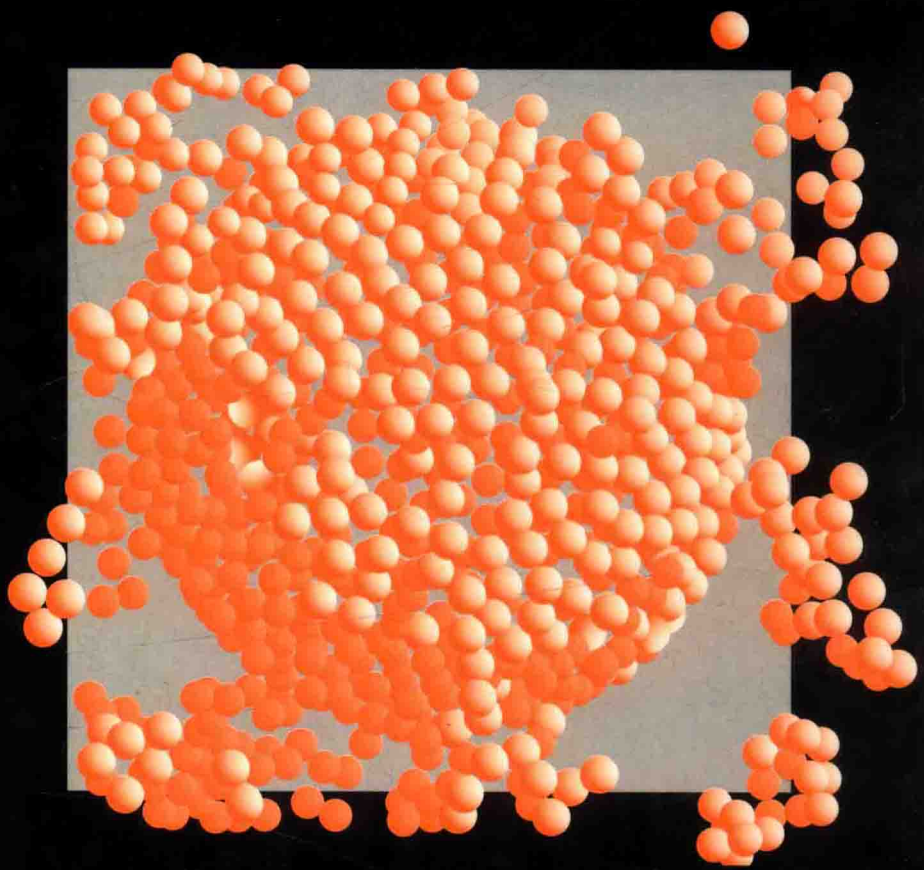
FOOD EMULSIONS AND FOAMS

Interfaces, Interactions and Stability



Edited by E.Dickinson
and J.M. Rodríguez Patino

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Edited by

E. Dickinson
Procter Department of Food Science, University of Leeds, UK

J. M. Rodríguez Patino
Universidad de Sevilla, Spain

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Preface

The underlying objective of this edited book is to record current progress in the development of fundamental understanding of the stability and rheological properties of food dispersions containing particles, droplets and bubbles. Examples of complex multiphase foods of this type are yoghurt, ice-cream, mayonnaise, *etc.* The properties of manufactured food emulsions and foams depend on the processing techniques used in their formulation and on the nature of the interactions involving the various constituent molecular ingredients—proteins, lipids and hydrocolloids. The structural and compositional complexity of food colloids generally necessitates a consideration of simpler model systems in order to elucidate the key mechanisms and principles contributing to texture, taste and shelf-life. Of particular importance are surface chemical properties of adsorbed protein layers and the nature and strength of the interactions between proteins and other molecular components, especially lipids and polysaccharides.

Every two years since 1986 an international conference in the area of food colloids has been held in Europe under the auspices of the Food Chemistry Group of the Royal Society of Chemistry (UK). The latest such conference, entitled 'Food Emulsions and Foams—Interfaces, Interactions and Stability', was held in Seville, Spain, on 16–18th March 1998. The three main themes of the Seville meeting were (i) dispersions, (ii) fluid–fluid interfaces, and (iii) rheology of food colloids. The programme included invited overview lectures, contributed oral presentations, and an exhibition of over 90 posters. The conference was attended by 190 participants from 19 different countries. Most of the invited lectures and contributed oral presentations are recorded in this volume. Research papers based on some of the poster presentations are being published separately in a special issue of the journal *Colloids and Surfaces B: Biointerfaces*.

The lecture programme of the conference, and hence the selection of contributions for this volume, was the responsibility of the International Organizing Committee comprising Dr Rod Bee (Unilever Research, Colworth Laboratory), Dr Björn Bergenståhl (Stockholm), Prof. Denis Lorient (ENSBANA, Dijon) and Prof. Pieter Walstra (Wageningen Agricultural University), as well as the editors of this volume. Detailed arrangements for

the conference were made by the members of the Local Organizing Committee: Prof. J. M. Rodríguez Patino (Chairman), Prof. J. de la Fuente Feria (Secretary), Prof. M. Ruíz Domínguez (Treasurer), Prof. M^{a}. R. Rodríguez Niño and C. Carrera Sánchez.

E. Dickinson (Leeds)
J. M. Rodríguez Patino (Seville)
May 1998

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Interfacial Structures and Colloidal Interactions in Protein-Stabilized Emulsions

By D. G. Dalgleish

CENTRE INTERNATIONAL DE RECHERCHE DANIEL CARASSO, 15 AVENUE GALILEE, 92350 LE PLESSIS-ROBINSON, FRANCE

1 Introduction

It is approximately 20 years since the publication of the important studies of Graham and Phillips,[1,2] which described for the first time many of the details of the behaviour of proteins adsorbed to oil–water and air–water planar interfaces. A few years later came the description by Oortwijn and Walstra[3] of the formation of emulsions from milk fat and the proteins of milk. Since that time there has been a considerable increase in our understanding of protein adsorption, and this paper will describe some of the developments during succeeding years, to demonstrate the progress which has been made, and perhaps also to suggest where additional research may be required.

Functionally, proteins are used in food emulsions because they confer stability. The fact that they are food emulsions means, of course, that the protein also provides nutritionally valuable material, as well as exercising its functional role in the formation and stabilization of the emulsion. At the same time, the fact that these proteins exercise their function (in real foods) in the presence of a number of other food components must also be taken into account, and it may be that the major challenge to the scientist of food emulsions today is to understand the behaviour, not just of simple systems which contain a protein and some lipid and an aqueous phase, but also the more complex mixtures which are found in real food preparations. Moreover, the manner in which these 'real' emulsions are processed affects the behaviour of the proteins in solution and on the interface, with consequent effects for the structure and stability of the product. For example, the properties of the emulsion in ice-cream has been extensively investigated and described,[4] but there are also products such as cream cheese, where milk-based emulsions are formed, heated, acidified and further processed, and yoghurts, in which similar processes occur. The additional complexity of the presence in these products of casein micelles, rather than simply monomeric proteins, adds factors to the texture, and, it must be admitted, a certain spice to the study of such complex systems.

We may conveniently divide the study of simple and complex food emulsions into a number of topics. First, we need to know what is on the interface, how it gets there, and what in its surroundings affects this interaction. Secondly, there is the need to know as exactly as possible the state of this adsorbed material; for proteins, this requires descriptions of their molecular conformations and how the adsorbed proteins interact. Accordingly, the discussion which follows will be divided into these two sections.

2 The Adsorption and Competition of Proteins at Oil–Water Interfaces

Exchange Reactions

It is evident that many proteins adsorb spontaneously to oil–water interfaces. However, the simple fact of adsorption conceals a number of important questions. Although it is easy to make an emulsion using oil and a single purified protein, it is evident even at the most simple level that different proteins have different emulsifying capacities (however one chooses to define this rather elusive quantity). That is, some proteins make finer or more stable emulsions than others. However, attempts to predict emulsifying capacities from some properties of the proteins—for example, from the surface hydrophobicity,[5] or by defining a scale based on the abilities of different proteins to displace one another from the interface[6]—seem to be doomed to failure. This is because, in the first case, the adsorption of a protein proceeds not simply via surface sites, and, in the second, free exchange between adsorbed and non-adsorbed proteins is rather difficult to achieve. Simply making an emulsion with one protein and adding a second protein afterwards does not ensure that the most surface-active protein actually ends up dominating the interface. With the two purified casein fractions, α_{s1} and β-caseins, there is indeed replacement of the former by the latter, suggesting that the β-casein is the more surface active of the two.[6] However, even for the native mixture of caseins (α_{s1}, α_{s2}, β, κ) found in sodium caseinate, the adsorption of the individual proteins is not as simple as the model systems suggest.[7]

It has never been unequivocally established whether an equilibrium exists between adsorbed and non-adsorbed protein in food emulsions, especially because the emulsion is formed under conditions which are far from equilibrium, using a homogenizer. In addition, as has been pointed out often, proteins adhere to the interface through many points of contact, and so the spontaneous desorption of the whole protein requires a number of events to happen simultaneously, which is unlikely. For caseins, and presumably for other proteins as well, the protein is used as efficiently as possible during emulsion formation; when emulsions are made using only small amounts of casein, the latter spreads across the surface of the emulsion droplets to cover as much of the surface as possible. Thus emulsions made with caseinate are stable[8] with a surface coverage as low as 1 mg m^{-2} although the surface coverage associated with a saturated monolayer is generally found to be *ca.* 3 mg m^{-2}. This

spreading occurs, but to a lesser extent, with the more rigid molecules of the whey proteins. Evidently, emulsions may be stable with less than maximal amounts of protein covering the surface, because it is possible for stable emulsions to exist with gaps between the adsorbed protein molecules (this would not be possible with any other type of surfactant, but the sheer size of the proteins and their capacity for steric stabilization may over-ride the undesirability of leaving gaps in the protein layer). This being the case, if an emulsion is prepared using less than saturating amounts of one type of protein, and then a second type of protein is subsequently added, it is possible to include a considerable quantity of this material in the interfacial layer without necessarily displacing any of the originally adsorbed protein.[8] In most of the experiments which have been described, there is little evidence that there is equilibrium between adsorbed and non-adsorbed protein; there may be changes in surface composition, but true equilibrium is a rare occurrence, if it occurs at all.

The exchange of protein between interface and bulk is often facilitated by the presence of a surfactant. The mechanism is the assisting of desorption by removing one by one the different points of contact of the protein with the interface, and replacing them with surfactant. This process is much more likely than the spontaneous simultaneous desorption of all of the points of contact of the protein. However, the surfactant must be chosen with some care, since not all surfactants behave in the same manner. For example, although water-soluble surfactants generally can remove proteins totally from the interface,[9] oil-soluble surfactants have often been found to remove only some of the protein,[10] presumably because the oil-soluble material is sufficiently hydrophobic that the protein may adsorb to it, even at the surfaces of the emulsion droplets. Even water-soluble surfactants may differ in their effects when different proteins are used; although we need to make allowances for the results from different laboratories, it appears more difficult for polyoxyethylene surfactants to displace whole casein than isolated β-casein.[9,11] This is similar to the competitive displacement of purified α_{s1}- and β-caseins, and suggests that the other caseins (α_{s2}- and κ-casein) may have an effect on the adsorption and structure of the adsorbed caseinate. The surfactants do not necessarily simply displace the protein; at low concentrations of surfactant, it is possible rather to loosen the protein on the interface, so that lateral diffusion may become easier.[12,13] Clear indications of this secondary effect of Tween-60 on caseins in adsorbed caseinate have been observed, and the suggestion may be either that the surfactant binds to the proteins, or that it allows the protein to take up a different conformation at the interface.[12]

In some cases, surfactants and protein do not compete at all. We have seen[14] that in oil-in-water emulsions made using caseinate and phospholipid as surfactants, caseinate is only slightly displaced by the phospholipid; indeed it may be considered uncertain whether the phospholipid adsorbs at all in some of these emulsions,[15] although it certainly enhances the emulsifying capacity of the caseins. It is possible that this latter effect arises from the formation of specific complexes between phospholipid and caseinate, either on or off the surfaces of the emulsion droplets; that such complexes are at least possible has been

demonstrated by measuring direct interactions between specific phospholipids and individual caseins.[16] This type of action is discussed in one of the other papers in this volume (Singh *et al.*), where it is shown* that the stabilizing action of peptides is enhanced by the presence of certain phospholipids, although there is no direct adsorption of the phospholipid to the interface, except when the emulsion is heated.

Effects of Heat on Surfactant/Protein Behaviour

The surface composition of emulsion droplets is defined not simply by the proteins which are present, but by other surfactant materials as well. However, it is also necessary to consider the effects of processing on these systems, since nearly all food preparations are processed, especially by subjecting them to heating of greater or lesser severity. With the exception of studies on homogenized milks,[17] few studies have been published on the effects of heat on the composition of the adsorbed layers of emulsion droplets. Some recent work in my own laboratory demonstrates that this may have some rather unexpected effects. It is well known[18] that heating milk causes the whey proteins α-lactalbumin and β-lactoglobulin to denature, and to interact, by the formation of disulfide bonds, with the κ-casein and possibly also the α_{s2}-casein of the micelle. By extension we would expect an emulsion droplet stabilized by whey proteins to interact with added caseins when the mixture was heated. This, however, has proved not to be the case;[19] the addition of sodium caseinate to the emulsion was found to cause no interaction at all, apart from the adsorption of some of the casein to saturate the interface, as described above. Surprisingly, there was no interaction between the sulfhydryl-containing caseins and the adsorbed whey proteins, either before or after heating. This seems to suggest that the adsorbed whey proteins were incapable of forming disulfide bonds with molecules from solution, although it is known that slow interactions between adsorbed whey proteins do occur via disulfide formation. The implication of these findings seems to be that the sulfhydryl groups of adsorbed β-lactoglobulin molecules are inaccessible to other proteins approaching the interface from solution.

On the other hand, the addition of whey protein to an emulsion initially made with caseinate has a considerable effect, in addition to the simple adsorption to ensure saturation of the interface.[19] During heating (85 °C, 2 minutes or more), β-lactoglobulin adsorbs to the oil droplets, and simultaneously some of the α_{s1}- and β-caseins are observed to desorb. Evidently the heating has the effect of facilitating the exchange of proteins. This is shown in detail in Figure 1, where the displacement of the α_{s1}- and β-caseins is balanced by the adsorption of β-lactoglobulin and α-lactalbumin. The greater the amount of added whey protein, the greater was found to be the observed effect. Originally, we believed that this effect resulted from the denaturation of the serum proteins, but subsequently we were able to demonstrate that the phenomenon occurred at

* See p. 117.

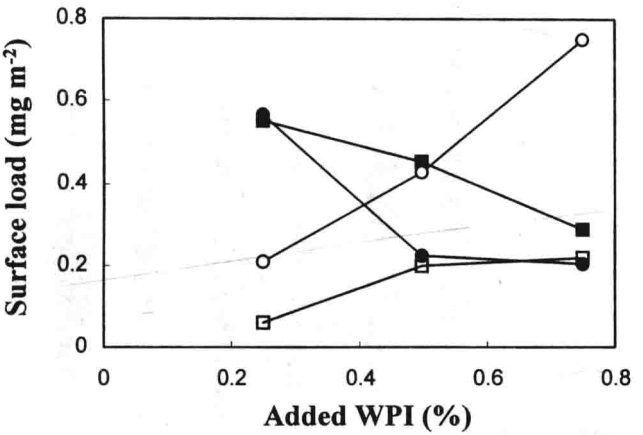

Figure 1 *Changes in the composition of adsorbed layers of protein in an emulsion (20% w/w soybean oil, 1% w/w sodium caseinate) during heating for 2 minutes at 85 °C in the presence of different amounts of added whey protein isolate (WPI), expressed as changes in the surface loads of the individual proteins:* ●, α_{s1}*-casein;* ■, *β-casein,* ○, *β-lactoglobulin;* □, *α-lactalbumin*

temperatures in the region of 40–50 °C, well below the normal temperature of denaturation of the whey proteins. This is illustrated in Figure 2, where the rates of adsorption of β-lactoglobulin and of desorption of α_{s1}-casein are shown for these moderate temperatures. Not only do the reactions occur at relatively low temperatures, they also do not depend on disulfide bond formation. A possible facilitating reaction may be the dissociation of the dimer of β-lactoglobulin, which takes place in this temperature range.[20] Therefore, it is not necessary for denaturation of the β-lactoglobulin to occur to cause the molecules be more surface-active, but just relatively small changes such as are defined by dissociation of multimers. This may also explain why earlier experiments at room temperature[21,22] failed to detect the phenomenon.

If we try to translate this observation into an industrial context, the implications may be that the surfaces of emulsions may not be at all stable during processing. Although an emulsion may be formed with one set of proteins on its interface, the influence of heating may be such as to change the proteins which adsorb, with some effects on either the stability or the more general properties of the emulsion. It may be partly for this reason that milks which are homogenized before heating have different behaviour from those homogenized after the milk has been forewarmed. The experiments described above were carried out on relatively simple emulsions made from caseinate; we do not know what is the effect if the casein is in micellar form—that is, if homogenized milk is heated in the presence of added whey proteins. Such evidence as exists[17] suggests that whey proteins in homogenized milk are less capable of displacing the casein micelles from the emulsion interface than are whey proteins in simple model emulsions.

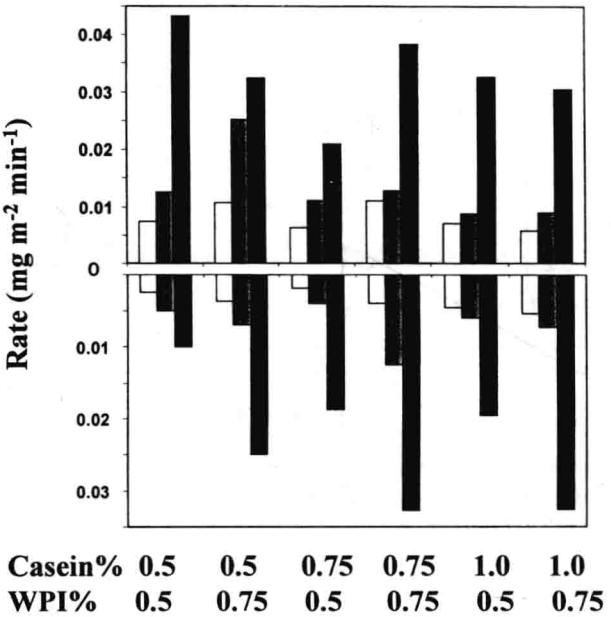

Figure 2 *Rates of the adsorption of β-lactoglobulin (upper plot) and of desorption of α_{s1}-casein (lower plot) during heating of emulsions (20% w/w soybean oil) originally made with sodium caseinate at different concentrations (0.5, 0.75, 1%), to which were added defined amounts of WPI (0.5, 0.75%). The rates are expressed in terms of the changes in surface loads of the different proteins during the heating. Open bars, heating at 40 °C; grey tone, heating at 45 °C; filled bars, heating at 50 °C*

Thus, heating may have the effect of creating or enhancing the exchange of proteins; another is simply to alter the emulsifying capacity of the proteins by denaturing them. Specifically, we may cite the case of the membrane fraction of buttermilk. By comparing the properties of membrane fractions isolated from buttermilk prepared from creams which had either been heat-treated or not, we have shown[23] that the heat-treated creams yield buttermilk membrane fractions in which the proteins are denatured, and which have low emulsifying capacity. Emulsions prepared using this material showed bridging flocculation, because the membrane material was present in the form of large particles, which evidently would not spread over the interface (Figure 3). On the other hand, the membrane fraction isolated from unheated buttermilk was much more functional in terms of its emulsifying capacity, and gave fat globules surrounded by thin membranes (Figure 4), so that evidently the native membrane structure is capable of being disrupted in the presence of lipid and to spread around the surfaces of the oil droplets.[24] Thus, although buttermilk is often considered to be an emulsifying agent, it is in general the casein micelles in it which provide the emulsifying capacity, rather than the membrane fraction, which denatures at a relatively low temperature (60–65 °C).

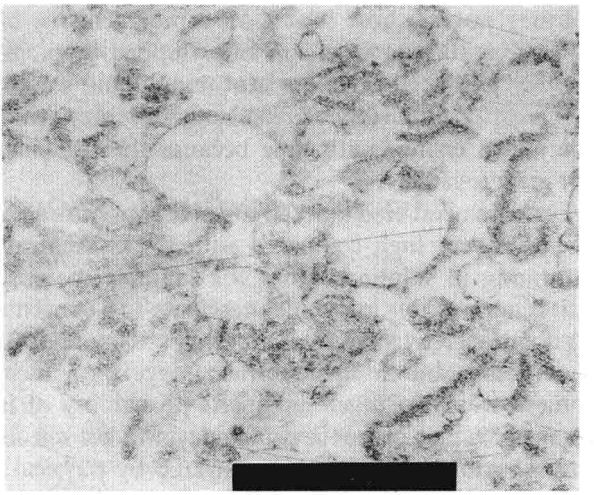

Figure 3 *Transmission electron micrograph of an emulsion (10% w/w soybean oil, 4% w/w milk fat globule membrane isolate). The membrane material was isolated from a commercial buttermilk derived from cream which had been pre-treated at a temperature of 85 °C. Scale bar = 1.1 μm*

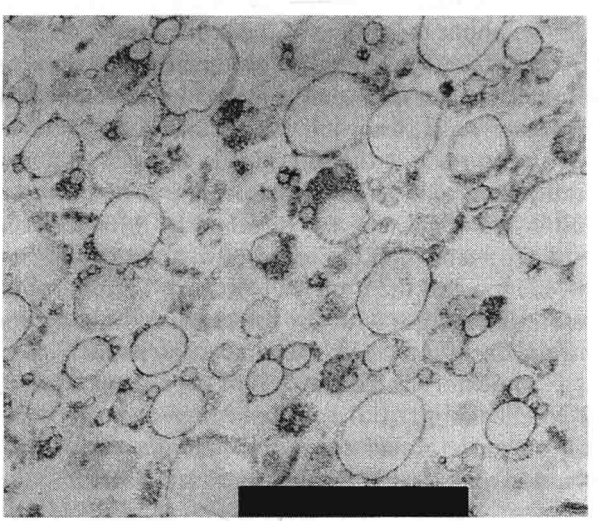

Figure 4 *Transmission electron micrograph of an emulsion (5% w/w soybean oil, 3% w/w milk fat globule membrane isolate). The membrane material was isolated from buttermilk derived from cream which had received no prior heat treatment. Scale bar = 0.7 μm*

Exchange and Changes During Processing

If, therefore, we consider the behaviour of emulsions during processing, we will find that the final composition, and therefore the structure and reactivities, of

the oil droplets may not be those which we expect. This is, of course, well understood in products such as ice-cream and whipped toppings, but it is less well defined in other products which are heat-treated and stored for extended periods of time thereafter. Quite apart from changes in composition, these types of emulsions may also change with time because the proteins change their conformation or react together.

It should always be remembered that the effective concentration of proteins in the adsorbed layer is very high compared with the concentrations typically regarded as saturating in solution. Thus, for example, because of viscosity considerations, it is not possible to dissolve caseinate to a concentration of, say, 25%; however, a surface coverage of 2.5 mg m^{-2} is more than sufficient to create such a concentration in the interfacial layer. At such extremes of concentration, there will be a much enhanced probability of intermolecular chemical reaction, which may cause the protein layer to become more rigid with time. While this behaviour has been demonstrated by physical and chemical means to occur in β-lactoglobulin,[25,26] it is also apparently possible even in such unstructured proteins as the caseins. Different experiments have shown that the interfacial viscosity of adsorbed α_{s1}-casein increases slowly with the age of the interface;[25] that β-casein becomes less susceptible to displacement by surfactant from an emulsion interface;[27] and that the calcium-sensitivity of caseinate-stabilized emulsions decreases with the age of the emulsion.[28] However, not enough is yet known about the causes of these different changes. Since neither α_{s1}-casein nor β-casein possess sulfhydryl groups, there is no possibility of the formation of disulfide bridges between the molecules, as happens with adsorbed β-lactoglobulin. However, it is not impossible that simply the very high local concentration of the protein at the interface is a factor, allowing some slow structural rearrangement and perhaps the formation of salt bridges to occur, especially if multilayers of protein are adsorbed to the interface. Certainly in emulsions it is quite possible that the speed of the non-equilibrium adsorption process may enforce some of the proteins to adsorb to non-optimal conformations for maximum interaction with the interface. A slow conformational re-equilibration is quite likely as a result. More recently (paper by Leaver *et al.*, this volume*) it has been demonstrated that a time dependent effect may also arise from chemical modification of the adsorbed protein by enals derived by peroxidation of polyunsaturated fatty acids in the oil phase. Clearly this covalent modification may have both functional and nutritional significance.

In all of these adsorption processes there is also an effect of the non-aqueous phase. The proteins adsorb slightly differently to interfaces of triglyceride oil or hydrocarbons.[29] Presumably, the hydrocarbons, being more hydrophobic than triglycerides, force stronger interactions with the hydrophobic portions of the proteins. However, there is another factor which makes the two non-aqueous phases difficult to compare, namely that they have different viscosities, and therefore produce emulsions with different particle sizes for the same homogenization pressure and the same concentrations of protein and non-aqueous

* See p. 258.