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Structure and antioxidant activity of whey protein isolate conjugated with glucose via the Maillard reaction under dry-heating conditions

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ABSTRACT

The aim of this study was to investigate the structural characteristics and antioxidant activities of the Maillard reaction products obtained from whey protein isolate (WPI) and glucose (1:1 weight ratio) after dry-heating (60 °C and 79% relative humidity) for 0–7 d. The results revealed that the color (redness and yellowness), the UV–vis absorption, and the fluorescence intensity increased as the reaction time increased ($P < 0.05$). In addition, marked increases in the reducing power and the ABTS radical scavenging activities of the WPI–glucose conjugates were obtained with an increase in the reaction time ($P < 0.05$). The sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis illustrated that WPI and glucose formed high molecular weight conjugates. The increased T_{max} demonstrated that the thermal stability of WPI was remarkably improved by its conjugation with glucose. The Fourier transform infrared (FT-IR) spectra suggested that the amide I, II, and III bands of WPI were altered by the Maillard reaction. All in all, these results suggested that WPI–glucose conjugates have high thermal stability and improved antioxidant properties.

1. Introduction

The Maillard reaction is a very complex reaction between the carbonyls and the amines of proteins; this reaction occurs spontaneously in the thermal process or storage of foods (Jing & Kitts, 2002) and is considered an important method for protein modification (Mastrocola & Munari, 2000). Because this reaction produces a variety of early, intermediate, and advanced compounds, the Maillard reaction products (MRPs) are a particularly complex mixture of various compounds with different molecular weights, including volatile compounds, non-volatile intermediates, and dark-brown melanoidins (van Boekel, 1998). The MRPs that are produced by such reactions give rise to fewer safety problems than chemically modified food proteins (Pan & Melton, 2007). Thus, these compounds can be added to foods as functional ingredients to improve the emulsion, gelation, appearance, and texture of food products (Honda & Huroda, 1999). MRPs not only can modify important food properties during processing and storage (Maillard, Billaud, Chow, Ordonaud, & Nicolas, 2007), but also may be associated with the formation of compounds with strong antioxidant activity (Morales & Babbel, 2002; Yoshimura, Iijima, Watanabe, & Nakazawa, 1997), antimicrobial ability (Rao, Chawla, Chander, & Sharma, 2011), and antihypertensive properties (Rufián-Henares & Morales, 2007).

Whey protein which is produced in large quantities as a by-product of the cheese manufacturing industry and is mainly

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comprised of globular protein has been widely used as a nutritious and high-protein food ingredient due to its excellent functional properties and high essential-amino-acid content. Kika, Korlos, and Kiosseoglou (2007) noted that the dry-heating of a whey protein–saccharide mixture under controlled temperature and relative humidity conditions generates a covalent protein–polysaccharide conjugate that exhibits improved functionality compared with the initial biopolymer mixture. Mishra, Mann, and Joshi (2001) also reported that such a conjugate exhibits significant functional improvements may be obtained from the dry-heating of a whey protein concentrate in an admixture with pectin. Moreover, glucose is a reducing sugar and its price is low. Many studies have shown that MRPs prepared by protein and glucose could significantly improve the protein functionality and the antioxidant capacity (Liu & Zhong, 2013; Wang, Bao, & Chen, 2013). Our previous studies also showed that MRPs prepared by heating porcine plasma protein hydrolysate and glucose had better antioxidant activity (Liu, Li, Kong, Jia, & Li, 2013).

Despite numerous observations on the functional properties of whey protein–saccharide conjugates, there are few reports that discuss the structural changes in the glycated whey protein induced by heating during the Maillard reaction. Liu and Zhong (2013) observed the thermal aggregation properties of whey protein isolate (WPI) and saccharides conjugates by differential scanning calorimetry. They found that glycation lowered the isoelectric point and enhanced denaturation temperatures of WPI. Liu, Ru, and Ding (2012) also summarized the primary, secondary, and tertiary/quaternary structure–function relationship of proteins which are influenced by glycation. Therefore, the study of the structural changes of these conjugates will aid the further understanding of the physicochemical properties and antioxidant activities of the MRPs formed by whey protein and saccharides.

In the present study, a dry-heated Maillard reaction of a WPI and glucose mixture under controlled temperature and relative humidity conditions was performed. The purposes of this experiment were (1) to identify some of the structural changes in the glucose-modified WPI, such as the subunit bands, thermal properties, non-fluorescent intermediate products, and browning products, and (2) to evaluate the antioxidant activities of the resulting conjugates.

2. Materials and methods

2.1. Materials

Whey protein isolate (WPI, 95% protein) was purchased from Davisco Foods International, Inc. (MN, USA). The 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), sodium dodecyl sulphate (SDS) and glucose were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All of the other chemicals and reagents used were of analytical grade.

2.2. Preparation of the WPI–glucose conjugates

The coupling of the protein and glucose was performed according to a method developed by Fechner, Knoth, Scherze, and Muschiolik (2007). WPI and glucose (1:1, w/w) were suspended in distilled water and stirred at room temperature for 1 h to form a uniform dispersion, and then freeze-dried. The dried mixtures were then incubated at 60 °C in a desiccator in the presence of supersaturated KBr solution (79% relative humidity). Each mixture was sampled after 0, 1, 4, or 7 d. The pure protein (WPI), the freeze-dried WPI–glucose mixtures (without incubation), and the WPI–glucose conjugates were then further characterized. The control group (only WPI) was heated under the same experimental conditions.

2.3. Measurement of color

The color of the protein samples was determined using a ZE-6000 colourimeter (Nippon Denshoku, Kogyo Co., Tokyo, Japan). The results are displayed as an a^* value (redness) and a b^* value (yellowness). The instrument was set to the reflectance mode with the standard illuminant D65, which corresponds to natural daylight (Recamales, Sayago, González-Miret, & Hernanz, 2006). A white standard plate (L^* = 95.26, a^* = −0.89, b^* = 1.18) was used for calibration.

2.4. Electrophoresis

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed using a 5.0% stacking gel and a 12.5% running gel according to the method described by Laemmli (1970) with the following modification: a vertical gel electrophoresis unit was used (Mini-Protein II; Bio-Rad Laboratories, Richmond, CA, USA). The protein samples (1.0 mL at 4.0 mg of protein/mL) were dissolved in 1.0 mL of the sample buffer (4% SDS, 20% glycerol, 10% β-mercaptoethanol, and 0.125 M Tris–HCl, pH 6.8), heated to 100 °C for 3 min, and centrifuged at 1800 × g to remove any particulates. Aliquots of 20 μL of the supernatants were loaded into each well on the gel. A molecular weight (Mw) standard, which was composed of a cocktail of proteins (6500–200,000) (TaKaRa Biotechnology Co., Ltd. Dalian, China) was also run. After electrophoresis, one gel was stained for protein by Coomassie blue R-250 and the other one was stained for carbohydrate by the Glycoprotein staining kit (Sangon Biotechnology Co., Ltd. Shanghai, China).

2.5. Differential scanning calorimetry (DSC)

Aqueous dispersions (10%, w/v) of the protein samples were prepared in 10 mM phosphate buffer (pH 7.0). The experiments were performed on a PE Pyris 6-DSC thermal analyser (Perkin-Elmer Optoelectronics, Fremont, CA, USA), as described by Bertan, Tanada-Palmu, Siani, and Grosso (2005). Aliquots (15 μL) of the protein dispersions were accurately injected into aluminum liquid pans. The pans were hermetically sealed and heated in the calorimeter from a temperature of 40 °C to a final temperature of 110 °C, and the temperature was increased at a rate of 10 °C/min. The transition temperatures (T_{max}) and total transition enthalpy changes (ΔH) were recorded. All of the DSC scanning experiments were performed in triplicate.

2.6. Fourier transform infrared (FT-IR) measurement

The infrared analysis was performed using the FT-IR technique according to the method described by van der Venc et al.