CALIFORNIA STATE SCIENCE FAIR
2014 PROJECT SUMMARY

Name(s)

Hope Lee

Project Number

S0616

Project Title

Optimization of Enzyme (Bromelain) Recovery Using Polymer-Salt Aqueous Two Phase Systems

Abstract

Objectives/Goals

The purpose of this study was to determine the optimization conditions of enzyme recovery in polymer-salt aqueous two phase systems (ATPS) by manipulating three factors: polyethylene glycol (PEG) concentration, PEG molecular weight, and phosphate solution concentration.

Methods/Materials

The equipment used were pipettes, a pipette gun, pipetmen, centrifuge tubes, a tube rack, syringes, filters, a balance, a volumetric flask, nalgene bottles, an UV spectrophotometer, a thermometer, a radiator, and a water bath. Materials used were PEG (three molecular weights), dibasic and monobasic potassium phosphate, bromelain powder, pineapples, L-cystein HCl#ªH2O, EDTA, NaOH, trichloroacetic acid, sodium acetate, acetic acid, casein, pure L-tyrosine, and water.

The study was divided into four phases. For the first phase, standard curves for L-tyrosine and enzymatic activity were created. In phase 2, ATPS partitioning behavior was tested to evaluate the effects of three factors on the ratio of PEG phase volume to total volume. In Phase 3, each factor was tested individually and its effects observed. In Phase 4, two design of experiment (DOE) quadratic models were created to optimize enzyme recovery. Casein Digestion Unit (CDU) analysis was used to test the enzyme reactivity of each sample.

Results

In phase 1, high precision standard curves for L-tyrosine and enzyme concentration were created with R2 values of 0.9986 and 0.9994, respectively. For phase 2, the partitioning ratios ranged from 0 to 0.57. PEG concentration and phosphate buffer concentration were identified as significant factors of partitioning behavior. In phase 3, the extraction percentage increased from 50% to 90% when PEG molecular weight increased, decreased from 43% to 6% when phosphate concentration increased, but showed no consistent trend when PEG concentration increased. In phase 4A (PEG10,000), a maximum of 45.607 mg protein was recovered from pineapple juice. In phase 4B (PEG3350 and 8000), a maximum of 11.416 mg bromelain was recovered.

Conclusions/Discussion

From the phase 4A model, a calculated maximum of 47.7 mg enzyme can be recovered at 166 mg/mL PEG and 1.35 M phosphate. From phase 4B model, a predicted maximum 10.5 mg bromelain can be recovered by using at 183 mg/mL PEG8000 and 2.19 M phosphate. Overall, my hypothesis was partially supported as empirical data contradicted my hypothesis but the calculated optimizations supported it.

Summary Statement

The purpose of this research was to determine the optimization conditions of enzyme recovery in polymer-salt aqueous two phase systems (ATPS) by manipulating three factors.

Help Received

Father helped scan samples with UV spectrophotometer at workplace lab.