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Separating a Mixture of Egg Yolk and Egg White Using Foam Fractionation

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Abstract

A mixture created by blending with a spatula, an egg yolk and an egg white from the same egg can serve as a binary system for testing to see how well foam fractionation can be used to separate two different groups of proteins naturally found together. This mixture of two phases is particularly attractive for such a study because the two phases can be visualized distinctly when in their separated states. It has been shown that air alone at a low flow rate and with little or no water added can effect visually clean separations of egg yolk from egg white, making this a "green" separation process. The white precedes the yolk in the process, which takes less than 10 min at a laboratory scale.

Index Entries: Egg albumin; egg protein; egg white; egg yolk; foam fractionation; protein separation; serum albumin.

Introduction

Foam fractionation processes are promising methods for separating proteins found in water solutions. Other than the addition of air or another carrier gas, such as carbon dioxide, no other substances are needed to remove the foaming (hydrophobic) proteins from the other proteins found in a solution mixture. Therefore, contamination by exogenous chemical separation agents such as organic solvents is minimized. Natural fractionation phenomena occur in various processes such as the protein foam head formation in beer glasses, the breaking waves along ocean shorelines, in biological waste treatment plants *(1)*, and in commercial fermentation processes such as those used to create antibiotics and industrial enzymes like cellulase.

Foam fractionation is a simple and low-cost technique for separating and concentrating surface-active chemicals such as hydrophobic proteins. In foam fractionation, hydrophobic proteins are attached by adsorption to the gas bubbles, which then rise to the top of the bulk liquid at the surface *(2)*.

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The proteins then become more concentrated as they rise because water is lost as they move upward through drainage.

Proteins are made up of long chains of amino acids. Chicken egg proteins are found in either the egg yolk or the egg white phases. The main constituent of an egg white is the protein, ovalbumin, which makes up 75% of the protein mass in the egg white *(3)*. Other proteins that make up the egg white include conalbumin, ovomucoid, lysozyme, globulins (G2, G3), ovomucin, flavoprotein, ovoglycoprotein, ovomacroglobulin, avoinhibitor, and avidin *(3)*. Egg yolk contains lecithin, a natural detergent and also apovitellenins I and VI and phosvitin *(4)*.

Previous studies on foam fractionation show its feasibility for concentrating and separating proteins that foam. In general, foam fractionation is a very promising method for removing proteins from dilute solutions, for example, kudzu proteins from a retting solution and potato protein wastes in the potato industry *(5)*. Many biopolymers including bovine serum albumin (blood), albumin, pepsin, and urease have been purified by foam fractionation. One of the key characteristics of this process is the speed with which the enrichment occurs because of the large gas-liquid interface generated in the bubbling process *(6)*.

When an egg is cracked into a bowl and is beaten lightly with a spatula or whisk, it can be seen that even though the egg white and yolk incorporate into one another nicely, there still exists a boundary between the two phases. Separating the egg white from the egg yolk is a membrane called the vitelline membrane *(4)*. When this mixture is placed into a foam fractionation column, a separation of the initial two phases, egg yolk and egg white, can be seen, suggesting a binary separation of the two phases. This two-phase system is a model system used here to explore how foam fractionation can be used to separate two optically different phases, each of which contain many proteins. To prove the binary separation of the egg white from egg yolk, the concentration of egg yolk and egg white of the foamate at various stages of foaming needed to be found. Optional spectroscopy used here to quantify the egg yolk and egg white separation, is a simple, noninvasive analytical technique with an infinitely broad range of applications in plant biotechnology and food processing.

Proteins are found in numerous other naturally occurring mixtures, which can be thought of as phases. In addition to water solutions of egg proteins, kudzu vine fermentation proteins, sweet potato proteins, and blood plasma proteins can be thought of as being consisting of visibly colored groupings or phases for analysis and for foam fractionation separation herein. Blood plasma contains groups of soluble proteins, the most abundant of which is serum albumin *(7)*. It is interesting that the hydrophilic and hydrophobic carrier properties of albumins in a water solution render them suitable for separation from other proteins or protein groupings (phases) using foam fractionation. Perhaps even foam/bubble fractionation could also be used to separate plant-derived mixtures of hydrophilic tannin from

hydrophobic cellulose. The effect of physio-chemical parameters on the separation of proteins from human placenta extract using a continuous foam fractionation column has also been examined in previous studies *(8)*.

Because the egg yolk membranes appear to be fractionated and left behind in the bulk residue consisting mainly of egg yolk for the case of moderate hand premixing, perhaps the egg shell membrane can also be separated from other egg components by using foam fractionation. If so, then it might be possible to recover and concentrate those egg membranes, which are used as the substrate to grow viruses. This low-cost recovery could then perhaps be used as a first step in producing vaccines more quickly.

Materials and Methods

Sample Preparation

Grade A large white chicken eggs (Weiss Lake Pride, distributed by LA-127 Weiss Lake Egg Col, Centre, AL 35960) were purchased from a local grocery store and refrigerated at 5°C. Each egg was allowed to warm to approx 20°C because it was found that warm eggs foam better than cold eggs. The egg was then cracked, and the yolk and white (albumin) were separated by decantation into separate glass beakers. Owing to the high viscosity of fresh egg white, which made it hard to split and dilute with water, grade II crude, dried chicken egg white purchased from Sigma-Aldrich (St. Louis, MO) as Lot 88H1447, was used in its place. To create the calibration curve for the egg white, the dried egg white was diluted with water to the desired concentrations. For the yolk calibration curve, the whole yolk was weighed and then diluted down with water. More stirring was required to fully incorporate the yolk into the water than was required for the dried egg white. For the foam fractionation experiment, a whole egg was placed in a beaker and beaten lightly with a spatula for approx 5 min. For a separate experiment, another egg was placed into a blender (Blend Master 10, with a 350 W motor, made by Hamilton Beach/Proctor-Silex Inc., Washington, DC) and mixed on high speed for 5 min.

Experimental Procedure

The experimental foam fractionation column used was made of glass with an inside diameter of 2 cm and a height of 10 cm. A porous ceramic (fritted) disk sparger with medium sized porosity was fitted inside the inner column wall near the bottom. Air from a compressed gas cylinder was introduced at a low rate (approx 35 mL/min) into the column after passing through a humidifying flask. The apparatus is shown in Fig. 1 and a typical spectrum of colors from a foaming experiment are described in Fig. 2. To test for the role of membranes in restricting the foaming process, the egg that was beaten by hand and an egg beaten vigorously in a blender to better break down the existing membranes were foamed in separate experiments. During the first experiment, the whole egg beaten by hand

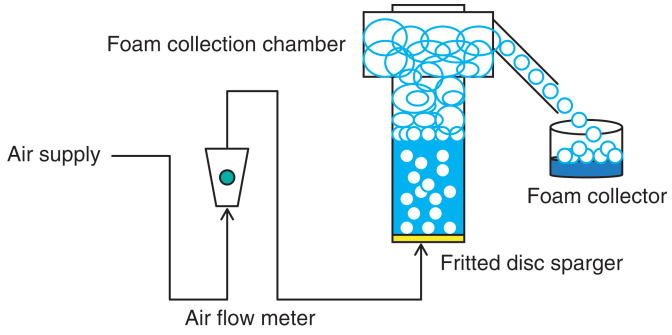

Fig. 1. Schematic drawing of the foam fractionation apparatus.

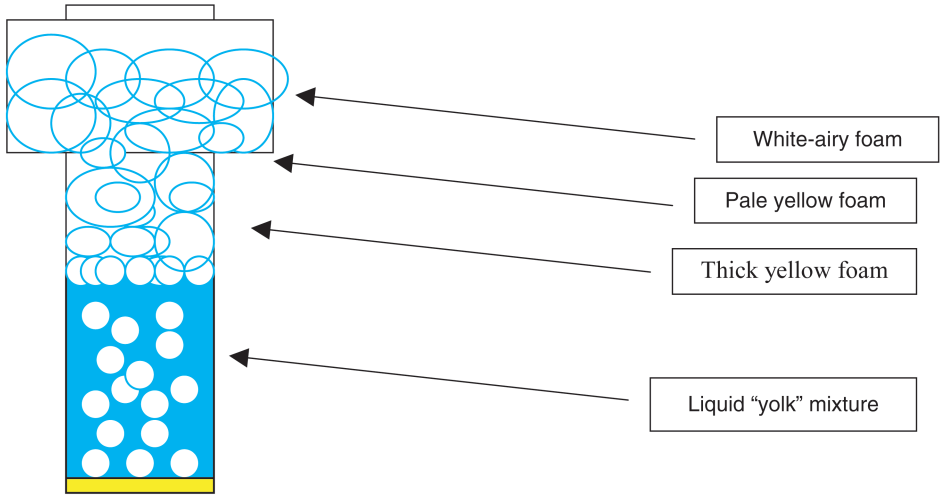

Fig. 2. Gradient of color in foaming within the column pictured in Fig. 1 of whole egg beaten by hand. This is observed before the collection of effluent foam to create the foamate in the foam collector.

was foamed. The second trial was done with the egg that was beaten by a blender. In each experiment foamate was collected at various time intervals and the absorbencies were read using a spectrophotometer.

Spectroscopy

To initially determine how the egg phases absorb in the spectrophotometer, two samples, one of pure egg yolk and one of egg albumin (mostly ovalbumin, *[9]*) powder were diluted with water to reach the Beer's law concentration range (≤0.94 g/L for egg yolk and ≤4.44 g/L for egg white led to linear absorbance vs concentration curves). The spectrophotometer (Bausch and Lomb, Spec 20) was calibrated according to the instructions and then absorbencies were read for the given concentration at a wide range of wavelengths. Only wavelengths in the visible light range (<600 nm) were used.

Once the two desired wavelengths were selected the absorbance vs wavelength scans data of each of the two phases, white and yolk, were taken for different concentrations of pure egg yolk and pure egg albumin powder using the previously prepared samples. Calibration curves were created for both yolk and white and used to determine the concentrations of yolk and white for the samples of the foamate, which were collected at various time intervals. To test the linearity range of Beer's Law and establish the associated linear coefficients correlating absorbance with the underlying phase concentration, the absorbance of each sample was found. The smaller the concentration, the better Beer's Law is expected to hold and meet the assumption of linear superposition of the component absorbencies of the yolk and white, so that these phase components will equal the measured total absorbance.

Results and Discussion

Visibly, when the egg was mixed by the blender on high speed for 5 min, the resulting mixture was "smoother" and more homogenous throughout as compared with the egg white and egg yolk that was blended by hand, mixing with a spatula. During the foam fractionation of a whole hand-beaten egg, the mixture was separated into two phases in the column. It took a few minutes for foamate to be produced, but once it started, the process went quickly. As seen in the schematic, shown in Fig. 2, the foamate at the top of the column was very white, and as foaming continued it increased in yellow color until the last of the foam left in the column was purely yellow. The yellow foam or yolk, could not make it out of the column at the flow rate we were using, but it remained in a foam-like state within the column, effectively creating a white foamate phase at the top of the column and a yellow residual liquid phase at the bottom of the column. The egg white foamed much more effectively than the yolk owing to the higher concentration of proteins; the lower concentration of fat in the white vs the yolk was especially because the fat in the yolk impedes foaming. It appeared that the egg mixture was reverting back into its original phases, egg white and egg yolk.

When the egg mixed by the blender was aerated, it began foaming immediately; however, there was no visible separation between the yolk and the white. Even when collected, the foam remained a pale yellow color as opposed to being separated out into white and yellow colors. The foamate was collected in a large container and after allowing it to sit for 5–10 min, the yellow yolk "dripped" to the bottom of the container and settled as a liquid. While doing so, the foamate on the top of the container became more and more white. Thus, a difference in the intensity of mixing before separation in the foam fractionation apparatus is a significant factor in the separation of the yolk and white phases. These experiments illustrate the ability of foam fractionation to separate visibly different

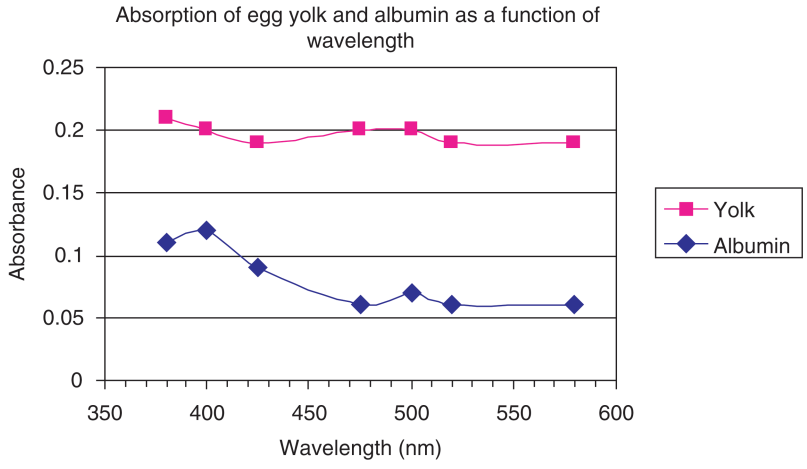

Fig. 3. The absorbance of a powdered egg albumin sample (concentration 4.44 g/L) and an egg yolk sample (concentration 0.94 g/L) scanned across the wavelength ranging from 380 to 580 nm.

phases from moderately mixed egg white and yolk solutions. The yellow color in the yolk mainly comes from the carotenoid, canthaxanthin *(10)*. This carotenoid most likely acts as a tracer, describing how the intensity of mixing allows spillover of the yellow color to the entire mixture of white and yolk when mixing is vigorous, and is retained in the yolk when mixing is gentle.

Figure 3 shows the results obtained by scanning a sample of egg yolk diluted with water and a sample of egg white powder diluted with water across a range of wavelengths. It can be seen that there are local peaks in the absorbance of egg white at 400 and 520 nm and a somewhat larger deviation in the absorbencies of egg white and egg yolk at 520 nm (than at 400 nm), wherein the white is close to its maximum absorbance. These two wavelengths were selected to create the calibration curves. In general, when both of the absorbing phases contribute to the total absorbance, the following set of equations can describe the system. For λ_1 = 520 nm, and λ_2 = 400 nm, the respective absorbencies are A_1 and A_2.

$$A_1 = k_1 C_w + k_3 C_y \tag{1}$$

$$A_2 = k_2 C_w + k_4 C_y \tag{2}$$

$$C_w = \frac{k_3 A_2 - k_4 A_1}{k_2 k_3 - k_1 k_4} \tag{3}$$

$$C_y = \frac{k_2 A_1 - k_1 A_2}{k_2 k_3 - k_1 k_4} \tag{4}$$

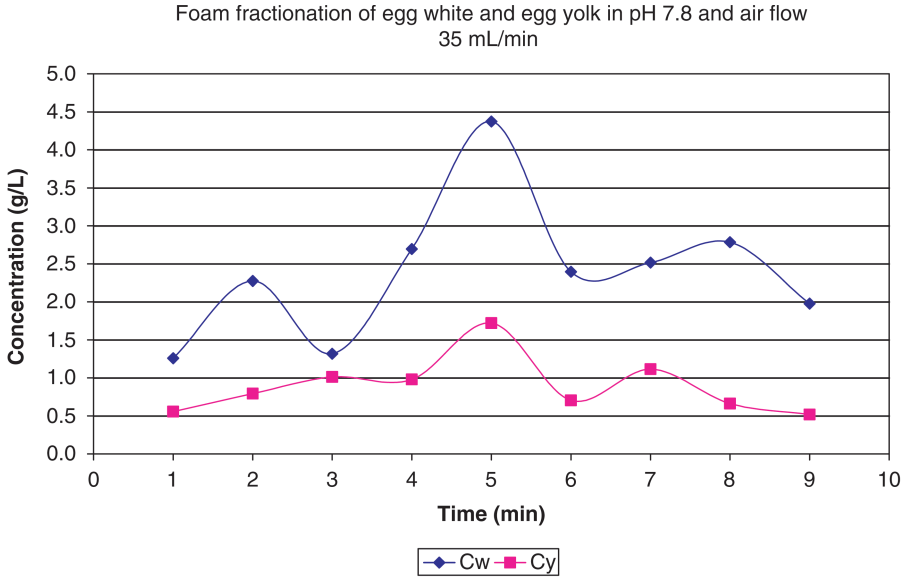

Fig. 4. The concentration of egg white and egg yolk foamate as a function of time at pH 7.8 and airflow rate of 35 mL/min.

The absorbance of an unknown sample found at either 520 nm or 400 nm, A_1 and A_2 respectively, can be found experimentally using a spectrophotometer for a sample with unknown concentration. It is imperative that λ_1 and λ_2 be selected to minimize the chance that the $(k_2k_3–k_1k_4)$ term (sometimes called the Wronskian in mathematics *[11]*) does not equal zero. Given k_1 and k_3 from the respective slopes from the developed calibration curves (consisting of three points each, in addition to the origin) for white and yolk at $\lambda_1 = 520$ nm, and k_2 and k_4 from the respective slopes of white and yolk (consisting of three points, respectively, at $\lambda_2 = 400$ nm [approx 420 nm], in keeping with earlier work with a different egg albumin absorbance-wavelength scan, absorbing at a local peak of 420 nm), Eqs. 1 and 2 can be combined to form Eqs. 3 and 4. Given A_1 and A_2 from experimentation, the concentration of egg white, C_w, and the concentration of egg yolk, C_y can be calculated. Here, $k_1 = 0.013$ L/g, $k_2 = 0.028$ L/g, $k_3 = 0.24$ L/g, and $k_4 = 0.26$ L/g. Typical values of C_w and C_y as functions of time are given in Fig. 4. Because the egg white tended to be produced in higher concentration than the yolk, the egg white concentration is depicted above the yolk concentration. Thus, the visual observation noted in Fig. 2 is verified by the absorption results in Fig. 4. The actual measured effluent samples are diluted down (1000X) with deionized water to the concentration levels in order to read the respective calibration graphs in the Beer's law range. The actual reported concentrations take the dilution factor into consideration. It is suggested that future work focus on measurement of the effect of

the airflow rate and the pH on the foam fractionation process. If the airflow rate is reduced then the separation will take longer, but perhaps, the separation between yolk and white may be more extensive.

Conclusions

The observed separation of a binary mixture from a whole egg using foam fractionation, demonstrates that it is possible to separate two phases of protein groups as a first step in more complete protein separations. This inexpensive method can possibly be used to separate other colored mixtures of proteins (such as seen in the separation of hydrophobic protein in beer foam from hydrophilic protein in the beer bulk phase, both within a glass of beer) found in natural substances. The similarity between egg albumin (ovalbumin) and blood albumin (serum albumin) might open up the possibility of less expensive separations before more complete chromatographic separations in the medical world. Blood albumin must be separated out of whole blood in order to use it in some medical applications such as the treatment of shock.

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