Food Hydrocolloids 28 (2012) 301–312

Contents lists available at SciVerse ScienceDirect

Food Hydrocolloids

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

Improvement of emulsifying properties of Maillard reaction products from β-conglycinin and dextran using controlled enzymatic hydrolysis

Jin-Bo Zhang, Na-Na Wu, Xiao-Quan Yang*, Xiu-Ting He, Li-Juan Wang

Research and Development Center of Food Proteins, College of Light Industry and Food, South China University of Technology (SCUT), Guangzhou 510640, PR China

ARTICLE INFO

Article history:
Received 14 October 2011
Accepted 3 January 2012

Keywords:
β-conglycinin
Dextran
Conjugates
Enzymatic hydrolysis
Emulsifying properties
Hydrolysates

ABSTRACT

A novel emulsifier was prepared by conjugating soy β-conglycinin and dextran (MW 67 kDa) under dry-heated Maillard reaction followed by trypsin hydrolysis with the degree of hydrolysis (DH) at 2.2% and 6.5%. The emulsifying properties of β-conglycinin, β-conglycinin–dextran conjugates and hydrolysates of β-conglycinin–dextran conjugates (DH 2.2% and DH 6.5%) were investigated using zeta-potential, droplet size and creaming index of the emulsions. The results showed that hydrolysates of β-conglycinin–dextran conjugates (DH 2.2%) were capable of forming a fine emulsion (d_{43} = 0.62 ± 0.04 μm, pH 7.0) which remained stable during 4 weeks of storage. A variety of physicochemical and interfacial properties of β-conglycinin, β-conglycinin–dextran conjugates and hydrolysates of β-conglycinin–dextran conjugates were investigated. Hydrolysates of β-conglycinin–dextran conjugates (DH 2.2%) had a much higher fraction of protein adsorption (F_{ads}) and a significantly lower saturation surface load (Γ_{sat}) compared with β-conglycinin, β-conglycinin–dextran conjugates and hydrolysates of β-conglycinin–dextran conjugates (DH 6.5%). This might be due to its higher molecular flexibility, which benefited the adsorption and unfolding of peptide molecules at the droplet interface. These might explain its markedly improved emulsifying capability. The conjugation of β-conglycinin and dextran effectively enhanced the hydrophilicity of the oil droplets surfaces and improved the steric repulsion between the oil droplets. Therefore the emulsions were still stable after 4 weeks of storage against pH, ionic strength and thermal treatment. This study demonstrated that controlled enzymatic hydrolysis of protein–polysaccharide conjugates could be an effective method for preparing favourable emulsifiers.

1. Introduction

Proteins have been used as emulsifiers in food products for many years (Dickinson & McClements, 1995, pp. 26–79). The ability of proteins to maintain stability is governed by their structures and properties at the interface. Proteins stabilize emulsions via their adsorption at the interface to provide a combination of electrostatic and steric repulsion between the oil droplets (Dalgleish, Dickinson, & Rodriguez Patino, 1999; Damodaran, 2005; Dickinson, 1999; McClements, 2004; Wilde, Mackie, Husband, Gunning, & Morris, 2004). However, protein stabilized emulsions are highly sensitive to environmental stresses such as pH, ionic strength, and temperature (Pongsawatmanit, Harnsilawat, & McClements, 2006). When the pH approaches the isoelectric point of the protein and/or the salt concentration is higher in the emulsion, the electrostatic repulsion of the protein adsorption layer decreases, and therefore coalescence and creaming occurs (Dickinson, 2008). The improvement of the emulsifying properties of protein will facilitate its utilization in the production of emulsion-based foods with desired functionalities.

Proteins derived from vegetable origin have recently gained attention due to the increased commercial production of protein ingredients with enhanced functionality. Soy protein is an abundant byproduct of the soybean-oil industry and has good functional properties for food processing because of the high nutritional value and the contribution to food texture and emulsifying properties (Comas, Wagner, & Tomas, 2006; Roesch & Corredig, 2002). Kobayashi, Kato, and Matsudomi (1990) first reported soy protein–polysaccharide conjugates prepared from the Maillard reaction. The studies of Kiosseoglou and co-workers demonstrated that soy protein–polysaccharide conjugates could improve emulsifying properties of soy protein, especially in the reduction of oil droplet size and emulsion stabilization against creaming (Diftis, Biliaderis, & Kiosseoglou, 2005; Diftis & Kiosseoglou, 2003, 2006a, 2006b). The conjugates adsorbed to the interface together with un-reacted protein constituents, enhancing steric stabilization forces of oil droplets.

* Corresponding author. Tel.: +86 20 87114262; fax: +86 20 87114263.
E-mail addresses: fexqyang@163.com, fexqyang@scut.edu.cn (X.-Q. Yang).

0268-005X/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodhyd.2012.01.006

Modification of soy protein by enzymatic hydrolysis for improved functionalities is a well accepted and safe method (Mahmoud, 1994). Hydrolysis of proteins causes changes such as an increase in protein solubility, a decrease in molecular size, and exposure of hydrophobic groups, which are the factors that influence emulsifying capability and emulsion-stabilizing ability of protein hydrolysates (Panyam & Kilara, 1996). Many studies have shown that the effects of enzymatic hydrolysis on protein functionalities are mainly dependent on the substrate characteristics, enzyme specificity, and the degree of hydrolysis (DH) (Jung, Roussel-Philippe, Briggs, Murphy, & Johnson, 2004; Lamsal, Jung, & Johnson, 2007; Ven, Gruppen, Bont, & Voragen, 2001). DH is defined as the percentage of peptide bonds cleaved per gram of protein compared with the total number of peptide bonds per gram of protein (Adler-Nissen, 1986, p. 427). Controlled enzymatic hydrolysis therefore means that the hydrolysis modification is regulated with a combination of enzyme/substrate (E/S) ratios, time, temperature, etc. for a desirable DH value (Qi, Hettiarachchy, & Kalapathy, 1997; Wu, Hettiarachcchy, & Qi, 1998). Trypsin is produced by the exocrine cells of the pancreas and its activity is maximized at alkaline pH (Qi et al., 1997). Because of the broad specificity to peptide bond cleavage, trypsin is commonly used for protein hydrolysis to improve functionality.

Therefore, a combination of Maillard reaction and controlled enzymatic hydrolysis might be a promising way for the functionality modification of globular proteins. However, limited information is available so far concerning the effects of Maillard reaction and the enzymatic hydrolysis pattern of soy protein on the emulsifying properties.

In this study, β-conglycinin, β-conglycinin–dextran conjugates and hydrolysates of β-conglycinin–dextran conjugates (DH 2.2% and DH 6.5%) were used as emulsifiers to obtain four kinds of emulsions. We investigated the properties of these emulsions against storage, heat treatment, pH and ionic strength changes. The emulsions were characterized by light scattering, zeta-potential, interfacial properties, and confocal laser scanning microscopy.

2. Materials and methods

2.1. Materials

Dextran (67 kDa) and trypsin were obtained from Sigma co. (St. Louis, MO, USA). β-conglycinin were purified from defatted soybean seed flour (commercially produced during soybean oil production by low-temperature technology; provided by XIANGCHI Cereal and Oil Co., Shangdong Province, China) using the method described by Nagano, Hirotsuka, Mori, Kohyama, and Nishinari (1992). The protein content of β-conglycinin was 91.21% (dry weight, $N \times 5.71$) as determined by the Dumas combustion method (Elemental Analyzer rapid N cube, Hanau, Germany). Corn oil was purchased from a local market. All solutions were prepared using deionized water. All other chemicals were of analytical grade.

2.2. Preparation of β-conglycinin–dextran conjugates

β-conglycinin, dextran and sodium phosphate buffer solution (10 mM, pH 6.8) were mixed to make a dispersion in which the concentration of β-conglycinin and dextran was 10% (w/v) and 10% (w/v), respectively. The mixture was stirred on a stirring plate for 2 h at room temperature, to dissolve the mixture. The pH of the mixture was adjusted to 6.8 by carefully adding 0.1 M HCl. The mixture was transferred to the environment of 4 °C with gentle stirring overnight to allow for the complete hydration of β-conglycinin and dextran, and then freeze-dried. The resulting β-conglycinin–dextran mixtures were incubated at 60 °C and 75% relative humidity (over saturated potassium bromide) for up to 6 days. After incubation, the samples were dispersed in deionized water for 2 h at room temperature and then centrifuged at 10,000 *g* for 15 min using a HITACHI CR22G centrifuge. The supernatant was then freeze-dried.

2.3. Hydrolysis of β-conglycinin–dextran conjugates

The β-conglycinin–dextran conjugates were resuspended in deionized water at a concentration of 1.5–2% (w/v) protein and stirred overnight at 4 °C. The pH was then adjusted to 8.0. The protein content of the solution was determined using the AOAC method (AOAC, 2000). Solutions were diluted to 1% (w/v) protein using deionized water. Hydrolysates with DH 2.2% and 6.5% were prepared from the β-conglycinin–dextran conjugates solutions by hydrolysis at pH 8.0 at 40 °C, using trypsin. The pH and DH were controlled using the pH-stat method by using a 719S Titrino (Metrohm ion analysis, Herisau, Switzerland) (Adler-Nissen, 1986, p. 427). The enzyme concentrations used were 3 or 13 units/g protein for the DH values of 2.2% and 6.5%, respectively. The enzyme was dissolved in deionized water and directly added to the solutions. The molarity of the NaOH solution used to maintain the pH at pH 8.0 varied from 0.1 to 0.3 M. When the desired DH was reached, the enzymatic hydrolysis was stopped by addition of a 100 mM phenylmethylsulfonyl fluoride stock solution in 2-propanol to a final concentration of 1 mM. The pH-stat experiment was stopped after the pH remained stable at pH 8.0. Hydrolysates were freshly prepared before each experiment.

2.4. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE experiments were performed according to the discontinuous buffer system of Laemmli (1970) at 5% stacking gel and 13% separating gel using Bio-Rad Mini-protein Tetra Electrophoresis System (Bio-Red Laboratories Ltd., California, USA). The conjugates samples were dissolved in a 0.125 M Tris–HCl buffer solution (pH 8.0) containing 1.0% (w/v) SDS, 0.05% (w/v) bromophenol blue, 30% (v/v) glycerol and 5% (v/v) β-mercaptoethanol. The samples were shaken using a vortex mixer (MS 3 basic, IKA, Germany) for about 5 s at 3000 rpm to disperse the samples homogeneously, and boiled for 5 min. Sample solution (15 μL, approximately 40 μg of protein) was loaded into each well. Electrophoresis was run at 20 mA in the stacking gel and at 40 mA in separating gel until the tracking dye reached the bottom of the gel. Two pieces of gels were run at the same time. After electrophoresis, one piece of gel was stained for protein by a Coomassies Blue stain kit (Biosafe, Bio-Rad Laboratories Inc.). The other piece of gel was stained for carbohydrate by the GelCode Glycoprotein staining kit (Pierce Biotechnology, Rockford, IL). The band patterns were then photographed.

2.5. Determination of molecular weight distribution (MWD) using high performance size exclusion chromatography (HPSEC)

SEC analysis was performed on a Waters Breeze system equipped with a Waters 1525 pump and Waters 2487 UV detector (Waters, USA). The β-conglycinin, β-conglycinin–dextran conjugates and hydrolysates of β-conglycinin–dextran conjugates (DH 2.2% and DH 6.5%) were dissolved in sodium phosphate (50 mM, pH 7.2) containing 50 mM NaCl for 2 h to achieve a protein concentration of 0.5% (w/v), centrifuged at 10,000 *g* for 20 min, and then filtered through 0.45 μm filters (Millipore, Fisher Sci.). The same buffer was used as mobile phase, and aliquots of the filtrate (10 μL) were injected into a prepacked TSK G4000SWxl column