Effect of Invertase on a Batch Foam Fractionation of Bromelain

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ABSTRACT

The method of foam fractionation can be applied to enrich proteins from a dilute protein solution if the proteins are hydrophobic and foam. If a protein, such as invertase, is hydrophilic, a dilute solution containing this protein may not foam. In that case, a batch foam fractionation process may not be appropriate for recovering a concentrated solution of that protein. In this paper, various concentrations of invertase were added to a dilute solution containing bromelain (a hydrophobic protein), in order to determine how the presence of a hydrophilic protein can affect the recovery of the desired hydrophobic protein. The effect of invertase on bromelain recovery was studied here at an initial bulk solution pH of 5 and an air superficial velocity of 4.6 cm/s.

INTRODUCTION

Foam fractionation is a simple and inexpensive process used to concentrate a dilute solution of a hydrophobic protein, such as bromelain. In this process, a stream of air bubbles flows from a sparger placed in the bottom of a glass column. The bromelain concentrates in the foam, which is then collected from the top of the column after the foam collapses to create a concentrated liquid foamate. This foamate is typically a small volume of relatively concentrated protein solution.

Another method for concentrating a dilute bromelain solution is to absorb the bromelain on polyphenol. In an earlier study, Liang, Huang, and Kwok used tea polyphenols obtained from Chinese green tea to complex bromelain molecules and to extract them from a solution of pineapple juice[1]. While this method is effective for concentrating bromelain, the foam fractionation method may be less expensive and it requires no chemical additives, which must be removed after recovery[3].

MATERIALS AND METHODS

Yeast invertase (lot no. I-9253), bromophenol blue (lot no. 83H3638), bromelain

(lot no. B-2252), gelatin (58H0150), sodium citrate (lot no. 66F0345), and sodium hydroxide (lot no. 873487) were purchased from Sigma (St. Louis, MO). Coomassie brilliant blue G-250 (lot no. 23242) was purchased from Bio-Rad (Richmond, CA). Sodium sulfite (lot no. 785778), and citric acid (lot no. 795790) were purchased from Fisher Scientific (Fair Lawn, NJ).

Foam Fractionation

A 100 mg/L stock solution of bromelain was used for the bromelain solution foaming experiments. A fresh solution was prepared for each set of experiments performed on a given day. The solution was prepared by dissolving powdered bromelain into deionized water. The pH was then set at values of 2, 3, 4, 5, 6, 7, 8 and 9 by the addition of 1N hydrochloric acid or 1N sodium hydroxide. A volume of 100 ml bromelain solution of the desired pH was then charged to the foam fractionation column and then foamed with air. An in-line rotameter was used to determine the constant flow rate set at 21 cubic centimeters per second. The supplied air was purified by bubbling through two water baths in separate beakers in series (to remove most of the proteins that are inherently present in the laboratory compressed air). The air entered the porous sparger at the bottom of the column, creating bubbles, ca. 0.5cm to 1.5cm in diameter, in the protein solution. The bubbles flowed through the solution from the bottom of the fractionation column and on to the top where they produced foam. The solution was allowed to foam until the foam could no longer leave the top of the column. The foamate was collected from the top part of the column through a side port. The liquid that did not foam was collected as the residue. The foamate volume was then measured using a pipette. ml samples of each solution: foam, initial, and residue were used to determine the concentration and activity of bromelain. If there was less than 3ml of foamate, the foamate sample was diluted two fold in order to provide the minimum volume of solution needed to test for bromelain activity.

Bromelain Activity

The bromelain activity was determined by the absorbance corresponding to the amount of bromophenol blue dye released from the gelatin, using a previously developed calibration curve [4], a linear equation comparing absorbance to the activity of bromelain. The concentration of blue dye is then determined from the absorbance, measured by the spectrophotometer, using the equation:

Concentration of bromophenol blue dye (g/L) = 0.01434 * (Absorbance @ 590nm)

This concentration of bromophenol blue dye is then converted to the defined

bromelain activity unit, where 1 unit corresponds to the hydrolysis of 5.2 mg of gelatin in 1 min at 37 C and pH 4.7, or: 1 unit = 4433.3 * Concentration of bromophenol blue dye (g/L)

These two relationships can then be combined to give [4]:

1 Bromelain Hydrolysis Unit(BHU) = 63.574 * (Absorbance @ 590nm) [4]

RESULTS AND DISCUSSION

The partition coefficient (the concentration of protein in the foamate divided by the concentration in the residue) varied with pH, with a low at pH 4 and a high at pH 8, as shown in Figure 1. Similarly, the enrichment ratio (the concentration of the protein in the foamate divided by the initial concentration) also varied with pH, from a low at pH 4 to a high at pH 8. These values were obtained using the Bradford (Coomassie Blue) Total Protein Method to determine bromelain concentration [5]. The total protein concentration measures bromelain in these invertase free experiments. The partition and enrichment coefficients, Kp and ER respectively, varied from about 1.0 to 2.5.

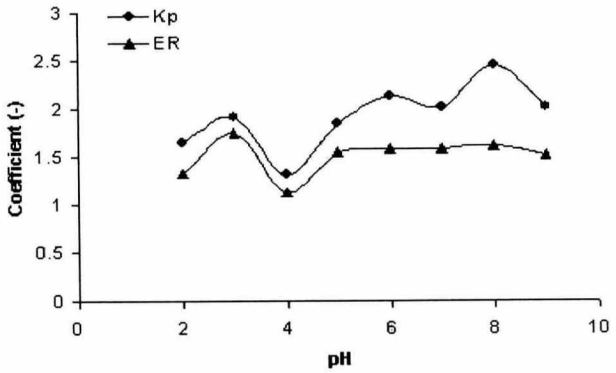

Figure 1. The effect of bulk solution pH on the partition coefficient (Kp) and enrichment ratio (ER) for a bromelain solution with an initial concentration of 100 mg/L.

Figure 2 shows the bromelain activity of each solution, initially, and in the foam and the residue after foaming. The points on Figure 2 represents the average activity values of two sets of data, where the upper error bar represents one set of data, and the lower error bar represents the other. This representation applies to Figures 4 and 5 as well. The activity is measured in bromelain hydrolysis units[4] discussed in the Materials and Methods section. The foamate activities are observed to increase in Figure 2 with an increase in pH. There may be a local maximum at pH 5. The initial values of bromelain activity are approximately equal up to pH 7, which is due to the fact that they

are measures of essentially identical solutions that have only undergone a pH change and returned to the pH 4.28 test condition (100mg/L solutions of bromelain buffered to a pH of 4.28 before the bromelain activity test).

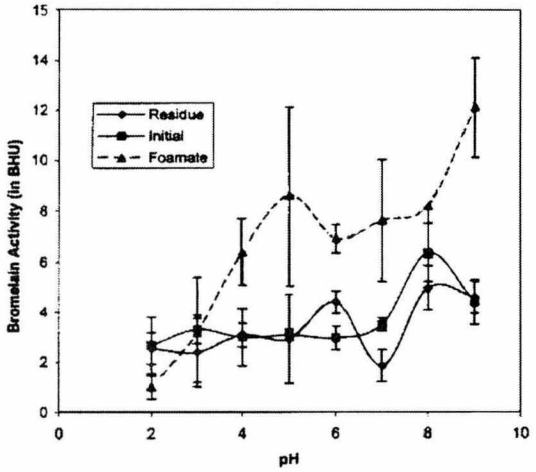

Figure 2. The effect of bulk solution pH on Bromelain Activity of Residue, Initial and Foamate solutions. Each pH experiment started with a 100 mg/L bromelain solution before foaming.

CONCLUSIONS

The addition of invertase to a 100mg/L bromelain (25mg/L of invertase) solution does not seem to cause the bromelain activity to drop. The addition of invertase does cause however, the foaming time to decreases about 60%. The size of the foam bubbles decrease by about 50% from a diameter of ca. 1.0cm to ca. 0.5cm while the volume of the foamate increases as invertase is added to the bromelain solution.

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