THE INTERFACIAL AND EMULSIFYING PROPERTIES OF BLOOD PLASMA PROTEINS.

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INTRODUCTION

Blood plasma, derived from slaughterhouse blood by centrifuging off the red blood cells, is mostly used as an additive in different sausage formulations. It is distributed either as frozen or spraydried. The functionality of the blood plasma proteins in sausage products has been found to be good. For example a certain exchange of the meat proteins for blood plasma proteins gives a product, which retain its fat and water binding properties, as long as the temperature in the product is raised to 76°C.

It is well established that the plasma proteins have very good gelling properties (1-6), and the optimum in waterbinding properties of these gels is obtained at 74-80°C (3). Investigations of the emulsifying properties of blood plasma proteins have also been performed (1,7-10), and according to these studies the plasma proteins are considered to be good emulsifiers. However, the elucidation of proteins as emulsifiers is not straight forward, and several contributing factors during the emulsification process can give conflicting results, if not kept under control. Firstly, the emulsifying apparatus, intensity and time are dominating parameters in governing the final oil droplet size distribution of the emulsions formed (11), which in turn influences the emulsion stability. Therefore the emulsification process has to be accurately standardized in order to make any comparison between proteins regarding their emulsifying properties. Secondly, the amount of protein surrounding the fat globule (protein load) is largely determined by the type of protein used and by the protein/fat surface area ratio (11). This latter fact has to be considered in the emulsifying capacity measurements as used by most authors. In these measurements the maximum amount of fat emulsified by a protein dispersion of a certain protein content is measured. The experiment is performed by adding oil at a given rate to a protein dispersion being constantly stirred, until the emulsion inverts into a water-in-oil emulsion. It has been found that the solubility of the protein correlates very well with the emulsion capacity of the protein, expressed as ml oil/100 mg protein (8). This is what is to be expected, as mainly the soluble part of the protein acts as an emulsifier. One can also express emulsion capacity as ml oil/mg soluble protein in order to exclude the influence of the insoluble part of the protein. It still turned out to be the most soluble protein that had the greatest ability to emulsify fat (8). From these findings one can not deduce that the less soluble protein contains soluble protein with a lesser ability to emulsify fat, because the more soluble protein has a higher protein/fat surface area ratio during the measurements. This results mostly in a thicker protein membrane around the fat droplets for the more soluble proteins, if the molecular weight of the proteins is polydisperse (12). This is the case for most protein products. The thicker the interfacial layer of the protein the more probable it is that the emulsion will be stable.

Evidently, the emulsifying properties of proteins are very much dependant on the emulsifying conditions and on the protein concentration. Therefore these factors have to be controlled and according to this, a procedure has been worked out, where the emulsifying properties of a soy protein isolate, a sodium caseinate and a whey protein concentrate have been studied (11, 13). The aim of this work is to make use of this procedure to study the emulsifying properties of blood plasma proteins and to investigate the interfacial behaviour of blood plasma proteins at the soybean oil/water interface as compared to the proteins studied before (19).

MATERIALS AND METHODS

Bovine serum albumin (BSA) from Sigma Chemical Co. was used.

Frozen blood plasma from Ellco Protein, Kävlinge, Sweden was used. Analysis: protein (N6.25) 7.0 wt%, fat 0.5 wt%, salt 1.5 wt%.

Spray dried blood plasma from Ellco Protein, Kävlinge, Sweden was used. Analysis: protein (N6.25) 68.7% (dry wt), salt 18% (dry wt). Solubility in distilled water and in 0,2 M sodium chloride solution at pH 7, denoted as (0-7) and (0,2-7), is 99.4% in both cases.

The source, protein content and solubility in (0-7) and (0,2-7) for the soy protein isolate, the sodium caseinate and the whey protein concentrate (WPC) used throughout this study are given fully elsewhere (14).

A commercially available soybean oil (AB Karlshams Oljefabriker, Karlshamn, Sweden) was used. The analysis is given elsewhere (11).

SDS-Polyacrylamide gel electrophoresis was carried out according to Neville (15).

The interfacial tension decay of the proteins at the soybean oil/water interface as a function of time was monitored with an apparatus based on the drop volume technique. A description of the apparatus used (17) and the procedure worked out for measuring time-dependent interfacial tensions (18) have been given earlier. The temperature was maintained at 25±0.1°C.

Protein stabilized emulsions were made up of 40% (w/w) soybean oil and 60% (w/w) protein dispersion of varying protein content. A quantity of 50 gram was emulsified in a valve homogenizer or a sonifier incorporated into a recirculating system as previously described (21). The flow rate of the emulsion through the recirculating system was held constant at 250±25 ml min^{-1}, and the temperature of the system was 25±2°C during processing. The power and energy input during the emulsification process have been measured (21). The emulsions were stored for 24 hr at 20°C and thereafter characterized in terms of fat particle size distribution and amount of protein adsorbed per unit area of fat surface ($mg/_{m}2$) (14). The interfacial area of the fat particles expressed in m^2 fat/ml emulsion is derived from the particle size distribution according to calculations given elsewhere (22).

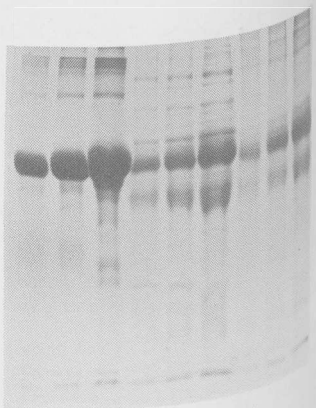

Figure 1. SDS-polyacrylamide gels showing the bands corresponding to 20, 40 and 80 µg of (from the left) BSA, frozen blood plasma and spraydried blood plasma

RESULTS AND DISCUSSION

SDS-Polyacrylamide gel electrophoresis

Separation of the proteins in BSA, frozen blood plasma and spray dried blood plasma can be seen in figure 1. The major protein in blood plasma is BSA, and this is consistent with the approximate content of 55-64% of albumin in blood as given by Feeney (16). The protein bands in the spray dried plasma are not as distinct as for the frozen plasma, and the amount of BSA has also decreased. Evidently, some changes occur to the proteins during spray drying, which also shows up when comparing the interfacial behaviour of the two differently treated plasma proteins (see below). Compared to BSA the two plasma protein products have a more polydisperse protein pattern, which is especially evident in the high molecular weight region. Besides albumin blood also contains lipoprotein 4-14% and immunoglobulines (1-10%), which are proteins of high molecular weight (16).

As a contrast sodium caseinate and WPC, both from milk, have a narrow molecular weight pattern. The pattern of soy protein isolate is more polydisperse but not as much as for the blood plasma protein.

Interfacial behaviour

The interfacial tension-time (γ-t) dependence resulting from the adsorption of BSA, frozen and spray dried plasma is presented in figure 2. The initial bulk phase concentration of the proteins was 10^{-2}% (w/w) based on the soluble content. They were dispersed in distilled water at pH 7 (0-7). In this concentration range the surface activity is highest for BSA, followed by frozen blood plasma and spray dried blood plasma, having the lowest interfacial depression effect. It is interesting to note that the difference in interfacial activity is greater between spray dried and frozen blood plasma than between frozen blood plasma and BSA. The small difference between the two latter proteins suggest that the serum albumin in the blood plasma contributes in this concentration range to a large extent to the surface activity of blood plasma. Tybor et al. (9) have shown that the solubility of the plasma proteins is reduced by spray drying, and our results show that also the interfacial activity of the proteins is altered during the spray drying operation.

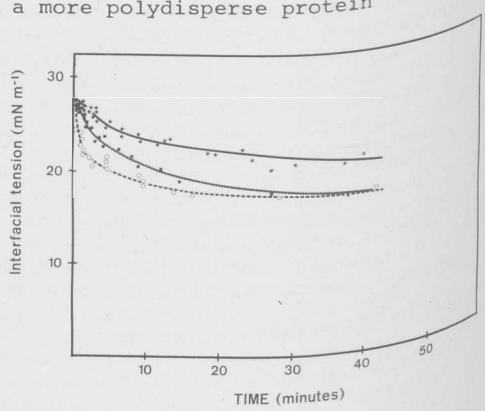

Figure 2. Time-dependence of interfacial tensions at the soybean oil-water interface for BSA (o), frozen blood plasma (■) and spraydried blood plasma (★) at (0-7) and at a subphase concentration of 10^{-2}% (w/w)

The concentration-dependence of the interfacial tension obtained at the soybean oil-water interface for the spray dried blood plasma at (0-7) and (0.2-7), the soy protein at (0.2-7) and (0-7), the sodium caseinate at (0.2-7) and (0-7) and the WPC at (0.2-7) and (0-7) can be followed in figure 3 (19). In this figure the surface pressure

tained after 40 minutes, $\pi_{40\ min}$ is plotted ainst the initial subphase concentration. The rface pressure, π, is defined as $\gamma = \gamma_0 - \gamma$, ere γ_0 is the initial interfacial tension. om the figure it can be stated for all the oteins studied that the addition of 0.2 M NaCl hances the interfacial activity over the whole ncentration range. Although the plasma proteins ntain 18% salt, which gives a salt concen- ation of 0.06 M in a 1% protein dispersion, ill salt addition has a large influence on eir surface activity. When salt is added the ectrical double layer surrounding the protein olloid is suppressed, and the electrostatic pulsion during adsorption and between the ad- rbed molecules is reduced. Therefore the otein molecules can pack more densly at the terface, causing a larger interfacial tension ecay.

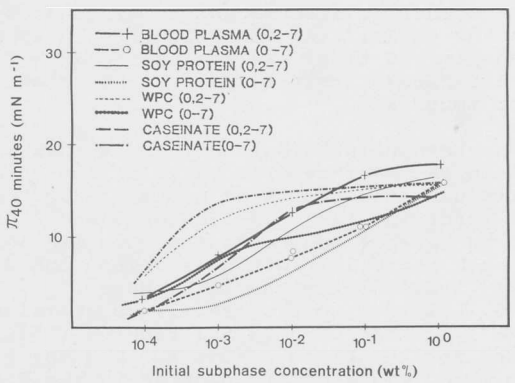

Figure 3. The surface pressure attained at the soybean oil-water interface after 40 minutes, $\pi_{40\ minutes}$, as a function of the initial subphase concentration for all the proteins studied at different ionic strengths (19)

mparing the concentration dependence of the terfacial tension for all the proteins studied he following can be stated. When salt is added, he sodium caseinate and the WPC have a curve rm similar to each other giving a plateau round 10^{-2}%, whereas for blood plasma and soy otein no such independence of concentration arts until a concentration of 1%. Furthermore, he interfacial tension decay caused by the milk oteins is much larger (caseinate always being superior) compared to the other two oteins at the lower concentrations, whereas the reverse is found at the highest concen- ations. This behaviour is especially pronounced for the blood plasma proteins, which ave greater surface activity than the soy proteins in the concentration range studied. ot only properties such as hydrophobicity, flexibility and charge density of the oteins, but also the average molecular weight, molecular weight distribution and the otein/surface area ratio influence the adsorption characteristics of the proteins. Cohen uart et al. (12) have concluded for polymer adsorption from polydisperse systems that as he polymer/surface area ratio increases a kind of fractionation occurs, with the high olecular weights on the surface and the lower molecular weights in the solution. The o-electrophoresis runs have shown that both the blood plasma and the soy proteins have a rger molecular weight and a wider distribution than the milk proteins. According to the eory outlined above this could explain the higher surface activity of the blood plasma nd the soy proteins at the very high concen- ations (large protein/surface area ratio). At he lower concentrations the importance of the drophobicity and the flexibility of the oteins increases in determining the interfacial activity. This might explain the superior inter- acial activity of the caseinate and the WPC, oth having higher hydrophobicity and being more lexible compared to BSA, having a more ordered tructure with 17 S-S-bridges and a helix content f 46%.

mulsifying behaviour

n figure 4 the fat surface area of the emulsions btained with 10 passes through the recirculating ystem are plotted versus power input during alve homogenization. The initial protein content f the continous phase was in all cases 2.5% (w/w) and the proteins used as stabilizers were pray dried blood plasma at (0-7) and (0.2-7) and aseinate (0.2-7). The latter protein was chosen s an example of non-flocculating emulsions, iving an almost linear increase of fat surface rea with power input, when valve homogenized (14). Tornberg (11, 14) has found that the fat urface area of a protein stabilized emulsion, roduced either in a sonifier or in a valve homo- enizer, is larger for flocculating than for onflocculating emulsions. By microscopical examination it could be stated that floccu- ation was observed around 40 W for blood plasma (0,2-7) and around 75 W for blood plasma (0-7). As can be seen in figure 4 these power consumptions coincide well with a sudden in- crease in surface area for the two curves representing blood plasma (0-7) and (0.2-7) tabilized emulsions. Otherwise the curves follow the more or less linear dependence with ower consumption.

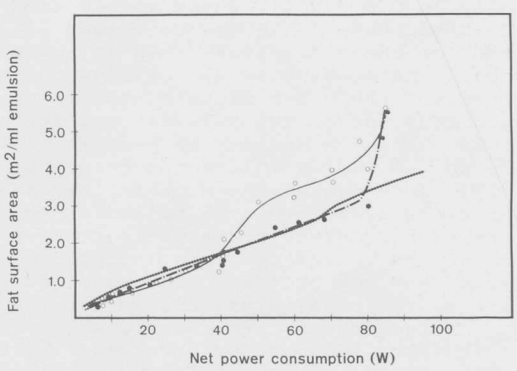

Figure 4. Fat surface area of protein stabilized emulsions as a function of net power input after emulsification with a valve homogenizer using 10 passes. Symbols: ○—○, Blood plasma (0.2-7), ●—●, Blood plasma (0-7), ••••••••••, Caseinate (0.2-7)

This further substantiates the findings by Tornberg (11) that the emulsifying conditions, such as emulsifying intensity and time, are dominating with regard to the final droplet size distribution, the choice of protein being of minor importance.

As has been pointed out throughout this article the protein/fat surface area ratio is a crucial factor in determining the amount of protein adsorbed on the fat droplets (protein load). In figures 5 and 6 protein load (mg adsorbed/m^2_{fat}) and percentage protein adsorbed from the bulk phase is plotted as a function of mg protein initially available in the bulk phase/m^2_{fat} (X) on a log scale. All the protein stabilized emulsions at (0-7) and (0,2-7) are included in the figures (13). The results obtained for the blood plasma and the soy protein (0,2-7) stabilized emulsion are plotted in the figures, whereas lines with error of limits are drawn for the other protein stabilized emulsions. Log X can be varied in two ways, either by having an initial constant protein concentration in the bulk phase and expanding the fat surface area by increased emulsifying intensity, or by varying the initial protein concentration and keeping the fat surface area more or less constant by the same emulsifying procedure. The former procedure has resulted in the lines with error of limits, whereas results obtained from the latter procedure are marked as points in the figures. The discrepancy between these two ways of making the emulsions will be discussed more thoroughly elsewhere (13). The main conclusion that can be drawn from this investigation is that the probability increases for a higher protein load when varying the protein content, due to more coalescence occuring during emulsification.

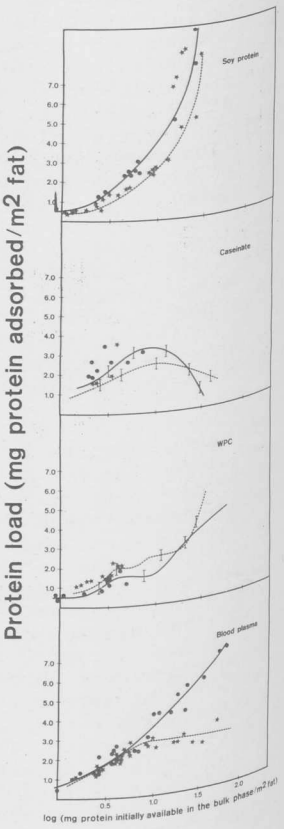

Figure 5. Protein load of all the protein stabilized emulsions at (0-7) (★---★) and (0.2-7) (●——●) as a function of log (mg protein initially available in the bulk phase/m^2 fat)

If we compare the different proteins in figure 5 at high log X, i.e. at a high protein/fat surface area ratio, very different behaviour is observed. The soy proteins, especially in 0,2 M NaCl, where aggregation of the proteins is favoured, have a high protein load ($\approx$10 mg/m^2), whereas for the caseinates it is as low as 0,5-1,5 mg/m^2. Moreover, the caseinates do not show the typical curve form observed for the other protein stabilized emulsions, where protein load increases or levels out for higher log X. There is instead a maximum in protein load around log X equals to 1,0. If we follow the theory of Cohen Stuart et al. (12) it suggests that protein load should increase with higher protein/surface area ratios as there is a preference for the higher molecular weight part of the proteins to adsorb at the interface. This is followed by the whey proteins, the soy proteins and the blood plasma proteins but not by the caseinates. Evidently, the caseinates have no high molecular part to adsorb, which further substantiate the proposal made by Tornberg (22) that it is the monomeric casein, which is in equilibrium with the casein aggregate, that migrates to the interface. Blood plasma (0,2-7) has a high protein load at large log X in between the soy proteins and the WPC. For blood plasma dispersed in distilled water the high level of protein load is not achieved, but instead the amount adsorbed stays around 2,8 mg/m^2 independent of log X.

The behaviour of the proteins at low log X can better be viewed in figure 6. There it can be seen that for the caseinates the percentage protein adsorbed increases with decreasing log X even to the lowest protein/surface area

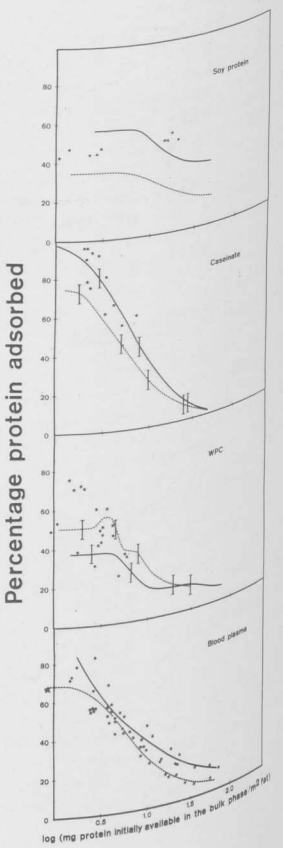

Figure 6. Percentage protein adsorbed from the bulk phase to the interface of all the protein stabilized emulsions at (0-7) (★---★) and (0.2-7) (●——●) as a function of log (mg protein initially available in the bulk phase/m^2 fat)

ratio, whereas for the soy proteins it levels out already at a log X around 1,0. This gives in this low log X-region thicker protein membranes for the caseinate stabilizedemulsions than for the soy protein stabilized emulsions. At high log X the reverse is found. The blood plasma proteins especially in (0,2-7) behaves more like the caseinates in the low log X-region. This is favourable, because the amount adsorbed for the blood plasma proteins is in this region higher than for the WPC and the soy proteins but less than for the caseinates. Thus, the blood plasma proteins seem to be good emulsifiers, anyhow when they are dispersed in 0,2 M NaCl solution, because comparatively thick protein membranes are achieved both at low and high protein/fat surface area ratios.

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