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# Performance of Aqueous Two Phase System for the Extraction of Lipase from Rice Bran

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## ABSTRACT

Lipases are ubiquitous enzymes of considerable physiological significance and industrial potential. Lipase catalyzes the hydrolysis of triacylglycerols to glycerol and fatty acids at oil-water interface. This investigation is focused on the extraction of lipase from rice bran using aqueous two phase system. Polymer-Salt Aqueous two phase system was evaluated in rice bran source at varying concentration of PEG 6000 and resulted in attaining a maximum enzyme concentration at 60% PEG. Higher enzyme activity was obtained pH 7.The performance of the aqueous two phase system was investigated through partition coefficient and obtained a maximum value of 1.44 was achieved at 60% PEG concentration

**Keywords**: Lipase; Rice bran; ATPS; Enzyme activity.

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## INTRODUCTION

Lipases (triacylglycerol acylhydrolases EC 3.1.1.3) act as a biocatalyst in various industrial processes, due to their multifaceted properties. They are known to promote a wide range of reactions such as hydrolysis, interesterification, esterification, alcoholysis, acidolysis, aminolysis and these enhances the industrial application in field of waste water treatment, nutrition, cosmetics, leather industry, and pharmaceutical processing [1]. Lipases play an important role in cell biology. Most lipases act at specific position on the glycerol backbone of a lipid substrate. Lipase acts to convert triglyceride substrates to monoglycerides and free fatty acids [2]. As most of the industrial processes operate at a temperature exceeding 45°C, lipase should be active and stable at a temperature around 50°C. Hence, alkalophilic and thermophilic microorganisms has been focused for lipase production [3]. *Aneurinibacillus thermoaerophilus*, thermostable and organic solvent-tolerant lipolytic bacterium is efficient micro organism as it can withstand harsh industrial processes. Ammonium sulphate precipitation technique was used for isolation of lipase from *B.stearothermophilus* [4]. Yeasts such as *Candida cylindracea*, *Saccharomycopsis lipolytica*, *Geotrichum candidum* and *Trichosporon fermentan* are microorganism with lipolytic activity that facilitates discovery of novel lipases [5]. Recently isolation of lipase from recombinant *B.licheniformis* shows remarkably stable at alkaline pH conditions [6].

In recent years liquid-liquid extraction (LLE) such as aqueous two-phase extraction is gaining attention for primary purification and concentration of enzymes/proteins. Aqueous two-phase extraction (ATPE) is one such potential technique where extraction, concentration and partial purification can be integrated in single step LLE [7]. Using ATPE the desired product could be selectively partitioned to one of the phases in a concentrated form, thus considerably reducing the volume to be handled in the subsequent purification steps [8]. The partitioning of proteins in a standard biphasic aqueous system depends on the concentration and molecular weight of the phase forming polymer(s), the concentration, pH and type of salt [9]. In order to reduce the processing and production cost for lipase, soil & rice bran can be used as main sources. Soils can also be used specifically to identify & isolate the microbial organisms as industrially important strains producing the secondary metabolites [10]. In this research work, rather than isolating lipase from microorganism, rice bran had been used as source for extraction. The present study involves in separation of lipase from rice bran using ATPE and in determining the enzyme activity by enzymatic assay.

## MATERIALS AND METHODS

### Material and Chemicals

Rice bran and olive oil were purchased from sathyamangalam region. Polyethylene glycol (PEG 6000), Nutrient agar were from Himedia Chemicals. Ammonium sulphate ($NH_3SO_4$), Di-potassium hydrogen phosphate ($K_2HPO_4$), Potassium di-hydrogen phosphate ($KH_2PO_4$), Petroleum ether (80-90$^o$ C), Calcium chloride ($CaCl_2$), Sodium hydroxide (NaOH), Acetone-Ethanol (1:1), 50mM Potassium phosphate buffer were procured from Rankem Chemicals.

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**Pretreatment of Rice Bran**

In order to remove the fat content present in rice bran, the material was mixed with petroleum ether and filtered. It was then dried for 30 minutes and dried substance had been treated with 50mM potassium phosphate buffer solution. To that $CaCl_2$ was added and centrifuged at 5000 rpm for 20 minutes. The supernatant sample was subjected to ATPE [8].

**Aqueous Two Phase Extraction**

Batch experiment was conducted in a 250 ml conical flask with 4ml of pretreated sample. PEG 6000 was formulated in various concentrations of (10-60%) with neutral pH and from each concentration 2ml of solution was mixed with 4ml of supernatant sample in test tubes. Titration was carried out against 50% $NH_3SO_4$ until the test tube solution turns into turbid form and was further confirmed from its retaining ability after adding 200µl of water [11].

These retained samples were allowed to attain partition into two layers for about 2-3hrs and both lower and upper layers were subjected to Lowry's assay for enzyme confirmation [4].The partition coefficient (K) was calculated as ratio of lipase in extract and raffinate phase [8].

$$K=C_t / C_o$$

$C_t$ - Concentation of lipase in extracted phase
$C_o$ - Concentration of lipase in raffinate phase

**Lipase Conformation**

**Qualitative Assay**

The Nutrient agar along with olive oil at ratio of (100:1) was prepared in petriplate. Four equal wells of 0.5cm diameter was punctured using well puncher and 20µl of extracted sample was added to each wells. Then the plate was treated 0.5% Congo red dye solution and allowed undisturbed until colour change was observed around the zone which indicates the presence of lipase [14].

**Enzyme Activity Assay**

A mixture containing extracted sample with of 50mM phosphate buffer solution at various pH conditions (3, 5, 7 and 9) to optimize lipase activity was treated with olive oil at a ratio of (1:2:2) was kept at magnetic stirrer (100 rpm) for 60 minutes at 37$^o$ C. 10 ml of emulsified sample was centrifuged and then supernatant was mixed with acetone-Ethanol mixture at a ratio of (1:1). The hydrolysed sample mixture was titrated with 0.05N NaOH using phenolphthalein indicator. Titration assay was performed to find out the enzyme activity for each pH conditions [9].

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**Unit Activity**

One unit of lipase activity is defined as the amount of enzyme liberating one Micro mole of fatty acid per minute under standard assay conditions.

**Lipase Unit Activity (U/ml/min)**

$$\text{Unit Activity} = \frac{\text{Normality (NaOH)} \times \text{Volume of NaOH Titrated} \times 1000}{\text{Time of incubation (60 min)}}$$

## RESULTS AND DISSCUSSION

**Aqueous Two Phase Extraction**

The partitioning behavior of lipase from rice bran was estimated using ATPE and binodal curve had been plotted (Figure 1). The enzyme concentrations were investigated for turbidity retained samples (10-60%) and it was observed that a maximum lipase enzyme quantity was extracted at 60% of PEG 6000. It was observed that as the concentration of PEG increases the volume of salt gets reduced. Similar effect was revealed during the separation of Lipase from *Burkholderia cepacia* by PEG/Phosphate ATPS [16]. Figure 2 shows various enzyme concentrations with respect to PEG 6000 concentration. It was observed that enzyme concentration increases with increase in PEG concentration. This effect was due to the solubility nature of PEG towards enzyme. Additionally partition coefficient (K) value reveals about the efficiency of ATPE as separation technique and the chemical potential of the solvent. From Figure 3 it was observed that obtained K value was about 1.27, 1.4 and 1.44 for PEG concentrations of 20%, 40% and 60% respectively. Higher value of the partition coefficient was 1.44 for 60%.The partition coefficient (K) is shown in the Figure 3 and average value is of 1.37, and about 12.97 average K value was reported in published data [13]. The efficiency in revealed work was higher because of the macro extractor used in that process.

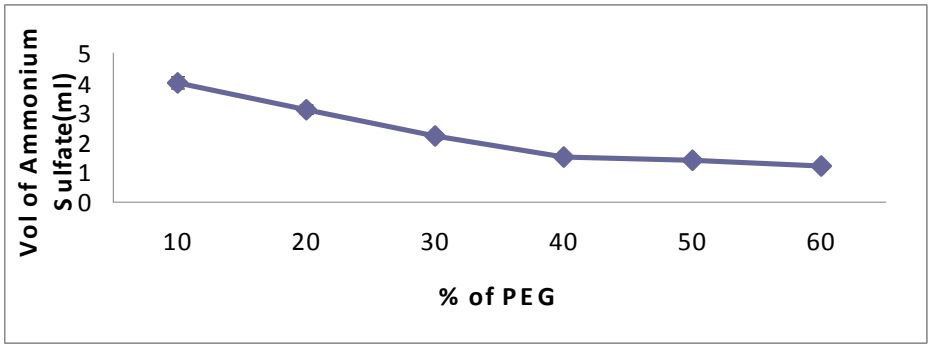


**Figure 1. Binodal curve for ATPE**

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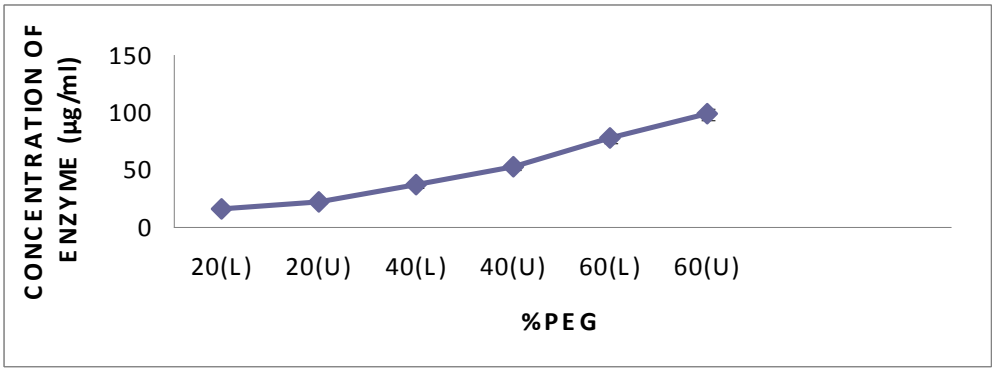


**Figure 2. Enzyme Concentrations (Turbidity Retained Samples)**

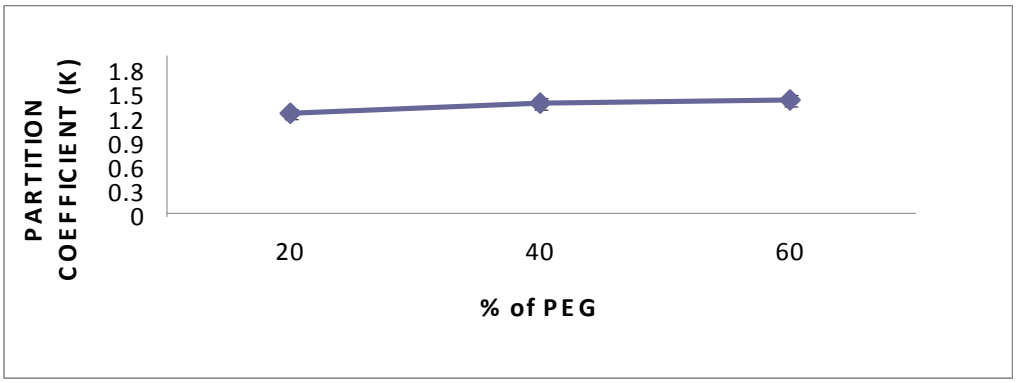


**Figure 3. Partition Coefficient (K) for ATPE**

**Lipase Assay**

Lipase enzyme extracted by ATPE was analysed using qualitative assay with congo red dye. Fig.4 shows the colour change around well (number markings) in the petriplate from red to yellow. This change in colour is due to the reduction of the fatty acids of olive oil which in turn leads to a reduction in pH range from value of below 4.4 to above 6. The zone of clearance with yellow was measured and to be 6mm, previously reported data for was about 12mm diameter zone around the agar well [5].

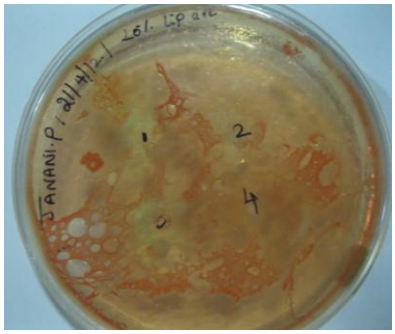

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**Figure 4. Qualitative Assay**

## Enzyme Activity

Enzyme activity checked (Figure 5) at various pH condition as an optimization process shows maximum activity at extraction buffer of pH 7 and activity value was 1.97U/ml/min. The appearance of pink color from colourless solution while titrating with NaOH indicates presence of lipase in extracted sample. The previously reported had maximum enzyme activity at pH of 7 and 7.5 [17]. Lactobacillus shows maximum lipase activity at pH 9, and this may be due to tolerating nature of species at alkaline nature [18].

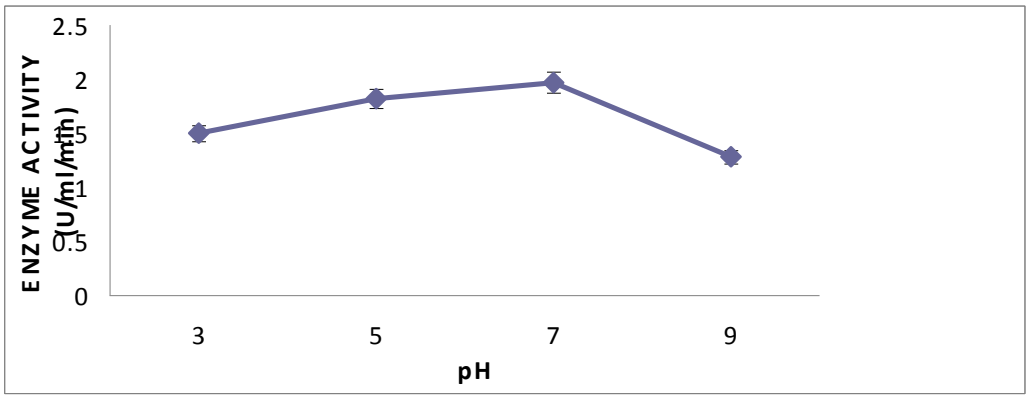


**Figure 5. Enzyme Activity at Different pH**

## CONCLUSION

Lipase is a biocatalyst mostly used in oleochemical industry. Sources such as oil cake, jack fruit waste, fungi, bacteria was also been used for synthesis of lipase. Microorganism is the better source for extracting lipase as it can tolerate various environmental factors, mostly preferred in laboratory conditions. ATPE is simple cost effective method for lipase extraction from rice bran. The extracted lipase reveals a partial coefficient of average value about 1.27 and the enzyme qualitative assay results in colour change from red dye into yellow 6mm diameter halo zone that conforms the presence of lipase in extracted sample. Optimized enzyme condition at various pH ranges shows a high value of activity at neutral pH and lipase unit activity was 1.97U/ml/min. Further the work can be carried out by optimizing the enzymatic conditions at various temperatures and the agitation speed.

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