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EMULSION STABILITY OF MICELLAR CASEIN; THE EFFECT OF ENVIRONMENTAL FACTORS

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The stability of emulsion prepared wit micellar casein and peanut oil was affected by energy input, the concentration of protein dispersion, pH nad ionic strenght. At pH 6 the emulsion stability was lower than at higher pHs i.e. pH > 6. The percent of emulsion separation increased as protein concentration was decreasing. Increasing the energy input and ionic strength (NaCl) improved the stability of emulsions formed in the value homogenizer. The results indicate marked structural changes in the interfacial protein under the conditions studied.

INTRODUCTION

Studies on the functional properties of milk proteins have been conduced by many workers, the emulsifying properties being among the most important for many food applications [26].

An emulsion has generally been described as a system containing two immiscible liquid phases, one of which is dispersed in the other in the form of droplets varying between 0.1 and 5.0 μm in diameter. The phase present in the form of liquid droplets and/or liquid crystals is called the „internal" or „dispersed" phase; the matrix in which the droplets are dispersed is termed the „external" or „continuous" phase. A typical food emulsion is a macro-emulsion (mostly oil in water) and is thermodynamically unstable [19] compared to micro-emulsions (in pharmaceutical products or cosmetics) which are considered to be more stable thermodynamically [7].

In all emulsions, the interface between two phases becomes very large and its integrity is critical to the stability of the whole system. Proteins stabilize food emulsions because, being amphiphatic and flexible, they form films and impart greater stability to the emulsion than smaller non-protein amphiphiles (Tweens, monoglycerides) [3]. The reduction of surface tension at the oil: water interface is a major function of an emulsifier. This decreases the energy required to disperse the oil phase into droplets and counteracts the Laplace pressure which resists further disruption of oil globules during emulsion formation. However, the reduction of surface tension does not ensure emulsion stability. Proteins, which

by forming cohesive films around droplets provide barriers that bestow long range steric hindrance [6], without significantly decreasing surface tension, are better emulsifiers in terms of imparting stability to emulsions than smaller surfactants which function primarily by lowering surface tension. Proteins form a cohesive film possessing a mechanical strength which reduces the rupture of the interfacial film when pressure is applied [11]. Therefore, proteins are very suitable for forming and stabilizing o: w emulsions [17].

Of the various methods that are available for making emulsions, the ultra turrax, colloid mill, liquid whistle and value homogenizer are commonly used [22] and of these the valve homogenizer is the only instrument that allows control and monitoring of the energy input [12, 21].

The objective of this study was to investigate the effect of environmental factors (concentration of protein, pH, ionic strenght, energy input) on emulsion stability of micellar casein-stabilized emulsion.

EXPERIMENTAL SECTION

MATERIALS

Micellar casein was prepared from raw skim milk (Holstein). The milk was centrifuged at 4068 g for 20 minutes in a RC-5 Superspeed Refrigerated Centrifuge (Dupont Instruments Sorvall) at 20°C. After filtration (Whatman No 1) casein micelles were separated from skimmed milk by centrifuging at 45440 g for 20 minutes at the same temperature. Wet micelles thus obtained were freeze-dried. Protein was estimated according to a modified Lowry method [24].

Peanut oil (921 kg/m^3) was of commercial edible grade. All other reagents were of analytical grade and distilled deionized water was used throughout.

METHODS

Protein dispersion: the concentration of micellar casein used for emulsification was 1, 3 and 5%. Freeze dried micellar casein was dispersed in a freshly prepared milk salt solution (Jenness-Koops buffer) [14] at the required pH (6.0, 6.7, 8.0, 9.0). The concentration of NaCl in ionic strength experiments was 0.05, 0.10, 0.15%.

Emulsion formation: a sinle-piston recirculating vale homogenizer [21] with a spring-loaded entrance valve was used for all the emulsifications, under conditions as described [12]. The stroke volume, V_s, was adjusted to a mean stroke volume of 4.2 cm^3 (n = 20) and the homogenizer was run at a constant inlet pressure of 414 kPa (60 Psi) with a stroke rate of one stroke per 0.9 sec. Constant temperature (25°C) was maintained by using a heat exhanger coupled to a Neslab (Endocal) water bath ($\pm$0.1°C). Calibration of the homogenizer was achieved with distilled water to a mean maximum pressure drop (ΔP_{max}) of 3.44 MPa and a valve opening time of 3×10^{-2} sec for a mean stroke volume (V_s) of 4.2×10^{-6} m^3.

Emulsion sample preparation: the protein dispersion was pipetted into a Janke-Kunkel TP 18-10 turbo blender and allowed to equilibrate to the desired temperature (25°C). The required volume of peanut oil was then placed in the same blender, and after equilibration to 25°C the oil : water : protein mixture (20 cm^3) was blended for 5 sec at approximately 3000 rpm to form a coarse dispersion and immediately transferred to the feed hopper of the valve homogenizer. The homogenizer was deaerated by running ten strokes with the inlet valve open to expel all the air from the system with-out causing any movement of emulsion through the head. The inlet valve was then closed, and the emulsion was circulated for a specified number of times.

Emulsion stability: the stability of emulsion was determined by centrifugation. The emulsion (10 cm^3) was centrifuged in 15 cm^3 tubes using a IEC Clinical Centrifuge at 868 g (25°C) for 15 and 30 minutes. The emulsion stability was expressed in terms of the height of the separate layer as a percentage of the initial height of the emulsion.

RESULTS AND DISCUSSION

The caseins (α-$_s$, α-$_{s2}$, β-, γ-, $\varkappa$-casein) exist as spongy spherical micellar aggregates (average diameter 120 nm, molecular weight $2\text{-}18 \times 10^8$ daltons) containing from 2 to 3 g of water per gram of protein and a net charge of -18 mV. Each micelle is made up of submicellar particles (10 to 20 nm) containing hydrophobically associated α_s- and β-caseins [15]. These are apparently held in the micelle matrix by colloidal calcium phosphate and the micelles are colloidally, electrostatically and sterically stabilized by calcium cross-bridging

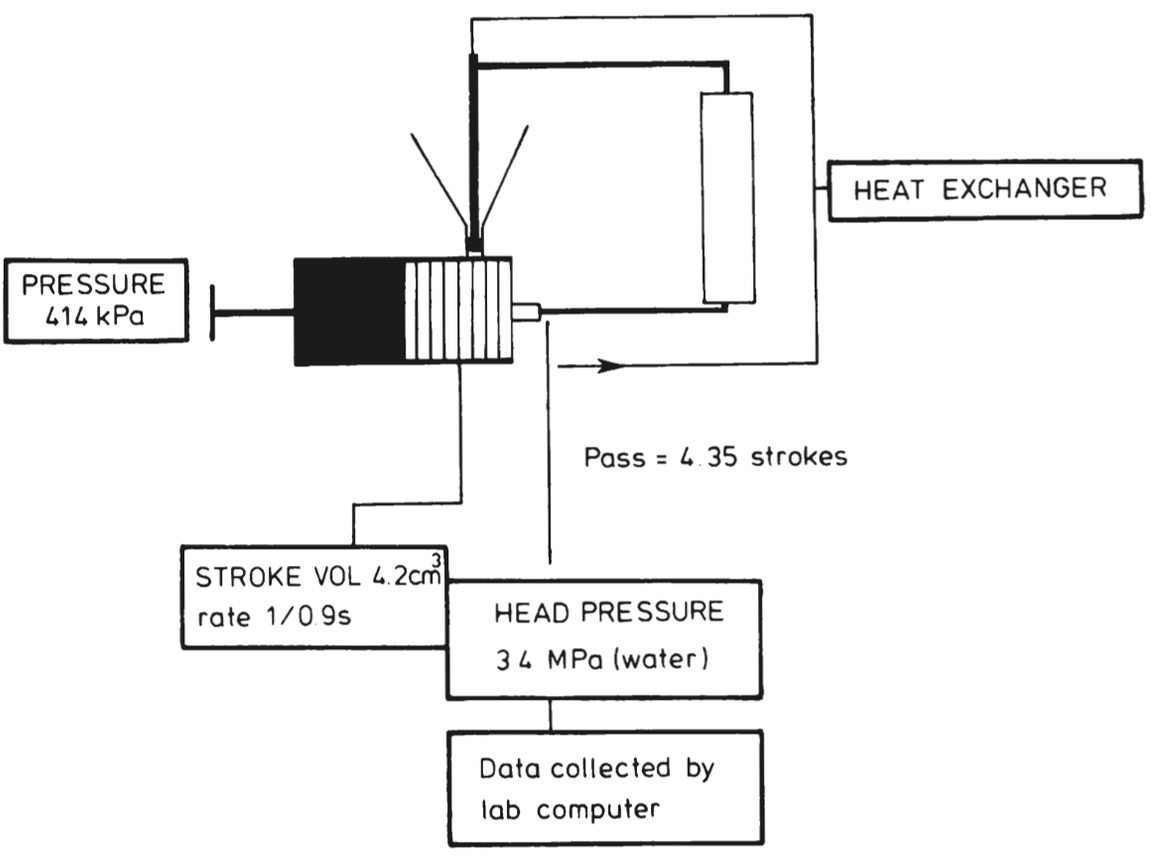

Fig. 1. Scheme of Emulsifying system

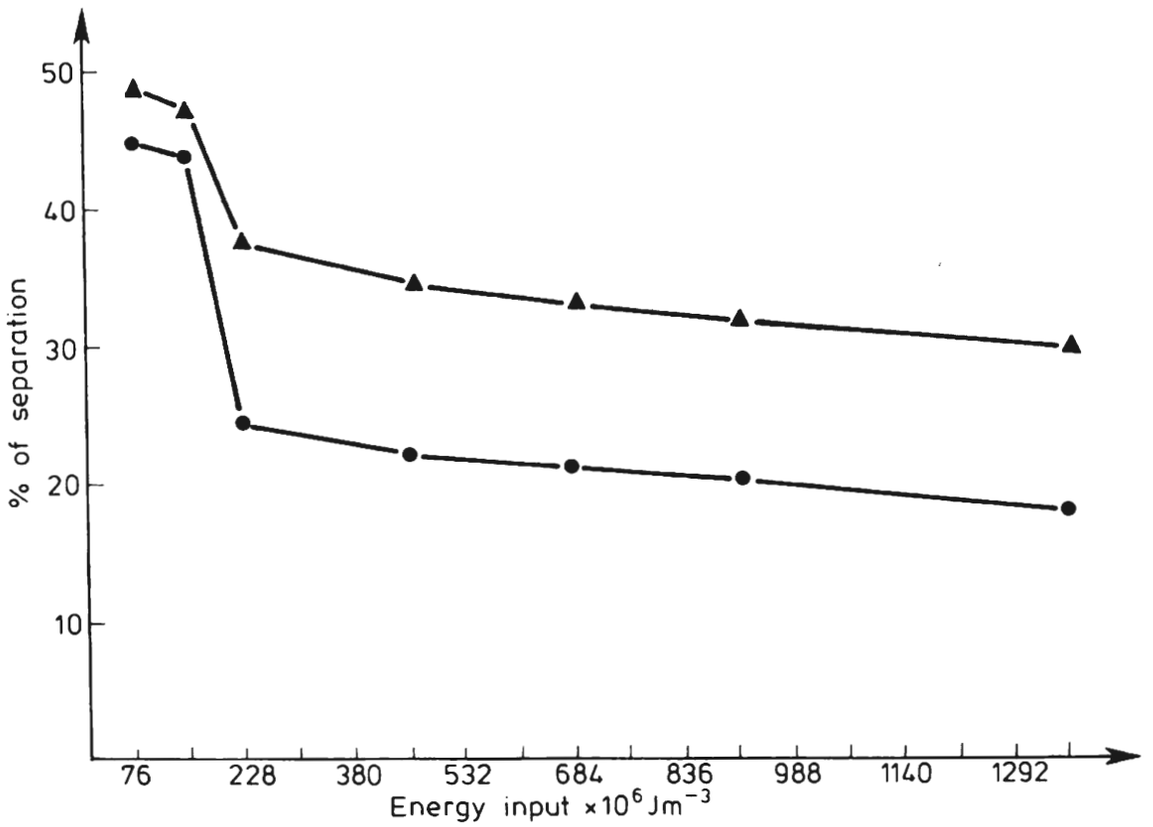

Fig. 2. Effect of energy input on stability of micellar casein emulsions. The o : w ratio was 4 : 6 and protein concentration in Jenness-Koops buffer was 3% at pH 6.7. Closed circle (●) and closed triangle (▲)denote 15 and 30 min of centrifugation, respectively

and a coating of χ-casein [2, 4, 5, 18]. Caseins posess hydrophobic domains that are discretely located in segments apart from the highly charged polar domains. Thus, caseins are remarkably dipolar and amphiphatic, which imparts good surfactant properties. Furthermore, caseins contain a relatively high frequency of prolyl residues which disrupt the secondary structures (α-helix, β-sheet) making caseins mostly random coil polypeptides, with a high degree of molecular flexibility and thus suitable for forming interfacial films. The highly charged regions are loose and highly hydrated [18]. In the formation of an emulsion, one questions the extent to which submicellar or monomers of casein dissociate at the interface. Presumably, the thermodynamics of the system are such that some monomers dissociate from the micelle and orient at the inerface, especially if the ionic strenght or pH is reduced. By virtue of their hydrofobic nature, caseins in solution tend to associate, however this is counteraced by their electronegativity, around pH 6 to 7. Thus, cations (e.g., calcium) facilitate association and depending upon the conditions (temperature, pH, chelating agents) the state of the casein micelle and its behavior in that system can be significantly affected. In designing model systems for evaluating the functional properties of casein micelles one must be aware of the factors affecting micellar stability.

EFFECT OF ENERGY INPUT ON EMULSION STABILITY

In this procedure, the protein concentration in Jenness-Koops buffer was taken at 3%, the pH at 6.7 and the oil volume fraction at 0.4. The emulsion stability of micellar casein-stabilized emulsions was plotted as a function of

energy input at constant level of power input (Fig. 2). Some features typical for all the protein-stabilized emulsions can be seen in this Figure: increasing the energy input improves the stability of the emulsions formed in the valve homogenizer.

Generally, when we used less energy in the process of emulsification a more separation was observed (45% of separation for 76 and 152×10^6 Jm^{-3}). The emulsion stability of freeze-dried micellar casein increased all the time with the degree of homogenization. In determining the characteristics of the formed emulsion the emulsifying time plays an important role. Prolonged emulsification gave more creaming stable emulsions. The time of emulsification, as reflected in an increased number of passes, causes in general an enhanced creaming stability of emulsions. This is in accordance with Walstra [23], who demonstrated that on repeated homogenization, the size distribution of milk becomes narrower and globule size smaller. Haque and Kinsella [13] showed that energy had a cumulative effect on the disruption of the dispersed oil phase. They concluded that repeated homogenization of the BSA emulsion under exactly identical conditions caused further disruption and decreased in the mean globule size (d_s). Gopal [8] states also that prolonging the emulsification beyond an optimum time interval does little to improve the quality of the emulsion.

EFFECT OF pH ON EMULSION STABILITY

In this procedure, the protein concentration in Jenness-Koops buffer was taken at 3%, the oil volume fraction at 0.4 and an energy input at 912×10^6 Jm^{-3}. The effect of pH on the stability of the emulsion was determined over a pH range

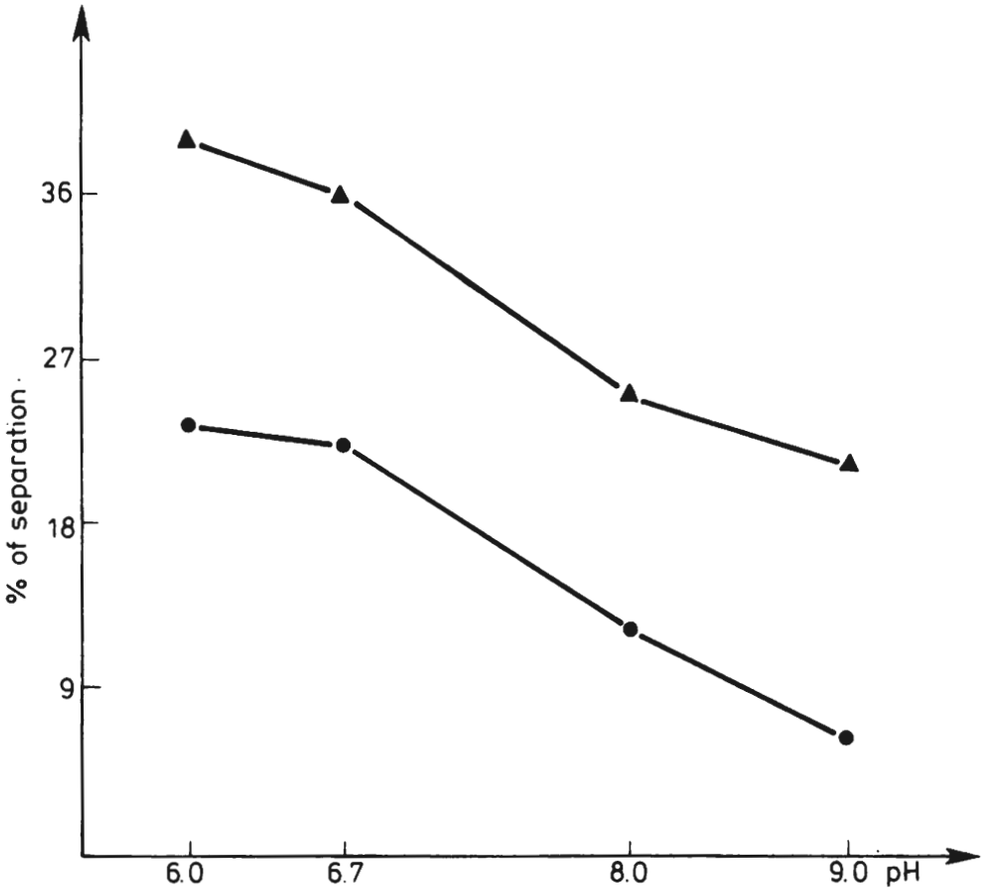

Fig. 3. Effect of pH on stability of micellar casein emulsions. Protein concentration in Jenness-Koops buffer was 3%, the o : w ratio was 4 : 6, energy input was 912×10^6 Jm^{-3}. Closed circle (●) and closed triangle (▲) denote 15 and 30 min of centrifugation, respectively

of 6-9. The results presented in Fig. 3 indicate that emulsion stability, expressed as a percent of separation, was minimal at pH 6 and that the alteration of pH from 6 to 9 increased the stability. At all pHs emulsion stability decreased with the time of centrifugation. Above pH 6.7, especially in the range of pH 8-9, the emulsions were resistant to separation.

Minimal stability of the emulsion at pH 6 suggests that stability strongly depends on the electrostatic nature of casein. At pH 6 the net charges of the protein are partly diminished and repulsive forces among the molecules are partly eliminated.

EFFECT OF PROTEIN CONCENTRATION ON EMULSION STABILITY

In this procedure, the pH was taken at 6.7 the oil volume fraction at 0.4 and energy input at 912×10^6 Jm^{-3}. Stability of emulsion strongly depended on the concentration of casein dispersion (Fig. 3). Generally, the percentage of separation of emulsion increased as protein concentration was decreasing from 5% to 1%. It was shown after 15 and 30 minutes of centrifugation. After 15 minutes of centrifugation the stability of the emulsion was very low when the casein concentration was 1% (48.78% of separation), whereas a stable emulsion could be formed by using 5% protein dispersion (0.50% of separation). Acton and

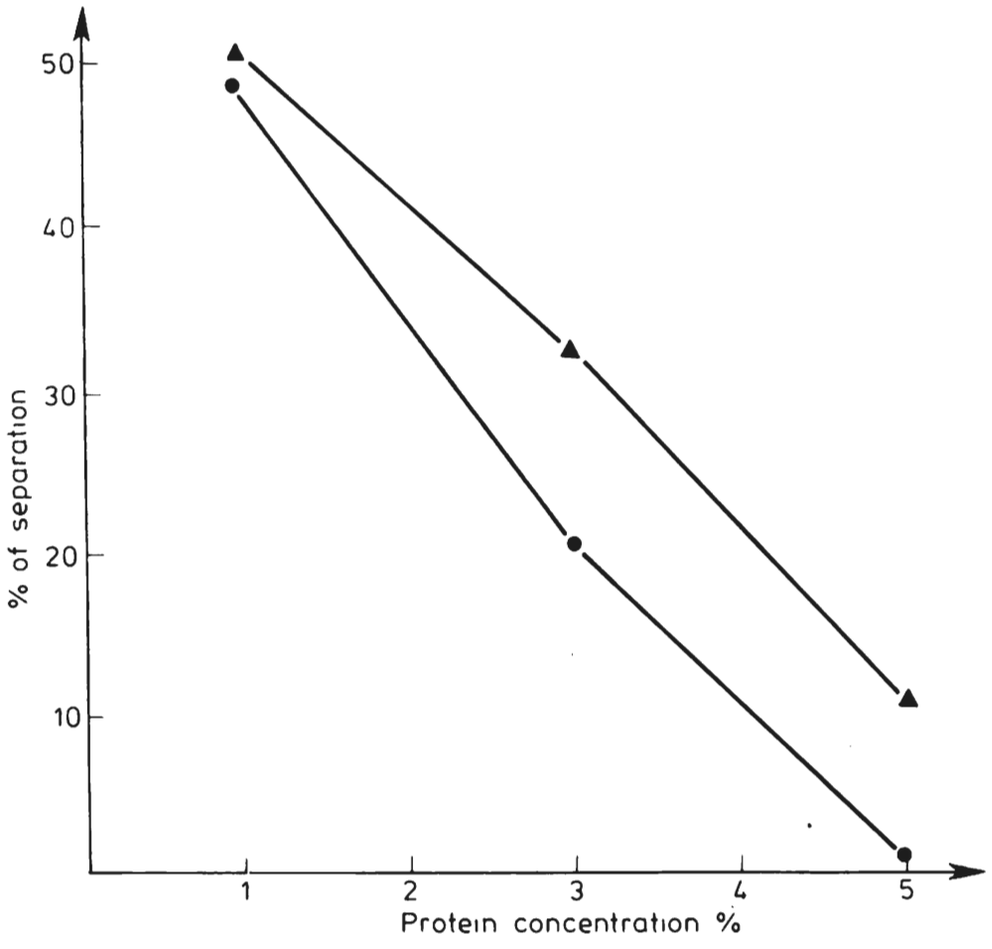

Fig. 4. Effect of protein concentration on stability of micellar casein emulsions. The o : w ratio was 4 : 6, energy input was 912×10^6 Jm^{-3} and pH was 6.7. Closed circle (●) and closed triangle (▲) denote 15 and 30 min of centrifugation, respectively

Saffle [1] have shown that in the case of meat proteins and corn oil emulsion the increase in protein concentration facilitates the adsorption of the protein and the increase in the adsorbed protein may cause a reduction of the interfacial tension which increases the emulsion stability. Yamauchi et al. [25] reported for emulsion of whey proteins and coconut oil that the amount of protein adsorbed on the fat globule surface was correlated to the concentration of the protein dispersion.

Graham and Phillips [9, 10] and Phillips [16] found that the viscosity of the continous phase increases with the increase of the number of suspended particles. Thus, this is one of the reasons why many emulsions are more stable in concentrated form than when diluted.

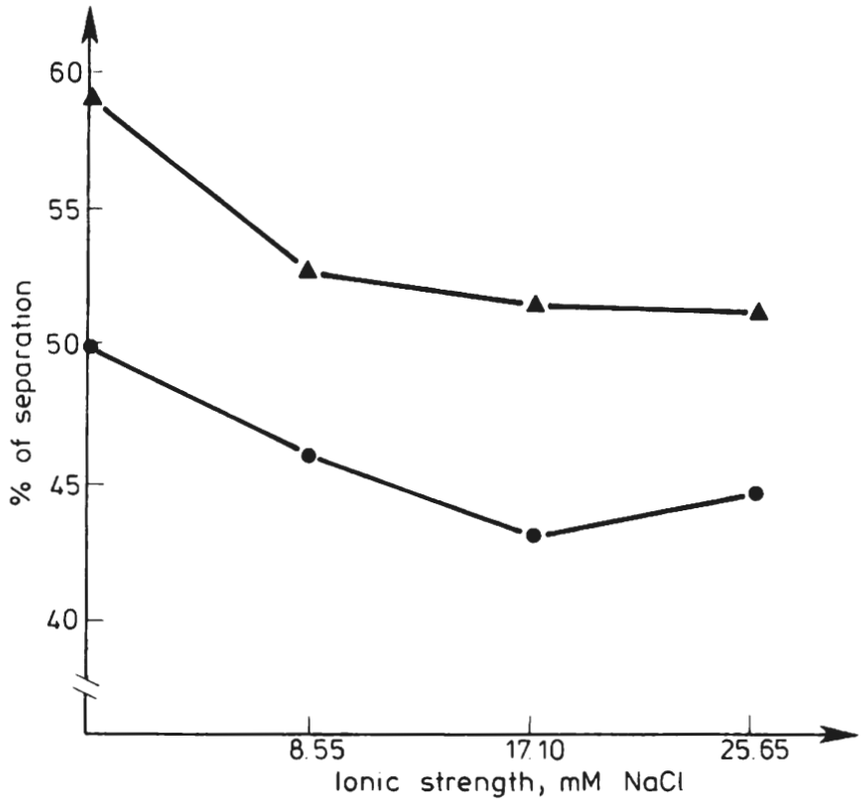

Fig. 5. Effect of ionic strength on stability of micellar casein emulsions. The o : w ratio was 4 : 6, energy input was 912×10^6 Jm^{-3}, protein concentration was 1% at pH 6.7. Closed circle (●) and closed triangle (▲) denote 15 and 30 min of centrifugation, respectively

EFFECT OF IONIC STRENGTH ON EMULSION STABILITY

Experiments were carried out in the presence of added NaCl (μ = 0-25.6 mM). The protein concentration in water was taken at 1%, the pH at 6.7, the oil volume fraction at 0.4 and energy input at 912×10^6 Jm^{-3} (Fig. 5). The stability of the emulsion was improved when NaCl was added. However, the beneficial effects of NaCl were lost at $\mu > 17.1$. The results suggest that charge masking by a small concentration of NaCl contributes to the stability of emulsion.

Tornberg [20] has found that the addition of NaCl up to 0.2 M reduces the creaming stability of the whey protein concentrates-stabilized emulsions, but improves it in the sodium caseinate-stabilized emulsions.

CONCLUSION

It is concluded that the stability of the emulsion prepared with micellar casein and peanut oil depended on the properties of the protein adsorbed onto the fat globule surface, which were affected by the concentration of protein dispersion, pH, ionic strength and energy input. The adsorption of micellar casein was remarkably influenced by pH, suggesting the importance of electrostatic nature and conformation of the protein in the adsorption at the oil : water interface. Our observations showed that emulsion stability of micellar casein increased markedly when pH changed from 6 to 9. The emulsion stability was the lowest at pH 6.

The percentage of emulsion separation increased as protein concentration was decreasing from 5% to 1%, and was decreasing all the time within the range of an energy input of $76\text{-}1444 \times 10^6$ Jm^{-3}. Kinsella [15] reported that during emulsification, proteins may be partially or wholly denatured. Thus, depending upon the type of protein, the stability may vary. Caseins, perhaps, are somewhat unique in that they have no tertiary structure and little secondary structure. Hence, extensive reemulsification does not cause excessive denaturation or overemulsification. Thus caseins or caseinates are useful in systems where repeated emulsification may be desirable.

Increasing ionic strength (NaCl) improved stability of the emulsion, particularly at NaCl 8.5-17.1 mM. At all concentrations of NaCl the stability was higher than it was for the control, even after 30 minutes of centrifugation. The results indicated that the conditions which favoured the increased surface area (i.e., smaller droplets) were associated with increased stability.

Scientists must continue research on the standard methods for determining emulsion stability. To minimize the time required to evaluate emulsion products we tried to use the centrifugation but the processes occuring during the centrifugation of the emulsion may be characteristic of those occuring in a stored or heated emulsion.

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STABILNOŚĆ EMULSJI STABILIZOWANEJ KAZEINĄ MICELARNĄ; WPŁYW CZYNNIKÓW ŚRODOWISKOWYCH

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Streszczenie

Określano wpływ nakładu energii, stężenia białka, pH oraz siły jonowej na stabilność emulsji stabilizowanej kazeiną micelarną. Stabilność emulsji badano po wirowaniu i wyrażano w procentach jako stosunek wysokości warstwy oddzielonej i wysokości początkowej emulcji.

Stwierdzono wyraźny wpływ analizowanych czynników na stabilność emulsji typu o/w stabilizowanej kazeiną micelarną. Zwiększenie nakładu energii do otrzymywania emulsji powyżej 228×10^6 Jm^{-3} znacznie poprawiało jej stabilność wskutek zmniejszenia średniego rozmiaru kropli, a tym samym zwiększenia powierzchni międzyfazowej. Dalszy wzrost nakładu energii w mniejszym stopniu wpływał na stabilność emulsji.

Stabilność emulsji była najniższa w środowisku o pH 6, wskutek zmniejszenia sił odpychania międzycząsteczkowego i zwiększała się waraźnie wraz ze wzrostem pH w zakresie 6-9, osiągając wartości 3-krotnie wyższe przy pH 9.

Stopień rozdziału faz w emulsji zwiększał się gdy stężenie białka w roztworze obniżało się; 5% stężenie kazeiny micelarnej w dyspersji zapewniało otrzymanie bardzo stabilnych emulsji typu o/w.

Dodatek NaCl w ilości do 17,1 mM poprawiał stabilność emulsji wskazując na maskowanie ładunków elektrycznych w środowisku o niewielkim stężeniu soli. Zwiększenie stężenia NaCl pogarszało stabilność emulsji.

Stwierdzone zależności wskazują na znaczne zmiany strukturalne białka międzyfazowego zachodzące podczas emulgowania w różnych warunkach środowiskowych.