FACTORS AFFECTING THE INTERFACIAL PROPERTIES OF SURFACTANT ABSORBED LAYERS ON AN OIL DROPLET SURFACE

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ABSTRACT

Proteins and low molecular weight (LMW) surfactants are widely used for the physical stabilization of many emulsions and foam - based food products. The formulation and stabilization of emulsions and foams depend strongly on the interfacial properties of the proteins and LMW surfactants. The effect of proteins and LMW surfactants, sodium caseinate concentration and calcium concentration on the interfacial tension and interfacial dilational modulus on the n-hexadecane/water interface was determined by an automatic drop tensiometer. The experimental data represented the interfacial tension and modulus as a function of the adsorption time, protein concentration in the bulk phase and calcium chloride concentration.

Keywords: interfacial tension, interfacial dilational modulus, sodium caseinate, whey protein isolate.

1. INTRODUCTION

Protein and low molecular weight (LMW) surfactants play a crucial role in the stabilization of emulsions and foams. Emulsion and foam stability strongly depends on the behaviour of these interfaces (Bos & van Vliet, 2001). The interfacial tension provides an indication of the film compactness: the lower the interfacial tension, the more compact the film becomes. The interfacial rheology can be described as the resistance of the interface to deformation and film strength (Burgess & Ozlen Sahin, 1997). Knowledge of the interfacial properties of adsorbed films is necessary to understand the role of protein and LMW surfactants in emulsion and foam stability.

Burgess and Ozlen Sahin (1997) investigated the effect of bulk concentration, temperature, pH and ionic strength on the interfacial properties by a Mark II surface rheometer. In the present study, the influence of different surfactants, protein concentrations and calcium chloride concentration on the interfacial tension and interfacial rheology was envestigated.

The aim of this research was to find a surfactant, a protein concentration and a calcium concentration on the absorbed layers on an oil droplet surface, which have a low interfacial tension and a high interfacial modulus.

2. MATERIALS AND METHODS

2.1. Materials

Sodium caseinate was supplied by Armor Proteins, France. This product contained 94.13% dry matter of which 94.77% was protein, 9750 ppm Na and 486 ppm Ca. Whey protein isolate (WPI) was supplied by Davisco International (USA). It contained 94.98% dry matter of which 97.53% is protein, 3950 ppm Na and 823 ppm Ca. The molecular weight of WPI was assumed to be the same as that of β-lactoglobulin (18363 g/mol). Tween 20 was

supplied from Sigma-Aldrich Chemie GmbH, Germany. It is a member of the polysorbate group and is a non-ionic surfactant.

Calcium chloride was obtained from MERCK, Germany.

Sunflower oil was purchased at the local supermarket in Gent, Belgium.

The n-hexadecane with purity of 99% was supplied by Sigma chemical Company.

For purified sunflower oil, the purification was carried out as follows:

10 g of silica gel was mixed with 250 ml sunflower oil into a 500 ml beaker. This mixture was stirred for 4 hours. At the end, it was centrifuged at 3000 rpm for 20 min. The purified oil was carefully withdrawn with a syringe or pipette.

2.2. Methods

2.2.1 Preparation of dispersions

Dispersion of sodium caseinate: 100mg sodium caseinate in 100 ml acetate buffer solution at pH 6.0 made from pure water was dispersed by magnetic stirring for 1 hour. The dispersed sodium caseinate was filtered through Whatman $N^0$1 filter paper to get the sodium caseinate dispersion of 1 mg/ml. Sodium caseinate dispersions with a concentration of 0.1 and 0.01 mg/ml were obtained by diluting the above mentioned sodium caseinate dispersion (1 mg/ml) in acetate buffer solution pH 6. 0 by a factor of 10 and 100, respectively.

Dispersion of WPI: 10 g whey protein isolate in 100 ml acetate buffer solution at pH 6.0 made from pure water was dispersed by magnetic stirring for 1 hour to make a concentration of those of 0.1 mg/ml.

Solution of Tween 20: 10 g Tween-20 in 100 ml acetate buffer solution at pH 6.0 made from pure water was dispersed by magnetic stirring for 1 hour to make a concentration of those of 0.1 mg/ml.

Sodium caseinate dispersions of 0.01 mg/ml containing 1, 10 and 20 mM $CaCl_2$ was prepared by diluting 2 ml of sodium caseinate dispersion of 1 mg/ml to 100ml by acetate buffer pH 6.0. This dispersion was mixed with 2, 20, and 40 mM $CaCl_2$-containing buffer solution at pH 6.0 at a ratio of 1:1.

2.2.2. Measurement of interfacial properties

The interfacial tension and interfacial modulus at the oil/water interface were measured by using the automatic drop tensiometer (Tracker, IT-Concept, Saint-Clementes Places, France). The experiments on the interfacial tension were carried out by adding 30 ml of water phase into the glass cuvette. The other parameters of the assays were fixed as follows: drop density (kg/dm^3) of 0.7733, bulk density (kg/dm^3) of 1.0000, rising drop, initial volume of 10 µl after expelling 1 drop, measurement time of 1800 or 3600 seconds, non measurement of sinusoidal profile.

The experiments on the interfacial modulus were carried out for 5 minutes after the interfacial tension was measured until the equilibrium stage. The main parameters were set to measure a volume sinusoidal profile as follows: amplitude of 1.5 µl, period of 10 seconds.

Statistical analysis: Data generated from different determinations were analyzed using the SPSS program.

3. RESULTS AND DISCUSSION

3.1. Effect of type of oil on the interfacial tension and modulus of the adsorbed film at the oil/water interface

In many food formulations the proteins are not the only emulsifiers present, because small molecular surfactants are also present in the food formulation. Commercial sunflower oil usually used in food emulsion production not only contain triacylglycerol, but also contains mono-, diacylglycerol and fatty acids. The latter can compete with proteins to adsorb into the fluid/fluid interface when making emulsions.

Table 1. The interfacial modulus at the oil/pure water interface after 30 min (n=2)

Type of oil	Modulus (mN/m)	Elasticity (mN/m)	Viscosity (mN/m)
Non purified sunflower oil	5.22	4.66	1.52
Purified sunflower oil	6.92	6.82	0.92

The interfacial tension and modulus are two important parameters to determine the purity of the oil. The effect of the type of oil on the interfacial tension and modulus is shown in Figure 1 and Table 1. Figure 1 shows that the interfacial tension in the sunflower oil/pure water interface and in the purified oil/pure water interface decreased very rapidly to an equilibrium value of 25 mN/m. On the other hand, the interfacial dilational modulus is 5.22 and 6.92 mN/m for non purified oil and purified oil, respectively.

The interfacial tension and modulus of the purified oil/pure water interface were expected to be 28 mN/m and near 0 mN/m, respectively (Van der Meeren, 2005). Hence, both sunflower oil and purified oil likely contain mono-, diacylglycerol and fatty acids.

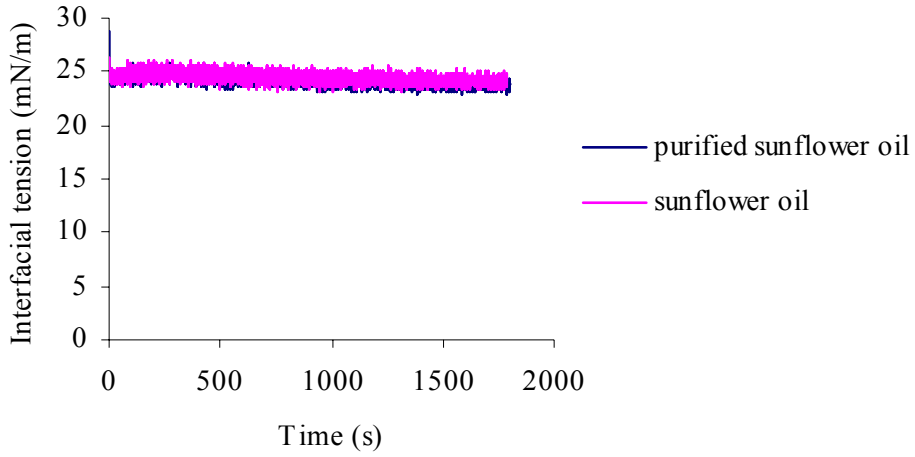

Figure 1. The interfacial tension at the oil/pure water interface

The interfacial tension and modulus of the oil/water interface was studied with a 0.1 mg/ml protein solution. Depending on the type of oil used, the interfacial tension varied between 8 and 22 mN/m (Bos & van Vliet, 2001). Figure 2 shows that decreasing the interfacial tension with time is significantly different between the three types of oil. For hexadecane, the interfacial tension decreased very rapidly from 48 to 17 mN/m, and remained constant during measuring. For sunflower oil and purified oil, the dynamic interfacial tensions were virtually the same and decreased very quickly from 26 to a plateau value of 12 mN/m.

Table 2. Interfacial dilational modulus of 0.1 mg/ml sodium caseinate in acetate buffer at pH 6.0 against the types of oil (n = 3)

Type of oil	Modulus (mN/m)	Elasticity (mN/m)	Viscosity (mN/m)
Non purified sunflower oil	6.67 ± 0.06^{a}	6.63 ± 0.01^{a}	1.27 ± 0.40^{a}
Purified sunflower oil	7.91 ± 0.28^{ab}	7.84 ± 0.28^{ab}	1.03 ± 0.09^{a}
n-Hexadecane	11.31 ± 1.55^{b}	10.51 ± 1.45^{b}	4.17 ± 0.53^{b}

(Different superscript letters indicate a significant difference at 95% among the values of the various oils)

While the dynamic interfacial tension of the sunflower oil/water interface and of the purified oil/water interface decreased more rapidly as compared to that of the n-hexadecane/water interface, the interfacial dilational modulus was lower after 30 min (Table 2). To eliminate the influence of mono-, diacylglycerol and fatty acids being present in vegetable oil for experimental data on the effect of calcium chloride on the interfacial tension and modulus at the oil/water interface, n-hexadecane was used as an oil phase for further experiments.

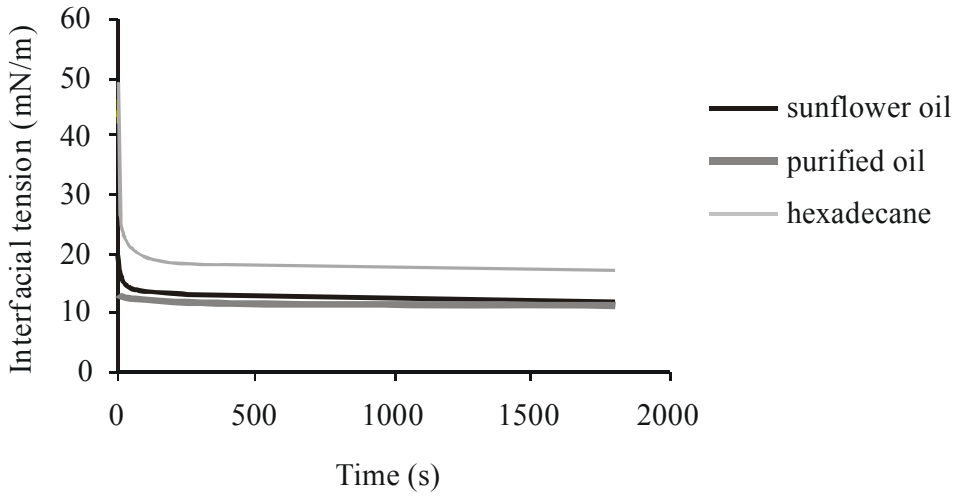

Figure 2. Effect of type of oil on interfacial tension on the oil/water interface (0.1 mg/ml sodium caseinate in acetate buffer solution at pH 6.0)

3.2. Effect of various surface-active compounds on interfacial properties on the n-hexadecane/water intesrface

Sodium caseinate (α, β and κ) comprises a family of proteins that lack a significant amount of ordered structure, in contrast to globular proteins such as WPI which often exhibits regular secondary structures (Beverung *et al.*, 1999). Tween-20, a water-soluble low molecular weight surfactant, is more surface-active than protein (Wilde *et al*., 2004).

The effect of different surfactants, consisting of sodium caseinate (NaCas), whey protein isolate (WPI) and Tween 20, on the interfacial tension and interfacial rheology was studied using a dynamic drop tensiometer. The interfacial rheology results of 0.1 mg/ml surfactant after aging for 30 min at pH 6.0 are shown in Table 3. The difference in interfacial rheology is very significant ($P = 0.00 << 0.05$). The interfacial dilational modulus of WPI was the highest (50.88 ± 2.17 mN/m) followed by Tween 20 (20.32 ± 1.92 mN/m), sodium caseinate (11.31 ± 1.55 mN/m) and pure water (5.28 ± 0.01 mN/m). From a rheological point of view, the interfacial modulus on the oil/pure water interface is nearly zero. In fact, it was observed that the interfacial modulus was 5.28 mN/m.

The interfacial tension decreased with time, reaching an equilibrium after about 10, 2 and 5 min for WPI, NaCas and Tween 20, respectively (Figure 3). The interfacial tensions of 0.1 mg/ml WPI, NaCas and Tween 20 and hexadecane decreased from 41 mN/m to 20 mN/m, from 48 mN/m to 17.5 mN/m and from 31 mN/m to 8 mN/m, respectively.

Table 3. Effect of various surfactants dissolved in acetate buffer (pH 6.0) on the interfacial dilational modulus at the n-hexadecane/water interface after 30 min (n =3)

Surfactants (0.1 mg/ml)	Modulus (mN/m)	Elasticity (mN/m)	Viscosity (mN/m)
Pure water	5.28 ± 0.01[a]	4.23 ± 0.95[a]	2.43 ± 1.30[a]
NaCas	11.31 ± 1.55[a]	10.51 ± 1.45[a]	4.17 ± 0.53[a]
WPI	50.88 ± 2.17[b]	50.11 ± 2.39[b]	8.75 ± 1.06[b]
Tween-20	20.32 ± 1.92[c]	18.86 ± 1.81[c]	7.57 ± 0.64[c]

(Different superscript letters indicate a significant difference at 95% among the estimated moduli of the various surfactants)

The rate of decrease in interfacial tension (or increase of surface pressure, $\pi = \gamma_0 - \gamma$) and interfacial modulus is determined by three processes: (i) the diffusion of surfactant molecules to attach to the interface; (ii) spreading or unfolding of already adsorbed molecules; and (iii) molecular rearrangements of adsorbed molecules (Rodríguez Patino *et al.*, 2001).

The kinetics of LMW adsorption is essentially determined by diffusion. The change in the surface pressure with time is expressed by Equation (1) (Rodríguez Patino *et al.*, 2001). However, several authors (MacRitchie, 1978) have used this equation to describe the adsorption of protein molecules from the bulk to the interface.

$$\pi = 2.C.k.T.(D.t/3.14)^{1/2} \quad (1)$$

Where: C is the concentration in the bulk phase
k is the Boltzman constant
T is the absolute temperature
D is the diffusion coefficient.

The time-dependent adsorption on the n-hexadecane/water interface is seen in Figure 3. It shows that Tween-20 reduced the interfacial tension more effectively than sodium caseinate, followed by WPI. The interfacial tension at the n-hexadecane/pure water interface was nearly 50 mN/m.

The difference in adsorption of sodium caseinate and that of WPI can be explained by the disordered structure of sodium caseinate in the bulk aqueous phase. It appears that the lack of regular structure promotes rapid adsorption and tension equilibration. The flexibility of casein allows it to seek an equilibrium conformation sooner than an ordered globular protein, such as WPI. This may be due to the strong adsorption of the hydrophobic C-terminus, leaving the negatively charged amino acid hydrophilic N-terminus suspended as loops and tails in the aqueous phase. Meanwhile, WPI with its ordered structure needs time for unfolding and rearrangement as it is adsorbed into the interface (Beverung *et al.*, 1999).

The difference in interfacial dilational modulus among the three surfactants is shown in Table 3. Theoretically, Tween 20 was expected to form the least viscoelastic adsorbed layer, followed by sodium caseinate and WPI. In fact the adsorbed layer by Tween 20 had a higher modulus than that of sodium caseinate. Girardet *et al.*, (2001) investigated the interfacial tension and interfacial rheology of Tween 20 on the triolein/water interface with the dynamic drop tensiometer (IT concept, Longessaigne, France). These authors found that the critical micellar concentration (CMC) was 3.74×10^{-6} M and the interfacial dilational modulus obtained was 25 mN/m and 18 mN/m at the CMC and 8.14×10^{-5} M (0.1 mg/ml), respectively.

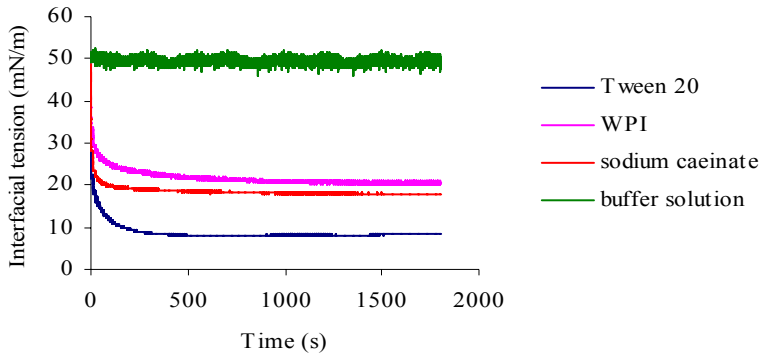

Figure 3. Effect of surfactants on the interfacial tension on the n-hexadecane/water interface

3.3. Effect of bulk concentration of sodium caseinate on interfacial properties on the n-hexadecane/water interface

The experimental transient surface dynamic properties such as interfacial tension and interfacial dilational modulus for sodium caseinate adsorbed on the n-hexadecane/water interface at pH 6 for 60 min, and at several protein concentrations in the bulk water phase are shown in Table 4 and Figure 4.

Table 4. Effect of the bulk concentration of sodium caseinate in acetate buffer (pH 6.0) on the interfacial dilational modulus at n- hexadecane/water interface after 60 min (n=3)

Sodium caseinate concentration (mg/ml)	Modulus (mN/m)	Elasticity (mN/m)	Viscosity (mN/m)
0.01	12.16 ± 0.83[a]	11.91± 0.84[a]	2.34 ± 0.58[a]
0.1	10.45 ± 1.12[a]	9.91± 0.97[a]	3.26 ± 0.92[a]
1	6.69 ± 0.58[b]	6.27 ± 0.55[b]	2.29 ± 0.42[a]

(Different superscript letters indicate a significant difference at 95% between the values at the various concentrations of sodium caseinate)

The transient surface dynamic properties of the adsorbed sodium caseinate film depend on the protein concentration in the bulk phase. As a general rule, it is expected that the interfacial tension decreases and the interfacial dilational modulus increases when the sodium caseinate concentration in the bulk phase is increased. As expected, the interfacial tension decreased with time (Figure 4), but the interfacial dilational modulus decreased as the protein concentration in the bulk phase was increased (Table 4).

The interfacial tension decreased from 23 mN/m to 17 mN/m, from 48 mN/m to 17.5 mN/m and from 50 mN/m to 18.5 mN/m for 1, 0.1 and 0.01 mg/ml of protein, respectively. The interfacial tension decreased with time, reaching equilibrium within the first minutes at 0.1 and 1 mg/ml, and by 15 min at 0.01 mg/ml. From these results we can deduce that the concentration of protein in the bulk phase does not affect the equilibrium interfacial tension value to a large extent, but only affects the dynamics of interfacial tension decrease if the oil drop is aged for a long time. At low protein concentrations, corresponding to 0.01 mg/ml, the dynamic interfacial tension can be divided into three time regimes: the first regime is an induction regime, where the interfacial tension remains relatively constant at the pure fluid value. The second regime is characterized by a sharp decline from this initial value. The final regime is a steady decline in interfacial tension, reaching a plateau value after several hours (Beverung *et al.*, 1999).

The reduction in interfacial tension is a result of protein adsorption and the ability of adsorbed protein to interact with both phases. Obviously, at high protein concentration in the bulk phase, the protein molecules are rapidly adsorbed into the interface to obtain a steady plateau value. At low sodium caseinate concentration in the bulk phase, the adsorption of protein molecules was slower. Thus, the interfacial tension did not significantly change during the first minute and was nearly equal to that of the n-hexadecane/pure water interface. During this period, a relatively small amount of protein molecules (insufficient to reach monolayer coverage of the oil drop) is adsorbed. After this time, more protein molecules are adsorbed into the interface and hence the interface becomes more saturated with protein during aging. Thus, the interfacial tension is reduced until a plateau value is obtained.

The effect of protein concentration on the bulk phase on the interfacial dilational modulus is shown in Table 4. When increasing the protein concentration from 0.01 mg/ml to 1 mg/ml, the modulus at 60 min decreased from 12.16 ± 0.83 mN/m to 6.69 ± 0.58 mN/m, respectively. The difference in the interfacial dilational modulus is very significant among the protein concentrations in the bulk phase ($P = 0.001 << 0.05$). This phenomenon can be explained by the collapse of the charged hydrophilic amino terminal tail of casein, especially β-casein, into the aqueous phase followed by readsorption onto the interface as the drop was alternately compressed or dilated (Girardet *et al.*, 2001). These results are in agreement with Girardet *et al.* (2001): when the concentration of β-casein was increased from 1 mg/l to 15 mg/l, they observed that the modulus decreased from 20 mN/m to 8 mN/m, respectively.

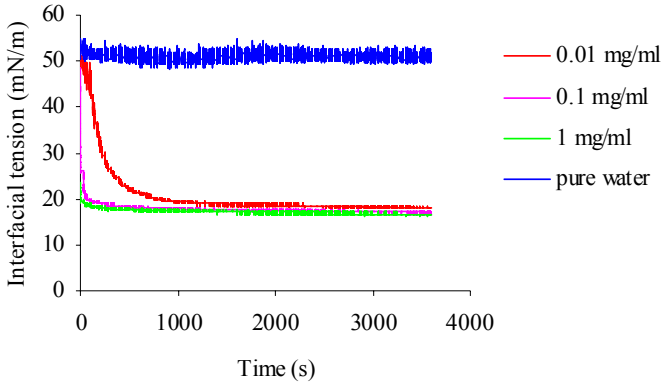

Figure 4. Effect of bulk concentration of sodium caseinate in acetate buffer (pH 6.0) on the interfacial tension on the n-hexadecane/water interface

3.4. Effect of calcium chloride concentration on interfacial properties on the n-hexadecane/water interface

An increase in ionic calcium concentration did not result in a variation of the interfacial tension as compared with a calcium-free protein solution (Figure 5), but caused a reduction in the interfacial dilational modulus (Table 5). The interfacial dilational modulus at different $CaCl_2$ concentrations has a slightly significant difference ($P = 0.038 < 0.05$). When adding 1, 10 and 20 mM $CaCl_2$ to 0.01 mg/ml sodium caseinate in acetate buffer, the modulus decreased from 13.51± 0.44 mN/m to 10.39 ± 2.21 mN/m and 9.62 ± 1.49 mN/m after 60 min, respectively (Table 5).

Table 5. Effect of the calcium chloride concentration on the interfacial dilational modulus at the n-hexadecane/water interface at 60 min (sodium caseinate concentration of 0.01 mg/ml in acetate buffer at pH 6.0) (n=3).

Concentration of $CaCl_2$ (mM)	Modulus (mN/m)	Elasticity (mN/m)	Viscosity (mN/m)
0	12.16 ± 0.83^{ab}	11.91± 0.84^{ab}	2.34 ± 0.58^{a}
1	13.51± 0.44^{a}	13.26 ± 0.37^{a}	2.61± 0.46^{a}
10	10.39 ± 2.21^{ab}	10.11± 2.14^{ab}	2.35 ± 0.64^{a}
20	9.62 ± 1.49^{b}	9.36 ± 1.53^{b}	2.08 ± 0.66^{a}

(Different superscript letters indicate a significant difference of 95% among the values at the various concentrations of calcium chloride added to 0.01 mg/ml sodium caseinate)

In general, an increase in calcium ions reduces the effective charge on the protein molecules as a consequence of counter-ion screening. The electrostatic repulsion between neighboring molecules is reduced and consequently adsorption and lateral interaction increase, which results in the reduction of interfacial tension and the increase in the interfacial dilational modulus. In fact the reverse was observed in these experiments. This phenomenon can be explained from the fact that the calcium chloride concentration (1 mM) added to the protein solution was not high enough to change the adsorption of protein molecules onto the interface. However, when calcium chloride significantly exceeds the critical concentration (10 mM), it results in the formation of aggregates. This aggregation would decrease the number of casein molecules available for adsorption. On the other hand, the binding of Ca^{2+} to casein partly collapses the adsorbed layer of casein around the oil drops, although the casein still provides a thick layer on the interface (Dalgleish, 1997).

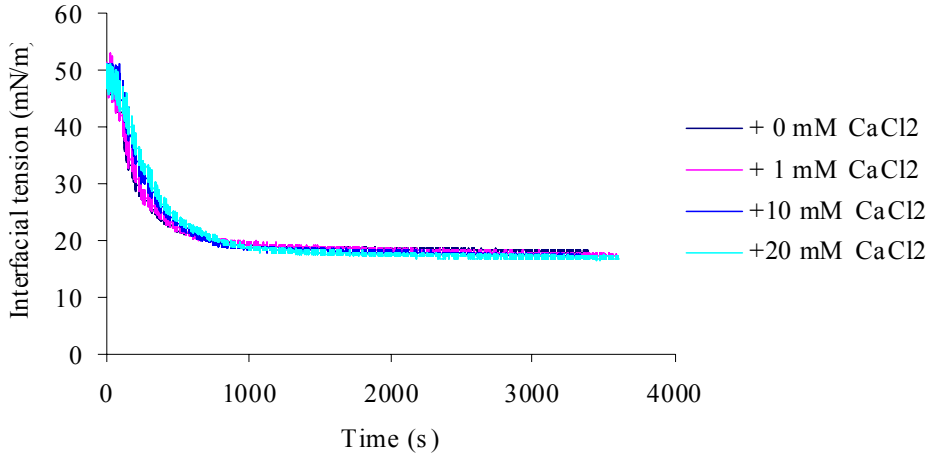

Figure 5. Effect of concentration of calcium chloride on the interfacial tension of 0.01 mg/ml sodium caseinate in acetate buffer (pH 6.0) on the n-hexadecane/water interface

4. CONCLUSION

The change in the interfacial tension and modulus were related to interfacial adsorption, interaction and configurational changes. The interfacial tension and modulus investigated were dependent on time, surfactant, concentration of protein and calcium ions. These experiments on interfacial properties have demonstrated that the interfacial tension of Tween 20 decreased with time more rapidly than sodium caseinate, followed by whey protein isolate, whereas the interfacial modulus was higher for whey protein isolate than that of Tween 20 and sodium caseinate.

Although the interfacial tension decreased more quickly with time for higher protein concentrations in the bulk phase, almost the same plateau values were obtained when aging for a long time (hours). In contrast, the interfacial modulus was higher for low protein concentrations, corresponding to 0.01 mg/ml, as compared to higher protein concentrations in the bulk phase.

The decrease in interfacial tension with time was not significantly affected by calcium, but the interfacial modulus decreased as the amount of $CaCl_2$ added to the protein solution was increased. As the coalescence stability of emulsions is known to be determined by the resistance of the interfacial film, it is affected by the presence of calcium ions: the stronger the interfacial film, the more stable the resultant emulsion. In practice, calcium ions may not always be avoided. Since calcium is important nutritionally, it has to be present in many food formulations. It is of course important to study an acceptable practical level of calcium that ensures emulsion stability during processing and storage.

REFERENCES

Beverung, C.J., Radke, C.J. and Blanch, H.W. (1999). Protein adsorption at the oil/water interface: characterization of adsorption kinetics by dynamic interfacial tension measurements. *Biophysical Chemistry* 81: 59-80.

Bos, M. A. and Van Vliet, T. (2001). Interfacial rheology properties of adsorbed protein layers and surfactants: A Review. Advances in Colloid and Interface Science 91: 437-471.

Burgess, D. J. and Ozlen Sahin, N. (1997). Interfacial rheological and tension properties of protein films. Journal of Colloid and Interface Science 189: 74-82.

Dalgleish, D.G. (1997). Adsorption of protein and the stability of emulsions. Trends in Food Science and Technology 8 (1): 1-6.

Girardet, J.M., Humbert, G., Creusot, N., Chardot, V., Campagna, S., Courthaudon, J.L. and Gaillard, J.L. (2001). Dilational rheology of mixed β-casein/Tween 20 and β-casein (f114-169)/Tween 20 films at oil-water interface. Journal of Colloid and Interface Science 243: 515-522.

MacRitchie, F. (1978). Protein at interface. Advances in Protein Chemistry 32: 283-326.

Rodriguez Patino, J.M., Rodríguez Niño, M.R., Sánchez C.C., Navarro García J.M., Rodríguez Mateo, G.R. and Fernández, M.C. (2001). The effect of temperature on food emulsifiers at fluid-fluid interfaces. Colloids and Surfaces B: Biointerfaces 21: 87-99.

Van der Meeren, P. (2005). Food Colloids. Ghent University, Belgium.

Wilde, P., Mackie, A., Husband, F., Gunning, P. And Morris, V. (2004). Proteins and emulsifiers at liquid interfaces. Advances in Colloid and Interface Science 108: 63-71.