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Effect of dynamic heat treatment on the physical properties of whey protein foams

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ABSTRACT

The influence of dynamically heat-induced aggregates on whey protein foams was investigated as a function of the thermal treatment applied with the aim of determining the optimal temperature for the production of heat-induced aggregates dedicated to foaming. The native protein solutions (2% w/v WPI; 50 mM NaCl) at neutral pH were heat-treated using a tubular heat exchanger between 70 °C and 100 °C. Protein denaturation and aggregation were followed by micro-differential scanning calorimetry, size exclusion chromatography, laser diffraction and dynamic light scattering. The protein solutions were whipped using a kitchen mixer to produce foams. Foam overrun, stability against drainage, texture and bubble size distribution were measured.

Experimental results showed that overrun slightly decreased with an increase of the temperature used to treat proteins, whereas foam rigidity was improved at the same time. An optimal temperature of thermal treatment was found at 80 °C for stability against drainage. Quantitative analyses showed that the formation of approximately 10% soluble aggregates in the WPI sample before whipping maintained a good level of overrun, lead to an increased stability against drainage and to an improved rigidity for whey protein foams. Conversely, they revealed that an increase of temperature above 80 °C before foaming did not improve anymore the functional properties of the proteins.

1. Introduction

In the recent years, whey proteins, especially whey protein isolates (WPI), have become the most employed functional food proteins in food formulations. β-lactoglobulin (β-Lg) and α-lactalbumin (α-La) account for 70% of the total protein content of the whey and are responsible for hydration, gelation, emulsifying and foaming properties of WPI (Tosi, Canna, Lucero, & Ré, 2007). For example, in typical food foams, the presence of adsorbed proteins at the fluid interface between the aqueous and the gas phases is responsible for the stabilization of the microstructure. For food manufacturers, a method to improve the stability of food foams consists in enhancing the functional properties of proteins. A similar method could also facilitate imparting the desirable features to a wide range of food products, including appearance, texture and consistency (Ibanoğlu & Karataş, 2001). Many factors affect the functional properties of food proteins. These include intrinsic properties, such as the amino acid sequence and composition, the secondary and tertiary structures, the hydrophilic/hydrophobic character of the protein surface, the molecular rigidity/flexibility of the protein, the net charge and the charge distribution; and also extrinsic factors such as pH, ionic strength, temperature and interactions with other food ingredients (Rodríguez Niño, Carrera Sánchez, Pizones Ruíz-Henestrosa, & Rodríguez Patino, 2005; Rodríguez Patino, Molina Ortiz, Carrera Sánchez, Rodríguez Niño, & Añón, 2003; Zhu & Damodaran, 1994).

Numerous treatments have been investigated for improving whey protein functionality, including enzymatic hydrolysis (Gauthier & Pouliot, 2003; Hamada, 1994), fractionation (Morr & Ha, 1993), dynamic high-pressure (Bouaouina, Desrumaux, Loisel, & Legrand, 2006; Grácia-Juliá et al., 2008; Ibanoğlu & Karataş, 2001) and heat treatments (Bernal & Jenel, 1985; Croguennec, Renault, Beaufils, Dubois, & Pezennec, 2007; Davis & Foegeding, 2004; De Wit, 1990). The effects of thermal treatment on the structural properties of whey proteins have been extensively studied these last years, especially for β-Lg. The initial steps of heat-induced denaturation at neutral pH involve the dissociation of β-Lg native dimers into native/modified monomers at a critical temperature close to 60 °C, accompanied by sulphydryl group exposure and

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thiol/disulphide exchanges (Croguennec, Bouhallab, Mollé, O'Kennedy, & Mehra, 2003; Iametti, de Gregori, Vecchio, & Bonomi, 1996), and then irreversible formation of polymers. Cairoli, Iametti, and Bonomi (1994) showed that hydrophobic-driven associations are also involved in aggregation mechanisms, depending on heating temperature and leading to larger aggregates.

Up to now, several authors (Davis & Foegeding, 2004; Leman & Dolgan, 2004; Zhu & Damodaran, 1994) attempted to correlate the influence of heat-induced denaturation and aggregation of whey proteins on the properties of protein foams, but this mechanism is still far from being well understood. For example, Zhu and Damodaran (1994) showed that mild heat treatments improved the foaming properties of whey proteins, whereas more severe heating conditions resulted in an impairment of these properties. Actually, the structure and the resulting functional properties of heat-induced aggregates depend on numerous factors, including heating temperature, protein concentration, ionic strength (De la Fuente, Singh, & Hemar, 2002). Molecular transition of proteins from native to the denatured state involves many conformational changes in the secondary, tertiary and quaternary structures, as well as constituent bond changes, such as hydrogen bonding, hydrophobic interactions, electrostatic linkages and disulfide bonds, and these have been shown to determine, for instance, both the final structure and the textural properties of whey protein gels (Ker & Toledo, 1992). For foaming properties, Zhu and Damodaran (1994) also showed that the ratio of monomeric to polymeric proteins in heat-denatured WPI played a critical role on its foaming properties. They reported that the monomeric species contributed to foam formation and that the polymeric species contributed to foam stability because the protein films formed by monomeric proteins did not appear to provide the required viscoelastic properties to stabilise foams. Similarly, Leman and Dolgan (2004) claimed that, depending on the degree of the protein denaturation and the nature of the newly developed protein structure, whey protein foams differed in the surface area of their interfacial film, and also in their mechanical resistance, viscosity, elasticity and ability for water holding.

However, in all the works mentioned above, denatured and aggregated proteins were always obtained using static heat treatments that differ significantly from the dynamic treatments applied in the food industry in heat exchangers, such as high temperature short time (HTST) pasteurisation or ultra-high temperature (UHT) sterilization. At the moment, there is no information available on the interplay between the physicochemical mechanisms of heat-induced denaturation and aggregation of WPI on one hand and the hydrodynamics in heat exchangers on the other hand. Besides, little is known on how much a dynamic heat treatment may affect the implication of the protein aggregates in foam formation. Similarly, the effect of dynamic heat treatment on protein foam properties remains unclear. The objectives of the present work are therefore:

(1) to investigate the influence of WPI denaturation and aggregation resulting from a dynamic HTST treatment on the foaming ability (foam overrun) and the foam properties (bubble size distribution, stability against drainage, rheology);
(2) to determine the optimal temperature for producing dynamically functional heat-induced aggregates dedicated to foaming;
(3) to better understand the role of protein denaturation and aggregation on foam formation and stabilization.

2. Materials and methods

2.1. Preparation of whey protein solutions

Commercial WPI Promilk® (83.35%, w/w total protein; 2.9% minerals; <1% fats) was provided by *IDI Ingredients* (Arras, France). WPI contained more than 68% w/w of native β-Lg. The powder was hydrated in deionised water for 2 h at 40 °C under mechanical stirring in order to prepare 2% w/v whey proteins solutions. The pH was adjusted to 7.0 by minute addition of a 1 M NaOH solution. To obtain the desired ionic strength of 50 mM, the required amount of sodium chloride was added in the protein solution. The solution was then kept at 4 °C for 12 h to achieve complete solubilisation. Unheated WPI solutions (native 2%-50 mM) were used as reference samples to analyze the influence of dynamic heat treatment. The protein solution was heat-treated at four different temperatures: 70 °C, 80 °C, 90 °C, 100 °C for 300 s (56 s represent the holding time) in a tubular heat exchanger Actijoule (Actini, France) composed of twelve tubes which represented the heating section (12 mm diameter) and one tube which represented the holding section (18 mm diameter). This exchanger was fed using a screw pump. The flow rate was about 20 L h^{-1} and laminar regime prevailed in the tubes (Re ~ 100). After heating, the samples were immediately cooled and kept at 4 °C; then, measurements for the characterisation of solution properties were immediately carried out. The pH shift due to heat treatment remained always negligible. Similarly, clogging was never observed during heat treatment and the amount of proteins lost in the heat exchanger could be neglected.

2.2. Particle size analysis

2.2.1. Laser light diffraction

Native and heat-treated WPI solutions were studied by laser light diffraction using a Mastersizer S apparatus equipped with a 300 reverse Fourier lens and a He–Ne laser ($l = 633$ nm) (Malvern Instruments, UK). Samples were diluted with deionised water in the sample dispersion unit under stirring (1500 rpm). Histograms of size distribution are given in volume percentage as a function of particle diameter, in the range of 0.05–900 mm. The analysis requires the relative refractive index of the dispersed phase (whey protein aggregates) and of water, 1.52 and 1.33, respectively.

2.2.2. Centrifugation step

To investigate the effect of the heat treatment on WPI solutions and particularly on the formation of soluble heat-induced aggregates, 20 mL of each sample studied were centrifuged at 10,000 g for 30 min at 20 °C in a Sigma 2K15 centrifuge (Osterode, Germany). Then, the soluble fractions were recovered and dry matter measurements were carried out using an oven (for 12 h at 102 °C). The dry matter for each sample was then compared to the total protein content in the WPI solutions to estimate the insoluble protein fractions. This centrifugation step was sufficient to remove all the particles larger than 1 mm, which separated roughly the soluble and the insoluble aggregated protein fractions. The size of the particles in supernatants was previously verified (data not shown) by laser light diffraction using a Mastersizer S apparatus (Malvern Instruments, UK) and this technique is in agreement with the one used by Bench, Johnson, Hamilton, Gooch, and Wright (2004).

2.2.3. Size exclusion chromatography (SEC)

The supernatants (i.e. the soluble fraction) were collected and 25 μL of each was analysed by size exclusion chromatography (SEC) on a Waters HPLC system (Milford, MA, USA) equipped with a TSK 6000 column of 30 cm length (Tosohaas, Montgomeryville, PA, USA). Proteins were eluted with a solution (pH = 7) containing 0.05 M Tris and 0.1 M NaCl; the eluted proteins were detected using UV absorption at 280 nm; the amounts of non-aggregated proteins and aggregates were calculated as percent area of each peak after deconvolution using the software PeakFit (Jandel Scientific Software, USA).