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Modification of soy protein hydrolysates by Maillard reaction: Effects of carbohydrate chain length on structural and interfacial properties

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ABSTRACT

This study investigated the effects of carbohydrate chain length on the structural and interfacial properties of the Maillard reaction conjugates of soy protein hydrolysates (Mw > 30 kDa). The covalent attachment of sugars to soy peptides was confirmed by amino acid analysis and examination of the Fourier-transform infrared spectra. The results suggested that the emulsion stability of the conjugates increased as the length of the carbohydrate chains increased. The surface activity measurement revealed that the soy peptide–dextran conjugates were closely packed and that each molecule occupied a small area of the interface. It was further confirmed that the soy peptide–dextran conjugates formed a thick adsorbed layer at the oil–water interface, as observed in the confocal laser scanning micrographs. The interfacial layer of soy peptides was rheologically complex with broad linear viscoelastic region and strong elastic modulus, and the soy peptide–dextran conjugates might form multilayer adsorption at the interface. This study suggested that the improved surface properties of the soy peptide–dextran conjugates were a result of the strong membrane formed by the closely packed molecular and multilayer adsorption at the interface, which provided steric hindrance to flocculation.

1. Introduction

Soy proteins are amphiphilic molecules that are commonly used as food ingredients to form and stabilize food emulsions and foams [1]. However, due to its large molecular weight and globular structure, soy protein exhibits poor surface activity compare to low molecular weight surfactants and flexible proteins, such as β-casein. Structural modification of soy protein has thus drawn considerable attention over the past two decades [2–5]. Limited enzymatic hydrolysis and the Maillard reaction are regarded as very effective ways to improve the surface activities of proteins [6–9].

The Maillard reaction, as a natural, nontoxic method of modifying protein or polysaccharide, is often used for improving the emulsifying properties of proteins. However, it has complicated effects on the surface properties of proteins. It has been suggested that the structure and surface hydrophobicity of proteins, the type of sugar, the reaction conditions (including time and temperature), and the degree of the reaction are related to the physicochemical and functional properties of the final Maillard reaction product. A large fraction (Mw > 3 kDa) of tofu whey conjugated to polysaccharides or oligosaccharides showed an improved emulsifying activity and emulsion stability, whereas the small fraction (Mw < 3 kDa)–oligosaccharide conjugates could only improve their emulsion stability [10]. Dunlap and Cote [11] reported that the emulsion stability of β-lactoglobulin – dextran conjugates increased as the size of the polysaccharides increased up to 150 kDa. Guo and Xiong [12] prepared five Maillard-type products by reacting buckwheat protein with five different saccharides. Their results regarding emulsion stability placed the five complexes in the following order: dextran > maltodextrin > xylose > glucose > fructose > control. In addition, the stability tended to increase with the duration of the reaction (except for dextran). However, a study of the functional properties of rice protein hydrolysates conjugated with monosaccharides, oligosaccharides, and polysaccharides through Maillard reaction showed no correlation between the emulsifying properties of the Maillard reaction products and the molecular weight of the saccharides [13]. Therefore, the effects of the protein structure and the length of the carbohydrate chain on the final functional

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properties of the Maillard reaction products are still poorly understood.

Limited enzymatic hydrolysis has been found to be an effective way of improving the functionality of soy protein, especially regarding its surface properties [8,14]. However, emulsions formed by the soy protein hydrolysates are usually unstable due to their small molecular size [15]. According to Dickinson [16], an effective emulsifier should be an amphiphilic molecule with the ability to quickly adsorb at the interface to resist flocculation and coalescence. It is hypothesized that limited enzymatic hydrolysis combined with Maillard reaction with a carbohydrate of suitable chain length would allow the individual soy peptides to form a hydrophilic tail, resulting in good emulsifying activity and emulsion stability.

The interfacial shear rheology has become very popular in recent years [17–19]. Work on interfacial rheology is often motivated by its relevance to the stability and flow behavior of foams and emulsions. A sensitive rheometer equipped with the Du Noüy ring geometry can be used to characterize and follow the evolvement of the adsorption of proteins to oil/water interfaces [20]. Interfacial adsorption of proteins such as β-casein, bovine serum albumin, lysozyme, and insulin [21], were studied. Working on the interfacial rheology could offer insights into the adsorption behavior and predict long term storage for emulsions.

The overall objective of this study, therefore, was to investigate the effects of the length of the carbohydrate chain on the structural and interfacial properties of the Maillard reaction conjugates of soy peptides. The specific molecular weights of soy peptides (Mw > 30 kDa) were obtained through limited hydrolysis of soy protein and ultrafiltration. The modified soy peptides were analyzed for net charge, hydrophobicity, surface properties, emulsifying and interfacial properties, and the microstructure of the emulsions.

2. Materials and methods

2.1. Materials

Soybeans were obtained from a local market. The soy protein isolates (SPI) were prepared following the procedure of Diftis and Kiosseoglou [22]. The protein content was determined to be about 92% ($N \times 6.25$) by the Kjeldahl method. Papain (600,000U/g) (EC 3.4.22.2) was purchased from Ruji Biotechnology Co. (Shanghai, China). Soybean oil was purchased from a local supermarket. All other chemicals used in the study were of analytical grade unless otherwise specified. All of the aqueous solutions were prepared using deionized water.

2.2. Preparation of soy peptides

A 5% (w/v) soy protein isolate dispersion was hydrolysed at 50 °C and pH 7.0 with papain (600,000U/g). The E/S ratio was 0.5%. The degree of hydrolysis of the hydrolyzed protein was determined to be 1.0% using the pH-stat method [23]. The hydrolysates were then heated at 90 °C for 10 min to inactivate the enzyme and cooled in ice water at room temperature for 1 h. After centrifugation (10,000 × *g* for 10 min), the supernatant was further fractionated by ultrafiltration with a Pellicon Mini membrane system (EMD Millipore, USA), following the steps of Wu et al. [24], with little modification. The inlet pressure was 10 psi and the outlet pressure was 5 psi. The hydrolyzate was separated by a 30 kDa molecular weight cutoff (MWCO) membrane (EMD Millipore, USA), which generated two parts: permeate and retentate. The separation was performed twice to obtain satisfactory separation. Each separation was performed until the volume of retentate reached to about 50 mL. Then the retentate was diluted to 1 L with deionized water and passed through the same membrane. Finally, the retentate peptide fraction (Mw > 30 kDa) was freeze-dried for further study.

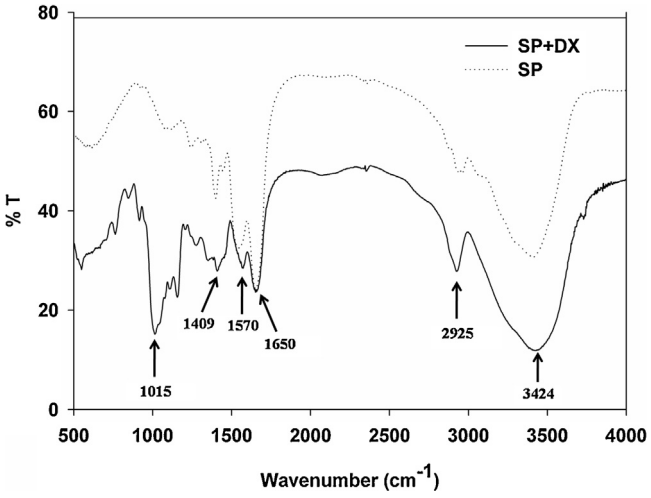

Fig. 1. Fourier transform infrared (FTIR) spectra of solid SP and SP + DX. (T: transmittance).

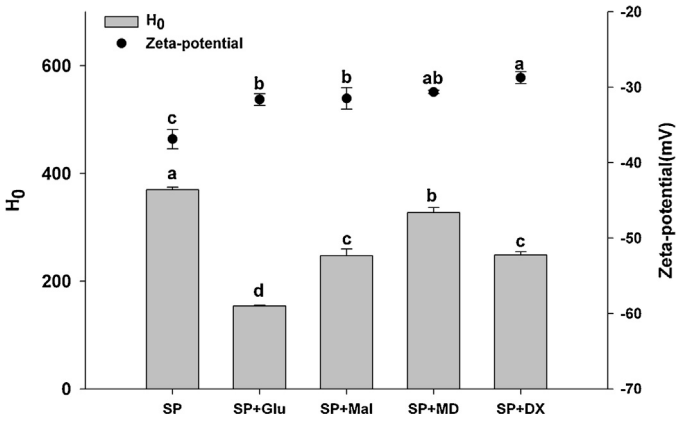

Fig. 2. Zeta-potential and surface hydrophobicity (H_0) of SP-sugar conjugates. Superscripts (a–d) represent significant differences at $p < 0.05$ level.

2.3. Preparation of soy peptide–sugar conjugates

The soy peptide–sugar conjugates were prepared by reaction of the soy peptides with glucose (180), maltose (342), maltodextrin (DE 20), and dextran (40 kDa). The wet-heating reaction was performed according to the method of Dan Zhu et al. [25], with some modification. The soy peptides and sugars were dissolved in distilled water at a weight ratio of 1:1. The pH was adjusted to 7.0 with 0.1 M NaOH. The mixtures were stored at 4 °C overnight to allow for complete hydration and then heated in a water bath at 60 °C for 3 days. The products were cooled in ice water before analysis. The control soy polypeptides were prepared using the same treatment.

2.4. Amino acid analysis

The sample was hydrolyzed in evacuated sealed tubes at 110 °C for 24 h with 6 M HCl. The amino acid analysis was performed on an Agilent 1100 series HPLC system (Agilent Technologies, Santa Clara, CA) with on-line pre-column derivation by o-phthaldialdehyde (OPA) and 9-fluorenylmethyl chloroformate (FMOC-Cl, for proline analysis) [13]. The analysis was performed on a Hypersil C_{18} column.