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Characteristics and antioxidant activity of water-soluble Maillard reaction products from interactions in a whey protein isolate and sugars system

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ABSTRACT

The objective of this study was to determine antioxidant activities of water-soluble MRPs (Maillard reaction products) from the reactions between whey protein isolate (WPI) and xylose (X), glucose (G), fructose (F), lactose (L), maltose (M) and sucrose (S) at different initial pH values (3, 4, 5, 6, 7, 8 and 9). MRPs derived from the WPI-X system with increasing of pH rendered the highest browning, reducing power and DPPH radical-scavenging activity. SDS–PAGE analyses indicated formation of cross-linked proteins of large molecular mass produced from WPI-X systems. Results of FT-IR analysis indicated that the amide I, II and III bands of WPI from the WPI-X and WPI-G systems were changed by the Maillard reaction. CD spectroscopy showed that β-sheet, β-turns and random coil were increased while the α-helix was decreased after the WPI-G and WPI-X system aqueous solutions were heated. In conclusion, MRPs obtained from the WPI-X system had high antioxidant activity.

1. Introduction

Improvement of functional properties of food proteins, using physical, chemical and/or enzymatic means, has been studied. Recently, some attempts were made to improve the functional properties of proteins through protein–saccharide graft reactions without using chemicals, which are based on Maillard reactions between the amino groups of proteins and the reducing-end carbonyl groups of saccharides (Guan, Qiu, Liu, Hua, & Ma, 2006; Kim & Lee, 2009). It has been reported that protein–saccharide grafts are useful as a new functional biopolymer having excellent emulsifying, antioxidant and antimicrobial effects for food applications (Guan et al., 2006).

Maillard reaction refers to the interaction initiated between the terminal α- or ε-amino group of lysine residues in peptides or proteins and the carbonyl moiety of reducing sugars. Maillard reaction may produce coloured or colourless reaction products, depending on the stage of the reaction, as well as other factors, such as pH, type of reactants, temperature, water activity and concentration of reactants (Billaud, Brun-Mérimée, Louarme, & Nicolas, 2004; Chang, Chen, & Tan, 2011). The antioxidative effect of Maillard reaction products was reported in 1954 (Franzke & Iwainsky, 1954). The antioxidant activities of Maillard reaction products have been extensively studied. In some studies, Maillard reaction products with antioxidative activity were identified, such as amino reductones, heterocyclic compounds, or high molecular melanoidins, but most of the active antioxidants in Maillard mixtures or foods rich in Maillard reaction products are still unknown (Dittrich et al., 2009).

However, most of the information available, so far, on Maillard reactions is based on the results of model systems in which less attention was paid to proteins, such as the whey protein than to the free amino acids. Whey proteins are a group of proteins recovered from cheese manufacturing, with β-lactoglobulin and α-lactalbumin being the most abundant. Functionality of whey proteins as food ingredients has been extensively studied (Bryant & McClements, 1998). Many methods have been developed to modify whey protein, including conjugation with carbohydrates for improved interfacial properties (Dickinson & Galazka, 1991), cross-linking (via thermal aggregation or enzymatic reaction) and hydrolysis (Foegeding, Davis, Doucet, & McGuffey, 2002). To our knowledge, there is no information about the antioxidant activity of the whey protein isolate and reducing sugar heated alone under the same reactive conditions as their MRPs.

The goal of this study was to improve the antioxidant activity of whey proteins by glycation under wet reaction conditions. The influences of pH, type of sugar and the concentration of MRPs on antioxidant activity were studied. The degraded carbohydrate

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structures bound to the exposed sites of the reacting protein and the change of whey protein isolate molecules and the space were also studied. Another aim was to supply basic theory for the glycated whey protein isolate used as an antioxidant in formulated foods (as a functional ingredient) due to its radical-scavenging activity and capacity to delay oxidative deterioration and find a new use for whey protein isolate.

2. Materials and methods

2.1. Chemicals

Whey protein isolate (WPI) was purchased from Fonterra Ltd. (New Zealand) with a protein content of 92.4%. The 2,2-diphenyl-1-picryl-hydrazyl (DPPH·), Coomasie Brilliant Blue R-250, D-(+)-xylose, sodium dodecyl sulphate (SDS) and Tris were purchased from Sigma Chemical Co. (St. Louis, MO, USA). D-(+)-glucose (analytical grade), D-(+)-fructose (analytical grade), D-(+)-lactose (analytical grade), D-(+)-maltose (analytical grade) and D-(+)-sucrose (analytical grade) were purchased from Tianjin Chemical Reagent Co., Ltd. (Tianjin, China). The other solvents/chemicals used were of analytical grade and obtained from Tianjin Chemical Reagent Co., Ltd. (Tianjin, China).

2.2. Preparation of Maillard reaction products

Whey protein isolate and sugar (equivalent to 2:1 m/m ratio of protein to sugar) was dissolved in distiled water at a total concentration of 60 mg/ml. Seven systems were prepared, including whey protein isolate (WPI), whey protein isolate-xylose (WPI-X), whey protein isolate-glucose (WPI-G), whey protein isolate-fructose (WPI-F), whey protein isolate-lactose (WPI-L), whey protein isolate-maltose (WPI-M) and whey protein isolate-sucrose (WPI-S). Six sugars (X, G, F, L, M and S) were dissolved in distiled water at a total concentration of 20 mg/ml. Every system was adjusted to different pH (3, 4, 5, 6, 7, 8 and 9). The solutions were kept in 25 ml screw-cap tubes and heated in oven at 50 °C for 7 d.

2.3. Measurement of the browning

The absorbance of the heated solutions was measured, using a spectrophotometer (UNICO UV-2100, Shanghai, China) at 420 nm, as marker at the final stages of the reactions (Kim & Lee, 2008). Samples were diluted to 10 mg/ml with distiled water to obtain an absorbance value at 420 nm.

2.4. Determination of reducing power

The reducing power of MRP samples was determined according to the method of Chawla, Chander, and Sharma (2009) with some modifications. 0.5 ml of MRPs sample was mixed with 2.5 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide ($K_3Fe(CN)_6$). The reaction mixtures were incubated in a temperature-controlled water bath at 50 °C for 20 min, followed by addition of 2.5 ml of 10% trichloroacetic acid after cooling to room temperature. The mixtures were then centrifuged at 750g, using a centrifuge (TGL-16C, Anting, Shanghai, China) for 10 min at 25 °C. The supernatant obtained (2.5 ml) was treated with 2.5 ml of distiled water and 0.5 ml of 0.1% $FeCl_3$. The absorbance of the reaction mixture was measured at 700 nm with a UNICO UV-2100 spectrophotometer. Results were the averages of three measurements and expressed as absorbance units (AU).

2.5. Determination of DPPH radical-scavenging activity

DPPH radical-scavenging activity was determined according to the method of Gu et al. (2009) with some modifications. An aliquot of MRPs sample (1.0 ml) was added to 4.0 ml of 0.12 mM DPPH· in ethanol (95%). The solution was then mixed vigorously and allowed to stand at room temperature in the dark for 30 min. The mixture was centrifuged for 5 min at 750g if there was sediment. The absorbance of supernatant was measured at 517 nm, using a UNICO UV-2100 spectrophotometer. Results were the averages of three measurements and expressed as radical-scavenging activity (%). The percentage of DPPH radical-scavenging activity was calculated as follows:

$$\text{Radical}-\text{scavenging activity}\% = [1-(A517\,\text{nm}_{\text{sample}} - A517\,\text{nm}_{\text{control}})/A517\,\text{nm}_{\text{blank}}] \times 100\%$$

where A517 $\text{nm}_{\text{sample}}$ is the absorbance of sample, A517 $\text{nm}_{\text{control}}$ is the absorbance of the control and A517 nm_{blank} is the absorbance of the blank.

2.6. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE)

SDS–PAGE was performed according to the method of Chicón, Belloque, Alonso, and López-Fandiño (2008, 2009) with slight modification, using 5% (pH 6.8) stacking gel and 12% (pH 8.8) separating gel. Samples (WPI, WPI-X, WPI-G, WPI-F, WPI-L, WPI-M, WPI-S, native pH) were mixed with sample buffer containing 2% SDS and 5% β-ME (ratio 1:8, m/v).The mixtures were then heated at 90 °C for 5 min before loading. The samples were run at 120 V and 80 mA in the Mini-PROTEAN II Electrophoresis Cell for 1.5 h. Subsequently, the gel was stained with 0.1% Coomassie Brilliant Blue R-250 in 40% methanol and 10% acetic acid mixed solution and destained in a solution containing 40% methanol and 10% acetic acid. The gel was then photographed with a CANON IXUS 430 digital camera. The protein fractions were identified, using Sigma's Prestained Protein Maker.

2.7. FT-IR measurements

FT-IR spectra were determined according to the method of Śmiechowski and Stangret (2008) with some modifications. FT-IR spectra were recorded in ATR mode on a Nicolet 8700 FT-IR spectrometer, using the Turbo mode of the EverGlo infrared source. 128 scans were made with a selected resolution of 8 cm^{-1}. A single-reflection Specac Golden Gate ATR system was used, equipped with heated tungsten carbide disc, fitted with a 45 horizontal diamond crystal. The measurement chamber, isolated with KBr windows from the optical compartment of the spectrometer, was purged with dry nitrogen to ensure low water vapour and carbon dioxide residues in the spectra. The purge gas was also directed inside the ATR accessory. The temperature of the sample was kept at 28.0 ± 1.0 °C with the aid of an external Specac West 6100+ controller.

2.8. Circular dichroism (CD) spectroscopy measurements

The secondary structures of modified proteins were determined at 25 °C, using a circular dichroism spectropolarimeter (Jasco J-600, Jasco Corporation Japan) with a spectral resolution of 0.5 nm. The spectrum (190–250 nm) was recorded using a 10 mm path length quartz cell at a scan speed of 100 nm/min and sensitivity of 20 mdeg. The samples (WPI-X, WPI-G, WPI-S and WPI, native pH) were diluted to 0.2 mg/ml. CD spectra were corrected for solvent contributions and were expressed in terms of specific ellipticities