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Preparation of chemically modified canola protein isolate with gum Arabic by means of Maillard reaction under wet-heating conditions

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ABSTRACT

The aim of this study was to produce covalently attached conjugate between canola protein isolate (CPI) and gum Arabic (GA) in aqueous solutions via the Maillard reaction at 90 °C in a model system consisting of 2% CPI and 1, 2 or 4% GA. Upon decreasing of free amino group content in the glycosylated CPI to 72%, a new band near the loading end of sodium dodecyl sulfate-polyacrylamide gel electrophoresis and a shift of CPI peak in high performance size exclusion chromatography confirmed that the covalent attachment of CPI to GA was successful. The results of secondary structure analysis suggested that grafted CPI had decreased α-helix and β-sheet levels and increased random coils level. The solubility of CPI at isoelectric point was improved remarkably after grafting with GA. The optimal conjugation conditions chosen from the further experiments were 1% of GA, 90 °C and reaction time 15 min.

1. Introduction

The protein-rich canola meal left after oil extraction has been recognized as a potential alternative protein source for human consumption due to its nutritional value and technological functionalities (Aider & Barbana, 2011; Tan, Mailer, Blanchard, & Agboola, 2011). The continuous growth in global production of canola signifies that more meal will be produced as a consequence of the increased oil extraction. This points out the need to reappraise the use of canola meal, possibly for the extraction products with high market value, such as functional protein extracts as an ingredient for human food utilization (Tan, Mailer, Blanchard, & Agboola, 2014). However, canola protein isolates are often prepared by direct alkaline extraction followed by acid precipitation (Tan et al., 2011) and the poor water solubility of such protein fractions (Yoshie-Stark, Wada, & Wasche, 2008) leads to isolates with unsuitable food technological functional properties.

During recent years, researchers have worked on the conjugation of protein with polysaccharide by means of Maillard reaction to improve physicochemical and functional properties of proteins in food systems (Diftis & Kiosseoglou, 2006; Jian, He, Sun, & Pang, 2016; Kato, 2002; Kim & Shin, 2015; O'Regan & Mulvihill, 2009; Yadav, Parris, Johnston, Onwulata, & Hicks, 2010) as a promising approach among the various modifications through physical, chemical and enzymatic treatments (Corzo-Martínez, Carrera Sánchez, Moreno, Rodríguez Patino, & Villamiel, 2012). However, most chemical modifications are unsatisfactory for food applications because of potential health hazards and/or deterioration of organoleptic properties (Guo & Xiong, 2013). With respect to the physical modifications, reactions depend on the mechanical forces, such as shear or high pressure (Galazka, Dickinson, & Ledward, 2000) and as for enzymatic modifications, the major drawbacks are the time consuming process of safety assessment of the products and the cost of enzyme, as well as the bitter tastes of the protein hydrolysates (Tian, Chen, & Small, 2011). Meanwhile, from an industrial viewpoint, dry-heating conditions, producing of Maillard reaction, is not feasible for large scale production (Zhu, Damodaran, & Lucey, 2008) owing to the long time required (up to several days or weeks), and the lack of control of the reaction, which may produce a combination of intermediate and/or advanced Maillard reaction products (MRPs), including insoluble aggregates that need to be removed by some technique (Qi, Liao, Yin, Zhu & Yang, 2010). Therefore, Zhu et al. (2008) found a wet-

Abbreviations: CPI, canola protein isolate; GA, gum Arabic; OPA, orthophthaldialdehyde; DUV, difference UV spectroscopy; SDS-PAGE, sodium dodecyl sulphate–polyacrylamide gel electrophoresis; MRPs, Maillard reaction products.

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heating method, which shortens the reaction time to only several hours thereby limiting the Maillard reaction to the initial stage of Schiff base formation and produce protein-polysaccharide conjugates with special functional properties.

Gum Arabic (GA), or Acacia gum, is a suitable polysaccharide in protein conjugation. GA is extracted from the stems and branches of acacia species trees and has a highly branched structure. GA is an anionic, hydrophilic heteropolysaccharide which has low viscosity compared to other polysaccharides of similar molar mass (Al-Assaf, Phillips, & Williams, 2005; Renard, Garnier, Lapp, Schmitt, & Sanchez, 2012; Wang, Wang et al., 2011).

However, on the one hand, works in the field of wet-heating Maillard reaction are scarce especially using of GA as a polysaccharide (Chen et al., 2013; Guo & Xiong, 2013; Jiang & Brodkorb, 2012; Li et al., 2009; Qi et al., 2010; Zhang et al., 2012; Zhu et al., 2008; Zhu, Damodaran & Lucey, 2010) and on the other hand, there are limited studies regarding the modification of canola protein isolate (CPI) only by electrostatic complexes (Stone, Cheung, Chang, & Nickerson, 2013; Uruakpa, & Arntfield, 2004, 2006). The major goal of this study was chemical modification of canola protein isolate with gum Arabic via wet-heating Maillard reaction to enhance its applications in food industry.

2. Materials and methods

2.1. Materials

Gum Arabic powder was obtained from Nexira Company (France). Industrial toasted meal was supplied from by-products of Golbahar Oil Company (Isfahan, Iran). Orthophthaldialdehyde (OPA), L-leucine, sodium tetraborate and sodium dodecyl sulphate (SDS) were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). All other chemicals used in this study were of analytical grade.

2.2. Preparation of canola protein isolate

Canola protein isolate (CPI) was prepared according to the direct alkaline extraction method of Tan, Mailer, Blanchard, and Agboola (2014) with some modifications. Briefly, defatted canola meal was dispersed in 0.1 M NaOH solution at 1:10 ratio (w/v) and stirred for 1 h at room temperature. The dispersion was then centrifuged (Hermle, Z36HK, Germany) at 10,000*g* for 30 min at 8 °C to collect the supernatant which was then filtered using Whatman No. 41 filter paper. The filtrate pH was then adjusted on 4 with 0.1 M HCl and centrifuged (10,000*g*, 30 min, 8 °C) to obtain the precipitates (CPI). The precipitate was washed with de-ionized water (pH 7.0), freeze-dried and kept at −20 °C until further analyzed. The protein content of the freeze-dried substrate was determined by the Kjeldahl method (N × 6.25), and the canola protein content was 82.5%.

2.3. Preparation of CPI–gum arabic conjugates

2% (w/v) CPI and 1, 2 or 4% (w/v) GA were dissolved in phosphate buffer solution (0.2 M, pH 7) and then were stirred with a magnetic stirrer at room temperature for 2 h, followed by gently stirring overnight at 4 °C to completely hydrate the mixture of biopolymers. The pH values of the solutions (pH 7) were adjusted by carefully adding 0.1 N HCl or 0.1 N NaOH. Aliquots of the solutions were heated at 90 °C for 0 min to 60 min and then immediately cooled in an ice-water bath to stop reaction. The reaction products were centrifuged at 4 °C (10,000*g*, 10 min) and the supernatant was freeze-dried. CPI, heated CPI at 90 °C for 15 min and mixture of CPI to GA without heating were used as controls.

2.4. Difference UV spectroscopy (DUV) and browning

DUV measurement was performed on a Beckman DU 530 UV–vis spectrophotometer (Beckman Coulter, Inc., Fullerton, CA, USA), in a 5 mm quartz cell at 25 °C. The samples were diluted appropriately, followed by centrifugation at 10,000*g* for 10 min at room temperature. The supernatant was used for a wavelength scan from 250 to 400 nm and as the result, the difference absorption spectra of conjugates at 294 nm (as an indicative of the formation of intermediate compounds of the Maillard reaction) were recorded against the control using a UV–vis spectrophotometer (T60 UV, PG Instruments Co., Ltd., UK). The absorbance of CPI-GA conjugate solutions at 420 nm as an indicative of the brown color development (Zhu et al., 2013) was also measured. Heated CPI and mixture of CPI and GA were used as the control for DUV measurement and absorbance at 294 nm or 420 nm, respectively.

2.5. Measurement of free amino groups

The content of free amino groups was determined by a modified OPA method. The OPA reagent was prepared freshly by mixing 80 mg of OPA (dissolved in 2 ml of methanol), 50 ml of 0.1 M sodium borate buffer (pH 9.85), 5 ml of 20% (w/w) sodium dodecyl sulfate, and 200 μl of β-mercaptoethanol and then diluting to a final volume of 100 ml with distilled water (Vigo, Malec, Gomez, & Llosa, 1992). The exact amount of 200 μl of sample solution (2 mg/ml in protein) was mixed with 4 ml OPA reagent in a vortex mixer and set at 35 °C for 2 min. The absorbance at 340 nm was recorded by UV–vis spectrophotometer. Free amino group contents were obtained using a calibration curve of L-leucine as a standard.

2.6. Fluorescence analysis

The fluorescence intensity of samples were measured at 350/420 nm (excitation/emission) (Sun, Hayakawa, Puangmanee, & Izumori, 2006) using a RF-5000 Shimadzu spectrofluorometer (Shimadzu, Corp., Japan). The sample was dissolved in 50 mM phosphate buffer (pH 7.0) at a protein concentration of 1 mg/ml.

2.7. SDS-polyacrylamide gel electrophoresis analysis

Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Laemmli (1970). The separating and stacking gel contained 12% and 5% acrylamide, respectively. The sample solutions were prepared in 0.125 M Tris–HCl buffer, consisting of 1% (w/v) SDS, 2% (v/v) β-mercaptoethanol (ME), 20% (v/v) glycerol and 0.025% (w/v) bromophenol blue, and heated in boiling water for 5 min before electrophoresis. After the electrophoresis, staining of gels were performed for protein with Coomassie Brilliant Blue R-250 and then destained with 10% acetic acid containing 20% methanol.

2.8. High performance size exclusion chromatography

High performance size exclusion chromatography (HPSEC) was carried out to provide information on the molecular size distribution of CPI using a GPC 500, 4.6 × 250 mm column (Synchropack, USA), connected to a Varian Pro-Star high performance liquid chromatography equipped with a Varian 3262 UV–vis detector operating at 280 nm and a Varian 210 HPLC pump (Varian, USA). The protein samples (20 mg/ml) were dissolved in 50 mM phosphate buffer (pH 7.2) containing 50 mM NaCl, stirred for 2 h and then stored overnight at 4 °C to complete hydration. After centrifugation of the protein solutions at 10,000*g* for 10 min at room temperature, the supernatant were filtered through a 0.22 μm filter. The elution