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Effect of pulsed-light processing on the surface and foaming properties of β-lactoglobulin

Estibalitz Fernández [a], Mari Luz Artiguez [a], Iñigo Martínez de Marañón [a], Maider Villate [b], Francisco J Blanco [b,c], Juan-Carlos Arboleya [a,*]

[a] AZTI-Tecnalia Food Research Institute, Parque Tecnológico de Bizkaia, Astondo Bidea, Edificio 609, 48160 Derio, Bizkaia, Spain
[b] CIC-BIOGUNE, Parque Tecnológico de Bizkaia, Edificio 801 A, 48160 Derio, Bizkaia, Spain
[c] IKERBASQUE, Basque Foundation for Science, 48011 Bilbao, Spain

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ABSTRACT

Pulsed-light processing was used to treat β-lactoglobulin (BLG) solutions. The impact of pulsed light (PL) on the structural properties of this protein was explored through far-UV, CD spectral analysis, size exclusion chromatography, surface hydrophobicity and NMR spectroscopy. Changes on these physico-chemical properties were related to surface rheology, surface tension, foam stability and foam capacity of the non-treated and treated BLG to elucidate adsorption mechanism and consequences on foaming capacity. Conformational modification of BLG was related with PL total fluence as important conformational changes increased when total fluence was higher. Consequently, adsorption rate of treated BLG at the air/water interface was faster than native BLG. Additionally, treated BLG formed highly elastic interfaces. This was found to have an impact on the foam stability. Pulsed-light treatment seemed to enhance the overall strength of the interface, resulting in more stable foams.

1. Introduction

Bubbles are desirable elements in a wide range of applications, particularly in food, since consumer perception of quality is strongly influenced by appearance. Aerated systems are however thermodynamically unstable and in a fluid system will eventually break down (Dickinson & Wasan, 1997). Therefore, the role of surface active ingredients like proteins is crucial for the formation and stability of food foams. Whey proteins are currently the most used food proteins in foodstuffs because of their excellent functional properties. Great effort has been made during the last years to improve those functional properties by applying different treatments, including chemical (Enomoto et al., 2007; Wooster & Augustin, 2007), physical (Phillips, Schulman, & Kinsella, 1990; Yang, Dunker, Powers, Clark, & Swanson, 2001) or enzymatic treatments (Davis, Doucet, & Foegeding, 2005). The effects of the applied treatment in the structural properties of whey proteins have been extensively studied, especially for β-lactoglobulin (BLG), mainly because all these treatments may have an impact in protein structure, modifying the kinetics of protein adsorption at the interface, the time needed for the protein to rearrange upon adsorption at the interface and the ability to interact with adjoining proteins (Croguennec, Renault, Bouhallab, & Pezennec, 2006). Proteins that can be altered to adsorb more rapidly, and produce stronger interfaces are generally capable of producing finer and more stable foams. The drawback of this is that some treatments can cause aggregation as interactions between proteins in the bulk are higher. This provokes a reduction in protein solubility and thus, in protein availability to be adsorbed and consequently results in poor foam production.

On the other hand, the dairy industry produces significant amounts of liquid wastes, mostly whey obtained during the cheese making process. This high-content-protein source must be treated for microorganism elimination by specific treatments while minimizing protein denaturation.

Pasteurization has been used to decontaminate whey. However, high temperature processes are known to denature whey proteins (Anema & Li, 2003) causing substantial changes in their nutritional, organoleptic or technological properties. For this reason, an important challenge would be to develop non-thermal technologies which can prevent adverse thermal effects and produce safe food products (Barbosa-Cánovas, Góngora-Nieto, & Swanson, 1998).

Among others, pulsed-light (PL) process consists of a successive repetition of short duration and high power pulses of broadband emission light (200–1000 nm) with a considerable amount of light

* Corresponding author. Tel.: +34 946 574 000; fax: +34 946 572 555.
E-mail address: jarboleya@azti.es (J.-C. Arboleya).

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in the short-wave UV spectrum (Wekhof, 2000). Pulsed-light process has been shown to be effective in inactivating a wide broad of microorganisms involved in food products spoilage (Lasagabaster, Arboleya, & Martínez de Marañón, 2011; Rowan et al., 1999). However, there is no research about the impact of pulsed-light processing in the functional properties of food proteins.

Only Elmnasser et al. (2008) studied the effect of PL treatment in milk proteins and lipids concluding that minor changes in protein structure were found, despite of changes in the polarity of the tryptophanyl residue microenvironment of BLG solutions and changes in the tryptophan indole structure and some protein aggregation. Therefore, the aim of this research is to study the conformational changes in BLG induced by applying a PL treatment and how these changes affect their surface properties and hence, their foaming properties.

2. Materials and methods

2.1. Materials

β-Lactoglobulin (BLG; L-2506 approximately 80% purity), derived from bovine milk, were purchased from Sigma chemicals (Gillingham, UK). Solutions were prepared at different concentrations in ultrapure water ($\gamma_0 = 72.6$ mN m^{-1} at 20 °C), allowed to equilibrate for 60 min, and used without further purification. Initial pH was 6.8 and it did not change after treatments. The concentration of the protein solution was varied from 0.5 mg mL^{-1} to 10 mg mL^{-1}. Heat treated BLG was prepared at 0.1 mg mL^{-1} in concentration and was heated in a water bath at a temperature of 80 °C during 35 min.

2.2. Pulsed-light treatment

PL treatments were performed using a SBS-XeMaticA-(L + L) device (SteriBeam Systems GmbH, Kehl, Germany). For the emission of light pulses, the electric power is stored in an energy storage capacitor and later released quickly to the Xenon lamps which emit then high intensity light pulses of 325 μs duration (Lasagabaster & Martínez de Marañón, 2006). The emitted light spectrum includes wavelengths from 200 nm to 1000 nm with a considerable amount of light (approximately 40%) in the UV-C spectrum (Wekhof, 2000). Samples at room temperature (20–23 °C) were placed at 8 cm from the upper Xenon lamp and received between 1 and 10 light pulses of 0.4 J cm^{-2}, up to a maximum total fluence of 4 J cm^{-2}. 10 mL of BLG solutions were poured in a quartz trough (16.6 × 9.8 cm) and stirred between pulses. No significant temperature increase was found at the maximum total fluence. Each experiment was repeated at least three times.

2.3. Determination of the surface hydrophobicity of glycoconjugates

The surface hydrophobicity of control (native) and treated BLG was investigated by binding of 8-anilino-1-naphthalenesulfonate (ANS). The relative fluorescence intensity (FI) of the ligand–protein conjugates was measured on a Shimadzu RF-1501 fluorescence spectrophotometer at room temperature. The wavelengths of excitation (λ_{exc}) and emission (λ_{em}) were 390 nm and 470 nm with slit widths of 10 nm.

10 μL of ANS solution (8.0 mM in 0.1 M sodium phosphate buffer, pH 7.4) was added to 1 mL of 0.1 mg mL^{-1} and 1.5 mg mL^{-1} of native and treated BLG samples, the resulting solution mixed and equilibrated for 2 min and, finally, the fluorescence intensity measured at room temperature. All measurements were performed in triplicate.

2.4. Size exclusion chromatography (SEC)

Size exclusion chromatography (SEC) was carried out under nondenaturing conditions (0.05 M sodium phosphate buffer, pH 7.3, containing 0.15 M NaCl) using a Superdex 75 column, HR 10/30 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden), on an AKTA system. A 100 μL volume of a 0.1 mg mL^{-1} and 1.5 mg mL^{-1} of native and treated BLG samples was applied to the column at room temperature. Elution was achieved in isocratic mode at 0.8 mL min^{-1} for 30 min, and detection of eluting proteins was performed at 214 nm. The standard proteins used for calibration were human serum albumin (67 kDa), ovalbumin (43 kDa), alpha-chymotrypsinogen (25 kDa), and ribonuclease A (13.7 kDa) (GE Healthcare Bio-Sciences AB). The void volume was determined with blue dextran 2000.

2.5. Circular dichroism (CD)

Circular dichroism (CD) measurements were performed at 25 °C on a Jasco J-810 spectropolarimeter using 0.2 cm or 0.01 cm path length quartz cuvettes. Protein samples were 0.1 mg mL^{-1} or 1.5 mg mL^{-1} in pure water. Thermal unfolding was induced by increasing temperature at a rate of 1 °C min^{-1} (using a programmable Peltier thermoelectric) and measuring the ellipticity at 205 nm or 217 nm.

2.6. NMR spectroscopy

One-dimensional ^{1}H NMR spectra were recorded at 800 MHz and 25 °C on 600 μL samples containing 0.1 mg mL^{-1} or 1.5 mg mL^{-1} in water with 5% (by vol.) ^2H_2O and 17 μL TSP (sodium trimethy-silyl propionate, used as internal chemical shift reference for protons at 0.0 ppm). The protein samples were irradiated with 0 and 10 pulses previously to the addition of ^2H_2O and TSP.

2.7. Surface tension

Surface tensions at the air–water interface of protein solutions were measured by using an FTA200 pulsating drop tensiometer (First Ten Ångstroms, USA). The capillary drop was formed with a tip of a syringe of 0.914 mm within an environmental chamber at room temperature, in which standing water increased the relative humidity to minimize drying effects. When required, changes in γ (surface tension) were monitored every one second. All measurements were made at room temperature (≈20 °C). Surface tension was monitored at room temperature for 30 min.

2.8. Surface rheology

Surface shear rheological measurements were carried out to study the mechanical and flow properties of adsorbed layers at fluid interfaces, which are sensitive to surface structure and composition (Ridout, Mackie, & Wilde, 2004). Experiments at the air–water interface were made using a stress controlled rheometer, AR2000 Advanced Rheometer (TA Instruments) and an aluminium bicone (diameter 60 mm, angle cone 4:59:13) as measuring geometry. The surface rheological response in 50 mL protein solution was tested by oscillation mode within the range of linear viscoelastic region at a frequency and strain of 0.1 Hz and 0.014, respectively. Measurements were monitored for 30 min at room temperature.

2.9. Foaming properties

Foam production was achieved by using a Foamscan TM apparatus (Teclis-ITConcept, Longessaigne, France). The principle is to