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Advances in Applied Science Research, 2011, 2 (2): 265-271

ISSN: 0976-8610
CODEN (USA): AASRFC

Aqueous two phase extraction – of Lipase from Rice Bran

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ABSTRACT

Due to various application of lipase in medical and industrial field, aqueous two-phase extraction for the downstream processing of lipase has been exploited. The influence of system parameters such as phase forming salts, molecular weight of the phase forming polymer, system pH, tie line length, and phase volume ratio on the partitioning behavior of lipase was evaluated. The aqueous two-phase system consisting of PEG 4000 and Magnesium sulphate and it has shown better results with the purification of 1.648 fold. As PEG 4000 with Magnesium sulphate has shown better results so further work can be carried out using PEG 4000 as phase forming polymer.

Keywords: Liquid–liquid extraction - Lipase - Aqueous two phase extraction - Partition coefficient, Lipase, Rice Bran.

INTRODUCTION

Lipases (triacylglycerol acylhydrolases, E.C. 3.1.1.3) are ubiquitous enzymes of considerable physiological significance and industrial potential. Lipase catalyze the hydrolysis of triacylglcerols to glycerol and free fatty acids at oil-water interface. They have extensive application in food, pharmaceutical, paper, cosmetic, detergent, and leather industries.

Recent developments in biotechnology have opened up new avenues towards the production of many enzymes/proteins of important in research, pharmaceutical and industrial applications. There has been an increased interest in the development of efficient down streaming processing methods for separation, concentration and purification of enzymes/proteins. In recent years liquid-liquid extraction (LLE) such as aqueous two-phase extraction is gaining attention for primary purification and concentration of enzymes/proteins.

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. Keeping this in view, the present study entitled "aqueous two-phase extraction of rice bran lipase" explores the possibility of ATPE.

MATERIALS AND METHODS

Materials:

Polyethylene glycol, PEG (Mol. Wt 600, 1000,1500, 4000, 6000) was procured from SRL, Mumbai, India. di-potassium hydrogen phosphate, Potassium di-hydrogen phosphate, di-sodium orthophosphate, sodium di-hydrogen orthophosphate, Magnesium sulfate, were procured from Ranbaxy Chemicals, India. Petroleum ether (60-80ºC) were procured from Qualigens fine chemicals, Potassium phosphate buffer (PPB), gum acacia, triton, Calcium chloride, Solution A and Solution B, Bradford's reagent was procured from Ranbaxy Chemicals, Rice bran samples procured from Local rice mill. All chemicals used were of analytical grade.

Requirements for the analysis:

1. Water bath
2. Spectrophotometer
3. Magnetic stirrer
4. Vortex mixer

Extraction of lipase from rice bran

- Take 25 grams of rice bran
- Defatted using petroleum ether and filter it
- Drying it for 30 mins
- Extraction of rice bran lipase using potassium phosphate buffer.
- Add $Cacl_2$ and centrifuge at 5000 rpm for 20 mins
- Supernatant (crude lipase) was subjected to ATPE

Lipase assay:

For measurement of lipase activity the spectrophotometric method was used and p-nitro-phenyl-acetate (pNPA) was used as substrate. The method is as follows:

Solution A: 40mg of pNPA dissolved in 12ml of acetonitrile

Solution B: 0.1 g of gum acacia and 0.4 g of Triton-X-100 dissolved in 90ml of 200 mM potassium phosphate buffer, pH 7.0.

The substrate solution was prepared by adding 0.4 ml of solution A to 3.0 ml of solution drop wise under intense stirring to get an emulsion which remained stable for 1.5 hours. 0.05 ml of enzyme sample was added to 3.4 ml of substrate solution and p-nitrophenol liberation was measured at 410 nm in a Hitachi-U2000 spectrophotometer. One unit of lipase activity was defined as the amount of the enzyme needed to liberate 1 µmole of p-nitrophenol per hour at the conditions described for each essay system.

Protein determination

Brad ford's method was used for determination of protein. It is a rapid and sensitive assay for determining protein content in a solution [29]. This is done by using Bradford Reagent and measuring the absorbance at 595 nm at 25°C in UV spectrophotometer (Read and Northcote, 1981).

RESULTS AND DISCUSSION

The Partitioning behavior of the rice bran lipase was studied for different systems, PEG 600/Potassium phosphate, PEG1000/Potassium phosphate, PEG 1500/Potassium phosphate, PEG 4000/Potassium phosphate, PEG 6000/Potassium phosphate, PEG 20000/Potassium phosphate, PEG 6000/ Sodium phosphate, PEG 4000/ Magnesium sulfate and PEG 6000/ Magnesium sulfate. In order to understand the partition behavior of rice bran lipase, experiments were conducted to study the effect of the PEG molecular weight and the different phase forming salts and are discussed in the following section.

PEG600/Potassium phosphate and PEG1000/Potassium phosphate system:

The total lipase activity recovery was found to be 68.57% and 31.42%. lipase purification fold in these system was less due to the precipitation of proteins.

PEG 1500/Potassium phosphate system:

The total lipase activity recovery was found to be 52.28% and lipase purification was 1.128 fold.

PEG 4000/Potassium phosphate system:

The total lipase activity recovery observed was 57.05% and lipase purification was 1.648 fold.

PEG 6000/Potassium phosphate system:

The total lipase activity recovery was found to be 32.55% and lipase purification was very less due to the precipitation of proteins.

PEG 20000/Potassium phosphate system:

The total lipase activity recovery observed was 48.96% and lipase purification was found to be 1.77 fold.

PEG 4000/ Magnesium sulfate system:

The total lipase activity recovery observed was 74.72% and lipase purification was 4.25 fold that is very good.

PEG 6000/ Magnesium sulfate system:

The total lipase activity recovery was found to be 61.49% and lipase purification was less due to the precipitation of proteins.

Table 1: PEG 600/Potassium phosphate system

	Crude	Top	Bottom	Activity recovery (%)
Lipase activity (U/ml/hr)	14.398	15.29	3.13	
Volume (ml)	17.0	11.0	10	
Total activity (U)	244.98	168.25	31.39	68.57
Protein (mg/ml)	1.81	2.20	1.03	
Total protein (mg)	30.86	24	10.3	
Specific activity (U/mg)	2.485	2.168	0.9524	

Table 2: PEG1000/Potassium phosphate system

	Crude	Top	Bottom	Activity recovery (%)
Lipase activity(U/ml/hr)	14.398	8.0869	2.668	
Volume (ml)	17.80	10.0	12.0	
Total activity (U)	257.42	80.869	32.016	31.415
Protein (mg/ml)	1.81	1.802	1.4187	
Total protein (mg)	30.86	18	17.024	
Specific activity (U/mg)	2.605	1.4038	0.5876	

Table 3: PEG 1500/Potassium phosphate system

	Crude	Top	Bottom	Activity recovery (%)
Lipase activity (U/ml/hr)	23.99	23.38	9.075	
Volume (ml)	18.60	10.00	12.00	
Total activity (U)	447.17	233.80	108.90	52.28
Protein (mg/ml)	1.795	1.55	1.81	
Total protein (mg)	33.45	15.50	21.816	
Specific activity(U/mg)	13.36	15.08	4.99	

Table 4: PEG 4000/potassium phosphate system

	Crude	Top	Bottom	Activity recovery (%)
Lipase activity(U/ml/hr)	17.808	19.306	12.702	
Volume (ml)	18.90	10.00	12.00	
Total activity (U)	338.35	193.06	152.42	57.05
Protein (mg/ml)	1.8460	1.2143	1.9568	
Total protein (mg)	35.074	12.143	23.481	
Specific activity (U/mg)	9.646	15.90	6.49	

Table 5: PEG 6000/Potassium phosphate system

	Crude	Top	Bottom	Activity recovery (%)
Lipase activity (U/ml/hr)	0.8420	0.120	0.396	
Volume (ml)	20.20	10.00	14.00	
Total activity (U)	17.067	1.02	5.556	32.55
Protein (mg/ml)	1.064	1.02	1.145	
Total protein (mg)	21.582	8.707	16.034	
Specific activity (U/mg)	0.791	0.118	0.35	

Table 6: PEG 20,000/Potassium phosphate system:

	Crude	Top	Bottom	Activity recovery (%)
Lipase activity (U/ml/hr)	24.68	20.154	21.22	
Volume (ml)	20.00	12.00	10.00	
Total activity (U)	493.97	241.85	212.20	48.96
Protein (mg/ml)	2.923	1.345	3.858	
Total protein (mg)	59.19	16.14	38.58	
Specific activity(U/mg)	8.44	14.98	5.50	

Table 7: PEG 4000/ Magnesium sulfate system

	Crude	Top	Bottom	Activity recovery (%)
Lipase activity (U/ml/hr)	11.872	10.645	9.91	
Volume (ml)	16.80	14.00	9.00	
Total activity (U)	199.44	149.03	89.15	74.72
Protein (mg/ml)	1.4618	0.308	1.905	
Total protein (mg)	24.55	4.311	17.145	
Specific activity(U/mg)	8.1214	34.57	5.196	

Table 8: PEG 6000/ Magnesium sulfate system

	Crude	Top	Bottom	Activity recovery (%)
Lipase activity (U/ml/hr)	3.135	0.2306	2.691	
Volume (ml)	16.70	10.00	12.00	
Total activity (U)	52.511	2.306	32.292	61.49
Protein (mg/ml)	1.816	0.354	1.715	
Total protein (mg)	30.34	3.539	20.58	
Specific activity (U/mg)	1.730	0.652	1.569	

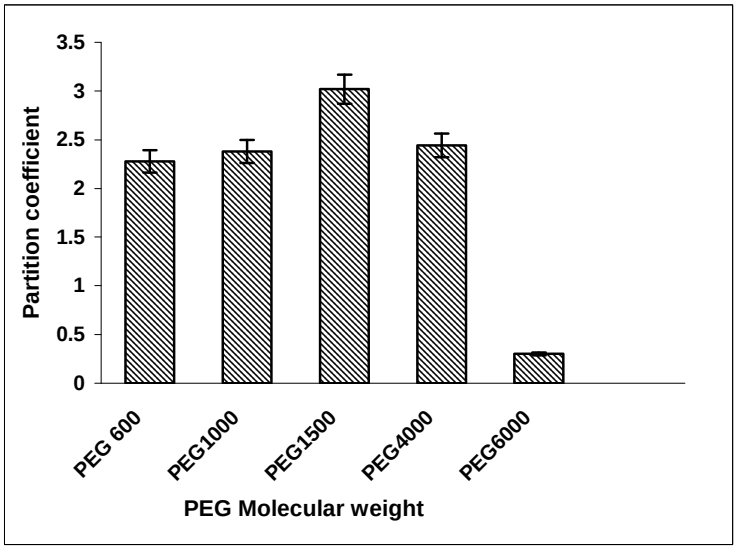

Fig 1: Effect of PEG molecular weight on lipase partitioning

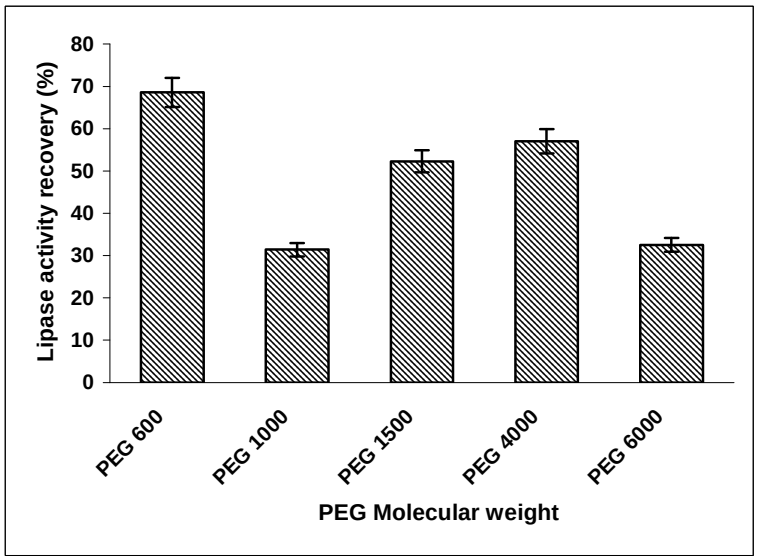

Fig 2: Effect of PEG molecular weight on lipase activity recovery

CONCLUSION

The Partitioning behavior of the rice bran lipase was studied for different systems. As observed from the above results there is a loss in activity in case of PEG 6000 phase system due to precipitation of the protein. The partitioning coefficient was high in case of PEG 4000 and it has shown better results with the purification of 1.648 fold. As PEG 4000 with Magnesium sulphate has shown better results so further work can be carried out using PEG 4000 as phase forming polymer with different salts.

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