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Partitioning of Porcine Pancreatic Lipase in a Two-Phase Systems of Polyethylene Glycol/Potassium Phosphate Aqueous

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Abstract The hydrolysis of triglycerides at the oil–water interface, synthesis of esters and transesterification in microaqueous conditions are catalysed by lipase. For its application, a proper purification method was necessary. This study examined the application of an aqueous two-phase system to partition porcine pancreatic lipase. The influence of molecular weight and concentration of polyethylene glycol (PEG), tie line length (TLL), potassium phosphate concentration, sodium chloride (NaCl) addition and temperature in the partition was studied. The enzyme was more efficiently purified in PEG 8,000 at 14.5 °C (PF=3.89-fold), presenting more recoveries at the top phase with shorter TLL and lower concentrations of PEG and potassium phosphate. Moreover, the increase of these variables repressed the purification and the further addition of NaCl did not promote the purification of the enzyme. These results demonstrated the efficiency of the aqueous two-phase system on lipase purification.

Keywords Aqueous two-phase system · Lipase · Enzymes · Purification · PEG

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Introduction

Lipases (EC 3.1.1.3) are a class of hydrolases that catalyse a wide range of reactions including hydrolysis, interesterification, alcholysis, acidolysis, esterification and aminolysis. They catalyse the hydrolysis of fatty acid esters bound in the triacylglycerol and release free fatty acids. The reaction is reversible and the direction of the reaction depends on the water content available in the reaction. In low-water media, lipase catalyses esterification, transesterification and interesterification [1, 2].

Since the mid 1980s, there has been a growing interest in lipases, especially in those of microbial origin, due to the different reactions they are able to catalyse and their region- and enantioselectivity [3]. Lipases present uses in numerous applications, for example in detergents, foods, pharmaceuticals, fine chemicals, leather, pulp and paper industries. Promising fields for application of lipases include the biodegradation of plastics, such as polyhydroxyalkanoates and polycaprolactone and the resolution of racemic mixtures to produce optically active compounds [4–6]. The critical factor for the use of lipase in the biotransformation process is the water activity present in the system because it effects the reaction's speed, production yield, enzyme activity and reaction selectivity [7–9]. More widespread industrial applications of these enzymes would depend on the development of low-cost processes for the production and purification of lipases.

As well as the desired components, the fermentation process generates by-products that frequently hinder the use of the fermented broth in industrial procedures. For the success of commercial production of enzymes and proteins, efficient downstream processing techniques are needed. When these processes are applied to biological materials, purification steps that are delicate enough to preserve the biological activity are required. However, these protocols increase the cost of the process and decrease the yield of the reaction [10]. The conventional procedure includes ammonium sulphate precipitation, chromatography, dialysis and filtration [11–13].

An effective and economically viable method for the separation and purification of lipase is the partitioning in an aqueous two-phase system (ATPS). The ATPS can be formed by combining either two incompatible polymers or a polymer and salt in water, above a certain critical concentration. These systems are attractive due to low costs, rapid phase disengagement and the availability of commercial separators, which allow faster continuous protein separation [14].

The goal of this work is to determine the application of an aqueous two-phase system (polyethylene glycol (PEG)/potassium phosphate) as a step to purify lipase, studying the influence of PEG and potassium phosphate concentrations, tie line length, NaCl addition and temperature in the partition of porcine pancreatic lipase.

Experimental Procedures

Materials and Enzyme

The reagents utilised include analytical grade PEG with nominal molecular weights of 1,500 and 8,000 g/mol, and porcine pancreatic lipase were purchased from Sigma (USA). Potassium phosphate and sodium chloride were also of analytical grade and were purchased from Sinth (Brazil). The polymer and salt were used without further purification. Water previously distilled and de-ionised by a Milli-Q device (Millipore, USA) was used throughout the experiments.

Phase Diagram

The phase diagram was obtained from the available literature [15, 16]. The mixture points were chosen by the phase diagram that promotes different lengths of tie line, which were defined by Eq. 1 [17]:

$$\mathrm{TLL} = \sqrt{(\Delta W_{\mathrm{T}} - \Delta W_{\mathrm{B}})^2_{\mathrm{PEG}} + (\Delta W_{\mathrm{T}} - \Delta W_{\mathrm{B}})^2_{\mathrm{Phosphate}}} \quad (1)$$

where TLL is the tie line length and ΔW is the weight percentages of phase-forming components in the top (T) and bottom (B), respectively.

Aqueous Two-Phase Systems

The systems were prepared from stock solutions of PEG (50%, *w*/*v*) by a phosphate buffer (at pH 7.0), which was formed as a mixture of potassium phosphate monobasic and bibasic (50%, *w*/*v*) in a ratio of 1.087 (*w*/*w*), and by sodium chloride (30%, *w*/*v*), all stored at 4 °C. The amount of the commercial enzyme preparation added to the systems was always 10% of the system's total volume (10 ml) and was the last component added to the system. All systems were prepared in graduated tubes. After 2 min of gently stirring, the systems were centrifuged at 4 °C and 3,000 g for 10 min. The tubes were brought to equilibrium in a thermostatic bath at 4 °C and local atmospheric pressure (1 atm) overnight (at least 12 h). After this treatment, the two phases became clear and transparent and the interface was well defined. The phases were carefully withdrawn using a pipette for the top phase and a syringe with a long needle for the bottom phase. The volumes were determined in graduated test tubes [10].

The partition coefficient is defined as the protein concentration (K_{p}) or enzyme activity (K_{e}) in the top phase, divided by the corresponding value in the bottom phase. In order to evaluate the purification process, the enzyme specific activity (expressed in units per milligram protein), the volume ratio (R_{v}), the enzyme recovered in the top phase ($R_{\mathrm{T}}-\%$) and bottom phase ($R_{\mathrm{B}}-\%$) and purification factor (PF) were calculated according Lima et al. [10].

The extraction efficiency of the enzyme was calculated by using the following Eq. 2:

$$E = \frac{EA_{\mathrm{T}}V_{\mathrm{T}}}{EA_{\mathrm{T}}V_{\mathrm{T}} + EA_{\mathrm{B}}V_{\mathrm{B}}} = \frac{K_{\mathrm{E}}R_{\mathrm{V}}}{1 + K_{\mathrm{E}}R_{\mathrm{V}}} \quad (2)$$

The partition experiments were carried out in triplicate and the average results are the values reported in the present work.

Enzyme Assay

Lipolytic activities were assayed using the oil emulsion method according to a modification used by Soares et al. [18]. The substrate was prepared by mixing 50 ml of olive oil with 50 ml of Arabic gum solution (7% *w*/*v*). The reaction mixture containing 5 ml of the oil emulsion, 2 ml of 100 mM sodium phosphate buffer (pH 7.0) and enzyme extract (1 ml) was incubated in a thermostated batch reactor for 60 min at 37 °C. Samples were taken at 5, 10, 15, 30 and 60 min. A blank titration was done on a sample where the enzyme was replaced with distilled water. The reaction was stopped by the addition of 10 ml of acetone–ethanol solution (1:1). The liberated fatty acids were titrated with 25 mM potassium hydroxide solution in the presence of phenolphthalein as an indicator. One unit of enzyme

activity was defined as the amount of enzyme that liberated 1 μmol of free fatty acid per minute (micromole per minute) under the assay conditions (37 °C, pH 7.0, 150 rpm).

Protein Assay

Total protein concentration was determined by Bradford's method [19] using bovine serum albumin as standard.

Results and Discussion

Influence of PEG Concentration on the Lipase Partition

The effects of PEG 1,500 and 8,000 g/mol on the partitioning of pancreatic porcine lipase are shown in Fig. 1 and Table 1. The volume ration was found to be above 4.0, which demonstrated that the top phase was formed in a bigger volume with PEG 1,500 g/mol and the trend of the curves was similar for PEG 8,000 and 1,500 g/mol. The R_v values were practically constant until 25% concentration of PEG 1,500 and 8,000.

Moreover, the partition coefficients were found below 1.0, indicating that the lipase accumulated in the bottom phase. These results suggest the high hydrophilicity of the enzyme. Similarly, Ribeiro et al. [20] observed the partition of glucose-6-phosphate dehydrogenase at the bottom phase in aqueous two-phase PEG and phosphate systems. The partition coefficients decrease with PEG concentration above 25%, in which the highest values were found (0.36±0.05 and 0.50±0.11 for PEG 1,500 g/mol and PEG 8,000 g/mol, respectively). According to Babu et al. [21] this decrease in partition coefficient of lipase is due to the influence of volume exclusion. In fact, the volume occupied by the PEG increases with an increase in polymer concentration, which resulted in a reduced space for biomolecules in the top phase, then the compound tends to partition in the bottom phase.

It was verified that the decrease in concentration of PEG enabled the increment of recoveries, efficiency and purification factors in the top phase. The values of the recovery and extraction efficiency were highest at 25% in PEG 1,500 g/mol (R_t=53.67 and E=64.08%) and 20% in PEG 8,000 g/mol (R_t=74.49% and E=74.33%), however the values of

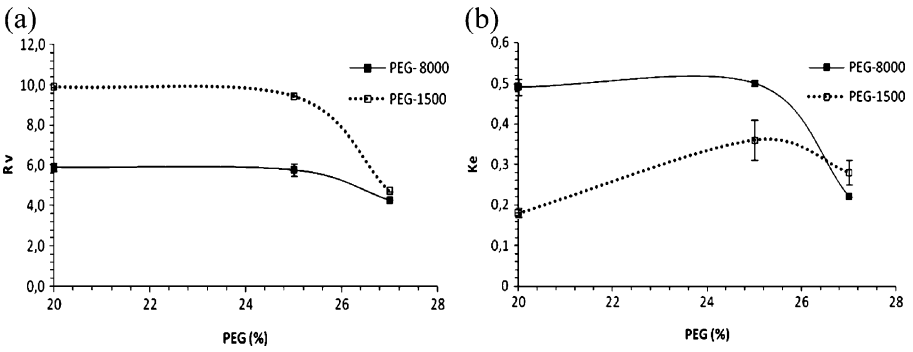

Fig. 1 Influence of PEG concentration on the volume ration between the phases (**a**) and partition of porcine pancreatic lipase (**b**) in the system containing 18% of potassium phosphate, without NaCl, at pH 7.0 and 4 °C

Table 1 Effects of PEG concentration on the partition of porcine pancreatic lipase in system containing 18% of potassium phosphate, without NaCl, at pH 7.0 and 4 °C.

PEG (%)	V_b (ml)	V_t (ml)	AS_b (mg/l)	AS_t (mg/l)	AS_i (U/ml)	Cp_b (mg/ml)	Cp_t (mg/ml)	Cp_i (mg/ml)	R_t (%)	E (%)	PF_t
PEG 1,500											
20	10.9±0.1	1.1±0.0	53.50±5.45	82.62±4.36	56.54±5.34	0.68±0.00	0.08±0.00	0.76±0.00	53.67±0.00	64.08	1.46±0.06
25	10.6±1.9	1.2±0.2	77.91±1.39	116.39±19.04	85.45±4.85	0.22±0.00	0.05±0.00	0.29±0.00	69.41±4.35	77.26	0.91±0.04
27	9.4±0.0	2.0±0.0	161.15±8.44	187.27±10.25	166.27±4.78	0.22±0.00	0.05±0.00	0.29±0.00	53.41±0.00	56.82	0.97±0.02
PEG 8,000											
20	9.8±0.1	1.6±0.1	156.83±10.98	42.25±1.01	55.73±2.18	0.24±0.00	0.03±0.00	0.27±0.00	74.49±1.56	74.33	2.81±0.09
25	10.1±0.1	1.8±0.1	87.19±7.30	29.98±3.80	38.25±2.25	0.41±0.00	0.07±0.00	0.46±0.00	73.91±3.05	73.29	2.29±0.33
27	9.4±0.0	2.2±0.0	175.97±10.01	195.54±11.35	191.63±11.08	0.21±0.00	0.05±0.00	0.26±0.00	49.01±0.03	48.44	0.92±0.00

AS_i specific activity in the entry system, Cp_i protein concentration in the entry system

these parameters at 20 and 25% of PEG concentration do not differ much. Associated to this fact, the biggest purification factors occurred at 20% for both molecular weights of PEG (1.46- and 2.81-fold at PEG 1,500 g/mol and PEG 8,000 g/mol, respectively). For this reason the concentration of PEG chosen for the following experiments was 20%.

Influence of Potassium Phosphate Concentrations

To observe the effect of differing potassium phosphate concentrations, a series of solutions containing 20% PEG (1,500 and 8,000 g/mol) and 18–28% of potassium phosphate were assessed. Figure 2 and Table 2 show the effect of varying concentrations of potassium phosphate on the partitioning of porcine pancreatic lipase.

The concentration of potassium phosphate significantly influenced the purification of porcine pancreatic lipase. The increase in phosphate concentration allowed the chaotropic capacity of the system to increase. Chaotropic salts have smaller negative or even positive values for Gibbs free energy of hydratation and thus formed the better system. Thus, the volume ration decreased from 9.91 to 2.19 and from 5.91 to 1.87 for PEG 1,500 and PEG 8,000 g/mol, respectively, with increasing phosphate concentrations. For the partition coefficients presented for PEG 1,500 g/mol, it was noted that the highest value was found at a concentration of 20% phosphate (0.52 ± 0.07), whereas for PEG 8,000 g/mol the lowest concentrations offered the highest partition coefficient (0.49±0.02 for the phosphate concentration of 18%). Overall, it appears that the increase in phosphate concentration made the bottom phase more hydrophilic and this enabled the enzyme to go through the hydrophilic phase and consequently the K_e to decrease.

The efficiency of lipase partition was negatively effected by the increase in phosphate concentration. The highest values were 66.22% and 74.33% for the 20% (PEG 1,500 g/mol) and 18% (PEG 8,000 g/mol) concentrations, respectively. The results of recoveries indicated two distinct regions, one with a higher recovery at the top and the other one at the bottom. The change occurred at a concentration of approximately 22% for both molecular weights of PEG. In the top phase, the highest recoveries were observed (66.19±3.07 at 20% of phosphate and PEG 1,500 g/mol and 74.49± 1.55 at 18% of phosphate and PEG 8,000 g/mol).

The purification factors were highest in the top phase when the lowest potassium phosphate concentration was used. The best result of lipase purification was obtained with 20% of phosphate for PEG 1,500 g/mol (1.46±0.06-fold) and 18% of phosphate for PEG 8,000 g/mol (2.81 ± 0.09-fold).

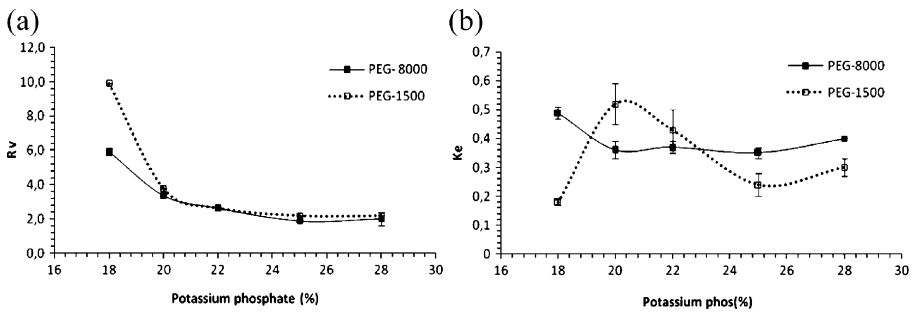

Fig. 2 Influence of potassium phosphate concentration on the volume ration between the phases (**a**) and partition of porcine pancreatic lipase (**b**) in system containing 20% of PEG, without NaCl, at pH 7.0 and 4 °C

Table 2 Effects of potassium phosphate concentration on the partition of porcine pancreatic lipase in system containing 20% of PEG, without NaCl, at pH 7.0 and 4 °C.

Phos. (%)	V_b (ml)	V_t (ml)	AS_b (mg/l)	AS_t (mg/l)	AS_i (U/ml)	Cp_b (mg/ml)	Cp_t (mg/ml)	Cp_i (mg/ml)	R_t (%)	E (%)	PF_t
PEG 1,500											
18	10.9±0.0	1.1±0.0	53.50±5.46	82.62±4.36	56.54±5.34	0.68±0.00	0.80±0.00	0.76±0.00	64.63±3.80	64.08	1.46±0.06
20	9.1±0.0	2.4±0.0	51.18±2.23	85.60±7.37	59.35±0.05	0.19±0.00	0.06±0.00	0.24±0.00	66.19±3.07	66.22	1.44±0.12
22	8.2±0.1	3.1±0.1	27.98±0.81	37.75±4.77	30.34±0.54	0.76±0.00	0.24±0.00	1.01±0.00	53.00±3.39	53.07	1.24±0.14
25	7.8±0.1	4.1±0.1	127.72±3.98	94.64±12.79	119.64±0.12	0.18±0.00	0.06±0.00	0.24±0.00	34.35±3.65	34.45	0.79±0.11
28	7.9±0.0	3.6±0.0	33.65±1.41	30.20±3.90	32.78±2.04	0.74±0.00	0.25±0.00	0.99±0.00	39.87±2.21	39.65	0.92±0.06
PEG 8,000											
18	9.8±0.1	1.6±0.1	42.25±1.01	156.83±10.98	55.73±2.19	0.24±0.00	0.03±0.00	0.27±0.00	74.49±1.56	74.33	2.81±0.09
20	9.1±0.1	2.7±0.0	75.04±7.53	144.21±4.21	86.00±7.01	0.18±0.00	0.03±0.00	0.21±0.00	54.97±2.15	54.82	1.68±0.09
22	8.4±0.1	3.2±0.0	30.58±3.26	37.22±1.46	36.75±2.78	0.75±0.00	0.27±0.00	1.02±0.00	48.91±1.46	49.13	1.01±0.04
25	7.6±0.2	4.1±0.0	92.31±4.96	123.19±13.07	98.78±6.66	0.17±0.00	0.05±0.00	0.22±0.00	39.73±0.59	39.56	1.25±0.05
28	7.9±0.0	4.0±0.8	34.37±0.69	38.81±0.80	35.53±0.72	0.73±0.00	0.26±0.00	0.99±0.00	44.47±4.89	44.27	1.09±0.00

Phos. phosphate, AS_i specific activity in the entry system, Cp_i protein concentration in the entry system

Due to these results, in further purification protocols of lipase, 18% of potassium phosphate concentration in the partition system was used because the highest PF_t was found in PEG 8,000 g/mol.

Influence of Tie Line Length

The effect of tie line length is shown in Fig. 3. The value of TLL was determined using Eq. 1. Increase in the tie line length is related to the increase in the hydrophobicity of the

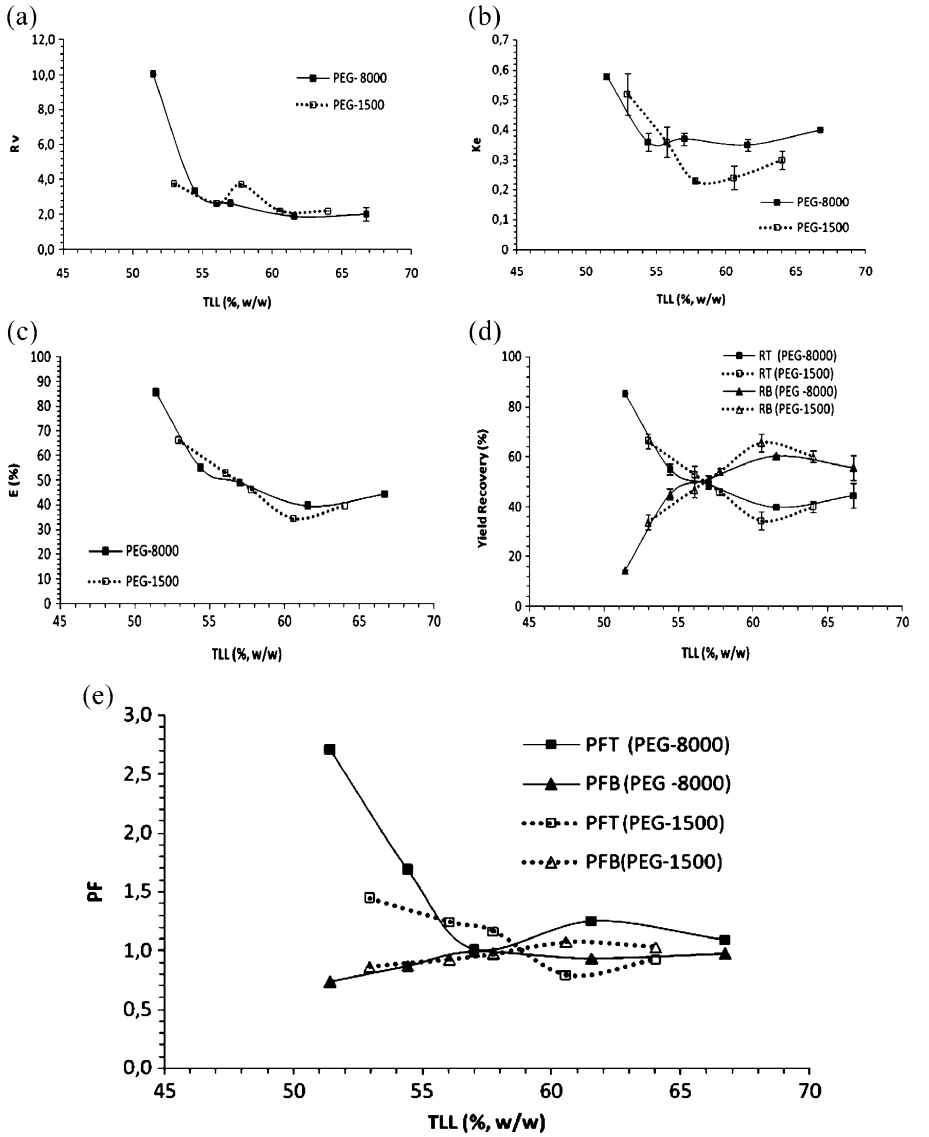

Fig. 3 Influence of tie line length in the volume ration between the phases (**a**), partition coefficient (**b**), efficiency (**c**), recovery (**d**), and purification factor (**e**) in the aqueous two-phase system applied to porcine pancreatic lipase without NaCl at pH 7.0 and 4 °C

top phase in an ATPS and also to the interface potential between the two phases [15]. This increase of the hydrophobicity favoured partitioning of porcine pancreatic lipase to the bottom phase. The same verification was obtained by Antov et al. [17] working on pectinase partitioning in an aqueous two-phase system using PEG and ammonium sulphate.

The TLL increase induced a decrease in the volume ration due to the decrease of the free volume of the bottom phase and furthermore promoted the protein partition to the top phase. The partition coefficients of bulk protein increased from 0.12 (TLL=50.17%) to 0.34 (TLL=66.74%) and from 0.13 (TLL=50.17%) to 0.36 (TLL=66.74%) for PEG 1,500 and PEG 8,000 g/mol, respectively (data not shown). Rito-Palomares and Hernández [22] showed similar observations in partitioning of cheese whey proteins. The enzyme was more partitioned to the bottom phase and the K_e decreased differently from TLL value. Malpiedi et al. [23] also showed that typsinogen from bovine pancreas partitioned to the bottom phase with the increase of TLL.

The efficiency of partitioning (E) followed the behaviour of R_t and the best values found were approximately 52% of TLL (E=66.22 and R_t=66.19% for PEG 1,500 and E=85.32% and R_t=85.35% PEG 8,000 g/mol, respectively). These results showed that the recovery of lipase was greater in the top phase for shorter TLL and in the bottom phase for longer TLL, with an inflexion point observed at 57% of TLL.

As the TLL increased, the volume of the bottom phase increased (R_v decrease), moreover, the phase became more hydrophilic and part of the enzyme went to the bottom phase, (K_e<0). Associated with this is the fact that K_p increased (protein went to the top phase), but this migration is not sufficiently to the PF_t increase with the increase of TLL. The highest values of PF_t were found in the shortest TLL.

Influence of the Addition of NaCl

Figure 4 and Table 3 show the effect of addition of NaCl on the purification process of porcine pancreatic lipase. The K_e decreased with the addition of NaCl. Lima et al. [10] also applied an aqueous two-phase system to purify pectinase and verified the reduction of the partition coefficient of the enzyme. In the system containing NaCl severe repression of the recovery, efficiency and purification factor were observed and the enzyme went to the bottom phase. Bradoo et al. [24] noted that the addition of NaCl to the system promoted the partitioning of lipase from *Bacillus stearothermophillus* to the top phase, whereas Bassani et al. [25] verified that addition of NaCl was implied in the reduction of the partition

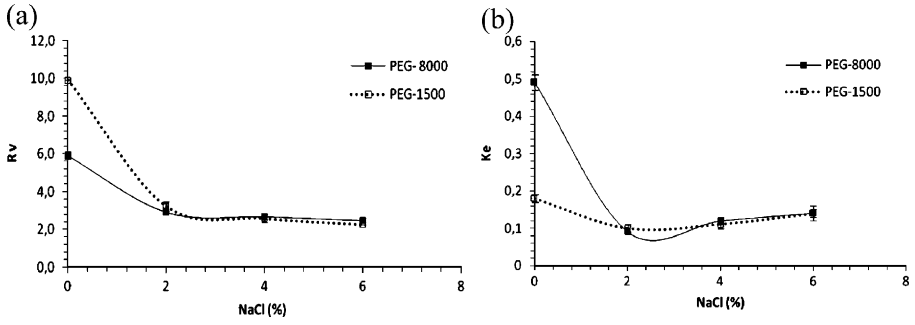

Fig. 4 Influence of NaCl concentration on the volume ration between the phases (**a**) and partition of porcine pancreatic lipase (**b**) in the system containing 20% of PEG and 18% of potassium phosphate at pH 7.0 and 4 °C

Table 3 Effects of NaCl concentration on the partition of porcine pancreatic lipase in system containing 20% of PEG and 18% of potassium phosphate at pH 7.0 and 4 °C.

NaCl (%)	V_b (ml)	V_t (ml)	AS_b (mg/l)	AS_t (mg/l)	AS_i (U/ml)	Cp_b (mg/ml)	Cp_t (mg/ml)	Cp_i (mg/ml)	R_t (%)	E (%)	PF_t
PEG 1,500											
0	10.9±0.0	1.1±0.0	53.50±5.46	82.62±4.36	56.54±5.34	0.68±0.00	0.08±0.00	0.76±0.00	64.18±1.14	64.08	1.46±0.06
2	8.6±0.2	2.7±0.1	137.45±10.23	74.54±0.52	127.12±7.06	0.24±0.00	0.05±0.00	0.29±0.00	25.03±0.81	24.03	0.59±0.03
4	8.1±0.0	3.1±0.0	194.83±32.53	101.15±5.16	177.69±23.98	0.18±0.02	0.04±0.00	0.22±0.01	22.62±0.83	22.04	0.58±0.11
6	7.7±0.0	3.4±0.0	335.23±29.58	65.65±12.29	223.27±30.25	0.10±0.00	0.07±0.01	0.17±0.00	23.89±0.37	24.04	0.29±0.02
PEG 8,000											
0	9.8±0.1	1.6±0.1	42.25±1.01	156.83±10.98	55.73±2.19	0.24±0.00	0.03±0.00	0.27±0.00	74.49±1.56	74.33	2.81±0.09
2	8.6±0.1	3.0±0.1	166.79±8.75	92.84±16.27	156.82±10.88	0.23±0.00	0.03±0.00	0.26±0.00	19.82±0.34	20.70	0.59±0.06
4	8.2±0.1	3.1±0.1	199.22±87.87	67.62±0.98	163.22±57.63	0.13±0.04	0.04±0.00	0.17±0.02	24.15±2.93	24.20	0.44±0.15
6	8.0±0.1	3.2±0.2	263.74±8.93	33.36±5.66	143.30±6.88	0.09±0.00	0.10±0.00	0.19±0.00	25.40±3.59	25.69	0.23±0.03

AS_i specific activity in the entry system, Cp_i protein concentration in the entry system

coefficient to PEG molecular weights over 4,000 g/mol. This corroborates the results of this study, however, the NaCl addition of 3% promoted a purification of eightfold. Due to these results, systems without the addition of NaCl were used in subsequent experiments.

Influence of System Temperature

The effect of temperature in the purification process of porcine pancreatic lipase using an aqueous two-phase system is shown in Fig. 5 and Table 4.

Lipase purification using PEG 1,500 and PEG 8,000 g/mol was carried out more efficiently at 14.5 °C. At this temperature, the best values for efficiency were verified (79.45% and 83.40% for PEG 1,500 and PEG 8,000 g/mol, respectively), recovery in the top phase (79.45±4.29% and 83.11±1.90% for PEG 1,500 and PEG 8,000 g/mol, respectively) and purification factor (1.50±0.01 and 3.89±0.42 for PEG 1,500 and PEG 8,000 g/mol, respectively). The increase in temperature did not promote the purification of lipase, probably due to deactivation of the enzyme.

Liu et al. [11] obtained a purification factor of 3.4-fold for lipase purification and recovery of 4.8% after using precipitation with ammonium sulphate, dialysis and application of a Sephadex G-75 column and DEAE Sepharose fast-flow anion-change column. The best purification factor found in the present work (3.89-fold) leads us to conclude that the aqueous two-phase system is a good process to use for lipase purification because a single step promoted a purification factor similar to that found in the pre-purification steps and the best enzyme recovery (83.40%).

Comparatively, the results obtained with the PEG 1,500 and 8,000 g/mol observed that the different degrees of PEG polymerization influenced the lipase purification efficiency. The increase of PEG molecular weight from 1,500 to 8,000 g/mol allowed us to find the best values of purification factor, recoveries and efficiency. Yang et al. [26] also observed the same effect when the aqueous two-phase system was applied to purify xylanase from *Paecilomyces thermophila*.

Conclusions

The purification process of porcine pancreatic lipase was more efficient when the system was formed with 20% PEG and 18% potassium phosphate (PF_t = 1.45 for PEG 1,500 g/mol

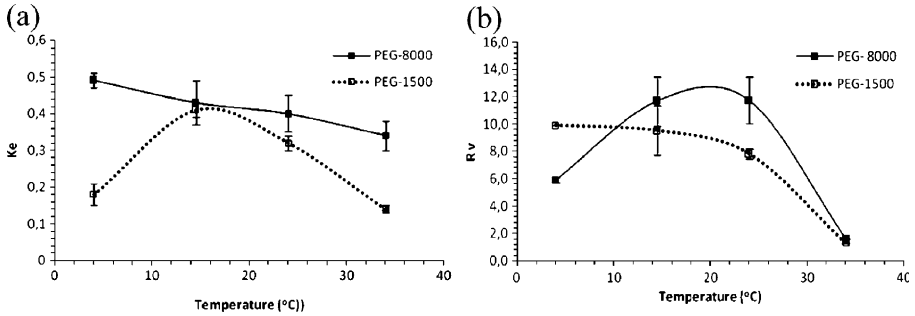

Fig. 5 Influence of temperature on the volume ration between the phases (**a**) and partition of porcine pancreatic lipase (**b**) in system containing 20% of PEG, 18% of potassium phosphate and without NaCl at pH 7.0

Table 4 Effects of temperature on the partition of porcine pancreatic lipase in system containing 20% of PEG, 18% of potassium phosphate and without NaCl, at pH 7.0.

T (°C)	V_b (ml)	V_t (ml)	AS_b (mg/l)	AS_t (mg/l	AS_i (U/ml)	Cp_b (mg/ml)	Cp_t (mg/ml)	Cp_i (mg/ml)	R_t (%)	E (%)	PF_t
PEG 1,500											
4	1.1±0.0	10.9±0.0	53.50±5.46	82.622±4.36	56.54±5.34	0.68±0.00	0.80±0.00	0.76±0.00	61.38±1.14	64.08	61.38±1.14
14.5	1.1±0.1	10.8±0.1	139.67±11.64	238.53±23.83	159.01±14.68	0.11±0.01	0.03±0.00	0.14±0.01	79.45±4.09	79.61	79.45±4.09
24	1.4±0.1	10.6±0.1	133.32±15.10	135.67±21.32	133.15 ± 6.39	0.10±0.00	0.03±0.01	0.13±0.00	71.53±1.97	71.45	71.53±1.97
34	4.8±0.1	6.3±0.1	367.21±41.06	190.33±30.59	327.52±18.93	0.05±0.01	0.02±0.01	0.07±0.00	15.58±0.59	15.40	15.58±0.59
PEG 8,000											
4	1.6±0.1	9.8±0.1	42.25±1.01	156.83±10.98	55.73±2.19	0.24±0.00	0.03±0.00	0.27±0.00	74.49±1.56	74.33	2.81±0.09
14.5	0.9±0.1	10.8±0.2	35.71±0.79	183.36±26.61	47.09±2.81	0.32±0.00	0.03±0.00	0.35±0.00	83.11±1.90	83.40	3.89±0.42
24	0.9±0.1	10.8±0.2	151.90±9.19	178.67±11.22	158.39±5.77	0.13±0.00	0.04±0.00	0.17±0.00	82.02±2.41	82.46	1.13±0.09
34	4.2±0.0	6.6±0.1	391.37±46.51	167.38±51.03	287.99±14.49	0.03±0.00	0.02±0.00	0.05±0.00	34.47±2.95	34.80	0.58±0.15

AS_i specific activity in the entry system, Cp_i protein concentration in the entry system

and PF_t = 2.81 for PEG 8,000 g/mol). Longer tie line length and NaCl addition in the aqueous two-phase system had a negative effect on the porcine pancreatic lipase. Temperature over 24 °C decreased the recovery, efficiency and purification factor of this enzyme, in fact, at 14.5 °C the best purification of lipase (1.50 and 3.89 for PEG 1,500 e PEG 8,000 g/mol, respectively) was observed.

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