JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

EXTRACTION AND ENZYMATIC ACTIVITY STUDIES FOR ARACHIS HYPOGAEA LIPASE

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ABSTRACT

This work reports a simple scheme for extracting plant based biocatalyst from cost effective source and then transform the pharmaceutical compound by utilizing this biocatalyst. Crude lipase extracted from *Arachis hypogaea* seed by liquid extraction method. Then protein content of *Arachis hypogaea* crude lipase extract was estimated by using Folin Lowry method. Enzymatic activity of crude lipase extract was estimated by the enzymatic hydrolysis of variety of oils namely as sunflower oil, coconut oil, soyabean oil, olive oil. The crude seed lipase extract activity is compared with commercially available fungal lipase. Comparably the highest seed lipase activity was found to be 0.326 (U/ml) for hydrolysis of coconut oil. *Arachis hypogae* seed crude lipase also used for hydrolysis of p-nitrophenyl acetate substrate and liberated p-nitrophenol and it is determined by UV Visible spectroscopy at λ_{max} 400 nm and after 180 min of reaction completed it showed 88 % consumption of p-nitrophenyl acetate.

KEYWORDS: Seed lipases, Protein content, Enzymatic hydrolysis, p-nitrophenyl acetate

INTRODUCTON

Enzymes are well-known as nature's biocatalyst, performing various biological processes. The foremost role of enzymes is to enhance the rates of these reactions and hence is called biocatalyst. Therefore, biocatalysts are going to be one of the important tools for implementing the green chemistry principles. [1]

Plant lipases present advantages over animal and microbial lipases due to quite interesting features such as specificity, low cost, availability and ease of purification, representing a great alternative for potential commercial exploitation as industrial enzymes. The modification of fats and oils by transesterification, for instance, can be performed by both chemical and enzymatic catalysis. In the enzymatic process, lipases can be used as biocatalysts to promote the exchange of triacylglycerols, showing greater efficiency and leaving no residues. However, several

studies have indicated that such processes are very expensive due to the high cost of purifying the microbial enzymes. [2,3,4]

We are elucidating here with simple extraction and enzymatic activity studies for seed lipase from *Arachis hypogaea.*

MATERIALS AND METHODS

Material

Peanut seeds, Olive oil, Soybean oil, Coconut oil and Sunflower oil was purchased from a local market and used without any further treatment. A commercially available *Aspergillus oryzae* fungal lipase was kindly supplied by Aumenzymes® (Gujarat, India). p-nitrophenyl acetate (p-NPA) were also purchased from Otto Chemie Pvt. Ltd. Mumbai, India.

Extraction of Lipase from *Arachis Hypogaea* Seed[5]

Uniform and healthy-looking seeds were taken from plant, soaked separately in distilled water for six hours, placed on moist filter paper inside petri dishes and seeds allowed to germinate for 72 h under dark condition at room temperature. The emergence of radicles was considered as an indication of germination. Seed coats were removed from the germinated seeds and 20 g of fresh weight of cotyledon homogenized for 5 minutes with distilled water (DW) solvents. The extracts of DW were filtered through four layers of cheese cloth and centrifuged at 10000 rpm using cooling centrifuge [4°C] for 30 min. The upper fat layer was removed by a spatula and the supernatant was taken as a source of crude lipases.

Fig. 1 Germinated seeds

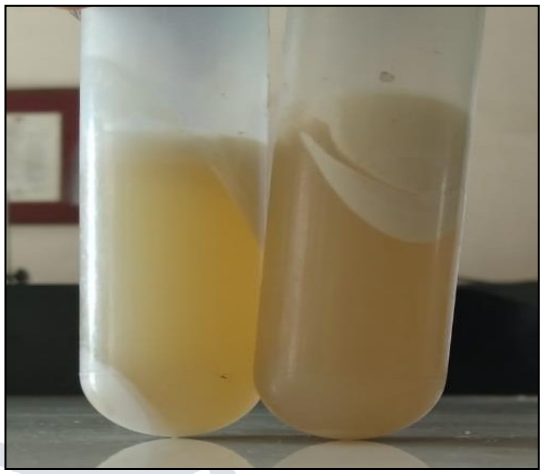

Fig. 2 Centrifuged extract

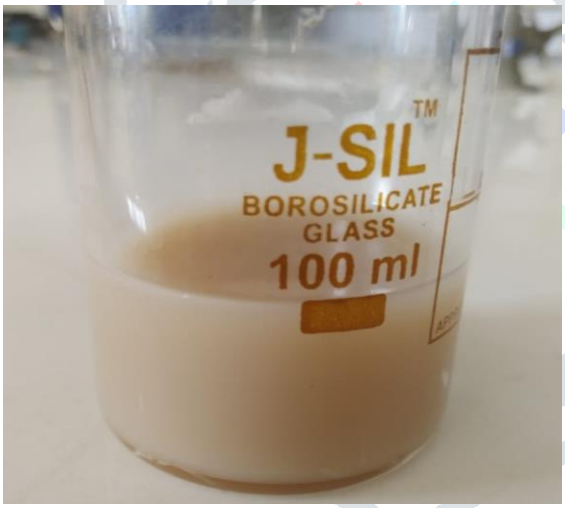

Fig. 3 *Arachis Hypogaea* lipase extract

Estimation of protein content of crude peanut lipase extract [6,7]

Estimation of protein was performed by Folin-Lowry method. The method involves using alkaline CuSO4 catalyzed oxidation of aromatic amino acids of protein with subsequent reduction of sodium potassium molybdate tungstate of Folin's reagent giving a purple color complex. Hence the protein sample can be estimated by colorimetry.

Preparation:

1. Albumin solution [Standard]: 5mg/ml:120mg albumin in 25 ml distilled water.
2. Lipase solution [Sample]:5 ml crude lipase extract in 25 ml distilled water.
3. Alkaline copper sulphate solution: [solution A]10 gram of sodium carbonate in 500 ml of 0.5 N sodium hydroxide. [Solution B] 2.5 gram of copper sulphate and 5gram sodium potassium tartrate in 500 ml distilled water. Mix solution A and B.

Procedure:

I. Prepare a series of test tube containing varying amount of standard Albumin in the range of 1 to 5 mg. Concentration of standard Albumin is 5 mg/ml.

II. To each of the test tube add 3 ml of alkaline copper solution and 0.5 ml of folin-Ciocaltean reagent and allow the test tube to stand for 30 minutes at room temperature and then make up the volume to 10 ml with distilled water.

III. Take 1 ml of sample solution and follow the same procedure as per the standard.

Determine the absorbance of standard as well as sample at 660 nm using a colorimeter against a blank. On the basis of standard plot determine the concentration of the sample of crude lipase

Fig. 4 Different concentration of standard Albumin and sample extract

Estimation of lipase extract activity by using different oils [8,9,10]

A buffer solution containing Na_2HPO_4/NaH_2PO_4 50 mM, pH=7, is prepared. Next an different oil emulsion (40 ml of oil added to 60 ml of gum Arabic – emulsifier solution 5%, w/v) is prepared and the mixture is homogenized in laboratory homogenizer. The substrate of the enzyme is composed of 50 ml of oil emulsion in 45 ml of buffer solution. 0.5 ml of crude enzyme solution is added into 9.5 ml of substrate. The mixture is incubated in a shaker for

1 h at room temperature. This is followed by titration of the mixture with NaOH solution 50 mM until color changes using phenolphthalein indicator. Amount of NaOH required to achieve end point (colorless to pink) was recorded.

Enzymatic activity is calculated by using following formula

$$EA = \frac{C\,(V - Vo)}{mt}$$

Where, C = Concentration of NaOH,

V = burette reading

Vo = Volume of Blank

t = Homogenization time

m = Quantity of enzyme

Hydrolysis of p-nitrophenyl acetate [11,12]

Hydrolysis of p nitrophenyl acetate was caried out by adding 100 mg p-nitrophenyl acetate into

20 ml of 100 mM phosphate buffer ph. 7.4 then 2 ml of peanut seed lipase extract was added into reaction mixture. Then reaction placed for stirring at 150 rpm in room temperature for 90 minutes. Then samples were withdrawn every 10 minutes time intervals and record the absorbance at λ_{MAX} = 400 nm for formation of p nitrophenol and the graph of p- nitrophenol absorbance versus time was plotted.

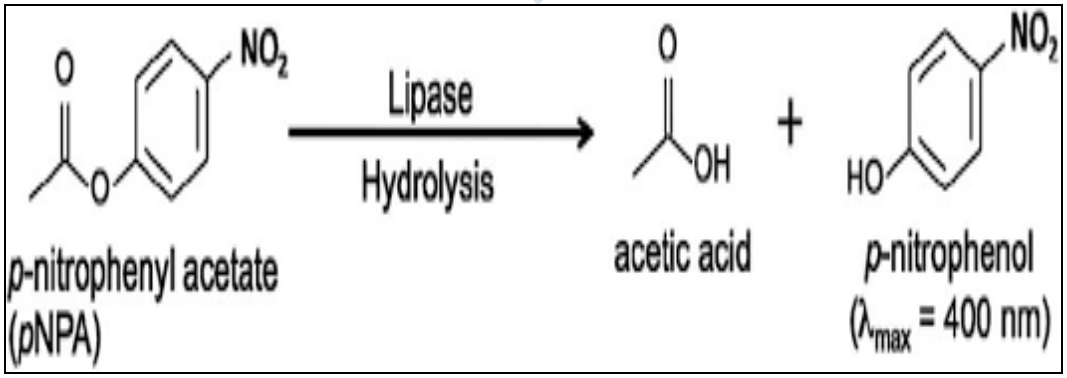

Fig. 5 Reaction of hydrolysis of p-nitrophenyl acetate

Determination of consumption of p-nitrophenyl acetate by hydrolysis

For determination of consumption of p-nitrophenyl acetate by calibration curve we added 10 mg p-nitrophenyl acetate into 10 ml of 100 mM phosphate buffer ph. 7.4 then peanut seed lipase extract was added into reaction mixture. Then reaction placed for stirring at 150 rpm in room temperature and samples were withdrawn every 30 minutes time intervals. then the concentration range of 16 to 80 ppm of p nitrophenyl acetate was used for preparation of standard curve in phosphate buffer and maximum absorbance is recorded at λ = 271 nm for p-nitrophenyl acetate.

RESULTS AND DISCUSSION

Calibration curve of albumin in distilled water

The concentration range of 1 to 5mg/ml of Albumin was used for preparation of standard curve in distilled water. The value of r^2 was found to be 0.997 indicating that the relation of albumin concentration and absorbance was linear. The equation obtained was y = 2.26x + 0.409, were y is absorbance and x are the concentration. Absorbance of crude lipase was found to be 0.64 at λ = 660 nm

TABLE 1: PROTEIN CONTENT

Test tube no	**Volume of standard solution in ml**	**Amount of protein**	**Volume of CuSo4**	**Volume of folin-Ciocalteau reagent**	**Distilled water in ml**	**Final volume in ml**	**Concentration of solution in mg/ml**	**Absorbance at 660 nm**
Blank	-	-	3	0.5	6.5	10	0	0
1	1	0.5	3	0.5	5.5	10	0.05	0.51
2	2	1	3	0.5	4.5	10	0.1	0.65
3	3	1.5	3	0.5	3.5	10	0.15	0.75
4	4	2	3	0.5	2.5	10	0.2	0.86

5	5	2.5	3	0.5	1.5	10	0.25	0.96
Lipase extract	1		3	0.5	5.5	10	**0.1**	**0.64**

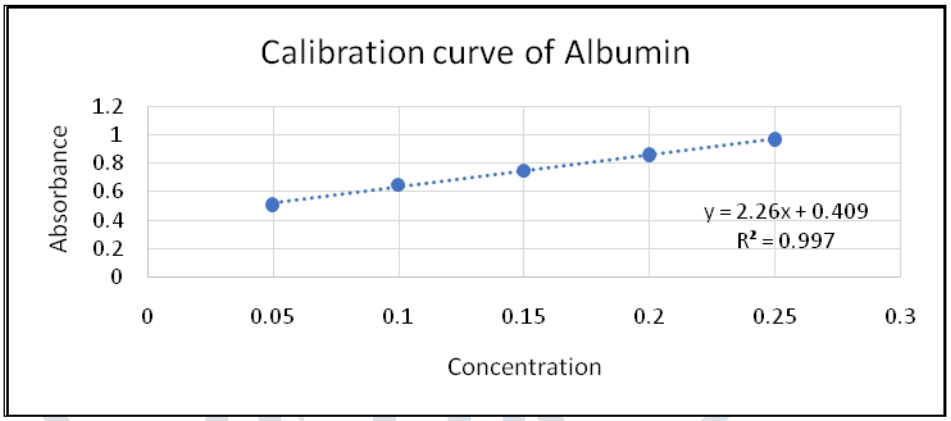

Fig. 6 Calibration curve of albumin

Calculation

From graph: concentration of dilute lipase extract sample is 0.10 mg/ml

$$\text{Dilution factor} = \frac{\text{Final volume}}{\text{Initial volume}} \times 100 = 10$$

Protein concentration = concentration from graph × dilution factor

= 0.10× 10

= 1 mg/ml

Concentration of protein contain in crude lipase was found to be 1 mg/ml

Optimization of hydrolyzed oils by titration method

Lipase acts on triacyl glycerides and releases fatty acids. Determination of lipase extract activity is calculated by titration release of free fatty acids.

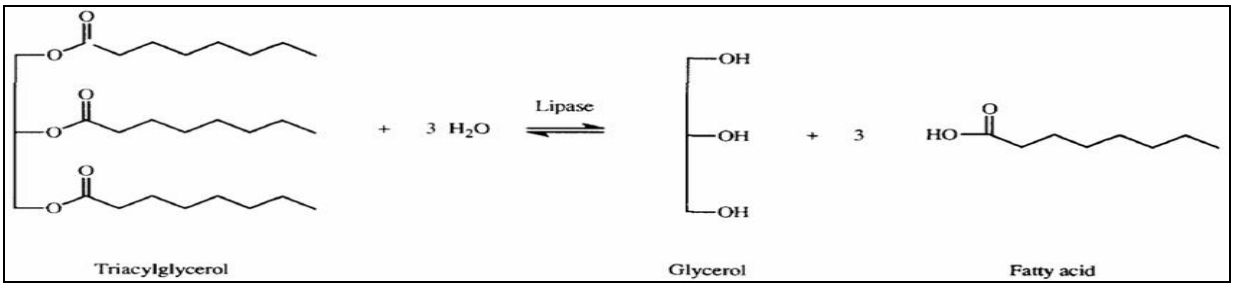

Fig. 7 Reaction of hydrolysis of tryacylglyceride

A unit of activity of lipase is the quantity of the enzyme that releases 1µmole of fatty acids in 1h at room temperature.

Comparison of plant and fungal lipase activity(U/ml) using different oils

Enzymatic activity of plant lipase extract and fungal lipase were determined by using hydrolysis of different type of oils as substrate of lipase. Table 2 shows the calculated activity of extracted Arachis hypogaea [Peanut] seed lipase and commercially available fungal lipase. Highest activity was found to be **0.326 (U/ml)** for hydrolysis of coconut oil by using Arachis hypogaea [Peanut] seed lipase and **0.446 (U/ml)** for hydrolysis of olive oil by using fungal lipase.

Table 2: Comparison between activity of two lipase

Sr. no	Oils as substrate	Vegetable extract of lipase	Marketed Fungal lipase
1	Olive oil	0.257 (U/ml)	0.446 (U/ml)
2	Sunflower oil	0.232 (U/ml)	0.388 (U/ml)
3	Coconut oil	0.326 (U/ml)	0.364 (U/ml)
4	Soya oil	0.258 (U/ml)	0.284 (U/ml)

Optimization of p-nitrophenol by UV Visible spectroscopy

In enzymatic hydrolysis using lipase, lipase activity plays an essential role in the catalytic profile. The study on lipase activity was conducted using the hydrolysis process of p-Nitrophenyl acetate and producing p-Nitrophenol as shown in the schematic reaction in Fig. 5.

Fig. 8 shows continuous increasing absorbance of p-nitrophenol at λ_{MAX} = 400 nm with respective to time due to liberated p-nitrophenol during hydrolysis of p-nitrophenyl acetate by using lipase extract and Fig 9 represent the spectra of p-nitrophenol λ_{MAX} = 400 nm.

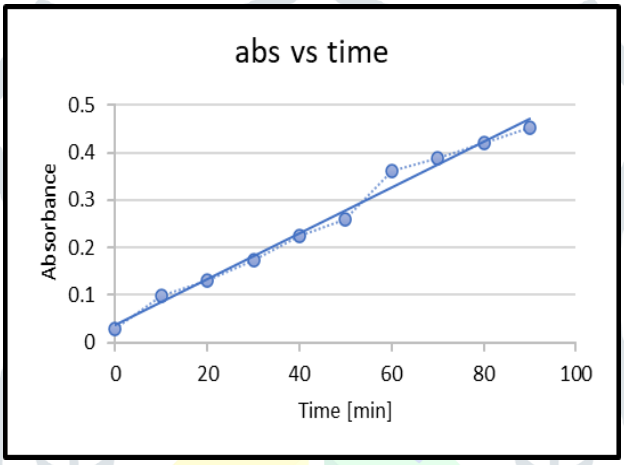

Fig. 8 Abs vs time

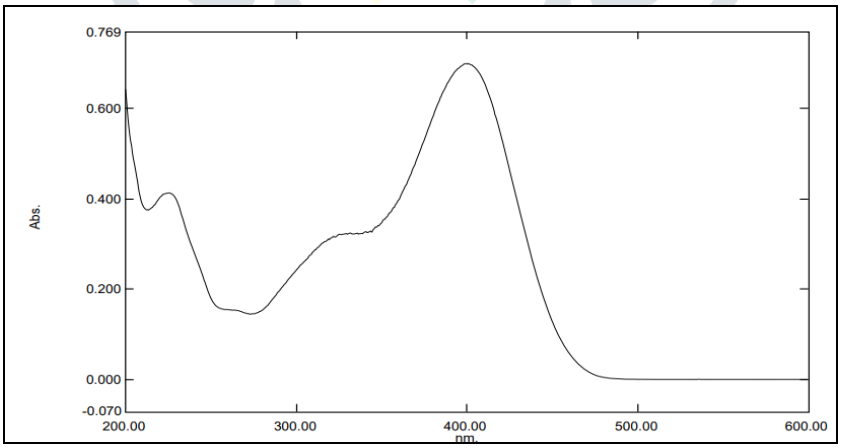

Fig. 9 Spectra of p-nitrophenol

Quantitative determination of consumption of p-nitrophenyl acetate

a) Calibration curve of p-nitrophenyl acetate in phosphate buffer pH 7.4

The calibration curve was prepared and value of r^2 was found to be 0.9982 indicating that the relation of p-Nitrophenyl acetate concentration and absorbance was linear. The equation obtained was y = 0.0105x + 0.04, were y is absorbance and x are the concentration. The result for the same are given in table no. 3 and fig. no. 10

Table 3: DATA OF CALIBRATION CURVE IN PHOSPHATE BUFFER

Concentration (ppm)	Absorbance at λ = 271 nm
16	0.205
32	0.385
48	0.547
64	0.695
80	0.891

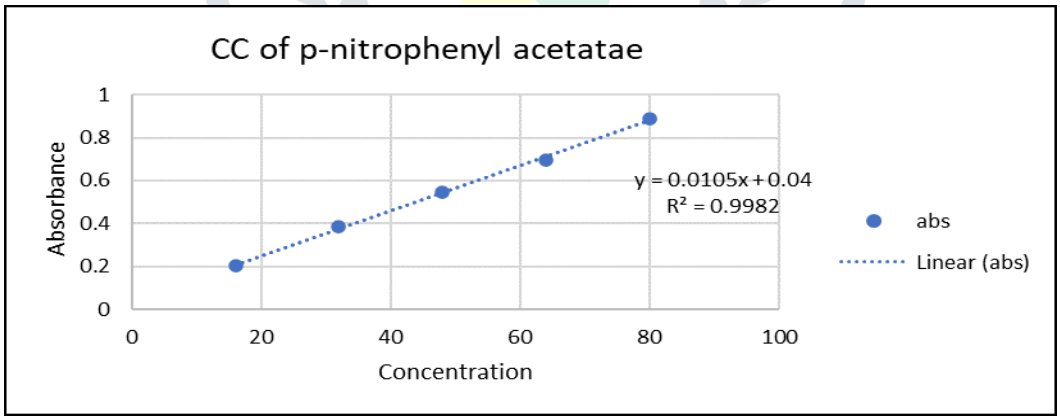

Fig. 10: Calibration curve of p-nitrophenyl acetate

b) Consumption of p-nitrophenyl acetate

Table 4: Consumption of p-nitrophenyl acetate

Sr.no	Time	Absorbance at λ = 271nm	Concentration in ppm	Consumption of p nitrophenyl acetate in percentage
1	0 min	0.937	85.4	0
2	30 min	0.592	52.5	38
3	60 min	0.360	30.4	64
4	90 min	0.245	19.5	77
5	120 min	0.207	15.9	82
6	150 min	0.171	12.4	86
7	180 min	0.156	11	88

Table 4 shows consumption of p-nitrophenyl acetate into reaction with respective time due to formation of product p-nitrophenol. Starting concentration at 0 min of p nitrophenyl acetate was found to be 85.4 ppm which is 100% and after 180 min of reaction completed concentration of p-nitrophenyl acetate was found to be **11 ppm** remaining into the reaction and which is represents **88 %** consumption of p nitrophenyl acetate.

CONCLUSION

From *Arachis hypogaea* (peanut) seed lipase extract was easily extracted by simple method. Protein content of this lipase extract was estimated by using Folin-Lowry method and which is found to be 1 mg/ml. Lipase extract hydrolyzed different oils such as olive oil, sunflower oil, coconut oil, soybean oil and it gives highest activity on hydrolysis of coconut oil. Its effectiveness was confirmed by using UV Visible spectroscopy on hydrolysis of p-nitrophenyl acetate and formation of product p-nitrophenol at λ_{MAX} = 400 nm and it gives 88 % consumption after 180 min of reaction completed.

ACKNOWLEDGEMENT

I would like to express my sincere gratitude to Dr. Parag Gide sir principal of Dr. L. H. Hiranandani college of pharmacy, Ulhasnagar-03 and I would like to thanks Aum enzymes® (Gujarat, India) for giving me fungal lipase enzyme as a gift sample.

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