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Food Emulsions: Their Structures and Properties

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I. INTRODUCTION

A. General Introduction

The taste and texture of a processed food perceived by the consumer depend on a variety of factors, important among which are the structures formed by the constituent materials. The molecules which make up the food interact to create assemblies of molecules which give the structure and hence to a large extent, determine the texture of the particular food. The ingredients are assembled during processing, and the structure created by the manufacturer is governed by the controlled application of one or more effects: physical (e.g., interparticle forces, phase separations), chemical (e.g., formation of specific covalent bonds between molecules and particles), or biological (e.g., fermentation, enzyme action). It is, of course, the aim of the processor to generate products of predictable properties from materials whose properties are themselves understood and to do this as economically as possible.

Among the structures and structure-forming units within foods, emulsions play a major part. They are known to impart desirable mouthfeel characteristics to the food, but, in addition, they are key ingredients in the formation of structures in certain products, such as whipped toppings and ice creams, and more complex products, such as processed cheeses. Therefore, the understanding of the formation, structures, and properties of emulsions is essential to the creation and stabilization of structures in foods. Food emulsions are widely used and are familiar to almost everyone. In addition to the products just mentioned, whole milk and cream are

emulsions, as are butter, margarine, spreads, mayonnaises and dressings, coffee creamers, cream liqueurs, some fruit drinks and whippable toppings. Many meat products depend on the presence of emulsions for their properties, as does bread dough, although in both cases the emulsion structures can be extremely complex.

The formulation and creation of a food structure involving emulsions is always a compromise, depending on the desired qualities of the food and the materials which can be used to create these qualities. In addition to the essential physical functionality of the materials, it is necessary to take into account nontechnological, but nonetheless important, factors. Foods contain ingredients which are subject to regulation by appropriate agencies. In some cases, the use of certain ingredients may be discouraged because of restrictions imposed by certain religious groups or by public perceptions of health issues. Furthermore, the processor is constrained by economics and cannot use expensive materials in product formulations. Last but not least, the product must be safe from a microbiological point of view; this may have important consequences because of the need for heat treatments, which may affect the stability of an emulsion during processing. All of these factors make the study of the efficient formulation and production of emulsions a key to the structure and behavior of processed foods.

B. Emulsion Types

An emulsion is a suspension of one phase in another in which it is immiscible. One of the phases exists as discrete droplets suspended in the second, continuous, phase, and there is an interfacial layer between the two phases which is occupied by some necessary surfactant material. There are three main types of emulsion which are important, or potentially so, in foods. In oil-in-water (o/w) emulsions, droplets of oil are suspended in an aqueous continuous phase. These are the most versatile of the emulsion types; they exist in many forms (mayonnaises, cream liqueurs, creamers, whippable toppings, ice cream mixes), and their properties can be controlled by varying both the surfactants used and the components present in the aqueous phase. Water-in-oil (w/o) emulsions are typified by butter, margarines, and fat-based spreads in general. These depend for their stability more on the properties of the fat or oil and the surfactant used than in the properties of the aqueous phase, and because of this, there are fewer parameters which can be varied to control their stability. The third of the emulsion types is water-in-oil-in-water (w/o/w), which is, in effect, an o/w emulsion whose droplets themselves contain water droplets (i.e., are w/o emulsions). These are the most difficult emulsions to produce and control, because the water

droplets contained in the oil droplets must be stable, as must the oil droplets contained in the continuous aqueous phase. These emulsions are described in detail in Chapter 5 and will not be described further here.

For convenience of description, we may divide o/w food emulsions into three classes, depending on how they are to be used. The first class contains emulsions which are end products in themselves. Coffee creamers and cream liqueurs are relatively simple emulsions whose only requirement is to remain stable toward creaming and coalescence during their shelf-life (which, however, may have to be considerable, so that sterility is also important). These emulsions present less of a challenge to the processor than do more complex emulsions; there are a few basic rules of formulation which allow successful products to be created. The second class of emulsions contains those which can be used as ingredients that participate in forming the structures of more complex products; that is, other components of the food (proteins, polysaccharides) form a matrix in which the fat globules are trapped or with which they interact. Examples are yogurts, processed cheeses, and other gelled systems containing emulsion droplets which must interact with other components in the food, but are not destabilized in the process. Their effect is to alter the rheological properties of the gel, thus creating texture and mouthfeel. In the third class of emulsion, the droplets are required to create new structures during processing, such as in ice cream or whipped products (1,2), where the emulsion is destabilized and further interacts as a means of creating structure in the product. Some emulsions themselves may also form gels during heating, to create new structures within foods (3). The requirements for the compositions and properties of the emulsion droplets are different in these different cases. However, it is generally necessary for the emulsion droplets to interact with themselves and/or with the other food components to give the required structures.

One of the ultimate goals in studying emulsions is to be able to describe their functionality well enough from first principles to allow the reduction of the amount of fat in a product without, at the same time, adversely affecting its texture and organoleptic properties. Similarly, it is important to anticipate the possibility that emulsions will be used as carriers of flavors or bioactive materials. To best achieve this, we need to understand how the fat functions in the original structure and how any material which is used to replace it should efficiently reproduce all of this function. Ideally, we would like to be able to define the properties of a product and then to construct it from a knowledge of the possible ingredients. At the present time, this is possible for only a few of the simpler systems.

In the text which follows, emphasis has been given to emulsions prepared using milk constituents. This is simply because the milk proteins are

by far the most studied of the food proteins (being easily prepared, soluble, and relatively well behaved once they are in solution) and the emulsions prepared using these materials have a correspondingly voluminous literature, especially on the subject of ice cream. Reports on the basic properties of other proteins in emulsions are more fragmentary; this is not to say that they are unimportant, as the egg-based mayonnaise, to name only one, is a very widely used product.

II. THE BASIS OF THE BEHAVIOR OF OIL-IN-WATER EMULSIONS

A. General Aspects of Emulsions

Oil and water do not coexist comfortably because of the surface energy (Gibbs free energy) of the oil–water interface. Because of the interfacial tension between oil and water, any emulsion will seek to minimize the interfacial energy by making the interfacial area between oil and water as small as possible. In the absence of surfactants, this is achieved by coalescence of the oil droplets, to give separated layers of oil and water. The presence of adsorbed surfactant molecules lowers the interfacial tension between the oil and water phases, so that the driving force for coalescence is reduced, although never to zero. Many surfactants (e.g., proteins) do not simply reduce interfacial tension, but actively inhibit coalescence by altering the viscoelastic properties of the interface. The adsorbed material can also prevent the close approach of oil droplets by causing the surfaces to have sufficient charge to repel one another or by creating an extended surface layer, which also prohibits close approach. Thus, although emulsions tend to be regarded as thermodynamically unstable, it is possible, by judicious use of surfactants, to control the kinetics of destabilization and to produce emulsions with very lengthy shelf lives. Surfactant molecules are amphiphilic; that is, they contain hydrophobic and hydrophilic domains. The former dissolves in, or interacts with, the hydrophobic surface of the fat or oil, whereas the latter dissolves in the aqueous phase. The surfactant therefore forms a layer on the surfaces of the emulsion droplets. Depending on the type of surfactant, the adsorbed layer may have a complex structure, examples of which are described in the following subsections.

The thermodynamic instability leading to coalescence is, however, only one way in which emulsions can be unstable. Coalescence has the most drastic consequences, because it can be reversed only by rehomogenizing the product, but other mechanisms which are important are creaming and flocculation. Both of these may promote coalescence and are generally not to be favored. In creaming, the emulsion droplets do not lose their identity; they simply redistribute in space and can be returned to their

original state by agitation. Flocculation or aggregation arises from a more permanent physical or chemical interaction between droplets. Flocs are often not easily redispersed and may, therefore, have a negative effect on product quality (in soups, sauces, etc.). Because flocs are have a long lifetime, the possibility that rupture of the interfacial layers and coalescence can occur is enhanced.

The functional behavior of oil-in-water food emulsions is related to their stability and is controlled by the three parts of the system: the fat or oil in the interior of the emulsion droplets, the interfacial material between this lipid material and the continuous aqueous phase, and the aqueous phase itself. Each of these "phases" may be chemically complex. The lipid may be partly or wholly crystalline and it may be subject to chemical change such as lipolysis or oxidation. The interfacial material can be composed of proteins or of small emulsifiers such as monoglycerides, esters of fatty acids, or phospholipids, or mixtures of a number of these components. Finally, the aqueous phase may contain ions, which may interact with and potentially destabilize the emulsions, or macromolecules such as polysaccharides, which may exert either stabilizing or destabilizing effects. Therefore, to understand the functional properties of the emulsions, it is necessary to understand the properties of these three parts, individually and collectively.

B. Lipids and Emulsion Functionality

In oil-in-water emulsions, the fat or oil used to form the emulsion affects the functionality of the emulsion mainly by its degree of crystallinity, or its ability to crystallize. Oils which are liquid at the temperature at which foods are produced and consumed have little effect on the behavior of the emulsion, because they act essentially as fillers. They can, of course, coalesce if the fat globules are destabilized and the interfacial layer is sufficiently weak, but they have little structural significance apart from that. On the other hand, it is possible for unsaturated liquid oils to undergo oxidation, and this, in turn, can lead to chemical reactions between the oxidized oil and a proteinaceous emulsifier (4). The overall effect of the reaction may be to alter functional properties of the emulsion and the nutritional value of the food, as well as creating undesirable flavors. The details of how the functional properties of the emulsions are altered by these reactions are not known, although it is known that the oxidized material is harder to displace from the interface than the original protein.

Emulsions are nearly always created (e.g., by homogenization) at a temperature at which all of the fat or oil is in a liquid state, and crystallization then occurs as the product is cooled to the temperature at which it is stored. Fats and oils which can crystallize in this way can be very important

in defining the functionality of the emulsion. By far the best known example of this is the involvement of partly crystalline fat in the mechanism of partial coalescence, which stabilizes air bubbles in whipped emulsion products or in ice creams (1). As the fat crystallizes, the growing crystals start to break the interfacial layer of the fat globules (5), and this weakening of the surfaces allows more efficient destabilization of the emulsion (which in ice cream mixes has often helped by weakening the interface by small-molecule surfactants). In addition, the emulsion droplets, because their surfaces are destabilized, are susceptible to being broken by the attachment to the air–water interfaces of the air bubbles as the mixture is whipped (6). The semiliquid fat cannot wholly coalesce as the interfacial layers are broken, because of the limited mobility of the crystalline material, but it will partially coalesce to form a sintered layer around the bubble (7). As long as the crystals are not melted, the layer will remain intact and stable around the bubbles. The fat may, of course, be completely crystallized after the partial coalescence has occurred, but it must be semiliquid for the original phenomenon to take place. Fats that are completely solid at the temperature at which whipping takes place are not efficient at stabilizing the foam. Equally, fat globule membranes that are too viscoelastic are too tough to permit the breakage essential for partial coalescence to occur.

Not all of the lipid components of a food are, however, neutral lipids in the form of triglycerides. Phospholipids (lecithins) are a second class of lipid materials that are important in defining the properties of emulsions. However, in contrast to the fats and oils, which are present in the interiors of the emulsion droplets, phospholipids are found in the interfacial layer (8) or may even not adsorb at all (9). It should be noted that although the lecithins are often described as "emulsifiers," they are not as efficient on their own as are other small or large molecular emulsifiers. They may, nevertheless, have a moderating effect on the properties of these other materials. It is also probable that although it is popular to designate a whole range of phospholipid materials under the heading of "lecithins," each individual phospholipid type (with different head groups and fatty acids) will behave in a way unique to itself. Thus, phosphatidylcholines may behave differently from phosphatidylethanolamines, and distearyl phosphatidylcholine will behave differently from dioleyl phosphatidylcholine. Hence, the source and composition of a lecithin sample will influence its functional properties.

C. The Interfacial Layer

The interfacial layers of many oil-in-water food emulsions contain proteins, which may be mixed with other surfactant materials (Fig. 1). Proteins are often present in the raw materials of the food, and the fact that they are

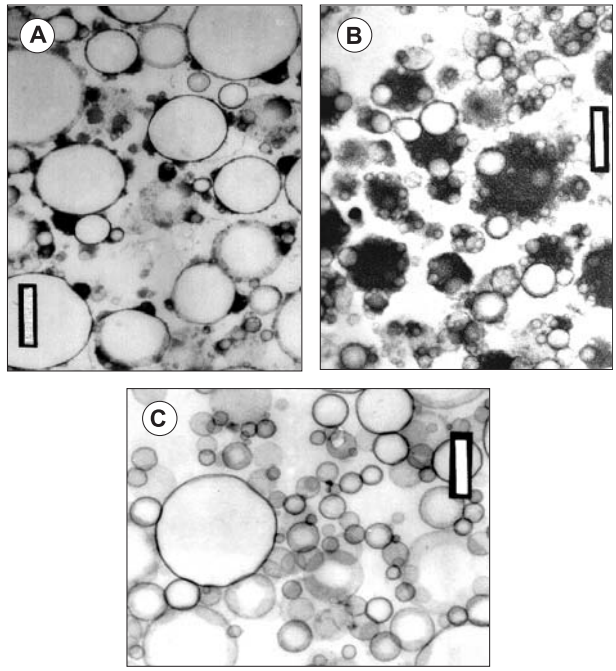

Figure 1 Transmission electron micrographs of emulsion systems. (A) and (B) show milk homogenized using a microfluidizer and centrifuged to separate different populations of particles. (A) represents the larger fat globules, which float during centrifugation (scale bar = 300 nm). (B) shows the sedimenting fraction from the same milk, where very small fat globules are complexed with protein particles (scale bar = 200 nm). (C) shows an emulsion made with soybean oil and sodium caseinate, with only thin protein layers around the fat droplets (scale bar = 200 nm).

excellent emulsifiers enhances their usefulness. The properties of the interfacial layers depend not only on the quantities of materials adsorbed but also on their properties, although we do not completely understand how the composition and structure of an interfacial layer affect the detailed properties of an emulsion.

The composition of the interfacial layer is governed mainly by what is present at the moment the emulsion is formed (10). If proteins are the only emulsifiers present, they will adsorb to the oil interfaces, generally in proportion to their concentrations in the aqueous phase (11). Certain mixtures of caseins are anomalous in this respect, because there is preferential adsorption of β-casein from a mixture of purified α_s- and β-caseins (12).

However, this preferential adsorption does not seem to occur in emulsions made with sodium caseinate, where the caseins adsorb approximately according to their ratios in the original caseinate (11). Why this should be is not clear, although it may be a consequence of the different aggregated states of the caseins in solution (13). It is possible also that the concentration of casein in the emulsion is important (14).

Small-molecule surfactants have important effects on the composition of the interfacial layer. Depending on their nature, they may completely displace adsorbed protein, as in the case of water-soluble surfactants added after the formation of the interface (15), or partially displace the protein, as found for oil-soluble surfactants, which must be added before the interface is formed (16). The effects of these surfactants may not be confined to simply displacing the proteins; there is evidence for binding to proteins (17) or a complex displacement reaction, which has been observed by atomic force microscopy (18). Between the small-molecule surfactants and proteins in size, there are peptides derived from the proteolytic breakdown of protein molecules. These are also capable of stabilizing emulsions, although it appears that larger peptides are more effective at this than smaller ones (19,20). Controlled proteolysis of proteins used as emulsifiers can give increases in their emulsifying efficiency; this has been observed for whey proteins (9,21) and soy proteins (22,23).

The direct or competitive adsorption processes during the formation and storage of an emulsion are, of course, time and path dependent, a subject on which there is little information. This leads to difficulties when interpreting the properties of emulsions produced in laboratory conditions, where it is often the case that ingredients are mixed one at a time, with the normal industrial situation, where many ingredients are mixed and processed at one time. Evidence of time dependence is manifest in the formation of networks of adsorbed whey proteins on the surfaces of emulsion droplets as the emulsion is aged (24). The formation of disulfide linkages between adsorbed proteins is probably responsible for the stability of that adsorbed layer, which is extremely difficult to displace (25). Strong rigid interfacial layers can also be created by deliberate enzymatic cross-linking of adsorbed proteins, the best known example of which is the use of transglutaminase (26).

Details of the structures of the adsorbed layers will be discussed in Section VI. Briefly, a number of methods has been used to estimate the dimensions of adsorbed protein monolayers, among them are dynamic light scattering (27), ellipsometry (28,29), and neutron reflectance (30). The results show that adsorbed layers of protein may be thick compared to molecular dimensions. By forming a hydrodynamically thick layer and because they are generally charged, adsorbed proteins can stabilize emulsion

droplets by both steric repulsion and electrostatic (charge-repulsion) mechanisms.

Many, if not all, adsorbed proteins exist in conformations that are different from their native states (31–33). This is a result of the tendency of hydrophobic parts of the molecules to be adsorbed to the hydrophobic interface, with a consequent distortion or disruption of their secondary or tertiary structures (34). As a result, the properties of the emulsion are not necessarily the same as those of the parent protein. A more drastic manipulation of the adsorbed layer is possible—by the action of proteolytic enzymes on the adsorbed proteins. Although it would be difficult to control on an industrial scale, the partial breakdown of adsorbed casein by trypsin can considerably enhance the stability of the emulsion toward Ca^{2+} (35).

D. The Continuous Phase

If the emulsion droplets are to contribute to the structure of a food, they must interact in some way with the other components which are present. Interactions can occur between the droplets themselves, leading to gelation or flocculation, but other reactions are possible. If the components of the food which are in the aqueous phase tend to form gels, then the emulsion droplets may act in the simplest case as filler particles (i.e., they take up space but do not interact physically or chemically with the gel) (36). On the other hand, the interfacial layer of the emulsion droplets may be capable of interacting with the aqueous-phase components as they gel (37); one obvious example of this is the gelation of whey protein-based emulsions during heating, where the protein in the aqueous phase interacts strongly with the adsorbed whey protein of the emulsion droplet surfaces (38). Similarly, in the acid gelation of milk, which is part of the manufacture of yogurt, the globules of milk fat are homogenized and end up with an interfacial layer that is composed mainly of disrupted casein micelles (39). This allows the droplets to interact with free casein micelles as the acidification proceeds. In yogurt, the interfacial layer remains intact after gelation, but in the related product, cream cheese, the protein–fat emulsion gel is further worked, with the result that the interfacial layers are partially broken down, to give a different structure to the final product.

Interactions between emulsion droplets and macromolecules in solution can be aided by the presence of certain ions, of which calcium is the most important. The presence of these ions may cause flocculation of the emulsions, or gel formation may be enhanced. A general increase in ionic strength can destabilize the emulsions (40), but calcium may form more specific bridges between emulsion droplets and materials in solution (41).

These effects can be greatly enhanced by inducing orthokinetic effects (i.e., by stirring). In such a case, added ions may have a very strong effect (42). The basis of these effects is the fact that proteins generally carry a negative charge in the pH region 5–7 and, therefore, are capable of binding the ions. In addition, the small ions alter the conformations and stabilizing properties of the adsorbed protein layer (43).

Polysaccharides in food systems containing emulsions behave in complex ways, which are being intensively researched at the present time. They may have at least three effects upon emulsions. First, there may be phase-separation effects, because of the thermodynamic incompatibility of the polysaccharides, on the one hand, and the emulsion droplets, on the other (44). This leads to "depletion flocculation" processes, where the emulsion droplets may be driven to form a concentrated emulsion phase, distinct from the aqueous phase containing the polysaccharide. Such depletion flocculation can also be caused by the presence of excess protein (45). This separation can be more pronounced as the concentrations of emulsion and polysaccharide are increased and may be rapid. Second, the polysaccharide may gel, trapping the emulsion droplets and, in effect, stabilizing the emulsion toward flocculation and coalescence (however, the presence of a sufficient quantity of polysaccharide to form a gel may also induce phase separation before gelation occurs). Third, the polysaccharides may interact directly with the adsorbed material on the surface of the emulsion droplet. Because polysaccharides are not hydrophobic in nature, they adsorb poorly, if at all, to lipid surfaces, although some galactomannans do show some surface activity (46). In some cases, such as gum arabic, there is sufficient protein associated with the gum to make it surface active (47–49); in addition, synthetic protein–polysaccharide complexes have been shown to be surface active (50,51).

In addition, charged polysaccharides may interact with adsorbed protein of the opposite charge. It is possible to stabilize caseinate-based emulsion droplets against acid precipitation by interaction with pectin (52), although the presence of pectin in emulsions can also lead to phase separation (53). Caseinate emulsions can also be stabilized against acidification by the presence of κ-carrageenan, which may bind to the κ-casein of the caseinate even though both are negatively charged (52).

It is apparent that the possible interactions between an emulsion droplet and the other components of a food can be very complex. Several ingredients are generally present and they may give rise to final structures which are dependent not only on overall composition, but also on the manner in which the ingredients are added and the time/temperature variations to which they are subjected during manufacture. Of the last two, we have little knowledge, because the kinetics of exchange, phase

separation, and binding reactions have been studied very little, because of the complexity of the reactions and the products.

III. SURFACTANTS

A. Small-Molecule Surfactants

A considerable variety of surfactants is permitted for use in food emulsions, and these are discussed more fully in Chapter 8. Small-molecule surfactants (monoglycerides and diglycerides, sorbitan esters of fatty acids, polyoxyethylene sorbitan esters of fatty acids, phospholipids, and many others) contain long-chain fatty acid residues, which provide the hydrophobic group which binds to the lipid phase of the oil–water interface and causes adsorption. The head groups of these emulsifiers are more varied, ranging from glycerol (in monoglycerides and diglycerides) and substituted phosphoglyceryl moieties (in phospholipids) to sorbitan highly substituted with polyoxyethylene chains. Such material can have hydrophile–lipophile balance (HLB) values from 3 (oil soluble) to 10 or higher (water soluble). As a general rule, emulsifiers of a low and high HLB are used to form w/o emulsions and o/w emulsions, respectively (54).

Because these molecules adsorb strongly to the oil–water interface and have few steric constraints to prevent them from packing closely, they generate low interfacial tensions (55) and are very effective at lowering the Gibbs interfacial energy. However, they do not generally give highly cohesive or viscous surface layers, so that adsorbed layers of these small molecules may be quite easily disrupted (relative to adsorbed proteins; see Section III.B). This property is indeed used in certain types of emulsion, where limited stability to coalescence is required.

B. Proteins

Proteins, on the other end of the scale of molecular complexity, act as emulsifiers but behave differently from the small molecules, because of their individual molecular structures, and, indeed, it is the particular proteins present which give many food emulsions their characteristic properties. Most, if not all, proteins in their native states possess specific three-dimensional structures (even though we may not know what they are) which are maintained in solution, unless they are subjected to disruptive influences such as heating (56). When proteins adsorb to an oil–water interface, the hydrophobic regions of their structures (created by clusters of appropriate amino acid side chains) lie on, or possibly partially dissolve in, the oil phase. Some structures may be considered as especially important; for example, it is

possible for an α-helical portion of a protein to have a hydrophobic side, created by the hydrophobic side chains which lie outside the peptide core of the helix. However, even proteins such as caseins, which lack large amounts of regular structure, possess many groups of amino acids with hydrophobic side chains which adsorb to the oil–water interface. When a protein is adsorbed, the structure of the protein itself (the polypeptide backbone) will prevent close packing of the points of contact with the interface (the side chains), and as a result, adsorbed protein reduces the interfacial tension less than do small molecules. Although some proteins are excellent emulsifiers, not all proteins can adsorb strongly to an o/w interface, either because their side chains are strongly hydrophilic or because they possess rigid structures that do not allow the protein to adapt to the interface [as in the cases of gelatin (57) and of lysozyme, which although it does adsorb to o/w interfaces, tends to be a poor emulsifier (58)]. However, even apparently very hydrophilic proteins may adsorb strongly, as shown by the egg protein phosvitin, which is a surprisingly good emulsifier (59–62) despite having more than 50% of its residues composed of phosphoserine (63), an amino acid which is charged and hydrophilic. In this case, even the relatively few hydrophobic residues in the protein are sufficient to cause adsorption, so that the protein can cover a large interfacial area with relatively few points of contact.

Because adsorption of proteins occurs via the hydrophobic side chains of amino acids, it has been suggested that a measurement of surface hydrophobicity (64) should allow prediction of the emulsifying power of a protein (65). However, surface hydrophobicity is an ill-defined parameter, which is determined by the binding of probe molecules to the protein in solution (66) and may be a poor predictor of adsorption, especially because adsorbed proteins change conformation during or after adsorption.

C. Adsorption and Protein Conformation

Much research has been aimed at determining the mechanism of protein adsorption, and it is likely that most of the proteins which adsorb well to interfaces are capable of changing conformation either as they adsorb or shortly afterward. The concept of surface denaturation is well established (67,68) because the protein as it adsorbs is affected by spreading pressure, which pulls apart the native structure to maximize the amount of hydrophobic contact with the oil interface (Fig. 2).

A number of methods have confirmed that proteins change their conformations when they adsorb to liquid or solid interfaces. Spectroscopic studies of lysozymes show that adsorption to polystyrene latex causes a decrease in the amount of secondary structure (69) and that the protein

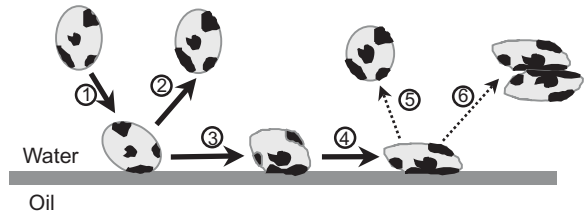

Figure 2 Schematic diagram of the adsorption and desorption of protein. The surface of the protein has hydrophobic (dark) and hydrophilic (light) regions. The protein molecule approaches the interface (1) and begins to adsorb. In principle, it is possible that very rapid desorption may take place (2) without the protein changing conformation. With time, the adsorbed protein changes its conformation to maximize hydrophobic contact with the oil, and this may pass through several stages (4). At this stage, the protein may be hard to displace (dotted arrows), and even if it is displaced, it will have an altered conformation (5) or even be denatured and aggregated (6). The adsorbed protein may itself react with its neighbors to form a network.

may pass through a number of conformational states as the adsorption process continues (70). The Fourier transform infrared (FTIR) spectra of adsorbed α-lactalbumin and β-lactoglobulin have both been shown to differ significantly from those of the native proteins (34,71). Proteins adsorbed to a surface and subsequently desorbed by the action of small molecules have been found to possess an altered conformation (72), showing that adsorption-induced changes may be irreversible; for example, lysozyme and chymosin lose their enzymatic activity on adsorption and do not regain it after being desorbed from the interface (32). It might be expected that the change in the conformation during adsorption is likely to destroy the secondary and tertiary structures of proteins, but it is possible to increase the ordered structure in some cases (73).

Once adsorbed, some proteins are capable of interacting chemically by forming intermolecular disulfide bonds to give oligomers, as has been shown for adsorbed β-lactoglobulin and α-lactalbumin (24,74), although such reactions do not occur in solution unless the proteins are denatured by heating (75). Further evidence for the denaturation of adsorbed proteins comes from differential scanning calorimetry (DSC), suggesting that unfolding on the surface has occurred (33,76,77). In some cases (e.g., lysozyme and α-lactalbumin), this surface denaturation appears to be at least partially reversible, but in others (e.g., β-lactoglobulin), adsorption causes irreversible changes in the protein molecules.

The casein proteins tend to be a special case. Because these proteins appear not to contain much rigid secondary structure (α-helix or β-pleated

sheet) (78) and because they possess considerable numbers of hydrophobic residues (79), they adsorb well (80). However, because of the lack of definition of their original native structures, it is impossible to determine whether conformational changes occur during adsorption, as neither spectroscopic changes nor DSC are capable of demonstrating conformational changes in these proteins.

Reactions between adsorbed protein molecules in emulsions (for instance, disulfide bridging interactions such as those mentioned earlier) will be encouraged by the very high local concentration of protein within the adsorbed interfacial layers. Generally, we know (81,82) that for monolayers of adsorbed proteins, the interfacial concentration (surface excess, Γ) is generally between 1 and 3 mg/m^2 and that the adsorbed layers are generally less than 5 nm thick (83), so it is simple to calculate that, in the interfacial region, a monolayer of protein has an effective concentration of about 500 mg/mL (i.e., 50%). This is, of course, very much higher than can be achieved by attempting to directly dissolve the proteins because of the extremely high viscosity generated, so direct comparisons between adsorbed and unadsorbed proteins at equal effective concentrations are not possible. However, the protein in the adsorbed layer may be in a favorable position for intermolecular interactions, because the molecules are very close to one another and adsorption holds them in position so that diffusion is slow. We should probably regard the adsorbed layer of protein as being more like a gel than a solution; this is at least partly the reason why many adsorbed proteins form highly viscous interfacial layers. These gels will be essentially two dimensional, with each molecule occupying approximately 11 nm^2 of interface [calculated on the basis of a molecule of 20,000 Da and a surface coverage of 3 mg/m^2; this agrees well with the expected dimensions of a globular protein of this weight (84) and is much larger than the 0.5–2.5 nm^2 per molecule which has been found for adsorbed modified monoglycerides (85)]. It is, therefore, not surprising that adsorption can alter the structures of proteins. The formation of such concentrated layers has relatively little to do with the overall bulk concentration of the protein in solution, which may give stable emulsions at relatively low bulk concentrations (although this depends on the amount of oil and the interfacial area to be covered).

Caseins form extended layers about 10 nm thick, and even at a Γ of 3 mg/m^2 have a "concentration" of about 300 mg/mL. Conversely, whey proteins form much thinner layers (about 2 nm thick) and must begin to form multilayers if Γ is more than about 2 mg/m^2, as there is no further space available for monolayer adsorption beyond that point.

With a few exceptions, most of the detailed research has been performed on relatively few proteins. Of these, the caseins (α_{s1}, α_{s2}, β, and κ)

and whey proteins (α-lactalbumin and β-lactoglobulin) predominate. This is principally because these proteins are readily available in pure and mixed forms in relatively large amounts; they are all quite strongly surfactant and are already widely used in the food industry, in the form of caseinates and whey protein concentrates or isolates. Other emulsifying proteins are less amenable to detailed study by being less readily available in pure form (e.g., the proteins and lipoproteins of egg yolk). Many other available proteins are less surface active than the milk proteins [e.g., soya isolates (86)], possibly because they exist as disulfide-linked oligomeric units rather than as individual molecules (87). Even more complexity is encountered in the phosphorylated lipoproteins of egg yolk, which exist in the form of granules (88), which themselves can be the surface-active units (e.g., in mayonnaise) (89).

IV. FORMATION OF EMULSIONS AND MEASUREMENT OF EMULSIFYING ACTIVITY OF PROTEINS

Food oil-in-water emulsions are generally produced using either colloid mills or high-pressure homogenizers. In the former, the oil–water–surfactant mixture is passed through a narrow gap between a rotor and stator, in which the stresses imposed on the mixture are sufficient to break up the oil into droplets, to which the surfactant adsorbs. This method tends to produce droplets of emulsion which are larger than those produced by high-pressure homogenization, being of the order of 2 μm in diameter. The technique is used to manufacture mayonnaises and salad creams, in which stability depends less on the presence of very small particles than on the overall composition and high viscosity of the preparation. In liquid emulsions, however, smaller particles are required to prevent creaming and possible coalescence.

High-pressure homogenization is used to produce these smaller droplets. A coarse emulsion of the ingredients is formed by blending, and this suspension is then passed through a homogenizing valve, at pressures which are generally in the region of 6.8–34 MPa (1000–5000 psi). This high-pressure flow through the valve creates turbulence, which pulls apart the oil droplets, during and after which the surfactant molecules adsorb to the newly created interface (90). If the adsorption is not rapid, or if there is insufficient surfactant present to cover the freshly formed interface, then recoalescence of the oil droplets rapidly occurs (91). The breakup and recoalescence occurs many times as the droplets pass through the field generated by the homogenizer (92). Apart from the mechanical design of the homogenizer, the sizes of the emerging droplets depend on, among other factors, the homogenization pressure, the viscosity of the suspension, the number of

passes (93), and the amount and types of surfactant present (94). Generally, when the surfactant is present in excess concentration, the particle size is limited by the characteristics of the homogenizer and of the suspension; on the other hand, if only small amounts of surfactant are present, the surfactant concentration limits the sizes of the particles, because insufficiently covered emulsion droplets will recoalesce. Generally, therefore, as the compositions of products are reformulated, the sizes of the emulsion droplets in them will change.

In addition to recoalescence and increased droplet size in the presence of insufficient surfactant, the phenomenon of bridging flocculation, in which the emulsion droplets form clusters during homogenization, can be observed. For the bridging to occur, it is necessary to have macromolecular surfactants with at least two sites by which they can adsorb to interfaces. At low surfactant concentrations, such molecules can become adsorbed to two separate oil droplets. Proteins can form bridges in this way (95), and even more commonly, natural aggregates of proteins such as casein micelles can induce clustering of the oil droplets (96). Bridging flocculation may be reversed by incorporating more surfactant (which need not be macromolecular) so as to provide enough material to cover the interface as it is formed. In the case of clustering by particles which themselves can be broken up, a second-stage homogenization at lower pressure can be sufficient to break down the bridging aggregates and to separate the clustered fat globules. Clearly, however, such treatment will be inapplicable to clusters bridged by single macromolecules, which cannot be broken up in this way.

One factor which can have considerable importance on the emulsifying properties of proteins is their quaternary structure. For example, in milk, the caseins exist in aggregates of considerable size (casein micelles) containing hundreds or thousands of individual protein molecules (97), held together by hydrophobic interactions and microparticles of calcium phosphate. The casein micelles act as the surfactants when milk is homogenized (98). During this homogenization, the micellar structure is disrupted, possibly by the forces within the homogenizer (99), but presumably also by the spreading forces which occur when the micelles violently encounter the oil surface. The result is that the oil surface is unevenly coated by partially broken up casein micelles, and not by a monolayer of casein (Figs. 1A and 1B).

In contrast, sodium caseinate (which is prepared by removing the calcium phosphate from the micelles by precipitation at acid pH and then washing the precipitate and redissolving at neutral pH) has much superior emulsifying properties compared to casein micelles (100) (i.e., the amount of oil which can be stabilized by a given weight of casein in either of the two

forms, under identical homogenization conditions). This effect is probably simply because the effective concentration of emulsifier is much less when the casein is in the micellar form, which is relatively resistant to disruption. Therefore, during homogenization, the nonmicellar casein will arrive at the interface more readily than the micelles. Interestingly, sodium caseinate at the concentrations generally used (above about 0.5% w/w protein) is not itself monomeric, but exists in the form of aggregates of the proteins containing about 30 molecules (13), which are held together probably by hydrophobic forces. In contrast to the intact micelles, these particles are believed to create monolayers of casein molecules around the fat globules; that is, the aggregates are pulled apart by the spreading pressure which they encounter as they bind to the interface. This cannot happen to casein micellar fragments, whose integrity is probably maintained by the presence of calcium phosphate.

Molecules such as β-lactoglobulin also show changes in quaternary structure as a function of pH (101), and these may be related to the changes in the protein's surfactant properties at different pH values (102). The denaturation of β-lactoglobulin by heat causes the protein to aggregate, and this decreases the emulsifying power to a considerable extent (103).

Because different proteins are more or less efficient at forming and stabilizing emulsions, and even the same protein may have different efficiencies in different circumstances (as has just been described for casein), it is essential to have methods for estimating the potential of given surfactants for forming emulsions. To achieve this, the required techniques should be method independent; that is, they should give absolute results, or at least give results applicable to specific methods for preparing emulsions. There are two widely used methods, Emulsifying Activity Index (EAI) and Emulsifying Capacity (EC). Neither of these methods is method independent, although they are simple to apply. To measure EC, a known quantity of surfactant is dissolved in water or buffer and then oil is added to it in a blender. This forms a crude emulsion, and further aliquots of oil are added until the emulsion inverts or free oil is seen to remain in the mixture. This ostensibly gives the weight of oil, which can be emulsified by the defined weight of protein. It is evident that this method is dependent on the particular blender because what is important in emulsion formation is not the weight of oil *per se* but its interfacial area. Thus, if the emulsion is made of large droplets, it will consume less surfactant than if small droplets are present. The conditions of emulsion formation are therefore critical to the method, as it is possible to obtain different results at different blender speeds, or with other homogenizing devices. Therefore, the method is not in any sense an absolute measure. As a quality control measure or as an internal method in a single laboratory, it may have considerable usefulness.

To measure the EAI, an emulsion is made and the particle size of the emulsion droplets is estimated. The assumption is then made that all of the protein is adsorbed to the interface, and so a measure of emulsifying potential can be measured. Although it provides more information than EC, the method has two major defects: First, it is very often the case that not all of the available protein is adsorbed, or adsorbed as a monolayer. Indeed, it is known that at concentrations of protein of more than about 0.5% (with oil concentration of 20%), some of the protein remains unadsorbed, even after powerful homogenization where the concentration of protein is the limiting factor in the determination of the sizes of the droplets (11,94). If homogenization is less extensive, then the proportion of protein which is adsorbed decreases. The second major problem in interpretation of the EAI is simply the difficulty of determining the particle sizes and their distribution. There are a variety of methods for measuring the size distribution of suspended particles, and care must be taken to avoid error in this measurement. Traditionally, the particle sizes in determinations of the EAI are measured by determining the turbidity of diluted suspensions of the emulsions, which is a method much subject to error.

Ideally, to fully describe the emulsifying capacity of a surfactant, the particle size distribution of the emulsion and the amounts of individual surfactants adsorbed to the oil–water interface need to be measured. It is possible to measure the amount of adsorbed protein by centrifuging the emulsion so that all of the fat globules form a layer above the aqueous phase and measuring by chromatography the concentration of surfactant left in the latter phase (12). Alternatively, the fat layer after centrifugation can itself be sampled, and the adsorbed protein can be desorbed from the interface by the addition of sodium dodecyl sulfate (SDS) and quantified by electrophoresis on polyacrylamide gels (104). In addition, although this is more difficult to determine, it is desirable to know the state of the adsorbed material (e.g., its conformation, which parts of the adsorbed molecules protrude into solution and are available for reaction, etc.). This represents an ideal which is rarely possible to achieve, but the explanation of the behavior of emulsions and perhaps the design of new ones may depend on this knowledge.

V. MEASUREMENT OF PARTICLE SIZES AND SIZE DISTRIBUTIONS IN EMULSIONS

Once an emulsion has been formed using homogenization or other means, it is often necessary to characterize it, specifically in terms of its average size and its size distribution. This is important in a number of

respects: Knowledge of the size distribution provides information on the efficiency of the emulsification process, and the monitoring of any changes in the size distribution as the emulsion ages gives information on the stability of the system. Regular measurement of particle size can be part of a quality control operation and can also be important when emulsion systems or processes are patented. However, the measurement of true size distributions or even the average sizes of emulsion droplets is not simple, despite the existence of a number of potentially useful and apparently simple methods.

The most direct method and one which is theoretically least subject to errors is electron microscopy (105). This technique measures the number-average size distribution, providing that (a) a fully representative sample of the emulsion is prepared, fixed, mounted, sectioned, and stained without distortion, (b) a sufficient number of particles is measured to ensure statistical accuracy of the distribution, and (c) proper account is taken of the effects of sectioning on the apparent size distribution. All of this requires considerable time, effort, and calculation, so that the technique cannot be used routinely to determine size distributions. It may be used as a standard against which to compare other methods, and it also finds a use in measuring systems where dilution causes changes in the particle sizes, as in microemulsions (106).

The most widely used of the rapid methods for particle sizing are based on light scattering. These tend to emphasize large particles in the distribution because larger particles scatter more light than smaller ones. The simplest of these methods depends on the measurement of turbidity at one or a number of wavelengths (107,108). Turbidity, or apparent absorbance of light, is a measure of the total amount of light scattered as it passes through a cuvette containing diluted emulsion (assuming that no component of the emulsion absorbs light of the wavelengths used). Although the method is rapid and may be performed in any laboratory possessing a spectrophotometer, it cannot be used to give the true distribution of particle sizes, but at best to give an average. It can be assumed that the particles form a distribution of known shape, but this, of course, assumes that the distribution is known beforehand.

A number of commercial instruments measure the distributions of particle sizes by determining the intensity of light scattered from a highly diluted sample at a number of specific scattering angles [integrated light scattering (ILS)]. With knowledge of the scattering properties (i.e., the Mie scattering envelope) of the particles (109), software is used to calculate the most probable distribution of particle sizes.

This does not always yield the true absolute distribution, for two main reasons. The first of these is that the angular range is often too restricted to allow measurement of small particles of diameters less than about 50 nm,

which scatter almost isotropically. Large particles preferentially scatter in the forward directions, so that to measure the distribution accurately, ideally measurements have to be made at a span of scattering angles between 0° and 150° (110). This is now available in modern particle sizers, but in older instruments, the angular range used limits the detection of small particles (smaller than 0.1 μm). Many food emulsions made by high-pressure homogenization contain particles of this size within their size distribution. A further problem with any light-scattering method is that the accuracy of the calculated distribution depends on how well the optical properties of the emulsion droplets (i.e., their real and imaginary refractive indices, which determine the scattering properties) can be defined. Generally, it is realistic to assume that the emulsion droplets are spherical, but it may be necessary to make assumptions about the structures of the interfacial layers. An emulsion droplet is essentially a coated sphere (111), which is characterized by refractive indices of the core and the coat, and these are likely to differ. Calculations based on of the scattering behavior of emulsion droplets may, therefore, depend on the presumed structures and compositions of the particles.

If the emulsion is unstable, the particle size distribution will change with time, and this will be detected by the light-scattering measurements. However, a simple measure of light scattering cannot distinguish between droplets which have flocculated and those which have coalesced, and other methods of measurement are needed to define which type of instability has occurred. Flocculation introduces another problem relative to the detailed interpretation of light-scattering measurements, because it produces particles which are neither homogeneous nor spherical. To determine the type of instability which has occurred, it is often possible to use light microscopy. Alternatively, the destabilized emulsion can be treated with SDS, which will dissociate any flocs. A second measurement of the particle size after the SDS treatment will show no change if the emulsion had coalesced, but it will revert to the original particle size distribution if the destabilization has been by flocculation (112).

Dynamic light scattering (DLS) offers an alternative means of measurement (113). This technique does not measure the total amount of light scattered, but the dynamics of the scattered light over very short time periods. Usually, the light scattering is measured at a fixed angle of 90° and a correlation function is measured. This is essentially a weighted sum of exponentials, which depend on the diffusion coefficients of the particles through the aqueous medium. As with ILS, the calculation of the true size distribution depends on the knowledge of the detailed light-scattering properties of the emulsion droplets. In addition, the fit of theory to the true correlation function is ill-conditioned (114), so that the size distribution obtained can

depend on the technique used to fit the correlation function. As with other light-scattering techniques, the contribution of larger particles to the size distribution is generally overestimated. This is partly because they tend to scatter more (i.e., have higher weighting factors), but also because of the nature of the correlation function itself, as the information about the small particles is contained only in the short-time part of the function, whereas information about the large particles is contained at all points.

An important demand of both ILS and DLS is that they require the suspensions of particles to be highly diluted. This is necessary because the theories used to calculate the particle sizes demand that the scattered photons have undergone only one scattering event. Multiple scattering distorts the photon statistics and leads to erroneous results. The dilution is not necessarily serious in the case of simple stable emulsions, but in more complex emulsions, it is necessary to ensure that no dissociation of particles is caused by the high dilution required for accurate light-scattering experiments. The dilution may lead to dissociation of flocculated material or to the breakdown of complex interfacial layers (e.g., those formed by casein micelles on the oil–water interfaces in homogenized milk). Conversely, it is possible that dilution into an inappropriate solution may promote aggregation or the emulsion droplets (e.g., dispersing casein-stabilized droplets in a solution containing large concentrations of calcium ions).

Recent advances in instrumentation have allowed some measurements of DLS to be made on more concentrated suspensions. Three methods have been developed. In the first, the optical paths of the incident and scattered beams of the instrument are positioned so that the measurements take place just inside the wall of the cuvette containing the sample. In such a case, the path lengths of the incident and scattered photons in the sample are very short, and up to a volume concentration of as much as 20%, the system may be considered as a simple DLS experiment with no multiple scattering. A second, more complex, but in principle more accurate, method is to use two scattering beams which are cross-correlated with one another (115). This effectively compares two scattering experiments on the same sample and allows the elimination of the contribution of multiply-scattered photons. This technique has been used to study milk (116) but not, so far, emulsions. Finally, a third technique uses the multiply-scattered light rather than trying to eliminate its effects. This is the technique of diffusing wave spectroscopy (DWS). The method is useful for concentrated suspensions such as emulsions (117), but although it can give average sizes of the particles, distributions are harder to obtain. It has, however, been more widely used than the other methods, especially in empirical ways, to detect gelation (118).

These three methods are not as yet widely used, because instrumentation is only now coming on to the market, and most DWS apparatuses tend

to be custom-built in a single laboratory. In using them, it is essential also to be aware of a problem that is not present in diluted solutions. All of the methods of dynamic light scattering depend on the presence of moving particles; in fact, they measure diffusion coefficients, which are then transformed into particle sizes. In turn, diffusion coefficients depend on the viscosity of the continuous phase. When concentrated suspensions are measured, the particles themselves contribute to the viscosity of the suspension, so that care must be taken when evaluating particle sizes from measurements made in concentrated suspensions.

Light-scattering methods are, at present, the most effective and most widely used means of obtaining information about the size distributions of particles in emulsion systems. Like many methods, they are especially useful in a comparative mode to measure changes that occur during processing or storage of the suspensions. All of the light-scattering methods can detect whether aggregation is occurring, so that they may all be used to detect instability of emulsions, and they are almost unique in allowing the kinetics of aggregation to be studied on a real-time basis (119). Simply, the fact that the particle size is increasing can be determined without any particular attributes of the particles needing to be known.

An alternative method of particle sizing, which shows considerable potential although it is not so widely used as light scattering, is based on ultrasonic acoustic spectroscopy. This measurement can easily be made on concentrated dispersions and depends on the fact that the attenuation and velocity of ultrasound of a defined frequency through a suspension depends on the sizes of the particles in the dispersed phase. By measuring the attenuation of sound at a series of different frequencies through the sample, it is possible to calculate the size distribution of the particles in the suspension (120,121). Instruments to perform the measurements are available and they are capable of being compared with the information available from light-scattering measurements (122). Just as the scattering of light depends on the relative refractive indices of the dispersed phase and the continuous phase, so particle sizing by ultrasound also depends on the physical properties of the dispersed phase; for example, the density, viscosity, thermal conductivity, and specific heat are all required to be known to permit the true size distribution to be determined (123). In the absence of these factors, ultrasonic attenuation spectroscopy can give only relative size distributions. However, because of the applicability of the method to real (undiluted) emulsion systems, it is likely that the method will increase in its usage. Ultrasound and its applications are discussed in detail in Chapter 10.

A related method which has been proposed is that of electroacoustic spectroscopy (124). Because the passage of ultrasound through a dispersion

disturbs the double layer which surrounds the dispersed particles, an electric current is set up. Measurement of this current allows the calculation also of the ζ-potential of the particles as well as their size distribution. The measurement may also be performed in reverse; an oscillating electric field causes the emission of ultrasound, which, in turn, permits the size distribution and the ζ-potential to be measured (125,126). Like the simpler ultrasonic spectroscopy, these methods require precise knowledge of the physical characteristics of the medium and the dispersed phase if they are to give absolute, rather than relative, results.

VI. STRUCTURES OF THE ADSORBED LAYERS ON THE SURFACES OF EMULSION DROPLETS

A. Simple Surfactants

The structures of the interfacial layers in emulsion droplets might be expected to be simple when small-molecule emulsifiers are used, but this is not necessarily the case, especially when not one but a mixture of surfactant molecules is present. Although simple interfacial layers may be formed where the hydrophobic moieties of the surfactants are dissolved in the oil phase and the hydrophilic head groups are dissolved in the aqueous phase, it is also possible for multilayers and liquid crystals or even crystals to form close to the interface. This depends on the nature and the concentrations of the different surfactants. Interactions between surfactants generally enhance the stability of the emulsion droplets, because more rigid and structured layers tend to inhibit coalescence. Also, mixtures of different surfactants having different HLB numbers appear to provide structured interfacial layers, presumably because of the different affinities of the surfactants for the oil–water interface. Specifically, phospholipids may form multilamellar structures around the oil–water interface, and presumably these layers will have different spacing depending on the amount of hydration (127). They also depend both on the nature of the oil and of the phospholipid (128). So, although the major adsorption of phospholipids at low concentration is likely to be in the form of monolayers, it may be possible to produce more complex structures when large amounts of phospholipid are present or when other surfactants are coadsorbed to the interface.

B. Interfaces with Adsorbed Proteins

Undoubtedly, the most complex interfacial structures are produced when proteins are used as the surfactants, because of the great range of conformational states accessible to such molecules. This is of interest because of the

implications of conformational change on the reactivity and functionality of the proteins [e.g., it appears that adsorbed β-lactoglobulin cannot form disulfide bonds with κ-casein when heated in the presence of caseinate (129), although this reaction is known to occur between the proteins in solution and in heated milk]. Flexible molecules such as caseins may be considered as adsorbing as if they were heteropolymers (130) because they are presumed to have high conformational mobility (78). As evidence of the presumed conformational change, adsorbed β-casein exhibits different susceptibility to attack by proteolytic enzymes, compared with the protein in solution (131). Further, adsorption to different hydrophobic materials causes differences in the conformation of the adsorbed molecule; for example, the protein seems to have somewhat different conformations when adsorbed to hydrocarbon (*n*-tetradecane) or triglyceride (soya oil)–water interfaces (132). As a result of these measurements, it appears that model hydrocarbon–water systems are not necessarily suitable for describing triglyceride–water systems.

Much is known about the structure of adsorbed β-casein, certainly more than is known for any other food protein. The first evidence from dynamic light scattering showed that β-casein can adsorb to a polystyrene latex and cause an increase in the hydrodynamic radius of the particle by 10–15 nm (133). Small-angle x-ray scattering confirmed this and showed that the interfacial layer was not of even density throughout and that the bulk of the mass of the protein was close to the interface (134). Neutron reflectance studies also showed that most of the mass of the protein was close to the interface (135). From these results, we can infer that a relatively small portion of the adsorbed protein molecule extends from the tightly packed interface into solution, but it is this part which determines the hydrodynamics of the particle and which must be the source of the steric stabilization which the β-casein affords to emulsion droplets (133). All of the studies just described were performed on polystyrene latex particles or on planar interfaces; however, it has also been demonstrated that the interfacial structures of β-casein adsorbed to emulsion droplets resemble those in the model particles (83,134). Although detailed control of emulsion droplets during their formation in a homogenizer is impossible, it is possible to break down the surface layers of protein once they are formed, by the use of proteolytic enzymes (135), and by comparison with the behavior of model systems under similar conditions, it is possible to demonstrate that the proteins seem to have similar conformations in the model systems and emulsions (27).

It is also possible to use proteolytic enzymes to demonstrate which part of the β-casein protrudes into the solution. There are many sites in the protein molecule (lysine and arginine residues) susceptible to attack by

trypsin, and these are almost equally susceptible to attack when the protein is free in solution. In the adsorbed protein, sites close to the N-terminal are most readily accessible, presumably because they form the part of the adsorbed layer which protrudes into solution (131). This region of the molecule is the one which would be expected to behave in this way, being the most hydrophilic and highly charged part of the protein. Thus, for β-casein, it is possible to predict some features of the conformation of the adsorbed protein from a study of its sequence (131). It seems that β-casein is perhaps the only protein for which this kind of prediction can be done; most other proteins (even the other caseins) have much less distinct hydrophilic and hydrophobic regions and, therefore, have conformations which are more difficult to predict (27). From studies of the structure of adsorbed α_{s1}-casein n model systems and in emulsions, it is established that neither the most accessible sites for trypsinolysis (136) nor the extent of protrusion of the adsorbed protein into solution (137) can be readily predicted.

Nevertheless, it is possible to calculate from statistical mechanical principles the approximate conformations of the adsorbed caseins, by assuming that they are flexible, composed of chains of hydrophilic and hydrophobic amino acids (138). The results of these calculations reproduce many of the features of the actual measured properties, especially the tendency of the adsorbed β-casein to protrude further from the interface than the α_{s1}-casein (139). These calculations have, in turn, been used to explain the differing stabilities of the two different types of emulsions toward added salts (140). These calculations have considerable success in explaining both the structure and stability of casein-coated emulsions, but they are less adaptable to explain the behavior of more rigid protein surfactants. However, the same principles have been used to explain the apparently anomalous adsorption of phosvitin (61).

The difficulty of ascertaining the structure of the adsorbed protein is greater for globular proteins. In these cases, the adsorbed layer is much thinner than it is for the caseins, so that layers of β-lactoglobulin appear to be of the order of 1–2 nm thick instead of the value of about 10 nm measured for the caseins (83,104,137). Hydrodynamic and scattering experiments suggest that the thickness of the adsorbed layers is smaller than would be expected from the protein in its natural conformation, so that these simple measurements of the size of the adsorbed protein already suggest that adsorption causes a conformational change. This is confirmed by other techniques. For example, adsorbed β-lactoglobulin forms intermolecular disulfide bonds (24), which does not occur when the molecules are in their native conformations in solution (although the high concentration of protein in the adsorbed layer will certainly enhance any tendency that the molecules have to aggregate). In addition, detailed studies of the DSC of

emulsions containing β-lactoglobulin (33) have shown that (a) the protein when adsorbed to the oil–water interface in emulsions loses its heat of denaturation (i.e., shows no intake of heat which can be associated with denaturation, presumably because the protein is already surface denatured) and (b) if the protein is desorbed from the interface by treatment with detergent (Tween-20), it can be seen to be denatured irreversibly (i.e., no recovery of the denaturation endotherm is seen). This may be contrasted with the behavior of α-lactalbumin, which loses its heat of denaturation when adsorbed but recovers its original thermal behavior when the protein is competitively desorbed by Tween (33,70). These studies confirm that different proteins show quite different degrees of denaturation when they are adsorbed to oil–water interfaces.

Similarly, the use of infrared spectroscopy (FTIR) shows that significant changes in the conformation of β-lactoglobulin occur as a result of adsorption. Detailed interpretation of the spectra is difficult, but changes in the contents of α-helix and β-sheet contents of the protein appear to occur (34). The protein α-lactalbumin is less affected by adsorption (71). However, nearly all of the evidence [chemical, physical, spectroscopic, and enzymological (32)] combines to show that adsorption-induced conformational changes occur. Moreover, these changes appear to be nearly always irreversible; that is, even if a protein is desorbed from the interface, it cannot recover its original conformational state.

When considering the structure of a protein-based interfacial layer, there are other factors to be considered, namely the possibility that multilayers, rather than monolayers, are formed and the possibility that specific proteins may exhibit variable behavior depending on the conditions. Caseins are capable of this; for example, it is possible to prepare stable emulsions containing 20% (w/w) of soya oil, with as little as 0.3% casein, and in these, the surface coverage has been measured to be slightly less than 1 mg/m^2. The hydrodynamic thickness of the adsorbed monolayer in these emulsions is about 5 nm. In emulsions prepared with larger amounts of casein (1–2%), the surface coverage increases to 2–3 mg/m^2 and the thickness of the adsorbed monolayer is about 10 nm (i.e., about twice that of the layer at lower surface coverage) (94). It has been suggested that this is a result of the adsorbed casein molecules adopting two different conformations: one at low coverage, where the proteins have to cover a maximum area of surface (about 48 nm^2 per molecule), and one at high coverage, where the molecules are more closely packed (about 13 nm^2 per molecule). The addition of more protein to the aqueous phase of emulsions made with low concentrations of caseinate results in the adsorption of some of the added protein and an increase in the thickness of the adsorbed layers. This is true, even if the added protein is not casein, and illustrates that, for caseins at least,

the proteins on the surface possess sufficient mobility to be moved as other proteins adsorb.

So far, we have considered monolayers. However, caseins and other proteins can form multilayers; this has been demonstrated for adsorption to planar interfaces, where large surface excesses are easily generated and multilayers are formed (80). There is less evidence for this in emulsions, although some high surface coverages (up to about $10\,mg/m^2$) have been measured (141), which can only arise from the presence of more than a single layer on the oil–water interface because it would be impossible to pack this amount of protein into a monomolecular layer. It is not clear why monolayers in some cases and multilayers in other cases are formed, although it is likely that the physical conditions of homogenization may be important, as well as there being a need for high concentrations of protein. Also, differences in the methods of preparing the caseins may be relevant; at neutral pH values, highly purified caseins have not generally been associated with multilayer formation, which seems generally to be associated with the use of commercial sodium caseinates.

Multiple layers seem to be more easily formed in emulsions containing whey proteins than with caseins. Because the whey proteins project less into solution than do the caseins, they may be less effective than caseins at sterically preventing the approach of additional molecules that go to form the multilayers. Finally, because they change conformation when they adsorb, they may offer new possibilities for interaction with incoming whey proteins from solution. There is evidence for multiple layers of whey proteins from both planar interfaces and emulsion droplets (11,142). However, although these multiple layers exist, there is no definite evidence that links them to changes in the functional properties of the emulsions. Nor is it well determined how stable the multiple layers are, compared with a monolayer. It is very difficult, or perhaps impossible, to simply wash adsorbed proteins from adsorbed monolayers formed on the interfaces of oil droplets (143). However, the outer portion of multilayers may be more readily displaced because it is held in place by protein–protein interactions only, which may be weaker than the forces which lead to adsorption. Generally, however, the properties of the outer parts of multilayers have been little studied.

C. Emulsions Stabilized by Particles

As a final degree of complexity, food emulsions may be stabilized by particles. Perhaps the most common are the protein "granules" from egg yolk, which play a role in the stabilization of mayonnaise (89), and casein micelles in products such as homogenized milk and in ice creams. Both of these

emulsifiers are known to be adsorbed to the oil–water interface as complex particles, which do not dissociate completely to their individual proteins either during or after adsorption (144,145). During the homogenization of milk, casein micelles are partially disrupted at the oil–water interface so that they adsorb either whole or in fragments. Indeed, once a micelle has adsorbed, it appears to be able to spread over an area of the interface (105,146,147). Thus, the fat droplets in homogenized milk are surrounded by a membrane that must contain some of the original fat globule membrane [phospholipid and protein (148)] but is primarily constituted of semi-intact casein micelles. If the milk has been heated before or after homogenization, whey proteins also form part of the layer surrounding the fat globules (39,149). Likewise, the oil–water interface in mayonnaise is partly coated by the granular particles formed from the phosphoprotein and lipoprotein constituents of egg yolk (150). In this high-lipid product, the granules also may act to keep the oil droplets well separated and prevent coalescence.

Natural fat globule membrane was mentioned earlier as a possible emulsifying agent (151). In its most native state (i.e., prepared in the laboratory from unheated milk), this membrane is an effective emulsifier (152), although little is known of its structure as it resurrounds oil droplets. However, heating at temperatures greater than about 65°C is sufficient to denature the proteins of the membrane. This appears to greatly diminish the efficacy of the membrane material as an emulsifying agent (153).

Emulsion formation by means of these aggregates of protein is generally less efficient than by the proteins when they are present in the molecular state, simply because the efficient formation of the emulsion depends on rapid coverage of the newly formed oil surface in the homogenizer. A particle containing many molecules of protein will encounter a fat surface less frequently than an equivalent amount of molecular protein. Thus, although it is possible to prepare homogenized milk with the proportions of casein and fat which occur naturally in milk (a ratio of about 1:1.5 w/w), it is not possible to use 1% w/w of micellar casein to stabilize an emulsion containing 20% oil (154). On the other hand, 1% casein in a molecular form (sodium caseinate) is quite sufficient to form a finely dispersed, stable emulsion with 20% oil. Therefore, unless the micelles are expected to confer some specific advantage on the functional properties of the emulsion or unless there are specific legislative reasons, it is generally more effective to use caseinate than casein micelles to stabilize an emulsion. With egg granules, the situation is different, inasmuch as the individual proteins from the egg granules are not readily available in a purified form analogous to caseinate. In this case, the choice between particulate and molecular forms of the protein does not arise.

VII. MODIFICATION OF THE INTERFACIAL LAYER AFTER EMULSION FORMATION

In many food emulsions, more than one surfactant is present, so that mixtures of proteins, small-molecule surfactants (oil soluble and water soluble), and lecithins may be on the interface or in the continuous phase. Under these circumstances, the interfacial layer will contain more than one type of molecule. The properties of the emulsion (the sizes of the droplets, the functionality, and the stability) will, in turn, depend on which types of molecule in the formulation are actually on the interface and what effect external factors have on the conformations of the adsorbed materials (Fig. 3).

A. Interfaces Containing Mixtures of Proteins

It has been shown that in mixtures of proteins in emulsions formed at neutral pH and moderate temperatures, there is generally no competition for the interface. For example, there is no preferential adsorption between the proteins when a mixture of α-lactalbumin and β-lactoglobulin is homogenized with oil; the amounts of protein adsorbed are in proportion to their concentrations (155). The same is true even when a mixture of sodium caseinate and whey protein is used as the surfactant in an emulsion (11). The only case where competitive adsorption has been truly observed is when

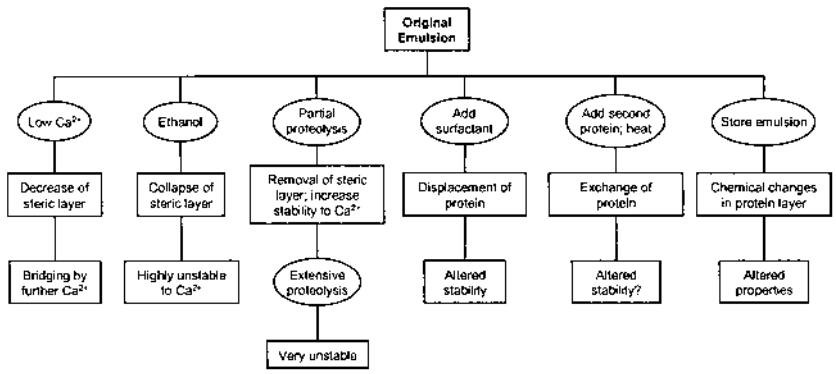

Figure 3 Examples of the potential modification of the adsorbed layer of protein during storage or processing of an emulsion stabilized by a layer of sodium caseinate. Various treatments (see text for details) can give rise to a number of modifications of the surface, which, in turn, affect the stability properties of the emulsion. The list of modifications is not exhaustive.

β-casein is used to displace adsorbed α_{s1}-casein, and vice versa, so that there is a possibility that these two proteins adsorb according to thermodynamic equilibrium (12). Even this observation is complicated, however, because it appears to apply only to mixtures of highly purified caseins; the displacement reactions with commercial sodium caseinate (where a similar result would *a priori* be expected), give much less clear results (16,156).

Rather than forming an emulsion in the presence of mixed surfactants, it is possible to form an emulsion using one protein and then to attempt to displace that protein from the interface with another. It is a general finding, however, that usually the protein which is first on the interface resists displacement (157). Because of its high surface activity and flexibility, β-casein appears to be the best displacing agent, but it is not always capable of displacing an already adsorbed protein. It can displace α_{s1}-casein or α-lactalbumin from an interface (12,158), but the process is more complex with adsorbed β-lactoglobulin (159), especially if the emulsion containing the β-lactoglobulin has been allowed to age before the β-casein is added.

The difficulty of replacing one protein with another is perhaps not surprising, because proteins are adsorbed to the interface by many independent points of contact (because they have several hydrophobic regions capable of binding to an interface). For all of these contacts to become desorbed at once is extremely unlikely, and so the spontaneous desorption of a protein molecule is very rare; this is why it is difficult to wash proteins from an oil–water interface (143). Replacement of an adsorbed protein molecule by one from solution must presumably require a concerted movement of the two molecules; as parts of one are displaced, they are replaced by parts of the other, until, finally, one of the two proteins is liberated into the bulk solution. Even this process, although more likely than spontaneous desorption, is not certain to succeed, especially if the adsorbed protein has been on the interface for some time and has been able to make bonds with neighboring molecules. Moreover, given the very high concentration of protein in the adsorbed layer (see earlier text), it may even be difficult for a second type of protein to penetrate the adsorbed layer to initiate the displacement process. Therefore, although thermodynamic considerations may favor one protein over another, kinetic factors militate against rapid exchange. Nonetheless, there do seem to be factors that influence the competition between proteins. As has been suggested earlier hydrophobicity and flexibility may be important criteria (because β-casein can be an effective displacing agent). Especially, whatever increases flexibility may lead to increasing competitiveness. The most obvious example of such a change is α-lactalbumin; in its native state, this protein has a globular structure partly maintained by the presence of one bound calcium ion (160). Removal of this Ca^{2+} leads to the protein adopting a ''molten globule'' state, whose tertiary structure is

altered (161), and this leads to increased flexibility and competitiveness at the interface (162). The removal of the Ca^{2+} can be achieved by chelation or by reducing the pH, and under these conditions, α-lactalbumin out-competes β-lactoglobulin for adsorption to the interface (102,104,162). To some extent, the competition can be reversed by reneutralizing in the presence of Ca^{2+}, so that in this case, there is dynamic competition between the proteins for the interface, not simply preferential adsorption during emulsion formation (104).

It should be noted that competition between proteins need not occur. For example, lysozyme (being positively charged at neutral pH) forms complexes with other egg-white proteins (negatively charged) on air–water interfaces. The complex may adsorb to the interface or the lysozyme may bind to already adsorbed proteins (163). This coadsorption is, however, not general, because it requires two proteins to be of opposite charge, which is relatively rare.

B. Addition of Small-Molecule Emulsifiers to a Protein-Stabilized Emulsion

Competition between adsorbed and free proteins can be considerably enhanced by the presence of small surfactant molecules (164). In such cases, there is competition between the proteins and small molecules as well as between the proteins themselves. However, in such a case, instead of the desorption of a protein requiring the inefficient process of simultaneous detachment at all points, or the slow creeping displacement of one protein molecule by another, it is possible for a number of small molecules to displace a protein by separately replacing the individual points of attachment. It is known that small-molecule surfactants are capable of efficiently displacing adsorbed proteins, although the details of the reactions depend on the type of surfactant and whether it is oil or water soluble (165–168). Water-soluble surfactants are capable of removing all of the adsorbed protein from the oil–water interface, although they may require a molecular ratio of about 30:1 surfactant:protein (164). At lower ratios, some, but not all, of the protein is displaced (Fig. 4). This displacement occurs either when the small-molecule emulsifier is present at the moment of homogenization or it is added later. Oil-soluble surfactants (low HLB numbers) are less effective at completely displacing protein or of preventing protein adsorption (16,168,169). For solubility reasons, these surfactants cannot be added to the emulsion after it has been formed, but must be incorporated during the homogenization step. In addition to competing with proteins for adsorption to the oil–water interface, both during formation of the emulsion and its subsequent storage, some small-molecule surfactants also facilitate the exchange

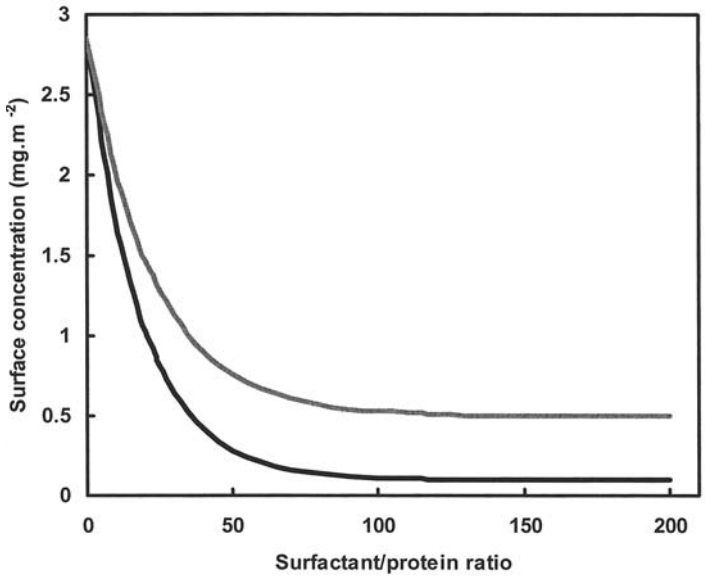

Figure 4 The displacement of caseins from an emulsion by the incorporation of the water-soluble surfactant Tween-20 (lower line) or the oil-soluble surfactant Span-20 (upper line). The water-soluble material can be added at any time before or after the emulsion is formed and can give almost complete displacement of the proteins. The oil-soluble surfactant must be present dispersed in the oil phase before emulsion formation takes place.

reactions of the proteins themselves. For example, although α-lactalbumin and β-lactoglobulin do not compete well with each other under normal circumstances at neutral pH (155), the presence of Tween causes the adsorption of α-lactalbumin to be favored over β-lactoglobulin (164). Presumably, the presence of surfactant enables a more thermodynamic equilibrium to be established, rather than the extremely slow kinetically determined exchange which normally occurs (if it occurs at all) between the two proteins. Alternatively, if the surfactant actually binds to the protein, its conformation may change so that it becomes more surface active, as was shown for the ''molten globule'' conformation of α-lactalbumin. Similarly, the presence of surfactants can alter the exchange between β-casein and β-lactoglobulin on an oil–water interface. Of the two proteins, β-casein is displaced first by both oil-soluble and water-soluble surfactants (18,170).

The displacement of proteins by small-molecule surfactants has been studied in some detail, using the technique of atomic force microscopy (AFM) which allows the detailed visualization of adsorbed films on a

planar interface. A series of reactions has been shown to occur when proteins are displaced by Tween. Rather than the protein molecules being displaced one by one, it is found that the surfactant pushes the protein aside from parts of the interface (171); in effect, a two-dimensional phase separation occurs. As this occurs, protein does not leave the surface, but forms multilayers over restricted areas of the interface (18). This ''orogenic'' displacement reaction explains some anomalous research results on the apparent thickness of adsorbed layers (17) and also offers the interesting possibility that the inhomogeneous structure of the interface may lead to areas of different reactivity. The results using AFM have been confirmed by studies using neutron reflectivity (172), which showed that the removal of the protein (β-lactoglobulin) by the surfactant is not a single continuous process. Modeling of the displacement reaction has been able to reproduce some aspects of the exchange behavior, using a fairly simple model (173,174). What must be critical to the behavior is the respective influence of interprotein and protein–solvent interactions. Because in practice the protein is displaced from the interface in an aggregated form, the changes in the protein structures and properties arising from adsorption and desorption can be qualitatively understood.

These observations lead to the question of whether the same type of behavior occurs when two proteins are present on an interface together. Here, the situation is not clear. Studies have been made on mixed proteins at air–water interfaces, and whereas in one case a slow phase-separation behavior has been seen over the course of some days (175,176), in another study by a different group, no such separation could be seen (18). It will be necessary to await further developments in this field before coming to a decision. Neither of the studies used emulsion droplets, which are not susceptible to the techniques, but it is intriguing to contemplate the possibility of producing controlled multifunctional surfaces by mixing appropriate proteins.

Lecithins represent a different type of small-molecule emulsifier. Although these molecules possess surfactant properties, they do not behave like other small-molecule emulsifiers. For example, they do not appear to displace proteins efficiently from the interface, even though the lecithins may themselves become adsorbed (8). They certainly have the capability to alter the conformation of adsorbed layers of caseins, although the way in which they do this is not fully clear; it is possibly because they can ''fill in'' gaps between adsorbed protein molecules (177). In actual food emulsions, the lecithins in many cases contain impurities, and the role of these (which may also be surfactants) may confuse the way that lecithin acts (178). It is possible also for the phospholipids to interact with the protein present to form vesicles composed of protein and lecithin, independently of

the oil droplets in the emulsion. The existence of such vesicles has been demonstrated (179), but their functional properties await elucidation.

C. Chemical Modification of the Interfacial Layer

Once a protein-stabilized emulsion has been formed, it is possible to modify the interfacial layer by chemical reactions. In fact, spontaneous reactions may occur during processing and storage of an emulsion, which change the structure and the properties of the interfacial layer. Both β-casein and β-lactoglobulin can suffer change in stored emulsions with soy oil (4,180). This may be attributed to the oxidation of the unsaturated fatty acid chains of the oil giving rise to enal molecules, which subsequently react with lysine or arginine residues of the proteins.

Changes in the conformations of adsorbed proteins can be induced by changes in the properties of the aqueous phase, especially for the caseins. Increases in ionic strength, or the presence of Ca^{2+}, cause the thickness of the adsorbed layers to decrease, because of smaller repulsive interactions between the charges of the protein molecules (43,181). Similarly, the addition of a poor solvent, such as ethanol, causes the adsorbed layer to collapse and lose its steric stabilizing properties (182). It is this effect that makes cream liqueurs so susceptible to the presence of Ca^{2+} (183).

The description in the previous section of the only moderate exchange between adsorbed and free proteins refers to results at room temperature, which is where nearly all of the detailed studies have been made. However, it appears that the exchange is temperature dependent. It has been demonstrated recently that whey proteins (especially β-lactoglobulin) can displace α_{s1}- and β-caseins from an oil–water interface during heating (this does not occur at room temperature) (129). If whey protein isolate is added to an emulsion prepared using oil and sodium caseinate and the mixture is heated to a temperature in excess of about 50°C, the whey proteins rapidly become adsorbed (Fig. 5), and as they do so, the caseins are desorbed, so the surface coverage by protein remains approximately constant (184). Only the major caseins are desorbed, however, with the α_{s2}- and κ-caseins remaining on the emulsion droplets. This behavior has been insufficiently studied to be fully understood. Interestingly, it is the whey protein which displaces β-casein; this is similar to the effect of surfactants described in the previous section. It is not known why the minor caseins resist displacement; the obvious possibility, that disulfide bonds are formed between these caseins and the whey proteins, does not seem to occur (185). It seems evident, however, that the exchange of proteins may occur more readily than was previously thought, especially in food preparations where an emulsion is added to a solution containing other proteins and the mixture undergoes a heat treatment.

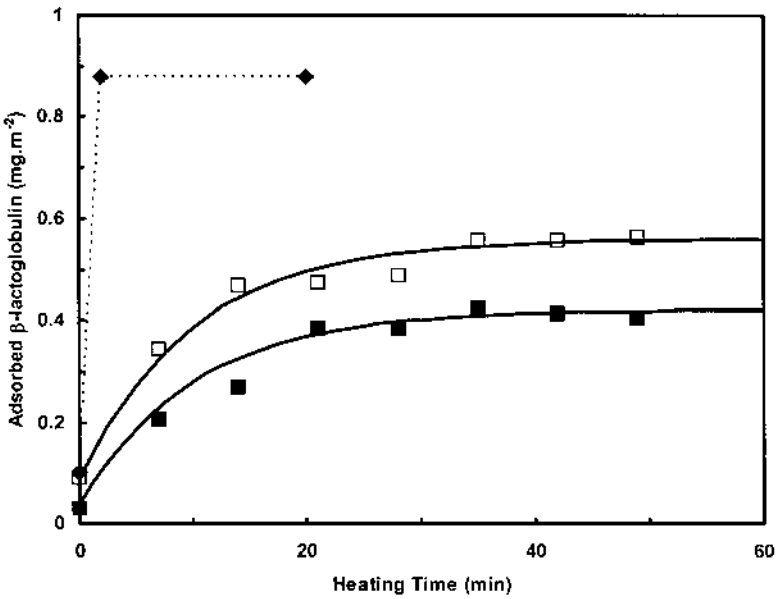

Figure 5 Kinetics of the exchange of whey protein with caseins at an emulsion interface. The figure shows the time-dependent adsorption of β-lactoglobulin from a whey protein isolate solution added to an emulsion originally made with sodium caseinate. Curves for the removal of the caseins from the surface are analogous. The results shown are for 45°C (■), 50°C (□), and 80°C (◆). The results show that not only is the rate of exchange temperature dependent, but so is the extent of replacement of one protein by another. The transfer at 80°C is too rapid to measure by the techniques employed in the research.

Mention has already been made of the disulfide-mediated polymerization of whey proteins (24). Evidently, the conformational changes produced as a result of adsorption allow the single sulfhydryl group of the protein to become accessible for reaction; normally, it is buried in the structure of the protein. The details of the reaction are not elucidated; evidently, the "activated" lactoglobulin molecules can react not only with one another but with α-lactalbumin as well, if it is present (74). It is even possible that the reactive adsorbed molecules can pull in molecules from the aqueous phase and that disulfide bonds can be formed between proteins on different particles. However, the free-sulfhydryl groups are not universally accessible, because β-lactoglobulin bound to an emulsion was not capable of reacting with κ-casein from caseinates added after the emulsion was formed, or from pure κ-casein added subsequently (184,185).

The sulfhydryl–disulfide exchange is a spontaneous phenomenon. It is also possible, however, to use cross-linking enzymes, such as transglutaminase, to polymerize the interfacial proteins (26). This polymerization, as may be expected, produces strong, rigid, interfacial layers (186), as long as the extent of cross-linking is not too large; these layers protect against coalescence and promote ethanol stability (187). Similar to the disulfide-induced polymerization, the cross-linking can be carried to an extent that causes the emulsions to destabilize (aggregate), presumably because cross-links begin to form between protein molecules adsorbed to different particles.

Finally, in this section, we mention the effects of ultra high pressure on systems containing emulsions. It seems probable that high pressure has an effect on the behavior of the proteins in emulsions and, consequently, the exchange between them. Thus, unlike the effect of heat, where β-lactoglobulin was shown to displace the major caseins, the application of pressure in such mixed-protein emulsion systems favors the adsorption of caseins (188), possibly because the high pressure denatures the β-lactoglobulin which is in solution, causes it to aggregate, and renders it incapable of adsorbing efficiently. Similar effects are found if a small-molecule emulsifier is mixed with the β-lactoglobulin-stabilized emulsion before pressure is applied. Further evidence of the effect of pressure-induced changes in β-lactoglobulin emulsions comes from the increased interaction of the protein with pectin as a result of the high-pressure treatment. Even at neutral pH, an interaction is observed (189). Clearly, the effect of pressure as a denaturing agent may influence the behavior even of adsorbed species.

It is worth noting that the structures of denatured proteins are different, depending on how they are denatured—whether by heat, by pressure, or by adsorption. The effects of combinations of these factors on the structures and reactivities of proteins are largely unknown; in fact, the effects of processing on emulsions are only now beginning to be studied in realistic systems, a trend which is much to be encouraged.

VIII. CONCLUDING REMARKS

The object of making food emulsions is to provide a stable and controllable source of food, whose texture, taste, and nutritional and storage properties are acceptable to the consumer. Although the number of possible ingredients is limited by the constraints of healthy nutrition, it is nevertheless evident that within the available range, there is a great deal of opportunity for variation in the properties of the emulsions—for instance, the particle size and the composition of the stabilizing layer of the interface, which, in

turn, influence the stability and functional behavior of the emulsion. On the other hand, many emulsions used in foods have their roots in established formulations, and an understanding of why certain emulsions behave as they do is still not established in a number of cases.

In the manufacture of many real food emulsions, the path followed is critical, emphasizing once again that emulsions, like many other food systems, are not in equilibrium states and that two products may be very different although they have the same overall composition. On this point, our knowledge is insufficient and needs to be extended. For example, the heat or high-pressure treatment of ingredient proteins either before or after the formation of an emulsion may critically affect the behavior of the emulsion. As foods containing emulsified material become more complex or sophisticated in their ingredients, the level of understanding required to control their formation and properties is increased. The challenge for the future is to be able to describe and control some of the most complex emulsions so as to enable greater functional stability for these food systems.

A further aspect, which is becoming of ever-increasing importance in the public mind, is that of the nutritional function of food emulsions. Reduced-fat formulations of traditional products are demanded, which, nevertheless, are required to possess textural and organoleptic properties as close as possible to those of the traditional material. This in itself provides a challenge to the emulsion technologist—to reproduce the properties while reducing the amount of "active" constituent. The demand for the incorporation of nutritionally beneficial lipid materials (phospholipids, specific fatty acids) also produces a challenge; the incorporation of these materials into foods, complete with antioxidants and other necessary ingredients, will require increased ingenuity on the part of the emulsion technologist. In addition, there is discussion of targeting the materials contained in a food. No longer is it sufficient simply to provide nutrition; ideally, it is necessary to define in which portion of the digestive tract the components of the food are to be liberated. Already there are encapsulated materials available whose coatings are designed for this purpose. With emulsions of specific oils being part of the "functional food" system, we may expect to see increased demand for emulsions which are controlled not only during manufacture but during consumption as well. This represents a real challenge for the emulsion technologist of the future.

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