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☘Film Forming and Foaming Behavior of Food Proteins

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ABSTRACT

The current state of understanding of protein structure as it relates to its function in foaming has proven to be of sufficient accuracy to predict the effects of particular modifications in soy proteins. Comparative whipping studies performed on egg white, casein, Bovine serum albumin and soy protein showed important differences both in the development and subsequent stability of foams produced from these proteins. Our understanding of the structures of soy proteins and the alterations induced by reductive modification and heating implied that similar modifications would have dramatic impact on the foaming properties specifically of the 11S protein. The foaming ability and stability of the 11S protein were enhanced dramatically by cleavage of intersubunit disulfide bridging. Computerized lamellar measurement techniques developed in this laboratory indicated that these modifications enhanced the ability of the protein to foam rapidly and then to stabilize surface films at the rate typically encountered in the whipping of foams.

INTRODUCTION

The unique structural properties of certain proteins is largely responsible for the proliferation of diverse foods with highly desirable texture, flavor and stability characteristics. Foods such as foams (whipped toppings, cakes, ice cream), gels (meats, cheeses) and emulsions (dressings, sausage) are dependent on specific protein components typically present at relatively low levels (1). As food sources, processing methods and consumer tastes evolve, the need to understand and predict the behavior particularly of the protein components of these foods becomes increasingly acute. Foams, in particular, constitute systems in which the protein component plays a highly 'functional' role (1). Considerable research in recent years has begun to unravel the structural basis of the foaming properties of different proteins and even to predict means by which this functionality can be improved.

A foam can be defined loosely as a two-phase system in which a distinct gas bubble phase is surrounded by a continuous liquid lamellar phase. A consequence of this dispersion is a very large gas-liquid interface. Since interfaces between non-miscible phases are under tension, the expansion of these interfaces requires energy, i.e., work is performed in forming them and energy is released on their relaxation, hence foams are highly unstable. Surfactants, being amphiphilic, orient at an interface and lower the energy or tension of that interface (2). In a dynamic, energetic system such as the formation of a foam, the ability of a foaming agent (surfactant) to rapidly reach an interface, effectively lower the interfacial tension and stabilize new surface determines its capacity to form foams. Once formed, each bubble is separated by a very thin column of liquid and must be stabilized by the film surrounding the gas. The role of a foaming agent at this stage must be to associate into a cohesive network or membrane which can withstand minor physical perturbations and repel the approach of adjacent films (1, 3,4). This introduces a basic paradox in the requirements of an effective foaming agent. That is, a perfect amphiphile should be soluble, small and flexible enough to rapidly absorb to and coat fresh surfaces as it is exposed, then interact immediately among adjacent molecules sufficiently to form a stable film.

Ideally, proteins as amphiphilic, structurally dynamic macromolecules are able to fulfill both roles in the chemistry of foams. They typically lower the surface tension of the air-water interface by up to 50%, facilitating rapid expansion of the surface (5). Also, by virtue of their ability to associate into a multimolecular matrix, proteins form surface films which retard the coalescence and collapse of the bubbles. Not all proteins possess this capacity, and different proteins of varying sizes, structures and flexibilities differ dramatically in their ability to form and stabilize foams (1, 3,6). It is the relationship between the structure of proteins and their ultimate role in the diverse processes of film and foam formation which new research techniques and more complete information on protein structure are beginning to elucidate.

Foam Formation

In terms of foam formation, certain general principles have been found. Several studies have described a parallel between a protein's foaming behavior and its capacity to lower surface tensions rapidly (1,3,6,7). This tendency is related to the ability of the protein to reach, absorb and 'unfold' rapidly at the interface. The rate at which a protein reaches a clean interface is related primarily to its diffusion coefficient; generally, the smaller the protein the faster it will move to an interface (4). A clean surface is very rapidly covered by a thin monolayer of protein. Subsequent association of proteins with the interface is related to their ability to adsorb onto and insert themselves into a preexisting film rather than disrupt the film's integrity via repulsive interactions or return to the bulk aqueous phase. This would re-

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quire a protein which exhibits a tendency to bind to hydrophobic surfaces. Work by Kato and Nakai (8) and Keshevarz and Nakai (9) has shown a clear correlation between the available hydrophobic surface on various proteins and their ability to produce foams. Finally, once at the interface a protein must be able to associate with and become part of the film. Since for proteins, which owe their native structure largely to the aqueous solvent, an interface is a strongly denaturing environment, this ability logically would require a protein which could be readily unfolded or denatured. Graham and Phillips (3) have shown that molecular flexibility or ease of unfolding of at least part of the proteins they studied paralleled their ability to produce stable films and generate foams with large surface areas.

These various lines of evidence suggest that a protein ideally suited to form foams should be relatively small and highly flexible, with significant exposed hydrophobic surface. This facilitates orientation and adsorption at the interface: molecular unfolding/rearrangement and protein-protein interaction during film formation (6).

Foam Stability

Once a foam bubble system is formed, its overall stability, that is the resistance of the lamella to drainage and of the bubbles to collapse, becomes dependent on the rheological and adhesive properties of the interfacial films surrounding the gas (6,4). Again, the structure and conformation of the particular protein(s) affect the physical properties of the membrane film: its surface topography, mechanical strength, viscoelasticity and water binding capacity. Studies on interfacial films suggest that these properties require proteins which retain extensive secondary and even tertiary structure at the interface (10,11). Proteins which have a high molecular weight exhibit greater film strength and foam stability; i.e., partial hydrolysis of various proteins almost invariably lower foam stability (12,13), and polymerization of proteins increases the film viscosity (14). Finally, proteins which possess a minimum of net charge have been observed to exhibit greater foam stability, and conditions which minimize the charge on a protein tend to increase the foam stability significantly (15,4).

The problem with predicting foaming behavior from protein structure, therefore, is in reconciling each of these disparate physical events with the conformational events induced in a particular protein when exposed to an interface. This involves both thermodynamic and kinetic parameters. For example, the seeming paradox of requiring a protein which both unfolds easily at the interface, and yet retains considerable secondary and tertiary structure at that interface, suggests that a critical balance must be met in forming and then stabilizing a film in which the forces and rates of molecular events at the surface are crucial (1). Unfortunately, solving these problems is confounded by the lack of unequivocal methods based on physical principles to measure precisely the various stages in a dynamic foaming system and the structural modifications in the protein. Perhaps even more disconcerting is the lack of agreement on a standardized method to simply describe the foaming ability of standard proteins.

METHODS OF FOAM ASSESSMENT

Foam Development

Several methods have been reported for the incorporation of air into a protein solution, including whipping, injection, sparging and shaking (6). Each has certain advantages depending on the specific research interests. However, important differences exist between the foams produced, and comparisons between data must be considered very carefully. Whipping highly concentrated protein solutions in a standard mixer most closely parallels the practical production of foams and can discern important differences between proteins. This method is also the most widely reported, thus the most easily incorporated into a standardized methodology. We have investigated various whipper geometries with the objective of establishing a standard method and found that the double beater configuration is faster, more repeatable and requires significantly less sample than single whipper units. In this study, protein solutions were prepared and 50 ml samples whipped for varying times in a double beater mixer (Sunbeam 1-250). Once the solution was completely incorporated into foam, overrun development was measured in 100 ml aliquots of foam sampled at 2-min intervals. Maximum overrun and the time required to reach maximum were the parameters used to compare the foaming ability of protein samples.

The simple generation of overrun, however, does not completely describe important differences between proteins even in terms of their ability to generate foams. In a whipped foam the ability of a protein to incorporate air into the solution, forming a fine bubble distribution, is the parameter which most closely describes its surface activity. Bubble size distributions of the foams whipped in the mixer were measured using a hanging drop slide and a microscope connected to a videotape camera. Foam samples were rapidly transferred to the slide, and several fields recorded on videotape. Size distributions were then measured from a CRT screen.

As an addendum, accurate measurements of the bubble size distribution and lamellar dimensions are essential for complete description of foams. The limiting step to this process currently is the time required to measure the bubbles. Image analysis using simple computer methods should be available soon and will be particularly useful in the accumulation of these very important data.

While whipping methods are adequate for describing the foaming properties of many protein solutions, the high concentrations, hence large quantities of protein required, make this technique impractical where highly purified and modified protein samples are of interest. For such studies the sparging method, in which gas bubbles are forced through a solution and a column of foam is allowed to form above the solution, are preferred. Interestingly, using the sparging method, foam density, that is, the inverse of overrun, correlates with improved foaming activity. This is a consequence of the dramatic difference between bubbling and agitation as the means to incorporate air into bubbles. In the whipping method, agitation is continued for a fixed amount of time and those proteins most able to rapidly stabilize films tend to produce a greater number of bubbles; hence a lower density, a higher overrun. Conversely, bubbling introduces a fixed amount of air and those proteins with better surface properties are able to stabilize these bubbles more rapidly. Hence, bubble collapse and drainage from the surrounding liquid phase are less as the foam column accumulates. In this case, where foam volume is constant, the quantity of solution retained by the foam corresponds to enhanced foaming ability and density increases, i.e., overrun is less. Foam columns were produced using the method of Waniska and Kinsella (16) modified to regulate the temperature of the column and the quantity of sample added during sparging. Columns were water jacketed and 30 ml of protein solution used as the sample. Foaming ability was determined from the density of the foam produced.

Foam Stability Measurement

While the ability of a protein to effectively generate a foam is necessary, its stability once formed is the principal criterion for its usefulness in whipping applications. Stability

of the foams produced using the whipping method was determined by the drainage rates. To minimize variability and possible artifactual destruction of the foams, drainage was measured directly from the bowl used to develop the foams. A 0.6 cm hole was drilled through the bowl and, immediately following the whipping phase, the hole was opened and drainage monitored continuously. The time required to drain 50% of the liquid in the foam was highly reproducible and accurately estimated the stability of foams of protein solutions.

Drainage from columns of foams has received much more theoretical treatment, and various attempts to model the process mathematically have been reported (17,6). Unfortunately, the striking differences between various laboratories in fitted models most probably reflect differences in equipment used to generate foams and measure drainage. While reports of the rate constants or half times of various protein foams are valid empirically for comparisons, the inherent complexity of foams limits the use of simple mathematical models to predict overall drainage behavior. In these studies, foam ability was determined from semi-log plots of liquid volume drained vs time. These plots proved to be linear in most cases, yielding a value corresponding to the half time of drainage.

While these drainage methods provide important information on the stability of foams in general, it is readily apparent that the overall process of drainage is the summation of several events. Initially in the development of a foam, the volume of liquid contained in the foam is very high. This liquid drains readily, leading to a stage in which bubbles approach, distort and produce thin liquid lamellae between them. Drainage then proceeds via two routes: from the lamellar region between two bubbles into the plateau borders at the intersection usually of three bubbles; further drainage proceeds through the plateau borders (6). Finally, approach of lamellae and collapse of bubbles further accelerate the process. The structure and microscopic flexibility of proteins will have impact on each of these events differently, hence approaches must be taken to isolate each effect.

Any attempt to characterize all of these component processes from an intact foam is faced with an overriding problem. Clearly, the size of the bubbles would be expected to influence the relative contribution of each event, the rate of drainage and the tendency to collapse. This parameter, i.e. the bubble size distribution, is dependent on several factors related to protein structure but which are not necessarily consistent with nor even related to stability. Nevertheless, these effects confound attempts to measure the stability of entire foams. Investigation of the stability and drainage of the actual protein films is confounded inexorably with the process of initial formation, the method used, environmental conditions, etc. Comparisons following modification in protein structure on the stabilization of the films cannot be separated from alterations of the foam per se, most particularly in the total area of surface initially formed. A means to study just the lamellar phase alone and the effect of protein structure on its thinning and collapse is required. While several methods have been developed to examine the properties of surface films of proteins, these differ from true foam films by orders of magnitude in terms of rates of formation and film dimensions.

Tensiolaminometry

Tensiolaminometry is a physical technique that measures solely the lamellar phase behavior of surfactants (18). The actual sampling unit is a square platinum wire frame (Fig. 1a). The 1 cm 'H' frame is moved mechanically through the surface of a protein solution. If the protein is able to form and stabilize new surface, a liquid film will form on the

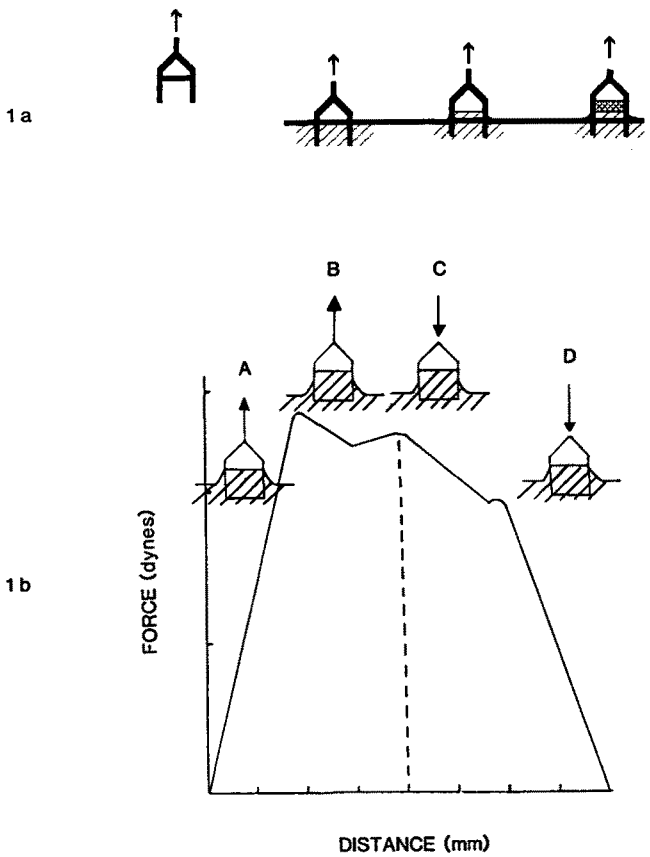

FIG. 1a. Diagram of the tensiolaminometer H frame and operation at a typical liquid surface. Arrows indicate measurement by a recording electrobalance. 1b. Idealized force distance/time run on the tensiolaminometer. Distance to the right of the dotted line is the actual mirror image shown for clarity; distance actually returns to zero.

frame. This is a physical analog to a foam lamella. The forces involved in the formation and collapse of this film can be measured continuously using a force transducer attached to the frame. The analysis of the system generates a force versus distance relationship of the film forming process which is the work function associated with the lamellar formation.

An idealized force versus time plot is shown in Figure 1b. As the solution is stretched over the frame (A) the force rises continuously. At this point, (B), the meniscus breaks and the film itself is formed over the frame. The direction is then reversed and the frame is pushed back into the solution, collapsing the film (C). The meniscus then reattaches and again the force returns continuously (D) to the starting point. For the analysis of film strength the regions (B) in which a film is formed and (C) in which the film is collapsed are of particular interest. In principle the method serves as a very useful model system for the formation, kinetic stability and rheological strength of the protein coated lamellar phase in foams, independent of the complications introduced by the bubble size differences in actual foams.

Previously, this apparatus has consisted of a variable speed motor acting to drive a platform containing the protein solution through the frame suspended from a force transducer. Technically, the method has proven difficult in terms of accurately positioning the surface and synchronizing the force-distance coordinates. This methodology has been improved significantly by digitizing the process and controlling both the platform movement and data acquisition from a single microcomputer. The schematic of the system is shown in Figure 2. The computer controller (Apple

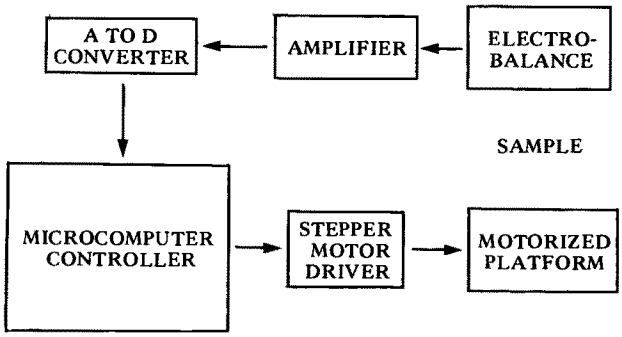

FIG. 2. Schematic of the tensiolaminometer operating system. Not shown though essential for accurate functioning are the H frame and a vacuum inlet positioned at a fixed height above the H frame.

microcomputer) simultaneously actuates the platform via a stepper motor (Fig. 3) and collects force data from a scanning electrobalance (Cahn Electrobalance, Cerritos, California). The primary advantage in the control of the system is afforded by the stepper motor driving the platform. With this motor driving the platform, the surface can be moved in any direction and eventually returned to precisely the same starting point.

The computer simultaneously stored the distance traveled by the surface and the force realized at this point. The film and the forces associated with its manipulation can be studied in a variety of ways. Films can be formed at variable rates, or several films can be made repeatedly from a single solution; a single film can be produced and allowed to collapse by itself or compressed at various rates. A single film also can be stretched and compressed at various rates, all with simple commands in software. Importantly, any method which measures the surface layers of protein solutions must consider the age of the film at the surface of the sample. In these studies the surface was cleaned immediately prior to each analysis by vacuum evacuation. A pipette was installed at a fixed distance above the measuring unit and, by calculating the distance from the unit and the rate of travel, the precise age of the clean surface could be regulated.

These measurements have been found to predict the effect of protein alterations on the various properties of foams made from them. The tensiolaminometric method was used to analyze 0.1% protein solutions (20 ml) of native and modified soy proteins.

RESULTS AND DISCUSSION

Foaming – Casein, Egg White

Subtle differences in the structure of proteins leading to significant differences in their foaming properties are readily apparent when simple whipping techniques are used to monitor them. Two traditionally successful foaming proteins, casein and egg white, can be shown to produce different foams. The effect of whipping time on the size distribution of bubbles is summarized (Fig. 4a,b). After 5 min whipping, even for these proteins which produce excellent foams, a significant difference was resolved in the bubble size distributions. The egg white bubbles were larger and more broadly distributed. Interestingly, when these foams were whipped for a further 5 min, the results reflected a change in the foams, i.e., the bubble distributions were very similar. This effect has been interpreted to reflect the additional time and energy required to unfold egg proteins sufficiently to associate at the interface and form a film. The relatively unstructured caseins tend to unfold more readily, while egg proteins are slightly more resistant; this property is reflected in the ease with which each protein is able to form a foam initially. These results demonstrate that foam formation per se represents a complex interplay of both the protein and the prevailing conditions. This also emphasizes the difficulties involved in comparing foams produced under different conditions or with different equipment. The macroscopic structure of the foam and the microscopic structure of the protein in the film undergo important changes within a very short time span. The egg white foam produced after 5 min whipping is different from that produced from the very same protein after 10 min whipping, let alone from that produced from casein.

While protein structure effects are important in forming a foam, these properties are even more apparent with respect to stability. The drainage rates of egg white and casein foams whipped for various lengths of time are summarized (Figs. 5a,b). The importance of protein structure is exemplified by the classic behavior of egg white protein (Fig. 5a). While the bubble size distribution and overrun continued to improve from 5 to 10 min, the optimum drainage stability was found at 5 min whipping. Further whipping actually destabilized the foam. This effect is again related to the unique structural stability of some of the egg white proteins. While some relaxation of the native structure favors film formation, excessive surface denaturation of

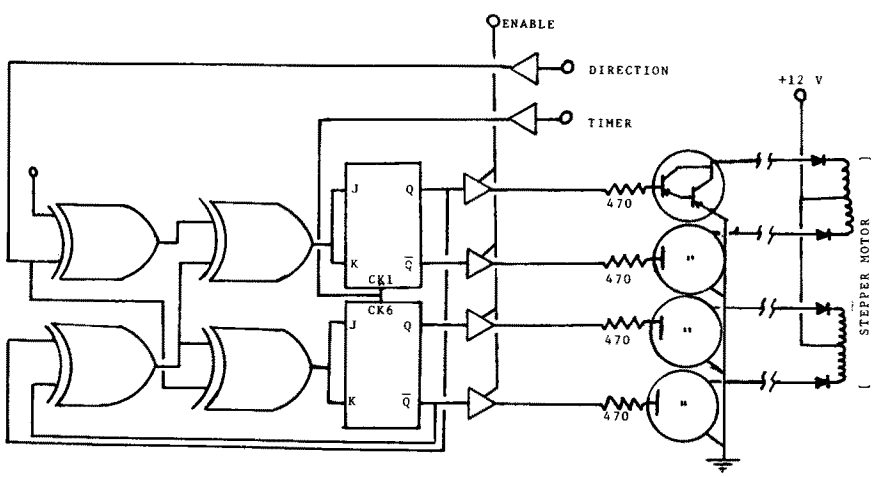

FIG. 3. Circuit design for the stepper motor logic and power board used to control the position and speed of movement of the platform supporting the sample for tensiolaminometric analysis.

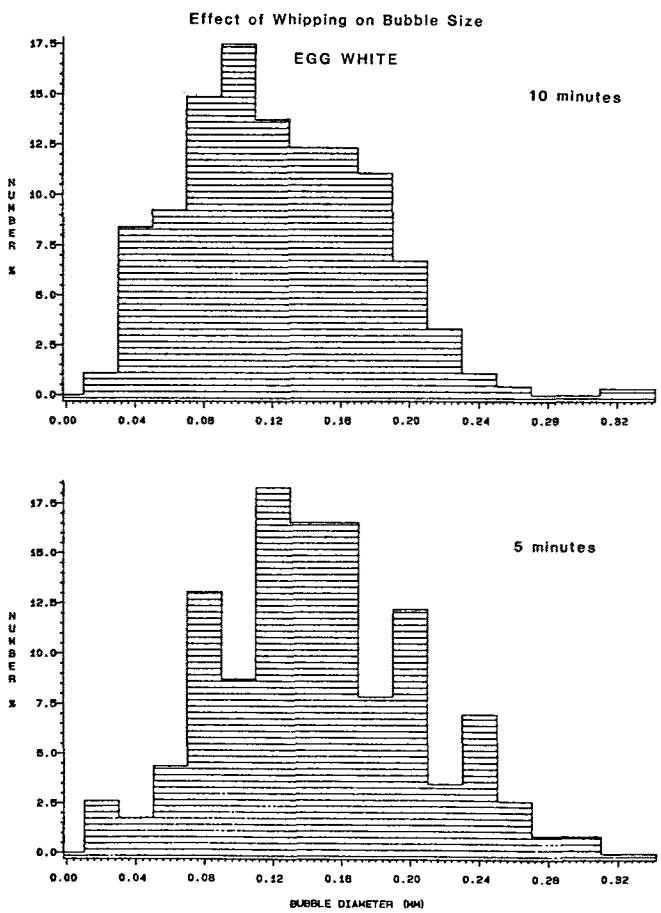

FIG. 4a.

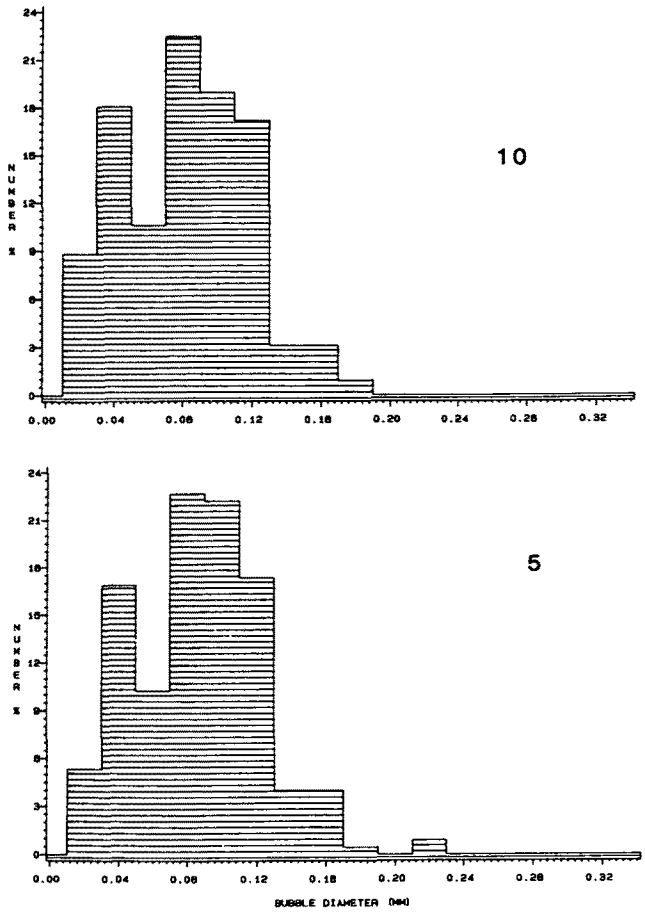

FIG. 4b.

FIG. 4. (a) Bubble size distributions for 5% egg white protein solution pH 7 whipped for 5 and 10 min in a Sunbeam 1-250 mixer (speed 13). Foam samples and bubble size measured as described in methods. (b) Bubble size distributions for 5% casein protein solution pH 7 whipped for 5 and 10 min as in (a).

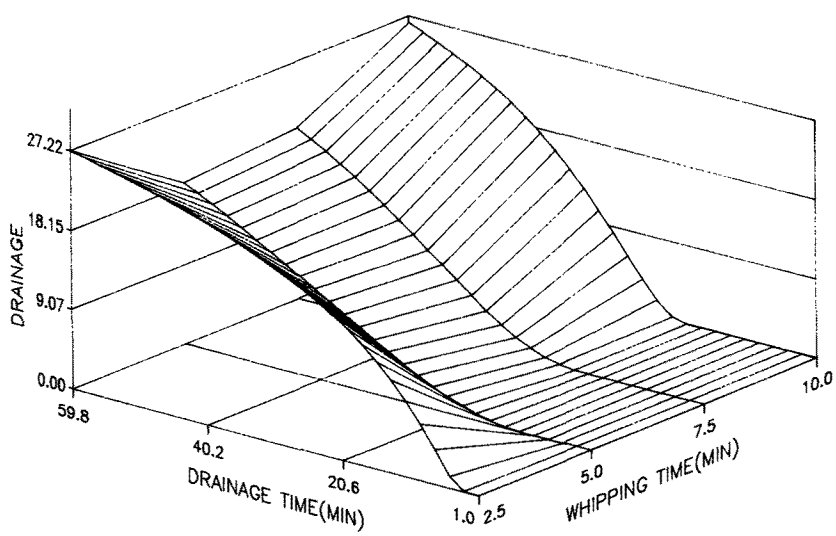

FIG. 5a. Accumulation with time of liquid drained from egg white foams whipped for varying lengths of time. Foams were whipped and drainage measured as described in methods.

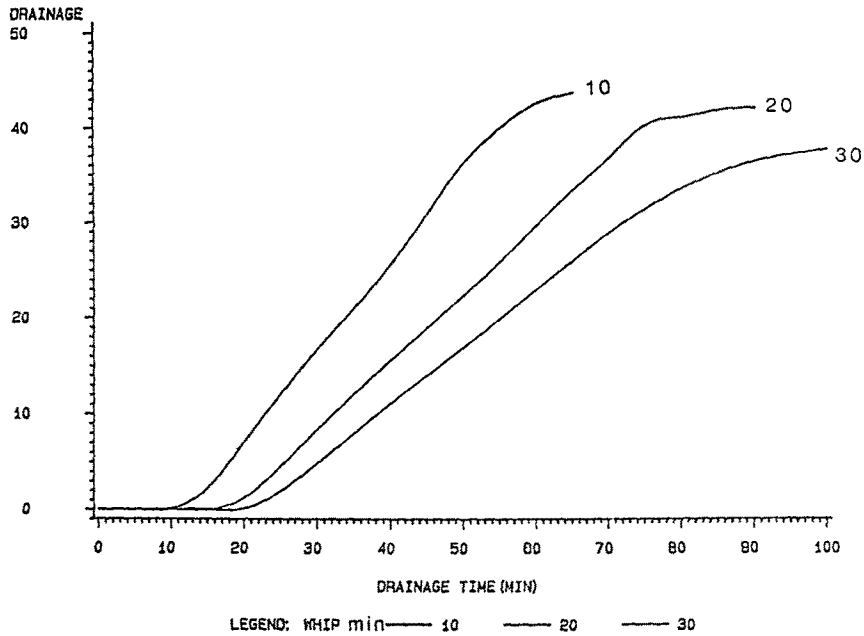

FIG. 5b. Accumulation with time of liquid drained from casein foams whipped for various lengths of time as in Fig. 5a.

some of the egg proteins actually results in proteins capable of destabilizing the films. Alternatively, the casein proteins do not show this effect, even when whipped for 30 min. In contrast to the egg white, continued whipping increased the stability of the casein foams.

Bovine Serum Albumin

The foaming properties of bovine serum albumin are particularly well studied, because this protein can be obtained in large qualities in high purity. While BSA can be shown to develop a foam after 10 min whipping, the overrun is relatively low and it is highly unstable, collapsing with a half time of approximately 10 min. Also using the sparging method, BSA forms a relatively low density foam column and again, the half time of drainage was relatively short. This instability is apparent in the bubble size distributions of BSA foams immediately after formation and following 10 min drainage (Fig. 6). The data illustrate the rapid collapse of small bubbles and accumulation of very large bubbles as the foam drained. These data suggest that a) BSA is unable to unfold facilely and stabilize new surfaces as they are formed, and hence resists forming a foam; or, b) whereas the protein readily lowers the surface tension favoring foam formation, as a consequence of an inability to successfully associate at the surface, BSA resists film matrix formation resulting in the rapid collapse of the lamellae. This was tested using the tensiolaminometer to examine the stability of BSA lamella. A typical run on the instrument is shown (Fig. 7). These data measure the development of force associated with the formation of a single lamella from 0.1% BSA solution. The surface tension is lowered, and a film forms readily on the frame. However, when this lamella was held fixed and the drainage monitored with time, the film broke after less than 5 seconds, and the force immediately returned to zero. In contrast, proteins forming stable foams, such as casein, continued draining for greater than 60 seconds without collapsing.

Soy Protein

Using this methodology and our understanding of the putative structural requirements for an ideal foaming protein, we investigated the foaming behavior of soy protein. Soy proteins have been suggested to be poor foaming proteins largely because of their large, compact structure which resists adsorption and unfolding at air interfaces, thereby preventing adequate film formation (19).

Previous work on the response of soy proteins to heating suggested several properties which were relevant to surface activity. In the native form, soy proteins, particularly the 11S, do not possess a high degree of surface hydrophobicity (8) nor significant hydrophobic binding sites (21). Additionally, this structure of soy 11S is normally stable to heating to 80 C. In the presence of reductant, however, the protein readily dissociates in response to heat or various denaturants, liberating acidic and basic subunit fragments. These fragments retain most of their native structure, yet the released basic subunits rapidly associate primarily via hydrophobic associations (20). In the presence of 7S subunits or, in fact, any acidic protein at neutral pH, the basic subunits being positively charged will associate with them electrostatically into multiple aggregates (20,21,24). These properties suggest that soy proteins, in fact, possess many of the structural properties consistent with good foaming. A possible hindrance is, however, the intersubunit disulfide bonds which stabilize associations between the native subunit fragments.

We investigated the foaming and the film forming properties of isolated soy proteins to determine if mild reduction of these disulfide linkages would allow the proteins to unfold at the interface and improve their surface properties. Soy proteins were fractionated into 7S and 11S using cold precipitation in Tris buffer, followed by exclusion chromatography (22). The 11S and 7S fractions were modified using either sulfite treatment or reduction with dithiothreitol (DTT) (23). In all cases the results were dramatic. Densities of foam columns made from these proteins are shown in Figure 8. Both 7S and 11S and an equal mixture of the two produced relatively light, weak foam columns. The cleavage of intersubunit disulfides, however, with either sulfite or especially DTT, resulted in a dramatic increase in the density of the foams.

The stability to drainage and collapse also was measured by the rate constant describing loss of liquid from the foam

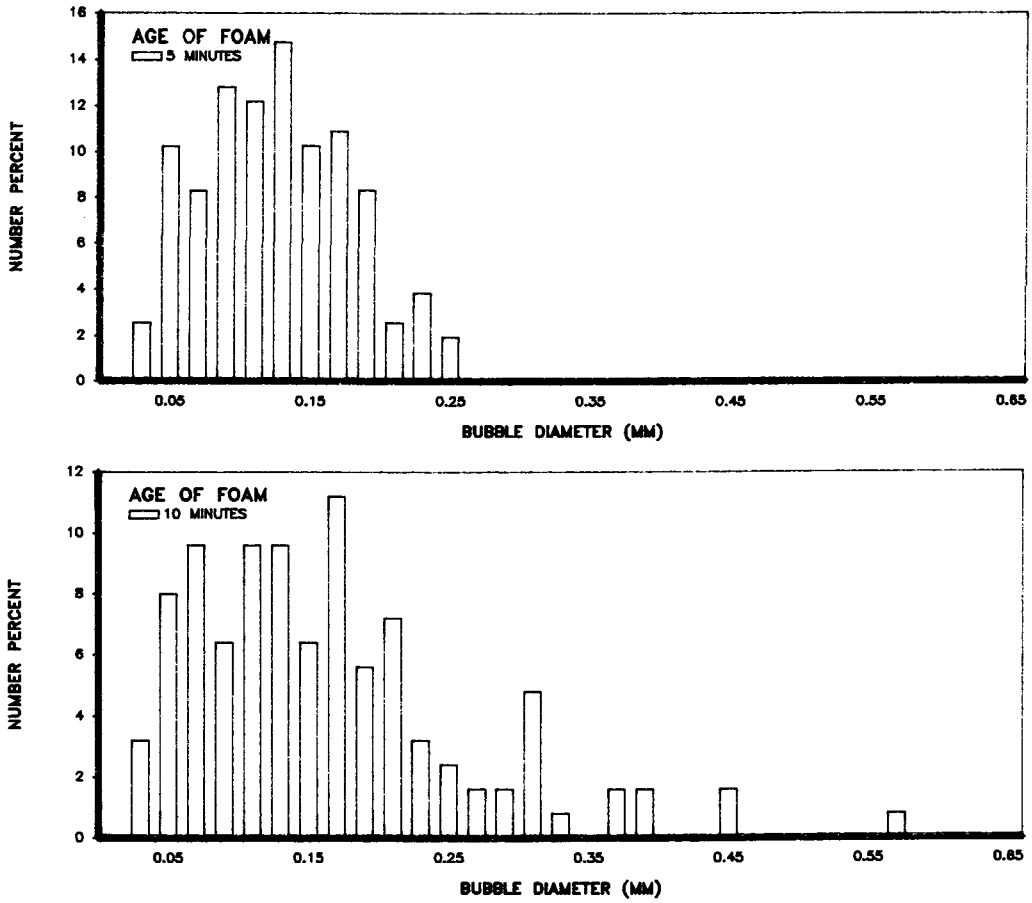

FIG. 6. Bubble size distribution of foam produced by whipping 5% BSA solution pH 7 for 10 min. Samples taken immediately following whipping and after 10 min drainage. Foams whipped and bubbles sampled as described in methods.

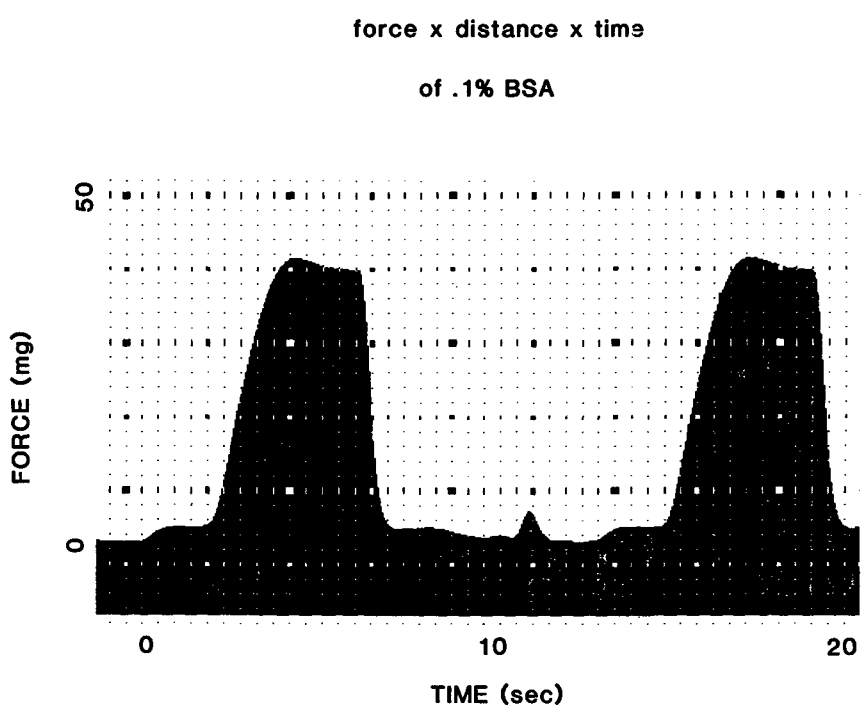

FIG. 7. Repeated tensiolaminometric analyses of BSA solution (.1%), pH 7. Traces shown are the actual computer output from the microcomputer controller.

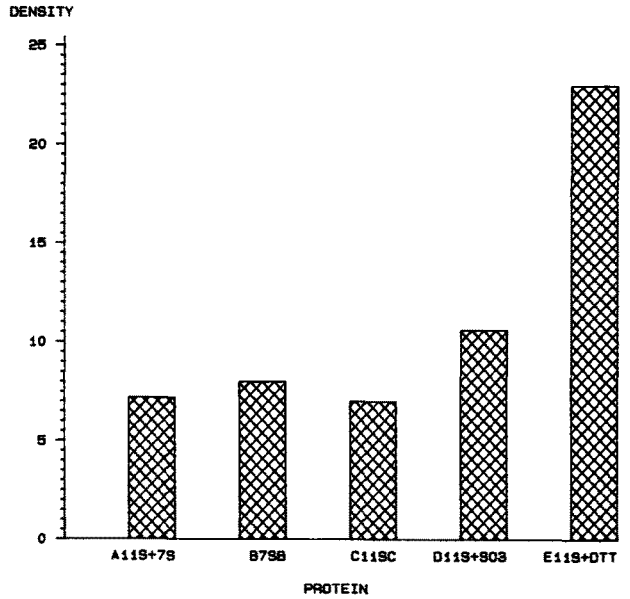

FIG. 8. Density in mg/ml of foam columns prepared from 0.1% protein solutions at pH 7. Foam columns produced by sparging protein solutions prepared as described and including 10 mM sodium metabisulfite or 10 mM DTT as indicated.

columns once formed. Typical data in this case for reduced 11S are shown in Figure 9. At pH 6 the stability to drainage was improved 10-fold.

Our original rationale proposed that modification of the structural stability of the protein would allow for more rapid and complete exposure of hydrophobic regions of the subunits at the expanding interface, and also permit more extensive intersubunit associations into an aggregated film network, yet retain sufficient secondary and tertiary structure for flexibility. These effects were tested using the single lamella techniques. The force distance relations for lamella produced by 11S and 11S reduced with dithiothreitol are shown in Figure 10. The slope and maximum force realized in the formation of the lamella measured the ability of the surfactant to reduce surface tension and permit lamellar formation. Reduction favored the expression of the film, and results were similar for sulfite treated proteins.

The relative strengths of lamella formed from these two proteins are also revealed by these data. While the film formed by native 11S was stable for just 3 seconds prior to collapse, the reduced protein film was still intact after 10 seconds, after which the film was returned to the solution. In fact, these same film conditions revealed that the disulfide-reduced protein films were greater than 10-fold more stable to breakage.

The improvement in film rheology also was measured by the force required to recompress the lamella once formed. The results of these analyses again for sulfite treated 11S are shown in Figure 11. At all pH's shown, reduction more than doubled the force per unit area of the single lamella. Interestingly, the introduction of additional negative charges via sulfite reduction lowered the effectiveness of the modification in both the rate of formation of the film and its ultimate stability (data not shown). These data are interpreted to suggest that while the proteins were able to unfold more rapidly the increased electrostatic repulsion inhibited protein-protein interactions in the film matrix. Alternatively, reoxidation of sulfite treated proteins may have restricted flexibility. These alterations in the rheology of the foam lamellae also were verified with measurements of the elastic modulus, surface viscosity and surface yield point of surface films produced from the same proteins (23). Work is ongoing in the laboratory to discover the nature of the subunit interaction induced at the interface suggested by these results.

The objectives of continued research in this area must address characterizing more fully the structures of food proteins and, importantly, changes that are induced by preparative and processing conditions. Additionally, the inherent complexity of the physical systems in which proteins function, i.e. foams, emulsion and gels, multiplied by the vast scope of proteins and conditions employed demand that standardized methodologies be adopted in order to validate comparisons between investigations. As more sophisticated techniques are developed, they should serve to describe and help interpret more fully a broad base of standardized, accepted data rather than simply obsolesce it.

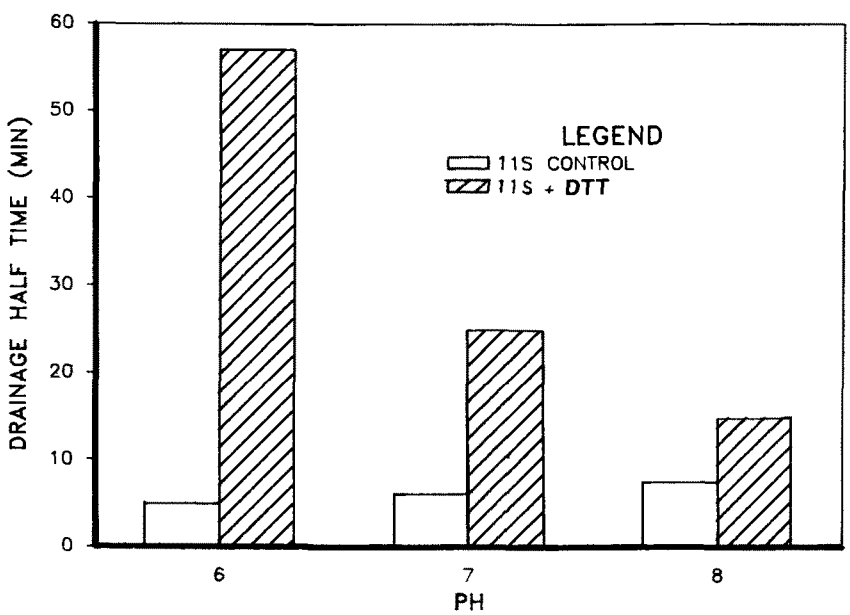

FIG. 9. Drainage half times in minutes for foam columns of soy protein prepared as described in methods (23). 0.1% solutions of soy 11S were sparged either in the presence (1) of absence (0) of 10 mM dithiothreitol (DTT).

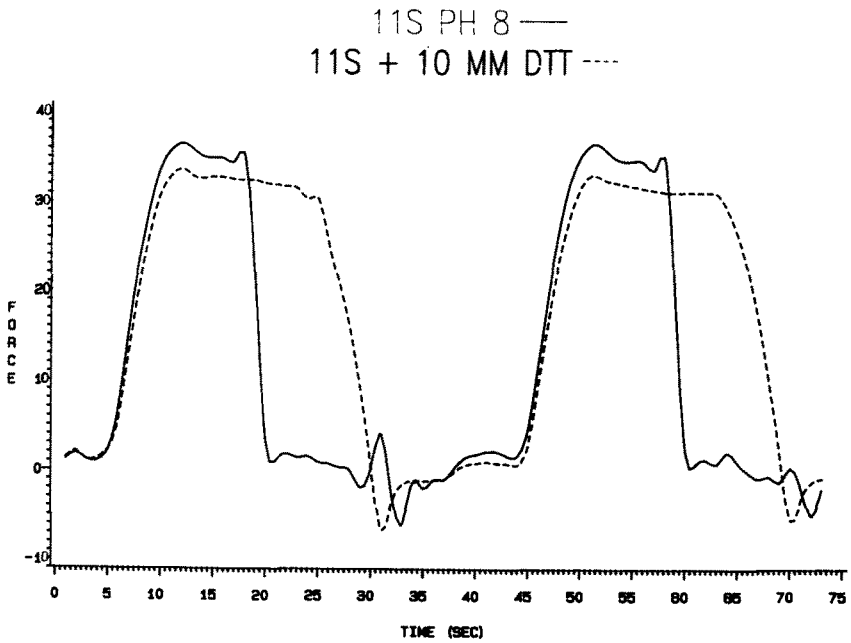

FIG. 10. Tensiolaminometer analysis of .1% soy 11S solutions pH 8 run in the absence or presence of 10 mM DDT.

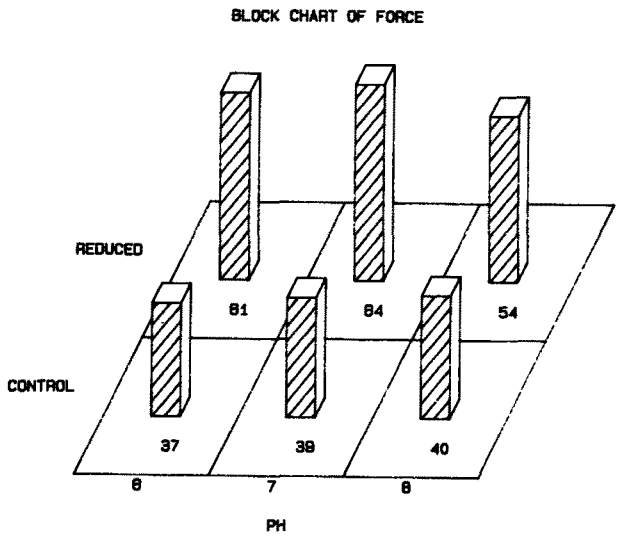

FIG. 11. Force required to compress protein lamella formed using the tensiolaminometer. Force reported in mg * 10 sec.

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