Relationship between the Method of Obtention and the Structural and Functional Properties of Soy Protein Isolates. 2. Surface Properties

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The surface properties of soy protein isolates that had been exposed to different neutralization conditions (pH 7 or 9), thermal treatments (98 °C, 5 and 30 min; 80 and 92 °C, 6 and 12 min), reduction conditions (0.05% Na_2SO_3; 0.05% Na_2SO_3–6 M urea or 2% Na_2SO_3), and enrichment on 7S or 11S fractions were studied. These treatments affected the 7S and 11S contents, degree of denaturation and aggregation, surface hydrophobicity, content of free sulfhydryl groups, etc. The behavior of isolates was evaluated by means of parameters that provide information about the different stages in the emulsion formation and stabilization processes. It was found that isolates having the best emulsifying properties were those exposed to a short thermal treatment, and the isolates chemically reduced in the presence of Na_2SO_3 and urea have a high surface hydrophobicity and solubility. Better emulsifying properties were found in isolates at pH 9 than at pH 7, as well as in those enriched in 7S. Electrophoretic analysis of the remaining components in the aqueous phase showed a lower migration to the emulsion interface of A–11S polypeptide than the rest of protein species (α, α′, and β–7S, and B–11S polypeptide). Nondialyzed isolates were better foaming agents than dialyzed isolates. Modifications of either 7S or 11S content had no effect on the foaming properties.

Keywords: *Surface properties; soy proteins; protein functional properties*

INTRODUCTION

The ability of a protein to act as an emulsifying and/or foaming agent depends both on its rate of migration to the interface and on its ability to form a stable film; structural requirements are different, however, in both cases, according to the function desired. The process of foam formation is slower than that of emulsification, since air is incorporated as migration toward the interface takes place, and is followed by the partial denaturation of the protein at the interface. Moreover, for a stable lamella to be formed, no electrostatic repulsions among the components of the film should occur (Poole, 1989). The more structured the protein is, the more rigid and cohesive the film formed will be (Graham and Phillips 1979a–c). The emulsifying properties of a protein depend on two factors: (a) the ability to reduce the interfacial tension because of its adsorption to the interface; (b) the ability to form a film which would act as an electrostatic, structural, and mechanical barrier. Emulsion formation depends on a fast desorption, unfolding in the interface, and reorientation, whereas stability is determined by a decrease of the interface free energy and also by the film rheological properties. The latter result from the hydration degree and the intermolecular interactions (Damodaran, 1989).

Methods currently used to measure the emulsifying properties of a protein characterize only some of those properties, so that the use of additional methods thus becomes imperative. Evaluation of the emulsifying activity index (Pearce and Kinsella, 1978) gives information mainly on the ability of the protein to become dispersed in the oil phase and to quickly provide enough coating on the interfacial area to avoid immediate coalescence. The emulsion is blocked at an initial step by SDS addition; then, not enough time is available for penetration and rearrangement at the interface. Instead, when determinations of the emulsifying capacity are evaluated, both the absorption and the mechanical resistance of the film are determined, since the system undergoes continuous deformation. The measurement of emulsion destabilization according to the method of Dagorn-Scaviner et al. (1987) evaluates processes of flocculation and creaming and, to a certain extent, also coalescence when the protein interfacial area had not been completely coated during homogenization. Such an instabilization depends on the different viscosities of both phases, the hydration capacity, the electric charge, and also on the viscoelastic properties of the film. The rate of adsorption is also evaluated by this method, since, if coalescence is to be avoided, it becomes necessary that the interfacial surface be completely coated by the protein. Instead, when the stability is determined by the centrifugation method, the mechanical resistance of the film is mainly evaluated.

In the present study, the behavior of different soy protein isolates obtained in our laboratory (Petruccelli and Añón, 1994) as emulsifying and foaming agents is analyzed. The relationship between structural characteristics and the capacity of interface formation is studied as well.

MATERIALS AND METHODS

Preparation of Isolates. Isolates were prepared according to the conditions described in the previous paper (Petruccelli and Añón, 1994).

Emulsifying Properties. *Measurement of the Emulsifying Activity.* Emulsifying activity (EAI) was measured according to the method of Pearce and Kinsella (1978). To this end, 6 mL of protein solution (1.0 mg/mL) was dispersed in 0.1 M phosphate buffer, pH 7, with 2 mL of sunflower seed oil in an

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Omnimixer for 30 s. The formed emulsion was immediately diluted with a stabilizing solution containing 0.1% SDS and 0.1 M NaCl, pH 7. Two serial dilutions, 1/10 and 1/25, were rapidly prepared; care was taken to spend the same length of time to prepare each dilution in all samples. The optical density of the last dilution was then recorded at 500 nm. Two emulsions were prepared from each sample; quadruplicate dilutions were done.

Destabilization by Flocculation–Creaming. This was carried out as described by Dagorn-Scaviner et al. (1987). The emulsion was prepared as described for the determination of the emulsifying activity; immediately after preparation, the emulsion was poured into a 10 mL cylinder. Separation into the aqueous phase and the cream phase was observed. Volumes of the aqueous phase were recorded at different times. Zero time corresponds to the end of pouring. Intervals between readings depended on the rate of separation of the two phases. The volume of the aqueous phase in equilibrium after 24 h of emulsion preparation was recorded. Determinations were made in duplicate or more. The destabilization process is close to a first-order kinetics, according to the equation

$$\ln[V_e/(V_e - V_t)] = kt \quad (1)$$

in which V_e is the volume of the aqueous phase at equilibrium (24 h), V_t is the volume of aqueous phase at time t (mL), and k is the destabilization rate constant (s^{-1}).

The line obtained when $\ln V_e(V_e - V_t) = f(t)$ is plotted represents a succession of segments, showing first-order kinetics. Slopes of these segments give the rate constants of the different steps of the flocculation–creaming process. Each step of the process can be considered approximately a straight line, but, to simplify the analysis of these curves, two straight lines were arbitrarily taken: the first line evaluates the destabilization during the first 500 s and the second line, the period ranging from 500 to 2500 s. Both slopes were determined by linear regression analysis.

Parameters used to characterize the emulsion are as follows: V_e, volume of aqueous phase in equilibrium; V_i, interstitial volume (water in cream phase); ϕ, volumetric oil fraction (oil volume/emulsion volume); k_1, rate of flocculation–creaming during the first 500 s (s^{-1}); and k_2, rate of flocculation–creaming between 500 and 2500 s (s^{-1}).

Determination of the Emulsion Destabilization by Coalescence. The emulsion was prepared as described previously. It was then centrifuged at 12000*g* for 90 min. The oil was separated and determined by weighing. When the amount of oil released was small, it was absorbed with filter paper; otherwise, it was removed by using a Pasteur pipet.

Determination of the Protein Remaining at the Interface. The amount of protein remaining at the interface, once the coalescence had been determined, was measured according to the method of Lowry et al. (1951). The protein remaining at the interface was obtained as the difference between the protein dissolved and that remaining in the aqueous phase after coalescence. An electrophoretic run was performed to verify whether the protein remaining in the aqueous phase holds preferentially a certain component of the interface. Duplicate determinations were carried out.

Foaming Properties. *Determination of the Foaming Capacity.* Solutions containing 1.5 mg/mL of the isolate were prepared in 0.1 M phosphate buffer, pH 7. These solutions were placed in a graduated glass column having a fritted glass plate at the base. Formation of foam was started by bubbling N_2 (0.60 $cm^3\ s^{-1}$). The volume of foam formed and the amount of liquid being incorporated into the foam were determined at different times. Bubbling was discontinued once the volume of foam had reached a constant value. Determinations were performed at least twice.

The volume of liquid incorporated to the foam decreases exponentially according to the equation

$$V_t = V_0 e^{-kt} \quad (2)$$

where V_t is the volume of liquid at time t (mL), V_0 is the initial volume of liquid (mL), and k is the constant of the rate of incorporation of liquid to foam (s^{-1}). Parameters used to characterize the foam formation capacity were the percentage of liquid incorporated in the foam ($(V_0 - V_F)/V_0 \times 100$), the volume of liquid at equilibrium V_e (mL), and the constant of liquid incorporation to the foam (k, s^{-1}).

Determination of Foam Stability. At the end of the 30 min bubbling period, both the liquid and foam volume were determined at different times; destabilization was determined by draining the liquid and by foam rupture.

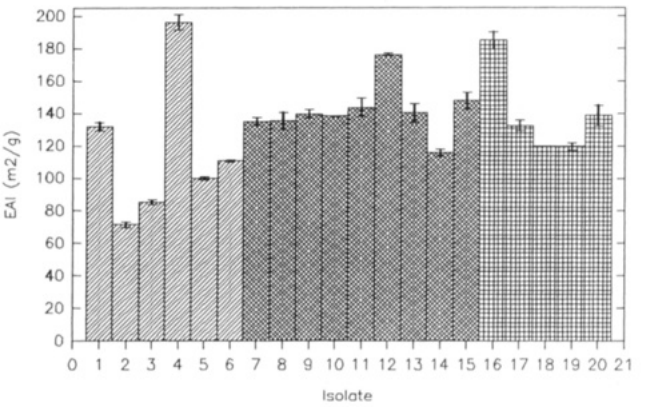
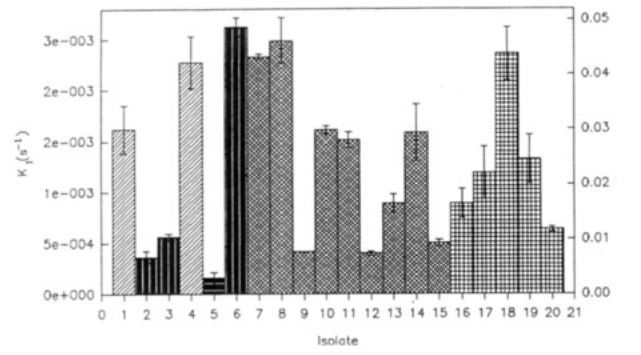


Figure 1. Emulsifying activity index (EAI m^2/g) of the isolates studied.

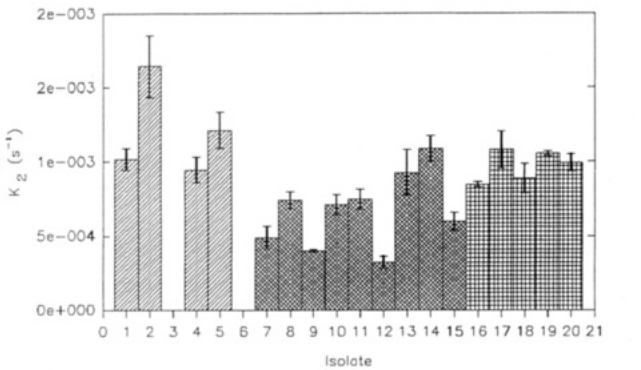
RESULTS AND DISCUSSION

The emulsifying properties of the isolates described in our previous paper have been evaluated (Petruccelli and Añón, 1994). Figure 1 shows the EAI of the isolates. Samples having the best emulsifying activity were the isolate at pH 9 (sample 4), the isolate enriched in 7S denatured with urea (sample 12), and the isolate containing denatured 7S and native 11S (sample 16). Samples 2, 3, 5, and 6 had low EAI values because of their low solubilities. Therefore, treatments leading to a better emulsifying activity are, for instance, increasing the pH to 9, which may result in oligomer dissociation. Those modifications should be permanent, since in all instances determinations were carried out in 0.1 M phosphate buffer at pH 7. The emulsifying activity was also favored by denaturation of the 7S fraction and by the denaturing and reducing treatments of isolates with a higher 7S content. The Pearce and Kinsella method allows mainly the evaluation of the rate of adsorption, since the emulsion is blocked at an early step of formation by the addition of a stabilizing solution; thus, these isolates are characterized by a higher rate of adsorption at the interface. Regarding isolate 16, the high adsorption rate can result from its greater surface hydrophobicity. Modifications of the adsorption rate can also arise as a result of changes in the mean molecular weight of the different components of the isolate. A factor that should be considered is the state of 7S globulin, since this effect is not present in isolates 12 and 15. With regard to isolate 15, this behavior may be imputed to its lower solubility. The fact that isolates having higher 7S content are better emulsifying agents has been reported previously (German et al., 1985; Yao et al., 1990).

Destabilization by flocculation and creaming was evaluated by means of two constants as was described under Materials and Methods. Figure 2 shows both rate constants, k_1 (2.1) and k_2 (2.2), for isolates studied. Phase separation was practically instantaneous in isolates 3 and 6. The most stable isolates, according to



2.1

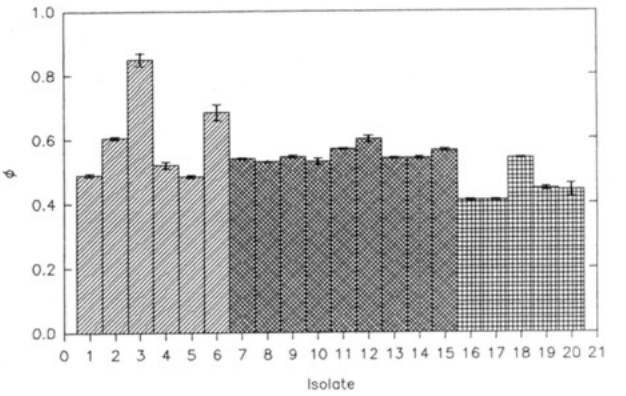


2.2

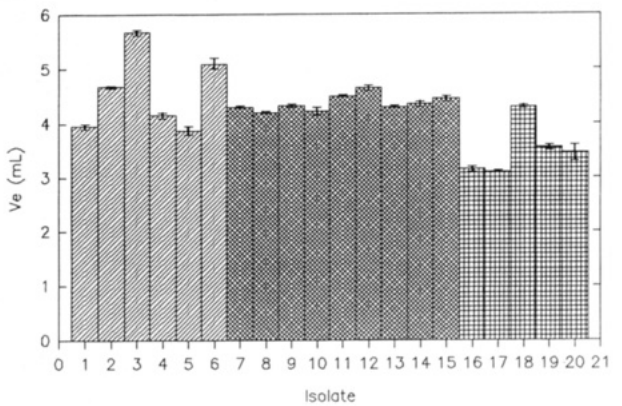
Figure 2. Constant of destabilization rate by flocculation–creaming corresponding to the first 500 s (k_1, 2.1) and to the period between 500 and 2500 s (k_2, 2.2) of the isolates studied. (♦, Use right-hand scale for isolates 2, 3, 5, and 6 shown in black.)

the values of their rate constants, were isolates 9, 12, and 15. These isolates were completely denatured, while glycinin experienced complete dissociation from A and B polypeptides. The fact that these isolates—with components of a mean molecular weight lower than those of other isolates—form more stable emulsions was unexpected, since it is generally thought that low molecular weight proteins can be more rapidly adsorbed on the surface but are unable to stabilize, because they form neither rigid enough films nor netlike structures able to resist deformation forces. The higher diffusion rates likely to be imputed to proteins of lower molecular weights is not a critical factor affecting their surface activity, since their incorporation into the interface is eased during homogenization (Halling, 1981; Phillips, 1981; Damodaran, 1989). Therefore, these isolates are able not only to be rapidly adsorbed but also to form a film which delays flocculation, perhaps as a result greater electrostatic repulsions.

Equilibrium values at 24 h may also be used as a measure of the emulsifying activity, once the determinations of flocculation and creaming have been performed. Figure 3 shows the volumetric fraction of oil at equilibrium (ϕ) (3.1), the volume of the aqueous phase at equilibrium (V_e) (3.2), and the interstitial volume of water in the cream phase (V_i) (3.3). Sample 3 was so unstable that coalescence took place. Samples 16 and 17 exhibited the best ϕ and V_i values; however, they did not show the best k_1 value, so the later destabilization steps were slower than the initial steps. Isolate 4 had ϕ and V_i values within the same range as those corresponding to most of the isolates. As far as isolates 9, 12, and 15 are concerned, these values were similar to those of the rest; then, they were destabilized at longer



3.1



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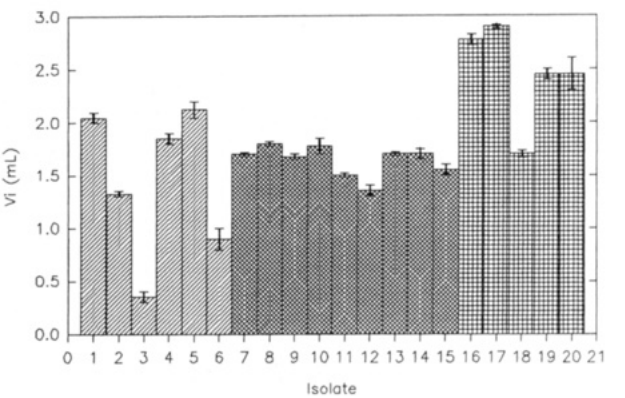


3.3

Figure 3. Oil volume fraction (ϕ) at equilibrium (3.1), volume of aqueous phase (V_e) at equilibrium (3.2), and water interstitial volume (V_i) in the cream phase (3.3) of the different isolates.

times. The highest equilibrium V_i value of isolate 16 was in agreement with the EAI determination; this is not the case, however, with regard to isolate 17.

Figure 4 shows the oil percentage released by coalescence (4.1) and the protein remaining at the interface after coalescence has occurred (4.2). The most insoluble samples (2, 3, and 6) exhibited over 60% coalescence. The rest of the isolates showed a coalescence below 20%. With the exception of samples 12, 13, and 16, there was a tendency toward lower destabilization by coalescence at higher protein contents at the interface. Isolates with the lowest oiling off were 11, 15, 16, and 20, which thus present the strongest films.

Electrophoretic analysis of the protein remaining in the aqueous phase after coalescence showed a high proportion of A polypeptide in relation to the rest of the components (α, α′, and β−7S and B−11S) in isolates 9,

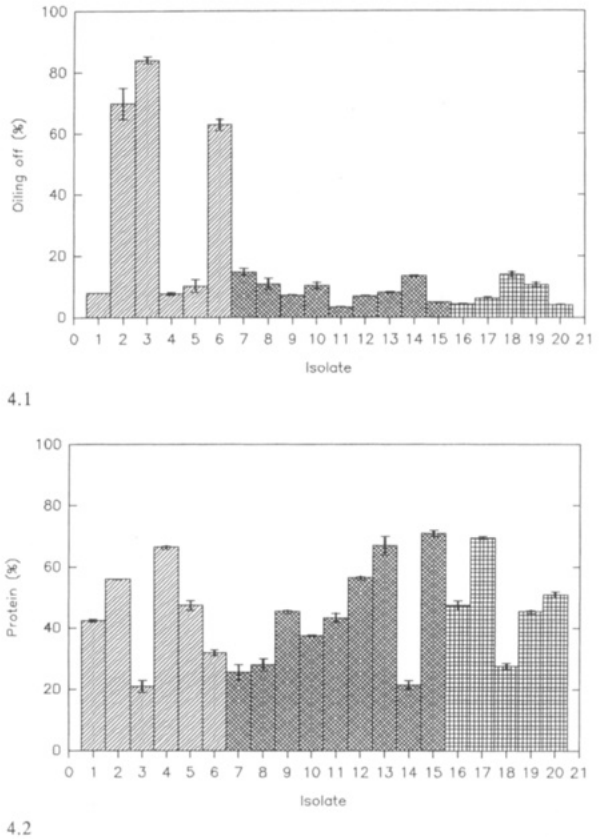


Figure 4. Percentage of oil released by coalescence (4.1) and percentage of protein remaining at the interface (4.2).

12, and 15, which would indicate that the polypeptide did not migrate to the interface. The rest of the isolates presented profiles similar to those observed when solubilization was carried out in phosphate buffer, except for B polypeptide, which was absent in most of the profiles; therefore, with the exception of the latter, the preferential migration of any component to the interface is unlikely.

According to the results obtained by all three methods, samples 2, 3, and 6 lack the capability of forming and stabilizing emulsions. This is in agreement with their low solubility; the remarkable emulsifying properties of isolate 9, of isolates reduced by Na_2SO_3 and urea, and of isolates exposed to smooth thermal treatments should be noticed. The treatments used affect differently the characteristics required to form emulsions, such as adsorption rate and capacity to form a stable film.

Values of the emulsifying activity—as determined according to the method of Pearce and Kinsella (1978)—and of the emulsifying stability were compared. The trend indicate that the greater the EAI, the greater the interstitial water volume is. Comparison of the EAI with the oil percentage released by coalescence (Figure 5.1) showed that samples having lower EAI values are more likely to coalesce. During destabilization of an emulsion by flocculation and creaming, a first stage of fast destabilization and then an equilibrium step are observed. Figure 5.2 shows the relationship between k_1 and V_i. As can be noticed, samples having a lower k_1 had a lower V_i, with the exception of samples 9, 12, and 15. These samples presented high hydrophobicity and the existence of a dissociated 11S fraction in A and B polypeptides. Figure 5.3 shows the relationship between the rate constant k_1 of the flocculation and

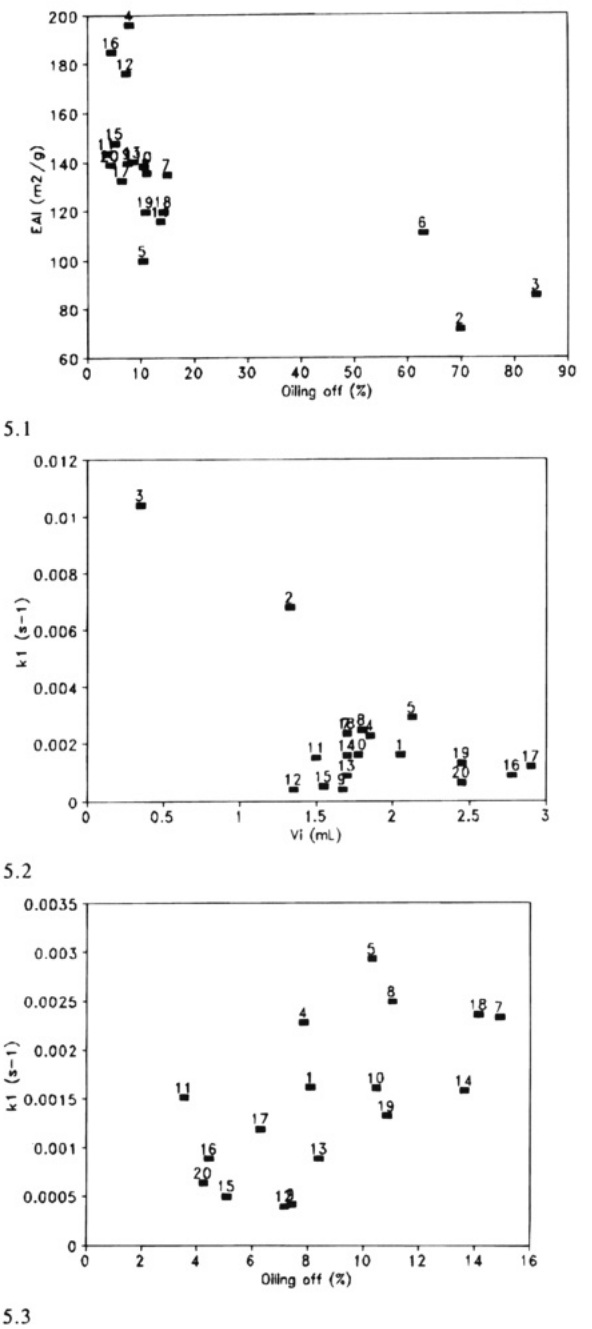


Figure 5. Relationship between the emulsifying activity index (EAI) and the percentage of oil released by coalescence (5.1), relationship between the constant of destabilization rate by flocculation and creaming (k_1) and the interstitial volume of water (V_i) in the cream phase (5.2), and relationship between the destabilization constant (k_1) and the percentage of oil released by coalescence (5.3).

creaming process and the destabilization by coalescence. The same trend is noticed between both destabilization processes.

Figure 6 shows the relationship among the three methods used to characterize the emulsifying properties; all of these methods show the same trend: the observed maximum corresponds to the values of the emulsifying activity index, the constant of the rate of destabilization by flocculation and creaming (k_1), and the oiling off of maximal instability. The dispersion observed when a correlation was sought shows the need to use more than one measurement method to characterize the emulsifying properties of an isolate. It should also be noticed that the treatments used affect the capacity both to form

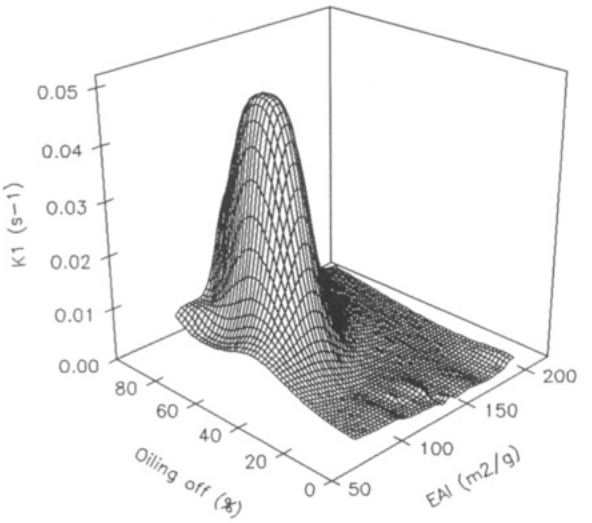


Figure 6. Relationship among the emulsifying activity index (EAI), the constant of destabilization rate (k_1), and the percentage of oil released by coalescence.

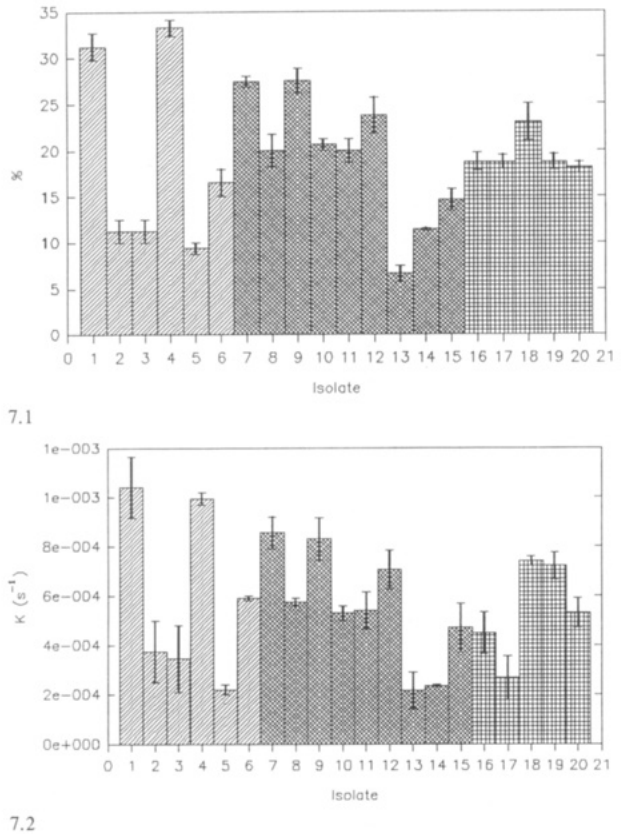


Figure 7. Percentage of fluid incorporated into the foam (7.1) and constant of incorporation rate of fluid into foam (7.2) of the isolates studied.

emulsions and to stabilize them; these changes occur sometimes in opposite directions.

Figure 7 shows the percentage of fluid incorporated into the foam (7.1) and the rate constant of incorporation of the fluid into the foam (7.2) corresponding to the isolates studied. The greatest foaming capacity was found in isolates 1 and 4; both were native isolates. When these isolates were compared with isolate 7, which was also native and had been dialyzed, the lower foaming properties seen in the latter can be ascribed to the salt content, since an increase of ionic strength favors foam formation. It should be taken into consideration that some whey proteins are lost during dialysis; those proteins could be responsible for the higher foaming activity observed in nondialyzed isolates. Isolate 8—which underwent treatment with Na_2SO_3—was not so good a foaming agent as isolate 7. The reducing treatment produced no changes of the foaming properties of isolates 11 and 14. Isolates 12 and 15—which had reducing treatment in the presence of urea were completely denatured and AB subunits were completely dissociated—are better foaming agents than samples 11 and 14. The sample 15 rich in glycinin was better than the untreated isolate 13. This result is in agreement with those of Kim and Kinsella (1986, 1987). When isolates 7, 10, and 13 were compared, it could be noticed that the obtention of an isolate with a high foaming activity required a glycinin/β-conglycinin ratio similar to that of a normal native isolate and that the isolates having a higher 7S or a higher 11S were the worst foaming agents.

The thermal treatment leading to solubility loss (isolates 2, 3, 5, and 6) produced a decreased capacity of foam formation. If no loss of solubility occurred, the decrease of foaming capacity persisted but it was not so pronounced (isolates 16 and 17).

None of the modified isolates had a better foaming capacity than the native one. This can be due to the fact that foam formation requires not only a high migration rate to the interface—which would be improved, for instance, by glycinin dissociation or by increased hydrophobicity—but also the presence of a particular protein structure. With regard to the latter consideration, it could be mentioned that globular proteins, such as bovine albumin and lysozyme, form rigid surface films having higher cohesion than those of a weakly structured protein, such as β-casein (Graham and Phillips, 1979a–c).

Isolates showing a decreased foam volume 30 min after bubbling had been discontinued are 2, 3, 5, 6, and 13–17, corresponding to decreases of 37%, 62%, 53%, 96%, 9%, 23%, 96%, 91%, and 92%, respectively. This shows that all of these foams are very unstable. All isolates lose fluid by draining. In the isolates exhibiting a decrease of foam volume the draining of 100%. All of these isolates lacked foaming properties.

The foaming capacity usually presents correlation with the exposed hydrophobicity, whereas the emulsifying capacity is related to the surface hydrophobicity (Townsend and Nakai, 1983; Nakai and Li-Chan, 1985). This occurs because during foam formation there is enough time for the protein to undergo denaturation at the interface, while the emulsion formation is a much faster process. This behavior is affected by the denaturing effect resulting from the difference of polarity between air and oil. Isolates obtained, except isolates 3 and 6, had the same exposed hydrophobicity. Differences should then be the result of other factors, such as the higher salt content or the loss of proteins with low molecular weights in the dialyzed isolates. These proteins, however, would also contribute to the total hydrophobicity. The salt content may affect the degree of association/dissociation, leading to a higher migration rate toward the interface of nondialyzed isolates at pH 7 and 9.

The relationships between emulsifying properties and solubility and between emulsifying properties and surface hydrophobicity were also studied. With regard to the former, it was found that highly insoluble isolates are poor emulsifiers; instead, no particular tendency was found in isolates having solubilities higher than

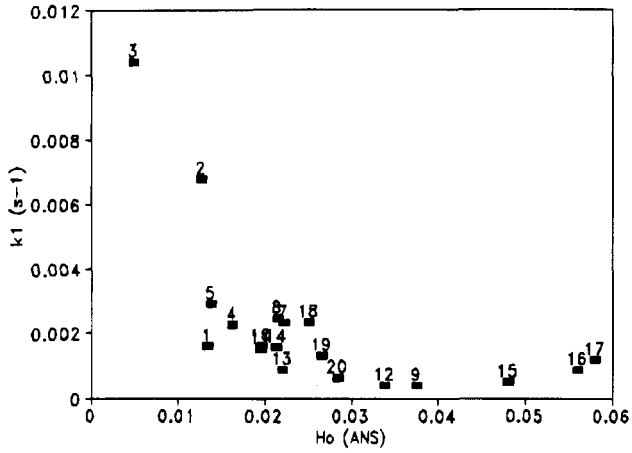
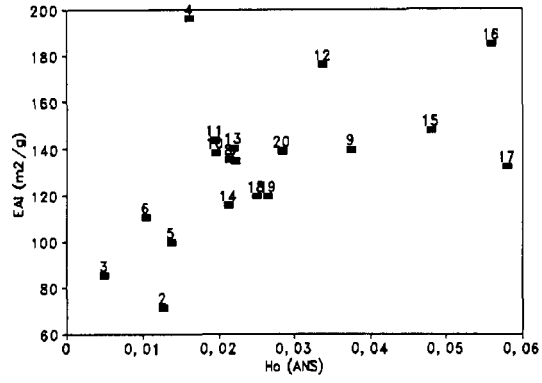
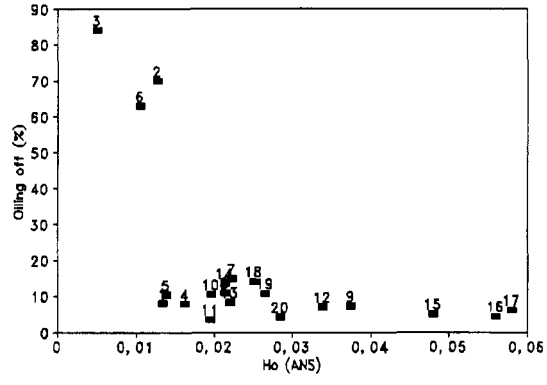


Figure 8. Relationship between the constant of destabilization rate of the flocculation–creaming process (k_1) and the surface hydrophobicity determined with ANS.



9.1



9.2

Figure 9. Relationship between the emulsifying activity index (EAI) and surface hydrophobicity determined with ANS (9.1) and between the percentage of oil released by coalescence and the surface hydrophobicity determined with ANS (9.2).

40%. When the relationship between these properties and surface hydrophobicity was studied, a good correlation was found between k_1 and H_0; the most hydrophobic samples showed low rate constants of destabilization by flocculation–creaming (Figure 8). The same trend could be noticed, although with a much larger dispersion, when the value of the emulsifying activity index or that of coalescence was plotted versus surface hydrophobicity and the plots were compared with each other (parts 1 and 2, respectively, of Figure 9). An inverse relationship was observed between the volumetric fraction of oil at equilibrium and the surface hydrophobicity (Figure 10). This relationship between emulsifying properties and

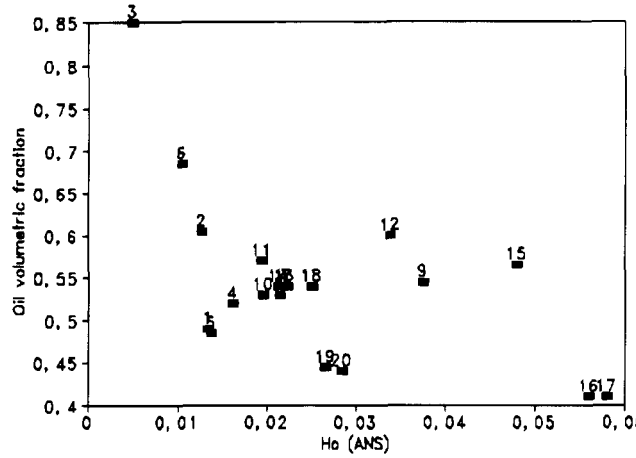


Figure 10. Relationship between the oil volumetric fraction (ϕ) at equilibrium and the surface hydrophobicity determined with ANS.

surface hydrophobicity had been already reported (Kato and Nakai, 1980; Li-Chan et al., 1985; Kato, 1991).

No correlation was found between foaming properties and the measurements of either solubility or H_0; the same negative results were encountered concerning the correlation between foaming properties and the content of either free sulfhydryl groups (SH_F) or incorporated sulfonate groups.

CONCLUSIONS

Isolates having the best emulsifying properties were those subjected to short thermal treatments (samples 16 and 17) and those reduced in the presence of Na_2SO_3 and urea. These isolates were characterized by high surface hydrophobicity and solubility. Increasing the pH of a native isolate from 7 to 9 did improve the emulsifying properties. Such modification may result either from changes of the association–dissociation degree or from the difference of salt content of the isolate, since one was taken to pH 7 and the other to pH 9 with NaOH.

Isolates enriched in 7S were better emulsifiers than those having either a normal composition or the 11S as a major constituent. This higher emulsifying activity of 7S-rich isolates is in agreement with their higher hydrophobicity with DPH. Normal isolates were better foaming agents than those in which a change of the proportion between 7S and 11S had been introduced.

Isolates having a greater degree of sulfonation and in which neither the solubility nor the hydrophobicity had been affected (18–20), and which exhibited different degrees of denaturation of 7S and 11S globulins, showed no changes of their emulsifying and foaming properties.

Nondialyzed isolates were better foaming agents than dialyzed isolates. Since dialysis was carried out against water, it is likely that the state of association–dissociation of 7S and 11S globulins had changed. In addition, some components of low molecular weight were also lost.

Evaluation of the emulsifying properties of the modified isolates requires the determination of more than one property, since the modifications produced by the treatment affect differently the adsorption capacity at the interface and the ability to form stable film. Therefore, the selection of a suitable method is dependent on food characteristics.

The only correlations observed were those between emulsifying properties and surface hydrophobicity, which had been already reported by other authors (Kato and Nakai, 1980; Voutsinas et al., 1983a,b; Hayakawa and Nakai, 1985a,b; Li-Chan et al., 1985; Li-Chan and Nakai, 1991; Nakai et al., 1991; Kato, 1991). The relationships among other structural properties such as degree of denaturation, type of aggregates present, incorporated sulfonate groups, SH_F and disulfide bonds, total hydrophobicity, modifications of the mean molecular weight, and the surface properties are difficult to quantify.

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