0273-2289/00/84–86/0835/$12.00

Effect of Protein Denaturation on Void Fraction in Foam Separation Column

Robert D. Tanner,* Tobias Parker, Samuel Ko, Yuqing Ding, Veara Loha,† Liping Du, and Aleš Prokop

Chemical Engineering Department, Vanderbilt University, 24th and Garland Avenues, Nashville, TN 37235, E-mail: rtanner@vuse.vanderbilt.edu

Abstract

Foam fractionation is a cost-effective process that uses air to extract protein from a liquid (in this case "crude" dilute egg-albumin solution). This article deals with how the void fraction (fraction of air in the aerated solution) of foam is affected by heat denaturation of the protein. A 2-mm glass tube was used to sample the foam-liquid interface fluid in a 35-mm-diameter column in order to detect small changes in void fraction and foam production, which are not easily detected directly from the bulk foam. The main control variable in this study was the protein solution preheating time. As the preheating time increased, the initial void fraction in the column decreased. The initial void fraction of the undenatured solution ranged from about 0.73 to 0.80, and the void fraction for significant preheating times of 5 min ranged from approx 0.68 to 0.72. Furthermore, the period of foam production increased from 5 to 7 min for undenatured proteins in solution to as long 15 min for 5-min preheated solutions. Side-port sampling through a small capillary tube has the potential to be used as a rapid and inexpensive way to determine the level of protein denaturation by directly determining the void fraction and then estimating the effect of denaturation from a protein denaturation calibration curve of the void fraction.

Index Entries: Egg albumin; protein denaturation; foam fractionation; hydrophobicity; void fraction.

Introduction

Change in protein structure is important to understanding the effect of protein denaturation *(1)*. Proteins are made up of polarized and nonpolarized ends or heads, which determine the manner in which they interact with water. If large amounts of nonpolarized heads are exposed to water,

*Author to whom all correspondence and reprint requests should be addressed.
†Presently at King Mongkut's Institute of Technology, Thonburi, Thailand.

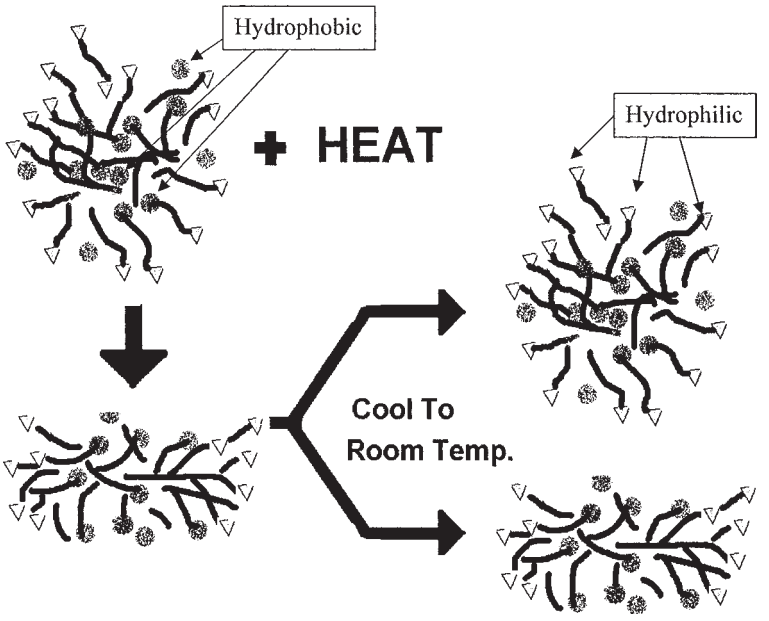

Fig. 1. Structural change owing to denaturation.

the protein is said to be more hydrophobic or resistant to being combined with water. If large amounts of polarized heads are exposed to water, the protein is then considered more hydrophilic or easily combined with water. By adjusting the amount of heads exposed to water, the water-interactive properties of the protein can be controlled. One way to make that adjustment is to expose the protein to adverse heat conditions (temperatures well above room temperature for animal or plant proteins), which change the structure. Heat can change the shape of the protein by breaking the bonds that give the protein its globular shape *(2)*. In this way, more hydrophobic heads can be exposed to the water, which can lead to more protein extracted during a foam fractionation process (which favors hydrophobic proteins) *(3)* (as shown in Fig. 1). In this article, we discuss how the void fraction is determined in order to characterize the degree of denaturation.

Materials and Methods

Dilute egg-albumin solution (approx 100 mg/L) was prepared by dissolving crude powdered egg albumin into deionized water and then filtering the solution (via gravity filtration) to remove large protein solids. The dilute albumin solution was preheated at 60°C for the desired preheating time, t_p (0, 3, and 5 min). The solution was then allowed to cool to room temperature. After cooling, 200 mL of the dilute protein solution was placed in the foam fractionation column. To operate the column, air was introduced into the bottom of the solution through a sparger, at a flow rate of 0.75 ft^3/min, or 386.4 cm^3/min. A small stream of the foam at the foam-liquid

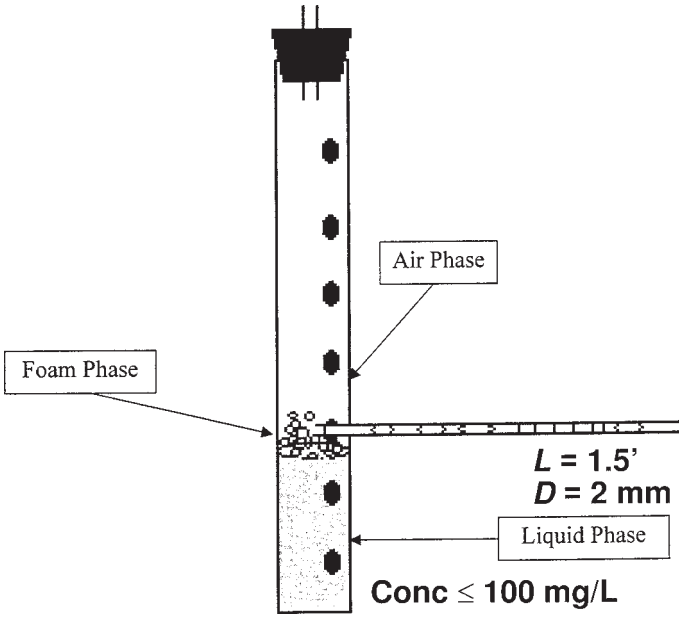

Fig. 2. Foam fractionation with the side bubble sampling tube. *L* = length of sampling tube (1.5 ft), and *D* = inside diameter of sampling tube (2 mm).

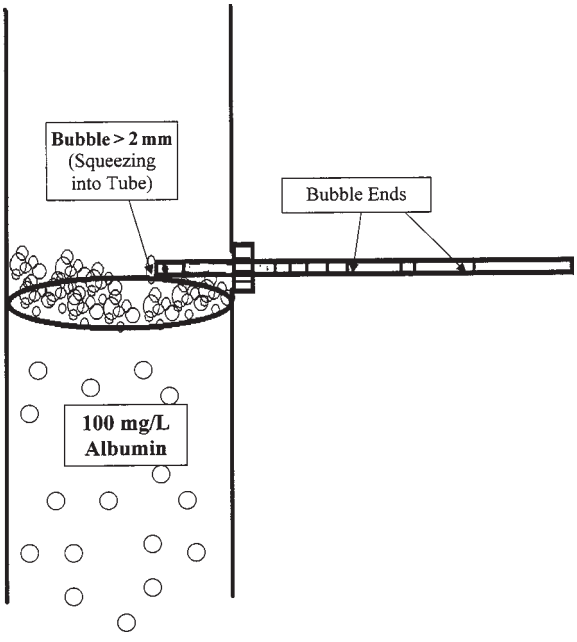

Fig. 3. Protein-enriched foam feeding into 2-mm diameter glass tube.

interface was then allowed to exit into a clear glass tube mounted perpendicularly to the column (*see* Figs. 2 and 3) *(4–6)*. Figure 3 presents a close-up of the side tube mounted in the foam fractionation column.

Determining Void Fraction Using Weight Method

The weight method (used for bubbles of diameter <2 mm) was conducted as follows.

First, the weight of the dry tube was subtracted from the weight of the same tube containing the protein-enriched foam (obtained from digital pan balance) in order to determine the foam weight (assuming that the air weight in the foam is negligible). Second, the volume of the liquid portion of the foam was determined from the measured foam weight and the density of water, ρ_{H_2O}, as follows:

$$\text{Volume of liquid portion of foam} = \text{weight of foam}/\rho_{H_2O}$$

Third, the volume of the air in the aerated solution was calculated by subtracting the volume of the liquid portion of the foam from the volume of the tube. Finally, the void fraction was determined from the following relationship:

$$\text{Void fraction} = \text{volume of air in the aerated solution}/\text{volume of the total tube}$$

Determining Void Fraction Using Photographic Method

The photographic method (used for bubbles of diameter <2 mm) was conducted as follows.

First, a photograph of the protein-enriched foam in the clear glass tube was taken every 30 s using a Nikon N90S camera (Japan) with a Nikon ED AF Micro Nikkor 200 mm 1:4D lens (a light-filtering lens was also used). Second, the number of bubble ends was counted from the photograph of an 8-cm length of the foam-filled tube (*see* Fig. 3). The bubble end weight was determined statically by measuring the weight of the foam-filled tube (similar to the weight test) and counting the total number of bubble ends (<15% variation in bubble-end weight calculations). Each bubble contains an aerated region and a liquid region. The void fraction was then determined from the following relationship:

$$\text{Void fraction} = \text{volume of aerated region within 8 cm length of tube}/\text{volume of glass tube with 8 cm length of tube}$$

Determining Bubble Size and Limitation

During the foam fractionation process, the protein was extracted with the bubbles. These bubbles increased in size until all the hydrophobic protein was extracted or when there was no further foam production. If the bubble size was <2 mm, several bubbles could pass through the tube in parallel, rather than in sequence, which made the photographic method ineffective, because the bubbles could not be calculated accurately. Therefore, the weight method was used to determine the void fraction for bubbles with diameters <2 mm. Conversely, if the bubble size was >2 mm, the photographic method was effective. There-

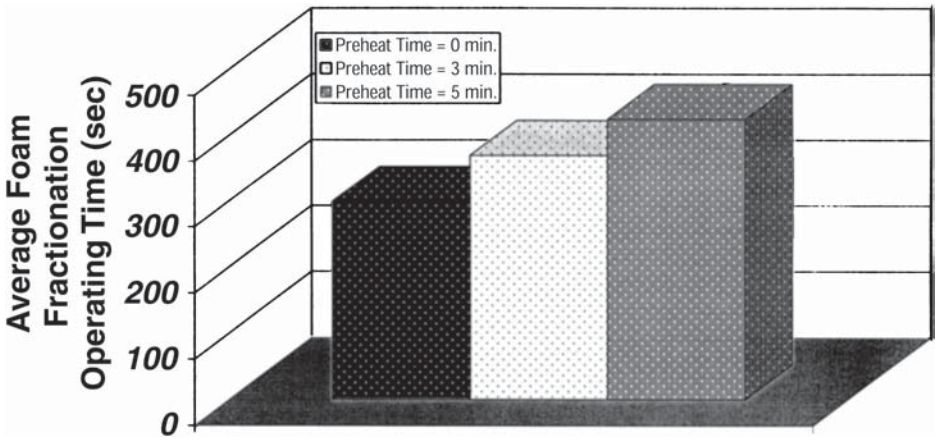

Fig. 4. Effect of protein preheating time on foam production time.

fore, the means for measuring bubble size was determined by the size of the (2 mm diameter) sampling tube.

Results and Discussion

As seen in Fig. 4, the foam production time increased as the preheating time of the dilute albumin solution increased. Figure 4 apparently indicates that as the preheating time increased, more protein was denatured. Denatured protein had more exposed hydrophobic heads, which, in turn, allowed for more protein-enriched foam. Figure 5 demonstrates that when the preheating time increased from 0 to 3 min; there was a negligible increase in the foam void fraction over the 30- to 120-s foam fractionation sampling times. Both the 0- and 3-min preheating time curves lead to void fraction trajectories, which are essentially the same. This seemed to indicate that there was negligible denaturation of at least part of the albumin protein, when the void fraction was calculated using the weight method.

The 0- and 3-min preheating curves separate after 120 s of foam fractionation, possibly indicating that the 3-min heating process broke the protein bonds in another region of the protein. Because the void fraction decreased by approx 10% when the preheating time was extended to 5 min during the first 120-s foam fractionation, this difference may be exploited to obtain a measure of this other type of denaturation/bond breaking. Interestingly, after 180 s of foam fractionation, the 3-min preheating trajectory follows the 5-min preheating curve trend, and after 240 s, the two curves tend to converge.

The overall trend of the void fraction seems to increase during foam fractionation or as the hydrophobic protein concentration decreases in the albumin solution. Because the internal pressures of a smaller bubble (lower

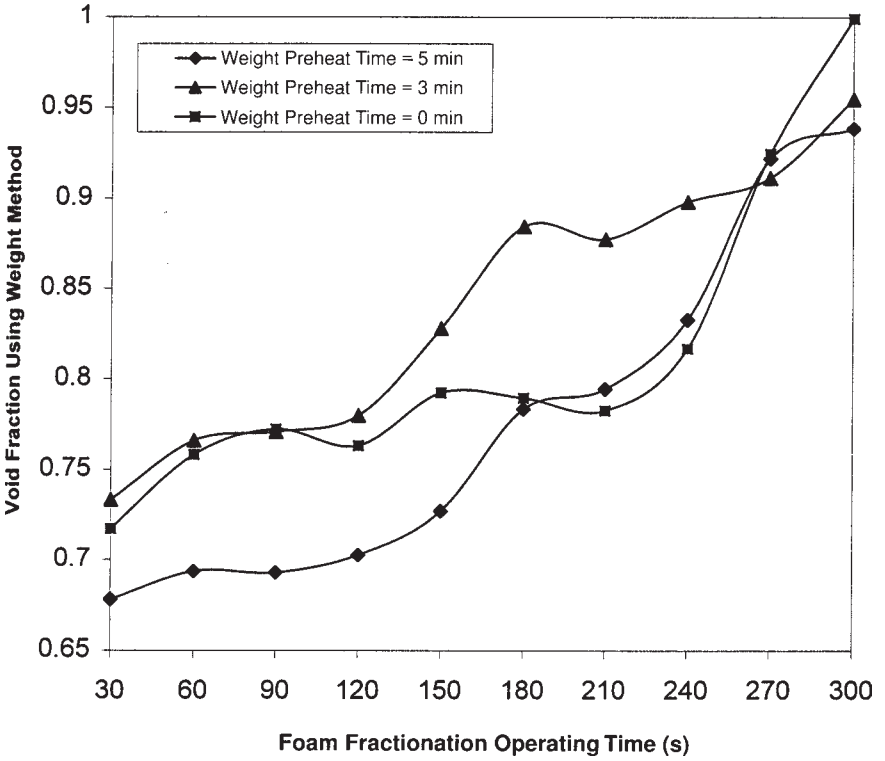

Fig. 5. Effect of foam fractionation operating time on the void fraction using the weight method.

void fraction foam) are higher than that of larger bubbles, increasing the concentration of protein within the foam must increase the strength of the bubbles. Also, as the liquid of the interface in the column gets sucked out of the tube, only air remains (to drive the void fraction to one).

A different, nonlinear correlation between the foam fractionation operating time and the void fraction is depicted in Fig. 6 for later times (after 150 s) using the photographic method. The photographic method gives additional information, which may be used to differentiate the degree of protein denaturation for later times. Note that the void fraction for the foam fractionation operating times before 150 s (for <2-mm diameter foams) cannot be calculated using the photographic method because the camera cannot detect bubbles with a diameter of <2 mm. As in Fig. 5, the curves tend to merge for late times.

Figure 7 shows a correlation between void fractions of the weight method and the photographic method for 0-min preheating times. Figure 7 indicates that under certain restricted cases a nearly linear relationship exists between the two methods. Such a correlation tends to strengthen a given type of measurement by corroborating one technique with another (independent) technique.

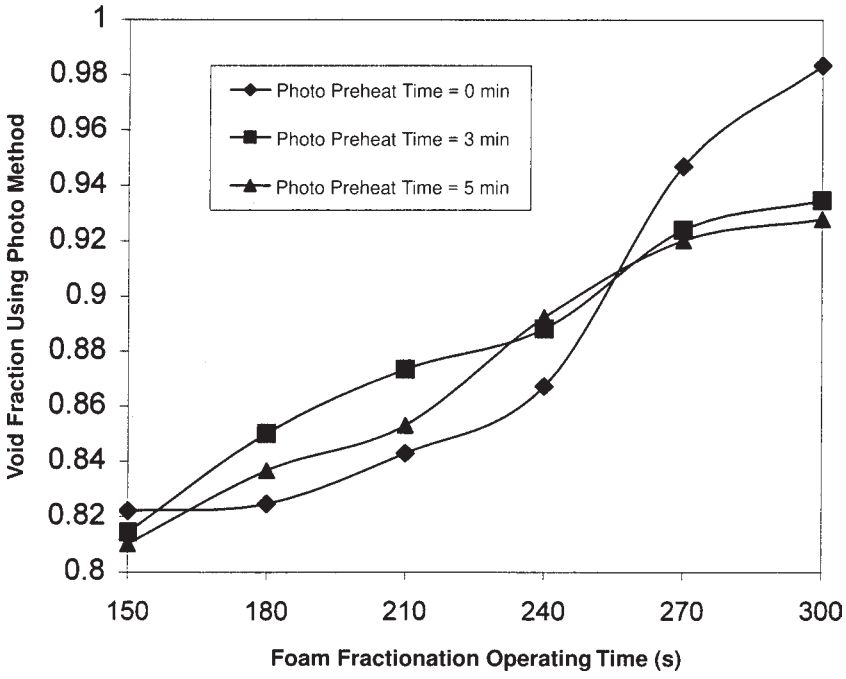

Fig. 6. Effect of foam fractionation operating time on the void fraction using the photographic method.

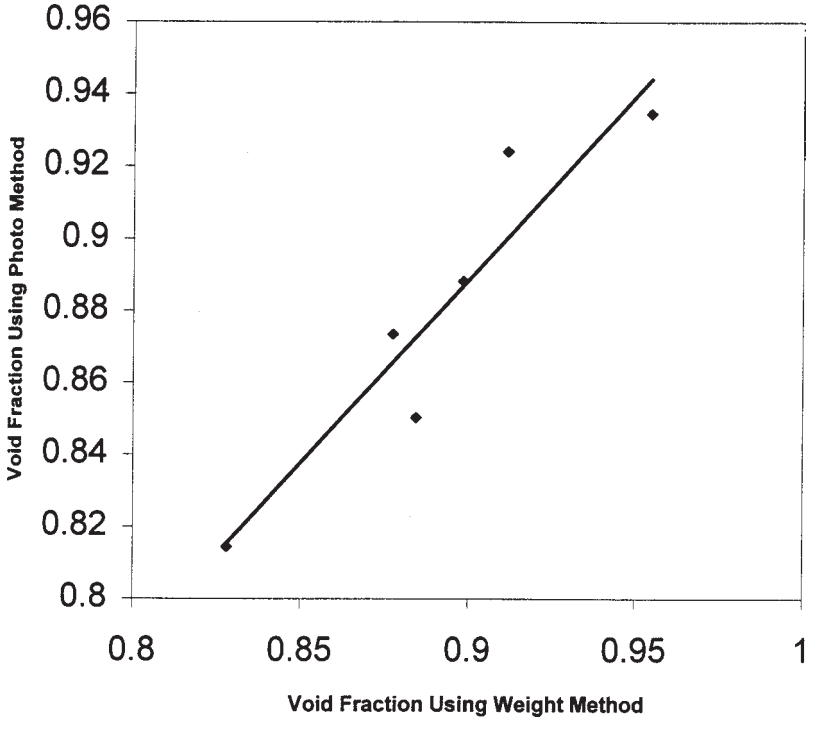

Fig. 7. Correlation of weight method with photo method at foam fractionation operating times between 150 and 300 s.

Conclusion

The protein-enriched foam production time increased as the preheating time of the dilute albumin solution increased. Protein denaturation, as presumably determined by the preheating time of the protein solution, affected the void fraction significantly, perhaps by changing the amount of hydrophobic and hydrophilic heads that interact with the water. The initial void fraction of the dilute albumin solution determined by the weight method decreased by approx 10% when the preheating time was extended to 5 min. Correlations using the void fraction for bubbles of about 2 mm in diameter appear to show the effect of denaturation with the weight method for early foam fractionation times. Additional correlations between the void fraction and denatured proteins in the foam fractionation processes were determined with the photographic method, offering an independent measure of the effect of preheating albumin solution. Clearly, these preliminary results are not complete, but they do indicate that void fraction–foam fractionation curves may offer a simple and rapid means to determine protein denaturation.

Acknowledgments

This work was supported by the National Science Foundation Grant No. CTS-9712486. We also acknowledge support from Veara Loha for the purchase of the special Nikon camera and from the Vanderbilt University Research Council through the Office of the Dean of the Graduate School.

References

1. Arntfield, S. D., Murray, E. D., Ismond, M. A. H., and Bernatsky, A. M. (1989), *J. Food Sci.* **54(6),** 1624–1631.
2. Allewell, N. M. (1993), *Trends Biochem. Sci.* **18,** 1–2.
3. Loha, V., Prokop, A., Du, L., and Tanner, R. D. (1998), *Appl. Biochem. Biotechnol.* **77/79,** 701–712.
4. Greaves, M. and Kobbacy, K. A. H. (1984), *Chem. Eng. Res. Des.* **62,** 3–12.
5. Barigou, M. and Greaves, M. (1991), *Meas. Sci. Technol.* **2,** 318–326.
6. Zhang, Z., Dai, G., and Chen, M. (1989), *J. Chem. Eng. Chin. Univ.* (Gaoxiao Huagong Xuebao) **3(2),** 42–49 (in Chinese).