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Effect of covalent modification by (−)-epigallocatechin-3-gallate on physicochemical and functional properties of whey protein isolate

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ABSTRACT

The physicochemical and functional properties of covalently modified whey protein isolate (WPI) by (−)-epigallocatechin-3-gallate (EGCG) were investigated. WPI was chemically modified by EGCG under alkaline conditions. The effect of modification on foaming and emulsifying properties was evaluated. The results of SDS-PAGE and size exclusion chromatography indicated that modification by EGCG induced cross-linking on proteins of WPI. Fourier transform infrared spectroscopy (FT-IR) analysis illustrated the incorporation of phenolic groups into the modified WPI and the changes in protein secondary structure. Intrinsic fluorescence spectra revealed that modified WPI had a more compact tertiary structure compared to unmodified WPI. The modified WPI exhibited better foaming and emulsifying properties than unmodified WPI. These results suggest that EGCG modification is a potential method for improving the functional properties of WPI.

1. Introduction

Whey proteins and their products are important food ingredients that are extensively utilized in manufacturing of food due to their high nutritional quality and versatile functional properties. WPI, one of the most important whey protein products, is a spray-dried powder with high protein content. Functional properties for WPI are particularly important in relation to the texture and structure of processed food. Therefore, increasing interest is directed toward modifying WPI to enhance functionality and thereby add value to the protein. Chemical modification is an effective way to improve the functional properties of WPI. During the past decade, a number of chemical modifications such as phosphorylation, glycation, deamidation, succinylation, and Maillard reaction have been proposed to improve the functional properties of WPI (Li, Enomoto, Ohki, Ohtomo, & Aoki, 2005; Liu & Zhong, 2012; Ma, Forssell, Partanen, Buchert, & Boer, 2011; Morand, Guyomarc'h, Legland, & Famelart, 2012).

Phenolic compounds represent the largest group of secondary plant metabolites and are widely distributed in plants. Dietary phenolic substances have received much attention due to their ability to scavenge reactive oxygen species. Additionally, dietary phenolic compounds are known to interact with proteins in the food matrix. The details of the interactions between target proteins and phenolic compounds are not well understood, but covalent interactions of food proteins with phenolic compounds have been proposed. Phenolic compounds may be oxidized in an alkaline solution to their corresponding quinones (Hurell & Finot, 1984). The electron-deficient quinones represent a species of highly reactive substances that normally react further with nucleophilic amino acid residues in a protein chain (Kroll, Rawel, & Rohn, 2003; Rawel, Rohn, Kruse, & Kroll, 2002). Many food proteins such as whey proteins, myoglobin, lysozyme, bovine serum albumin, and soy proteins could interact with phenolic compounds in this manner. Covalent modification by phenolic compounds produces food protein derivatives that have different physicochemical and conformational properties compared with unmodified proteins. Rawel, Czajka, Rohn and Kroll (2002) reported that soy protein derivatives exhibited different characteristics compared with unmodified soy protein, including isoelectric points, solubility, digestibility, secondary structure, and thermal stability. Ali, Homann, Khalil, Kruse, and Rawel (2013) reported that modification of β-lactoglobulin with coffee-specific phenolic compounds resulted in

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changed structural properties, and alteration in solubility, surface hydrophobicity, and emulsifying properties were observed.

Tea is one of the most widely consumed beverages in the world, and is a rich source of health protective phenolic compounds (Kim et al., 2011; Thielecke & Boschmann, 2009). The predominant bioactive phenolic component of green tea, (−)-epigallocatechin-3-gallate (EGCG) is known to possess antioxidant, anti-inflammatory, and anti-cancer cell proliferation properties (Katiyar, Afaq, Azizuddin, & Mukhtar, 2001; Lestringant, Guri, Gülseren, Relkin, & Corredig, 2014; Zhong, Chiou, Pan, & Shahidi, 2012). On the basis of previous literature, we hypothesized that EGCG could covalently bind to nucleophilic amino acid residues in whey protein under alkaline conditions, which could lead to conformational changes. It is well known that the functional properties of food proteins are closely related to their physiochemical and structural characteristics. Accordingly, functional properties of whey protein may be altered by modification with EGCG through covalent reaction. However, to our knowledge, no studies have investigated the effects of covalent modification by EGCG on functional properties of WPI, particularly the foaming and emulsifying properties. Therefore, the main objective of the present study was to investigate the effect of covalent modification by EGCG on the physicochemical characteristics of WPI. Furthermore, the foaming and emulsifying properties of modified WPI were evaluated and compared.

2. Material and method

2.1. Chemicals

WPI was obtained from Hilmar Cheese Co. (Hilmar, CA, USA). According to the product bulletin, the typical composition of the WPI was 89% protein, 1.5% lactose, 0.5% fat, 2.5% ash, and 4.5% moisture. EGCG, KBr, and electrophoresis reagent were purchased from Sigma–Aldrich (Shanghai) Trading Co., Ltd. (Shanghai, China). Corn oil was purchased from Wumart Stores, Inc. (Hangzhou, China). All other chemicals were of reagent grade and obtained from Mike Chemical Co., Ltd. (Hangzhou, China).

2.2. Preparation of modified WPI with EGCG

WPI powder was dispersed in deionized water (35 g into 900 mL), and the pH value of the protein dispersion was adjusted to 9.0 using 0.5 M NaOH. EGCG solution (50 mL of 5 mg/mL) was mixed with the protein dispersion and then the volume was adjusted to 1000 mL with deionized water. After 12 h of reaction time under continuous stirring at 25 °C, the pH value of the protein dispersion was adjusted to 6.8 using 0.5 M HCl. To remove the free phenolic compounds in the protein dispersion, an ultrafiltration was performed using a Millipore Pellicon cassette module (Bedford, MA, USA), containing Biomax-5 membrane with a molecular weight cut-off (MWCO) of 5 kDa, with a membrane area of 0.1 m^2. The ultrafiltration was operated in the mode of batch ultrafiltration with full recycle of the retentate. Until the final volume reduced to 200 mL, 800 mL of deionized water was added into the retentate. The ultrafiltration was repeated five times, and then the retentate was lyophilized. In addition, a control experiment was performed to obtain the unmodified WPI without EGCG during the same period.

2.3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed by a slab gel made from 5% stacking gel and 12% separating gel, according to the method of Laemmli (1970). WPI samples were dissolved in loading buffer (50 mM Tris–Cl, pH 6.8, with 2% SDS, 10% glycerol and 0.1% bromophenol blue). Each well in the SDS-PAGE gel was loaded with 10 μg of WPI sample. Middle range unstained protein standard (catalog no. BM525, Sangon Biotech, Shanghai, China) was applied. The gel was stained with Coomassie Blue R-250, destained, and scanned.

2.4. Size exclusion chromatography (SEC)

Size exclusion chromatography (SEC) experiments were performed using the GE Healthcare ÄKTA Purifier 100 FPLC system equipped with a monitor UV-900 (Uppsala, Sweden) on a column of Sephacryl S-100 HR (1.6 × 70 cm). The column was equilibrated and eluted with 100 mM phosphate buffer (pH 7.8) at a flow rate of 1 mL/min. A 500 μL aliquot of WPI solution (5 mg/mL) was loaded on the column. The absorbance at 280 nm was used for detection of protein elution.

2.5. FT-IR spectroscopy

Infrared spectra were recorded at room temperature using a Thermo Scientific Nicolet 380 spectrometer (Madison, WI, USA) equipped with a deuterated triglycine sulfate detector. The WPI samples were mixed with KBr and then laminated. The resolution and scanning time were 4 cm^{-1} and 32 times, respectively.

2.6. Intrinsic fluorescence spectroscopy

The fluorescence spectra were recorded using a Shimadzu RF-5301PC spectrofluorometer (Tokyo, Japan) in a 1 cm path length quartz cell. The excitation wavelength was 285 nm. Both the excitation and emission slit widths were set at 5 nm. The concentration of sample solutions prepared in 50 mM phosphate buffer (pH 7.0) was adjusted to 0.3 mg/mL. The emission spectra were collected between 300 and 450 nm.

2.7. Determination of foaming properties

Foaming properties were determined by using the method described by Aewsiri, Benjakul, and Visessanguan (2009) with some modifications. WPI dispersions (5, 20 and 35 mg/mL) were prepared in graduated test tubes by dispersing WPI powder in 20 mL of 100 mM phosphate buffer (pH 6.8). Whipping treatment was conducted at 12,000 rpm for 2 min at 25 °C using the IKA T25 homogenizer (Staufen, Germany). The sample was allowed to stand for 15 min at 25 °C. Both foaming capacity (FC) and foaming stability (FS) were calculated from the following equations:

$$\mathrm{FC}\ (\%) = \frac{V_1}{V_0} \times 100$$

$$\mathrm{FS}\ (\%) = \frac{V_2}{V_1} \times 100$$

where, V_0 is the liquid volume before whipping, V_1 is the initial foam volume after whipping and V_2 is the final foam volume after leaving at 25 °C for 15 min.

2.8. Emulsion preparation and particle size determination

WPI dispersions (10, 20, and 40 mg/mL) were prepared in beakers by dispersing WPI powder in 150.0 mL of 100 mM phosphate buffer (pH 6.8) containing sodium azide (0.1 mg/mL). The emulsion was formed by transferring 50.0 mL of corn oil into the sample dispersion. The mixture was then pre-homogenized with the IKA T25 homogenizer (Staufen, Germany) at 12,000 rpm for 1 min at