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Foam Fractionation of a Dilute Solution of Bovine Lactoferrin

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Abstract

Lactoferrin (Lf), a protein found in human and bovine milk, tears, blood, and other secretory fluids, has been used to prevent infection from potential microbial pathogens by its ability to bind with iron (Fe^{3+}). Currently, bovine lactoferrin can be purified from milk using ion exchange resin, which is a costly procedure making lactoferrin expensive. The purpose of this work was to investigate a low-cost foam fractionation process as the first step in separating lactoferrin from milk.

Index Entries: Lactoferrin; foam fractionation; milk; surface tension.

Introduction

Lactoferrin is an iron binding protein found in human milk, tears, blood, and other secretory fluids. Bovine lactoferrin (bLf) is found in cow's milk, and has been consumed by humans for centuries. It is believed to prevent infection by its ability to bind with iron (Fe^{3+}) from potential pathogens *(1)*. In addition, apo-lactoferrin (iron free lactoferrin also found in milk) has been shown to bind to microbial membranes, kill bacteria and inhibit tumors *(2)*. These antimicrobial abilities are known to be effective against *Escherichia coli* O-157, which causes numerous deaths and illnesses in the United States every year. *E. Coli* O-157 is of particular concern in undercooked hamburgers. The medical benefits of lactoferrin are not limited to *E. coli*. It has been shown to control the microbial activity of organisms such as *Salmonella*, *Staphylococcus*, *Listeria*, and *Candida*. Currently, bovine lactoferrin can be purified from milk using ion exchange resin *(3)*, which is a costly procedure making lactoferrin expensive, approx $1/gram, 98% pure.

Foam fractionation is an adsorptive bubble separation process, which can be used to concentrate dilute protein solutions *(4)*. This process is an

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inexpensive procedure that can easily be scaled up or scaled down. Foam fractionation shows promise in being an inexpensive first step in concentrating a specific protein in a dilute solution. This separation technique involves a gas, air in this case, being pumped into the bottom of a fractionation column containing the dilute mixture. The gas creates bubbles in the bulk liquid solution. Above the liquid phase surface these air bubbles can form concentrated protein foam layers that rise up and dry in the column. The foam layers can then be collapsed and collected to produce a liquid foamate *(4)*. The bulk liquid that remains in the fractionation column is called the residue. The concentrations of the proteins in both the foamate and the residue are then determined using a dye along with a spectrophotometer. From these measured protein concentrations and volumes, the separation ratio (SR) and the mass recovery (MR) can be determined.

The highest concentration in a produced foam typically occurs when the pH of the bulk liquid is at the isoelectric point of the protein. At the isoelectric point the protein has no net charge, thus making it more hydrophobic. A hydrophobic protein tends to concentrate at the gas–liquid interface of the liquid phase. An increase in concentration of the protein at this surface will generally decrease the surface tension of the solution. Thus, typically at the lowest surface tension the pH approaches the isoelectric point, and the highest concentration in a foam.

When the foamed protein solution has more than one protein, as in milk, gel electrophoresis can be used to determine the concentration of those proteins in that mixture. Gel electrophoresis is used to separate proteins in solution by their molecular weights and the concentration of a given protein can be determined by comparing the darkness of the band to a sample of known concentration. Thus, if the molecular weights of several marker proteins are known, a given protein can be identified by molecular weight and from its intensity, its concentration can be estimated.

Objectives

There were several objectives to this research. The first was to determine if a dilute solution of bovine lactoferrin would foam, then to determine the foaming ability of bovine lactoferrin within a wide pH range. The surface tension of a dilute solution of bovine lactoferrin over a pH range of 2–10 (Fig. 1) is very interesting, and will be examined in depth later in this paper. The final objective of the research was to determine the highest separation ratio (SR) and the mass recovery (MR), and then determine the optimal conditions for separation using an objective function ($\Phi = SR \times MR$).

Materials and Methods

Bovine lactoferrin (bLf) came from DMV International Nutritionals (Frazer, NY). Gels and buffers for the gel electrophoresis came from Pharmacia BioTech Inc. (Uppsala, Sweden), Coomassie® Blue Reagent G250

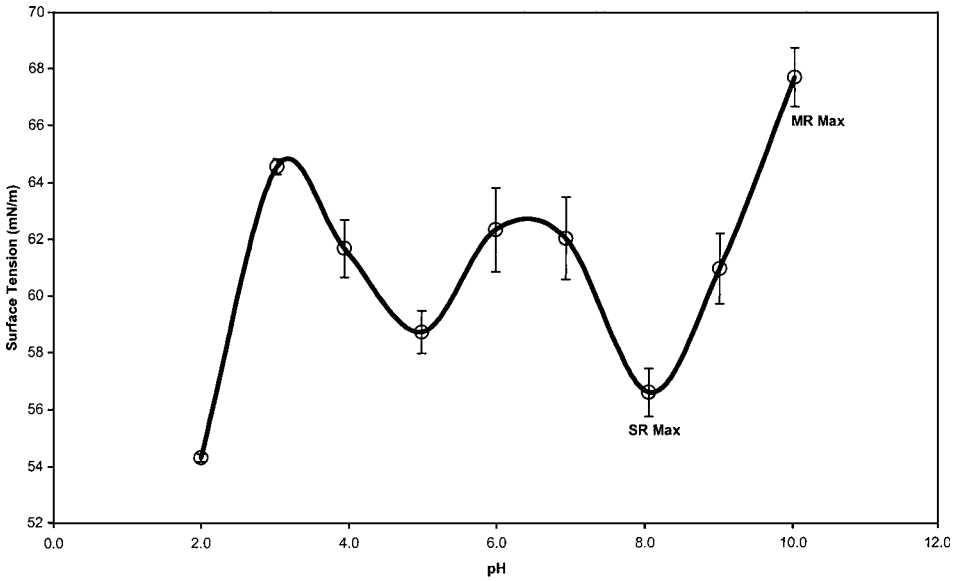

Fig. 1. The surface tension of a dilute solution of a 0.1 mg/mL concentration of bovine lactoferrin over a pH range of 2–10.

for Bradford Assay from Sigma-Aldrich (St.Louis, MO), and Skim milk from Kroger (Brentwood, TN).

Experimental Procedure

To determine the surface tension of dilute bLF solution, a 0.1 mg/mL protein solution was prepared by dissolving 100 mg of bovine lactoferrin in 1 L of deionized water. The pH of the protein solution was adjusted for various pH studies using 0.1 *N* NaOH, or 0.1 *N* NaCl prepared from concentrated acid. A 20 mL sample of the protein solution was placed in a 50 mL glass cylinder, which was placed inside the KSV Sigma70 surface tension apparatus. The Wilhelmy Plate-prototype surface tension apparatus was then used to probe the solution and record the force needed to break the surface on a computer. The first five trials were then averaged to determine the surface tension of the tested liquid at a specific pH. These data were then recorded and the isoelectric point of bovine lactoferrin was determined at the lowest part of the surface tension–pH profile to be 8.0 (see Fig. 1), as reported by Saito *(3)*.

For the foam fractionation experiment, a bLF protein solution of 0.1 mg/mL was prepared. 125 mL of this protein solution was placed in a foam fractionation column, and the solution was foamed for 10 min at different superficial air velocities and bulk pHs. After foaming, the Bradford method was used to determine the total protein concentration in the both the foamate and the residue positions *(5)*. For each trial, 1.6 mL of Coomassie Brilliant Blue solution were added to 2.4 mL of the protein solution sample,

transferred into a 10 mL cuvet. This disposable cuvet has a square cross section and was read for absorbance in a Hitashi Model 100-10 spectrophotometer at a wavelength of 595 nm. The concentrations were then recorded in a spreadsheet on a computer to calculate the separation ratio (SR) and mass recovery (MR) for each trial.

The foaming of milk is more complex due to the many different proteins found in milk. To prepare the milk for foaming, the pH of a 125 mL sample was adjusted to a pH of 8.0 using 0.1 *N* sodium hydroxide. The milk was then placed inside the foam column and foamed for 10 min. After foaming, the total protein concentration of the foamate and the residue were then determined using the spectrophotometer. This determined the total amount of protein in each sample. The concentration of bovine lactoferrin was determined by analyzing samples of the foamate and the residue using gel electrophoresis to determine the amount of bovine lactoferrin separated. The data were then recorded on a spreadsheet in the computer to calculate the separation ratio (SR) and the mass recovery (MR).

Results and Discussion

The optimum separation conditions of bLf were determined in terms of the separation ratio (SR) and the protein mass recovery (MR). The separation ratio quantifies the degree of concentration that occurs and the mass recovery states how much of the protein is recovered. The separation ratio is defined as the concentration in the foamate divided by the concentration in the residue. The concentrations of the residue and the foamate were determined by a Coomassie Blue total protein assay. A mass balance was performed after the concentrations were determined. The protein mass recovery is defined as the mass of bLf in the foamate divided by the mass of bLf initially in the bulk fluid. An objective function (Φ) was defined as $\Phi = SR \times MR$ to determine the bulk pH and superficial air velocity for optimal separation.

The surface tension for a dilute solution of bovine lactoferrin was lowest at a pH of 8.0 (Fig. 1), which was the reported value of the bovine lactoferrin's isoelectric point *(3)*. It was expected that the greatest separation would occur at this pH. It is also noted that when the pH equals 2, the surface tension is less than that at 8. However, since bovine lactoferrin is most likely denatured at pH 2, the isoelectric point is probably not this low. Surface tension was at its highest when the pH was equal to 10. When the surface tension is high, the forces holding the protein to water are stronger. These stronger forces bind the now hydrophilic protein more strongly to water and cause more water to foam out with the protein. Thus, a smaller amount of lactoferrin foams out.

As seen in Fig. 2, the largest foam volume recovered occurred when the bulk pH equaled 10 and the superficial air velocity was 21 cm/min. The largest mass recovery (MR) of 40% is observed (Fig. 3) at these settings. It is interesting to note that the slope of the curve for pH 10 is similar in both

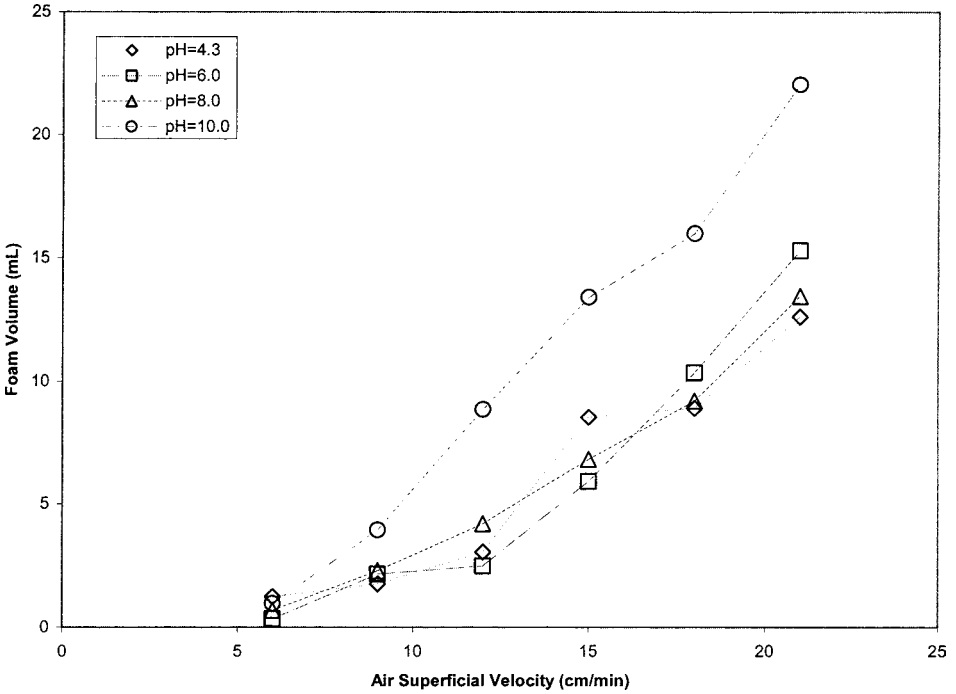

Fig. 2. The effect of air flow rate on the foam recovery of bovine lactoferrin solution.

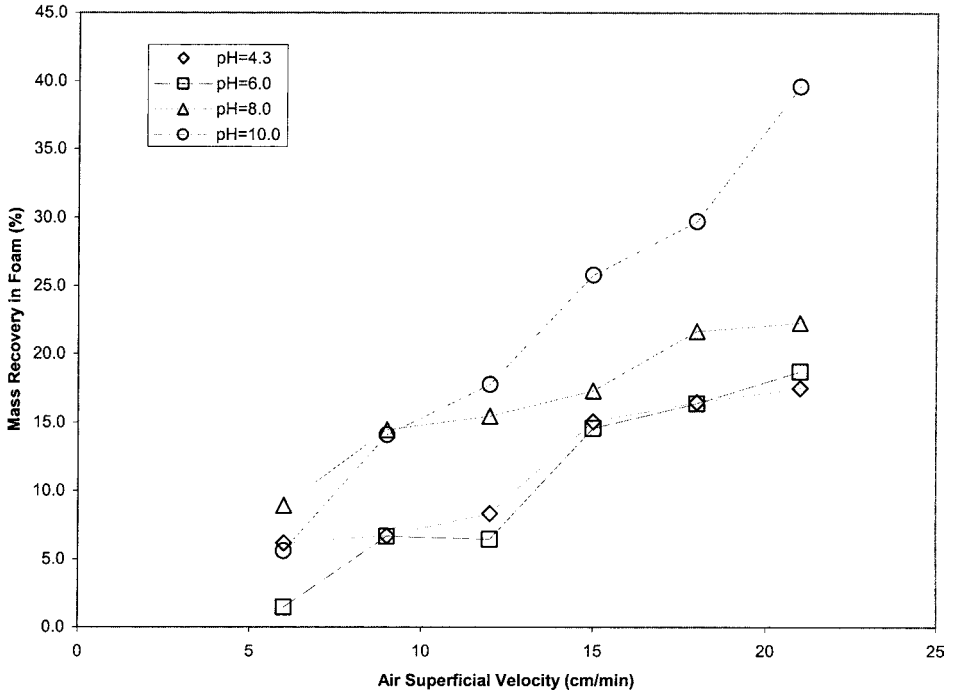

Fig. 3. The effect of superficial air velocity on the mass recovery of bovine lactoferrin in milk.

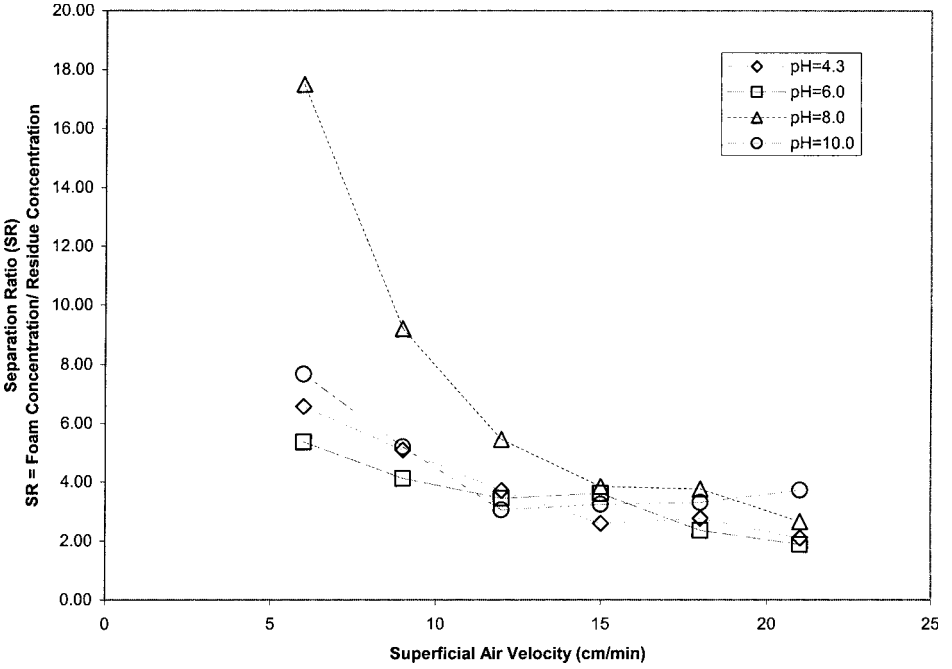

Fig. 4. The effect of superficial air velocity on the separation ratio of bovine lactoferrin.

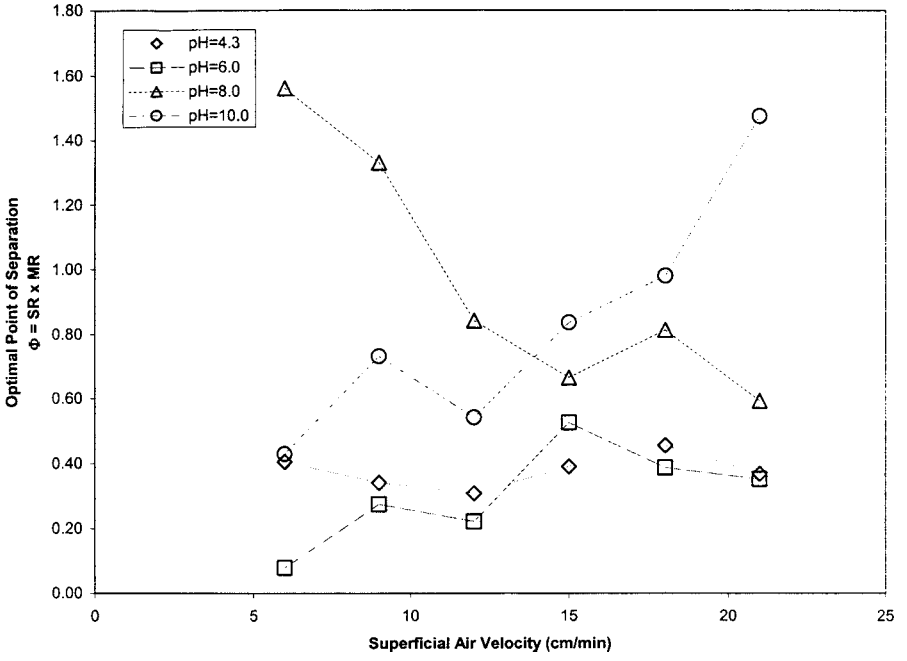

Fig. 5. Determination of the optimal point of separation with respect to the superficial air velocity.

Figs. 2 and 3. This indicates that the concentration of lactoferrin in the foamate remained about the same at higher superficial air velocities for a bulk pH of 10.

The largest SR occurred when the pH was equal to 8 and the superficial velocity was equal to 6 cm/min (Fig. 4). This agrees with the argument stated earlier, that the highest separation ratio generally occurs at the isoelectric point of the protein. However, it should be noted that at the superficial air velocity of 6 cm/min, only a small amount of volume is recovered. The mass recovery that occurred at pH 8 was small compared to the amount of mass recovered at pH 10, owing to the small amount of volume recovered.

To determine the bulk pH and superficial air velocity for optimal separation, an objective function (Φ) was defined as $\Phi = SR^{\alpha} \times MR^{\beta}$ where α and β are exponential weightings. For the case of equal exponential weightings presented here, α and β are set equal to one, as shown in Fig. 5. The condition with the highest objective value was at a bulk pH of 8 and a superficial air velocity of 6 cm/min. The second highest objective value occurred at a bulk pH of 10 and a superficial air velocity of 21 cm/min. The difference between these two values is relatively small. To determine which condition is better, it must first be decided if one would like to recover a high concentration or more mass. If neither is more important than the other, the condition with the lowest superficial air velocity would be preferred to minimize energy costs. The activity must also be checked to ensure that the protein is not denatured.

A 100 mL sample of skim milk, purchased in Brentwood, TN, was aerated at pH 8, and at a superficial air velocity of 6 cm/min. The milk was foamed for 10 s. Using gel electrophoresis, the concentration of bovine lactoferrin was determined to increase about 50%. This corresponds to a separation ratio of approx 1.5.

Conclusions

1. The maximum mass recovery of 40%, occurred at pH 10 and at an air superficial velocity of 10.
2. The maximum separation ratio of 17.8 occurred at pH 8 and at an air superficial velocity of 6 cm/min.
3. The best operating condition, according to the objective function (Φ), is at a bulk pH of 8 and a superficial velocity of 6 cm/min.
4. Two local minima of 57 mN/m at pH 8 and 59 mN/m at pH 5 were found in the surface tension experiment.

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