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Characteristics and antioxidant activity of hydrolyzed β-lactoglobulin–glucose Maillard reaction products

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ABSTRACT

Maillard reaction products (MRPs) were prepared by reacting hydrolyzed β-lactoglobulin (LH) with glucose (Glu) at 90 °C for up to 18 h of heating. Solutions of LH and glucose were also heated alone as controls. The chemical properties of MRPs (heated LH–glucose) and their antioxidant activity were investigated. Besides the Maillard reaction, the browning intensity and absorbance at 294 nm of heated LH–glucose was partially due to the caramelisation reaction of glucose. Fluorescence intensity of heated LH–glucose were mainly associated with the Maillard reaction. Heated LH–glucose increased DPPH radical scavenging activity, reducing power and iron chelating activity, which was partially related to the caramelization of glucose. Both the 2 h and 4 h heated LH–glucose showed the strongest antioxidative activity in fish oil-in-water emulsions, and longer heating times (>4 h) decreased their ability to inhibit lipid oxidation, but it was not related to the caramelization of glucose. Therefore, Mallaird reaction was a potential method to improve the antioxidant activity of hydrolyzed β-lactoglobulin.

1. Introduction

The Maillard reaction (MR) is involved in the formation of brown pigments via the condensation of a carbonyl group of reducing sugars, aldehydes or ketones with the amine group of amino acids (such as amino acids, peptides and proteins) or any nitrogenous compound (Kim & Lee, 2009). The complexity of the MR lies in the fact that it is influenced by numerous factors, such as temperature, pH, time, water activity, type and concentration of buffer, protein, amino acids, peptides or protein hydrolysates and sugar involved (Ajandouz, Desseaux, Tazi, & Puigserver, 2008; Ames, 1990; Lu, Hao, Payne, & Ho, 2005; Sumaya-Martinez, Thomas, Linard, Binet, & Guerard, 2005). Therefore, the changes of any factors influencing MR will each time lead to a new case study (Laroque et al., 2008).

The MR has been employed for improving functional properties of proteins (Jing, Melissa, Wong, & Kitts, 2009; Liu et al., 2010). In recent years, some research has shown that MR is a chemical method to improve the antioxidant activity of protein hydrolysate. MRPs derived from hydrolysates of mechanically deboned chicken residue and soy protein hydrolysate exhibited good antioxidant activity, such as reducing power, DPPH radical scavenging and Fe^{2+} chelating activity (Sun, Zhao, Cui, Zhao, & Yang, 2010). Guérard and Sumaya-Martinez (2003) reported that the antiradical scavenging effect was improved by 75% when casein peptone and cod viscera hydrolysate were heated in the presence of glucose.

In addition, the caramelization of sugar also contributes to the chemical properties and antioxidant activity of MRPs. The caramelisation reaction was found to account for 40–62% and 10–36% of UV absorbance and browning, respectively, when solutions of either glucose, bovine serum albumin (BSA), casein, glucose–BSA or glucose–casein were heated at 60 to 100 °C at pH 8.0 and pH 9.7 (Ajandouz et al., 2008). The ribose caramelization products from sugar–tuna stomach hydrolysate model system contributed to the antiradical activity and browning reactions at 95 °C and 115 °C (Sumaya-Martinez et al., 2005). Heated fructooligosaccharides (FOS) and soy protein isolate inhibited low density lipoprotein oxidation induced by copper mainly through the thermal degradation of FOS (caramelization), since the heating of FOS induced the formation of neocompounds able to delay *in vitro* LDL oxidation (Mesa, Silván, Olza, Gil, & del Castillo, 2008).

To our knowledge, there is little information about the antioxidant activity of both the proteins and reducing sugar heated alone under the same reactive conditions as their MRPs. Our previous studies have shown that β-lactoglobulin hydrolysates could effectively decrease oxidative rancidity in fish oil-in-water emulsions (Elias et al., 2006). The objectives of this research were to elucidate the contribution of glucose (caramelisation reactions) and hydrolyzed β-lactoglobulin (LH) to the antioxidant activity of their MRPs by analyzing radical scavenging activity, the ferric-reducing power, metal ion-chelating capacity and their

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effects on lipid oxidation in fish oil-in-water emulsions. In addition, the changes of chemical properties of LH, glucose and LH–glucose with heating time were also investigated.

2. Materials and methods

2.1. Materials

β-lactoglobulin was donated by Davisco Foods International, Inc. (Eden Prairie, MN). Food-grade deodorized, refined and bleached Menhaden oil without added antioxidants was supplied by Omega Protein (Reedville, VA) and contained 10–17% of EPA and 7–12% of DHA. Oil was stored in the dark at −80 C and thawed in cold tap water immediately before use. Rosemary extracts were provided by Kalsec Inc. (Kalamazoo, MI). Cumene hydroperoxide, Alcalase 2.4 L, propionaldehyde, Tween 20, ammonium thiocyanate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), mixed tocopherols, propyl gallate (PG), butylated hydroxy toluene (BHT), Trolox, 2,4,6-tris(2-pyridyl)-*s*-triazine 100 (TPTZ), 2,4,6-trinitro-benzenesulfonic acid (TNBS), D-glucose and 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4′,4″-disulfonic acid sodium salt (Ferrozine), were from Sigma-Aldrich Co. (St. Louis, MO). Distilled and deionized water was used in all studies. All other reagents were of analytical grade or better.

2.2. Preparation of hydrolyzed β-lactoglobulin

Hydrolyzed β-lactoglobulin (LH) was prepared by the hydrolysis of β-lactoglobulin using the proteolytic enzyme Alcalase. β-lactoglobulin (100 g) was dissolved in 2.0 L deionized water to give a starting protein concentration of 5% (w/v). The solution was adjusted to pH 8.0 using 3 M NaOH, and hydrolyzed (0.3/100 enzyme/substrate ratio) at 55 °C in a stirred bath. The pH of the mixture was maintained constant during hydrolysis using 3 M NaOH. After 3 h of hydrolysis, the solutions were heated at 90 °C for 10 min to inactivate the enzymes. The LH were frozen, lyophilized and kept at −18 °C before further analysis.

The protein concentration of LH was determined by the Lowry method (Lowry, Rosebrough, Farr, & Randall, 1951).

2.3. Preparation of MRPs from a LH–glucose model system

LH at a protein concentration of 50 mg mL^{-1} was dissolved in 50 mM phosphate buffer solution (pH 8.0) and glucose was added at the concentration of 22.6 mg mL^{-1} (equivalent to approximately 1:1 mole ratio of free amino group residues to sugar carbonyl groups). The solution was kept in 25 mL screw-cap tubes and heated in a water bath at 90 °C for up to 18 h. Control experiments with LH (50 mg mL^{-1}) heated without glucose, glucose (22.6 mg mL^{-1}) heated without LH, were also conducted.

2.4. Absorbance measurements

The absorbance of samples was measured using a Hitachi UV-2500 spectrophotometer at 294 nm and 420 nm, as markers of the intermediate and final stages of the reactions, respectively (Ajandouz, Tchiakpe, Ore, Benajiba, & Puigserver, 2001). Appropriate dilutions were prepared in order to obtain an absorbance value of less than 1.5.

Fluorescence intensity of samples was determined by the method adapted from Jing and Kitts (2004). Fluorescence spectra were recorded using F-2500 spectrofluorometer (Hitachi, Co. Japan) with a slit width of 5 nm at 347/421 nm (excitation/emission). The MRPs were diluted (1:100, v/v) with 10 mM phosphate buffer (pH 7.0) for fluorescence spectra analysis. A quinine sulfate solution of 1 μg mL^{-1} in double distilled water was prepared daily as an internal standard for relative fluorescence.

2.5. Free amino group determination

The free amino groups were determined using TNBS, as described by Fields (1971). One milliliter of sample (0.05 mg/mL in 0.1 M sodium borate containing 0.1 M NaOH) was reacted with 20 μl of TNBS (1 M in water) for 5 min. The reaction was stopped by adding two volumes of 1.5% sodium sulfite (1.5 mM in water) and 98.5% of dibasic sodium phosphate (0.1 M). The absorbance was measured at 420 nm. Absorbance readings were converted into free amino content (in mM glycine equivalent) using a calibration curve obtained with L-glycine (0–0.2 mM) as a standard. Data were finally expressed as relative concentrations (%) in comparison with the content of non-heated samples.

2.6. Size measurements of heated LH–glucose

The size of samples were measured by dynamic light scattering measurements (Zetasizer Nano-ZS, model ZEN3600, Malvern Instruments, Worchester, U.K.) and expressed as *z*-average mean diameter. Samples were diluted approximately 10-fold with the same buffer, mixed, and immediately transferred into plastic cuvettes for size determination.

2.7. Ferric reducing/antioxidant power (FRAP)

The reducing ability of samples was measured using the ferric reducing/antioxidant power (FRAP) assay (Benzie & Strain, 1996). In the assay, 3.0 mL of freshly prepared FRAP reagent (mixture of 2.5 mL of 10 mM TPTZ, 2.5 mL of 20 mM $FeCl_3 \cdot 6H_2O$, and 25 mL of 30 mM acetate, pH 3.6) was warmed to 37 °C and 0.1 mL of sample, along with 0.3 mL of H_2O was added. Absorbance (593 nm) of samples as well as blank solutions was measured for up to 10 min. Trolox stock solutions were used to perform the calibration curves. Results were expressed as μmol equivalents of Trolox per gram protein.

2.8. DPPH radical-scavenging activity

DPPH radical scavenging activity was measured according to the procedure reported by Morales and Jiménez-Pérez (2001) with a slight modification. Test samples in 4 mL of water were mixed with 1 mL of 99.5% ethanol containing 0.25 mM DPPH. This mixture was shaken and kept at room temperature for 30 min, and the absorbance of the mixture was measured at 517 nm. The results are calculated as the percentage inhibition according to the following formula:

$$\%\ \text{inhibition} = \left[\frac{C-(S-SB)}{C}\right] \times 100$$

where S, SB, and C are the absorbance of the sample, the blank sample, and the control, respectively.

2.9. Metal-chelating activity

The ability of samples to chelate ferrous ions was assessed using the method of Decker and Welch (1990). One milliliter of peptide solution (2.5 mg mL^{-1}) was first mixed with 3.7 mL of distilled water. Then it was reacted with a solution containing 0.1 mL 2 mM $FeCl_2$ and 0.2 mL of 5 mM Ferrozine. After 10 min, the absorbance of the reaction mixture was measured at 562 nm. Chelating activity was calculated as a percentage using {1 − (absorbance of the sample at 562 nm − absorbance of the blank sample at 562 nm) / (absorbance of control at 562 nm)} × 100.

2.10. Inhibiting against lipid oxidation in fish oil-in-water emulsions

2.10.1. Preparation of emulsion

Stock surfactant solution for emulsion preparation was prepared by dispersing surfactant (1.0 wt % Tween 20) in phosphate buffer (10 mM) adjusted to pH 7.0 with 1 M HCl. Stock sample solutions