Innovative Food Science and Emerging Technologies 38 (2016) 221–230

Contents lists available at ScienceDirect

Innovative Food Science and Emerging Technologies

journal homepage: www.elsevier.com/locate/ifset

Effects of high pressure processing on the structural and functional properties of bovine lactoferrin

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ARTICLE INFO

Article history:
Received 26 June 2016
Received in revised form 14 October 2016
Accepted 17 October 2016
Available online 19 October 2016

Keywords:
Bovine lactoferrin
High pressure processing
Protein structure
Solubility
Foaming properties
Emulsifying properties

ABSTRACT

High pressure processing (HPP) is an emerging non-thermal processing technology and highlights a vast prospect in the food industry. Bovine lactoferrin (LFb), a representative protein in nutraceutical supplement, was investigated by HPP in this study with focus on the changes in structural and functional properties. LFb was treated by HPP at different pH (4.0 and 6.0) and at 300–700 MPa for either 30 or 60 min. The results showed that the tertiary structure of LFb was significantly modified with increased intensity of HPP, indicating partial denaturation and aggregation of LFb. The decrease in the unordered structure indicated that HPP resulted in a looser secondary structure of LFb. HPP also improved the solubility, foaming and emulsifying properties of LFb, and the samples treated at 400 MPa for 30 min and 300 MPa for 60 min at pH 4.0, or 600 MPa for 30 min and 400 MPa for 60 min at pH 6.0, had the most enhanced functionality.

Industrial relevance: In recent years, high pressure processing (HPP) has been used in food industry as an alternative technology to improve functional properties of various kinds of proteins, including milk whey proteins such as β-lactoglobulin, α-lactabumin and bovine serum albumin, soy protein isolate, walnut protein isolate and so on. It was found that HPP produces a variable degree of protein denaturation, which leads to aggregation and dissociation of polypeptides, and modifies their surface hydrophobicity, solubility, and other physiochemical properties. Lactoferrin (LF) possesses a broad spectrum of functional properties towards humans and animals such as antimicrobial, anti-inflammatory, antitumoral and immunomodulatory activities. These functions make LF a valuable protein to industry. Thus, it is necessary to investigate the effect of HPP on the structure of LF under varying pH conditions. Furthermore, because the functional properties such as solubility, foaming and emulsifying properties of proteins are important to its application in food industry, the effect of HPP on these properties of LF was also been studied in this manuscript.

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1. Introduction

In recent years, many innovative food processing technologies involving the use of high pressure processing (HPP), microfluidization and homogenization, etc., have been developed, and the effects of these methods on protein emulsifying properties have been widely investigated (Chen, Chen, Yu, & Wu, 2016; Khan, Mu, Zhang, & Arogundade, 2014; Lee, Subirade, & Paquin, 2008). High pressure processing, an alternative non-thermal method for food processing, is being investigated to replace heat treatment in the processing of certain products (Franco, Castillo, & Pérez, 2012). In HPP, pressures in the range of 300–700 MPa are commonly applied to the food, and water is used as pressure transmitting fluid (San Martín, Barbosa-Cánovas, & Swanson, 2002). HPP has been commercially applied in fruit juice production and experimentally applied in protein-containing products. It was reported that HPP could improve the quality of cheese and yoghurt (Trujillo, Buffa, Casal, Fernández, & Guamis, 2002; Harte, Luedecke, Swanson, & Barbosa-Cánvovas, 2003; Penna, Subbarao-Gurram, & Barbosa-Cánvovas, 2007), while the side effects of HPP on proteins was also published. In recent years, HPP has been used in food industry as an innovative technology to improve functional properties (especially emulsifying properties) of various kinds of proteins, including milk whey proteins, such as β-lactoglobulin, α-lactalbumin and bovine serum albumin (Fandiño, 2006), soy protein isolate (Li, Zhu, Zhou, & Peng, 2012; Wang et al., 2008), walnut protein isolate (Qin et al., 2013) and others. It was found that HPP could result in a variable degree of protein denaturation, which could induce aggregation and dissociation of polypeptides, and modify their surface hydrophobicity, solubility, and other physicochemical properties (Condés, Speroni, Mauri, & Anon, 2012).

Lactoferrin (LF), an iron binding glycoprotein distributed in many external secretions of mammals, is characterized by its capacity to

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http://dx.doi.org/10.1016/j.ifset.2016.10.014
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bind and transfer iron ions, antimicrobial, anti-inflammatory, anti-tumoral and immunomodulatory activities (Brock, 2002). Bovine lactoferrin (LFb), recognized functional food ingredient, has been used in a wide variety of products such as infant formula, probiotics, supplemental tablets, pet food, and cosmetics and as a natural solubilizer of iron in food (Stănciuc et al., 2013). However, only few studies have considered the effects of HPP on the structural and functional properties of LF. Franco, Pérez, and Castillo (2013) investigated the effect of HPP (400, 500 and 650 MPa for 15 min at 20 °C) on the structure and the immunoreactivity of LFb, and found that the antimicrobial activity of LFb could be retained after the treatment at 400 MPa for 15 min. When LFb was subjected to HPP over 500 MPa, the structure of LFb was modified. Mazri, Sánchez, Ramos, Calvo, and Perez (2012) studied the effect of HPP (450–700 MPa at 20 °C) on the denaturation of lactoferrin in skim milk, whey and phosphate buffer. They reported that the denatured proportion of lactoferrin increased with the increase in pressure and holding time, and protein was denatured more slowly in buffer and milk than in whey system. Mayayo et al. (2014) studied the effect of high pressure (300–650 MPa at 20 °C) and heat treatment (65–90 °C) of human milk on immunoreactivity of lactoferrin and kinetic parameters for its denaturation process were calculated. The results suggested that HPP could be a potential alternative to thermal pasteurization, giving greater retention of native lactoferrin. To the best of our knowledge, no previous research has been reported on the effect of HPP on the emulsifying property, solubility and foaming properties of LFb. Therefore, the objective of the current study was to investigate the effect of HPP on the functional properties of LFb under varying pH conditions, with an emphasis on the changes in molecular structure and emulsifying properties of LFb. The relationship between HPP-induced structural changes and functional properties of LFb was also established.

2. Materials and methods

2.1. Materials and chemicals

Bovine lactoferrin (LFb, purity ≥96.2%) was obtained from Hilmar Ingredients (California, USA), and the composition reported was 99.3% protein, 0.4% moisture, 0.3% fat, and 0.2% ash. The iron saturation of the protein was 9.85%. Canola oil was obtained from Canola Harvest (Lethbridge, Alberta, Canada). All other chemicals and reagents were of analytical grade.

2.2. High pressure processing (HPP)

LFb solutions (1% w/v) were prepared by dissolving LFb powders in sodium phosphate buffer (PBS, 10 mmol/L) at different pH (4.0 and 6.0), and stirred overnight to ensure complete dissolution. As most food products have pH values ranging from acidic to neutral, two representative pH, that is 4.0 and 6.0 were chosen to study the effects of pH on the functional and structural properties of LFb. Sodium azide (0.02 wt%) was added as an antimicrobial agent. Prior to pressure processing, equivalent volume of LFb solutions were vacuum-packed in polyethylene bags. Samples were treated by HPP in the pressure range of 300–700 ± 10 MPa, at 25 °C for 30 and 60 min in a laboratory-scale high pressure processor (HPP L2-700/1, Tianjin Huatai Senmiao Biological Engineering Technology Ltd., Tianjin, China), with water as transmitting medium. Untreated samples (0.1 MPa) were used as controls. Pressure was raised at an approximate rate of 6.5 MPa/s and released at 20 MPa/s. The treatment time reported in this study did not include the pressure increase and decrease time. Compression was accompanied by an increase in temperature of about 3 °C 100 MPa^{-1} (Balasubramaniam, Farkas, & Turek, 2008). But when the pressurization was completed, the sample temperature quickly dropped to its initial temperature due to heat transfer from the samples to the stainless steel of the vessel (Chen & Hoover, 2003). Thus, the temperature of the samples remains 20 °C during the holding time. HPP were performed at least twice on different days and samples then were kept at 4 °C until analyzed.

2.3. Determination of structure changes in LFb

The structure changes of LFb can be reflected from physical and chemical characteristics, such as fluorescence spectra, zeta-potential and circular dichroism spectra (Jiang et al., 2014; Shevkani, Singh, Kaur, & Rana, 2015; Soares, Mateus, & De Freitas, 2007).

2.3.1. Fluorescence spectroscopy measurement

LFb samples before and after HPP were diluted to a concentration of 0.1 mg/mL in PBS (10 mmol/L). Fluorescence measurements were carried out using a fluorescence spectrophotometer (F-7000, Hitachi, Ltd., Tokyo, Japan). Fluorescence emission spectra of LFb were recorded with an excitation wavelength of 292 nm and emission wavelengths of 300–450 nm, with a slit width of 10 nm.

2.3.2. Determination of zeta-potential

Zeta-potential of LFb solutions before and after HPP was determined by dynamic light scattering using a Zetasizer Nano-ZS90 (Malvern Instruments, Worcestershire, UK). Zeta-potential was determined by measuring the direction and velocity of droplet movement in a well-defined electric field. Samples were equilibrated for about 60 s in the machine after loading, and the reported data was collected mean of over 30 continuous readings.

2.3.3. Circular dichroism measurement

Samples of LFb before and after HPP were diluted to a concentration of 0.1 mg/mL in PBS (10 mmol/L). The CD spectra were recorded using a CD spectrapolarimeter (Pistar ΠI-180, Applied Photophysics Ltd., Leatherhead, UK) at far-UV (190–260 nm) region under constant nitrogen flush, with a path length of 0.1 cm. Ellipticity was recorded at a speed of 100 nm/min, 0.2 nm resolution, 20 accumulations and 2.0 nm bandwidth. The collected data was analyzed using Dichroweb: the online Circular Dichroism Website http://dichroweb.cryst.bbk.ac.uk (Lobley, Whitmore, & Wallace, 2002; Whitmore & Wallace, 2004).

2.4. Measurement of the solubility of LFb

Solubility of LFb was determined according to the method described by Qin et al. (2013), with few modifications. Volumes of 10 mL HPP treated and HPP untreated LFb solutions (1% w/v) were centrifuged at 10,000 × *g* for 30 min at room temperature using a high-speed freezing centrifuge (3K15, Sigma-Aldrich Co., St. Louis, Missouri, USA). Protein content of supernatants was determined by micro-Kjeldahl and the nitrogen content was multiplied by 6.25. Solubility of LFb was calculated as grams of soluble protein per kilogram of total protein. All determinations were performed in triplicate.

2.5. Measurement of foaming properties of LFb

Foaming capacity (FC) and foaming stability (FS) were determined according to the method described by Qin et al. (2013), with few modifications. Volumes of 50 mL (V_1) HPP treated or HPP untreated LFb solutions (1% w/v) were stirred for 1 min using an Ultra-Turrax T25 high-speed blender (IKA, Staufen, Germany) at 10,000 rpm at room temperature, and the resulting samples were poured into 250 mL graduated cylinders. Volumes of the whole mixture were recorded before and after whipping. FC and FS were calculated using the following equations:

$$\mathrm{FC}\ (\%) = \frac{V_2 - V_1}{V_1} \times 100$$

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