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Effect of dynamic high pressure on functional and structural properties of bovine serum albumin

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ABSTRACT

Dynamic high pressure (DHP) has been investigated as an innovative suitable method to induce protein modifications. This work evaluated the effect of DHP (up to three passes at 100, 150 and 200 MPa, with an inlet temperature of 20 °C) on functional and structural properties of bovine serum albumin (BSA). Results indicated that DHP process applied up to an energy limit of 100 MPa increased the protein foaming capacity (FC) ($p < 0.05$ - increase up to 63% after 1 pass at 100 MPa) and the utilization of multiple passes at high pressure promoted a reduction in this property ($p < 0.05$ - reduction up to 31.6% after 3 passes at 200 MPa). Similar results were observed for sulfhydryl group, indicating an influence of free thiol groups on FC. Complementarily, DHP process promoted an increase of proteins particles size, suggesting a new rearrangement of their conformational structure. DHP did not affect tryptophan microenvironment in BSA; however, this process induced the rearrangement of secondary structure elements. In the first cycle, the pressure increase resulted in a loss of secondary structure, while in the second and third cycles the DHP process resulted in the gain of secondary structure elements. These results indicated that the second and third passes triggered a molecular rearrangement of the protein structure, giving rise to a novel and more stable conformational state. This conclusion was also supported by thermal unfolding studies (melting temperature reduction from 67.5 to 54.6 °C after 1 pass at 200 MPa), in which the additional cycles of DHP caused the occurrence of an initial denaturation at high temperatures, compared to the first cycle.

1. Introduction

Proteins are characterized by a native structure (primary, secondary, tertiary, and quaternary structure), which is the most thermodynamically stable and is associated to the lowest value of the free energy (Damodaran, 2008). The chemical properties of the environment (pH, molarity and composition) influence the protein native state, resulting from the interaction of the amino acid side groups of proteins and the solvent, as well as from the balance of repulsive and attractive forces. Variations of chemical properties of the environment, in which the protein is in, namely pH and ionic strength, as well as the application of physical stresses, namely heating, pressure increase, shear force, may modify the native structure of the protein reversibly (unfolding) or irreversibly (denaturation). Protein denaturation, which indicates the irreversible change occurring in its secondary, tertiary, and quaternary structure, may be desirable, since generally the proteins are consumed in their denatured state (egg, meat, fish proteins), being more digestible and palatable.

The modification of the protein structure, moreover, influences its functional properties, namely solubility, binding and surfactant properties, water and oil absorption capacity, emulsifying and foaming capacity. The exposure of hydrophobic groups on protein surface controls the interactions with oils (emulsions), air (foam) or other proteins (gels) (Li-Chan & Nakai, 1989). The hydrophobic amino acids are usually buried inside the globular proteins but, because of the unfolding of the native structure, these hydrophobic groups can be involved in

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intermolecular interactions (Kato, Ibrahim, Watanabe, Honma, & Kobayashi, 1989; Monahan, German, & Kinsellat, 1995). Food, chemical and pharmaceutical industries, utilizing proteins as technological ingredients in their products, are potentially interested in controlling the functional properties, with the aim of improving the stability of their formulations or developing novel foods (McClements, 1999).

Nonthermal technologies, such as high hydrostatic pressure (HHP), pulsed light (PL) and pulsed electric fields (PEF), dynamic high pressure (DHP) are able to induce significant changes in protein structure and properties (Barba, Esteve, & Frigola, 2012; Sun, 2014). During the DHP process, the fluid is forced to pass through a homogenizing valve at high pressures (Campos & Cristianini, 2007). The passage through the narrow gap and the abrupt decompression of the fluid generate an increase in speed and an increase in temperature (about 1.5–2.5 °C every 10 MPa pressure increase) due to the intense friction in the homogenizing valve region (Diels & Michiels, 2006). In addition to shear effects, the fluid is submitted to an intense drop of pressure, turbulence and cavitation, that leads to changes in protein structure (Campos & Cristianini, 2007; Diels & Michiels, 2006; Dumay et al., 2013

The pressure and temperature levels applied during DHP processing determine the extent of the induced changes in proteins. The increase of these variables magnitude favors structural changes and denaturation of proteins (Keerati-U-Rai & Corredig, 2009). In this context, the DHP has been found to induce more significant changes in the interfacial protein layer (Lee, Lefèvre, Subirade, & Paquin, 2009). These changes in protein structure are associated with modifications of hydrogen and hydrophobic interactions, disrupting the tertiary and/or quaternary structure (Iucci, Patrignani, Vallicelli, Guerzoni, & Lanciotti, 2007; Lanciotti, Patrignani, Iucci, Saracino, & Guerzoni, 2007; Liu, Zhang, Liu, Xie, & Tu- Z.; Liu, J., & Liang, R., 2010).

At the current state of the art, the effect of DHP on proteins is not clearly understood and very little is known about the changes occurring to proteins during homogenization (Molina, Papadopoulou, & Ledward, 2001; Torrezan, Tham, Bell, Frazier, & Cristianini, 2007; Zhang, Li, Tatsumi, & Isobe, 2005). During the dynamic high pressure processing, the protein solutions experience high shear forces, cavitation, impact on solid surfaces and high pressure. However, the few studies available on globular proteins processed by DHP showed that protein functional properties can be enhanced up to pressures of 200 MPa (Bouaouina, Desrumaux, Loisel, & Legrand, 2006; Floury, Desrumaux, & Legrand, 2002; Paquin, 1999). Irreversible unfolding and gelation of proteins processed in DHP process have been observed (Floury et al., 2002; Roesch & Corredig, 2003). The changes of functional properties as well as the variation of the conformational structure may be particularly relevant in case of allergens, which are mainly specific glycoproteins able to activate the reaction of the human immune systems (Penãs, Préstamo, Baeza, Martínez-Molero, & Gomez, 2006).

According to these findings, the aim of the current work was to investigate the effects of DHP on the functional properties and structure of a globular protein, the bovine serum albumin (BSA). BSA was chosen to carry out the experimental trials since it represents a very interesting case study. In fact, BSA, which is a globular protein present in of bovine meat and whey, is an allergen responsible for several allergic cross-reactions, mainly in children suffering for beef allergy. In several cases the same children show allergic reactions to cow's milk and in general to dairy products, as reported in several papers available in the literature (Martelli, De Chiara, Corvo, Restani, & Fiocchi, 2002). The effects of DHP processing on BSA conformational structure and functional properties were described in detail in previous papers (De Maria, Ferrari, & Maresca, 2015; De Maria, Ferrari, & Maresca, 2016). However, no investigations on the effects of DHP on unfolding and/or aggregation of BSA protein, and in general on oligomer proteins, are present in the literature. Therefore, this study proposed a characterization of the DHP processed BSA, aiming to determining the mechanism by which dynamic pressure could affect the functional and structural properties of this protein.

2. Materials and methods

2.1. Sample's preparations and dynamic high pressure equipment

Bovine serum albumin (BSA, Sigma-Aldrich, Italy) samples were prepared by dissolving the protein ($50\ mg \cdot mL^{-1}$) in a Tris-Glycine buffer (0.05 M - pH 8.0), kept at 25 °C under gentle mixing until a homogenous solution was obtained. The pH of the protein solutions was reduced up to the desired value (pH 8.0) with HCl and measured with a laboratory pH-meter (Mettler-Toledo GmbH, Schwerzenbach, Switzerland). This pH was chosen due to the distancing of the pH equivalent to the isoelectric point of the BSA (pH 4.7). Thus, at higher pH occurs the increase of the electrostatic repulsion minimizing the effects of aggregation. Consequently, the changes promoted by the DHP process would be better elucidated. The protein solutions were stored under refrigerated conditions (4 °C) before being processed by DHP.

A Panda Plus High-Pressure Homogenizer (model 2000, GEA-Niro-Soavi, Parma, Italy) was used in the experimental trials. This equipment has a single acting intensifier pump that amplifies the hydraulic pressure up to 200 MPa and operates at a flow rate of $9\ L\ h^{-1}$.

2.2. Dynamic high pressure processing of BSA protein solution

A volume of 1 L of the BSA solution ($50\ mg \cdot mL^{-1}$) prepared in 0.05 M Tris-Glycine buffer pH 8.0 was homogenized up to three passes at 100, 150 and 200 MPa, with an inlet temperature of 20 °C, totaling 9 samples (100 MPa for 1, 2 and 3 passes; 150 MPa for 1, 2 and 3 passes; 200 MPa for 1, 2 and 3 passes). For each sample, 50 mL of the BSA solution were collected. The maximum pressure level was chosen considering the operational capacity of the equipment and the level of pressure and passes that caused a significant change in the structural conformation of this protein.

The inlet temperature of the protein solution was 19.2 ± 1.7 °C for all passes. The increase in pressure promoted an increase in temperature of approximately 12.8 ± 1.7 °C/100 MPa, reaching a final temperature of 33.0 ± 0.9; 36.0 ± 1.0 and 46.4 ± 2.5 °C after processing at 100, 150 and 200 MPa, respectively.

After each pass, samples were immediately cooled to 20 °C using a shell and tube heat exchanger, in order to ensure the uniformity of the final temperature after each homogenization step. The residence time at the temperature reached after the DHP valve (< 10 s) was estimated considering the equipment flow and the distance between the homogenization valve and the heat exchange inlet. The time between consecutive processes was < 4 min and the final processing time was around 12 min. The temperatures were measured using a digital T-type thermocouple (Multithermometer®, Porto Alegre, Brazil). After processing, processed samples and control sample (non-processed sample) were stored under refrigerated conditions (4 °C) before being analyzed.

2.3. Sample analyses

2.3.1. Foaming capacity (FC)

Foaming capacity was carried out according to De Maria et al. (2016). For this, a volume of 5 mL of the sample, placed in a glass beaker of 100 mL, was stirred in a rotor/state device (Ultra-turrax® T-25 Basic, IKA Labortechnik, Germany) at 18000 rpm for 5 min at ambient temperature (25 °C). The produced foam was immediately and gently transferred into a graduated glass cylinder (diameter = 2 cm, height = 16 cm, graduated volume = 25 mL) to determine the foam volume (Vf). The Foaming Capacity (FC) was calculated as the ratio between the measured foam volume (Vf) and the initial volume of liquid solutions (V_0), according to Eq. 1:

$$FC = Vf/V_0\% \tag{1}$$

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