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Comparative studies on the physicochemical properties of soy protein isolate-maltodextrin and soy protein isolate-gum acacia conjugate prepared through Maillard reaction

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ABSTRACT

Dry-heated Maillard reaction was applied in the preparation of protein–polysaccharide conjugates. Reaction mixtures containing soy protein isolate (SPI) and maltodextrin (1:1 weight ratio) were dry-heated at 60 °C and 79% relative humidity for three days. The mixtures of SPI and gum acacia (GA) were dry-heated at the same condition for one week. The conjugate of SPI–MD showed lower levels of free amino groups and higher degree of graft, which indicated that reaction between SPI and MD developed much faster than reaction between SPI and GA. The solubility of SPI at isoelectric point was improved remarkably after grafting with MD or GA. The grafted SPI showed significantly higher levels of emulsifying properties than SPI and the emulsifying properties of SPI–GA conjugate were much better than SPI–MD. Decreases of lysine and arginine contents after the graft reaction indicated that these two amino acid residues attended the covalent linkage between SPI and MD or GA. The graft reaction reduced surface hydrophobicity and fluorescence emission maximum value because of a shielding effect of the polysaccharide chain bound to proteins. The results of secondary structure suggested that grafted SPI had decreased the levels of α-helix, β-sheet and β-turn and increased unordered coils level.

1. Introduction

Generally, the functional properties of proteins are of key interest to manufacturers of pharmaceutical, food, and cosmetic products. Many modifications have been used to improve the functional properties of proteins, such as chemical, physical or enzymatic treatments (Achouri, Zhang, & Shiying, 1999; Apichartsrangkoon, 2003; Klompong, Benjakul, Kantachote, & Shahidi, 2007; Lawal, Adebowale, & Adebowale, 2007; Molina, Papadopoulou, & Ledward, 2001; Ramezani, Aminlari, & Fallahi, 2003; Wang et al., 2008). However, most chemical modifications have not been applied for food industry, because of the potential health hazard or the appearance of detrimental products. For the physical modifications, reactions need to depend on the mechanical forces, such as high pressure or shear (Galazka, Dickinson, & Ledward, 2000; Haykawa, Linko, & Linko, 1996). Therefore, an idea different from a conventional approach is desirable to improve the functional properties of proteins in food systems.

Recently, some researchers have attempted to improve the functional properties of proteins through protein–polysaccharide graft reactions, which are based on Maillard reactions between the ε-amino groups of proteins and the reducing-end carbonyl groups of polysaccharides (Kato, 2002). The most striking characteristic of the resulting protein–polysaccharide conjugates is the excellent emulsifying properties which are superior for industrial applications (Akhtar & Dickinson, 2007; Diftis & Kiosseoglou, 2006). In addition to the improvement of emulsifying properties, this method of modification was efficient in improving the solubility, antibacterial effect and antioxidant effect of proteins (Kato, Shimokawa, & Kobayashi, 1991; Nakamura, Kato & Kobayashi, 1991, 1992). It has also been proposed that conjugation of the allergen protein with polysaccharides may be effective in reducing the allergenicity (Arita, Babiker, Azakami, & Kato, 2001; Babiker et al., 1998). Moreover, this method utilized a naturally occurring reaction and no chemical reagent was applied in this approach. Therefore, this method could be one of the most promising approaches for food applications, because of its safety.

In this paper, a dry-heated Maillard reaction between soy protein isolate (SPI) and maltodextrin (MD) or gum acacia (GA) under controlled temperature and relative humidity conditions was carried out. The solubility, emulsion capability, and surface hydrophobicity of grafted SPI were investigated. Moreover, the amino acid composition and secondary structure were determined.

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2. Materials and methods

2.1. Chemicals

Crude soy protein isolate was purchased from Shandong Yuwang Industrial Co. Ltd. The powder was purified by the method described as follows. The powder was dispersed in distilled water and adjusted to pH 8.0. The dispersion was stirred for 2 h to extract proteins and then centrifuged at 4000 *g* for 30 min at 4 °C. The supernatant was adjusted to pH 4.5 and centrifuged at 4000 *g* for 20 min at 4 °C. The pellet was washed twice with distilled water. Thereafter, the protein pellet was resuspended in distilled water and neutralized to pH 7.0 (Jiang, Chen, & Xiong, 2009). The samples were freeze-dried and stored in a 4 °C cooler. Protein content in the prepared SPI powder was 95% (w/w) as determined with the biuret method (Gornall, Bardawill, & David, 1949) using bovine serum albumin (Huamei Biotechnology Co., Ltd., Luoyang, China) as standard. Gum acacia (GA) was purchased from Sinopharm chemical reagent Co., Ltd. (Shanghai, China). Maltodextrin, with a dextrose equivalent of 10, was purchased from Xiwang Co., Ltd. (Shandong, China). Soy oil was obtained from a local supermarket and used directly without further purification. 1, 8-anilinonaphthalenesulfonate (ANS) reagent was purchased from Sigma (St Louis, MO, USA). Bovine serum albumin (BSA), o-Phthaldialdehyde (OPA), and all other reagents were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China).

2.2. Preparation of the SPI–GA and SPI–M conjugates

Protein–polysaccharide conjugates were prepared through Dry-heated Maillard reaction described as follows. A mixture of SPI–MD or SPI–GA of a weight ratio of 1/1 was prepared by mixing solutions of the two biopolymers and the dispersion was adjusted to pH 7, followed by freeze-drying and comminution of the dried sample. The powder of SPI–MD was then incubated at 60 °C in a desiccator, in the presence of saturated KBr solution, for a period of three days. The powder of SPI–GA was then incubated at the same dry-heating conditions for a period of one week. In order to study the effect of dry-heated treatment on native SPI, the native SPI was also incubated at the same dry-heating conditions for one week. Samples, drawn after incubation, were freeze-dried again.

2.3. Determination of free amino groups

The levels of free amino groups of samples were determined by a modified OPA method. The OPA reagent was prepared by mixing 40 mg of OPA (dissolved in 1 mL of methanol), 25 mL of 100 mM sodium tetraborate, 2.5 mL of 20% (w/w) sodium dodecyl sulfate (SDS), and 100 μL of β-mercaptoethanol and then diluting to a final volume of 50 mL with distilled water (Vigo, Malec, Gomez, & Llosa, 1992). Sample solution (2 mg/mL in protein, 200 μl) was incubated with 4 mL of OPA reagent at 40 °C for 2 min. The absorbance at 340 nm was measured by UV–vis spectrophotometer (Shanghai Spectrum Instruments Co., Ltd., China). A calibration curve was obtained using 0.25–2 mM L-leucine. Degree of graft (DG) was calculated using the equation: $DG = [(A_0 - A_t)/A] \times 100\%$, where A_0, A_t, and A are the absorbance of sample solution of SPI–MD/GA conjugates, SPI–MD/GA mixtures and native SPI, respectively.

2.4. Protein solubility at different pH levels

Protein solubility was determined by dispersing the samples in distilled water to obtain a final solution of 2 mg/mL in protein. The pH values of the protein solution were adjusted from 7 to 4 with 0.5 M HCl or 0.5 M NaOH and then centrifugated at 12,000 g for 30 min (4 °C). The content of protein for the resulting solution was analyzed according to the Lowry method (Lowry, Rosebrough, Farr, & Randall, 1951).

2.5. Emulsion activity and stability

The emulsifying activity index (EAI) and emulsifying stability index (ESI) were measured according to the method of Wang et al. (Wang, Zhao, & Jiang, 2007). For emulsion formation, 5 mL of soy oil and 15 mL of sample solution (2 mg/mL in protein) in de-ionized water (pH 7.0) were homogenized in a PT-MR 2100 (KINEMATICA AG, Switzerland) for 1 min at 24,000 rpm. Fifty microliters of emulsion was taken from the bottom of the homogenized emulsion, immediately (0 min) or 10 min after homogenization, and diluted (1:100, v/v) in 0.1% (w/v) SDS solution. After shaking in a vortex mixer for 5 s, the absorbance of diluted emulsions was recorded at 500 nm using UV–vis spectrophotometer (Shanghai Spectrum Instruments Co., Ltd., China). EAI was calculated using the equation: $EAI\ (m^2/g) = \{(4.606 \times A_0)/[C \times \varphi \times \theta \times 10^4]\} \times$ dilution factor, where A_0 represents the absorbance of the diluted emulsions at 0 min, C is the protein concentration (g/mL) before emulsification, φ is the oil volume fraction (v/v) of the emulsion and θ is the optical path (1 cm). ESI was defined as: $ESI\ (min) = [A_0/(A_0 - A_{10})] \times 10$, where A_0 and A_{10} represent the absorbance of the diluted emulsions at 0 and 10 min, respectively.

2.6. Surface hydrophobicity (H_0)

Surface hydrophobicity was measured using the 1-anilino-8-naphthalenesulfonate (ANS) as the fluorescence probe (Liu, Zhao, Zhao, Ren, & Yang, 2012). Protein dispersions were diluted (0.05, 0.1, 0.2, 0.5, 1, and 2 mg/mL) in phosphate buffer solution (0.2 M, pH 7.5). Then, an aliquot of ANS solution (20 μL, 8.0 mM in the same buffer) was added to 4 mL of sample. Fluorescence intensity (FI) was measured with a Hitachi F-7000 fluorescence spectrometer (Hitachi, Ltd., Tokyo, Japan) at wavelengths of 390 nm (excitation) and 470 nm (emission). The initial slope of the FI versus protein concentration plot (calculated by linear regression analysis) was used as an index of protein hydrophobicity.

2.7. Intrinsic fluorescence emission spectroscopy

Intrinsic emission fluorescence spectra of the protein samples were obtained by a Hitachi F-7000 fluorescence spectrophotometer (Hitachi, Ltd., Tokyo, Japan). Protein solutions (0.15 mg/mL) were prepared in 10 mM phosphate buffer (pH 7.0). The protein solutions were excited at 290 nm (slit width 5 nm) and emission spectra were recorded from 300 to 400 nm at a 10 nm/s scanning speed.

2.8. Analysis of amino acids

The native SPI and SPI–polysaccharides conjugates were acid hydrolyzed at 110 °C for 24 h in 6 M HCl in vacuum-sealed tubes. The lysine and arginine levels were determined by Agilent 1100 high performance liquid chromatograph (Agilent technologies Co., Ltd, Santa Clara, USA) equipped with an ODS Hypersil column (5 μm, 250 × 4.6 mm).

2.9. Analysis of circular dichroism spectrum

Conformational changes in the secondary structure of protein samples were analyzed using a Mos-450 CD spectropolarimeter (Biologic, Claix, France) at 25 °C. Secondary structure determination was performed by scanning diluted protein samples (0.2 mg/mL in protein, diluted with 10 mM phosphate buffer solution, pH 7.0) between 190 and 250 nm. The same phosphate buffer solution was used as blank solution for all samples. Five scans were averaged to obtain one spectrum. Secondary structure was interpreted by visual