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# Emulsifying and foaming properties of β-lactoglobulin modified by heat treatment

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ABSTRACT

The effects of heat treatment on emulsifying properties of beta-lactoglobulin were studied in order to compare them with previous studies on foaming properties. Both of them are closely linked to the structural changes on the protein. Aliquots from 5.5% (w/v) beta-lactoglobulin solution in 20 mM phosphate buffer at pH 6.8 were heated at 85 °C for different time periods, from 1 to 15 min. Protein solubilities were measured for unheated and heated beta-lactoglobulin samples. Protein-stabilized O/W emulsions were prepared with these samples and corn oil. Droplet size distribution in the emulsions and emulsifying activity index were determined for each system, as parameters of the emulsifying ability of the protein. Emulsion stability was estimated from three different methods: backscattering, determination of the remaining protein concentration after creaming and monitoring the oiling off process. With the assayed methodology, heat treatment of beta-lactoglobulin led to different effects on foaming and emulsifying properties of the protein, depending on the time of heating. For shorter times of heating, both foamability and foam stability improved, while emulsifying properties diminished. After 10 min of heating at 85 °C, both foaming and emulsifying properties diminished. Formation time scales, as well as size of the sedimentable aggregates and their steric effect on the interfacial film, play an important role in explaining these differences between foaming and emulsifying properties.



## 1. Introduction

Proteins play an important role as macromolecular surfactants in foam and emulsion-type food products. The functioning of proteins is determined by their structure and properties in the adsorbed layers at air–water or oil–water interfaces. Since typical food proteins are mixtures of several proteins, interaction between them in the adsorbed layer also impacts their ability as surfactants to stabilized dispersed systems (Damodaran, 2005).

Both emulsions and foams share many common features: two phases, energy requirement in order to be formed and subsequent thermodynamic instability, which makes them liable to separation into their two original phases over time. Although destabilization is an unavoidable process that the system undergoes with time, environmental conditions and/or previous treatments of the emulsifying/foaming agent can be controlled in order to enhance the stability of the emulsion/foam, achieving better food uses (Damodaran, 2005; Dickinson, 2009; Euston & Hirst, 1999; Foegeding, Luck, & Davis, 2006).

The factors affecting the stability of foams are very similar to those that affect emulsion stability. They are disjoining pressure, viscoelasticity of the surfactant film and its interfacial tension (Damodaran, 2005). Liquid drainage and gas disproportionation (Ostwald ripening) are two macroscopic processes that contribute especially to instability of foams (Monsalve & Schechter, 1984; Yu & Damodaran, 1991). Foam stability is conditioned by several different factors like interfacial film properties, such as its rheology and surface tension, as well as viscosity of the continuous phase and bubble size. Finally, rupture of the film leads to a decrease of the foam column (Wilde & Clark, 1996).

On the other hand, the physical mechanisms of emulsion destabilization include: gravitational processes (creaming or sedimentation), flocculation, droplet coalescence, Ostwald ripening and phase inversion (McClements, 1999). For an oil-in-water emulsion, in quiescent conditions, the most obvious initial manifestation of instability is creaming, which leads to macroscopic phase separation into discernible regions, separating cream from serum. This may then be followed by droplet coalescence within the cream and oiling off (the complete separation of phases) at the top of the sample (Dickinson, 1992, 2001, 2003). Creaming is influenced by several factors such as droplet size, emulsion polydispersibility, continuous phase viscosity and floc formation (McClements, 1999).

In the formulation of any emulsion or foam, two types of ingredients are usually needed: some emulsifying/foaming agent and some stabilizer (Dickinson, 1992, 2003). While the first ones promote dispersion formation in both systems and their short-term stabilization by interfacial action, the stabilizers confer long-term stability by a mechanism of adsorption. Proteins are able to fulfil both roles: as emulsifying/foaming agents and as stabilizing ones. An ideal emulsifying/foaming protein must contain hydrophobic groups that are numerous enough and relatively accessible on a short time scale to enable the adsorbing molecules to adhere to and spread out at the interface, thereby protecting the newly formed droplets/bubbles. Proteins must move to the interface,

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adsorb on it, unfold and rearrange themselves in order to produce a viscoelastic film which protects droplets or bubbles. This process can be enhanced depending on some protein properties like their amphipathic structure, tensoactive capacity and surface hydrophobicity (Dickinson, 1992; Graham & Phillips, 1979; Walstra, 1993). Protein surface active properties are governed by size, shape, net charge and distribution of charges, surface hydrophobicity, stability, flexibility, amino acid composition and structure (Cayot & Lorient, 1997; Dalgleish, 1996). Therefore, disordered, smaller and more flexible proteins are better surface agents than ordered, larger and rigid ones. Besides, an emulsifying/foaming agent capable of making small droplets/bubbles is typically composed of species of relatively low molecular mass with good solubility in the aqueous continuous phase (Dickinson, 2003).

Protein denaturation often improves the surface activity of proteins. Thermal denaturation produces a pronounced structural change with the exposure of hydrophobic sites (Damodaran, 1994; Kinsella & Whitehead, 1989; Phillips, Whitehead, & Kinsella, 1994). This implies a practical significance since heating is an important processing step for many products that consist of protein foams (Foegeding et al., 2006).

In a recent work (Moro, Báez, Busti, Ballerini, & Delorenzi, 2011), we have shown that the time of previous heating is a crucial variable for the features of beta-lactoglobulin (β-LG) as a foaming agent. In this cited work, 3 min was pointed out as the critical time when 5.5% (w/v) β-LG solution was heated at 85 °C, since the most significant conformational change and aggregation processes occurred, producing non-native monomers and the greatest amount of dimers and trimers (monomer 51%, dimer 33% and trimer 16%). Heat treatment affected foamability and even more, foam stability. Both foaming properties are closely linked to structural changes of the protein. The increase in surface hydrophobicity was considered a decisive factor in the improved foamability, in spite of the presence of aggregates of higher molecular weight. On the other hand, volume foam stability was increased in a much higher degree than foamability. The best foam stabilization was achieved at 3 min of heat treatment, with an increment of ~800% higher than that corresponding to foam formed with unheated β-LG. This is coherent with the most significant conformational changes observed at this time. The greater stability was attributed to an increase in protein solution viscosity because of the presence of aggregates of low molecular weight, which slows the drainage rate, and mainly to rheological factors such as the stiffening of the interfacial film, which makes the bubbles more resistant to disproportionation and collapse. The presence of larger aggregates, formed over 10 min of heat treatment, could be responsible for the observed opposite effect, the decay of stability.

In this paper, recent progress in our understanding of molecular mechanisms and conformational changes involved in thermal treatment of β-LG has been reviewed, to achieve a further description and explanation over its effects on formation and stability of protein-stabilized foams and emulsions. In view of our prior experiences with foams (Moro et al., 2011), the aim of this work was to study the effects of heat treatment on emulsifying properties of β-LG, in order to compare them with the already known effects of this treatment on the protein foaming properties.

## 2. Materials and methods

### 2.1. Materials

β-LG was purchased from Sigma Chemicals Co. (St. Louis, MO, USA) and used without further purification. All other chemicals were of analytical grade.

### 2.2. Heat treatment of β-LG

A stock 5.5% (w/v) β-LG solution was prepared in 20 mM phosphate buffer at pH 6.8. Aliquots of 3 mL from this solution were placed in small glass tubes and heated in a water bath at 85 °C for different periods of time, from 1 to 15 min. The samples were cooled to room temperature and analyzed as described in the following sections. All the following measurements were carried out at 25 °C.

### 2.3. Protein solubility

Unheated and heated β-LG samples, for different time periods (1, 3, 5, 7, 10 and 15 min), were centrifuged for 10 min at 15,000 *g* (Presvac EPF-12 microcentrifuge, Argentina) in order to sediment insoluble proteins. Concentrations of β-LG that remained in solution after centrifugation were determined through measurements of absorbance at 280 nm using a Jasco V-500 spectrophotometer (Jasco International Co., Ltd., Tokyo Japan), in the presence of 1% sodium dodecyl sulfate (SDS). This strong denaturing agent was added in order to unfold the protein and expose aromatic residues, such as tryptophan and tyrosine, to the solvent uniformly in the different β-LG species. A calibration curve was plotted using a standard sample of β-LG, neither heated nor centrifuged, in the presence of 1% SDS. When turbidity affected absorbance measurements, a correction was made. The presence of turbidity is usually revealed by an apparent absorbance gradually decreasing toward longer wavelengths in nonabsorbing regions (i.e., >320 nm). A linear plot of the logarithm of the absorbance vs. the logarithm of the wavelength can be extrapolated through the 250–300 nm region and subtracted from the experimentally observed spectrum to obtain corrected values (Mach, Volkin, Burke, & Russell Middaugh, 1995). This procedure was only necessary for longer times of heat treatment, 10 and 15 min, in which turbidity was more intense.

Protein solubility in percentage, PS (%), was calculated as:

$$\mathrm{PS}(\%) = \frac{pcs}{pcc} \cdot 100 \quad (1)$$

where *pcs* is the protein concentration in the supernatant of each heated sample and *pcc*, the protein concentration of the unheated sample.

### 2.4. Emulsion formation

Protein-stabilized emulsions (O/W protein) were prepared by intensive stirring of 20 mL of 0.1% (w/v) β-LG solution in 20 mM phosphate buffer at pH 6.8 with 5 mL of corn oil (volume fraction of the dispersed phase, $\phi = 0.20$) using an Omni GLH homogenizer (Omni International, Marietta, GA) operating at 20,000 rpm for 1 min. Samples of the protein, both unheated and heated for different times, were used to form each corresponding emulsion.

### 2.5. Emulsifying properties

#### 2.5.1. Particle size distribution

Immediately after homogenization, aliquots of emulsions formed with solutions of β-LG, both unheated and heated for different time periods, were analyzed using a Laser Diffraction Mastersizer 2000 Particle Size Analyzer (Malvern Instruments, Worcestershire, U.K.). Oil droplet size distribution was recorded and D[4,3] (also known as De Brouckere Mean Diameter) was determined as droplet mean value for volume distribution. Determinations were carried out in triplicate.

#### 2.5.2. Emulsifying activity index

Aliquots of the emulsion were diluted (1:100) in 0.1% (w/v) SDS, immediately after emulsion formation (Pearce & Kinsella, 1978). The flasks containing the diluted emulsions were shaken in vortex in order to obtain homogeneous mixtures, and the absorbance (A) was read at 500 nm.

Turbidity was calculated from Eq. (2):

$$\tau = \frac{2.303 \cdot A \cdot f}{1} \quad (2)$$

where f is the diluting factor and l, the optical path length (0.01 m).

The emulsifying activity index (EAI) was calculated from Eq. (3) (Cameron, Weber, Idziak, Neufeld, & Cooper, 1991; Silva, Morais, & Silvestre, 2003; Tang, Yang, Chen, Wu, & Peng, 2005):

$$EAI = \frac{2 \cdot \tau}{(1-\phi) \cdot C} \quad (3)$$

where $\tau$ is the turbidity of the sample, $\phi$ is the volume fraction of the dispersed phase and $C$, the initial concentration of protein ($10^3$ g $m^{-3}$).

EAI ($m^2$ $g^{-1}$) of the different samples were plotted versus heating time, as ratios in reference to the initial value (EAI°), the EAI value for the emulsion formed with unheated β-LG.

Although particle sizing is recognized as a more accurate method, EAI is a widely used parameter due to the availability of the laboratory equipment required and because it is a simple and useful method when comparisons between different emulsifying agents are made (Cameron et al., 1991). It has been noted that while laser diffraction generates the D[4,3] or volume moment mean, EAI is related to the surface area moment mean or Sauter mean diameter, D[3,2].

### 2.5.3. Emulsion stability

*2.5.3.1. Backscattering measurements.* Backscattering (BS) measurements of an emulsion sample over time provide a good quick estimate of its stability. Changes in BS values are associated with changes in homogeneity, droplet particle size and concentration, thus giving an appropriate estimation for emulsion stability (Durand, Franks, & Hosken, 2003). Emulsion stability was estimated using a vertical scan analyzer (Quick Scan, Beckman Coulter, Fullerton, CA). The samples were placed in a cylindrical glass measurement cell (total height = 60 mm) and BS profiles were obtained, in quiescent conditions, at different times after emulsion preparation. The zone of the cell between 10 and 20 mm high was chosen to follow the destabilization kinetics of the emulsion as a function of time. Each profile represents the variation of BS values (BS %) with the sample height, over time.

The time required for the initial mean BS value (t = 0 min) to decay 20% is defined as $t_{0.2}$, being the emulsion stability parameter considered in this work. This time is plotted versus heating time, as ratios in reference to the initial value, $t_{0.2}$°, corresponding to the emulsion formed with unheated β-LG.

*2.5.3.2. Remaining protein concentration.* In order to determine the remaining protein concentration at the end the creaming process ended, the different emulsion samples were left in quiescent conditions for 24 h. Then, in order to separate the oil phase from the aqueous phase, each sample was centrifuged for 30 min at 1000 *g*. The aqueous phase was withdrawn from the bottom with a syringe and the β-LG concentration in the aqueous phase was determined by measuring absorbance at 280 nm.

*2.5.3.3. Oiling off.* The oiling off process, in which the oil phase ends up separated in the creaming layer, was observed by taking photographs with a digital camera (Olympus D-580 Zoom, Olympus Corporation, Tokyo, Japan). Emulsions were placed in transparent plastic tubes (diameter 1.5 cm, height 12.0 cm) in quiescent conditions for days. Images for these systems were taken after 15 days.

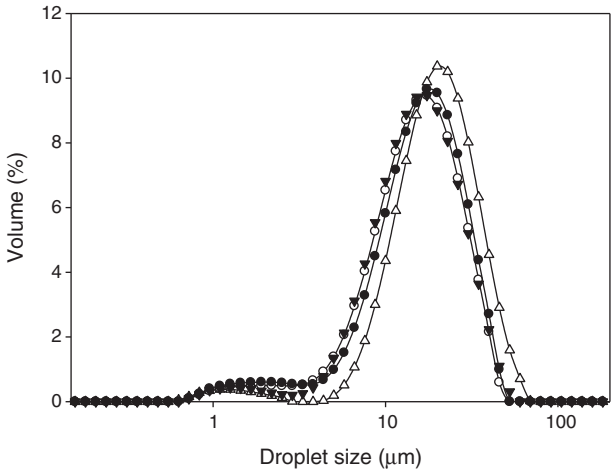


**Fig. 1.** Volume particle size distribution for emulsions made with unheated and heated β-LG for different times: (●) unheated β-LG, (○) β-LG heated for 7 min, (▼) β-LG heated for 10 min, and (Δ) β-LG heated for 15 min.

## 3. Results and discussion

### 3.1. Emulsifying properties of heated β-LG

Fig. 1 shows the results obtained by laser diffraction, the volume particle size distribution for emulsions made with unheated and heated β-LG for different times. Although the emulsions made from all the described heating times have been tested, only four times (0, 7, 10 and 15 min) are shown in this figure, as representative times of the heating process. It has been noted that these observed droplet sizes are greater than 1 μm, particle sizes for which it has been demonstrated that creaming is the most important destabilizing process (McClements, 1999). With the heat treatment, the distribution curve corresponding to 15 min was moved to relatively greater droplet sizes. D[4,3] values, obtained from the volume particle size distributions, increased with heat treatment time, from 15.8 ± 0.3 μm to 18.7 ± 0.7 μm, for unheated and 15 min heated samples, as it can be seen in Fig. 2. Similar particle size distributions were reported for emulsions prepared with different proteins (Comas, Wagner, & Tomás, 2006; Palazolo, Sorgentini, & Wagner, 2004) using a high speed homogenizer like the one used in the present work. In Fig. 2, a weak trend could be observed up to 10 min of heat treatment, and a significant increment in D[4,3] values was observed for 15 min of treatment. D[4,3] is an important parameter of emulsifying activity; however, both emulsifying properties – activity

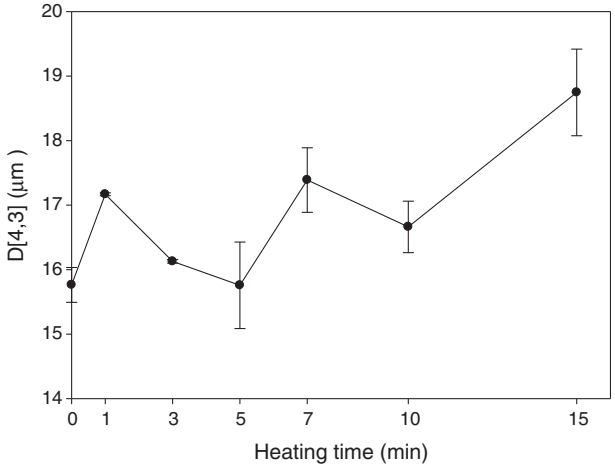


**Fig. 2.** D[4,3] vs. heating time of β-LG. Error bars were calculated from the standard deviation of three replicates.

and stability – are closely linked for particles of these sizes: a poor emulsifying leads to larger oil droplets and then, to a more unstable emulsion. Therefore, for these droplet sizes, the observed trend for D[4,3] can be considered as an estimation of emulsion stability, but the following studies of EAI, related to the surface area moment mean, should be considered another useful parameter to complete the understanding of the stability phenomena.

Fig. 3 shows the way in which relative emulsifying activity index (EAI/EAI°) decayed with heat treatment times of β-LG. EAI is directly related to the interfacial area generated during the emulsifying process. Thus, low EAI, for the same $\phi$ and protein concentration, implies larger droplet sizes which will be a decisive factor for emulsion destabilization.

Surface hydrophobicity is another significant factor, which generally improves emulsifying activity, since a direct correlation between surface hydrophobicity and emulsifying activity has been found for a wide range of proteins, both in the native and in the denatured state (Kato & Nakai, 1980). The surface hydrophobicity of β-LG increased with heating times (Moro et al., 2011). Thus, an EAI increment with heating should have been expected. On the contrary, in the present set of experiments, EAI diminished with heating time (Fig. 3), suggesting that other factors should be involved. One of these factors could be the larger sizes of the protein aggregates, which are significantly formed after 10 min of heating at 85 °C, when they represent more than 80% of the present species (Moro et al., 2011). Although homogenization generates turbulence, which allows these aggregates to reach the interface through a convective mass transport (Dickinson, 2009), their poor flexibility and/or their large size could be the responsible factors for avoiding the proper sticking to the oil droplet interface, and then progressively minor values of EAI resulted.

It has also been noted that another important factor is the time in which the interfacial film is formed. The film is more efficient at protecting droplets against collisions as long as it is formed in a quite short-time scale. This implies the protein must contain enough quantity of hydrophobic groups and they have to be relatively accessible on a short-time scale to enable the anchorage and spreading of the adsorbed molecules in the interface. The aggregates of higher molecular weights could not be able to develop this whole process fast enough as the other smaller species (non-native monomers, dimers, trimers and oligomers of low molecular weight) can do.

At the moment of studying emulsion stability, it should be taken into account that under quiescent conditions, creaming is the main destabilization process, in which oil droplets migrate from the bottom to the top with time (Dickinson, 2001). In Fig. 4, a particular BS profile, corresponding to O/W emulsions prepared using unheated β-LG is shown. BS profiles of the other samples (not shown), emulsions formed with β-LG heated for different periods of time, also followed the same pattern, which corresponds to a creaming process of destabilization, where individual droplets and/or flocs without difference in the distribution of the droplet size can be found. Moreover, in all profiles the convergence of the different time curves in an isosbestic point can be seen, which indicates that there is no particle size variation in the sample (Mengual, Meunier, Cayre, Puech, & Snabre, 1999; Palazolo et al., 2004).

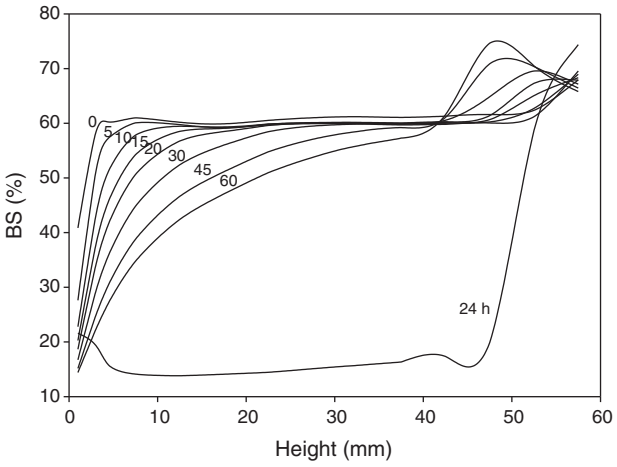


**Fig. 4.** Backscattering, BS, profiles for O/W emulsions prepared using unheated β-LG. Profiles were obtained at 0, 5, 10, 15, 20, 30, 45, 60 min and at 24 h.

Fig. 5 shows that the relative parameter of emulsion stability ($t_{0.2}/t_{0.2}°$) decayed with the heating time of β-LG. This might be due to the greater droplets generated as a result of the heat treatment, as it has already been discussed in this section. Stokes' law states that creaming rate depends on the square of the radius of the droplet. Thus, the greater the radius of the droplets, the greater the destabilization of the emulsion is.

### 3.2. Protein solubility

Protein solubility has been reported to be the most important factor in determining functionality (Kinsella, 1976). The limited emulsifying activity of some biopolymers can be attributed to their poor solubility and/or insufficient amphiphilic character to produce rapid and substantial lowering of the interfacial tension during droplet break-up (Dickinson, 2003).

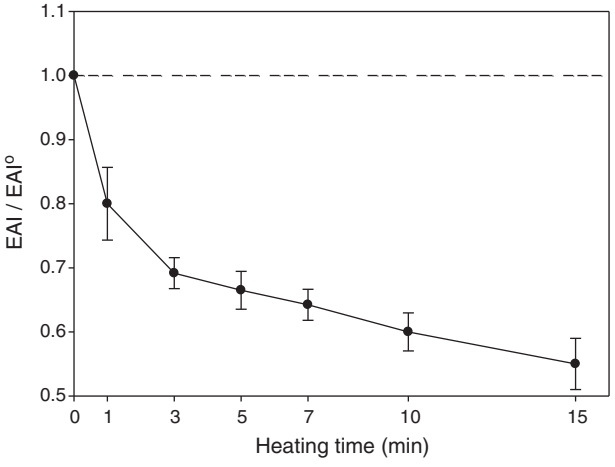


**Fig. 3.** EAI/EAI° vs. heating time at 85 °C. EAI° = 37.1 ± 0.31 $m^2\ g^{-1}$. Error bars were calculated from the standard deviation of three replicates.

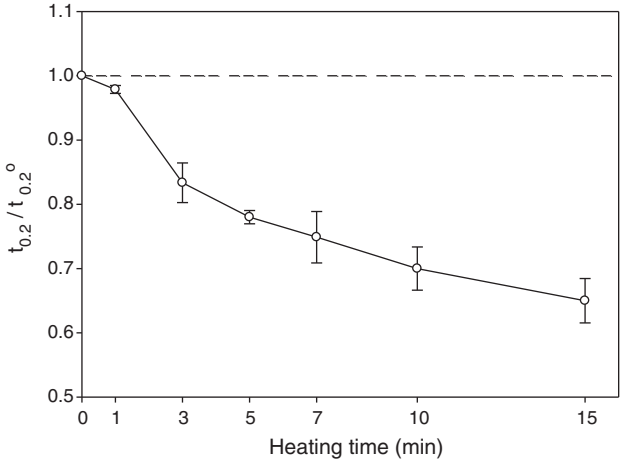


**Fig. 5.** $t_{0.2}/t_{0.2}°$ vs. heating time at 85 °C. $t_{0.2}° = 33.6 \pm 1.0$ min. Error bars were calculated from the standard deviation of three replicates.

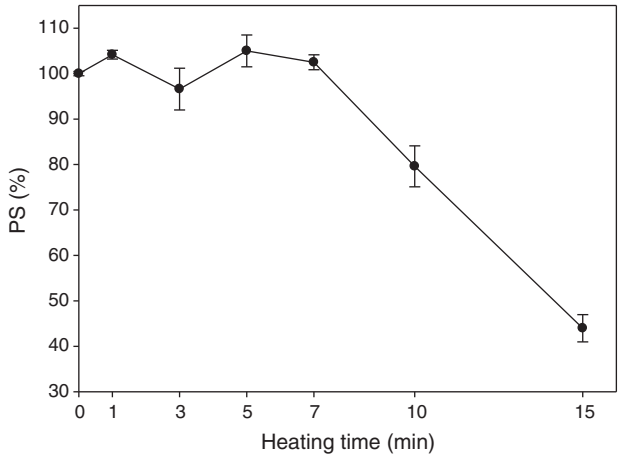


**Fig. 6.** Protein solubility, PS (%), vs. time of previous heating of β-LG at 85 °C. Error bars were calculated from the standard deviation of three replicates.

In this work, protein solubility of β-LG heated for different time periods was measured. Fig. 6 shows constancy in protein concentration, with a first decreased value at 10 min of heating and the tendency of decrease continues after this time. As it has been verified (Moro et al., 2011), aggregates such as oligomers and polymers of β-LG represented more than 80% of the species in solution after 10 min of heating. These larger species sediment after centrifugation and then, lower protein solubility values were obtained. EAI and $t_{0.2}$ diminished with heating time (Figs. 3 and 5), although protein solubility remained constant up to 7 min. Therefore, in view of these results, protein solubility does not seem to be an important factor for emulsifying properties, for the assayed systems and conditions.

### 3.3. Remaining protein concentration

Fig. 7 shows the remaining protein concentration in the bottom aqueous phase for several emulsions formed with β-LG heated for different time periods. This concentration was measured 24 h after the creaming process ended; hence, it can be assumed that the proteins that have disappeared from the solution were adsorbed at the droplet interfacial surface. Up to 3 min of heating, an increase in the concentration of the remaining protein was verified, indicating a lesser adsorption than that corresponding to emulsion with unheated protein. Up to this critical time, dimers and trimers are formed (Moro et al., 2011), and as it was explained in Section 3.1, they were not able to develop the whole process fast enough; therefore, they remained in solution and can be measured as soluble protein. For heating times longer than 3 min, with the appearance of oligomers and polymers, the remaining protein concentration decreased. An attempting explanation for this can be that these larger aggregates were dragged to the top by the oil droplets that were creaming during the 24 h the emulsion was left in quiescent conditions. Thus, even though the aggregates were formed, they were not measured and the remaining protein concentration decreased.

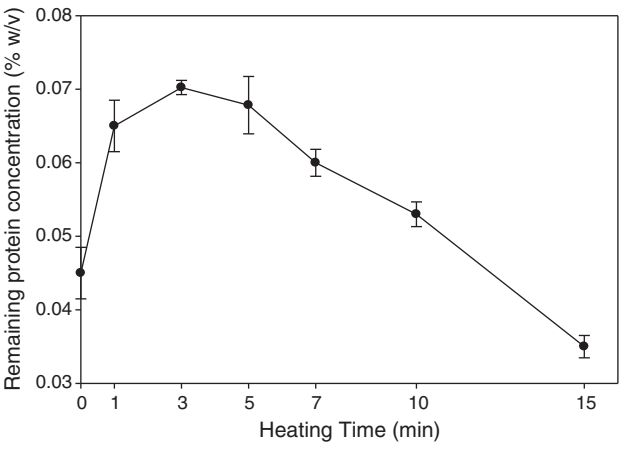


**Fig. 7.** Remaining protein concentration (% w/v) after emulsion vs. time of previous heating of β-LG at 85 °C. Error bars were calculated from the standard deviation of three replicates.

### 3.4. Oiling off monitoring

Fig. 8 shows the images of emulsions formed with unheated and heated β-LG, left in quiescent conditions for 15 days. These images may be a visual monitoring of the oiling off process emulsions have suffered. In tubes corresponding to emulsions formed with unheated β-LG and β-LG heated up to 3 min (Tube 0, 1 and 3, in Fig. 8), the oil phase is clearly separated. As regards the system formed with β-LG heated for 5 min, the oil phase could hardly be seen (Tube 5 in Fig. 8), and for further times of protein heating, emulsions did not show the separated oil phase. Considering that the quantity of larger aggregates increased with β-LG heating time and that they were dragged to the top during the creaming process, it can be assumed that these aggregates play a protective steric role against oiling off, placing themselves among oil droplets and then, avoiding droplet contact and coalescence.

Coalescence is a destabilization process, which is the essential first step of oiling off. It takes place according to the region of emulsion considered: while "coalescence in bulk" is favored for Brownian motion, "coalescence in cream" is caused by the permanent proximity of droplets to each other in creamed layer (Kumar, Narsimhan, & Ramkrishna, 1996). Therefore, the arrangement of the larger aggregates must be an important factor for oil droplet coalescence and their resulting oiling off phenomenon. The aggregates, once dragged to the top by the oil droplets, might arrange as blocks among them, can locate as multilayer arrangements or even they can be linked among themselves through hydrophobic interactions between their hydrophobic patches, which were exposed by heating denaturation. In any case, the presence of the aggregates can make droplet contacts more difficult, avoiding coalescence and enhancing even more their protective role against oiling off.

### 3.5. Comparison between foaming and emulsifying properties of heated β-LG

Comparing foaming and emulsifying properties, the opposite effects were observed, depending on the length of heating. Up to 10 min, while thermal treatment at 85 °C improved both foamability and foam stability (Moro et al., 2011), emulsifying properties diminished. For longer heating times, both foaming and emulsifying properties diminished. These facts can be due to the different formation time scales foams and emulsions require: while foams need longer times to be formed, emulsions imply faster processes. In foams, the longer time scale involved allows a better arrangement of the foaming agent in the bubble

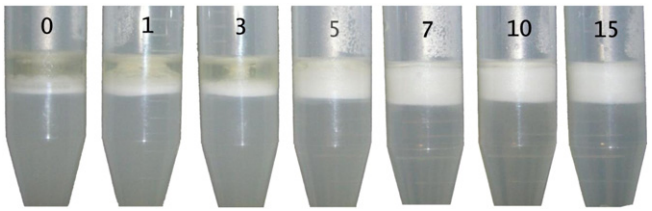


**Fig. 8.** Photograph of the 15-day-oiling off of emulsions formed with unheated β-LG and β-LG heated at 85 °C for different times: 1, 3, 5, 7, 10 and 15 min.

interface and more hydrophobic interactions (Mitchell, 1982), which improve foam stability through rapid formation of a stiffer viscoelastic film, which eventually leads to improvement on both foam properties. On the other hand, when an emulsion is formed, the faster processes hinder an appropriate adsorption of the aggregates of the emulsifying agent to the droplet interface, leading to worse functional properties with heating time. For times longer than 10 min, the same results remain in emulsions, while in foams larger sedimentable aggregates work against foamability and foam stability, producing a steric impediment which leads to an open and weaker interfacial film (Moro et al., 2011).

Some authors pointed out the same conclusions when they studied the behavior of large fractal aggregates of β-LG formed by heating, showing that these aggregates were not able to improve foam stability (Rullier, Axelos, Langevin, & Novales, 2009; Rullier, Novales, & Axelos, 2008).

In the case of emulsions, the presence of low molecular weight aggregates is enough to cause the decay of the emulsifying properties. During homogenization, most of the process occurs on time scales of milliseconds or less: emulsifier adsorption, emulsifier spreading, droplet deformation and droplet collision. The final droplet size distribution is a result of the combination of the time taken for the interface to be covered with emulsifier, and the average time interval between droplet collisions. In order to make the emulsifying process more efficient, the distribution of the emulsifier in the interface should be a faster process than the collision between droplets. When the emulsifier is adsorbed slowly and/or is present at too low concentration, most of the individual droplets, formed during the intense energy dissipation of emulsification, are not retained in the final emulsion (Dickinson, 2009). To retain small droplets during emulsification, the time between droplet collisions should be longer than that needed for the emulsifier to adsorb at the new oil–water interface and to create a transient stabilizing layer (Dickinson, 2003).

Surface hydrophobicity has a different impact on foamability and emulsifying activity. While in our previous study on foams, surface hydrophobicity of β-LG was recognized as the main influential factor to enhance foamability (Moro et al., 2011); in this work on emulsion, emulsifying activity was not affected in the same degree and surface hydrophobicity was not important enough to compensate the presence of aggregates of higher molecular weights, which can be the responsible ones for the resulting minor emulsifying activity (Section 3.1.).

## 4. Conclusions

Both foaming and emulsifying properties are closely linked to the structural changes suffered by the foaming/emulsifying protein and factors affecting foam stability are similar to those affecting emulsion stability.

Both emulsifying activity and emulsion stability are closely related, since a poor emulsifier protein leads to larger droplets in the emulsion and therefore, to a more unstable emulsion over time. At the assayed conditions, creaming is the most important destabilizing process.

Emulsifying activity decayed with β-LG heating time, when the increasing quantities of aggregates avoid the proper sticking of the protein to the interface. The relative emulsion stability decayed with the β-LG heating time, due to the greater droplet size generated.

The larger aggregates produced by longer heating times and dragged to the top by the oil droplets during creaming process, playing a protective role against oiling off. These aggregates can locate as blocks among oil droplets, build a multilayer arrangement or link themselves through hydrophobic interactions between the hydrophobic patches exposed during thermal denaturation. As a result, they avoid droplet contact, coalescence and subsequent oiling off.

While surface hydrophobicity of β-LG was recognized as the main influential factor to enhance foamability, it does not seem to be an important enough factor when emulsions are studied.

The different formation time scales, larger for foams than for emulsions, are a critic factor at the time of analyzing the behavior of the functional properties in function of the heating treatment.

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## References

Cameron, D. R., Weber, M. E., Idziak, E. S., Neufeld, R. J., & Cooper, D. G. (1991). Determination of interfacial areas in emulsions using turbidimetric and droplet size data: Corrections of the formula for emulsifying activity index. *Journal of Agriculturaland Food Chemistry*, *39*(4), 655–659.

Cayot, P., & Lorient, D. (1997). Structure–function relationships of whey proteins. In S. Damodaran, & A. Parf (Eds.), *Food proteins and their applications* (pp. 473–502). New York: Marcel Dekker.

Comas, D. I., Wagner, J. R., & Tomás, M. C. (2006). Creaming stability of oil in water (O/W) emulsions: Influence of pH on soybean protein–lecithin interaction. *Food Hydrocolloids*, *20*(7), 990–996.

Dalgleish, D. G. (1996). Conformations and structures of milk proteins adsorbed to oil–water interfaces. *Food Research International*, *29*(5–6), 541–547.

Damodaran, S. (1994). Structure–function relationships of food proteins. In N. Hettiarchchy, & G. R. Ziegler (Eds.), *Protein functionality in food systems* (pp. 1–38). New York: Marcel Dekker.

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

Dickinson, E. (1992). *An introduction to food colloids* (pp. 66–70). Oxford, U.K.: Oxford University Press.

Dickinson, E. (2001). Milk protein interfacial layers and the relationship to emulsion stability and rheology. *Colloids and Surfaces. B, Biointerfaces*, *20*, 197–210.

Dickinson, E. (2003). Hydrocolloids at interfaces and the influence on the properties of dispersed systems. *Food Hydrocolloids*, *17*(1), 25–39.

Dickinson, E. (2009). Hydrocolloids as emulsifiers and emulsion stabilizers. *Food Hydrocolloids*, *23*(6), 1473–1482.

Durand, A., Franks, G. V., & Hosken, R. W. (2003). Particle sizes and stability of UHT bovine, cereal and grains milks. *Food Hydrocolloids*, *17*(5), 671–678.

Euston, S. R., & Hirst, R. L. (1999). Comparison of the concentration-dependent emulsifying properties of protein products containing aggregated and non-aggregated milk protein. *International Dairy Journal*, *9*(10), 693–701.

Foegeding, E. A., Luck, P. J., & Davis, J. P. (2006). Factors determining the physical properties of protein foams. *Food Hydrocolloids*, *20*(2–3), 284–292.

Graham, D. E., & Phillips, M. C. (1979). Proteins at liquid interfaces. 1. Kinetics of adsorption and surface denaturation. *Journal of Colloid and Interface Science*, *70*(3), 403–415.

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

Kinsella, J. E. (1976). Functional properties of proteins in foods: A survey. *CRC Critical Reviews in Food Science and Nutrition*, *7*(3), 219–280.

Kinsella, J. E., & Whitehead, D. M. (1989). Proteins in whey: Chemical, physical and functional properties. *Advances in Food and Nutrition Research*, *33*, 343–427.

Kumar, S., Narsimhan, G., & Ramkrishna, D. (1996). Coalescence in creaming emulsions. Existence of a pure coalescence zone. *Industrial and Engineering Chemical Research*, *35*(9), 3155–3162.

Mach, H., Volkin, D. B., Burke, C. J., & Russell Middaugh, C. (1995). Ultraviolet absorption spectroscopy. In B. A. Shirley (Ed.), *Protein stability and folding* (pp. 91–114). New Jersey: Humana Press Inc.

McClements, D. J. (1999). *Food emulsions. Principles, practice and techniques.* New York: CRC Press (Chapter 7).

Mengual, O., Meunier, G., Cayre, I., Puech, K., & Snabre, P. (1999). Characterisation of instability of concentrated dispersions by a new optical analyzer: The TURBISCAN MA 1000. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *152*(1), 111–123.

Mitchell, J. R. (1982). Foaming and emulsifying properties of proteins. In B. J. F. Hudson (Ed.), *Development in food proteins* (pp. 291–338). London: Elsevier Applied Science.

Monsalve, A., & Schechter, R. S. (1984). The stability of foams: Dependence of observation on the bubble size distribution. *Journal of Colloid and Interface Science*, *97*(2), 327–335.

Moro, A., Báez, G. D., Busti, P. A., Ballerini, G. A., & Delorenzi, N. J. (2011). Effects of heat-treated β-lactoglobulin and its aggregates on foaming properties. *Food Hydrocolloids*, *25*(5), 1009–1015.

Palazolo, G. C., Sorgentini, D. A., & Wagner, J. R. (2004). Emulsifying properties and surface behavior of native and denatured whey soy proteins in comparison with other proteins. Creaming stability of oil-in-water emulsions. *Journal of the American Oil Chemists' Society*, *81*(7), 625–632.

Pearce, K. N., & Kinsella, J. E. (1978). Emulsifying properties of proteins: Evaluation of a turbidimetric technique. *Journal of Agricultural and Food Chemistry*, *26*(3), 716–723.

Phillips, L. G., Whitehead, D. M., & Kinsella, J. E. (1994). *Structure–function properties of food proteins.* San Diego: Academic Press.

Rullier, B., Axelos, M. A. V., Langevin, D., & Novales, B. (2009). β-Lactoglobulin aggregates in foam films: Correlation between foam films and foaming properties. *Journal of Colloid and Interface Science*, *336*(2), 750–755.

Rullier, B., Novales, B., & Axelos, M. A. V. (2008). Effect of protein aggregates on foaming properties of β-lactoglobulin. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *330*(2–3), 96–102.

Silva, J. G., Morais, H. A., & Silvestre, M. P. C. (2003). Comparative study of the functional properties of bovine globin isolates and sodium caseinate. *Food Research International*, *36*(1), 73–80.

Tang, C. H., Yang, X. Q., Chen, Z., Wu, H., & Peng, Z. Y. (2005). Physicochemical and structural properties of sodium caseinate biopolymers induced by microbial transglutaminase. *Journal of Food Biochemistry*, *29*(4), 402–421.

Walstra, P. (1993). Principles of emulsion formation. *Chemical Engineering Science*, *48*, 333–349.

Wilde, P. J., & Clark, D. C. (1996). Foam formation and stability. In G. M. Hall (Ed.), *Methods of testing protein functionality* (pp. 110–152). London: Blackie Academic & Professional.

Yu, M. -A., & Damodaran, S. (1991). Kinetics of protein foam destabilization: Evaluation of a method using bovine serum albumin. *Journal of Agricultural and Food Chemistry*, *39*(9), 1555–1562.