Carbohydrate Polymers 187 (2018) 26–34

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Transglutaminase-treated conjugation of sodium caseinate and corn fiber gum hydrolysate: Interfacial and dilatational properties

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ARTICLE INFO

Keywords:
Corn fiber gum
Sodium caseinate
Transglutaminase
Hydrolysate

ABSTRACT

This study compliments previous work where peroxidase was successfully used to crosslink corn fiber gum (CFG) with bovine serum albumin and improve CFG's emulsifying properties. Herein, an alternative type of enzyme, transglutaminase, was used to prepare conjugates of CFG and sodium caseinate. Additionally, the CFG was partially hydrolyzed by sulfuric acid and its crosslinking pattern with caseinate was evaluated. The interfacial crosslinking degree between caseinate and CFG increased after hydrolysis according to high performance size exclusion chromatography. The equilibrium interfacial tension of CFG hydrolysate-caseinate conjugate was lower than that of CFG-caseinate conjugate as the rearrangement rate of the CFG hydrolysate-caseinate conjugate was higher. The dilatational modulus of CFG hydrolysate decreased from that of CFG.

1. Introduction

Proteins and some polysaccharides are natural biopolymer emulsifiers, widely used in food emulsion systems to encapsulate bioactive ingredients (Dickinson, 2009). In order to improve the functional properties of these two biopolymers, people have used physical and chemical methods to form complexes or conjugates between proteins and polysaccharides. The interfacial properties of polysaccharide-protein complexes or conjugates are of great interest because they are related to the stability, texture and industrial production of emulsions prepared by such conjugates or complexes (Karbowiak, Debeaufort, & Voilley, 2006; Szczesniak, 1963). Introducing intermolecular linkages can strengthen the interfacial layers and the associated enhancement of colloid stability can lead to more stable emulsions or foams (Dickinson, 1999a); this is facilitated by the hydrophobic fractions of proteins anchoring the polysaccharide-protein conjugate or complex strongly to the interface and the hydrophilic carbohydrate polymer providing stabilization at the interface by steric repulsion (Dickinson, 2008; Patino & Pilosof, 2011).

Complexes of biopolymers formed by physical interactions, such as electrostatic forces, are susceptible to environmental changes of pH and ionic strength. Thus, interactions between proteins and polysaccharides through chemical covalent conjugations have been developed during the past two decades. Maillard and enzymatic reactions are two commonly used strategies to construct covalent conjugations. Pioneered by Kato, Minaki, and Kobayashi (1993), Maillard reaction has been widely utilized to prepare protein and polysaccharide conjugates in the last quarter century (Einhorn-Stoll, Ulbrich, Sever, & Kunzek, 2005; Hou, Wu, Xia, Phillips, & Cui, 2017; Pirestani, Nasirpour, Keramat, Desobry, & Jasniewski, 2017; Yadav, Parris, Johnston, Onwulata, & Hicks, 2010; Yadav, Strahan, Mukhopadhyay, Hotchkiss, & Hicks, 2012). Besides the Maillard reaction, enzymatic crosslinking methods have also been frequently used to conjugate protein and polysaccharide and require relatively milder reaction conditions and shorter reaction times (B. Chen, Li, Ding, & Suo, 2012; Chen et al., 2003; T. Selinheimo, Lampila, Mattinen, & Buchert, 2008). The most commonly used food enzymes for crosslinking polysaccharide and protein include peroxidases, laccases and transglutaminases (Zeeb, McClements, & Weiss, 2017).

Different types of crosslinking enzymes will induce the crosslinking of protein and polysaccharide at specific but different reaction sites. Peroxidases and laccases are able to catalyze the formation of covalent bonds between phenolic acids in feruloylated polysaccharides and the amino acids in proteins (Figueroa-Espinoza & Rouau, 1998; Osma, Toca-Herrera, & Rodríguez-Couto, 2010). Jung and Wicker (2012) prepared conjugates of sugar beet pectin (SBP) and β-lactoglobulin (H-BLG) via ferulic acid in SBP and tyrosine in H-BLG using laccase. Boeriu et al. (2004) used horseradish peroxidase to catalyze the crosslinking of

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https://doi.org/10.1016/j.carbpol.2018.01.034
Received 23 October 2017; Received in revised form 22 December 2017; Accepted 10 January 2018
Available online 11 January 2018
0144-8617/

arabinoxylan with β-casein. The problem with using peroxidase and laccase is that they lead to the consumption of phenolic acids in the polysaccharides, which are important active antioxidant groups (Mathew & Abraham, 2004). Transglutaminase can catalyze the formation of new peptide bonds between one or more proteins through γ-carboxyamine group of glutamyl residues and various primary amino groups on proteins, such as lysine or lysyl residues (Zeeb et al., 2017). VY this means, transglutaminase can couple added protein to the proteins already associated with polysaccharides and provides an alternative reaction method to modify the proteinaceous portion of the natural protein-containing polysaccharide complexes. Ovomucin, a glycoprotein, was conjugated with food protein through the transglutaminase reaction (Kato, Wada, Kobayashi, Seguro, & Motoki, 1991). Flanagan and Singh (2006) prepared conjugates of gum Arabic and sodium caseinate via transglutaminase treatment and confirmed the formation of conjugates through size exclusion column chromatography.

Sodium caseinate is an excellent food-grade emulsifying agent, composed of four different proteins (α_{s1}, α_{s2}, β, κ-caseins). β-Casein, the most surface active protein, will preferentially adsorb at the interface with a long tail at the *N*-terminus extending into the aqueous solution and α_{s1}-casein can adsorb at the interface through its hydrophobic *N*-terminal peptide; α_{s2} and κ-caseins, however, are not as surface active as the other two fractions (Dickinson, 1999b; Robson & Dalgleish, 1987; Shimizu, Takahashi, Kaminogawa, & Yamauchi, 1983) Transglutaminase is commonly used to improve the emulsifying properties of sodium caseinate (Færgemand, Murray, Dickinson, & Qvist, 1999; Nonaka et al., 1992; Schorsch, Carrie, & Norton, 2000; Tang, Yang, Chen, Wu, & Peng, 2005). The cross-linked sodium caseinate forms a highly dense and viscoelastic network and has been shown to improve the chemical and physical stability of oil-in-water emulsions (Ma et al., 2012; Partanen, Forssell, Mackie, & Blomberg, 2013).

An arabinoxylan extracted from corn fiber/bran, corn fiber gum (CFG) may act as an emulsifying agent when there is a significant proteinaceous fraction associated with the polysaccharide backbone, which enables stabilization at the oil-water interface. However, CFGs extracted from certain varieties of corn fiber have shown poor emulsifying properties due to inferior amounts of associated protein. To solve this problem, we have previously cross-linked bovine serum albumin (BSA) to the phenolic acid residues present on CFG (Liu et al., 2015, 2017). The results showed that the CFG-BSA conjugates had better emulsifying performance, but as the phenolic acid groups were consumed, the antioxidant activity of CFG could be compromised. The objective of current study is to evaluate the effect of hydrolysis of CFG on the transglutaminase-catalyzed crosslinking between CFG and sodium caseinate as well as their interfacial properties. We hypothesized that CFG and hydrolyzed CFG would form intermolecular conjugation interactions with sodium caseinate and hydrolyzed CFG may show higher degree of crosslinking with sodium caseinate.

2. Materials and methods

2.1. Materials

Sodium caseinate was purchased from Sigma-Aldrich Chemical Company (Lot # SLBQ9222V, MO, NY). Microbial transglutaminase was purchased from Ajinomoto Food Ingredients (Activa® TI, Itasca, IL, USA) and it contained 100 Units of enzyme activity per gram of powdered preparation, which was determined by a spectrophotometric assay using *o*-phtaldialdehyde described by Dinnella, Gargaro, Rossano, and Monteleone (2002). The product has 0.9% enzyme content, 94.4% maltodextrin and other inactive components of sodium, calcium, magnesium, phosphorus, and iron salts.

2.2. CFG preparation

The identical batch of corn fiber gum (CFG) used herein was extracted from corn bran using an alkali extraction method and fully characterized as reported in our previous work (Liu et al., 2015). Briefly, de-oiled and de-starch corn fiber was mixed with NaOH and $Ca(OH)_2$ and then boiled at 100 °C for 15 min. The corn fiber-alkali slurry was centrifuged and supernatant was collected and stirred at room temperature with the addition of hydrogen peroxide. CFG was obtained by adding absolute ethanol to the supernatant collected from the corn fiber-hydrogen peroxide mixture solution after centrifugation. The molecular weight of CFG was 1.4×10^5 g/mol (Mw) as measured by the high performance size exclusion method (Liu et al., 2017).

2.3. Acid hydrolysis of CFG

CFG stock solution was prepared at the concentration of 10 mg/mL. CFG solutions were mixed in equal volume with different concentrations of sulfuric acid (0.25 M, 0.5 M, 1 M and 2.5 M). The hydrolysis was performed at 90 °C for 30 min. The effect of hydrolysis time was tested and the hydrolysis was conducted at the acid concentration of 0.25 M for 10, 20, 30 min and 1, 2 h.

2.4. Reducing end sugar determination

Reducing end sugar contents of CFG before and after hydrolysis were determined by bicinchoninic acid (BCA) assay (Kongruang, Han, Breton, & Penner, 2004). BCA reagent was prepared freshly by mixing equal volumes of solution A (27.14 g of Na_2CO_3, 12.1 g of $NaHCO_3$ and 0.971 g of BSA disodium salt hydrate, pH 9.7) and solution B (0.624 g of $CuSO_4 \cdot 5H_2O$ and 0.631 g of L-serine, pH 3.4). Then equal amounts of the BCA reagent and samples were mixed, vortexed and incubated at 75 °C for 30 min in a water bath. The BCA reagent-sample mixtures were cooled for an hour at room temperature and their absorbance was measured at 560 nm. A calibration curve was built using 1 μM–70 μM glucose solutions.

2.5. Preparation of enzymatically crosslinked CFG/hydrolyzed CFG and sodium caseinate

The enzymatic crosslinking of CFG/hydrolyzed CFG and sodium caseinate was performed based on the method applied by Flanagan and Singh (2006). Sodium caseinate solution (5 mg/mL) was prepared. CFG and hydrolyzed CFG solutions of different concentration were mixed in equal volume with each protein solution to obtain polysaccharide-protein mixtures with weight ratios of 2:1, 1:1, and 1:2. The formation of CFG-sodium caseinate was catalyzed by adding 10 mg transglutaminase powder per mL sample solution. The CFG-protein mixtures were incubated in a water bath at 40 °C for 2 h. Transglutaminase was deactivated by heating for 10 min at 100 °C (Nonaka et al., 1992). CFG-protein mixtures without enzymes were also prepared to use as control.

2.6. High performance size exclusion chromatography of CFG hydrolysates and CFG-protein conjugates

The molecular weight distribution of CFG hydrolysates and CFG-protein conjugates were obtained by size exclusion chromatography using an Agilent 1200 liquid chromatography system (Agilent Co., California, USA). The HPLC-SEC method was described by Qi, Chau, Fishman, Wickham, and Hotchkiss (2017) with some modifications. Briefly, samples (2 mg/mL) were prepared by dissolving them in 50 mM, pH 6.0 phosphate buffer at room temperature. The samples were fully soluble. The sample solutions dialyzed against a 1 L solution of 50 mM, pH 6.0 phosphate buffer and the dialysis tubing had a molecular weight cut-off of 6.0–8.0 kDa and solutions were changed four times. The recovery after filtration through 0.22 um filter was 90% for