0273-2289/01/91–93/0405/$11.75

# Effect of a Natural Contaminant on Foam Fractionation of Bromelain

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## Abstract

Foam fractionation is a simple, inexpensive method for separating and purifying proteins. Typically, a dilute bromelain solution with a pH ranging from 2.0 to 7.0 foams very well when bubbles are introduced into a foam fractionation column. It was observed, however, that the dilute enzyme solution only foamed between approximately pH 2.0 and 3.0 when the inner wall of the fractionation column was coated with a natural contaminant (okra residue). We studied the separation ratio and the protein mass recovery to explore the effect of a natural antifoaming agent on the foam fractionation of a dilute bromelain solution. The control variables used in this process were the initial bulk solution pH, which ranged from 2.0 to 7.0, and the superficial air velocity, which varied between 1.7 and 6.2 cm/s.

**Index Entries:** Bromelain; foam fractionation; protein; protein separation; protease recovery; antifoaming agent; natural antifoaming agent.

## Introduction

Generally, naturally occurring foams in biologic processes (such as fermentation processes) are not desirable and are suppressed by antifoaming agents such as silicones and non-charged polymeric antifoaming agents. However, the addition of synthetic antifoaming agents to a process may raise the level of contamination and introduce new problems to the system. The substitution of a contamination problem in place of a foaming problem can perhaps be readily ameliorated if a bioreactor is operated such that the generated protein foam is continually removed from the process rather than suppressed with the antifoaming agents. Another approach is to use antifoaming additives that are compatible with the process and that, in turn, may be degraded by the process if they are natural materials.

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In this study, we examined one such natural material, okra solution residue, as a possible antifoaming agent. This natural material, however, could create a problem if it were present in a foam fractionation process used to recover proteins.

Currently, foam fractionation can be used to remove dissolved organic wastes from water while increasing dissolved oxygen levels *(1)*. Foam fractionation is a simple and relatively inexpensive procedure that can also be used to separate and purify proteins *(2)*. This process has great potential for reducing protein recovery costs in the pharmaceutical and food industries. Nevertheless, when proteins are extracted from living organisms such as plants, existing natural contaminants in these organisms may act as antifoaming agents and suppress the desired foaming during foam fractionation.

Thus, it is important to investigate how natural contaminants can affect the foam fractionation of proteins when natural contaminants are inadvertently introduced into the fractionation column. This investigation may lead to a possible screening method for antifoaming agents. In this article, we report on the effect of the bulk solution pH and air superficial velocity on a dilute bromelain solution at two different conditions: (1) when the cylindrical fractionation column is coated with a natural contaminant (from okra), and (2) when the column is scrubbed clean prior to fractionation.

The enzyme we used is bromelain (a foaming protein), found in pineapples and other fruits. Bromelain is a group of proteolytic enzymes that are often used by people who suffer from malabsorption of food *(3,4)*. Recovery with minimal denaturation of this enzyme is an important first step in the industrial processing of bromelain from food wastes.

## Materials and Methods

### *Materials*

Bromelain (lot no. B-2252) 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). Frozen okra (Kroger brand; Cincinnati, OH) was purchased from a local grocery store.

### *Experimental Procedure*

A 100 mg/L bromelain solution was prepared by dissolving bromelain powder in deionized water. The dilute bromelain solution (originally at pH 4.5) was adjusted initially to the desired pH between 2.0 and 7.0 by adding HCl or NaOH. The initial volume of bromelain solution used for the batch experiments was 100 mL. The foam fractionation apparatus comprised an elongated glass column with a porous fitted glass sparger (pore size 40–60 μm) fitted flush to the bottom of the column, with a port at the top of the column for the foam to exit, as shown in Fig. 1. An air supply entering at the base of the column created bubbles in the bulk solution and

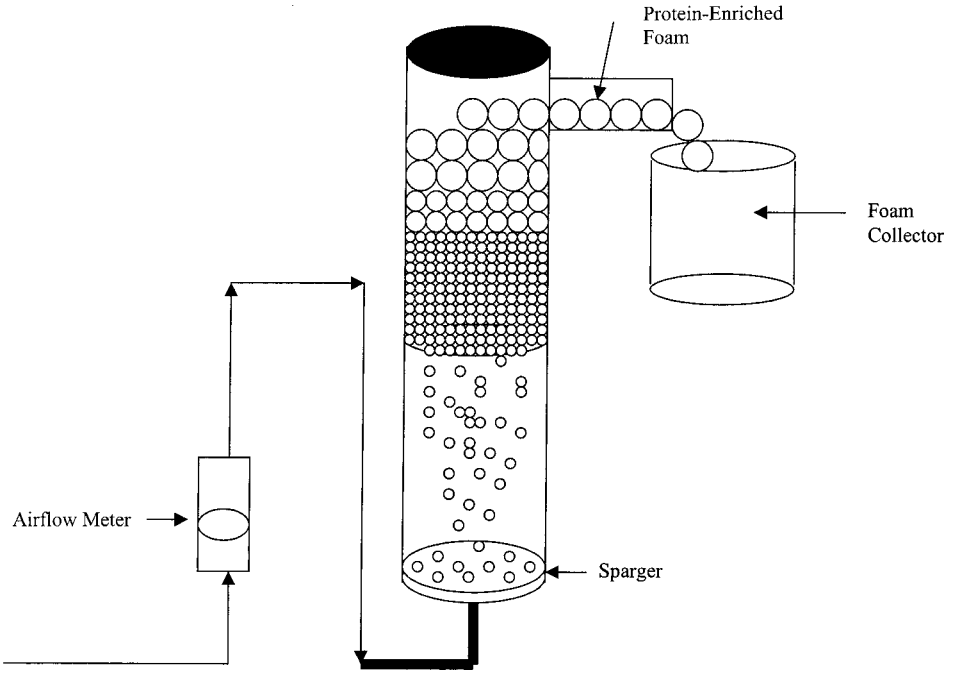


Fig. 1. Schematic of foam fractionation of a dilute bromelain solution.

foam at the air/liquid interface, which, in turn, rose up to the top of the column. The bubbles and foam were enriched with bromelain as the bubbles rose in the column. The air was humidified by bubbling it through water before feeding it into the column. The humidifier also served to trap undesired proteins found in the dust in the air. The air superficial velocity was measured with an inline rotameter. The foamate from the top of the column and the residue were collected and their volumes measured. The superficial air velocity was varied between 1.7 and 6.2 cm/s. Although this process was a rate-based collection, the recovery with varying time was not explored. The mass recovery (MR) of the total protein was analyzed with the collected foam until no more foam reached the top of the column. The okra coating on the column came from a previous experiment in which proteins from an okra solution were foamed. The column was cleaned with several rinsings of distilled water, but the residual protein coating the glass column was not scrubbed off with a laboratory brush. For the okra protein-free experiment, the glass column was scrubbed thoroughly.

### *Total Protein Assay*

The total protein content in the bromelain solution was determined using the Coomassie blue (Bradford *[5]*) method with a Bausch and Lomb Spectronic 20 spectrophotometer set at 595 nm *(5,6)*. In all the assays performed, 2 mL of Coomassie blue reagent was added to 3 mL of each sample solution. The optical absorbance was read 5 min after adding the reagent.

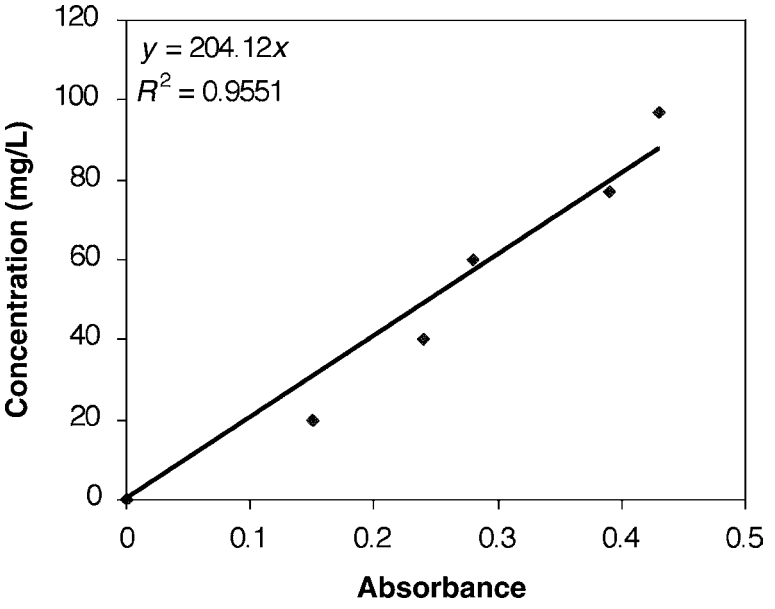


Fig. 2. A linear correlation between concentration of Coomassie blue (g/L) and absorbance at 595 nm.

The relationship between the optical absorbance and the known total protein content was correlated to determine the unknown total protein content during the experiment. The following calibration relationship was developed to determine the total protein content, as shown in Fig. 2 (drawn through the origin), and used for the bromelain assay:

$$\text{Bromelain (protein) concentration (mg/L)} = 204.12 \times (\text{absorbance @ 595 nm})$$

## Results and Discussion

Without a contaminant on the glass wall of the foam fractionation column, bromelain foam was readily created in the column over the entire studied pH range of 2.0–7.0. The air superficial velocity was set at the intermediate value of 2.8 cm/s, as well as other values. The MR is one measure of the amount of foam produced; Figure 3 shows the okra-coated case. This foam fractionation can also be described by the partition coefficient, as shown in Fig. 4. A partition coefficient value of 1 indicates that there is no concentration of proteins in the foam, regardless of the amount of foam produced. The partition coefficient, $K_p$, is defined as the ratio of the protein (here, bromelain) concentration in the overhead collapsed foam ($C_{foam}$) to the remaining protein concentration in the remaining residual solution ($C_{residue}$):

$$K_p = [(C_{foam})/(C_{residue})]$$

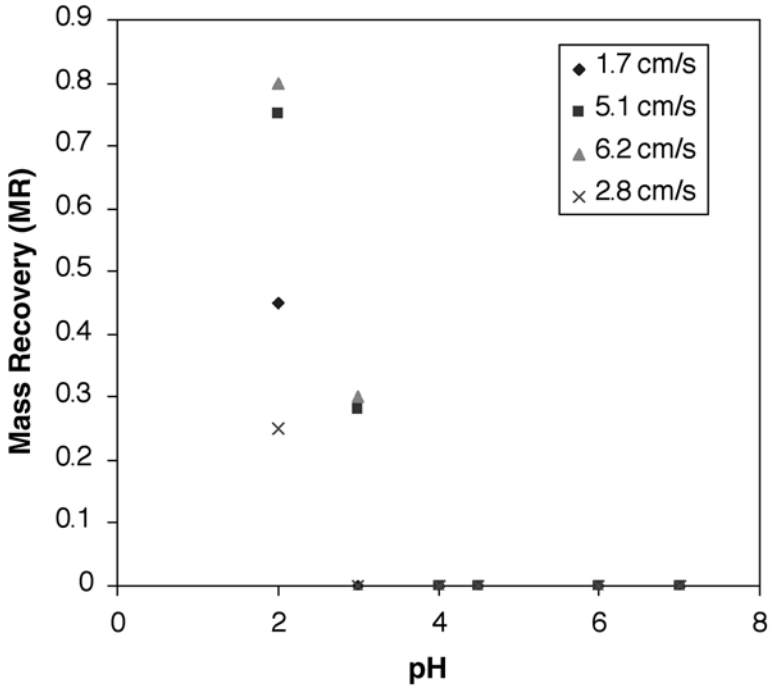


Fig. 3. Total partition coefficient, $K_p$, for the foam fraction at pH 2.0–7.0. The initial concentration of the bromelain-invertase mixed solution was 200 mg/L of total protein (100 mg/L of bromelain and 100 mg/L of invertase).

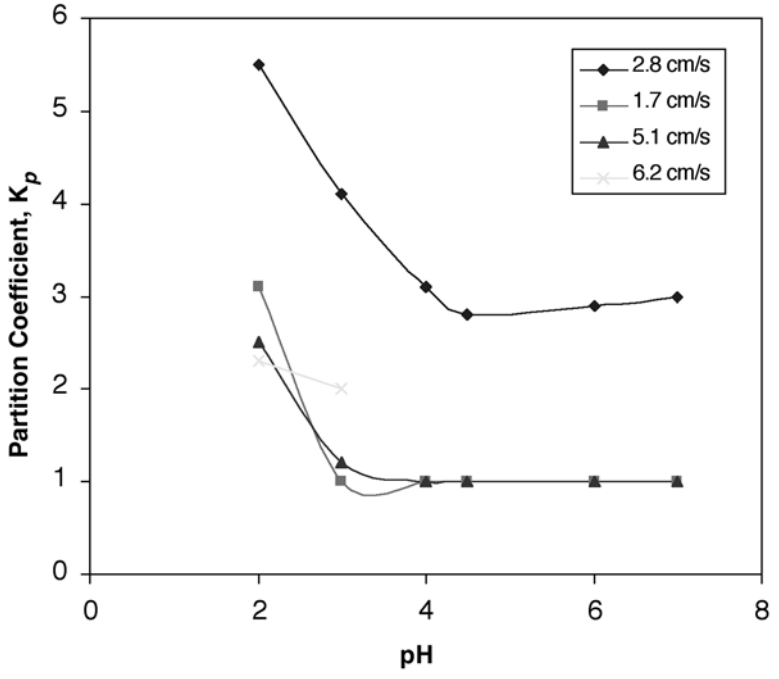


Fig. 4. Partition coefficient of proteins from the droplet fractionation experiment conducted at pH 2.0–7.0.

Here *C* is determined in milligrams of protein/liter. The maximum partition coefficient of 5.5 occurred at the low pH of 2.0, at which the bromelain may be denatured by adding an excessive amount of acid.

On the other hand, when okra residue (the natural contaminant/antifoaming agent) coated the foam fractionation column wall, foam was created only in a narrow pH range (between 2.0 and 3.0) at air superficial velocities ranging between 1.7 and 6.2 cm/s. With the okra residue, the partition coefficient reached a maximum value of 3.5 between pH 2.0 and 3.0, as shown in Fig. 4. In the pH 2.0–3.0 range, therefore, the partition coefficient with okra residue decreased by approx 50% relative to the partition coefficient without okra residue.

Figure 3 illustrates the effect of the bulk solution pH on the protein MR when the okra residue coats the foam fractionation glass column. MR is defined as the ratio between the recovered protein mass in the foamate and the initial protein mass in the foam fractionation column:

$$\text{MR} = (\text{protein mass in foamate})/(\text{protein mass in initial bulk solution})$$

Here, the MR decreased with increasing bulk solution pH in the range between pH 2.0 and 3.0. It was observed that MR was negligible outside that range, at low airflow rates, since the bromelain solution did not foam significantly at pHs above 3.0 and because of the lower superficial air velocities. MR increased as the superficial air velocity increased. This appears to be owing to the enhanced foaming created by the additional aeration.

## Conclusion

Based on our foam fractionation experiments, bromelain can be concentrated in the foamate phase from a dilute protein solution. When a natural contaminant coats the foam fractionation column, however, the foaming is significantly suppressed. The okra residue (natural contaminant) acts like an antifoaming agent and decreases the amount of foaming, leading to both a lower mass recovery and a lower partition coefficient in the pH 2.0–7.0 range. Negligible foaming occurred above pH 3.0 at air superficial velocities between 1.7 and 6.2 cm/s for the okra-inhibited system. A foam fractionation apparatus such as the one used here could be used to screen for natural antifoaming agents, which, in turn, could be compatible with commercial fermentation and other microbial processes.

## Acknowledgment

This article is based on work supported by the National Science Foundation under Grant No. CTS-9712486.

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