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Peroxidase-mediated conjugation of corn fiber gum and bovine serum albumin to improve emulsifying properties

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ABSTRACT

The emulsifying properties of corn fiber gum (CFG), a naturally occurring polysaccharide–protein complex, was improved by kinetically controlled formation of hetero-covalent linkages with bovine serum albumin (BSA), using horseradish peroxidase (HRP). The formation of hetero-crosslinked CFG–BSA conjugates was confirmed using ultraviolet–visible and Fourier-transform infrared analyses. The optimum CFG–BSA conjugates were prepared at a CFG:BSA weight ratio of 10:1, and peroxidase:BSA weight ratio of 1:4000. Selected CFG–BSA conjugates were used to prepare oil-in-water emulsions; the emulsifying properties were better than those of emulsions stabilized with only CFG or BSA. Measurements of mean droplet sizes and zeta potentials showed that CFG–BSA-conjugate-stabilized emulsions were less susceptible to environmental stresses, such as pH changes, high K ionic strengths, and freeze–thaw treatments than CFG- or BSA-stabilized emulsions. These conjugates have potential applications as novel emulsifiers in food industry.

1. Introduction

Proteins and polysaccharides are important macromolecules in food systems, and they are widely used to stabilize oil-in-water emulsions. There is particular interest in the use of polysaccharide–protein complexes instead of plain polysaccharides or proteins (Evans, Ratcliffe, & Williams, 2013). Polysaccharide–protein complexes could be formed through electrostatic interactions or covalent bonds (Schmitt, Sanchez, Desobry-Banon, & Hardy, 1998). The newly formed complexes will exhibit combined functional properties of each component and their improved emulsifying abilities is receiving heated attention. (Galazaka, Dickinson, & Ledward, 2000; Li, Fang, Phillips, & Al-Assaf, 2013). Although electrostatic interactions are widely used, complexes formed by this method are susceptible to the variation of pH values and easily dislocated from each other. Compared with electrostatic interactions, covalent bonds could render the complexes more irreversible and stable to low pH and high ionic strength (Evans et al., 2013).

Covalent bonding between a polysaccharide and a protein is commonly achieved through Maillard reactions and enzymatic mediations. Maillard reactions usually take several days or weeks to conjugate the ε-amino groups in proteins and the reducing-end carbonyl groups in polysaccharides (Flanagan & Singh, 2006; Wong, Day, & Augustin, 2011). Enzymatic methods are more cost-effective than Maillard reactions, because milder conditions, lower quantities of reactants, and shorter reaction times are needed, they are less likely to produce toxic byproducts, and are acceptable to consumers (Poutanen, 1997).

Corn fiber gum is usually extracted from the byproducts of corn-milling processes, using the alkaline hydrogen method (Gaspar, Juhasz, Szengyel, & Reczey, 2005; Hespell, 1998). It has attracted increasing interest because of its potential use as a novel industrial emulsifier with certain health benefits, as shown by in vitro fermentation studies (Nino-Medina et al., 2009; Rose, Patterson, & Hamaker, 2010). It consists mainly of a β-(1→4)-xylan backbone with arabinofuranosyl substituents attached through O-2 and/or O-3 (Martinez-Lopez, Carvajal-Millan, Miki-Yoshida, et al., 2013; Martinez-Lopez, Carvajal-Millan, Rascon-chu, Marquez-Escalante, & Martinez-Robinson, 2013). In addition to the major arabinoxylan

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fraction, there are minor functional components, namely phenolic acids and proteins. These two fractions play important roles in the emulsifying properties and modification of CFG. Phenolic acids, namely ferulic acids, a small amount of p-coumaric acids and diferulic acids, are esterified to arabinosyl residues (Saulnier, Vigouroux, & Thibault, 1995). According to current studies, a small fraction of proteins may covalently bond, or physically precipitate, with polysaccharide fractions in CFG (Yadav, Johnston, Hotchkiss, & Hicks, 2007; Yadav, Nunez, & Hicks, 2011). This protein fraction anchors the CFG molecules at the oil–water interface, and carbohydrate fractions stretch into the aqueous phase, providing an electrosteric barrier (Randall, Phillips, & Williams, 1988); CFG can therefore stabilize oil-in-water emulsions, as can other naturally occurring complexes such as gum arabic and sugar beet pectin. However, not all CFGs have excellent emulsifying properties. The poor emulsifying properties of some CFGs are partly the result of insufficient protein contents (Yadav, Parris, Johnston, Onwulata, & Hicks, 2010).

In this study, we used horseradish peroxidase (HRP) to catalyze hetero-conjugation between CFG and bovine serum albumin (BSA), and used the hetero-crosslinked CFG–BSA conjugates to stabilize oil-in-water emulsions. HRP can use various aromatic components as substrates, e.g., aromatic phenols, phenolic acids, and amino acids (Veitch, 2004). It is therefore an ideal enzyme for achieving hetero-conjugation between phenolic acids in CFG and tyrosines in BSA. Previous studies showed that HRP can catalyze homo-conjugation of polysaccharides or hetero-conjugation between polysaccharides and open-chain proteins such as casein, or peptides (Boeriu et al., 2004; Martinez-Lopez, Carvajal-Millan, Rascon-chu, et al., 2013; Ng, Greenshields, & Waldron, 1997; Oudgenoeg et al., 2001). However, previous attempts to achieve oxidation between polysaccharides and compact proteins have been unsuccessful (Figueroa-Espinoza et al., 1999).

In this investigation, we selected BSA as a model protein, because it has a compact structure with tyrosine moieties mostly located on the outer surface (Zhao, Li, Carvajal, & Harris, 2009), so the tyrosyl residues are readily accessible to peroxidase oxidation to form dityrosines (Aeschbach, Amadoo, & Neukom, 1976; Chen et al., 2003). BSA also emulsifies oil-in-water emulsions, and has been used in the food industry for many years (Haque & Kinsella, 1988).

The objective of this study was to conjugate CFG with BSA. The emulsifying abilities of the conjugates were investigated, with the aim of broadening the range of CFG applications in the food industry.

2. Materials and methods

2.1. Materials

BSA (fraction V, M_w 68,000) was purchased from Roche (Nutley, NJ, USA). HRP was purchased from Sigma–Aldrich [St. Louis, MO, USA; 1000 U/mg solid, using 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)]. Heat-resistant α-amylase was purchased from the Aladdin Industrial Corporation (Shanghai, China). CFG was extracted from corn fiber processed by wet-milling, using the procedure described in Section 2.3. p-Coumaric and ferulic acid standards were obtained from Sigma Chemical Co., St. Louis, MO. All other reagents were analytical grade. The wet-milled corn fiber was provided by the local corn-milling factory.

Stock phosphate stock buffer solution (PBS, pH 7.0, 50 mM) was prepared by adding sodium dihydrogen phosphate (50 mM) to disodium phosphate (50 mM), and then adjusting the pH to 6.0 using 1 M HCl. BSA and CFG solutions were prepared separately by dispersing a dry powder in PBS (pH 6.0), stirring at room temperature until completely dissolved, and storing at 4 °C overnight for complete hydration. Peroxidase solution (100 U/mL) was prepared using PBS (pH 6.0), stored at 4 °C, and used within 7 days. H_2O_2 solution (30%, v/v) was diluted with PBS (pH 6.0) to 0.06% (v/v); it was prepared freshly prior to the CFG–BSA conjugation experiments.

2.2. CFG isolation

Corn fiber and corn bran are byproducts from corn wet milling and dry milling processes, respectively. During the wet milling process, corn grains are soaked in warm water containing sulfur dioxide and corn fiber is collected in the step of wet sieving (Rose, Inglett, & Liu, 2009). In the investigation, corn fiber from wet-milling was dried in an oven and ground to 40 mesh. The ground fiber was de-oiled by adding hexane at a weight ratio of 1:7, and stirred for 2 h using a magnetic stirrer. The de-oiled fiber was mixed with distilled water at the weight ratio of 1:10 and boiled for 10 min. Heat-resistant α-amylase (3 mL/50 g) was added and the mixture was incubated in water at 40 °C for 2 h. The absence of starch was confirmed by staining the de-oiled and destarched corn fiber with I/I_2. CFGs were extracted from the de-oiled and destarched fiber using a modified alkaline hydrogen method (Yadav et al., 2007). The de-oiled and destarched corn fiber was stirred with an alkaline solution containing 2 meq/g fiber of NaOH and $Ca(OH)_2$, and boiled for 15 min. The reaction mixture was cooled to room temperature, and centrifuged at 8000 × *g* for 5 min. The supernatant was decanted from the residue, 30% H_2O_2 was added, the pH was adjusted to 11.5 with 6 mM NaOH, and the mixture was stirred at room temperature for 2 h. The pH of the alkaline H_2O_2 extract was adjusted to 4.0–4.5 with concentrated HCl and centrifuged at 8000 × *g* for 20 min. The supernatant was decanted and two volumes of ethanol were gradually added to it, with stirring. The supernatant–ethanol mixture was allowed to settle for 15 min; CFG was obtained as a white flocculent precipitate. The water–ethanol mixture was decanted, and the precipitate was transferred to plastic boxes to be lyophilized.

2.3. Compositional analysis

The isolated CFG samples were subjected to moisture, ash content, lipid, protein and phenolic acid analysis. Moisture and ash contents were determined using "AACC Approved Methods" 44-19 and 08-01, respectively (AACC International, 1995). The total protein content was measured using BCA protein assay reagent kit, which detects protein using bicinchoninic acid (BCA) (Smith et al., 1985). Bovine serum albumin (BSA) was used as the standard to construct a calibration curve. The total lipid content was measured using fat analyzer (ZF-06, Ruizheng Equipment Corporation, Shanghai). The total phenolic acid content was measured using UV spectroscopy (UVmini-1240, Shimadzu Corporation, Japan) at the wavelength of 325 nm. The total amount of phenolic acids was calculated from known standard curves of p-coumaric acids and ferulic acids and expressed as a sum of p-coumaric acids and ferulic acids ($y_1 = 12.246x - 0.2596$, $R^2 = 0.9979$; $y_2 = 8.523 - 0.3342$; x is absorbance, y_1 and y_2 is the concentration of ferulic acid and p-coumaric, respectively).

2.4. Optimum conditions for CFG–BSA conjugation using peroxidase

Oxidative crosslinking between CFG and BSA using a peroxidase/hydrogen system was performed using a modified version of the methods reported by Boeriu et al. (2004) and Oudgenoeg et al. (2001).

The optimum oxidation conditions for CFG–BSA conjugation were identified by performing the reaction at various weight ratios