Correlation of average hydrophobicity, water/air interface surface rheological properties and foaming properties of proteins

A Medrano, C Abirached, AC Araujo, LA Panizzolo, P Moyna and MC Añón

Food Science and Technology International 2012 18: 187 originally published online 13 March 2012
DOI: 10.1177/1082013211415137

Article

FOOD SCIENCE AND TECHNOLOGY INTERNATIONAL

Correlation of average hydrophobicity, water/air interface surface rheological properties and foaming properties of proteins

A Medrano[1], C Abirached[1], AC Araujo[1], LA Panizzolo[1], P Moyna[1] and MC Añón[2]

Abstract

A comparative study on the behavior in the air–water interface of β-lactoglobulin, α-lactoalbumin, glycinin and β-conglycinin was performed. The behavior at the interface was evaluated by equilibrium surface tension and surface rheological properties of adsorbed films. There were significant differences ($\alpha \leq 0.05$) in the values of the constants of adsorption to the interface of the four proteins. The glycinin had the slowest rate of adsorption, due to its low average hydrophobicity, low molecular flexibility and large molecular size. Smaller proteins like β-lactoglobulin and α-lactoalbumin tended to greater equilibrium pressure values than the larger proteins because of its higher rate of adsorption to the interface. The foam capacity of proteins showed a positive correlation with the average hydrophobicity; the maximal retained liquid volume or the initial rate of passage of liquid to foam were significantly lower ($\alpha \leq 0.05$) when protein was glycinin. The dilatational modulus of glycinin was the lowest, which implies lowest resistance to disruption of the film. Glycinin protein has lower proportion of gravitational drainage and higher disproportionation having perhaps a less resistant film. In conclusion, β-conglycinin and whey proteins showed a similar behavior, so β-conglycinin might be the best soybean protein to replace milk proteins in food formulations.

Keywords

Soy proteins, milk whey proteins, interfacial rheology, foams

Date received: 28 November 2010; revised: 12 April 2011

INTRODUCTION

Of all the functional properties of proteins, the formation of foam is of great interest because they provide the airy texture of many products (Campbell and Mougeot, 1999). The foams are present in many foods either in the finished product or incorporated during its production stage, in a preliminary process, which may be subject to subsequent processing steps. Therefore, knowledge of the mechanisms of formation and stability of foam is essential if we are required to produce foam with certain characteristics.

Foam destabilization process consists on the tendency of the discontinuous gaseous phase to form a continuous phase by approaching and fusion of the bubbles, in order to reach a minimum surface area (minimum free energy). To this process, there is an opposition of the surface protein film, a mechanical barrier that is more effective as it is stiffer and viscoelastic (Wagner, 2000). The mechanisms for foam destabilization are liquid drainage (as a consequence of gravity force and liquid transfer from the interbubble

Food Science and Technology International 18(2) 187–193

DOI: 10.1177/1082013211415137
fst.sagepub.com
SAGE

[1]Cátedra de Ciencia y Tecnología de los Alimentos, Facultad de Química, Universidad de la República, Uruguay
[2]Centro de Investigación y Desarrollo en Criotecnología de Alimentos (UNLP – CONICET La Plata), La Plata, Argentina

Corresponding author:
A Medrano, Cátedra de Ciencia y Tecnología de los Alimentos, Facultad de Química, Universidad de la República, Uruguay
Email: amedrano@fq.edu.uy

lamella to the Plateau border), foam collapse by lamellar rupture and disproportionation. Foam collapse is usually a consequence of liquid drainage, and disproportionation also involves liquid drainage (Walstra, 1989).

Therefore, it is reasonable to consider the existence of two different processes for liquid drainage from foam, one due to liquid drainage itself and other due to a disproportionation (Panizzolo et al., 2012). Panizzolo et al. (2012) proposed a second-order kinetic model of foam destabilization with two terms, showing the existence of two simultaneous mechanisms of foam destabilization (liquid drainage by gravity and disproportionation) which predominate alternatively according to foam age. These authors also indicate that the kinetic constant k is the appropriate parameter for comparing the stabilizing properties of different proteins, since it is not influenced by the initial volume of liquid in the foam.

To be a good foaming agent, a protein should possess the following attributes: first, it should be able to rapidly adsorb at the air–water interface during whipping or bubbling; second, it should undergo fast conformational change and rearrangement at the air–water interface and rapidly reduce the surface tension and third, it should be able to form a cohesive, viscoelastic film through intermolecular interactions. The first two criteria are essential for better foamability; whereas, the third criterion is important for the foam stability (Damodaran, 1997).

No correlation was found among the foaming capacity of proteins and their surface hydrophobicity (So; Townsend and Nakai, 1983), instead, foaming capacity exhibits a positive correlation with the average hydrophobicity of proteins (Kato et al., 1983). At the high free energy air–water interface, proteins are denatured to a greater extent, which exposes buried hydrophobic residues to the interface. Thus, the properties of the unfolded protein rather than the native protein dictate the behavior of the protein at the air–water interface (Damodaran, 2005). Despite the above, very few studies have correlated the average protein hydrophobicity and foam properties.

So it is essential in designing new aerated products using soybean protein (glycinin and β-conglycinin) and whey protein (β-lactoglobulin [β-Lg] and α-lactoalbumin [α-La]), to evaluate the drainage and disproportionation and relate them with dynamic properties of adsorbed protein films on air–water interface to know better the way in which this affects the formation and stability of foams. Therefore, the objective of this work was to carry out a comparative study between these proteins evaluating foaming properties, the proportion in which the gravitational drainage and disproportionation occurs, their behavior at the air–water interface and relate them with their average hydrophobicity.

MATERIALS AND METHODS

Materials

α-La and β-Lg isolate, both with 90.0% of purity, from Davisco (Le Sueur, MN) were used in all experiments performed.

The isolation of the native fractions of β-conglycinin and glycinin was made from defatted soy flour with the procedure proposed by Nagano et al. (1992), followed by the dispersion of the precipitate on alkaline medium (pH 8.0) and freeze-drying.

In all cases, protein content was determined by Lowry method (Lowry et al., 1951).

All other chemicals and reagents were of analytical grade from Sigma Chemical Co. (St. Louis, MO).

Methods

Protein solubility. The solubility index of all the samples was calculated as the ratio between the soluble protein concentration (mg/mL) and the total protein concentration (mg/mL). Soluble protein concentration and the total protein concentration were determined by Lowry method (Lowry et al., 1951). Samples were dispersed in 0.01 M phosphate buffer pH 7.5 for 30 min at room temperature and centrifuged for 15 min at 10,000 g at 5 °C before measuring protein concentration in the supernatant.

Average hydrophobicity. The average hydrophobicity was calculated according to the method of Bigelow (1967).

Surface hydrophobicity. It was evaluated using 8-anilino-1-naphthalenesulfonic acid (ANS) as fluorescent probe (Hayakawa and Nakai, 1985). Spectrofluorometric measurements were taken at pH 7.5 on an Aminco-Bowman Series 2 Luminescence spectrometer. The fluorescence intensity of the blank (FIb) and of the ANS—protein conjugate (FIe) were recorded at λ_{ex}: 363 nm and λ_{em}: 475 nm, using 5-nm emission and excitation slit widths. The So was obtained graphically using the Kato and Nakai (1980) equation. A plot of FI% versus PC was drawn; where PC is the protein concentration, FI% = (FIN)/(FImax), FIN = FIe – FIb, FImax is the maximum fluorescence measured from the total binding of ANS in methanol.

Interfacial tension and interfacial rheology. The interfacial tension at the air–water interface was measured by using the automated drop tensiometer (Tracker, IT-Concept, Saint-Clémenttes Places,

Table 1. Solubility index, average hydrophobicity and surface hydrophobicity of β-lactoglobulin, α-lactoalbumin, β-conglycinin and glycinin. The solubility index and surface hydrophobicity were measured in dispersions of 1 mg/mL of the protein in 0.01 M sodium phosphate buffer pH 8

	Solubility index* (%)	Average hydrophobicity (kJ/mol)	Surface hydrophobicity*
β-Lg	86 ± 5 a	5.482	37 ± 1 b
α-La	84 ± 2 a	5.041	12 ± 2 a
β-conglycinin	88 ± 4 a	4.653	14 ± 6 a
Glycinin	88 ± 6 a	4.041	17 ± 2 a

β-Lg: β-lactoglobulin; α-La: α-lactoalbumin.
*Means in the same column followed by different letters are significantly different ($p \leq 0.05$).

France). Measurements were performed at room temperature (25 ± 3 °C). A dispersion of 1 mg/mL of the proteins in 0.01 M phosphate buffer pH 8 was introduced in the bucket of the tensiometer and the air was added in a syringe from which a bubble of 3 μL was formed. The surface pressure (π) is $\pi = \gamma_0 - \gamma$, where γ_0 and γ are the surface tension of pure water in absence of any surface-active component and in presence of protein, respectively.

The interfacial rheological properties were also determined by the oscillating bubble technique as described by Benjamins et al. (1996). The volume of the air bubble was modified in sinusoidal way with a constant amplitude ($\Delta A/A = 0.1$) and oscillation frequency of 0.2 Hz.

The surface dilatational modulus (E) derived from the change in interfacial tension (γ; equation (1)) as a result of a small change in droplet surface (A), equation (2), according to equation (3) (Lucassen and van den Tempel, 1972).

$$\gamma = \gamma_0 \times \sin(\varpi t + \phi) \quad (1)$$

$$A = A_0 \times \sin(\omega t) \quad (2)$$

$$E = \frac{\mathrm{d}\gamma}{\frac{\mathrm{d}A}{A}} = \frac{d\pi}{d \ln A} \quad (3)$$

where γ_0 and A_0 are the stress and strain amplitudes, respectively, t is time, ω is frequency, ϕ is the phase angle between stress and strain, $\pi = \gamma_0 - \gamma$ is the interfacial pressure and γ_0 is the interfacial tension in the absence of emulsifier.

The dilatational modulus, E, is a complex quantity and is composed of real and imaginary terms equation (4). The real term is related to the elastic or storage component (E_d) and the imaginary term to the viscous or loss component (E_v). For a perfectly elastic material, stress and strain are in phase ($\phi = 0$) and the imaginary term is zero. In the case of a perfectly viscous material ($\phi = 90°$), the real term is zero. The loss-angle tangent is defined by equation (5). If the film is purely elastic, the loss-angle tangent is zero.

$$E = \left(\frac{\gamma_0}{A_0}\right) \times (\cos\phi + i \sin\phi) = Ed + iEv \quad (4)$$

$$tg\phi = \frac{Ev}{Ed} \quad (5)$$

Foam properties. The foam properties of samples were determined by conductimetry using the method and device developed by Loisel et al. (1993). Foam was formed by air sparging into the protein solution in a column with fritted glass disk at the bottom. The foaming solutions were prepared at 1 mg/mL in 0.01 M sodium phosphate buffer pH 8. The level of the solution as a function of time was measured by conductimetry with a pair of electrodes located at the base of the column. To evaluate the foaming capacity, the maximal volume of liquid retained in the foam (VLE_{max}) and the initial rate of liquid transfer to the foam (v_o) were measured (Wagner et al., 1996). In order to estimate the stability of the foams formed, experimental data were adjusted to the second-order equation of two terms proposed by (Panizzolo et al., 2012).

$$V(t) = \frac{V_g^2 k_g t}{V_g k_g t + 1} + \frac{V_d^2 k_d t}{V_d k_d t + 1} \quad (6)$$

where k_g and k_d are the rate constants corresponding to the gravitational draining and gas diffusion or disproportion, respectively, and t is time. V_g and V_d are the maximum volume drained because of the gravitational drainage and gas diffusion or disproportion, respectively. By this model, it can be distinguish between two processes that occur simultaneously such as gravity drainage and disproportion.

Statistical analysis. All the experiments were performed in triplicate. The statistical analysis was established by variance analysis and test of minimum significant difference, using statistical program StatgraphicPlus7.0.

RESULTS AND DISCUSSION

Characterization of proteins

Several functional properties, such as thickening, foaming, emulsification and gelation of proteins are affected by protein solubility. Solubility of a protein is mainly related to its hydrophilicity/hydrophobicity balance.

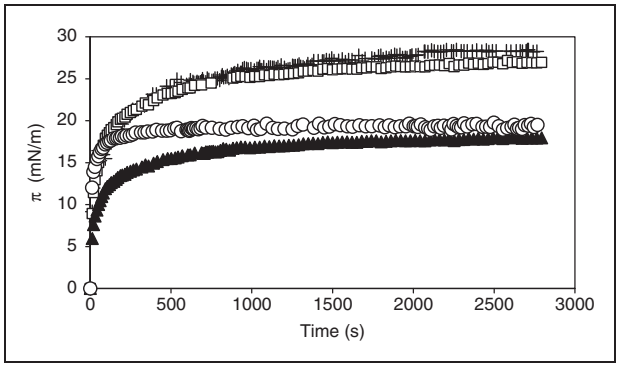

Figure 1. Surface pressure (π) as a function of adsorption time of the α-lactoalbumin (+), β-lactoglobulin (□), glycinin (▲) and β-conglycinin (○) in dispersions of 1 mg/mL of the protein in 0.01 M sodium phosphate buffer pH 8 at the air–water interface.

Table 2. Parameters related to the foaming capacity corresponding to the different samples assayed in dispersions of 1 mg/mL of the protein in 0.01 M sodium phosphate buffer pH 7.5

	VLE_{max} (mL)	$V_o \times 10^3$ (mL/s)
β-Lg	7.6 ± 0.6 a	2.7 ± 0.3 a
α-La	6.1 ± 0.7 b	2.7 ± 0.5 a
β-conglycinin	6.3 ± 0.4 b	3.0 ± 0.2 a
Glycinin	4.3 ± 0.3 c	2.2 ± 0.3 b

β-Lg: β-lactoglobulin; α-La: α-lactoalbumin.
Means in the same column followed by different letters are significantly different ($p \leq 0.05$).

Thus, the amino acid composition of a protein inherently affects its solubility characteristics.

The values of solubility of the β-Lg, α-La, β-conglycinin and glycinin in 0.01 M phosphate buffer pH 7.5 had no significant differences between them (Table 1). However, the values of So of the β-Lg were significantly higher than those from α-La, β-conglycinin and glycinin with no significant differences ($p \leq 0.05$) between the last ones. It is also observed that the β-Lg had the highest average hydrophobicity followed by the α-La and β-conglycinin, while glycinin had the lowest value.

Surface behavior

The diffusion of protein onto the air–water interface can be inferred from the rate of change of the surface pressure upon adsorption (Figure 1). There was a significant increase in surface pressure with time of adsorption at the interface for the four proteins studied. This increase was related to the behavior of proteins at the interface which can be evaluated through three consecutive stages: the diffusion of protein molecules, adsorption to the interface and the unfolding of the adsorbed molecules (Rodríguez Patino et al., 2008). It is observed that milk whey proteins have the highest values of equilibrium surface pressure which is consistent with data from average hydrophobicity. Rodríguez Niño et al. (2005) observed that the milk whey protein isolate presented higher surface pressure than 7S and 11S soy globulins. The same behavior was achieved in this work as shown in Figure 1.

The experimental data were adjusted with a first-order equation with two exponential components developed by Panizzolo (2005) and the first-order rate constants for the adsorption (k_a) and rearrangement (k_r) processes of the proteins in the air–water interface were estimated

$$\gamma_t = A_a e^{-k_a t} + A_r e^{-k_r t} + \gamma_e \quad (7)$$

where γ_t is the tension at the time t, k_a and k_r are first-order rate constants for the processes of adsorption (involving penetration and possible unwinding of the protein molecule at the interface) and rearrangement of proteins at the air–water interface, respectively. A_a and A_r are the amplitude parameters of these kinetic phases and γ_e the equilibrium tension.

Glycinin had the slowest rate constant for adsorption in the air–water interface (k_a) due to its low average hydrophobicity (Table 2), low molecular flexibility and large molecular size. These results are consistent with those found by Wagner and Guéguen (1995). These authors showed that the adsorption rate at the beginning of the process was highly dependent on conformational state. Consequently, smaller proteins like β-Lg (MW: 18 kDa) and α-La (MW: 14 kDa) tend to have greater equilibrium pressure values than the larger proteins (Table 2) because its rate of adsorption is related to their higher rate of diffusion to the interface (Martin et al., 2002). With respect to k_r, there were no significant differences between the samples (results not shown). The same result was achieved by Rodríguez Niño et al. (2005) with the milk whey protein isolate and the 7S and 11S soy globulins.

The surface viscoelasticity is a measure of the ability of the film to adapt to a change in surface area, and therefore it is expected to play a crucial role in the foam capacity of the protein. In this case, it was found that the dilatational modulus (E), E_d and E_v of glycinin were significantly lower than those corresponding to other proteins, which implies a lower resistance to rupture of the film formed (Table 2).

Foam properties

Regarding foaming capacity, no significant differences were found in the maximal retained liquid volume

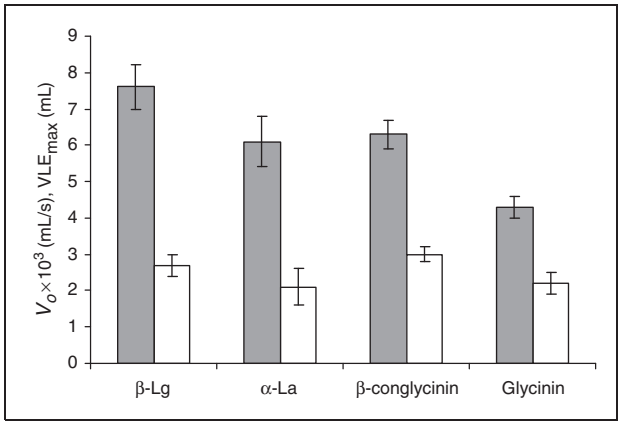

Figure 2. Parameters related to the foaming capacity, (□) V_o* and (■) VLE_{max} corresponding to the different samples assayed in dispersions of 1 mg/mL of the protein in 0.01 M sodium phosphate buffer pH 8.

Table 3. The rate constant of adsorption to air–water interface, K_a, surface dilatational properties E (dilatational modulus), E_d (dilatational elasticity), E_v (surface dilatational viscosity) of the studied proteins in dispersions of 1 mg/mL of the protein in 0.01 M sodium phosphate buffer pH 7.5

	K_a (s^{-1})	E (mN/m)	E_d (mN/m)	E_v (mN/m)
β-Lg	0.051 ± 0.002 a	36 ± 4 c	35 ± 4 c	7 ± 2 b
α-La	0.041 ± 0.002 b	57 ± 4 a	56 ± 4 a	13 ± 2 a
β-conglycinin	0.010 ± 0.002 c	48 ± 4 b	47 ± 4 b	5 ± 2 b
Glycinin	0.002 ± 0.002 d	21 ± 2 d	21 ± 2 d	2.2 ± 0.3 c

β-Lg: β-lactoglobulin; α-La: α-lactoalbumin.
Means in the same column followed by different letters are significantly different ($p \leq 0.05$).

(VLE_{max}) or the initial rate of liquid to foam passage (v_o) between β-Lg, α-La and β-conglycinin. However, VLE_{max} and v_o were significantly lower ($p \leq 0.05$) when protein was glycinin (Figure 2).

The foamability of proteins shows a positive correlation with the average hydrophobicity (Kato et al., 1983). The results shown in Figure 2 and Tables 1 and 2 suggest that the surface hydrophobicity (SO) allowed the proteins to successfully anchor to the air/water interface during bubbling. However, once the protein is adsorbed to the interface, its ability to expose all the hydrophobic residues to the interface depends on the average hydrophobicity, as it was previously mentioned by Damodaran (2005). Experimental evidence indicates that proteins indeed undergo substantial conformational changes at the air/water interface (Phillips et al., 1995). Thus, the rate of reduction in surface tension and the expansion of the interfacial area during foaming are only limited by the total number of hydrophobic groups available in the protein, not by the number of hydrophobic patches on the protein surface.

Although flexibility, which is defined as the relative movement of various domains in a protein or the reorientation relaxation rates of amino acid residues in a polypeptide chain, has been recognized as the most important criterion for functionality, especially for foaming properties, the relationship between initial conformation of a protein in solution and its foaming properties is not fully understood (Damodaran, 1997, 2005). This is important, especially in technological applications, because if such a relationship exists, proteins can be physically or chemically modified to assume a certain structure prior to foaming.

The foaming capacity of a protein is intimately related to the rate of diffusion onto the surface and to the rheological properties of the protein adsorbed layer. Hence, small, flexible and random proteins have higher foaming capacity than large and compact proteins due to their more rapid diffusion onto the surface. The surface viscoelasticity of the protein layer is a key parameter in foamability according to numerous studies (Langevin, 2000; Stubenrauch and Miller, 2004; Wilde, 2000). Explicitly, the process of formation of foam implies the creation of surface area that should be rapidly covered by protein to prevent rupture of the film.

In the foams obtained with the four proteins, the values of the k_g (gravity drainage) were higher by an order regarding k_d (disproportionation). Glycinin protein presents a higher k_g than the other proteins ($p \leq 0.05$), which implies lower foam stability. Foams formed from β-conglycinin, α-La and β-Lg had no significant differences in stability with respect to gravity drainage (k_g), probably related to a more resistant film as confirmed in the study of interfacial rheology (Table 3). Pizones et al. (2009) found that, for a particular protein, the overall foam destabilization (the half-life time of the foam) may be related to the interfacial characteristics of the protein film adsorbed around the bubbles, which, in turn, depend on the aggregation of the protein molecule in dissolution and at the interface. A relationship exists between the overall foam stability and surface pressure. On the other hand, the combined effects of interfacial adsorption and interfacial interactions between adsorbed proteins, which are reflected in surface dilatational modulus, may also correlate with the foam stability, but this correlation was not observed by them. However, we found that a better foam stability implies higher values of the elastic component or the viscous component of the elastic dilational modulus.

One important factor that retards film drainage is the Marangoni effect. The magnitude of the

Marangoni effect is a function of the dilatational modulus, E, of the protein film and the ability of the surfactant layer to stretch toward the high-tension region is greater the larger is E, E_d and E_v (Damodaran, 2005).

The viscoelastic properties of the surfactant film can exert control of the shrinkage of the small bubbles because of the solubilization of the gas in the continuous phase in the disproportionation. For instance, if the surfactant molecule in the film can be readily displaced or dissolved into the bulk phase as the bubble shrinks, then disproportion can proceed without any change in the interfacial tension. In the case with proteins, the increase in the concentration of surfactant in the adsorbed layer as the bubble shrinks increases its surface rheological properties, notably, the surface dilatational modulus of the adsorbed layer. This significantly retards the rate of disproportion (Damodaran, 2005).

The mechanisms of foam destabilization also occur during the formation of the foam so that to have good foaming properties, the proteins must be able to counter these mechanisms of destabilization. The presence of protein at the interface is a key factor in the formation of foams and, hence, it seems reasonable to find that only the solutions that provide a fast decrease of the surface tension provide stable foams. The Marangoni effect plays a vital role during foam formation, where constant stretching of foam lamellae occur (Damodaran, 1997), with the consequent relationship with the interfacial rheological parameters noted above.

The study of the rate constants is interesting, to see in what extent the gravitational drainage and disproportionation mechanisms contribute to the total volume of liquid drained. For this reason, the proportions of volume of liquid drained by gravity drainage (V_g) and drained volume because of the disproportionation (V_d) was determined. In all tested cases, the proportion of drained liquid by gravity drainage was significantly higher (never less than 60%) than the drained volume because of the disproportionation. For the calculation of V_g and V_d, it was assumed that all the liquid has drained (infinite time). As to the proportions of the contribution of each mechanism, glycinin has a lower proportion of gravitational drainage and higher disproportionation having perhaps a less resistant film. The glycinin has the lowest values of surface dilatational modulus (E), elastic component (E_d) and viscous component (E_v), then, the Marangoni effect is diminished, and therefore the foam stability phenomena.

CONCLUSIONS

The results obtained indicate that in the working conditions glycinin presents worse behavior in the air–water interface and foaming properties than other proteins studied. Glycinin presents the slowest rate of adsorption, v_o and VLE_{max} due to its low average hydrophobicity and the lowest film resistance at the interface. β-conglycinin presented a similar behavior to those corresponding to α-La and β-Lg, so β-conglycinin might be the best soybean protein to replace milk proteins in food formulations.

FUNDING

The authors acknowledge the financial support from the Plan de Desarrollo Tecnológico (PDT) del Ministerio de Educación y Cultura (Proyecto S/C/O/34/02, S/C/O/66/12 and Beca S/C/BE/33/03) and Plan de Desarrollo de las Ciencias Básicas (PEDECIBA), Uruguay.

ACKNOWLEDGMENTS

The authors wish to thank Luis Mussio, Álvaro Gancharov, Eduardo Aguilar, and Heinkel Bentos-Pereira (Departamento de Instrumentos, Facultad de Química) and Daniel Acosta y Lara (Taller de Aparatos de Vidrio, Facultad de Química) for their help in the design and construction of the apparatus to evaluate foaming properties, TIAV 2002.

REFERENCES

Benjamins J, Cagna A and Lucassen–Reynders E. (1996). Viscoelastic properties of triacylglycerol/water interfaces covered with proteins. *Colloid and Surfaces* 114(1): 245–254.

Bigelow CC. (1967). On the average hydrophobicity of proteins and the relation between it and protein structure. *Journal of Theoretical Biology* 16(2): 187–211.

Campbell GM and Mougeot E. (1999). Creation and characterisation of aerated food products. *Trends in Food Science & Technology* 10(9): 283–296.

Damodaran S. (1997). Protein-stabilized foams and emulsions. In: Damodaran S and Paraf A (eds) *Food Proteins and Their Applications*. New York: Marcel Dekker, 57–110.

Damodaran S. (2005). Protein stabilization of emulsions and foams. *Journal of Food Science* 70(3): R54–R66.

Hayakawa S and Nakai S. (1985). Relationships of hydrophobicity and net charge to the solubility of milk and soy proteins. *Journal of Food Science* 50(2): 486–491.

Kato A and Nakai S. (1980). Hydrophobicity determined by a fluorescence probe method and its correlations with surface properties of proteins. *Biochimica et Biophysica Acta* 624(1): 13–20.

Kato A, Osako Y, Matsudomi N and Kobayashi K. (1983). Changes in the emulsifying and foaming properties of proteins during heat denaturation. *Agricultural and Biological Chemistry* 47(1): 33–37.

Langevin D. (2000). Influence of interfacial rheology on foam and emulsion properties. *Advances in Colloid and Interface Science* 88(1–2): 209–222.

Loisel W, Guéguen J and Popineau Y. (1993). A new apparatus for analyzing foaming properties of proteins. In: Schwenke KD and Mothes R (eds) *Food Proteins. Structure and Functionality*. Weinheim: VCH, 320–323.

Lowry OH, Rosebrough NJ, Farr AL and Randall RJ. (1951). Protein measurement with the folin-phenol reagent. *Journal of Biological Chemistry* 193(1): 265–275.

Lucassen J and Van den Tempel M. (1972). Dynamic measurements of dilational properties of a liquid interface. *Chemical Engineering Science* 27(6): 1283–1291.

Martin AH, Bos MA and Van Vliet T. (2002). Interfacial rheological properties and conformational aspects of soy gycinin at the air/water interface. *Food Hydrocolloids* 16(1): 63–71.

Nagano T, Hirotsuka M, Kohyama K and Nishinari K. (1992). Dynamic viscoelastic study on the gelation of 7S globulin from soybeans. *Journal of Agricultural and Food Chemistry* 40(6): 941–944.

Panizzolo LA (2005). *Modificación de proteínas por vía enzimática. Análisis de la relación estructura-funcionalidad de los productos de hidrólisis*. Doctoral thesis, Facultad de Química, Universidad de la República, Uruguay.

Panizzolo LA, Mussio LE and Añón MC. (2012). A kinetic description for the destabilization process of protein foams. *International Journal of Food Properties* 15(1): 60–68.

Phillips LG, Hawks SE and German JB. (1995). Structural characteristics and foaming properties of β-lactoglobulin: effects of shear rate and temperature. *Journal of Agricultural and Food Chemistry* 43(3): 613–619.

Pizones Ruíz–Henestrosa V, Carrera Sánchez C, Pedroche JJ, Millán F and Rodríguez Patino JM. (2009). Improving the functional properties of soy glycinin by enzymatic treatment. Adsorption and foaming characteristics. *Food Hydrocolloids* 23(2): 377–386.

Rodríguez Niño R, Carrera Sánchez C, Pizones Ruíz-Henestrosa V and Rodríguez Patino JM. (2005). Milk and soy protein films at the air–water interface. *Food Hydrocolloids* 19(3): 417–428.

Rodríguez Patino JM, Rodríguez Niño MR and Carrera Sánchez C. (2008). Implications of interfacial characteristics of food foaming agents in foam formulations. *Advances in Colloid and Interface Science* 140(2): 95–113.

Stubenrauch C and Miller R. (2004). Stability of foam films and surface rheology: an oscillating bubble study at low frequencies. *Journal of Physical Chemistry B* 108(20): 6412–6421.

Townsend A and Nakai S. (1983). Relationship between hydrophobicity and foaming capacity of proteins. *Journal of Food Sciences* 48(3): 588–594.

Wagner JR. (2000). Propiedades superficiales. In: Bartholomai W and Pilosof AMR (eds) *Caracterización funcional y estructural de proteínas*. Argentina: EUDEBA, 41–74.

Wagner JR and Guéguen J. (1995). Effects of dissociation, deamidation and reducing treatment on structural and surface active properties of soy glycinin. *Journal of Agricultural and Food Chemistry* 43(8): 1993–2000.

Wagner JR, Sorgentini DA and Añón MC. (1996). Thermal and electrophoretic behaviour, hydrophobicity and some functional properties of acid-treated soy isolates. *Journal of Agricultural and Food Chemistry* 44(7): 1881–1889.

Walstra P. (1989). Principles of foam formation and stability. In: Wilson AJ (ed.) *Foams: Physics, Chemistry and Structure*. London: Springer-Verlag, 1–15.

Wilde P. (2000). Interfaces: their role in foam and emulsion behavior. *Current Opinion in Colloid & Interface Science* 5(3): 176–181.