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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.