The Emulsifying Properties of Commercial Milk Protein Products in Simple Oil-in-Water Emulsions and in a Model Food System

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ABSTRACT: The emulsifying properties of six commercial milk protein products were studied. The products were separated into one of two groups depending on whether they contained aggregated (micellar) casein or disordered protein (casein or whey protein). Disordered proteins had a greater emulsifying ability than aggregated proteins. Dispersion of aggregated protein in dissociating buffer improved the emulsifying ability. Comparison of emulsion properties in simple oil-in-water emulsions with those in a model coffee whitener formulation showed that the lower emulsifying ability of aggregated protein could be partially compensated by other ingredients.

Key Words: emulsion, milk protein, casein, whey protein, coffee whitener

Introduction

PROTEINS THAT ARE GOOD AT FORMING emulsions do not necessarily confer long-term coalescence stability on the oil drops. The ability of a protein to form and stabilize an emulsion droplet is related to its ability to adsorb and unfold rapidly at the nascent oil-water interface (that is, its surface activity). Protein molecules that are good at forming emulsions and giving short-term stability within the homogenizer are the flexible milk caseins and the globular whey proteins. Milk proteins in this form are found in products such as sodium caseinate, total milk proteinate and whey protein concentrate. More structured milk protein aggregates, such as the micellar casein aggregates found typically in milk protein concentrates, skim milk powders, and buttermilk powder, are poor at forming emulsions because they are inflexible (the structure is held together by calcium bridges) and cannot unfold rapidly at the oil-water interface. Dalgleish (1996) has also attributed the poor emulsifying ability of micellar caseins to a concentration effect. When large aggregates of protein are present, the effective number of protein "particles" is lower than for free dissociated caseins. This leads to less casein being available for adsorption. It is also likely that protein aggregates have an increased proportion of hydrophobic groups at the core of the aggregate, and the surface of the aggregate is less hydrophobic. This would lead to a reduced tendency for adsorption.

The stability of an oil-in-water emulsion is controlled by many factors. If we concentrate on those factors contributed by the stabilizing protein molecule, we can identify three important parameters: the surface coverage, the adsorbed layer dimensions, and the surface viscosity. More aggregated proteins, in general, give emulsions with a higher surface coverage, a higher surface viscosity and greater adsorbed layer dimensions, and so might be expected to have a greater stabilizing effect on the emulsion droplets. Mulvihill and Murphy (1991) have found that this is indeed the case with caseins, where calcium caseinate and micellar casein give more stable emulsions than sodium caseinate, but are not as good at forming emulsions. Leman and others (1988) have also shown that at neutral pH, the creaming stability of milk protein-stabilized emulsions decreases in the order micellar casein > skim milk > whey protein.

In reality, milk proteins are not found in isolation in food emulsions, but in combination with a range of other functional ingredients. The interactions between these other ingredients and the milk protein emulsifiers are important in determining the structure and stability of the final product. Of the ingredients other than proteins that are found in food emulsions, low-molecular-weight emulsifiers and polysaccharides are the most widely studied. It is well known that low-molecular-weight surfactants compete for interfacial area with proteins in emulsions and displace them from the surface. Work on model systems using pure individual milk proteins (Dickinson and Woskett 1989, Dickinson and Tanai 1992) and commercial milk protein products (Euston and others 1995, 1996) has shown that water-soluble surfactants are better than oil-soluble surfactants at displacing protein from the oil-water interface, and that the mechanism and degree of displacement depends on the protein type (globular or disordered). Other researchers have shown that some surfactants can form interfacial complexes with milk proteins without necessarily displacing them (Doxastakis and Sherman 1984). At concentrations of surfactant lower than are needed to cause protein displacement, the low-molecular-weight emulsifiers are able to disrupt interactions within the adsorbed protein layer (Dickinson and others 1990).

Polysaccharide stabilizers are also known to interact with adsorbed protein molecules (Dickinson and Euston 1991, Dickinson and McClements 1995). However, because they are not surface-active, this interaction may lead to a cooperative adsorption. The most common type of interaction is between oppositely charged groups on the proteins and polysaccharides. This can lead to enhanced stability of the emulsion to coalescence (Dickinson and Euston 1991). Alternatively, several protein-polysaccharide mixtures exhibit thermodynamic incompatibility (Dickinson and McClements 1995). This is a situation in which two molecules exhibit an unfavorable enthalpy of mixing. Under these conditions a protein-stabilized emulsion will spontaneously flocculate (phase separate) to minimize unfavorable protein-polysaccharide interactions.

Other ingredients will also influence the surface chemistry of milk proteins. Simple sugars and salts (phosphates, halide anions, and so on) will alter the way in which proteins interact with the oil-water interface. Many of these molecules have an influence through their effect on the

structure of water, that is, they are chaotropes or kosmotropes (Damodaran and Kinsella 1982). Sugars in solution increase the ordered structure in bulk water (by cooperative hydrogen bonding) which results in an increased tendency for protein hydrophobic aggregation. Early studies have shown an increased protein surface activity in the presence of sugars (MacRitchie and Alexander 1961a,b) and have demonstrated an increased protein surface coverage at the oil-water interface in sugar containing systems. Salts, such as phosphate (PO_4^{3-}) have a similar effect to sugars, while other salts such as the chloride anion (Cl^-) have the opposite effect, that is, they favor protein unfolding (weaker hydrophobic interaction) and lead to reduced surface coverages (Damodaran 1990). We can see that, far from being a simple case of protein adsorbing at the oil-water interface, food emulsions are stabilized by a range of competitive and cooperative interactions between food ingredients. A systematic study of these ingredient interactions in real food emulsions is clearly required.

It is for this reason that we choose to compare the emulsifying properties of milk protein products both in simple oil-in-buffer systems and in a model coffee whitener formulation. Our aim was to ascertain how good a model the simple buffered protein/oil-in-water emulsion is for a real food system.

Materials & Methods

Materials

Sodium caseinate (SCN), milk protein concentrate (MPC), micellar casein (MCN), low-heat skim milk powder (SMP), whey protein concentrate (WPC,) and total milk proteinate (TMP™) were obtained from the New Zealand Dairy Board, Wellington, New Zealand. Imidazole (ultrapure), di-sodium ethylenediaminetetraacetic acid (EDTA), glycerol monostearate (GMS) (technical grade), and K_2HPO_4 (Analar grade) were purchased from BDH Chemical Co., Poole, U.K. Maltrin M-100 (maltodextrin) was obtained from New Zealand Starch, Auckland New Zealand, carrageenan (commercial grade) from Sigma Chemical Co., St. Louis, Mo., U.S.A., Panodan (diacetyl tartaric acid esters of monoglycerides) from Danisco, Brabrand, Denmark, and soya (soybean) oil from Andrews Foods Ltd., Auckland, New Zealand.

Preparation of soya-oil-in-buffer emulsions

Six protein products were chosen for study in imidazole buffer: MPC, MCN, SMP, SCN, WPC and TMP™. A known mass of protein product was dissolved in 100 g of imidazole buffer (0.05 M, pH 7.0) at 55 °C in a shaking water bath. The weight of protein used was sufficient to give a protein solution of approximately 0.5 wt% (0.4 wt% in the final emulsion). The solutions were held at 55 °C for 2.5 h to allow complete solubilization of the protein. At the same time, a portion of soya oil was heated to 55 °C. Homogenization was carried out using a Jet homogenizer (Labplant Ltd., Huddersfield, U.K.) at a pressure of 200 bar. Three replicate emulsions were made for each protein product.

Preparation of soya-oil-in-dissociating-buffer emulsions

Emulsions were made using the same procedure as for emulsions with imidazole buffer as the aqueous phase, except that the buffer system used was imidazole (0.05 M)/EDTA (0.05 M), pH 7.0. The same six protein products were used as for emulsions made with imidazole buffer.

Preparation of model coffee whitener formulations

The model coffee whitener formulation used in this work is listed in Table 1; it was based on a formulation given by Si (1991). Three replicate emulsions were made for each protein product. The same six protein products as were used for emulsions with an imidazole buffer were used in the model coffee whitener formulation.

One hundred g of each emulsion were made up according to the formulation in Table 1. All ingredients except the oil were weighed into a screw-cap glass bottle, and the formulation was left to dissolve at 55 °C in a shaking water bath for 2.5 h. Homogenization was carried out with a Jet homogenizer (Labplant Ltd., Huddersfield, U.K.) at a pressure of 200 bar.

Measurement of particle size and specific surface area

The particle size distribution of each emulsion was measured using a Malvern Mastersizer E, (Malvern Instruments, Malvern, U.K.) with presentation code 2NAD as defined by the Malvern software. This presentation code assumes the following optical parameters: a refractive index for the dispersed oil phase of 1.456, a particle absorbance of 0.00, and a continuous phase refractive index of 1.33.

Measurement of emulsion stability

The accelerated stability of the emulsions was measured using the emulsion volume index method devised by McDermott and others 1981. This is a centrifugal stability test. It separates the emulsion into two phases: a "cream" phase containing the emulsion droplets, and an aqueous phase. The stability of the emulsion, that is, the emulsion rating or ER (%) is defined as the ratio of the volume of the cream phase to the theoretical volume of a totally stable emulsion.

Table 1 – Formulation for liquid coffee whitener emulsion (based on Si 1991)

Ingredient	Composition (wt %)
Soya oil	20.0
Milk protein	0.4
Maltodextrin (Maltrin M-100)	10.0
Glycerol monostearate (GMS)	0.2
Tartaric acid esters of monoglycerides (Panodan A-100)	0.2
Carrageenan	0.05
K_2HPO_4	0.2
Water	to 100%

Determination of surface protein coverage

The surface coverage of protein on the emulsion droplets was determined by the method developed by Hunt and Dalgleish (1994) with minor modifications. This procedure involves centrifugal separation of the emulsion, and direct analysis of the cream layer by sodium dodecyl sulfate polyacrylamide gel electrophoresis and laser densitometry (Molecular Dynamics Personal Densitometer, Sunnyvale, Calif. U.S.A.). The method has been detailed elsewhere (Euston and others 1999.)

Results

Emulsifying properties in imidazole buffer

Fig. 1 presents data for the mean particle size ($d_{3,2}$) of emulsion droplets made with the 6 milk protein products in an imidazole buffer system. The obvious feature

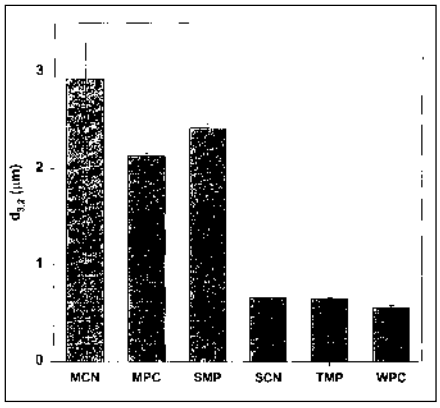

Fig. 1—Mean particle size for soya oil-imidazole buffer emulsions made with commercial milk protein products. Error bars are expressed as plus or minus one standard deviation.

of this graph is that the protein products fall into 2 groups. One group with relatively large $d_{3,2}$ contains the products MPC, MCN, SMP, that is, those products with aggregated (micellar) or partially aggregated casein. The second group contains the products SCN, WPC and TMP™, that is, the disordered caseins and the ordered, globular, but nonaggregated, whey proteins. It is clear that aggregation of the caseins severely affects their emulsifying properties.

When the stability of the emulsions is considered (Fig. 2), the differences between the two protein groups identified in Fig. 1 are markedly less, although all proteins that fall into the group with high $d_{3,2}$ have a lower ER than those that form emulsions of a smaller particle size.

Fig. 3 presents data for the surface coverage of protein at the emulsion droplet interface. Again it is possible to differentiate the 2 groups of milk proteins on the basis of the amount of protein at the surface of the oil droplets. The aggregated caseins have a high surface coverage, whereas the second group is characterized by a lower surface coverage.

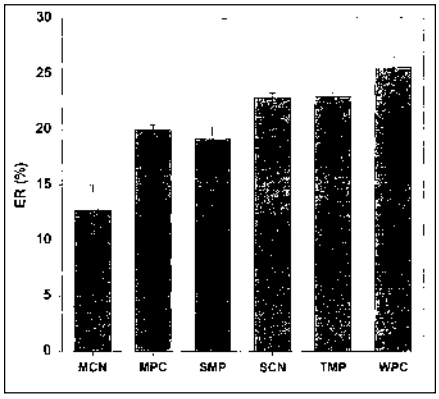

Fig. 2—Creaming stability, expressed as the emulsion rating (ER) of soya oil-imidazole buffer emulsions made with commercial milk protein products. Error bars are expressed as plus or minus one standard deviation.

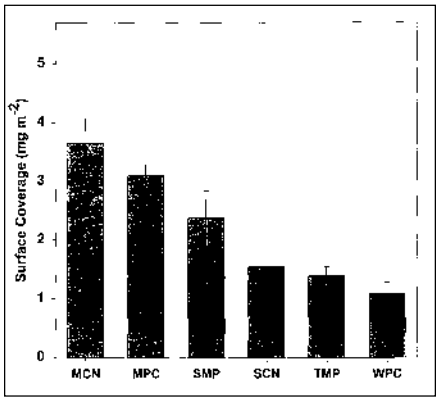

Fig. 3—Protein surface coverage of soya oil-imidazole buffer emulsion droplets made with commercial milk protein products. Error bars are expressed as plus or minus one standard deviation.

Emulsifying properties in a dissociating buffer

Fig. 4 is a comparison of the $d_{3,2}$ values of the 6 milk protein products in both imidazole buffer and imidazole/EDTA buffer. The emulsifying ability of the aggregated proteins is improved considerably in the dissociating buffer, with the $d_{3,2}$ decreasing from 2-3 m to about 0.6 m. Interestingly, the effect on the dissociated caseins in TMP™ is the opposite, with a slight increase in $d_{3,2}$. For SCN Fig. 4 shows no significant difference for $d_{3,2}$ in imidazole and imidazole/EDTA buffers. This probably indicates that the dissociating buffer environment is less favorable for proteins (a poorer solvent) than imidazole buffer alone and reduces the ability of the protein to spread at the oil-water interface. The effect of the dissociating buffer on WPC is even more marked, with a large increase in $d_{3,2}$ from 0.56 to 0.87 m.

A similar observation is made for the emulsion stability measured as the ER (Fig. 5). Dissolving the aggregated caseins in a dissociating buffer leads to an increased emulsion stability, whereas the emulsion stability of the nonaggregated proteins is decreased.

The opposite effect is observed for the surface coverage of emulsion droplets (Fig. 6) made up in the dissociating buffer. The aggregated proteins show a considerable reduction in protein surface coverage, down to values typical of protein monolayers. In contrast, the nonaggregated proteins show a slight increase, although the values found would still suggest monolayer coverages and are comparable to the surface coverages for the aggregated protein products. The exception is, again, WPC, which shows a large increase in surface coverage in the dissociating buffer. This is possibly indicative of protein aggregation. It would also explain the reduction in emulsifying ability when the whey protein is dissolved in the dissociating buffer, as we have already seen that the aggregated proteins have a lower emulsifying ability. We do not know why EDTA should have this large effect on whey proteins. It is possible that EDTA is a kosmotrope, that is, it alters the water structure in such a way as to promote hydrophobic interactions and aggregation. This would affect the emulsifying proper-

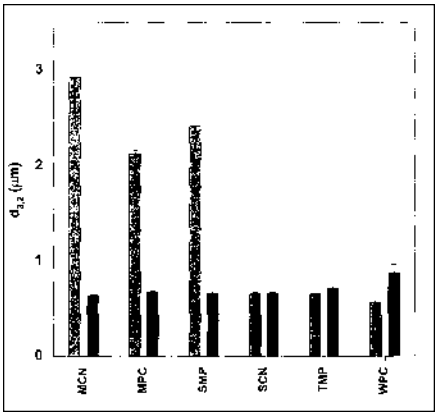

Fig. 4—Mean particle size for soya oil-imidazole/EDTA buffer emulsions made with commercial milk protein products. The left-hand bar (light shading) represents the imidazole buffer emulsions; the right-hand bar (darker shading) represents the imidazole/EDTA buffer emulsions. Error bars are expressed as plus or minus one standard deviation.

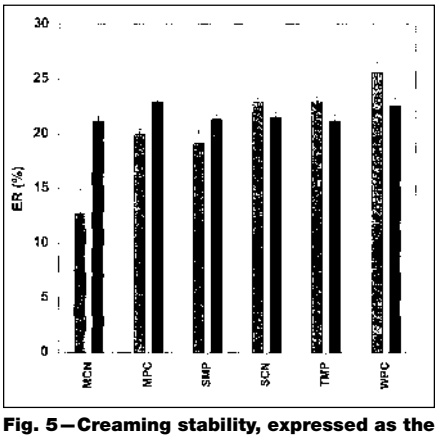

Fig. 5—Creaming stability, expressed as the emulsion rating (ER) of soya oil-imidazole/EDTA buffer emulsions made with commercial milk protein products. The left-hand bar (light shading) represents the imidazole buffer emulsion; the right-hand bar (darker shading) represents the imidazole/EDTA buffer emulsion. Error bars are expressed as plus or minus one standard deviation.

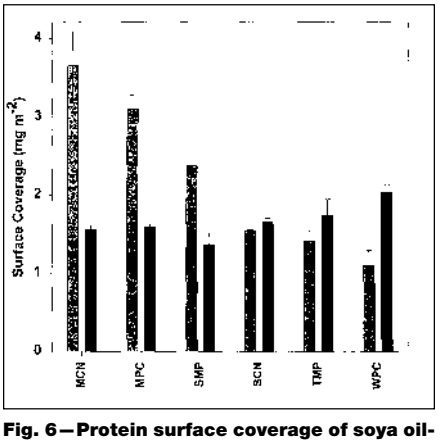

Fig. 6—Protein surface coverage of soya oil-imidazole/EDTA buffer emulsion droplets made with commercial milk protein products. The left-hand bar (light shading) represents the imidazole buffer emulsion; the right-hand bar (darker shading) represents the imidazole/EDTA buffer emulsion. Error bars are expressed as plus or minus one standard deviation.

ties.

It is clear from this study that a lower emulsifying ability in the aggregated micellar proteins is strongly linked to the structure of the constituent molecules. Breaking this structure restores the emulsifying ability. The opposite effect appears to occur when whey protein is dissolved in the dissociating buffer. With this protein, the dissociating environment may promote protein aggregation that lowers emulsifying ability. The mechanism for this is not calcium chelation, but it could be an increase in hydrophobic interaction that promotes denaturation and aggregation. It is interesting that the same buffer system can have the opposite effect in the two different protein systems.

Emulsifying properties in a model coffee whitener formulation

Fig. 7 presents a comparison of the $d_{3,2}$ values of the 6 milk protein products in a coffee whitener formulation and an imidazole buffer system. The $d_{3,2}$ is lower in the coffee whitener formulation than in the simple buffer system for all protein products.

A similar situation is observed in Fig. 8, which shows the emulsion stability (ER). For all products, with the exception of WPC, the emulsion stability is greater in the coffee whitener formulation.

When the surface coverages of the emulsion droplets are compared (Fig. 9), an interesting situation is observed. In the coffee whitener formulation there is a lower coverage of protein at the surface for the nonaggregated protein products, whereas the surface coverage is increased for the aggregated protein products.

Discussion

In this work we used the $d_{3,2}$ as a measure of how efficient a particular protein is at forming an emulsion. To form an oil droplet from a bulk oil phase requires the input of energy. This energy requirement arises from the tendency for demixing of oil and water, a phenomenon that is driven, entropically, by unfavorable changes in the structure of water in the presence of included hydrophobic droplets. To overcome this immiscibility of oil and water, energy must be put into the system to create the nascent oil-water interface (the smaller the droplet, the more surface area is formed and thus the higher is the energy input). Then the oil-water interface has to be stabilized against subsequent recoalescence. This is achieved by using surface-active molecules, such as milk proteins, which adsorb at the surface and stabilize the droplets against coalescence. We can distinguish between two types of emulsion droplet stability, which depend on different characteristics of the protein molecules. These are the initial stabilization of droplets in the homogenizer, which determines the initial droplet size distribution, and the long-term stability of the droplets to coalescence, flocculation, and creaming.

The initial stability of the oil-water interface, and thus the oil-droplet size distribution, depends on 2 apparently competing mechanisms. In the homogenizer, the ability of a protein to come into contact with the oil droplet and to adsorb at the surface increases as its size increases (Walstra and Oortwijn 1992). However, the ability of a protein to spread at the oil-water interface is hindered by increases in the size, structure, and aggregation state. So the relatively small, disordered SCN, TMP™, and WPC are able to spread rapidly at the oil droplet surface to stabilize the droplets when they are small. The larger, more aggregated, MCN, MPC, and SMP do not spread as easily, because their structures are held together more tightly by calcium bridges and hydrophobic/noncovalent interactions. This leads to significant recoalescence of the droplets in the homogenizer before they are stabilized by the adsorbing protein. This explains the large differences in the $d_{3,2}$ values for the 2 groups of protein product types seen in Fig. 1. When the structure of the protein products containing aggregated caseins is disrupted by the presence of a calcium chelator such as EDTA, the surface-active species in the emulsion have a structure that is more random and much closer to that found in the SCN emulsions. The result is that, when aggregated protein products are dissociated, their emulsifying properties improve and they are virtually indistinguishable from disordered SCN.

For the model coffee whitener formulations, there is an improvement in the emulsifying ability of the aggregated protein products, but for a different reason. In coffee whiteners, the protein is not the only surface-active molecule. Low-molecular-weight emulsifiers (GMS and tartaric acid esters of GMS) are also present. These will also have an effect on the particle size. Low-molecular-weight emulsifiers are able to stabilize small emulsion droplets because they can spread very rapidly at the oil-water interface. For this reason they are often used as a functional ingredient in

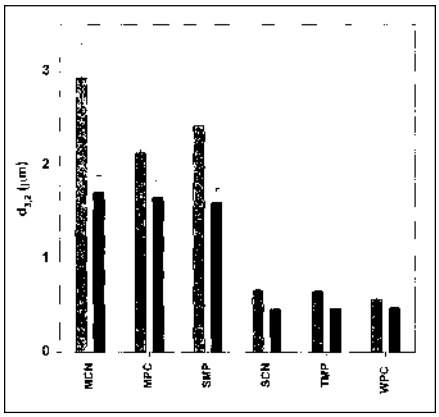

Fig. 7—Mean particle size for coffee whitener formulation emulsions made with commercial milk protein products. The left-hand bar (light shading) represents the imidazole buffer emulsions; the right-hand bar (darker shading) represents the coffee whitener emulsions. Error bars are expressed as plus or minus one standard deviation.

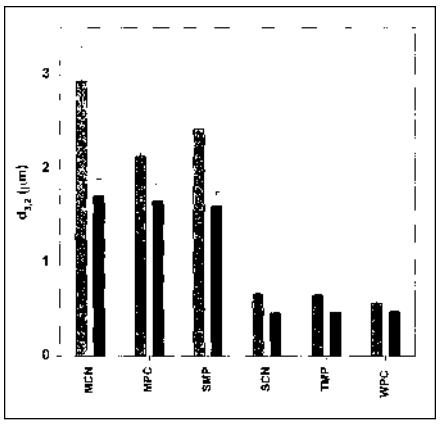

Fig. 8—Creaming stability, expressed as the emulsion rating (ER) of coffee whitener formulation emulsions made with commercial milk protein products. The left-hand bar (light shading) represents the imidazole buffer emulsion; the right-hand bar (darker shading) the coffee whitener emulsion. Error bars are expressed as plus or minus one standard deviation.

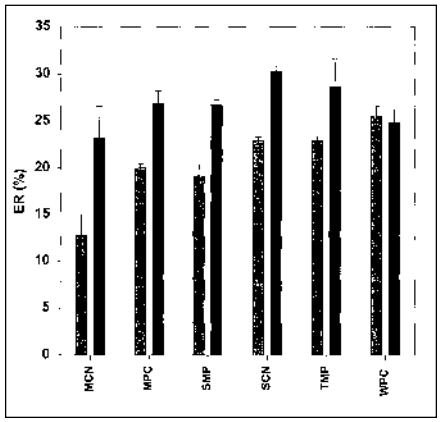

Fig. 9—Protein surface coverage of coffee whitener formulation emulsion droplets made with commercial milk protein products. The left-hand bar (light shading) represents the imidazole buffer emulsions; the right-hand bar (darker shading) represents the coffee whitener emulsion. Error bars are expressed as plus or minus one standard deviation.

dairy emulsion formulations (Euston 1997).

The second form of emulsion stability, the long-term stability under quiescent conditions outside of the homogenizer, also has a dependence on the aggregation state of the protein. This form of emulsion stability can be divided into 3 types: coalescence, flocculation, and creaming. In this study we looked at the creaming stability of the emulsions, as it is a phenomenon that is a common problem in fluid milk products (Walstra and Jenness 1984).

There are 3 ways in which proteins can contribute to the creaming stability of an emulsion droplet. The extent of creaming is highly dependent on the emulsion droplet size, the continuous phase viscosity and the density difference between the oil droplet and the continuous phase. At the protein concentrations used (0.4 wt%), the differences between the viscosities of solutions of the aggregated and nonaggregated proteins are likely to be small, and will not play a large role in determining the creaming stability. However, particle size and droplet density is likely to be more important.

If we look at Fig. 2, which presents data for the ER, we see that emulsions made with aggregated proteins (which have a larger particle size) are less stable to creaming than those made with disordered proteins. When we consider the dependence of the creaming stability (ER) on particle size (Fig. 10), we can see obvious differences between the aggregated and nonaggregated protein products. The creaming stabilities of emulsion droplets containing nonaggregated protein fall on a straight line. Those containing nonaggregated protein in imidazole and EDTA buffers, and containing aggregated protein after dissociation in EDTA buffer, form a close cluster in Fig. 10. In a coffee whitener formulation, the SCN, WPC and TMP™ emulsion droplets are more stable but this is associated with a smaller $d_{3,2}$. A separate grouping of products is formed by the aggregated proteins in imidazole buffer and in a coffee whitener formulation. These again form a group with a linear relationship between particle size and ER. This second grouping has a higher creaming stability for a particular particle size than the nonaggregated proteins. It is clear that a second factor other than particle size must control the stability.

The surface coverage of protein around the emulsion droplets shows a distinct variation between the aggregated and nonaggregated proteins. The more aggregated the protein, the higher is the surface coverage. However, it is likely that the majority of this protein is not physically adsorbed to the oil-water interface, but is cross-linked to other proteins that are adsorbed. This would probably result in a thick interfacial adsorbed layer containing partially spread-out casein micelles and micellar fragments, as opposed to a thin adsorbed layer composed of spread-out individual milk proteins, which would be found for the nonaggregated proteins (Dickinson and others 1993a, Fang and Dalgleish 1993). The structure and thickness of the adsorbed layer would be expected to contribute to the emulsion stability. This is because, on a weight per unit surface area basis, there is more protein at the oil droplet surface in an emulsion containing aggregated caseins. Therefore, it is reasonable to suggest that these emulsion droplets have a greater effective density than those stabilized by the nonaggregated caseins. In a recent study the protein concentration-dependence of the emulsifying properties of SCN, MPC, SMP, and WPC has been determined in imidazole buffer/soybean oil/protein emulsion (Euston and Hirst 1999). We calculated the contribution that an adsorbed protein layer would make to the creaming stability using Stokes equation by assuming that the emulsion droplet is a sphere of oil surrounded by a shell of protein. The calculation required a number of assumptions such as the thickness of the adsorbed protein layer, the protein solution density as a function of concentration, and the viscosity of the protein solution as a function of concentration. It was observed that only for aggregated protein products did the adsorbed layer make a large enough contribution to the density to increase the creaming stability of the emulsion droplets, and this only became significant at protein concentrations of 2.0 wt% and above. In this study we have used a protein content of only 0.4 wt%.

Comparing the ER as a function of surface coverage for the 6 protein products in different formulations (Fig. 11), differences are apparent. There is a linear dependence of the creaming stability on surface coverage for all systems, apart from the aggregated proteins in a coffee whitener formulation. These have a greater stability at the same particle size than the same proteins in imidazole buffer alone. Emulsions made with aggregated protein in imidazole buffer are less stable to creaming than those made with disordered protein, but still appear to be part of the linear relationship between ER and surface coverage for this group. If the aggregated protein contributed to a higher density of the emulsion droplets, we would have expected imidazole buffer-soybean oil emulsions to be more stable than they are. Thus the higher ER as a function of $d_{3,2}$ observed for the aggregated protein in imidazole buffer cannot be explained by the increase in the surface coverage of protein. Given that the protein content of the emulsions is only 0.4 wt% it is not surprising that the contribution of the adsorbed layer to droplet density and creaming is not significant in our present study. The most likely explanation for the increased creaming stability of emulsions made with aggregated protein in a coffee whitener

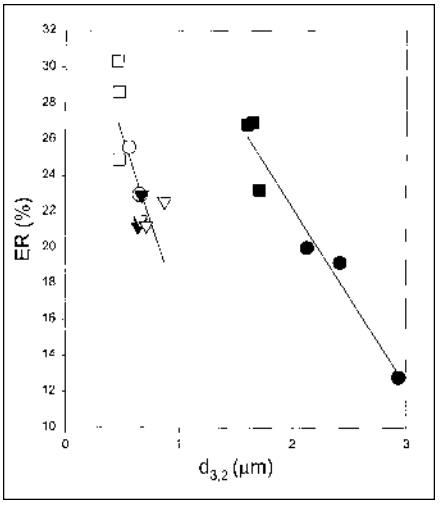

Fig. 10—Dependence of the creaming stability (ER) on the mean particle size ($d_{3,2}$). ●, aggregated proteins in imidazole buffer; ○, disordered proteins in imidzole buffer; ▼, aggregated proteins in EDTA buffer; ▽, disordered proteins in EDTA buffer; ■, aggregated proteins in coffee whitener formulation; □, disordered proteins in coffee whitener formulation

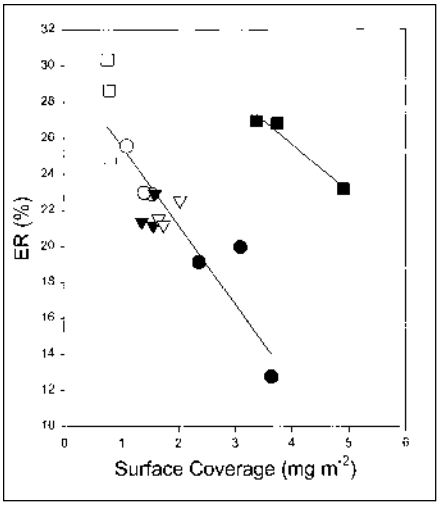

Fig. 11—Dependence of the creaming stability (ER) on the protein surface coverage. ●, aggregated proteins in imidazole buffer; ○, disordered proteins in imidzole buffer; ▼, aggregated proteins in EDTA buffer; ▽, disordered proteins in EDTA buffer; ■, aggregated proteins in coffee whitener formulation; □, disordered proteins in coffee whitener formulation

formulation is a synergistic interaction between the protein and other ingredients. The interaction between micellar casein and carrageenan is well documented (Samant and others 1993), and is known to lead to a synergistic increase in solution viscosity. The interaction is via the κ-casein at the surface of the micelle (Ozawa and others 1984, Dalgleish and Morris 1988, Samant and others 1993). The effect on viscosity is less for products containing disordered casein, since the κ-casein is free in solution and not attached to the other caseins in the micelle. No interaction between carrageenan and whey protein has been reported. The interaction between casein micelles and carrageenan could explain the increased creaming stability of aggregated proteins in the coffee whitener formulation.

Recently, Dickinson and others (1997) have shown that emulsions stabilized with sodium caseinate can exhibit depletion flocculation. This has been attributed to the formation of micelles of nonadsorbed κ-casein in the aqueous phase. The interaction of these micelles with the emulsion droplets gives rise to the depletion flocculation. In a recent study (Euston and Hirst 1999), we have shown that this form of depletion flocculation does not appear to be important in emulsion systems where the protein is highly aggregated, such as in MPC and SMP, or for whey protein emulsions.

Several reviews have claimed to explain the function of different ingredients in coffee whitener (Knightly 1969, Si 1991). The protein emulsifier is assigned the function of aiding in the formation of fat droplets and of providing long-term stability. Similarly, low-molecular-weight emulsifiers are believed to aid in the formation of fine emulsion droplets through their ability to lower the interfacial tension more rapidly than a protein. The finer emulsion droplets give a greater whitening power to the coffee whitener. This is related to the larger interfacial area from which light can be scattered (Leo and Betscher 1971, Si 1991). It is evident from Fig. 7 that there is a component in the coffee whitener formulation that helps in the formation of smaller emulsion droplets. This is likely to be the role of the low-molecular-weight emulsifiers. Being more surface-active than the proteins, these molecules are able to lower the interfacial tension rapidly, and they can give short-term stability to the small emulsion droplets formed in the homogenizer. Longer-term stability is provided by the more viscous cohesive surface films provided by the protein. Studies (Dickinson and Tanai 1992) have shown that the emulsion droplet size is reduced when mixtures of proteins and GMS are used as the emulsifiers.

The increase in emulsion stability observed for the coffee whitener formulation is probably a consequence both of the reduced particle size and of the modifications to dispersion rheology resulting from the addition of the carrageenan. Polysaccharides are traditionally added to increase the viscosity of the continuous phase. This has the effect of slowing creaming in the emulsion. Carrageenan is also known to have a strong interaction with caseins, particularly κ-casein (Ozawa and others 1984, Dalgleish and Morris 1988, Samant and others 1993). This may also have an effect on the stability of the emulsion droplets if the carrageenan interacts with adsorbed casein. Protein-polysaccharide interactions are known to lead to increased emulsion stability (Dickinson and Euston 1991, Samant and others 1993). It is conceivable that this could explain the lack of increase in emulsion stability when WPC is used as the protein stabilizer in the coffee whitener formulation (Fig. 8). Another possible explanation for the increased emulsion stability arises from a consideration of the rheology of polysaccharide solutions. Polysaccharides are hydrophilic molecules that can have a significant effect on solution rheology at low concentration. Many are able to form weak gel-like network structures, which have a low yield stress. This means that they will behave as a weak solid as long as they are not subject to a stress above a certain critical value (the yield stress). Above this yield stress, the solution flows like a fluid. In rheological terms, they have a behavior similar to a Bingham material. If a polysaccharide stabilizer forms a weak network structure in an emulsion, then, as long as the forces exerted on the weak gel generate a stress below the yield stress, the emulsion droplets should be trapped immobile in the gel. Creaming will then stop. Dickinson (1988) states that a yield stress of only 10 mPa is sufficient to prevent creaming under gravitational forces of droplets of several microns in diameter.

Another consequence of the presence of low-molecular-weight emulsifiers in the coffee whitener formulation is that competition for interfacial area will occur between these molecules and the milk protein. Because the low-molecular-weight surfactants are more surface-active than the proteins, they will tend to displace protein from the oil-water interface. This is indeed what is observed for the nonaggregated protein products, where a reduced surface coverage is observed in the coffee whitener formulation compared with the simple buffer system. This is consistent with previous work by other groups on model systems (Dickinson and others 1993b, Euston and others 1995, 1996). Surprisingly, though, the surface coverage for aggregated protein products is higher in the coffee whitener formulation than in the imidazole buffer, despite the presence of the emulsifier. It is possible that the adsorbed layer formed from highly aggregated partially micellar casein leaves gaps at the interface, which allows emulsifier to adsorb without displacing the protein. Alternatively, the protein and emulsifier may adsorb cooperatively via the formation of a protein-emulsifier complex. The increased surface coverage in the presence of the emulsifier may support this hypothesis if the complex has a higher surface activity than the protein alone. Whatever the mechanism, it is clear that aggregated and nonaggregated proteins display different adsorption behavior in the presence of monoglyceride-derived low-molecular-weight emulsifiers. More work in this area is needed to clarify the origin of this difference.

It is apparent from this comparison of milk protein product emulsifying properties that a simple buffered oil-in-water emulsion model is sufficient for predicting trends in the emulsifying ability of the products. Although the emulsifying ability in a coffee whitener is higher than in the imidazole buffer, the trend is consistent, and the imidazole buffer system is able to distinguish between the two protein groupings. However, the limitations of the simple buffer system are clearly delineated when the ERs of the two systems are compared. In the coffee whitener system, the ER of the WPC-stabilized system does not increase over that of the imidazole buffer model, unlike for all 5 other protein products tested. It appears that the simple imidazole buffer model is not a good representation of the emulsion stabilizing ability of WPC. Similarly, in Fig. 9, whereas the surface coverage for all the nonaggregated protein products decreases in the coffee whitener formulation, as would be expected if competition with the low-molecular-weight emulsifier were occurring, that for the aggregated proteins increases; this result was unexpected.

Using a simple model system has some limitations if we wish to use the results to predict the functional properties of milk proteins in a food application. Some surface chemical properties of milk protein products do correlate well between the 2 types of system, such as the emulsifying ability. Other properties correlate well only for some of the proteins. The presence of other functional food ingredients (polysaccharides, emulsifiers, and others) has a large influence on the emulsifying characteristics. It may also be true to say that the emulsifying behavior of a protein

in the coffee whitener formulation may not correlate well with the behavior of the same protein in a different food or dairy emulsion where the nonprotein functional ingredients may be different.

Conclusions

THIS WORK HIGHLIGHTS 2 IMPORTANT points. First, the use of very simple model systems may not be entirely appropriate when trying to predict the emulsifying characteristics of milk proteins in food systems. However, useful information can still be gained from these systems. They can be used to gain a basic understanding of milk protein adsorption without the complicating effects of other ingredients, and they could be used to screen possible emulsifiers for further study in more complex systems. Second, although the interaction of milk proteins can have a large effect on the emulsifying properties of the milk protein, there are gaps in our knowledge of which interactions are important, how they occur and how they can be exploited. There is a need for a systematic study of this area to allow us to use this information to better select ingredients for a particular application.

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