

Isolation, Purification and Analysis of Pancreatic Lipase from '*Gallus gallus domesticus*'

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Abstract:- This article discusses the isolation of pancreatic lipase enzyme from the pancreas of *Gallus gallus domesticus*. Whereas lipase catalyses the hydrolysis and the synthesis of esters formed from glycerol and long-chain fatty acids. Lipases occur widely in nature, it involves applications like organic syntheses, hydrolysis of fats, oils, modification of fats, flavor enhancement in food processing, detergent industries, pharmaceutical industries, chemical analyses, and biodiesel production. Pancreatic lipase was purified to the homogeneity by 70% saturated Ammonium sulphate further, it was dialysate using the dialysis membrane and then gel filtration chromatography was carried out by Sephadex G-75 and DEAE cellulose. The molecular weight of purified lipase sample was determined by SDS-PAGE, it was found to be 98KDa. The lipase was active in the pH range of 5-10 with an optimum pH of 6.0. The optimum temperature for the hydrolysis of olive oil was 37°C in the range of 25°C - 50°C.

Keywords:- *Gallus gallus domesticus*, *Lipases*, *Enzyme kinetics*, *Column chromatography*, *SDS-PAGE*.

I. INTRODUCTION:

Lipases (triacylglycerol acyl hydrolases, E.C. 3.1.1.3) are lipolytic enzymes involved to catalyze the hydrolysis of long-chain triglycerides into free fatty acids and glycerol at the interface of emulsified lipid substrates. The fatty acids are oxidized endogenously to get energy available for doing mechanical work while the glycerol moiety produces energy in some specific tissues through oxidation procedure. Lipases are widely used for biotechnological applications in such dairy industry, oil processing, etc. lipases from aquatic animals are even less stable and well known than a mammalian, plant, and microbial sources.

So far evidence suggested the specification and importance of lipase in producing various essential and nutritional products. Lipase can hydrolyze lipids to produce undesirable sour flavor in milk products, meat, fish, and other food products contain oil or fat. Lipases have been used extensively in the dairy industry, household detergent, and in the oleochemical industry (Verger et al., 1982). Recently, most of the lipases applications are identified starting from their use in laundry detergents, the

modification of the nutritional, sensory, and physical properties of the triglycerides in foodstuffs, and the production of optically pure enantiomers (Hemachander et al., 2000; Underage et al., 2001).

❖ *Model organism – Gallus gallus domesticus*

- Kingdom - Animalia
- Phylum - Chordata
- Class - Aves
- Family - Phasianidae
- Genus - *Gallus*
- Species – *gallus domesticus*

Chickens are specially grown for their meat and egg purpose. Hence they're classified into two types, egg-type chickens and meat-type chickens. Here egg-type chicken is mainly composed of egg production and they are maintained for the purpose of chicks' production for high egg production in a basic need for human consumption. Breeds of meat-type chickens are primarily included broilers, roasters, and fryers.

- **Broiler:** Broilers are genetically selected for fast growth and raised for meat instead of eggs. Here chickens are raised for its meat and marketed at an age of 6-8 weeks.
- **Layer:** The management of birds during 21-72 weeks aged for the aim of laying eggs (egg production).
- **Giriraja:** This is the local ancient breed of chicken that produces both eggs and meat. Typically, poultry is reared specifically for the important purpose like laying eggs and meat. They produced eggs while they were alive, meat once they were dead, and lived on household scraps. Also, male laying birds needn't be slaughtered since this breed is often wont to produce both meat and eggs. Animal- welfare experts believe that using this breed is going to be more human since the birds won't develop health problems due to intensive breeding.

The function of the pancreas of chicken is to provide digestive fluid which may be a mixture of digestive enzymes, which are involved within the metabolism of carbohydrates.

II. MATERIALS AND METHODS:

A. Collection of the pancreas tissue sample from chickens:

Pancreas tissue of *Gallus gallus domesticus* was collected during a commercial chicken stall, which wasn't consumed by humans and left for animal feed. These collected pancreas tissues were homogenized with the assistance of mortar and pestle within the addition of tris HCl buffer for homogenization. After fine homogenization, it had been centrifuged at 4°C at 6000rpm for 10 minutes. Later supernatant was collected in a separate beaker and the sample was left to perform enzyme assay.

B. Enzyme assay of lipase:

Pipette (in milliliters) the following reagents into suitable containers (Test: Blank). Deionized Water - (2.50: 2.50), Buffer - (1.00: 1.00), Olive Oil - (3.00: 3.00). Swirling well and equilibrate to 37°C for 10 minutes. Then add Enzyme extract 1.00 only in the test. Mix well and incubate at 37°C for 30 minutes. Immediately after the incubation is over, add 95% Ethanol 3.00 each. Shake well and then add 4 drops of phenolphthalein indicator to both the Test and Blank solutions. Titrate each solution with NaOH till it reaches a pale pink color.

C. Estimation of Protein by Lowry's method:

Different aliquots of 0.2, 0.4, 0.6, 0.8, and 1.0mL of the working standard BSA was pipette out into a sequence of test tubes, 1.0mL of crude extract was pipette out into test tubes. The overall volume was made up to 1.0mL in all test tubes, with the addition of distilled water. A test tube with only distilled water served as blank. 5.0mL of Reagent C was added to all the test tubes including blank, the contents of the test tubes were mixed well and incubated for 10min at room temperature. Then 0.5mL of Reagent D (Folins - Ciocalteu Reagent) was added, mixed well, and incubated at room temperature for 30min and the absorbance was measured at 640nm.

D. Purification of pancreatic lipase:

➤ Ammonium sulphate precipitation:

The extracted solution was centrifuged at 6000 rpm for 10 mins and the cells free supernatant was collected in a beaker, then it was placed on magnetic stirrer in ice-cold condition. As the supernatant sample collected was measured through a measuring cylinder, which was approximately 88ml. Here 70% saturation salt was used, hence 38.82g of ammonium sulphate was added pinch by pinch and after salt precipitation, the solution was stored in 4°C overnight. (Verger, R., F. Ferrato, C.M. Mansbach, and G. Pieroni. 1982).

➤ Dialysis:

The dialysis membrane activated by heating the membrane in 2% NaHCO₃ solution for 10 min, transferred to boiling water for 10 mins and cooled naturally. Salt precipitation sample was centrifuged at 1000 rpm for 10 min and the pellet was collected in 10ml of Tris HCl and poured into dialysis bag and the ends were sealed and dialyzed for 2

to 3 hours in a magnetic stirrer by changing the distilled water every half an hour for 3 hours.

➤ Ion exchange chromatography:

In the first step, the sample loaded into the column by loading buffer. The charged samples were bound to the column due to ionic interactions. In the second step, 5mL of 1M HCl and NaCl were prepared. Into a series of the test tube, 250 of 1M HCl and (250-1500) of NaCl and 5mL of autoclaved water were added to prepare the elution. Then the sample collected in dialysis was transferred into the column and elution was transferred accordingly. (Gjellesvik et al., 1992; Iijima et al., 1998).

➤ Gel filtration chromatography:

The elution selected from the ion exchange chromatography was transferred to a column, separated, and collected in 20-25 Eppendorf tubes. (Iijima et al. (1998)

E. Characterization of pancreatic lipase enzyme kinetics:

➤ Effect of pH on enzyme activity:

Enzyme is affected by changes in pH. The high favorable pH value point where the enzyme shows its activity is known as the optimum pH. Here three different buffer is used in different pH and blanks correspondingly. Sodium acetate buffer (200mM) for pH 5.0 and 6.0, Phosphate buffer (200mM) for pH 7.0 and 8.0, Glycine buffer (200mM) for pH 9.0 and 10.0. The enzyme assay was carried out by the titration method, the graph was plotted with the pH of buffer along x-axis and enzyme activity along the y-axis.

➤ Effect of substrate concentration on enzyme activity:

A substrate is a substance that is used to make a final product. The enzyme binds to and changes substrates into their final product. Here Olive oil is used as a substrate for the purified enzyme sample and buffer used was tris HCl, where the concentration of substrate used was 0.5, 1, 1.5, 2, 2.5, and 3. To this substrate concentrations, blanks were taken correspondingly. The enzyme assay was conducted by titration method, the graph was plotted with substrate concentration along x-axis and enzyme activity along the y-axis.

➤ Effect of temperature on enzyme activity:

The rate of an enzyme-catalyzed reaction increases as the temperature is raised. Variations in reaction temperature show changes of 10 to 20% in the result. Here tris HCl buffer is used in incubation time of different temperatures 25°C, 30°C, 35°C, 40°C, 45°C, and 50°C with corresponding blanks. The enzyme assay was conducted by the titration method, the graph was plotted with temperature along x-axis and enzyme activity along the y-axis. (Abbas, H., A. Hiol, V. Deyris, and L. Comeau. 2002).

➤ Effect of time on enzyme activity:

The longer an enzyme is incubated with the substrate, the greater the amount of product that will be formed. The rate of formation of the product is a simple linear function of the time of incubation. Here tris HCl buffer is used in

different incubation of time 10, 20, 30, 40, 50, and 60. For these different time incubations, different blanks were taken correspondingly. The enzyme assay was conducted by the titration method, the graph was plotted with incubation time along x-axis and enzyme activity along the y-axis.

F. Molecular weight determination by SDS-PAGE:

The glass plate was cleaned with methanol to make it grease-free, and clamped for making leak-proof, then freshly prepared to resolve gel was added and was allowed to polymerize. Later on, stacking gel was poured over the resolving gel and comb was inserted and was allowed to polymerize for 1 hour. Meanwhile, the samples (25µL sample + 15µL gel loading buffer) were denatured at 80°C for 10min, after polymerization the comb was removed and the wells were cleaned with filter paper clipped in methanol, the plates were placed in electrophoresis unit with buffer and

40µL of processed enzyme sample was loaded into the wells. The sample was electrophoresed until the dye reaches the base of the gel, then the gel was removed carefully and placed in a staining solution overnight. The excess stain was removed by placing the gel in destaining solution, the protein bands were visualized. (Iwai et al. (1975), while (Aloulou et al. (2007) reported the MW of the lipase from *Yarrowia lipolitica* a was 38.48 KD)

III. RESULTS AND DISCUSSION:

Enzyme activity calculation

$$\begin{aligned} \text{Units per ml of enzyme} &= (\text{NaOH}) (\text{Molarity of NaOH}) \\ & (1000) (2) / (1) \\ &= 11.6 \times 0.05\text{M} \times 1000 \times 2 / (1) \\ &= 1.160 \text{ units/ml} \end{aligned}$$

➤ *Purification table:*

Samples	Protein concentration	Enzyme activity (units per ml)	Specific activity (units per mg)	Fold
Crude	636.13	1.160	0.657	1
Salt precipitation	270.96	1.540	1.969	2.996
Dialysis	131.65	0.300	0.793	1.207
Ion-exchange chromatography	40.96	0.370	3.490	5.312
Gel filtration	31.31	0.430	17.20	26.179

Table 1

➤ *Protein estimation: Lowry's method*

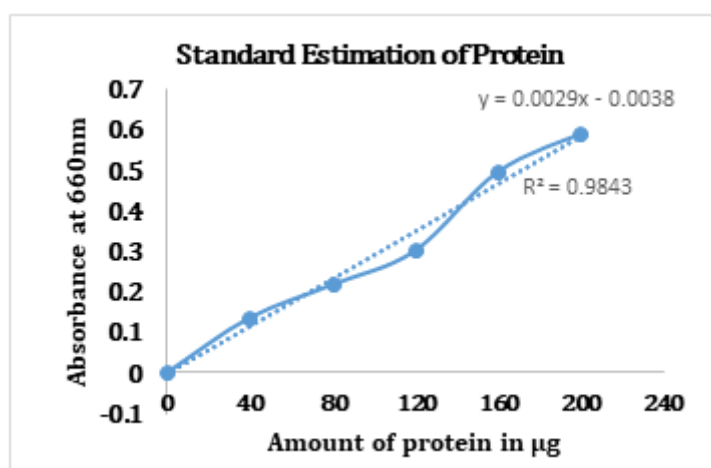


Fig 1

➤ *Enzyme kinetics:*

Enzyme activity affected by various parameters like pH temperature, substrate concentration, incubation time etc. Pancreatic lipase enzyme shows optimum activity in basic pH and temperature at pH 6.0 and 37°C, substrate concentration is 1% and stability increase in 30 minutes.

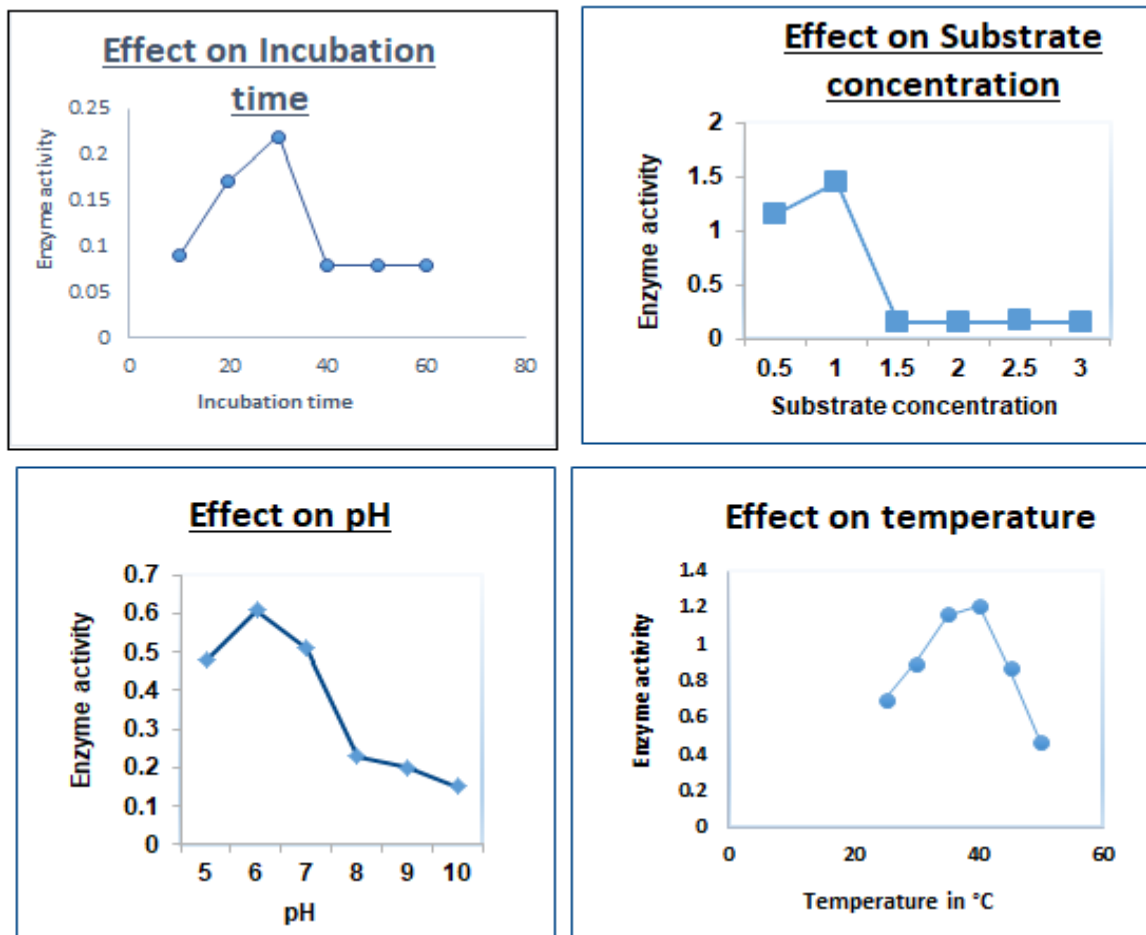


Fig 2

➤ **SDS PAGE:**

The molecular weight of pancreatic lipase enzyme of Gallus gallus domesticus was determined to be approximately 98kDa, with the help of sea blue marker using the method of SDSPAGE.

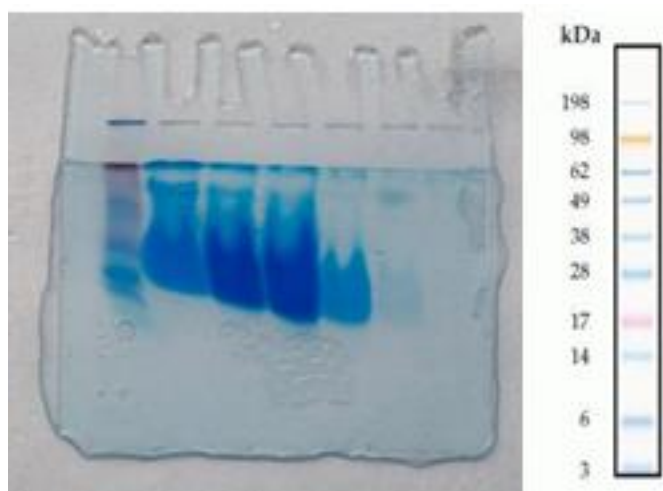


Fig 3

IV. CONCLUSION:

As an overview of the present article, lipases catalyze both the hydrolysis and the synthesis of esters formed from glycerol and long-chain fatty acids. A lipase is an important group of biocatalysts in organic chemistry. Lipases include facts which are stable in organic solvents, possess broad substrate specificity, it does not require any cofactors, it acts over a wide range of pH and temperature, and lipases exhibit high enantioselectivity. In contemporarily lipases are produced by animals, plants, and microorganisms. Animals have been found to produce high activity of lipases compared to plants and microorganisms. The present study is expected to provide us new insights by using chicken pancreas tissue for the extraction of the pancreatic lipase enzyme. As pancreas tissue is non-consumable by humans hence it was not used further, so it is inexpensive to work with.

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