Food Hydrocolloids 35 (2014) 209–216

Contents lists available at SciVerse ScienceDirect

Food Hydrocolloids

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

The effects of pH and high hydrostatic pressure on the physicochemical properties of a sweet potato protein emulsion

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ARTICLE INFO

Article history:
Received 7 January 2013
Accepted 22 May 2013

Keywords:
Sweet potato protein emulsion
High hydrostatic pressure
Emulsifying properties
Rheology

ABSTRACT

The effect of high hydrostatic pressure (HHP) treatments on the physicochemical properties of sweet potato protein (SPP) emulsions at three pH values (3, 7 and 8) was investigated. The emulsifying activity index (EAI) and the emulsifying stability index (ESI) of all emulsions at the different pH values were significantly increased by the HHP treatments ($P < 0.05$). The oil droplet sizes were significantly decreased, whereas the volume frequency distribution of the smaller droplets was markedly increased when the pressure was increased from 200 to 600 MPa ($P < 0.05$). A significant increase in the interfacial protein concentration in the pH 3, 7 and 8 emulsions was observed when the pressure was increased from 200 to 600 MPa ($P < 0.05$). Under non-reducing conditions, higher molecular weight aggregates formed by disulfide bonds were observed in the pH 7 emulsions by SDS-PAGE. However, HHPs did not change and/or displace the main electrophoretic bands of SPP. The SPP emulsions had shear-thinning behaviors at pH 3 and 7 with and without HHP treatment, whereas a Newtonian behavior was observed in the pH 8 emulsion subjected to the 200 and 400 MPa treatments. These results suggest that HHP-treated emulsions stabilized by SPP could have various applications in the food industry.

1. Introduction

The production of proteins from sweet potato starch wastewater has attracted attention in China due to its economic benefits and environmental concerns. Sweet potato protein (SPP) is one of the major components of starch wastewater generated from the processing of sweet potato starch and has nutritional benefits, a balanced amino acid composition and good functional properties (Arogundade & Mu, 2012; Mu, Tan, & Xue, 2009). The application of vegetable proteins in foods for bi-functional purposes (supplement and functional agent) is of interest in the food industries.

Proteins are amphiphilic molecules that can be used as emulsifiers to stabilize emulsions (Damodaran, 1996). Proteins being surface-active agents, can preferentially migrate to the oil-water interface, form a protective adsorption layer around an oil droplet and prevent oil droplets from coalescing. The interfacial film at the oil-water interface can be enhanced by increasing the interfacial protein concentration, thereby increasing the physical stability of the emulsions. SPP has good emulsifying activity but low stability (Mu et al., 2009). The maximum interfacial protein concentration reported for SPP was 1.81 mg/m^2 with 2% (w/v) protein concentration; however, it has a poor stability compared to legume protein (Guo & Mu, 2010).

High hydrostatic pressure (HHP) is an effective tool to destroy the microorganisms in foods; compared to treatments such as pasteurization and sterilization, HHP has a less severe effect on the stability and the gelation properties of protein emulsions (Anton, Chapleau, Beaumal, Delépine & de Lamballerie, 2001). Dickinson and James (1998) reported that an emulsion treated at 800 MPa for 60 min destabilized the emulsion system to a much lesser degree than a 65 °C thermal treatment for 5 min. Protein emulsions stabilized by soybean, β-lactoglobulin, and egg yolk have been subjected to HHPs at different pH values to modify the functional properties of the food formulation (Anton et al. 2001; Dickinson & James. 1998; Puppo et al., 2008). Puppo et al. (2008) observed a low degree of flocculation, no coalescence, and an increased viscosity for a soybean protein isolate (7%w/v) emulsion at pH 8 that was processed with a combined temperature/HHP treatment. However, Anton et al. (2001) observed an increased viscosity at pH 7 compared to pH 3 with no effects on the interfacial protein concentration and on the droplet sizes in an HHP-treated egg yolk emulsion.

Our recent study has shown the potential of SPP as a functional additive during food product development (Arogundade, Mu &

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0268-005X/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodhyd.2013.05.011

Añón, 2012). Hence, the appropriate structure modification of an SPP emulsion by HHPs might lead to enhanced functionality, which could increase the applications of SPP emulsions in the food industry.

The objective of this study was to evaluate the effects of HHP treatment on the stability, viscosity, interfacial protein concentration and droplet sizes of SPP emulsions (1% w/v) subjected to different HHPs and at different pH values (3, 7, and 8) for a better understanding of SPP as a functional agent in food industries.

2. Materials and methods

2.1. Materials

The sweet potatoes (variety: Mixuan No.1) used in this study were cultivated in Miyun County, Beijing, China. They were harvested during the early month of October and then stored at 10–14 °C. All the reagents used in this study were of analytical grade.

2.2. Preparation of SPP

SPP was prepared according to the method described by Guo & Mu (2010). The sweet potato roots were washed, cut into small pieces, added to a 0.1% (w/v) sodium bisulfite solution (1 kg of sweet potato per liter of solution) to reduce oxidative browning and then ground and filtered using a double-layered cheese cloth. The slurry was centrifuged at 10,000 *g* for 60 min. The supernatant was ultra-filtered, lyophilized. The SPP was composed of 90.20% protein, 0.63% fat, 0.24% crude fiber, 3.2% ash and 5.9% moisture, using standard methods (A.O.A.C., 1990). One percent (w/v) solutions of SPP were prepared at three different pH values (3, 7 and 8) using 50 mM glycine and Tris buffers. Our previous study considered SPP emulsion at pH 7 (Guo & Mu, 2010), while the present study was design to evaluate the effect of HHP in neutral, acidic and alkali condition of emulsion.

2.3. Emulsion preparation

The emulsions were prepared with 2 mL of corn oil and 6 mL of the SPP solutions (pH 3, 7 or 8); the oil volume fraction was 0.25. The mixtures were then homogenized at 21,000 rpm for 60 s using a high-speed homogenizer (FJ-200, Shanghai Specimen Co, China) equipped with a 12-mm working head.

2.4. HHP treatment

The HHP treatments were carried out using a high-pressure device (model HHP.L3-600/0.6; Tianjin Huatai Senmiao Engineering and Technique Co. Ltd, Tianjin, China) and a hydraulic type cell with an inner capacity of 1 L and a water jacket for temperature control. Before the high-pressure treatment, 8 mL of each emulsion were packed under vacuum in polyethylene bags. The emulsions were then pressure-treated at 25 °C for 15 min at 200, 400, and 600 MPa. The target pressure was reached at a rate of approximately 250 MPa/min and released at approximately 300 MPa/min.

2.5. Determination of the emulsifying activity index and the emulsifying stability index

The emulsifying activity index (EAI) and the emulsifying stability index (ESI) of the emulsions were determined by turbidimetric methods (Pearce & Kinsella, 1978). After the HHP treatment, the emulsions (20 μL) were immediately diluted in a 0.1% SDS solution (5 mL). The absorbance of the diluted emulsions was measured at 500 nm using a spectrophotometer (Hitachi U- 3010, Tokyo, Japan). The absorbance values after the HHP treatment were used in the calculation of the EAI. Similarly, the emulsions (20 μL) were diluted with 5 mL of 0.1% SDS solution after 10 min of HHP treatment and the absorbance values were used in the calculation of the ESI. The turbidity of the emulsion was calculated using the following equation:

$$T = 2.303 \times \frac{A}{L} \times D$$

where T is the emulsion turbidity (m^{-1}), A is the absorbance value, D is the dilution factor (250), and L is the light path length (0.01).

The emulsion activity index (EAI, expressed in m^2/g) was calculated as:

$$\mathrm{EAI} = \frac{2 \times T_0}{\varphi \times C \times 1000}$$

where T_0 is the emulsion turbidity after the HHP treatment, ϕ is the oil volume fraction (0.25), and C is the concentration of the protein present in the protein dispersion (10 mg/mL).

The emulsifying stability index (ESI, expressed in min) was calculated as:

$$\mathrm{ESI} = \frac{A_0}{A_0 - A_t} \times t$$

where t is the time interval (10 min), and A_0 and A_t are the absorbance of the emulsion after HHP treatment for 0 and 10 min, respectively.

2.6. Measurements of droplet size and distribution

The emulsion droplet sizes were determined by laser diffraction in a Baite particle size analyzer (BT-9300, Dandong Baite Instrument Co., Ltd, Dandong, Liaoning, China). Immediately, after the HHP treatment, an emulsion aliquot (0.5 mL) was added to 5 mL of a 1% SDS solution (w/v). The volume-weighted mean diameter ($D_{4,3}$), the volume-surface mean diameter ($D_{3,2}$) and the droplet size distribution were determined. Additionally, the specific surface area (m^2/mL emulsion) was calculated according to the method reported by Walstra (1983):

$$\mathrm{Sv} = 6\varphi/D_{3,2} \ \left(\mathrm{m^2/mL\ emulsion}\right)$$

where ϕ is the oil volume fraction and $D_{3,2}$ is the volume-surface average diameter of the particles suspended in the SDS solution.

2.7. Emulsion microscopy

The freshly HHP-treated emulsions were carefully placed between a microscope slide and a cover slip while ensuring that no air gaps or bubbles were trapped between the sample and the cover slip. The effects of the HHP treatment on the droplet flocculation were then assessed using an optical microscope (equipped with a 20 × magnification objective and a 10 × magnification eyepiece; CX41, Olympus Corporation, Tokyo, Japan). The images of the emulsions were captured using a CCD camera (EOS 450D, Canon, Japan).

2.8. Interfacial protein concentration

The adsorbed and non-adsorbed proteins at the o/w interfaces of the HHP-treated emulsions were obtained according to the method described by Patton and Huston (1986). Two-milliliter aliquots of the fresh emulsions were diluted with 2 mL of a 50%