should be 1.4% or less if the standards are assayed in duplicate (Table III). The over-all precision for the entire procedure was estimated to be 1.6%, if duplicate meat samples are used. Thus, duplicate assays should give a precision sufficient for most purposes.

The method described was used to determine relative mucoprotein content of representative muscles from different animals. The samples also were assayed for collagen and elastin, as well as total nitrogen (Table IV). Within muscles, mucoprotein hexosamine content paralleled elastin content very closely, while the correlation between collagen and mucoprotein was less consistent. The data indicate a negative correlation between the amount of the three connective tissue components and the relative tenderness of representative muscles within an animal.

The veal muscles were all considerably higher in elastin and mucoprotein (and in some instances, collagen) than corresponding muscles in the 2-year-old Hereford and Texas Longhorn steers. These results indicate that age may influence the content of these connective tissue components in skeletal muscle. Although not directly comparable, it is interesting to note that Shetlar and Masters (*18*) found the acid mucopolysaccharide content of human cartilage to be maximum in the fetus and new born, and to decrease with age.

The close correlation which appeared to exist between mucoprotein hexosamine content and the other connective tissue components is consistent with results of studies reported by Banga and Baló and others (*4*), which indicate that mucoprotein is intimately associated with both elastin and collagen fibers.

The data reported represent results from single animals and therefore do not constitute conclusive evidence for the relationships mentioned above. However, the data do show trends which should be investigated further, using more animals.

The results of this study indicate that the mucoprotein fraction of skeletal muscle is indeed worthy of consideration in the assessment of the relation of connective tissue to meat tenderness. The data suggest that the inherent tenderness of a given muscle may be influenced by its mucoprotein, as well as by collagen and elastin contents.

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INTERFACIAL ENERGIES IN MILK

Influence of Milk Proteins on Interfacial Tension between Butter Oil and Various Aqueous Phases

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THE INTERFACE between the fat and aqueous phase of milk is in a state of dynamic equilibrium (*7*). During the handling and processing of milk, there is evidence that the interfacial constituents migrate to the plasma (*4, 8, 11*) and vice versa (*6*). Unfortunately, little is known about the fat-plasma interface in milk, and any information pertaining to the composition and arrangement of the constituent components at this interface would contribute to the resolution of this problem.

To gain such information, this study utilized an indirect approach of comparing the influence of milk proteins on the interfacial tension between butter oil–water and butter oil–protein-free milk plasma. The energy-reducing activity exhibited by the proteins in the model systems used in this study indicates a preferential order of adsorption of the proteins by a milk fat surface.

Materials

Butter Oil. Freshly churned butter was washed with an equal volume of water at 50° C. and separated in a Model 619 DeLaval cream separator. This operation was repeated four times with subsequent centrifugation at 25,000 × g until an absolutely transparent oil was obtained.

Protein-Free Plasma. Two liters of distilled water in cellulose casings were dialyzed against 38 liters of skim milk for 48 hours. The dialyzate was protein-free but otherwise identical to the aqueous system of milk (*1*). The model systems which used protein-free plasma as the aqueous phase presented a native environment in which to study the interfacial behavior of milk proteins.

Protein:

Prepared Proteins. κ-CASEIN, prepared by Fox's method (*2*).

MONODISPERSED CASEIN. Calcium caseinate was centrifugally sedimented from skim milk and washed twice with protein-free plasma. Each washing consisted of completely dispersing the casein in protein-free plasma prior to centrifugation. A constant volume was maintained throughout the preparation. The protein not sedimented after the second washing was taken as monodispersed casein. All separations were completed in a gravitational field of 44,330 × g for 120 minutes.

This study was conducted to gain information regarding the fat-plasma interface in milk and the composition and arrangement of the constituent components at this interface. The interfacial activity of the large casein micelles of milk is low. The individual whey proteins are influenced by the salts of milk, the albumins and globulins showing interfacial behavior with group similarities. A protein isolated from the natural interface in milk reduced the interfacial tension more than any other protein used in this study. In protein-free plasma, the preferential order of adsorption of the proteins at the butter-oil interface provides an index of probability which can be used to predict the presence of individual proteins on newly created fat surfaces in milk. Such information would be useful in determining changes in interfacial composition as influenced by milk processing.

INTERFACE PROTEIN, prepared by a modified method of Herald and Brunner (*5*). This protein corresponds to the soluble membrane fraction but is more homogeneous, because of a single extraction with dilute salt solution rather than the exhaustive procedure recommended by these authors. The complete method of isolation will be presented in a future publication dealing solely with the interface protein.

β-LACTOGLOBULIN, prepared and crystallized from solution four times as described by Gordon, Semmett, and Ziegler (*3*).

Purchased and Procured Proteins. BOVINE SERUM ALBUMIN. A crystalline preparation purchased from Armour and Co., Chicago, Ill.

α-CASEIN, β-CASEIN, α-LACTALBUMIN, AND THE IMMUNE GLOBULINS, prepared and made available by the Animal Proteins Section, Eastern Regional Research Laboratories, Wyndmoor, Pa.

Methods

All interfacial tension measurements were made with a Cenco-duNouy 70540 Tensiometer (*12*), modified by replacing the sample table with a Model 19089 Cenco-Lerner lab-jack, which provided more rigid support for the vessel in which the measurements were made and more precise movement of the platform in a vertical plane. The vessel was cylindrical and made entirely of glass to permit observation of the ring and the interface at all times. It was designed with an internal diameter of 3 inches, which allowed ample room to make measurements with disregard to meniscus effects. A half-inch glass jacket permitted circulation of water from a large bath and thereby maintained selected temperatures in the measuring chamber within 1° C.

Results and Discussion

There is a paucity of data in the literature dealing with the interfacial tension activity of individual, purified milk proteins. Some of the data reported by Mohr and Brockmann (*9*) and Powell (*10*) are compared in Table I with values obtained in this study.

The calcium caseinate system in milk comprises approximately 80% of the total protein. If this concentration advantage is coupled with a high interfacial activity, casein would undoubtedly dominate the composition of adsorbed proteins on new fat surfaces in milk. In Figure 1, curve *A* shows the effect of removing calcium caseinate on the interfacial tension of butter oil and skim milk. Curve *B* is a plot of the removal of sedimentable nitrogen (read on right ordinate) with time in a gravitational field of 14,830 × g. A comparison of *A* and *B* shows that while removing 97% of the sedimentable nitrogen, the change in interfacial tension was limited to less than 2 dynes cm.$^{-1}$ Since the larger casein micelles comprise the bulk of the protein being removed, the small change $\left(\frac{d\gamma}{dt}\right)$ in curve *A* indicates that the large

Table I. Comparison of Interfacial Tension Measurements

(Dynes cm.$^{-1}$ at 40° C.)

	Mohr and Brockmann, 1930	Powell, 1932–34	Jackson and Pallansch, 1960
Butter oil–water	22.6[a]	25.5[b]	19.2[a]
Butter oil–skim milk	14.1[b]	14.5[b,c]	14.6[a]

[a] Ring method.
[b] Drop-weight method.
[c] Taken from graphical data.

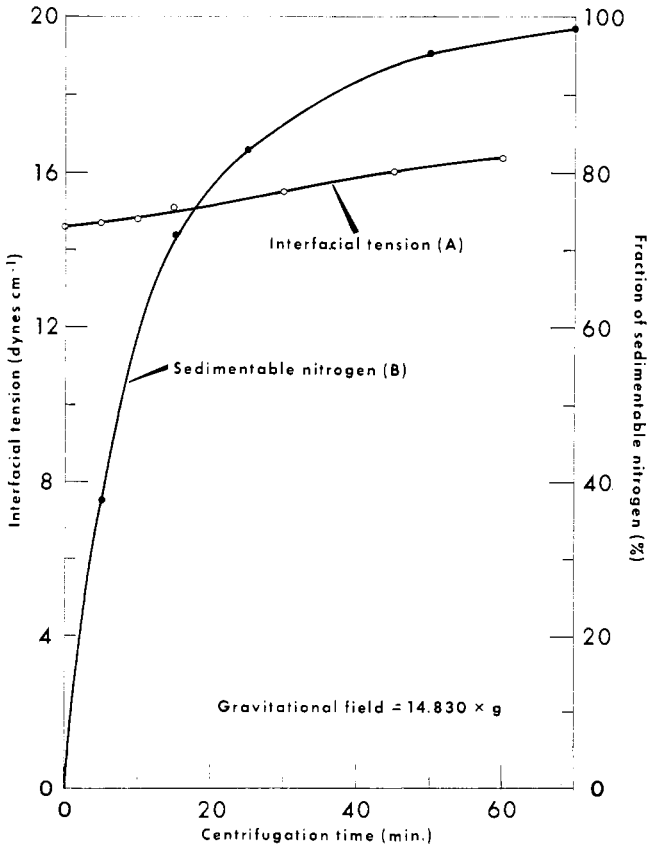

Figure 1. Effect of calcium caseinate removal on butter oil–skim milk interface at 40° C.

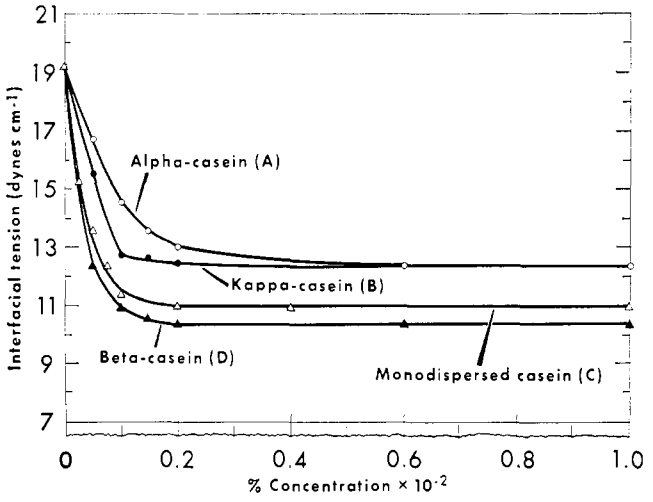

Figure 2. Caseins at butter oil–water interface at 40° C.

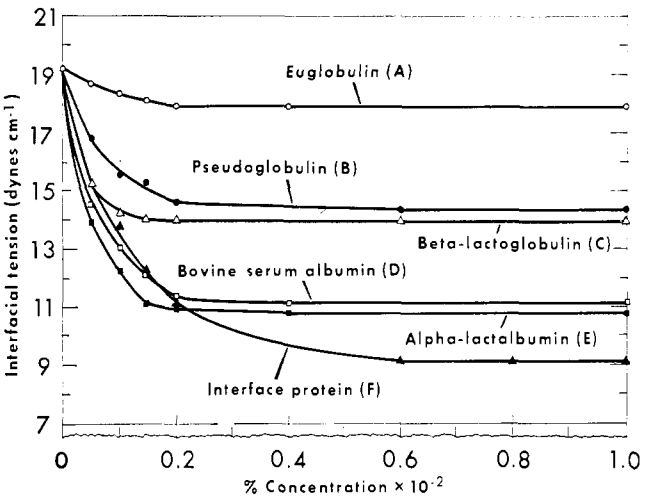

Figure 4. Plasma proteins at butter oil–water interface at 40° C.

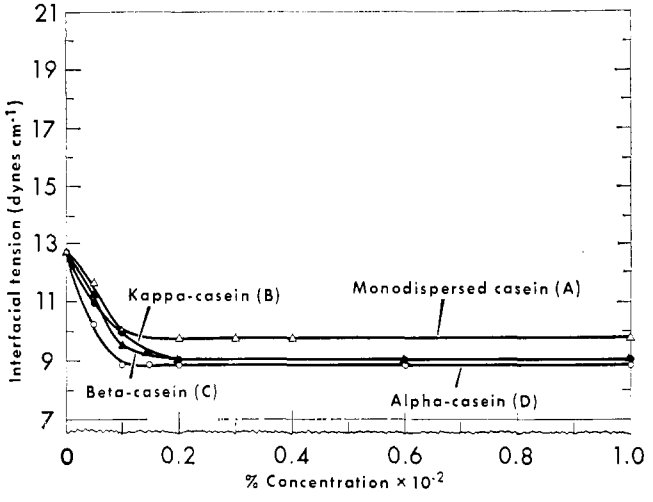

Figure 3. Caseins at butter oil–protein-free plasma interface at 40° C.

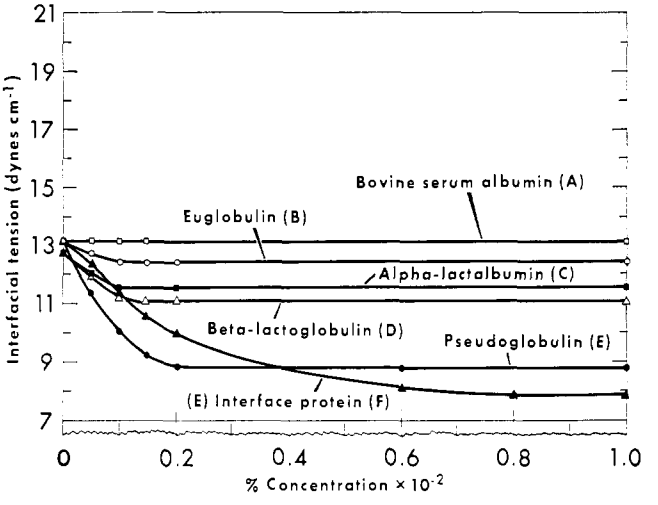

Figure 5. Plasma proteins at butter oil–protein-free milk plasma interface at 40° C.

micelles do not appreciably depress the free energy at the butter oil–skim milk interface and would not be expected to concentrate there.

In a system of butter oil and water, Figure 2 compares the α-, β-, and κ-casein moieties of isoelectrically precipitated casein and the monodispersed casein isolated from calcium caseinate. The monodispersed casein was relatively interfacially active in this system and presented a curve which is entirely unique from any of the moieties prepared from isoelectrically precipitated casein.

Figure 3 shows a different order of levels of reduction of interfacial tension by the same proteins shown in Figure 2. Measurements for Figure 2 were made in a butter oil–water system, while those for Figure 3 were made in a butter oil–protein-free plasma system. All the proteins in the latter system depressed the interfacial tension to a lower level than in the former system. Even more obvious is the grouping of the α-, β-, and κ-casein values. Monodispersed casein again maintained a unique curve. The difference in the interfacial tension at zero protein concentration of butter oil–water (Figure 2) and butter oil–protein-free plasma (Figure 3) was attributed to low molecular weight materials which were capable of passing through the cellulose casing in the preparation of the protein-free plasma.

As a group, the plasma proteins showed more divergent interfacial behavior than the casein family, especially at the butter oil–water interface (Figure 4). Euglobulin (curve *A*), pseudoglobulin (curve *B*), and β-lactoglobulin (curve *C*) behaved ineffectively, whereas by comparison, bovine serum albumin (curve *D*) and α-lactalbumin (curve *E*) markedly reduced the interfacial tension. The deficiency of ions in the system is probably responsible for the similarity in the group behavior of the albumins and the globulins. This seems logical, since the differentiation of these two classes of proteins is based on their solubility in dilute salt solutions. The interface protein (curve *F*), which was isolated from the natural interface of milk, reduced the interfacial tension more than any other protein used in this study.

Figure 5 shows the interface protein to be even more effective at reducing the free energy at the interface in protein-free plasma than in water. The comparative tension-reducing ability of the globulins increases, while that of the albumins decreases, especially bovine serum albumin (curve *A*). Bovine serum albumin is known to react readily with a variety of ions, which may explain its poor interfacial activity in the ion-rich, protein-free plasma.

Figure 6 shows the effect of hydrating bovine serum albumin and measuring its interfacial activity in the presence of lactose (curve *B*) and the ash of milk (curve *C*). Although the salt system in milk is not truly represented by milk ash, the concentration of ash used in this experiment approximates the concentration of the salts in milk. Curves *A* and *B* were taken from the previous two

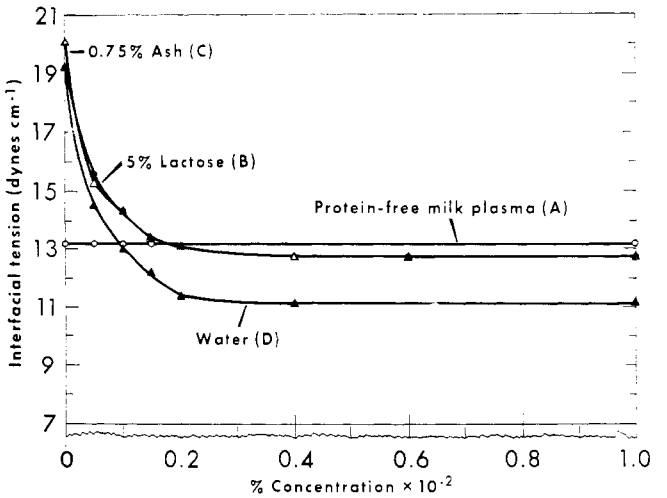

Figure 6. Bovine serum albumin in various aqueous phases at 40° C.

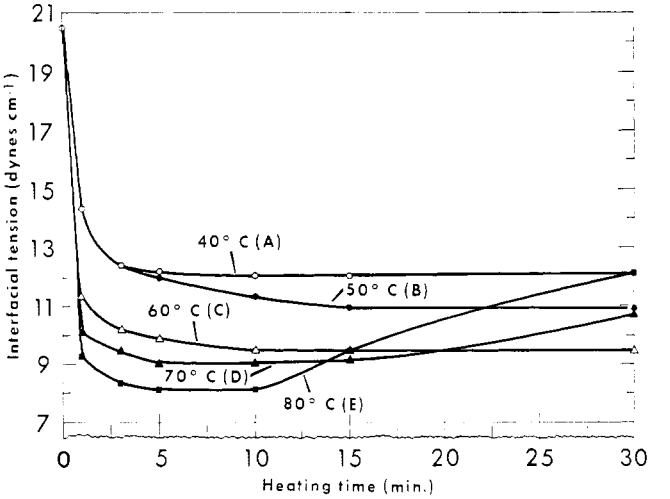

Figure 7. Effect of heat on 0.01% β-lactoglobulin at butter oil–water interface

figures for purposes of comparison. Curve *A* shows the behavior of bovine serum albumin in protein-free plasma and curve *D* shows the activity of the same protein in water. It would appear that bovine serum albumin loses its high capacity to reduce free interfacial energy in the presence of the salts and principal carbohydrate of milk, both of which influence the behavior of bovine serum albumin. This trend is shown by the levels of curves *B* and *C*, both approaching that of curve *A*, which was drawn from measurements in protein-free plasma.

Figure 7 shows the effect of heat on the interfacial activity of heat-labile β-lactoglobulin at the butter oil–water interface. A trace of NaCl was used to induce the solubilization of this protein initially. Interfacial tension was decreased with increasing temperatures at any given protein concentration. This did not hold true at 70° and 80° C. (curves *D* and *E*), where 10 minutes at these temperatures caused the interfacial tension to increase. After a few minutes, the solutions in the measuring vessel began to appear cloudy, because of the heat-denatured protein. Perhaps as the protein began to aggregate, its ability to maintain a reduced interfacial tension was diminished.

Conclusions

The data presented demonstrate the capacity of purified milk proteins to reduce the free energy in model systems of butter oil–water and butter–oil protein-free milk plasma. In protein-free plasma, the preferential order of adsorption of the proteins at the butter oil interface provides an index of probability which can be used to predict the presence of individual proteins on newly created fat surfaces in milk. Certainly, these predictions must be tempered by such factors as the relative concentrations of the milk proteins.

The protein isolated from the fat-plasma interface in milk is present in a minor concentration, but this study shows it to have such unique energy-reducing properties that it may be predicted to remain with the fat phase throughout normal processing changes of temperature and pressure.

β-Lactoglobulin had increasing energy-reducing properties with increasing temperatures to the point of denaturation, whereupon its interfacial activity was depressed. As a group the globulins were more effective than the albumins at reducing interfacial energy in an aqueous medium of protein-free plasma. The reverse was true when water was used as the aqueous medium.

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