Effect of Acidification and Heating on the Rheological Properties of Oil-Water Interfaces with Adsorbed Milk Proteins

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ABSTRACT

The behavior of casein and whey proteins at the oil-water interface was studied using a dynamic drop tensiometer (DDT). The dilational modulus of the interface was measured for aqueous solutions of skim milk powder (SMP) and whey protein concentrate (WPC) with various additions (salt, calcium, lactose) and (order of) various processing steps. Acidification or heating was performed before or after creation of the interface. The elastic properties of oil-water interfaces with adsorbed milk proteins could partly determine the rate of partial coalescence and resulting product instability.

For WPC, preacidification slows down the adsorption, but the modulus is not affected. This is probably because, although the whey proteins change conformation more slowly at the interface, still a homogeneous film is formed. If postacidification is applied, coarsening of the protein film leads to loss of interfacial rigidity. Preheating of the aqueous phase with WPC leads to denaturation and aggregation, but the aggregates formed are still surface active and give high moduli. If preheating of a WPC solution is followed by postacidification, the resulting modulus is high (~60 mN/m).

The oil-water interfacial properties of SMP are only minimally affected by preheating or by choice of powder (low, medium, or high heat). At low pH, however, aggregates are formed that are less surface active, and interfacial moduli are lower.

If measurements are performed at high temperature (i.e., if postheating is applied), for both SMP and WPC systems, moduli became much lower (~10 mN/m). This is probably because of accelerated rearrangements, leading to the formation of inhomogeneous film structures.

(Key words: milk protein, adsorption, emulsion, interfacial rheology)

Abbreviation key: DDT = dynamic drop tensiometer, IEP = isoelectric point, SMP = skim milk powder, WPC = whey protein concentrate.

INTRODUCTION

Typical processing steps in the production of many dairy emulsions are acidification, heating, and stirring (or any other treatment leading to the creation of oil-water interfaces). Such conditions are typically found in the preparation of dairy protein stabilized oil-in-water emulsions such as quark, yogurt, sour cream, cream cheese, and some types of dairy drinks. In the experience of the food industry, it is known that these processing steps usually require a specific order and intensity to give the desired end result. Apart from oil, water, and dairy proteins, a typical dairy emulsion contains lactose and salts, and they may be enriched in calcium. The composition may also vary in the total amount or ratio of casein and whey proteins.

The choices taken in the production of the products mentioned previously depend at least partly on the viscoelastic properties of the oil-water interfaces formed. In this study, the dynamic drop tensiometer (DDT) (Benjamins et al., 1996) is used to measure the interfacial rheological properties of oil-water interfaces with adsorbed milk proteins under the realistic processing and ingredient conditions mentioned.

The rheological properties of oil-water interfaces with adsorbed milk proteins have been studied extensively [for an overview, see Dickinson (2001, 2004)]. Only a few have focused on complex emulsions with commercial milk powders containing both casein micelles and whey proteins, under realistic processing conditions of heating and acidification and/or in the presence of sugars and salts (Sharma and Dalgleish, 1993; Sharma and Singh, 1998; Brum and Dalgleish, 1999; Bryant and McClements, 2000; Euston and Hirst, 2000; Kulmyrzaev et al., 2000; Dalgleish et al., 2002; Segall and Goff, 2002; Sourdut et al., 2002; Liveney et al., 2003; Kiokias et al., 2004). In contrast, the effect of these conditions on aqueous dispersions of similar milk powders and the structure and rheology of the resulting bulk gels has been studied extensively (Roefs and van

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Vliet, 1990; Roefs et al., 1990; Cooney et al., 1993; Lupano et al., 1996; de Kruif, 1997; Sherwin and Foegeding, 1997; Ju and Kilara, 1998; Lucey et al., 1998; Verheul and Roefs, 1998; Spiegel, 1999; Bryant and McClements, 2000; Kulmyrzaev et al., 2000; Vasbinder et al., 2001; Mellema et al., 2002).

The interfacial rheological properties are important for the control of emulsion stability. Lack of emulsion stability can lead to flocculation or partial coalescence (Boode et al., 1991; Kiokias et al., 2004). Partial coalescence occurs particularly upon temperature cycling (e.g., taking in and out of refrigeration) of products with considerable amounts of (solid) fat and leads to product thickening or firming. Stability is largely determined by the quality of the protein film at the oil-water interface. This quality depends on the degree at which the protein molecules protrude in the aqueous solution. In addition, it depends on the lateral interactions between the proteins and the homogeneity of the film. These latter two properties are both expressed in the dilational modulus of the interface. Increased electrostatic and steric repulsion reduces chances of flocculation (Shimizu et al., 1981; Masson and Jost, 1986; Dickinson, 2004), which usually precedes coalescence. A higher modulus contributes to increased stability of the interface against coalescence (Dickinson et al., 1988) and partial coalescence (Rousseau, 2000).

The 2 main types of milk proteins, caseins and whey proteins, exhibit different properties at the oil-water interface. Individual caseins, such as β -casein, have high surface activity and are relatively flexible. Native whey proteins are globular and more slowly adsorbing. Upon heating (>70 to 75°C), whey proteins denature. The surface activity of the resulting aggregates of denatured proteins is largely unknown and highly dependent on the conditions. The native form of the major whey protein, β -lactoglobulin, is known to change conformation after adsorption and to form a relatively elastic film at the interface (Shimizu et al., 1981; Boerboom, 2000). This change of conformation bears some resemblance with the unfolding upon denaturation, although it is probably less extensive. Also, because they are mechanically induced changes, they are more likely changes of the tertiary structure than the secondary structure.

Acidification usually leads to a decrease in stability. This is mainly because aggregation violates the amphiphilic nature of the proteins. With respect to the whey proteins, at pH 6.7 (milk pH), β -lactoglobulin and α -lactalbumin will adsorb in the proportions present in bulk; at pH 4.6 (isoelectric point, **I**EP, of proteins), α -lactalbumin will adsorb preferentially (Shimizu et al., 1981, 1985; Masson and Jost, 1986; Hunt and Dalgleish, 1994).

In milk powder, casein resides in the form of casein micelles. Hence, the micelles exhibit a low affinity to hydrophobic surfaces compared with caseinate. However, submicelles (Walstra, 1999) or any other submicellar structure (down to the molecules) lack this stabilization mechanism. Hence, casein micelles can also accumulate at oil-water interfaces by falling apart into submicellar structures (Courthaudon et al., 1999). A schematic model for this behavior, which also occurs in homogenized milk, has been proposed by Walstra et al. (1999). Adsorbed amounts are 3 times higher for skim milk protein (**SMP**) than for caseinate or whey powder (Sharma and Singh, 1998).

Despite the fact that a lot is known about the emulsifying properties of the individual proteins, less is known of their behavior in combination (Oortwijn and Walstra, 1979, 1982; Britten and Giroux, 1991; Sharma and Dalgleish, 1993). Generally caseinates give more stable emulsions than whey proteins. However, if the casein is in micellar or aggregated state, whey proteins probably give more stable emulsions. Euston and Hirst (2000) compared the emulsifying properties of commercial milk protein products, such as micellar casein, SMP, and whey protein concentrate (**WPC**). They found that both droplet sizes and creaming stability of the formed emulsion decreased in the following ranking: $\text{WPC} > \text{SMP} > \text{micellar casein}$. It should be noted that emulsifying capacity here means stability against flocculation followed by coalescence. This emulsifying capacity does not necessarily correlate with the interfacial rheological properties. Another factor that can influence the emulsifying properties of milk proteins is thermal processing, because of denaturation of whey protein. At low pH, denatured whey proteins adsorb at the surface of the casein micelles (Vasbinder et al., 2001). At neutral pH, denatured whey proteins can displace caseinates from oil-water interfaces (Dalgleish et al., 2002).

As already mentioned, in contrast to the effect of these parameters on milk proteins at interfaces, a considerable amount of knowledge is available on the effect of processing steps, such as heating and acidification on casein micelles and whey proteins in bulk. Assuming that the protein film can be approximated as the 2-D analogue of the bulk gel, the results of these studies can also be used to explain our interfacial data.

MATERIALS AND METHODS

Commercial sunflower oil, ex Albert Heijn, was used as the oil source. It was not silica-treated, so minor amounts of surface-active impurities are not excluded. The water used was distilled. The following other ingredients were used: WPC (Nutrilac QU7560); low, medium, and high-heat SMP (Friesland Coberco Dairy

Foods, The Netherlands); calcium chloride (calcium chloride dihydrate; Fluka Chemie GmbH); lactose (D(+)-lactose monohydrate; Mallinckrodt Baker, Holland); NaCl; GDL (D-gluconic acid lactone; Sigma Chemical Co., Ireland); and lactic acid (BDH Laboratory, England).

The protein content in the WPC and SMP are, respectively, 75 and 35%. Note that the protein in WPC is mainly whey protein, and the protein in SMP is approximately 80% casein and 20% whey protein. The heat history of milk powder manufacture is measured by the whey protein nitrogen index. It is expressed as the quantity of undenatured whey/serum protein per gram of SMP. The whey protein nitrogen index values for the various types of SMP investigated are ≥ 6 (low heat), 1.5 to 6 (medium heat), and (1.5 (high heat). In the experiments, the concentrations of milk protein powders were chosen such that protein concentration was constant at a 0.5 weight percentage on aqueous phase (except where stated otherwise), which resulted in the following concentrations of protein powder: SMP, 1.4 weight percentage; WPC, 0.7 weight percentage.

The IT Concept DDT used was a modified version of the automated drop tensiometer (Benjamins et al., 1996). This equipment was used to measure the interfacial elastic dilatational modulus, which is the elastic response of the interfacial tension to periodic changes in interfacial area. Standard frequency and amplitude were 0.1 Hz and 10% (note: for 1.4% whey powder, elastic modulus was shown to be independent of amplitude in the range of 5 to 20%, suggesting a linear regimen). Interfacial tensions and elasticities were taken at certain time intervals to get an idea of the adsorption rate of the proteins.

The temperature was kept either at room temperature (25°C) or at an elevated temperature (45 or 85°C). Heating was performed for 20 min before creation of the oil droplet (preheating) or inside the measuring cell itself after creation of the oil droplet (postheating). The pH of the aqueous solution was set at 6.7 (neutral) or 4.6 (acidified). This was done either by addition of lactic acid to the aqueous phase before creation of the oil droplet (preacidification) or by using glucono-delta-lactone after creation of the oil droplet (postacidification).

Other experimental parameters of interest include the following: syringe, 0.25 μL ; needle, U-shaped #21; and droplet volume, 4 μL . An error analysis using the reference solutions of 1.4% SMP and 0.7% WPC showed that the error of the equipment was ± 2 mN/m, which means that any difference between data of < 4 mN/m cannot be considered significant.

In addition to the interface dilatation modulus, $|E|$, and the interfacial tension, the equipment also monitors the interfacial dilational elasticity, ε_d ; the interfa-

cial dilational viscosity, η_d ; and the parameter, $\tan \delta$. The relationship among these parameters is as follows:

$$|E| = \sqrt{(\varepsilon_d^2 + \eta_d \omega^2)}, \text{ and } \tan \delta = \eta_d \omega / \varepsilon_d,$$

where ω is the frequency of deformation and δ is the phase difference between the applied sinusoidal variation on the surface area and the resulting interfacial tension signal. If the parameter $\tan \delta$ is low, the viscosity is low compared with the elasticity at a given frequency. The modulus $|E|$ is a measure of the total interfacial rigidity. For a more detailed explanation of these rheological terms see Mellema et al. (2002), (Williams and Prins, 1996), and Cooney et al. (1993).

A general remark on the interfacial tension data is that, assuming an insoluble protein layer (Mellema et al., 1998), there should be a correlation with the interfacial concentration. We did not include measurement of the actual interfacial concentration, but, for the discussion, we can and use trends in the surface tension data to deduct trends in interfacial concentration.

RESULTS AND DISCUSSION

Effect of Ingredient Parameters on Interfacial Properties

In this section, we present the effects of ingredient variations on the elastic response of the interface of sunflower oil and an aqueous solution of milk proteins. Figure 1 shows the effect of the type of milk protein source (SMP or WPC) and concentration on interfacial modulus (a) and interfacial tension (b). Note that 0.5 and 1% protein solutions were tested (i.e., 0.7% WPC/1.4% SMP, and 1.4% WPC/2.8% SMP).

A clean oil-water interface has a modulus and tension of 0 and 27 mN/m, respectively. Figure 1(a and b) shows that, in the presence of protein, a gradual increase in modulus and decrease in interfacial tension is obtained after creation of the interface at time = 0. Both for SMP and WPC, slightly higher values of the oil-water modulus are found compared with purified fractions of milk proteins [e.g., β -casein and β -lactoglobulin (Williams and Prins, 1996)]. Final moduli of the WPC and SMP systems are quite similar.

The SMP gives a lower final interfacial tension compared with WPC; the final modulus for WPC is slightly higher. The former shows that casein micelles co-adsorb with the whey proteins (Walstra et al., 1999); the latter suggests that whey proteins are capable of yielding slightly higher interfacial moduli.

For SMP, the concentration does not affect the behavior of the modulus at all. Even if the concentration is set at 0.7% SMP (results not plotted), the development

Figure 1. A) Oil-water interfacial modulus as a function of time for aqueous solutions of 2 types of protein powders [whey protein concentrate (WPC) and medium-heat skim milk powder (SMP)] at 2 concentrations (corresponding to 0.5 and 1% actual protein content, respectively). B) Oil-water interfacial tension as a function of time for aqueous solutions of 2 types of protein powders (WPC and medium-heat SMP) at 2 concentrations (corresponding to 0.5 and 1% actual protein content, respectively).

of the modulus is almost identical, which suggests that multilayer adsorption (if occurring at all) does not contribute to the film interfacial rheology.

The development of the modulus at high concentrations of WPC is slower. This result suggests that, immediately after the first adsorption step, rearrangement or unfolding of the whey proteins is determining the development of the interfacial rheology. This well-known, two-step adsorption behavior of globular proteins, such as β -lactoglobulin (Boerboom, 2000), is clearer from the high concentration data.

In an additional experiment, the type of SMP (low, medium, or high heat) was varied. These protein powders vary in the degree of denaturation of the whey proteins. The results are not shown here, because type

Figure 2. A) Oil-water interfacial modulus as a function of time for aqueous solutions of 1.4% skim milk powder (SMP) with various additions as indicated. B) Oil-water interfacial tension as a function of time for aqueous solutions of 1.4% SMP with various additions as indicated.

of SMP did not significantly affect the modulus or surface tension. Apparently different degrees of denaturation [i.e., different degrees of coating of the casein micelles (Vasbinder et al., 2001)] is not of major influence on the adsorption behavior. For the remainder of this study, 0.7% WPC and 1.4% (medium heat) SMP are used as reference.

In Figures 2 (SMP) and 4 (WPC), the effect of various additions to the aqueous phase on the interfacial modulus (a) and tension (b) are shown.

From Figures 2 and 3, it can be concluded that all 3 additions have impact on the interfacial properties. Ranking from high to low impact (similar for SMP and WPC), the results are salt > calcium > lactose. The reader should take into account the high scatter in the elasticity data for the WPC system with added salt,

Figure 3. A) Oil-water interfacial modulus as a function of time for aqueous solutions of 0.7% whey protein concentrate (WPC) with various additions as indicated. B) Oil-water interfacial tension as a function of time for aqueous solutions of 0.7% WPC with various additions as indicated.

which is why we will refrain from any further interpretation for these data.

The effect of salt seems to be stronger in the case of WPC; at an early stage, already the interfacial tension had become so low that the droplet became unstable and detached from the syringe. The same happened for the addition of calcium chloride at a somewhat later stage (Figure 3b). The interfacial modulus also decreases as the interfacial tension approaches zero. We assume that this is a measurement artefact resulting from the strong distortion of the droplet right before detachment. Up to the point of detachment, for calcium and lactose, the modulus is not much different from the WPC reference.

In the case of WPC with lactose and SMP with salt and or calcium, there is no significant change in interfacial tension, suggesting that no major changes in in-

Figure 4. A) Oil-water interfacial modulus as a function of time for aqueous solutions of 0.7% whey protein concentrate (WPC), neutral (reference, pH 6.7) or acidified with lactic acid (to pH 4.6) before the interface was created. B) Oil-water interfacial tension as a function of time for aqueous solutions of 0.7% WPC, neutral (reference, pH 6.7) or acidified with lactic acid (to pH 4.6) before the interface was created.

terfacial concentration occurred. The decrease in modulus could result from a minor coarsening of the interfacial protein film.

The large effect of calcium (and salt), especially on whey proteins, is well known from studies on the behavior of these proteins in solution and gels (Roefs and van Vliet, 1990; Sherwin and Foegeding, 1997; Ju and Kilara, 1998; Verheul and Roefs, 1998; Bryant and McClements, 2000). This can be explained as follows. By addition of electrolytes, such as sodium or calcium chloride, charges on the proteins are screened, and the proteins become more hydrophobic. Casein micelles are less sensitive to addition of electrolytes because they are stable in solution by steric repulsion; whey proteins

rely on electrostatic repulsion only. Loss of repulsion leads to aggregation and a coarser interfacial film structure. The resulting network is more brittle and is usually weaker. However, at the same time, the proteins with screened charges tend to accumulate stronger at hydrophobic interfaces. The resulting net effect is that the weakening of the protein film is compensated by a higher total concentration of protein in the film.

Finally, lactose and other sugars are known to slightly change the solubility of proteins (Spiegel, 1999; Kulmyrzaev et al, 2000). As a result, processes such as adsorption will be affected. In our case, the net result is a lower modulus at similar interfacial concentration, which suggests that solubility has decreased upon addition of lactose. The effect is less pronounced for SMP, possibly because this protein powder contains a lot of lactose already.

Effect of Processing Parameters on Interfacial Properties

In Figure 4 (a, modulus; b, tension), the results of preacidification (i.e., acidification down to pH 4.6, mild stirring, and putting the aqueous dispersion in the DDT for measuring) on the properties of the oil-water interface are given.

Acidification to pH 4.6 brings the system close to the IEP of both whey proteins (5.3) and caseins (4.6). Particularly, the casein micelles are sensitive to pH changes and will lose their colloidal stability. The general expectation is that acid-aggregated casein micelles would be less amphiphilic and, hence, less surface active. In Figure 4b, we indeed observe that preacidified solutions have a higher equilibrium value of the interfacial tension, suggesting lower adsorbed amounts. Lower adsorbed amounts at or around the IEP has been reported before (Masson and Jost, 1986). This behavior has already been reported before. Interestingly, the aggregated state does not affect the elasticities too much. In the case of WPC, preacidification even led to a higher equilibrium modulus compared with the reference.

We can interpret the results as follows. For the SMP case, at least 2 effects are counteracting each other at low pH. On the one hand, the interfacial tension data suggest that adsorbed material close to the interface is lower (although initial adsorption rates seem to be higher, probably because of increased hydrophobicity) and the film structure is coarse and brittle. On the other hand, the film structure is quite firm because the (lateral) interactions are quite strong. For the WPC, the former process is less pronounced because the majority of the whey proteins is in native form and, hence, less acid-sensitive than casein micelles (i.e., they do not form such large aggregates at pH 4.6). Also, for

Figure 5. A) Oil-water interfacial modulus as a function of time for aqueous solutions of 0.7% whey protein concentrate (WPC) or 1.4% skim milk protein (SMP), not heated (reference) or heated to 45 or 85°C for 20 min before the interface was created. B) Oil-water interfacial tension as a function of time for aqueous solutions of 0.7% WPC or 1.4% SMP, not heated (reference) or heated to 45 or 85°C for 20 min before the interface was created.

preacidified WPC, the interfacial tension does not reach equilibrium but continues to decrease. This result is probably because unfolding rearrangements occur more slowly at low pH. In the end situation, the proteins will be relatively closely packed at the interface, leading to somewhat elevated interfacial elasticities.

Note that from previous studies (Shimizu et al., 1985; Hunt and Dalgleish, 1994), it is known that at low pH, α -lactalbumin is more dominant at the interface than β -lactoglobulin. Hence, we could also explain our results assuming that α -lactalbumin adsorbs more slowly but gives a fairly rigid interface.

Next, in Figure 5 (a, modulus; b, tension), we plotted the effect of preheating the SMP and WPC solutions for 20 min at 85°C (actual rheological measurements

performed at room temperature, 25°C). Such heat treatment is sufficiently severe to assume maximum denaturation of the whey proteins.

In these figure, it can be seen that preheating does not have a major impact on the interfacial properties (most of the changes are within experimental error). From Figure 5b, it can be concluded that aggregation is somewhat accelerated, but similar equilibrium values for both modulus and tension are attained. Preheated WPC may be the only exception, with some indications of lower interfacial tension, and clearly elevated values for the modulus.

The observations can be explained by the denaturation of the whey proteins. In the case of SMP, this should not affect interfacial properties. (There was no effect of low- or high-heat SMP either.) Apparently, there is a difference with bulk gels, because, for the latter, the effect of heating leads to considerable changes in microstructure and rheology (Lucey et al., 1998). The reason for this difference between bulk and interface behavior is not known. The DDT technique is probably not accurate enough to probe differences at the given conditions.

In the case of WPC, the effect is more pronounced. Denatured whey proteins are not stable in solution and will have a high tendency to aggregate or adsorb. Apparently, the adsorbed aggregates can spread out to form an elastic protein film.

Note that the combination of preheating and preacidification before creation of the interface gives results similar to preacidification (results not shown here). Thus, preacidification dominates the interfacial behavior over preheating. This is not surprising for SMP, because of the minor influence of heating, but, for WPC, this was not expected. For WPC, the combination of heating and acidification leads to extensive aggregation. Apparently, denatured and aggregated whey proteins still yield considerable interfacial moduli (even if the amount of protein close to the interface—which is the only thing that can influence the DDT results—is lower). Note that the positive effect of denatured whey proteins in interfacial elasticity is more generic; it is also found for air-water interfaces (Bals and Kulozik, 2003).

In Figure 6, we have plotted the effect of acidification after creation of the interface, (on both interfacial tension and modulus) for SMP (a) and WPC (b). To obtain a gradual decrease in pH, use was made of glucono-delta-lactone.

The results presented in Figure 6 confirm most of our findings from the preacidified samples (Figure 4). Generally, acidification leads to decreased moduli and increased interfacial tension. The change in interfacial properties starts at or around the IEP of the proteins

Figure 6. A) Oil-water interfacial tension and modulus as a function of time for aqueous solutions of 1.4% skim milk protein (SMP), not acidified or postacidified to approximately pH 4.0. B) Oil-water interfacial tension and modulus as a function of time for aqueous solutions of 0.7% WPC, not acidified or postacidified to approximately pH 4.0.

and becomes more pronounced as the pH decreases further. The results can be understood by assuming that the proteins tend to aggregate or rearrange upon acidification and disturb the homogeneous film structure, which leads to a decrease in modulus. Some of the proteins may desorb to form larger aggregates (that are still anchored at the interface). This protein material, which is further away from the interface, is not probed by the DDT.

Figure 4a showed that the behavior of the modulus upon preacidification is opposite that postacidification. We can speculate that upon preacidification, the native whey proteins with the highest tendency to disturb the modulus are inactivated by the acidification before they can adsorb. To add some further detail to this selection

Figure 7. Oil-water interfacial tension and modulus as a function of time for preheated (20 min, 85°C) aqueous solutions of 0.7% whey protein concentrate, not acidified or postacidified to approximately pH 4.0.

mechanism, it could well be that, in the case of WPC, α -lactalbumin, which is known to adsorb preferentially above β -lactoglobulin at low pH, remains and is responsible for the elevated moduli.

Postacidification can also be combined with preheating (results not shown). For SMP, the effect of this was similar to postacidification without preheating (Figure 6a), which is in accordance with experiences described previously. For WPC, the behavior is plotted in Figure 7.

From Figure 7, it can be concluded regarding the modulus of WPC films, that preheating and postacidification yields a reverse effect compared with postacidification only (Figure 6b). The modulus is higher already at an early stage, which is in accordance with Figure 6a, but, upon acidification, it continues to increase. For the interfacial tension, the observed effect is similar (increase of tension).

We speculate that, despite the loss of amphiphilic character of both native and denatured whey proteins, the decrease in the modulus upon postacidification is only for the native whey proteins. We conclude that denatured whey proteins yield the highest oil-water interface moduli, before native α -lactalbumin at low pH, and next, native β -lactoglobulin.

The only sequence of processing steps that remains to be discussed is postheating. Interestingly, postheating leads, in all cases (SMP or WPC, preacidified or not, twice the protein concentrations), to a considerable decrease in modulus to approximately 5 to 10 mN/m (results not shown). Interestingly, the equilibrium surface

tension was not affected too much, which is an indication that the protein would remain at the interface upon postheating, but the rearrangements leading to a coarsening of the network structure were accelerated by the high temperature. This effect of temperature on the modulus has also been observed for bulk gels made from WPC (Cooney et al., 1993) and SMP (Roefs and van Vliet, 1990; Mellema et al., 2002). Continuation of the high temperature is known to be accompanied by an increased permeability and reduction of water-holding capacity (i.e., increased syneresis).

From previous data, we concluded that native whey proteins give the highest moduli of all proteins investigated. The decrease in modulus by postheating could, therefore, be explained in the measurement temperature or by a selection mechanism (similar as used to explain the difference between pre and postacidification). High temperatures increase the hydrophobic interactions between certain fractions of proteins, yielding compact structures. For postheating, this disturbs the protein film to such an extent that interfacial moduli are almost completely lost.

The latter observations on postheating are not in accordance with measurements by Kiokias et al. (2004) on the stability of partly crystalline fat-based, whey protein-stabilized oil in water emulsions. They found that heat-treatment or acidification before emulsification leads to unstable emulsions during temperature cycling, whereas heat treatment after acidification results in stable emulsions. The reason for the inconsistency with our data could be because the sensitivity of an emulsion to partial coalescence cannot fully be explained by the interfacial modulus. The modulus is a parameter that is determined by the behavior of protein close to the interface. The presence of protein molecules further away from the interface, still anchored to the interface via other protein molecules, could well contribute to stability of the emulsion. In other words, the contribution of protein film thickness to emulsion stability is not fully captured by measuring the interfacial dilational modulus. Apparently bulk protein gel properties cannot be neglected in explaining the stability of protein-stabilized emulsion gels to (partial) coalescence.

Finally, we offer some comments on the behavior of $\tan \delta$. As already mentioned, this parameter is a measure of the degree of liquid behavior of gel-like structures. It has been shown to be related to the tendency of a gel to rearrange and form locally compact structures, at first leading to increased rigidity, but eventually leading to a more weak (low moduli) and brittle gel (Mellema et al., 2002). For the systems presented here, $\tan \delta$ was found to be fairly low (approximately 0.05 to 0.15), confirming the elastic behavior of the

films. There were no major changes as a function of the ingredient and processing parameters of interest. Only postheating (0.2) and the addition of salt (0.25) lead to considerably higher values, which is in line with the expectations, as these were the parameters leading to the largest decreases in moduli.

CONCLUSIONS

Measurement of oil-water interfacial moduli and tension using the DDT is a useful technique to study the behavior of milk proteins at oil-water interfaces. The addition of electrolytes and the order of various processing steps were of major importance on the final interfacial properties.

Decreasing the pH before creation of interface leads to a slower unfolding of the native whey proteins at the interface, although final moduli are higher. Upon postacidification, coarsening of the film structure occurs, leading to loss of interfacial rigidity. Preheating of the aqueous phase leads to denaturation and subsequently aggregation of the whey proteins. It turns out that these aggregates are still surface active and give high moduli. If preheating of a WPC solution is followed by postacidification, the resulting modulus is highest of all systems investigated (~60 mN/m).

The oil-water interfacial properties of SMP are only minimally affected by preheating, which is in accordance with the interfacial data for low-, medium-, and high-heat SMP, where we did not see a significant effect either. At low pH, however, caseins aggregate extensively. The resulting aggregates are less surface active, and interfacial moduli are low (regardless of order; pre or postacidification).

If measurements are performed at high temperature (i.e., if postheating is applied), for both SMP and WPC systems, moduli became much lower. This is probably because of increased hydrophobic interactions and accelerated rearrangements, leading to the formation of inhomogeneous film structures that are weaker.

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