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Effects of high hydrostatic pressure on the conformational structure and the functional properties of bovine serum albumin

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ABSTRACT

Non-thermal technologies, such as High Hydrostatic Pressure (HHP), are able to induce extensive changes in the structure of biological macromolecules, namely proteins. HHP treatments disrupt the electrostatic interactions, which stabilize the quaternary and the tertiary structure of the proteins, and activate the reactions of sulfhydryl-disulfide bond exchange. These structural changes result in the dissociation and refolding of proteins during HHP treatments, and consequently in the modification of protein functional properties, namely physico-chemical properties (solubility, binding and surfactant properties, water and oil absorption capacity, emulsifying and foaming properties). The technological behavior of the proteins in food preparation, processing, storage, as well as their contribution to determine quality perception of foods mainly depends on these functional properties.

This work aims at investigating the effects of HHP treatments on the conformational (quaternary, tertiary and secondary) structure and the functional properties of a globular water soluble protein, the Bovine Serum Albumin (BSA). BSA (50–100 mg/mL) solutions in Sodium Phosphate Buffer were processed at different pressure levels (100–500 MPa) and treatment times (15, 25 min). BSA unfolding and refolding were analyzed in terms of free sulfhydryl (SH) groups, changes of secondary structure, foaming and emulsifying properties.

Analyzing the experimental data it can be concluded that the unfolding of BSA samples with a concentration of 50 mg/mL occurred in the pressure range between 100 and 400 MPa. In fact an increased number of the free SH groups as well as an improved foaming and emulsifying ability were detected in the treated samples. Pressure levels above 400 MPa promoted the interactions between adjacent polypeptide chains and the formation of soluble high molecular mass aggregates. The concentration of the protein in the samples, also, controlled the occurrence of unfolding and aggregation. Extensive changes in BSA secondary structure were observed at pressure level above 300 MPa, for longer processing times and higher protein concentrations. In these processing conditions β-sheet aggregates were likely to replace the initial α-helixes.

Industrial relevance: The paper consists in the study of the Effects of High Hydrostatic Pressure processing (HHP) on the conformational structure and the functional properties of Bovine Serum Albumin.

he work proposes an innovative technology for Food Industries that can be widely used for food conservation and to induce protein modifications in the same time. For this reason the technology represents a good tool for the production of hypoallergenic compounds especially in the field of dairy products and infant formula companies.

1. Introduction

In the last years proteins, the major structural and functional components of several foodstuffs, such as dairy products, meat, bakery products, have been widely investigated, with the aim of formulating novel foods or innovate the texture of traditional foods.

The functional properties of proteins, namely physicochemical properties (solubility, binding and surfactant properties, water and oil absorption capacity, emulsifying and foaming properties) influence food preparation, processing and storage, and contribute to the quality perception during food consumption. The exposure of hydrophobic groups on protein surface controls the interactions with oils (emulsions), air (foam) or other proteins (gels and coagula) (Li-Chan & Nakai, 1989). The hydrophobic amino acids are usually buried inside the globular proteins but, as a consequence of the unfolding of the native structure, these hydrophobic groups can be involved in the intermolecular interactions (Kato, Ibrahim, Watanabe, Honma, & Kobayashi, 1989; Monahan, German, & Kinsellat, 1995).

Food processing can affect the functional properties of proteins since it can induce unfolding and aggregation, depending on the technology used, the processing conditions applied and the type of product. The

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knowledge of the mechanism underlying the structural arrangement of protein molecules may be useful to predict and control protein functionality in food formulations. Consequently, processing methods, able to modify the functional properties of proteins, are of interest to the food, chemical and pharmaceutical industries utilizing proteins as functional ingredients in their products.

Non-thermal technologies, namely High Hydrostatic Pressure (HHP), Pulsed Light (PL) and Pulsed Electric Fields (PEF), are able to induce changes in functional properties of food protein similarly to the traditional thermal treatments (pasteurization, sterilization). HHP technology is widely applied to inactivate spoiling microorganism and increase the shelf life of foods, preserving their nutritional and sensorial properties. HHP treatments, which have an irrelevant effect on small nutrients, such as vitamins, amino acids and flavour compounds, are instead able to induce changes in macromolecules, mainly proteins and carbohydrates, whose extent is dependent on the treatment temperature, the applied pressure level and the treatment time (Barba, Esteve, & Frigola, 2012; Galazka, Dickinson, & Ledward, 2000).

HHP processes are able to affect the protein quaternary structure by modifying the hydrophobic interactions, the hydrogen bonds, the disulphide interactions, the metal-ion ligations and salt bridges observed at the interfaces of protein–protein and protein–peptide complexes, the tertiary structure by reversible unfolding and the secondary structure via irreversible unfolding (Tauscher, 1995). The quaternary structure dissociates at moderate pressures (150–200 MPa), the tertiary structure is also significantly affected at pressure level above 200 MPa and secondary structure changes take place at very high pressure (300–700 MPa) (Ahmed, Ramaswamy, Kasapis, & Boye, 2010). Protein unfolding, induced by HHP treatments in the pressure range between 100 and 500 MPa, allows the inaccessible SH groups to be exposed. Consequently, the number of the free sulfhydryl (SH) groups and disulfide (SS) bonds also undergo changes during HHP treatments. Under severe processing conditions (above 500–600 MPa, depending on the protein and the temperature applied), the number of free SH group, instead, decreases, probably due to the formation of disulfide bonds by oxidation, especially at alkaline pH where the thiolate anions are more reactive. For instance, the SH-SS interchange reactions induced by HHP treatments were observed for β-lactoglobulin by Funtenberger, Dumay, and Cheftel (1995). The high degree of exposure of sulfhydryl groups, and the subsequent oxidation and sulfhydryl-disulfide bond exchange reactions result in insoluble and/or soluble aggregates and gel formation. Tang and Ma (2009), who investigated the occurrence of aggregates formation in soy protein isolate (SPI) treated by HHP processes at 200–600 MPa, observed combined insoluble (IA) and soluble (SA) aggregates.

The number of the disulfide bonds, moreover, affects the texture of the HHP induced gels, being the storage modulus, related to the elasticity of a solid, proportional to the cross-linking density in the gel network (Quin et al., 2012).

HHP treatments affect also the proteins functional properties: the viscosity and the surface tension are increased, as observed for egg white (Yang, Li, Zhu, & Zhang, 2009) and the foaming capability is increased, as demonstrated for ovalbumin (Denda & Hayashi, 1992) and egg white (Knorr et al., 1992; Yang et al., 2009). Yang et al. (2009) observed a direct correlation between the egg white foaming ability and the number of free SH groups. At the lowest hydrophobicity level of egg white foaming capacity and foam stability of egg white reached the maximum value (Yang et al., 2009).

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 (Gross & Jaenicke, 1994; Penãs, Prestamo, Baezac, Martinez-oleroc, & Gomez, 2006; Shriver & Yang, 2011). Bovine Serum Albumin (BSA), a globular protein soluble in water, represents a very interesting case of investigation, being a protein component of whey and blood and a common allergen of bovine meat and whey proteins. Moreover, BSA physicochemical and structural properties have been investigated and measured, making it a desirable model for the research on food protein. BSA is a protein responsible for several allergic cross-reactions: most children with beef allergy are also allergic to cow's milk and should avoid the consumption of dairy products. Sensitization to bovine serum albumin is a marker of cow's milk allergy in children with beef allergy (Martelli, De Chiara, Corvo, Restani, & Fiocchi, 2002). Furthermore, cooking or other processing methods do not necessarily eliminate the allergenic epitopes from milk and meat products. Nevertheless few papers, which investigated the effects of HPP processing on BSA, demonstrated that the solution properties of the protein can be affected by pressure (Buchow, Wendorff, & Hemar, 2011).

According to these findings, this paper proposed an extensive characterization of a HHP treated allergenic protein, Bovine Serum Albumin (BSA), with the aim of individuating the mechanism of action of HHP technology on the structure as well as on the functional and biological properties of this compound and determining to which extent the physical and structural changes occurring to the proteins previously described can be observed for this particular case. An extended experimental campaign was carried out to assess the effects of HHP process parameters, namely pressure level and treatment time, and protein concentration on the structural characteristic of the allergens.

The effect of processing conditions, namely pressure level and treatment time, on the conformational structure and the functional properties of BSA solubilized in a buffer solutions at different concentration was analyzed. BSA unfolding and refolding were analyzed in terms of free sulfhydryl (SH) groups, changes of secondary structure, foaming and emulsifying properties. The experimental data were analyzed in order to identify the role of the process parameters in the transition from reversible unfolding to irreversible unfolding/aggregation of this protein. A deeper understanding of the mechanisms governing the conformational changes will be very useful to design novel processes addressed to the reduction and/or elimination of food allergenicity.

2. Materials and methods

2.1. Preparation of the samples

Different amount of BSA protein (Sigma-Aldrich, Italy) to reach the concentration of 50 and 100 mg/mL was dissolved, at a temperature of 25 °C, in Sodium Phosphate Buffer (50 mM, pH = 8), according to the protocols reported by Penãs et al. (2006). After a gentle mixing, a homogenous solution was obtained. The pH of the protein solution was measured with a pH-meter (S400 SevenExcellence, Mettler Toledo International Inc.). The protein solutions were stored under refrigerated conditions before HHP treatments.

2.2. Experimental apparatus

The HHP system U22 (Institute of High Pressure Physics, Polish Academy of Science, Unipress Equipment Division, Poland), which is a laboratory scale unit provided with a vessel which has a maximum processing volume of 50 mL, was used during the experiments.

The system can be operated in a wide pressure range (0–1400 MPa) under thermal controlled conditions (25–120 °C).

Operating pressure, ramp rate and processing time are set up on a control panel, which, in turn, allows the opening and the closure of the HHP vessel. A portable Temperature Power and control Unit (TCU) connected to the main unit with electrical cables and thermocouples (K-type) permits the set up and control of the operative temperature in the HHP vessel. The vessel is heated with electrical heaters and cooled withcompressed air. The pressurizing medium is Plexol (Bis (2-ethylhexyl) sebacate from Sigma-Aldrich, Italy) and the estimated temperature increase due to pressure build-up is 2–3 °C/100 MPa.

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