Response surface methodology modelling of an aqueous two-phase system for purification of protease from Penicillium candidum (PCA 1/TT031) under solid state fermentation and its biochemical characterization

ABSTRACT

Penicillium candidum (PCA 1/TT031) synthesizes different types of extracellular proteases. The objective of this study is to optimize polyethylene glycol (PEG)/citrate based on an aqueous two-phase system (ATPS) and Response Surface Methodology (RSM) to purify protease from *Penicillium candidum* (PCA 1/TT031). The effects of different PEG molecular weights (1500-10,000 g/mol), PEG concentration (9%-20%), concentrations of NaCl (0%-10%) and the citrate buffer (8%-16%) on protease were also studied. The best protease purification could be achieved under the conditions of 9.0% (*w*/*w*) PEG 8000, 5.2% NaCl, and 15.9% sodium citrate concentration, which resulted in a one-sided protease partitioning for the bottom phase with a partition coefficient of 0.2, a 6.8-fold protease purification factor, and a yield of 93%. The response surface models displayed a significant ($p \leq 0.05$) response which was fit for the variables that were studied as well as a high coefficient of determination (R^2). Similarly, the predicted and observed values displayed no significant ($p > 0.05$) differences. In addition, our enzyme characterization study revealed that *Penicillium candidum* (PCA 1/TT031) produced a slight neutral protease with a molecular weight between 100 and 140 kDa. The optimal activity of the purified enzyme occurred at a pH of 6.0 and at a temperature of 50 °C. The stability between different pH and temperature ranges along with the effect of chemical metal ions and inhibitors were also studied. Our results reveal that the purified enzyme could be used in the dairy industry such as in accelerated cheese ripening.

Keyword: Aqueous two phase systems; Penicillium candidum; RSM; Protease; Solid state fermentation