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Antioxidant activity of Maillard reaction products from a porcine plasma protein–sugar model system

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Abstract

Maillard reaction products (MRPs) were prepared by heating 2% porcine plasma protein (PPP) and reducing sugars (glucose, fructose and galactose) at the levels of 1% or 2% at 100 °C up to 5 h without pH control. Browning and intermediate products, as monitored by absorbance at 420 nm and absorbance at 294 nm, increased as heating time increased ($P < 0.05$). However, fluorescence (Ex 347 and Em 415 nm) sharply increased within 1 h and subsequently decreased when heating time increased ($P < 0.05$). Increase in browning and formation of intermediate products was observed with a concomitant decrease in free amino groups. Among sugars and concentrations used, galactose at 2% rendered the highest browning and intermediate products. MRPs derived from galactose, especially at a level of 2% possessed greater reducing power and DPPH radical-scavenging activity than those prepared from fructose and glucose. MRPs derived from fructose or galactose at the level of 2% showed the increase in reducing power and DPPH radical-scavenging activity in a concentration-dependent manner. In general, antioxidative activity of PPP–sugar MRPs was coincidental with the browning development and the formation of intermediate products.

Keywords: Maillard reaction; Browning; Antioxidant; Radical scavenging; Reducing power; Porcine plasma protein

1. Introduction

Non-enzymatic interaction between a reducing sugar and an amino acid, peptide or protein has been known as the Maillard reaction. Glycosylation or glycation is an important reaction, which induces the covalent attachment of sugars to α- or ε-NH_2 groups of amino acids and protein to form glycated proteins (Friedman, 1996). The Maillard reaction produces a variety of intermediate products and finally brown pigments (melanoidins) are formed (Van Boekel, 1998). The Maillard reaction is influenced by many factors, including reactant concentration, temperature, time, initial pH and water activity (Baxter, 1995; Ashoor & Zent, 1984; Naranjo, Malec, & Vigo, 1998; Tanaka, Chiba, Ishizaki, Takai, & Taguchi, 1994; Wijewickreme & Kitts, 1997).

The Maillard reaction produced from an amino acid–sugar model system has been associated with the formation of compounds with strong antioxidant activity (Tanaka, Chui, Nagashima, & Taguchi, 1990; Yen & Hsieh, 1995; Yoshimura, Iijima, Watanabe, & Nakazawa, 1997). Antioxidant activity of MRPs derived from a protein–sugar system has been also studied (Jing & Kitts, 2002; Yeboah, Alli, & Yaylayan, 1999). However, Lingnert & Eriksson (1980) found a lower antioxidative activity of MRPs derived from protein–sugar model systems than amino acid–sugar model systems. Additionally, antioxidative activities of MRPs are affected by

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pH and temperature used (Alaiz, Hidalgo, & Zamora, 1999; Mastrocola & Munari, 2000). MRPs have been used to prevent lipid oxidation in many products. MRPs exhibit an antioxidative activity in meat products (Alfawaz, Smith, & Jeon, 1994; Bedinghaus & Ockerman, 1995). Furthermore, MRPs derived from a fructose–tryptophan system also prevent the oxidation of sardine lipid (Chiu, Tanaka, Nagashima, & Tagushi, 1991).

Porcine blood is an abundant by-product in the slaughtering process in Thailand with an estimated amount of 30,000 metric tons per year (Benjakul, Visessanguan, & Srivilai, 2001a). Blood plasma contains a variety of bioactive compounds, including a proteinase inhibitor and plasma transglutaminase (Benjakul et al., 2001a, Benjakul, Visessanguan, & Srivilai, 2001b). This could increase the breaking force and deformation of bigeye snapper surimi gels (Benjakul et al., 2001a). Apart from utilization as a surimi gel enhancer, porcine plasma can be used as the potential source of proteins or peptides for Maillard reactions in the presence of an appropriate sugar. However, no information regarding the MRPs prepared from porcine plasma protein on its antioxidative activity has been reported. The objectives of this investigation were to study the Maillard reaction products derived from porcine plasma protein and different reducing sugars and to determine the antioxidative activity of MRPs obtained under different conditions.

2. Materials and methods

2.1. Chemicals

2,4,6-Trinitrobenzenesulfonic acid (TNBS), L-leucine, 1,1-diphenyl-2-picryl-hydrazyl (DPPH) and potassium ferricyanide were purchased from Sigma–Aldrich (St. Louis, MO, USA). Trichloroacetic acid was obtained from Riedel-deHaen (Seelze, Germany). Ferric chloride, sodium sulfite, trisodium citrate and glucose were purchased from Merck (Damstadt, Germany). Fructose and galactose were obtained from Fluka (Messerchmittstr, Switzerland).

2.2. Preparation of porcine plasma protein

Porcine blood was collected from a slaughter house in Hat Yai, Thailand. The blood was mixed with 3.8% trisodium citrate at a ratio of 1:9 (v/v) to prevent blood coagulation and transported to the Department of Food Technology, Prince of Songkla University, Hat Yai. The blood was then centrifuged at 1000*g* for 30 min at 4 °C using a Sorvall Model RC-5B Plus centrifuge (Newtown, CT, USA) to remove red blood cells. The resultant supernatant was dialysed with 10 volumes of distilled water at 4 °C for three times. Dialysed plasma protein was freeze-dried and kept at 4 °C until used. The dry powder was referred to as porcine plasma protein (PPP).

2.3. Effect of sugars and heating time on the characteristics and antioxidative activity of Maillard reaction products (MRPs)

PPP (2 g) was mixed with different reducing sugars (glucose, fructose and galactose) at two different levels (1 and 2 g). Distilled water was added to dissolve the mixture and the volume was adjusted to 100 ml. The mixture was then transferred to screw-sealed tubes, tightly capped and heated in oil bath (Buchi labortechnik AG, Flawil, Switzerland) at 100 °C. The samples were taken after heating for 0, 1, 2, 3, 4, and 5 h. The heated samples were cooled immediately in iced water. MRPs samples obtained were kept at 4 °C until analysed.

2.4. Effect of MRP amount on the antioxidative activity

Sugars of the type and level exhibiting the highest antioxidative activity were used to prepare MRP. PPP–sugar mixtures were heated at 100 °C for 5 h. MRPs were then cooled in iced water. Different amounts of MRPs were determined for reducing power (0, 25, 50, 100, 150 and 200 μl) and DPPH radical-scavenging activity (0, 10, 20, 40, 60 and 80 μl).

2.5. Analyses

2.5.1. Measurement of UV-absorbance and browning

The UV-absorbance and browning of MRP samples were measured according to the method of Ajandouz, Tchiakpe, Ore, Benajiba, & Puigserver (2001). Appropriate dilution was made using distilled water and the absorbance was measured at 294 and 420 nm using a UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan) for determining UV-absorbance and browning intensity, respectively.

2.5.2. Measurement of fluorescence

Fluorescence of MRP samples with an appropriate dilution was determined as described by Morales & Jimenez-Perez (2001) with a slight modification. The fluorescence intensity was measured at an excitation wavelength of 347 nm and emission wavelength of 415 nm using a RF-1501 Fluorescence spectrophotometer (Shimadzu, Kyoto, Japan).

2.5.3. Determination of free amino group content

Free amino group content was determined according to the method of Benjakul & Morrissey (1997). MRP samples (5-fold dilution) (125 μl) were mixed with 2.0 ml of 0.2125 M phosphate buffer, pH 8.2 and 1.0 ml