rpm for 10-15 mins
- Collect the supernatant carefully.
- Add 4 ml Biuret reagent in each tube.
- Incubate for 30 mins at room temperature.
- Take the O.D in colorimeter at 530 nm.

B. Effect Of Substrate

Take 5 tubes and add the given substrate amount 0.5, 1.0, 1.5, 2.0 ml. The substrate used is 1 % casein prepared in phosphate buffer

- 1 ml of enzyme extract was added to all tubes.
- Add 2 ml phosphate buffer in the above tubes
- Incubate the above tubes at room temperature for 30 mins.
- Centrifuge the tubes at 4°C at 10,000 rpm for 10-15 mins.
- Collect the supernatant carefully.
- Add 4 ml Biuret reagent in each tube.
- Incubate for 30 mins at room temperature.
- Take the O.D in colorimeter at 530 nm.

C. Effect Of Inhibitors

- Take 5 tubes with 2 ml 1% Casein substrate in each of them
- Add 2 ml phosphate buffer in the above tubes .
- 1 ml of enzyme extract was added to all tubes.
- Add the inhibitor 1% CuSO_4 in the following volume 0.2, 0.4, 0.6 ml.
- Incubate the tubes at room temperature for 30 mins.
- Centrifuge the tubes at 4°C at 10,000 rpm for 10-15 mins
- Collect the supernatant carefully.
- Add 4 ml Biuret reagent in each tube.
- Incubate for 30 mins at room temperature.
- Take the O.D in colorimeter at 530 nm.

Observations:



Fig. 1. Zone of Clearance observed around Chickpea seeds indicating Protease production

A. Effect Of Temperature: Chart 1

Temperature (°C)	O.D at 550 nm
BLANK	0.0
4	0.02
28	0.03
37	0.04
55	0.06
100	0.01

B.SubstConcentration:,Chart 2

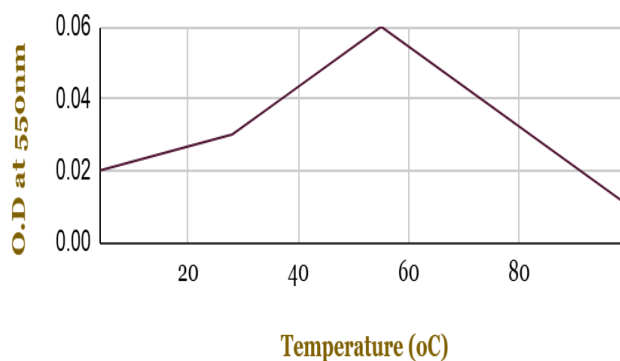
1% Casein (Substrate) (ml)	O.D at 550 nm
BLANK	0.0
0.5	0.01
1	0.02
1.5	0.04
2.0	0.05

C.Effect of Inhibitor: Chart

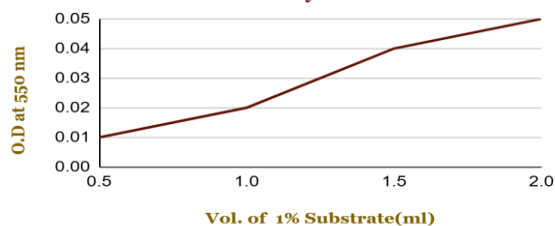
1% CuSO ₄ (Inhibitor) (ml)	O.D at 550 nm
BLANK	0.0
0.2	0.13
0.4	0.07
0.6	0.05
0.8	0.02

Chart 1

Effect of Temperature on Protease Activity



Effect of Substrate Concentration on Protease Activity



Results And Discussions: Chart 2

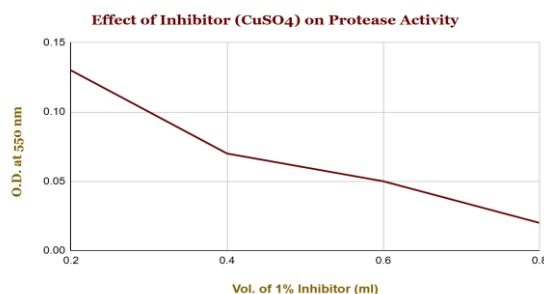


Chart 3

From the observations obtained for determining the optimum Protease activity considering the different parameters like effect of Temperature, substrate concentration and concentration of inhibitors that decreases the enzyme activity it was found that -

The Protease extracted from *Cicer arietinum* showed optimal activity at 55 °C, which was found not consistent with the study carried out by **Tooba Naz Shamsi et al.**, in which the optimum temperature obtained was between 4^o C to 37^oC. In the present study it was found that as the substrate concentration increases enzyme activity also increases when the maximum substrate concentration taken was 1% Casein. These findings coincided with the results of Guowei SHU* et al in 2016.

The concentration of inhibitor that decreased the protease activity in the present study was found to be 0.06. At a concentration 0.02ml of 1% CuSO₄ was found to be maximum. Further as the concentration of the inhibitor increased, the protease activity decreased. The present study results were found to be consistent with the results obtained by S. V. Lagade et al .

Conclusions:

The Protease enzyme was extracted from *Cicer arietinum* by chemical and biophysical methods. The enzyme extracted was then processed for determining the optimum conditions where it will prove beneficial to maintain the stability of the extracted enzyme from *Cicer arietinum* .

The optimum temperature determined was 55 °C, wherein it can be proved that it is a thermostable enzyme and can be used in the food and textile industry.

It was always found that as the concentration of Casein that is substrate increases the enzyme activity also increases.

From the study above, it can be said the protease extracted from *Cicer arietinum* is thermostable. As the volume of substrate increases the protease activity also increases. The Protease activity in presence in 1% CuSO₄ solution showed the peak activity at 0.2 ml. Enzyme activity decreased with the increased volume of CuSO₄ . For inhibitor , it was observed that as the concentration of the inhibitor goes on increasing the activity of protease also decreases in the present study.

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Conflict Of Interest

The authors declare no conflict of interest.

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