Kinetics of Destabilization of Soy Protein Foams

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The kinetics of destabilization of soy protein foams was studied by monitoring the decay of interfacial area of foams as a function of time. The decay of soy protein isolate, soy 11S, and soy 7S foams followed biphasic first-order kinetics. These two kinetic phases were related to gravitational liquid drainage and interbubble gas diffusion processes. The relative magnitude of the contribution of these two microscopic processes to foam decay was dependent on environmental factors such as pH, temperature, ionic strength, and protein concentration. Soy isolate and soy 11S foams were more stable than soy 7S foams. The poor stability of soy 7S foam was mainly due to its inability to retard gravitational drainage.

INTRODUCTION

Soy proteins are increasingly being used in fabricated food products, such as processed meats. However, the successful and extended use of soy protein in other types of foods, such as emulsion and foam-type food products, requires extensive investigation of their surface properties under various experimental conditions.

Soybean contains two major storage proteins, viz., 11S and 7S globulins. The 11S globulin has a quaternary structure composed of 12 subunits. It is a dimer of two identical hexamers. Three of the subunits in the hexamer are acidic and the other three are basic in nature. Each pair of acidic and basic subunits is linked via a disulfide bond. The quaternary structure of 11S is affected by environmental factors such as ionic strength, pH, and temperature (Kinsella et al., 1985). For example, soy 11S globulin forms an associated polymer at pH 7.6 as the ionic strength of the solvent is reduced from 0.5 to 0.1. Like the 11S globulin, the 7S globulin also undergoes reversible association–dissociation reactions depending on the ionic strength of the solvent (Koshiyama, 1968; Neilsen, 1985). The quaternary structure of 7S globulin is made up of three subunits, with a molecular weight of about 180 000. In contrast to 11S, the 7S globulin is a glycoprotein, does not contain disulfide bonds, and has a very low sulfur content (Neilsen, 1985).

Several studies have been reported on the foaming and interfacial properties of soy proteins (German et al., 1985; Kim and Kinsella, 1987a,b). However, in most of these studies the liquid drainage method has been used to study the stability of soy protein foams. As pointed out by Halling (1981), measurement of liquid drainage from the lamellar phase of foams provides only limited information on the molecular processes that affect foam stability. In contrast, measurement of the rate of decay of the interfacial area of foams, which is a more fundamental variable than the liquid drainage, would provide more insight into the microscopic processes that affect the stability of food protein foams. In the present study the kinetics of destabilization of soy protein foams under various conditions was studied by using the foam apparatus described in the preceding paper (Yu and Damodaran, 1991).

MATERIALS AND METHODS

Soy protein isolate, soy 11S, and soy 7S fractions were prepared from defatted soybean flour (Central Soya, Chicago) according to the method described by Thanh and Shibasaki (1976).

Protein solutions were prepared in 20 mM sodium phosphate buffer (pH 7.0, $\mu = 0.05$). Protein concentration was determined by the biuret method. Unless otherwise indicated, all foaming studies were performed with 1% (w/v) protein solutions.

Kinetic Studies. The kinetics of destabilization of soy protein foams was studied according to the method described in the preceding paper (Yu and Damodaran, 1991). The rate of pressure change in the foam column was monitored by using a differential pressure transducer. The pressure change inside the foam column was transformed to changes in the interfacial area of the foam according to the relation

$$A_t = \frac{3V}{2\gamma}(\Delta P_\infty - \Delta P_t)$$

where A_t is the interfacial area of the foam at time t, V is the total volume of the foam apparatus, γ is the surface tension of the protein solution, ΔP_t is the net change in pressure at time t, and ΔP_∞ is the pressure change when the foam completely collapses at infinite time.

A dimensionless fractional interfacial area at any given time t during foam decay was calculated by using the relation

$$A_t/A_0 = (\Delta P_\infty - \Delta P_t)/\Delta P_\infty$$

where A_0 is the initial interfacial area of the foam, which is given by $A_0 = 3V\Delta P_\infty/2\gamma$. The decay of soy protein foams was analyzed according to the biphasic first-order kinetics equation (Yu and Damodaran, 1991)

$$A_t/A_0 = Q_g \exp(-k_g t) + Q_d \exp(-k_d t)$$

where k_g and k_d are first-order rate constants for the decay due to gravitational liquid drainage and interbubble gas diffusion, respectively, and Q_g and Q_d are the amplitude parameters of the two kinetic phases. Unless otherwise indicated, each kinetic curve represents an average of three runs.

RESULTS AND DISCUSSION

Since soy protein isolate contains two major protein fractions, viz., 7S and 11S globulins, the foaming properties of soy isolate should be related to the interfacial properties of its constituent protein fractions. To elucidate which of the two protein fractions exerts a major influence on the stability of soy isolate foam, the behaviors of 11S and 7S foams were studied.

In the preliminary studies it was found that while it was easy to generate the required amount of foam from a 1% (w/v) solution of soy isolate and soy 11S, in the case of soy 7S at a 1% concentration it was difficult to generate any reasonable volume of foam even after an extended period of bubbling. It was observed that at 1% soy 7S concentration, the rate of breakage of the foam was almost equal to the rate of formation of foam, which resulted in no

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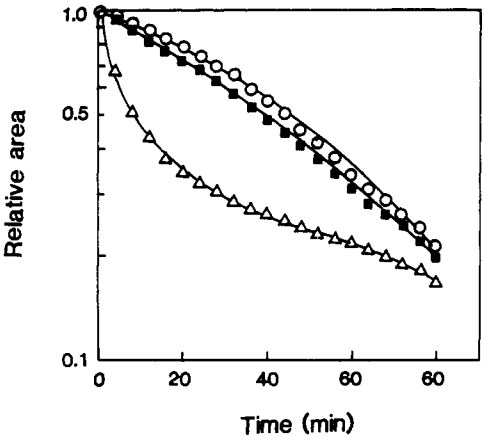

Figure 1. Surface area decay of soy protein foams (2%, pH 7.0) at 25 °C: (○) 11S globulin; (△) 7S globulin; (■) soy isolate. Each curve is an average of three experiments.

Table I. Initial Surface Area, A_0, of Three Soy Protein Foams (2.0%, pH 7.0) at 25 °C

soy protein	$A_0 \times 10^{-3}$, cm^2
11S	27.7 ± 2.2
7S	19.5 ± 2.5
soy isolate	16.0 ± 1.8

foam formation. However, when the protein concentration was increased to 2% (w/v), it was possible to generate the volume of foam required for monitoring the kinetics of destabilization of the foam by using the foam apparatus.

The initial surface area, A_0, of soy isolate, soy 11S, and soy 7S foams generated from a 2% solution is presented in Table I. The purities of the 11S and 7S protein preparations used in this study were about 95% and 75%, respectively, as judged by SDS–PAGE. The soy protein isolate used in this study contained about equal amounts of soy 11S and soy 7S. The A_0 of soy 11S foam was much greater than those of soy 7S and soy isolate, indicating that the average size of bubbles in soy 11S foam was the smallest. It should be noted that even though the 11S content of soy isolate was greater than that of the crude 7S preparation, the A_0 value of soy isolate was smaller than that of soy 7S. This suggests that in a mixture of soy 7S and 11S the foamability in terms of interfacial area generation is not a simple additive function of the foamabilities of the 7S and 11S components. It is known that native soy 11S and 7S proteins do not interact with each other in aqueous solutions. Hence, it is reasonable to assume that the relative extent of adsorption of these proteins at the interface is solely dependent on their individual molecular characteristics in the solution state. Previously, it has been reported that when soy isolate is heated at 90 °C, the dissociated basic subunits of 11S interact with and form a soluble complex with the subunits of 7S (German et al., 1982; Damodaran and Kinsella, 1982). It is quite possible, therefore, that in the adsorbed state at the air–water interface, dissociation and denaturation of these proteins, caused by surface denaturation, may facilitate conditions for intermolecular interaction between these proteins. Under these conditions, the dynamics of creation of interfacial area of the foam could be influenced by the extent of these interactions and the composition and relative distribution of 11S and 7S proteins in the film rather than by the composition in the bulk phase.

The kinetics of surface area decay of 11S, 7S, and soy isolate foams are shown in Figure 1. Among these three soy protein preparations, soy 11S foam exhibited the highest stability and the soy 7S foam exhibited the least stability. All three soy protein preparations exhibited biphasic first-order kinetics, indicating that two microscopic processes, presumably liquid drainage and interbubble gas diffusion, are involved in foam decay. It should be noted that while the nonlinear first-order curves of soy 11S and soy isolate were convex in shape, that of soy 7S was concave in shape. As discussed previously (Yu and Damodaran, 1991), in the case of concave first-order plots, the decay due to gravitational drainage is much faster than that due to gas diffusion. In other words, in concave-type first-order decay, the liquid drainage process is not the rate-limiting step in the decay of foam. On the other hand, in the case of convex-type first-order plots, the decay due to liquid drainage is slower than that due to gas diffusion. Since interbubble gas diffusion cannot be significant above a critical thickness of the lamella film, the gravitational drainage in a convex-type first-order plot is the rate-limiting step for foam decay. The data in Figure 1 suggest that whereas the gravitational liquid drainage is the rate-limiting step in soy protein isolate and soy 11S foams, it is not the rate-limiting process in the decay of soy 7S foam. This suggests that soy 11S films exhibit better rheological and water-holding properties than does the soy 7S protein film. This is surprising, because it has been reported that in the range of 1–12% protein concentration the viscosity of soy 7S dispersion was significantly greater than that of soy 11S (Rato et al., 1986). Furthermore, since soy 7S is a glycoprotein with a carbohydrate content of 4% (Hermansson, 1978), the water-binding capacity of soy 7S would be expected to be greater than that of soy 11S. The apparent disagreement between the solution behavior and the interfacial behavior of soy 7S and 11S indicates that factors other than water-holding capacity and bulk rheological properties may have greater influence on the stability of 7S and 11S foams. These may be related to differences in the electrostatic properties of soy 7S and 11S globulins. The subunits of soy 7S are highly negatively charged: the pI of the subunits ranges from 4.9 to 5.7 (Kinsella et al., 1985), and the net pI of the 7S protein is in the neighborhood of 4.8. On the other hand, soy 11S consists of acidic and basic subunits; the pI of the acidic and basic subunits ranges from 4.6 to 5.4 and from 8.0 to 8.5, respectively. This results in an apparent pI of 6.4 for the 11S oligomer (Thanh and Shibasaki, 1976). In addition, about a 0.47 fraction of the total amino acid residues in soy 7S are charged at pH 6.0, whereas only a 0.2 fraction of the residues of 11S are charged under the same conditions (Kinsella et al., 1985). These differences clearly indicate that the electronegativity and hydrophilicity of soy 7S at pH 7.0 are much greater than those of soy 11S. This strongly suggests that the electrostatic potential energy barrier for the adsorption of soy 7S at the air–water interface during bubbling would be very high and hence the amount of protein adsorbed would be very low. Furthermore, the strong electrostatic repulsion between the adsorbed molecules would hinder formation of a cohesive protein network at the interface. These adverse interactions would accelerate drainage of the lamella fluid and the eventual rupture of the protein film. In contrast, because of the low charge density of 11S at pH 7.0 (pI = 6.4), the electrostatic potential energy barrier for adsorption at the interface would be very low. Furthermore, the absence of strong electrostatic repulsion between the 11S molecules at the interface would facilitate formation of a viscous multilayer protein network that would retard drainage of lamella fluid.

It is noteworthy that even though the foamability of soy isolate is poorer than that of soy 7S (Table I), the stability

Table II. Gravitational Drainage (k_g) and Gas Diffusional (k_d) Rate Constants for the Decay of Soy Protein Foams (2%, pH 7.0) at 25 °C

soy protein	$k_g \times 10^{-2}$, min^{-1}	$k_d \times 10^2$, min^{-1}
11S	1.349	2.362
7S	15.566	1.032
soy isolate	1.750	2.198

of soy isolate foam is significantly greater than that of soy 7S foam (Figure 1). Previous studies have shown that proteins that exhibit good foamability (e.g., β-casein) often lack the molecular properties that impart stability, and proteins that produce stable foams (e.g., lysozyme) often lack the ability to create foams (Graham and Phillips, 1976). It appears that foamability and stability are two different properties of protein foams which are influenced by two different sets of molecular properties. In the case of soy isolate foam, while interactions between various protein components apparently decrease its foamability, they seem to impart stability to the foam.

The first-order rate constants of decay due to the drainage and gas diffusion processes for the 11S, 7S, and soy protein isolate foams are presented in Table II. The drainage rate constant of soy 7S was severalfold greater than that of soy 11S and soy isolate. It should be noted that even though the initial surface area created by 7S was slightly greater than that of soy isolate (Table I), the rate of decay of 7S foam due to liquid drainage was much faster than that of soy isolate. This implies that the single most important factor that affects the stability of soy 7S foam is its inability to retain the lamella fluid. It should be pointed out that in terms of composition the main difference between soy isolate and crude 7S is that while the former contained 50% 7S globulin, the latter contained 75% 7S globulin. Therefore, the remarkable difference in the stability of soy isolate and crude 7S protein foams should be attributable to the higher content of 7S globulin in the crude 7S preparation. These results also might imply that up to a certain ratio of 7S to 11S, the positive effect of the 11S component might overcome the negative effect of the 7S component on foam stability. Above a critical ratio of 7S to 11S, however, the deleterious effect of the 7S fraction might have a greater influence on foam stability.

Effect of Ionic Strength on Soy Isolate Foam. The effect of NaCl concentration on the kinetics of destabilization of soy isolate foam formed from a 1% solution, pH 7.0, at 25 °C is shown in Figure 2. The stability increased with increase of ionic strength up to 0.1, indicating that electrostatic interactions play an important role in the integrity of the foam. Increase of ionic strength above 0.1 did not cause further increase in the stability of the foam. This is not surprising because it is known that electrostatic forces in proteins are effectively neutralized at about 0.1 ionic strength (Eagland, 1975). It is interesting to note that while the first-order plots at 0 and 5 mM NaCl were essentially linear, they were slightly convex in shape at and above 0.1 M NaCl. This suggests that the increased stability of soy isolate foam in these more concentrated NaCl solutions was predominantly due to a decrease in the gravitational drainage rate. The relationships between ionic strength and the drainage rate constant, k_g, and gas diffusional rate constant, k_d, are shown in Figure 3. The k_g dropped precipitously at 0.1 M NaCl and remained constant up to 1.0 M NaCl. However, the k_d dropped initially up to 0.1 M NaCl and slightly increased at 1.0 M NaCl.

The initial surface area, A_0, of soy isolate foam at various NaCl concentrations is presented in Table III. Unlike

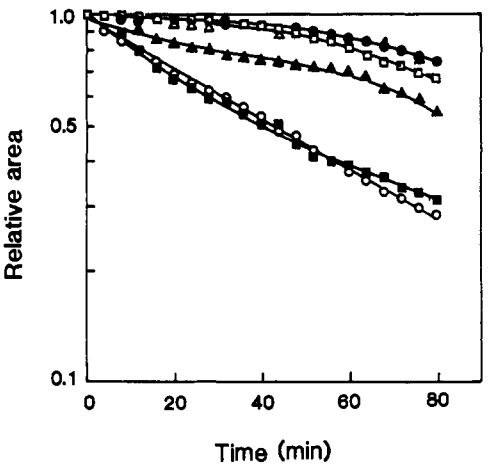

Figure 2. Effect of ionic strength on surface area decay of soy protein isolate foam (1%, pH 7.0) at 25 °C: (○) no salt; (■) 0.005 M NaCl; (▲) 0.05 M NaCl; (△) 0.1 M NaCl; (●) 0.5 M NaCl; (□) 1.0 M NaCl. The data represent a single measurement at each salt concentration.

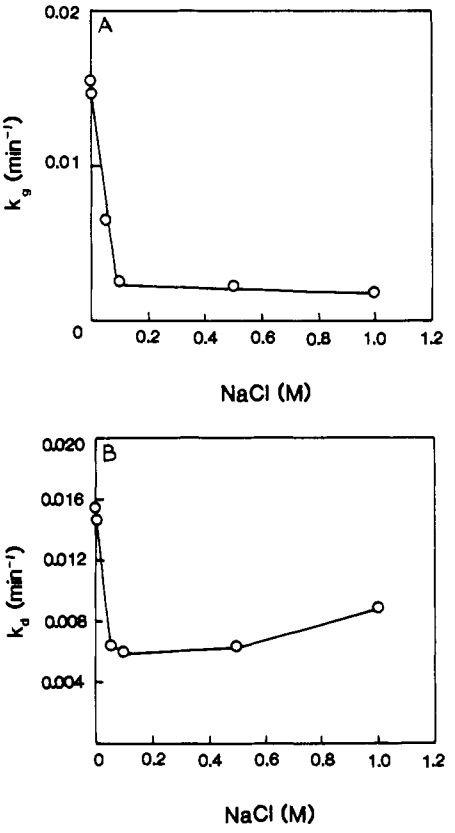

Figure 3. Effect of NaCl on (A) the gravitational rate constant and (B) the diffusional rate constant of decay of soy protein isolate foam (1.0%, pH 7.0) at 25 °C.

Table III. Initial Surface Area, A_0, of Soy Isolate Foam (1.0%, pH 7.0) at Various Salt Concentrations at 25 °C

concn of NaCl, M	$A_0 \times 10^{-3}$, cm^2	concn of NaCl, M	$A_0 \times 10^{-3}$, cm^2
0.0	10.8	0.1	21.4
0.005	15.2	0.5	18.9
0.05	31.8	1.0	25.9

stability, A_0 reached a maximum at 0.05 M NaCl and decreased at higher NaCl concentrations; however, a slight increase was observed at 1.0 M NaCl. The lack of a parallel between the behaviors of A_0 and stability as a function of ionic strength indicates that the factors that govern the creation of interfacial area of foams are not exactly the ones that determine the stability of foams.

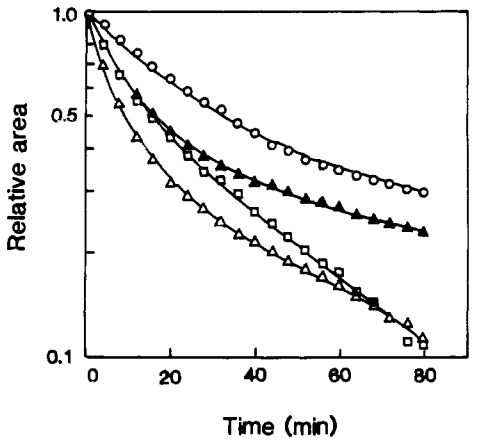

Figure 4. Surface area decay of soy 11S foam (1.0%, pH 7.0) at various temperatures: (○) 15 °C; (▲) 25 °C; (□) 25 °C; (△) 30 °C. Each curve is an average of three experiments.

Table IV. Initial Surface Area, A_0, of Soy 11S Foam (1.0%, pH 7.0) at Various Temperatures

temp, °C	$A_0 \times 10^{-3}$, cm^2	temp, °C	$A_0 \times 10^3$, cm^2
15	20.2 ■ 2.5	25	21.4 ± 4.1
20	18.0 ± 0.7	30	18.6 ± 2.5

Table V. Gravitational Drainage and Gas Diffusional Rate Constants for the Decay of Soy 11S Foam (1.0%, pH 7.0) at Various Temperatures

temp, °C	$k_g \times 10^2$, min^{-1}	$k_d \times 10^2$, min^{-1}
15	5.286	0.767
20	19.225	0.859
25	16.152	2.308
30	17.619	1.632

Effect of Temperature on Soy 11S Foam. The effect of temperature on the stability of soy 11S foam is shown in Figure 4, and the values for drainage and gas diffusional rate constants are presented in Table IV. The stability of soy 11S foam decreased with increases in temperature. At all temperatures studied, the shapes of the first-order plots were concave, indicating that the gravitational drainage was not the rate-limiting step for the decay process. This is in contrast to the behavior of BSA foam, which exhibited a convex curve at 15 °C (Yu and Damodaran, 1991). In phenomenological terms, the increased stability of soy 11S foam at lower temperatures is related to an increase in the viscosity of the lamella liquid as well as the film. Since the denaturation temperature of soy 11S is about 85 °C (Damodaran, 1988), the effect seen in the range of temperature studied cannot be attributed to temperature-induced conformational changes in the protein. It is probable, however, that a small increase or decrease in temperature, even in the range of 15–40 °C, may have a greater modulating effect on the surface/interfacial denaturation of soy 11S and thus on its interfacial behavior. However, there is no evidence in the literature to substantiate this speculation.

The initial surface area of soy 11S foam formed at various temperatures is presented in Table V. The A_0 was not affected significantly by temperature in the range studied ($p < 0.05$). This is in contrast to the behavior of BSA foam, which exhibited a 2-fold increase in A_0 at 40 °C compared to that at 15 °C (Yu and Damodaran, 1991). This demonstrates that the foaming activity (i.e., the amount of interfacial area created) of proteins under similar sets of foaming conditions is related to their unique physicochemical properties. The stability of the foam, however, is greatly affected by the rheological properties of the lamella which very much depend on environmental conditions as well as the physicochemical properties of the proteins.

The results presented here on the stability of soy protein foams clearly indicate that at least two microscopic processes, namely, gravitational liquid drainage and interbubble gas diffusion, are involved in the decay of soy protein foams. The inability of soy 7S to foam at 1% protein concentration and the rapid decay of the 7S foam formed at 2% protein concentration are related mainly to its inability to retard gravitational liquid drainage.

The results also indicate that the decay behavior of soy protein foams is affected by environmental conditions. For example, whereas the decay of soy 11S foam generated from a 2% protein solution exhibited a convex-type curve (Figure 1), the soy 11S foam formed from a 1% protein solution exhibited a concave-type decay curve (Figure 4) at 25 °C. It is also interesting to note that the decay rate constant k_g (related to gravitational liquid drainage) for the 2% 11S foam was more than 1 order of magnitude greater than that for the 1% soy 11S foam, whereas the decay rate constant k_d (related to interbubble gas diffusion) was almost the same for the 1% and 2% soy 11S foams (Tables II and V). This indicates that while the bulk-phase protein concentration affects the rheological properties of the lamella fluid very significantly, it does not affect the physical properties of the interfacial protein film. The effect of protein concentration on k_g might be attributable to an increase in viscosity of the protein solution. However, it should be pointed out that the difference in viscosity between 1% and 2% soy 11S solutions is only marginal (Rao et al., 1986). This clearly suggests that a slight increase in viscosity of protein solution has profound influence on the liquid drainage rate and thus on the stability of the foam. In other words, the results presented here suggest that the stability of soy protein foams can be improved by improving the rheological properties of soy proteins.

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