# Surface Functional Properties of Native, Acid-Treated, and Reduced Soy Glycinin. 2. Emulsifying Properties

Jorge R. Wagner*,† and Jacques Guéguen‡

Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA), Facultad de Ciencias Exactas, Universidad Nacional de La Plata, calle 47 y 116 (1900) La Plata, Argentina and Laboratoire de Biochimie et Technologie des Protéines (LBTP), Institut National de la Recherche Agronomique, Rue de la Géraudière, B.P. 527, 44026 Nantes Cedex 03, France

Emulsifying properties of native and chemically modified soy glycinins were studied. The influence of ionic strength, protein sample composition and concentration, and assay conditions on the flocculation−creaming process and coalescence resistance was analyzed. Differences in these emulsifying properties were exhibited by native glycinins, which have a variable content of 4S, 11S, and 15S forms. Structure and functionality of native glycinin were modified by means of combined treatments: mild acidic treatments without heating or with heating at variable time and with or without disulfide bonds reduction. Modified glycinins presented different degrees of deamidation, surface hydrophobicity, and molecular mass. A slight enhancement of emulsifying stability at moderated deamidation degrees was observed. In different protein samples, a positive relationship between the flocculation−creaming rate constant and equilibrium oil volume fraction of emulsions with surface hydrophobicity was detected. A remarkable difference was observed between reduced and nonreduced samples, mainly with respect to behavior at low or high ionic strength.

**Keywords:** *Glycinin; 11S globulin; soy protein; surface properties; emulsifying properties; chemical modification*

## INTRODUCTION

Vegetable proteins are used in food technology because of their interesting role as functional agents. The functionality of proteins is derived from physicochemical characteristics such as molecular size, amino acid composition and sequence, conformation, net charge, and surface hydrophobicity (Kester and Richardson, 1984). Any modification capable of altering one or more of these characteristics may also change one or several functional properties.

Emulsifying properties of proteins depend basically on two effects: (1) a substantial decrease in the interfacial tension due to the adsorption of the protein at the oil−water interface and (2) the electrostatic, structural, and mechanical energy barrier caused by the interfacial layer that opposes destabilization processes. The relationship between physicochemical characteristics, interfacial behaviors, and emulsifying properties of proteins was extensively investigated on bovine serum albumin, $\beta$-casein, and lysozyme as model proteins (Graham and Phillips, 1976; Phillips, 1981). They emphasized the importance of the thickness and charge of the protein interfacial layer in preventing coalescence of emulsion. Current hypotheses suggest that surface hydrophobicity is one of the characteristics of the protein most likely to define its surface behaviors and consequently its emulsifying properties (Graham and Phillips, 1979; Nakai, 1983; Nakai et al., 1980, 1986). These authors have reported a positive correlation between surface hydrophobicity, surface tension, and emulsifying activity index. Other authors concluded that to prevent flocculation and to increase resistance to coalescence the interfacial layer has to be as thick as possible, heavily hydrated, and charged (Graham and Phillips, 1976; Dagorn Scaviner et al., 1987; Guéguen et al., 1990).

Glycinin (protein 11S-type), one of the major globulins of soybean seeds, has a hexameric structure $(AB)_6$ with a molecular weight around 360 kDa in its native state. Its quaternary structure is stabilized by electrostatic and hydrophobic interactions and by disulfide bridges between A and B polypeptides, constitutive of the subunits (Peng et al., 1984). The surface behavior and, as consequence, the foaming and emulsifying properties of native glycinin were shown to be limited by its close packed globular conformation, low surface hydrophobicity, and low molecular flexibility (Kinsella, 1979; Kim, 1985; Wagner and Guéguen, 1995). Appropriate dissociation of the oligomeric structure of glycinin and simultaneous unfolding of the polypeptide chains of AB subunits markedly improves its solubility and surface-active properties (Kim and Kinsella, 1987a,b). This structural modification could be induced by varying environmental factors such as ionic strength, pH, or temperature (Peng et al., 1984) or by chemical treatments such as reduction of component disulfide bonds (Kim, 1985; Kim and Kinsella, 1987a) and succinylation or acetylation (Kim and Kinsella, 1987b). Matsudomi et al. (1985) studied the conformational changes and alteration of surface functional properties of soy protein caused by mild acid treatment. The improvement of foaming and emulsifying properties of acid-modified soy

* To whom correspondence should be addressed. Fax: 54 221 4249287. E-mail: jrwagner@nahuel.biol.unlp.edu.ar.
† Universidad Nacional de La Plata.
‡ Institut National de la Recherche Agronomique.

10.1021/jf9809784 CCC: $18.00 
Published on Web 05/04/1999

proteins may be mainly due to an increase in surface hydrophobicity induced by deamidation and acid-induced denaturation. Recently, Wagner and Guéguen (1995) have reported that the surface behavior of soybean glycinin could be greatly improved by mild acidic treatments, with or without reduction. The protein species arrived at by these chemical modifications had good adsorption kinetics in the interface, as a result of their decreased molecular size, and increased surface hydrophobicity and charge. As it was expected, these structural modifications improve the ability of the protein to adsorb at the interface and to act as foaming agent (Wagner and Guéguen, 1999).

The objective of the present study was to analyze the influence of the structural changes of glycinin (from progressive dissociation−deamidation and reducing treatments) on emulsion forming and stabilizing capacities. The influence of glycinin composition and emulsifying conditions on its functionality was also studied.

## MATERIALS AND METHODS

Chemical reagents of analytical grade were obtained from Merck, Pharmacia, and Sigma Chemical Co. Seeds of soybean varieties "Kador B10" and "Alaric", obtained from the Station de Génétique et D'amélioration des Plantes (INRA, Montpellier), were employed. The seeds were dehulled, ground, and defatted by Soxhlet extraction with hexane, which was then removed at room temperature. All solutions were prepared with Millipore water, and to them were added 1 mM benzamidine as protease inhibitor and 0.02% sodium azide as antimicrobial agent, even to those used for dialysis.

**Preparation of Glycinin.** Crude glycinins (CGI and CGII) were prepared from defatted soy flour, varieties Kador B10 and Alaric, respectively, according to the procedure described previously (Wagner and Guéguen, 1999). The purity of the crude glycinins was higher than 90% (determined by SDS−PAGE and affinity chromatography in ConA-Sepharose 4B).

**Purification of Glycinin.** Purified glycinin (PGI) was prepared from crude glycinin (CGI) by the procedure described in previous work (Wagner and Guéguen, 1999). The purity of the PGI sample was higher than 99% (determined by SDS−PAGE).

**Preparation of Modified Glycinins.** Acid dissociation and deamidation and reducing treatment of crude glycinin were carried out, as described previously by Wagner and Guéguen (1995). CGII sample (10 mg/mL) was treated in 0.1 M HCl without heating (4 °C; 9 h: treatment 1) and with heating (70 °C; 1.5, 3, 4.5, 6, and 9 h: treatment 2). The resulting solutions were neutralized with 1 M NaOH, dialyzed against water, and freeze-dried. The deamidation degree of resulting samples was 0, 1.7, 5.9, 10.6, 16.1, and 25.0, respectively. The acid-treated samples were reduced (treatment 3) as follows: the acid-treated samples were solubilized at 0.25−0.5% in 0.03 M Tris-HCl buffer, pH 8, with 0.2% $Na_2S_2O_5$ as the reducer agent of disulfide bonds. The protein solutions were then heated at 100 °C for 5 min. After treatment, solutions were cooled in ice water and centrifuged at 10000*g* for 15 min. The supernatants were dialyzed against water and then freeze-dried. The degree of deamidation of the resulting reduced samples was the same as those in nonreduced ones.

**Degree of Deamidation.** The degree of deamidation was determined by measurement of the ammonia released due to a moderated protein hydrolysis (Matsudomi et al., 1985).

**Gel Filtration.** An exclusion−diffusion superose 12 HR column connected to LKB HPLC equipment was used. The procedure was as described by Wagner and Guéguen (1995). Proteins were detected at the column outlet at 280 nm. The percentage of protein species was determined by the relationship between the area of each peak and the total area.

**Surface Hydrophobicity.** Surface hydrophobicity was determined by the hydrophobic fluorescence probe, using 1-aniline-8 naphthalene sulfonate (ANS). Measurements were performed according to the ANS method of Kato and Nakai (1980) as described by Wagner and Guéguen (1995).

**Emulsifying Properties.** Emulsifying properties were studied according to Dagorn-Scaviner et al. (1987) with slight modifications. Protein solutions (0.3−3 mg/mL) were prepared by solubilizing native and modified glycinins in sodium phosphate buffers, pH 7.6, with different ionic strengths (0.02−0.5). The emulsions were prepared by homogenizing 30 mL of a protein solution with 10 mL of paraffin oil (condition 30/10) or 15 mL of a protein solution with 5 mL of paraffin oil (condition 15/5), using a Polytron PT 10 homogenizer (30 s at 20 000 rpm). In the 15/5 emulsifying condition, the foam formation was possible because the total volume employed (20 mL) was not enough to cover the lateral orifice of the rotor of the Polytron homogenizer, thus allowing air incorporation into the emulsion. In the emulsification condition 30/10, this phenomenon was controlled because the orifice was always covered by the emulsion (40 mL). Emulsion stability was determined by following the flocculation−creaming process during aging of the emulsion. Emulsion was poured into a 10 mL graduated vessel. Emulsion destabilization was followed by plotting the volume of separated aqueous phase, $V_t$ (mL) vs time. The flocculation−creaming process can be considered a succession of first-order kinetics, which can be visualized by plotting $\ln(V_e/(V_e - V_t))$ vs $t$, $V_e$ being the equilibrium value of $V_t$ after 24 h. The flocculation−creaming rate constant $k_1$ (an indicative parameter of the emulsion stability) was estimated by determining the slope of first linear step. The equilibrium volume fraction, $\phi$, of the oil in the creamed phase was calculated by $\phi = 2.5/(10 - V_e)$. Resistance to coalescence was studied as follows: 12 mL of emulsion (prepared as above) was immediately centrifuged for 100 min at 1000*g*. The volume ($V_s$) of the separated oil phase was determined for different concentrations of proteins in the aqueous phase in the range $10^{-2}$ to 1 mg/mL. The quantity $100V_s/V_i$ ($V_i = 3$ mL, initial volume of the oil in the emulsion), taken as a measure of coalescence, was plotted vs protein concentration.

## RESULT AND DISCUSSION

**Emulsifying Property of Native Glycinin.** *Influence of Ionic Strength.* It had been observed that the ionic strength of the medium, by inducing association−dissociation of the glycinin, influences the adsorption kinetics in the air−water interface (Wagner and Guéguen, 1995). Being a surface property, the emulsifying property of native glycinin is also modified by the ionic strength of the medium. Figure 1 shows the effect of ionic strength on the flocculation and creaming processes of an emulsion prepared with 15 mL of paraffin and 5 mL of a 0.3 mg/mL solution of CGII (condition 15/5, see Materials and Methods). The lower the parameters $k_1$ and $\phi$ are, the greater the emulsion stability and the hydration of the creamed phase will be, respectively. At low ionic strengths ($\mu < 0.2$), the parameter $\phi$ gets the lowest value. For increasing ionic strengths ($\mu > 0.2$), an increase in $\phi$ is observed, which is more noticeably caused by phosphate ion than by chloride. At $\mu > 0.5$, $\phi$ values reach their maximum and constant values (Figure 1a). The parameter $k_1$ also tends toward a minimum value at low ionic strength ($\mu < 0.2$) and then increases for higher ionic strengths. Concerning the effect of ion chloride, the parameter $k_1$ increases up to $\mu = 0.5$; in contrast, when ion phosphate is considered, the effect of ionic strength extends up to $\mu = 1.4$ (Figure 1b). This saline effect on the $k_1$ and $\phi$ values could be related to changes in electrostatic properties of the interfacial layer and steric effects and in the hydration capacity of the layer, respectively (Guéguen et al., 1996). According Wagner and Guéguen (1995), the dissociation of oligomeric structure of 11S-glycinin

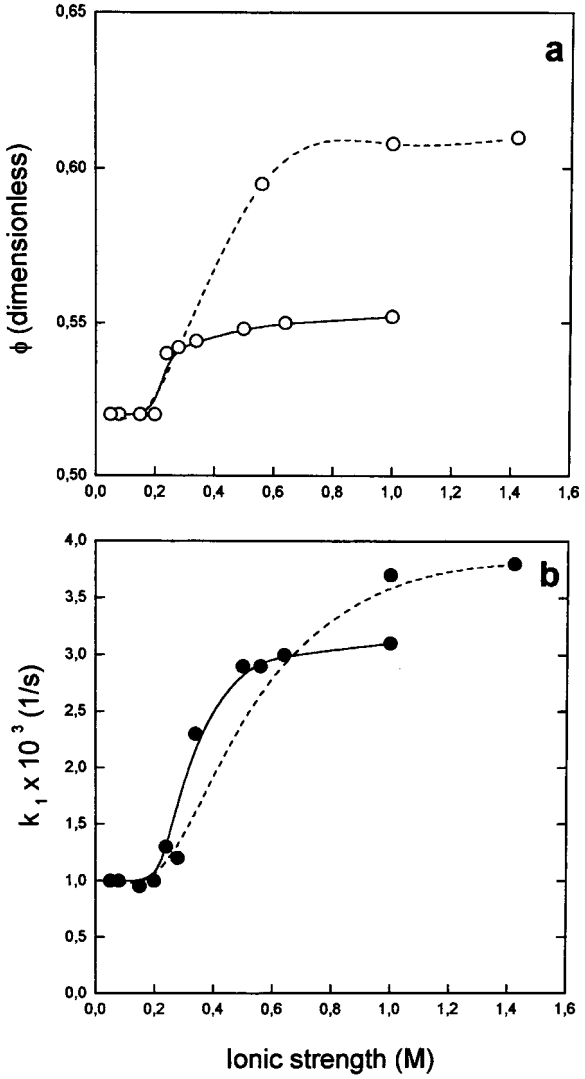


**Figure 1.** Emulsifying properties of crude glycinin II solution (0.3 mg/mL, pH 7.6) at different ionic strengths (variable sodium phosphate concentration (- - -); 0.05 M sodium phosphate plus variable sodium chloride concentration (—)). Emulsions were performed at condition 15/5 (see Materials and Methods). Parameters are as follows: $k_1$ (●), flocculation−creaming rate constant; $\phi$ (○), equilibrium volume fraction of oil in the creamed phase. Values correspond to mean values of two assays as minimum. Maximum standard deviation: 5% for $k_1$; 3% for $\phi$.

at low ionic strength, and consequently the improvement of surface behavior, could explain the higher emulsion stability and hydration for glycinin under this condition. From Figure 1, it is apparent that, basically, there are two ionic strength ranges in which emulsion stabilization or destabilization is favored; they are $\mu \leq 0.2$ and $\mu \geq 0.5$, respectively. To compare the emulsifying property of native and modified glycinins, under these clearly different conditions, we used two solutions: 0.02 M phosphate ($\mu = 0.06$) and standard phosphate buffer ($\mu = 0.5$).

**Influence of Protein Concentration.** For native glycinin (CGII sample), the effect of protein concentration on the emulsifying property, both at low and at high ionic strengths, was studied (Figure 2). The 15/5 condition was also employed for these tests. Figure 2a shows that $\phi$ values decrease as protein concentration increases. The changes in $\phi$ as a consequence of the ionic strength are observed only at low protein concentration

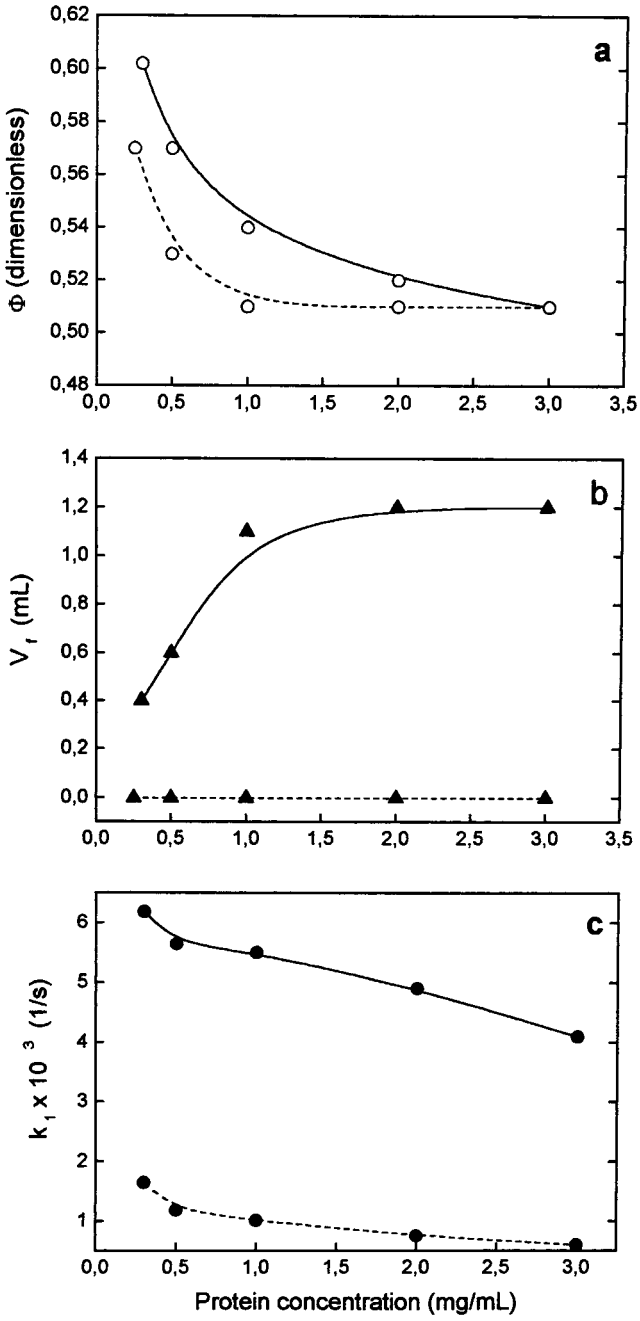


**Figure 2.** Emulsifying properties of crude glycinin II solutions (standard phosphate buffer, $\mu = 0.5$, pH 7.6 (—); 0.02 M sodium phosphate, pH 7.6 (- - -)) at different protein concentrations. Parameters $k_1$ (●) and $\phi$ (○) are as described in Figure 1; $V_f$ (▲) is the foam volume in the emulsion. Emulsions were prepared at condition 15/5 (see Materials and Methods). Values correspond to mean values of two assays as minimum. Maximum standard deviation: 5% for $k_1$ and $V_f$; 3% for $\phi$.

(0.3−1.0 mg/mL). In the tests carried out with standard phosphate buffer ($\mu = 0.5$), the creation of an additional foam phase was detected. As protein concentration increases, the volume of the foam formed by the emulsification process ($V_f$) becomes more important. Concerning the 0.02 M phosphate solution, no foam formation was observed in any of the concentrations assayed. Then, in the emulsion of native glycininsolution, foam forming and stabilization was favored by high ionic strength and high protein concentration. These results are congruent with those obtained in the study of the foaming property of native glycinin. On the other

hand, it is observed in Figure 2b that $k_1$ values decrease as the protein concentration increases. The improvement in emulsifying stability as a consequence of the increase in protein concentration was explained by an increase of rigidity of interfacial lamella (Halling, 1981). In all the concentrations tested, the best emulsion stability (lower $k_1$) is obtained at low ionic strength.

*Influence of Native Glycinin Composition and Emulsifying Condition.* By HPLC measurements, it was observed that the samples CGI, CGII, and PGI differ in percentages of 15S, 11S, and 4S forms: 24.9, 61.6, and 13.4% for CGI; 21.9, 71.5, and 6.5% for CGII; 23.4, 76.6, and 0% for PGI, respectively. The emulsifying properties of these three native glycinin samples, both at low and high ionic strengths, were studied in conditions 15/5 and 30/10, as described in Material and Methods. Results are shown in Figure 3a−c (for 15/5 condition) and in Figure 3d−f (for 30/10 condition). It can be observed that the three 11S-type globulins do not differ significantly from each other in their emulsion stability (Figure 3b,e) although they have distinct compositions.

In both emulsifying conditions, the expected differences caused by the ionic strength of the medium are observed. A comparison of parts b and e of Figure 3 also shows that, in the 0.02 M phosphate solution, the emulsifying condition does not introduce appreciable differences in the $k_1$ values. On the contrary, in standard phosphate buffer, the condition 30/10 leads to more stable emulsions (low $k_1$) than condition 15/5. A similar result was obtained for $\phi$ in standard phosphate buffer (comparison of parts a and d of Figure 3). This decrease of emulsifying properties observed for the 15/5 condition in standard phosphate buffer can be explained by the presence of foam in the emulsion (Figure 3c,f). The foam formation would imply adsorption of glycinin molecules in the air−water interface, thus their action as an emulsifying agent in the oil−water interface is weaker. Moreover, the process of air bubbles creaming (or floatation), which occurs at a higher velocity than for oil drops creaming (Walstra, 1989), could produce a destabilizing effect on emulsion by enhancing the flocculation process. Going back to Figure 3a,d, it is observed that, unlike in the parameter $k_1$ (Figure 3b,e), the parameter $\phi$ in 0.02 M phosphate gives different values for the three glycinins tested ($\phi$ value: PGI > CGII > CGI). The partially dissociated oligomeric structure in CGI samples, plus the mentioned dissociating effect of low ionic strength, could explain the observed differences with purified glycinin. Results would show that the purity degree of glycinin affects the hydration of the creamed phase more than the emulsion stability.

**Emulsifying Properties of Modified Glycinin.** Having observed that the 15/5 condition produces emulsion with foam when carried out in standard phosphate buffer, even with samples of low foaming properties as native glycinins, we expected a greater amount of foam when using samples of better foaming properties (as the modified glycinins). We confirmed that assumption by emulsification tests conducted in the 15/5 condition on reduced and deamidated glycinins, which showed an elevated foam formation (>1 mL). To avoid this foam formation during emulsification, the emulsifying property of native and modified glycinins was compared by measuring in the 30/10 condition.

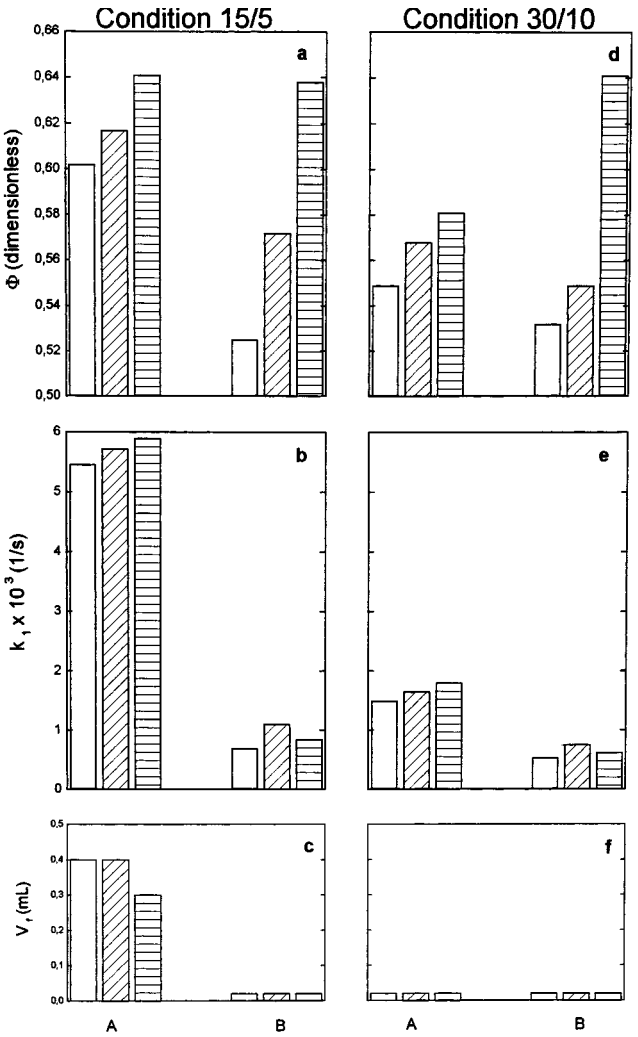


**Figure 3.** Emulsifying properties of glycinin crude I (empty bar), glycinin crude II (diagonally striped bar), and purified glycinin I (horizontally striped bar). Emulsions were performed with sample solutions at 0.3 mg/mL in (A) standard phosphate buffer, $\mu = 0.5$, pH 7.6. or (B) 0.02 M phosphate, pH 7.6, using the conditions 15/5 (a, b) or 30/10 (d, e) (see Materials and Methods). Parameters $k_1$, $\phi$, and $V_f$ are as described in Figures 1 and 2. Values correspond to mean values of two assays as minimum. Maximum standard deviation: 5% for $k_1$ and $V_f$; 3% for $\phi$.

*Effect of Chemical Modification and Influence of Ionic Strength.* The values of $\phi$ and $k_1$ as a function of the deamidation degree are shown in Figure 4a,b (native and nonreduced modified glycinin samples) and Figure 4c,d (reduced modified glycinin samples). For nonreduced glycinins in standard phosphate buffer, the volume fraction $\phi$ decreases slightly with the treatment in cold acidic medium (%$D = 0$), keeping almost constant as the deamidation degree increases (Figure 4a). In 0.02 M phosphate, the value of $\phi$ tends to increase at high deamidation degrees (%$D$ > 15). The interfacial concentration of these extensively deamidated samples would be low if their high molecular charges were taken into account, which could induce electrostatic repulsion between adsorbed molecules and high steric ability to cover the interface. In this case, the amount of fixed water, which depends on number of adsorbed proteins, would also be low. Moreover, the difficulty of highly charged proteins to form a protein matrix capable of holding water in the interface could be another possible explanation for the behavior of deamidated glycinins.

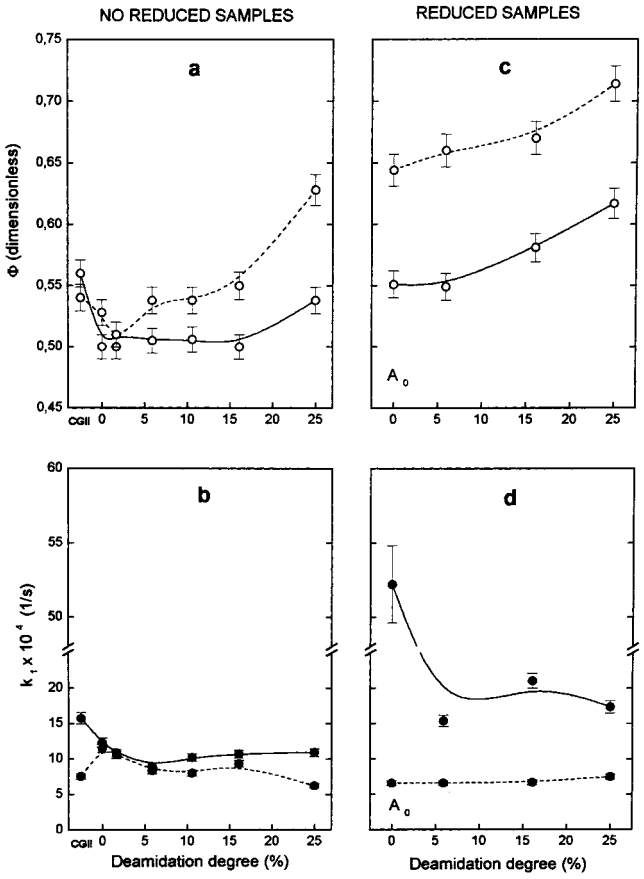


**Figure 4.** Emulsifying properties of native (N, crude glycinin II) and modified glycinin samples (soluble proteins resulting from treatment of crude glycinin II in cold acidic medium (deamidation degree, $D$ = 0%) or hot acidic medium ($D$ = 6−25%) without (a, b) and with (c, d) reducing treatment). (See Materials and Methods for details.) Parameters $k_1$ (●) and $\phi$ (○) as described in Figure 1. Emulsions were performed with (—) sample solutions at 0.3 mg/mL in standard phosphate buffer, $\mu$ = 0.5, pH 7.6, or (- - -) 0.02 M phosphate, pH 7.6, using the conditions 30/10. (See Materials and Methods.) Values correspond to mean values of two assays as minimum.

With respect to emulsion stability, Figure 4b shows that the glycinin treated in cold acidic medium exhibits, in standard phosphate buffer, a $k_1$ value lower than that of native glycinin. On deamidated glycinin, this value reaches a minimum for %$D$ = 5.9. Wagner and Guéguen (1995) showed that treatments of glycinin in cold acid medium or in hot acid medium during a short time enhance the surface behavior of protein, mainly due to dissociation of the 11S form (appearance of 4S form), denaturation of subunit AB, and, as a consequence, exposition of hydrophobic sites. In 0.02 M phosphate, $k_1$ starts from a low value for native glycinin, it increases with the acid treatment (%$D$ = 0−1.7), and then it decreases for higher %$D$ values until reaching values comparable to those of native glycinin. It is interesting to observe that in the range %$D$ = 0−6 there was practically no difference in emulsifying properties due to ionic strength. This behavior could be considered an advantage if one takes into account that, for food applications, good functional properties at a wide range of salt concentration are desirable. For increasing deamidation degrees (%$D$ > 6), both $\phi$ and $k_1$ reflect the differences caused by both ionic strengths (Figure 4a,b). This result would be attributed to variation in the protein net charge as a consequence of deamidation. For high ionic strength (standard phosphate buffer) an important part of this charge would be counterbalanced, while at low ionic strength (0.02 M phosphate) the protein molecules would have almost all their charge exposed. Low ionic strength, then, would lead to more stable emulsions ($< k_1$) by electrostatic repulsion of the oil drops, since they are surrounded by a highly charged protein film, but with a low water retention ($> \phi$) by the creamed phase.

On the other hand, for reduced glycinin samples, noticeable differences in $\phi$ and $k_1$ values, compared at high and low ionic strengths, could be seen (Figure 4c,d). In standard phosphate buffer, the named $A_0$ sample (soluble protein resulting from the reducer treatment of the glycinin treated in cold acid medium) exhibited a very high $k_1$ value ($k_1$ = 52.2). As we have already seen, this sample is mostly formed by the A polypeptide which, by high ionic strength action, is found partially associated to aggregates of higher apparent molecular weight (Wagner and Guéguen, 1995). This association state of A polypeptide and its low surface hydrophobicity ($S_0$ = 2.5) delayed its interfacial adsorption and anchorage. Figure 4d shows that soluble samples that result from the reduction of glycinins previously treated in hot acid medium give emulsions in standard phosphate buffer with $k_1$ = 15−18, which indicate a lower emulsifying stability than that of the nonreduced samples (Figure 4b). This result would be due to the fact that the reduced−deamidated samples contain distinct proportions of A and B polypeptides in variable deamidation degrees which, even when they are more charged, do not possess a high surface hydrophobicity ($S_0$ between 2.2 and 1.4) (Wagner and Guéguen, 1995). In 0.02 M phosphate, the same samples give more stable emulsions (with $k_1$ values similar to those of nonreduced samples), due to both the dissociating effect of the low ionic strength and the noncounterbalanced high charge density of deamidated proteins. On the other hand, it can be seen in Figure 4c that, under both ionic strengths, the value of $\phi$ increases markedly with the deamidation degree. In this case, the loss of hydration capacity due to the effects of deamidation, hydrolysis, and disulfide bond reduction (all processes that contribute to increasing the charge and diminishing the molecular size) is more evident than in the respective nonreduced samples (Figure 4a). Comparison of parts a−c with parts b−d of Figure 4 allow us to see that the $k_1$ and $\phi$ values do not follow the same behavior, both against the deamidation degree and against the ionic strength of the medium. In this way, it is observed that the values of $k_1$ are higher (lower stability) in standard buffer phosphate than in 0.02 M phosphate. This is opposite to the trends presented by $\phi$. The concept arising from this is that, for deamidated glycinins (with or without reduction), low ionic strengths favor emulsion stability (owing to the dissociating effect and for keeping protein charge in the interfacial layer) while high ionic strengths would improve the hydration capacity of the layers. If this later behavior is compared with that of native glycinin, there is an apparent contradiction (see Figures 1a and 4b,d). At higher ionic strengths, a decrease in hydration capacity of emulsion was observed for native glycinin (higher $\phi$ values, Figure 1b). This behavior could be explained by the formation of a major oligomeric 11S form at $\mu \geq 0.5$ (Wagner and Guéguen, 1995). This hexameric structure has a low hydration ability due to its close packed globular conformation (Peng et al., 1984; Kim, 1985).

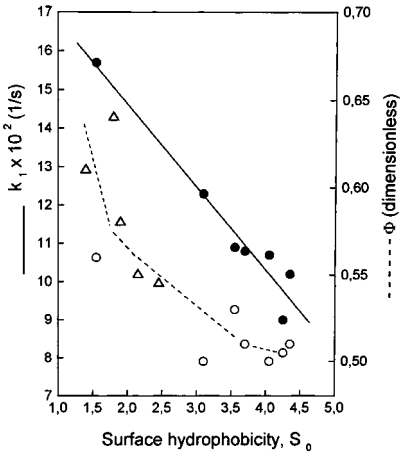


**Figure 5.** Relationship of $k_1$ (●, nonreduced samples) and $\phi$ (○, nonreduced; △, reduced samples) with surface hydrophobicity ($S_0$). Emulsions were performed with sample solutions at 0.3 mg/mL in standard phosphate buffer, $\mu = 0.5$, pH 7.6, and condition 30/10. (See Materials and Methods for details.) Values correspond to mean values of two assays as minimum. Maximum standard deviation: 5% for $k_1$; 3% for $\phi$.

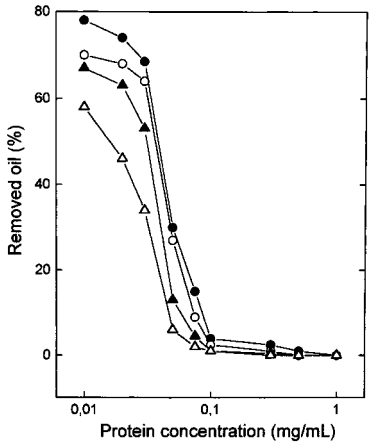


**Figure 6.** Coalescence of emulsions expressed as the removed oil volume percent of the total oil phase at different protein concentration for native (●, CGII) and modified glycinin samples (soluble proteins resulting from treatment of CGII in cold acidic medium (deamidation degree, $D = 0\%$) without (○, $AB_0$) and with (△, $A_0$) reducing treatment or hot acidic medium ($D = 5.9-10.6\%$, corresponding to a treatment for 3−4.5 h) without reducing treatment (▲) (See Materials and Methods for details.) Values correspond to mean values of two assays as minimum. Maximum standard deviation for removed oil: 6%.

The results seem suggest that a combination of different structural characteristics of proteins determine their surface activity. Nevertheless, the surface hydrophobicity ($S_0$) would be one of the most preponderant factors that affect the surface properties of glycinin samples. As it was previously reported by other workers (Graham and Phillips, 1976, 1979; Nakai et al., 1980, 1986), the hydrophobicity exposed by the protein would allow a better molecular anchorage to be established in the oil−water interface, giving more stable emulsions. Matsudomi et al. (1985) reported that the surface hydrophobicity of soy protein increased at an early stage of the mild acid treatment, then gradually increased until a deamidation degree of approximately 10% was reached, and decreased slightly after that. The changes of both the emulsifying activity and emulsion stability were very similar to those of the surface hydrophobicity. In our study, this structural property would also explain the values of $k_1$ and $\phi$ obtained for the acid-modified glycinins. In Figure 5 can be observed that, for nonreduced samples, the $k_1$ value decreases for increasing $S_0$. The lower $k_1$ values (higher emulsion stability) correspond to acid-treated glycinin with 6−10% degree of deamidation (corresponding to hot acid treatment for 3−4.5 h. Matsudomi et al. (1985), for mild acid-treated soy isolates, reported that at deamidation levels higher than 10%, the hydrolysis of peptide bonds began to be important. By gel filtration measurement, we determined that in the modified glycinins with $\%D > 6$ the percentage of species with molecular mass lower than 20 kDa is superior to 10%, indicating an advanced hydrolysis process. It was demonstrated that these species, which come from the hydrolysis of A and B polypeptides, possess tensioactive action since they are able to diffuse and be adsorbed in the air−water interface (Wagner and Guéguen, 1995). However, they lack the capacity to interact and anchor in such interface to form a rigid protein film around the oil droplets. Halling (1981) has shown that the presence of small molecule surfactants (as low molecular weight peptides) could modify the surface rheology of the protein film. These agents will tend to penetrate or even displace the protein into the interfaces, usually reducing the rigidity of the surface film.

With respect to $\phi$ values, the same Figure 5 shows that this parameter correlates also with $S_0$, when all samples (nonreduced and reduced) are included. It can be observed that the less hydrophobic reduced samples, as a whole, form emulsions with $\phi$ values higher than those respective acid-treated but nonreduced glycinins. It would seem that the deamidated glycinins are capable to form highly hydrated films, mediated by hydrophobic interaction between their polypeptide chains, only when A−B disulfide bonds are preserved.

To complete the present study, we determined the resistance to coalescence of emulsions in standard phosphate buffer for protein concentrations in the range 0.01−1 mg/mL. Four, well-different samples were used: native glycinin, CGII; glycinin treated in cold acid medium (deamidation degree, $\%D = 0$) with reduction ($A_0$) and without reduction ($AB_0$); and deamidated glycinin with $\%D = 5.9-10.6\%$ ($AB_3-AB_{4.5}$). Figure 6 shows that at protein concentrations above 0.3 mg/mL no coalescence was observed (almost 0% oil separated) in almost all samples tested. At 0.3 mg/mL, coalescence was observed only with native glycinin. Below 0.1 mg/mL, all emulsions coalesce, but in varying degrees, depending on the sample. The resistance to coalescence increased in the following order: CGII < $AB_0$ < $AB_3$ (or $AB_{4.5}$) < $A_0$. These results are in agreement with Graham and Phillips (1976), who concluded that "for optimum prevention of coalescence the interfacial protein layer had to be as thick as possible and heavily hydrated and charged". The $A_0$ sample, mostly composed of A polypeptide, has the ability to associate at the interface and to increase the rigidity and hydration of the protein lamella (Wagner and Guéguen, 1995), which is congruent with its capacity to give highly stable foams (Wagner and Guéguen, 1999). Thus, the resistance to coalescence would be determined, to a great extent, by the resistance of the protein network formed during the emulsification process.

## CONCLUSIONS

In this work, the influence of the soy glycinin composition and the degree of structural modification on its

emulsifying properties were shown. By means of moderated deamidation it was possible to prepare modified glycinins with slightly enhanced emulsifying properties at low as well as high ionic strength. When disulfide bond reduction was included, modified proteins exhibited poor emulsion stability in front of the creaming−flocculation process at high ionic strength. However, in the same condition, these samples form emulsions with high stability toward coalescence. This result suggests that flocculation−creaming and coalescence processes are controlled by different mechanisms, perhaps electrostatic repulsion in the first and hydrophobic interactions in the second. Therefore, it is not always possible to attain an enhancement in both emulsifying properties at the same time by means of chemical modification of the glycinin structure.

ACKNOWLEDGMENT

We thank the LBTP personnel for the technical assistance. Dr. Jorge R. Wagner is affiliated with CONICET researcher Career.

LITERATURE CITED

Dagorn-Scaviner, C.; Guéguen, J.; Lefebre, J. Emulsifying properties of pea globulins as related to their adsorption behaviors. *J. Food Sci.* **1987**, *52*, 335−341.

Graham, D. E.; Phillips, M. C. The conformation of proteins at interfaces and their role in stabilizing emulsions. In *Theory and Practice of Emulsion Technology;* Smith, A. L., Ed.; Academic Press: London. 1976; pp 75−93.

Graham, D. E.; Phillips, M. C. Proteins at Liquid Interfaces. I. Kinetics of Adsorption and surface denaturation. *J. Colloid Interface Sci.* **1979**, *70*, 415−426.

Guéguen, J.; Bollecker, S.; Schwenke, K. D.; Raab, B. Effect of succinylation on some physicochemical and functional properties of the 12S storage protein from rapessed (*Brassica napus* L.). *J. Agric. Food Chem.* **1990**, *38*, 8, 61−69.

Guéguen, J.; Popineau, Y.; Anisimova, I. N.; Fido, R. J.; Shewry, P. R.; Tatham, A. S. Functionality of the 2S albumin seed storage proteins from sunflower (*Helianthus annuus* L.) *J. Agric. Food Chem.* **1996**, *44*, 1184−1189.

Halling, P. J. Protein-stabilized foams and emulsions. *CRC Crit. Rev. Food Sci. Nutr.* **1981**, *15*, 155−203.

Kato, A.; Nakai, S. Hydrophobicity determined by a Fluorescence Probe Methods and its Correlation with Surface Properties of Proteins. *Biochim. Biophys. Acta* **1980**, *624*, 13−20.

Kester, J. J.; Richardson, T. Modification of whey proteins to improve functionality. *J. Dairy Sci.* **1984**, *67*, 2757−2774.

Kinsella, J. E. Functional properties of soy proteins. *J. Am. Oil. Chem. Soc.* **1979**, *56*, 242−258.

Kim, S. H. Structure, Surface Properties and Foam Stability of Native, Reduced and Succinylated Soy 11S Globulins. Ph.D. Thesis, Cornell University, 1985.

Kim, S. H.; Kinsella, J. E. Surface Active Properties of Food Proteins: Effects of Reduction of Disulfide Bonds on Film Properties and Foam Stability of Glycinin. *J. Food Sci.* **1987a**, *52*, 128−131.

Kim, S. H.; Kinsella, J. E. Surface Active Properties of Food Proteins: Effects of Progressive Succinylation on Film Properties and Foam Stability of Glycinin. *J. Food Sci.* **1987b**, *52*, 1341−1352.

Matsudomi, N.; Sasaki, T.; Kato, A.; Kobayashi, K. Conformational changes and functional properties of acid-modified soy protein. *J. Agric. Food Sci.* **1985**, *49*, 1251−1256.

Nakai, S.; Ho, L.; Helbig, N.; Kato, A.; Tung, M. A. Relationship between hydrophobicity and emulsifying properties of some plant proteins. *Can. Inst. Food Sci. Technol. J.* **1980**, *13*, 23−27.

Nakai, S.; Li Can, E.; Hayakawa, S. Contribution of protein hydrophobicity to its functionality. *Narhung* **1986**, *30*, 327−336.

Nakai, S. Structure−function relationship of food proteins with emphasis on the importance of protein hydrophobicity. *J. Agric. Food Chem.* **1983**, *31*, 676−683.

N Guyen Thi Quynh, A.; Biyaoula, D.; Demeaux, M.; Lorient, D. Amélioration des propriétes tensioactives des protéines de soja par hydrolyze chimique limitée. *Sciences des Aliments*, **1992**, *12*, 117−130.

Peng, I. C.; Quass, D. W.; Dayton, W. R.; Allen, C. E. The Physicochemical and Functional Properties of Soybean 11S Globulin − A Review. *Cereal Chem.* **1984**, *61*, 480−490.

Phillips, M. C. Protein conformation at liquid interfaces and its role in stabilizing emulsions and foams. *Food Technol.* **1981**, *35*, 50−59.

Thanh, V. H.; Shibasaki, K. Major proteins of soybeans seeds. A Straight Forward Fractionation and Their Characterization. *J. Agric. Food Chem.* **1976**, *24*, 1117−1121.

Wagner, J. R.; Guéguen, J. Effects of dissociation, deamidation, and reducing treatment on structural and surface active properties of soy glycinin. *J. Agric. Food Chem.* **1995**, *43*, 1993−2000.

Wagner, J. R.; Guéguen, J. Surface functional properties of native, acid treated and reduced soy glycinin. 1. Foaming properties. *J. Agric. Food Chem.* **1999**, *47*, 2173−2180.

Walstra, P. Principles of foam formation and stability. In: *Foam: Physics, chemistry and structure*; Wilson, A. J., Ed.; Springer-Verlag: London, 1989; Chapter 1, pp 1−15.

Received for review September 2, 1998. Revised manuscript received March 18, 1999. Accepted March 19, 1999.

JF9809784