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Investigation into the Maillard reaction between ε-polylysine and dextran in subcritical water and evaluation of the functional properties of the conjugates

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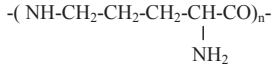
ABSTRACT

The effects of temperature and pressure on the Maillard reaction between ε-polylysine and dextran in subcritical water were investigated. Browning index was determined at 420 nm, and degree of graft was estimated by *O*-phthaldialdehyde (OPA) method. The formation of conjugates by Maillard reaction was testified by UV–vis spectrum and SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The emulsifying activity of the conjugates was greatly improved with the rise of temperature. The antimicrobial activity of the conjugates formed below 110 °C remained the same as ε-polylysine, and then decreased obviously when the reaction temperature was above 120 °C. The reaction pressure (0–10 MPa) had no effect on the emulsifying and antimicrobial activities, but higher pressure could effectively inhibit the formation of browning compounds.

1. Introduction

ε-Polylysine (ε-PL), a natural homopolymer of L-lysine with a structure as below,



was produced by *Strptomyces albulus*. (Shima & Sakal, 1977). The L-lysine residues are linked between ε-amino and α-carboxyl groups, and the degree of its polymerization (n) is usually 25–30. ε-PL is a cationic surface active agent, because its free ε-NH_2 has positive charges in solution, and has a wide antimicrobial spectrum against gram-positive, and gram-negative bacteria, yeast and molds (Geornaras & Sofos, 2005; Hiraki, 2000). At the same time, it is stable under acidic and alkaline environment and resists heat treatment. Additionally, the safety of ε-PL was evaluated by toxicity studies in rats (Hiraki, 1995a, 1995b; Hiraki et al., 2003; Koichi, Takayuki, Michiko, Masamine, & Masahito, 1999).

Because of some desirable advantages, ε-PL is commercially produced in Japan as a natural food preservative (Yoshida & Nagasawa, 2003) and has been applied in boiled rice, noodles and cooked vegetables. However, one practical problem with the application of ε-PL in food products is that it tends to interact with food components such as protein and acidic polysaccharides, possibly lowering the antimicrobial activity (Hiraki, 1995a, 1995b). Moreover, due to lack of hydrophobic groups, ε-PL has poor emulsifying properties. Therefore, ε-PL is mainly added in starch-based foods (Otsuka, Kuwahara, & Manabe, 1992).

Many attempts have been made to improve emulsifying properties of proteins by conjugating with reducing sugars through Maillard reaction (Wooster & Augustin, 2006). The covalently modified protein with sugar often exhibit better emulsifying ability and solubility (Kato, Minaki, & Kobayashi, 1993; Oliver, Kobayashi, & Stanley, 2006). Nevertheless, it takes several hours or even days to obtain desired Maillard reaction products under the traditional condition, which limits its practical application. Many investigations have been carried out to accelerate Maillard reaction coupled with an external physic field. So far, researchers have studied the effects of microwave (Tsubokura, Fukuzaki, Noma, Igura, & Shimoda, 2009), high hydrostatic pressure (Schwarzenbolz, Klostermeyer, & Henle, 2002), ultrasound (Guan et al., 2011; Mu et al. 2010), and supercritical carbon dioxide (Casal, Ramírez, Ibañez, Corzo, & Olano, 2006) on Maillard reaction.

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Subcritical water is a technique that maintains water in liquid state by exerting appropriate pressure while the temperature ranges from 100 to 374 °C during the whole procedure (Ramos, Kristenson, & Brinkman, 2002). As a new and environmentally clean extraction technique, the subcritical water technique has been widely used for the extraction of functional components from plants, such as polyphenol (Ju & Howard, 2005), essential oil (Jiménez-Carmona, Ubera, & Luque de Castro, 1999), protein (Sereewatthanawut et al., 2008), polysaccharide (Moreschi, Petenate, & Meireles, 2004). During the process of extraction, some components from raw material may interact to form new substance, which has different structures and properties from the target compound. For example, Maillard reaction may occur between proteins and carbohydrates in the raw material at high temperature. However, the information on the effect of subcritical water on Maillard reaction is scarcely reported. Only Merichel (Plaza, Amigo-Benavent, del Castillo, Ibáñez, & Herrero, 2010) reported the occurrence of Maillard reaction between amino acids and glucose during the extraction with subcritical water, but the further research could not be found.

Thus, in this study, the effects of different parameters in subcritical water on Maillard reaction between ε-polylysine and dextran were investigated. The emulsifying and antimicrobial activities of the conjugates formed under different conditions were evaluated.

2. Materials and methods

2.1. Chemicals

ε-Polylysine (average molecular weight: 4300) was purchased from Silver Elephant Bio-engineering Co., Ltd., (Zhejiang, China). Dextran (molecular weights: 35–45 kDa) and *O*-phthaldialdehyde (OPA) were purchased from Sigma Aldrich (St. Louis, MO, USA), Sodium dodecyl sulfonate (SDS) and sodium hydroxide from Sinopharm chemical regent (Shanghai, China). All the chemicals were of analytical grade.

2.2. Preparation of ε-PL-dextran conjugates

A mixture of ε-polylysine and dextran was prepared in a ratio of 1:15 (w/w) and then dissolved in pH 8.5 phosphate buffer, at a concentration of ε-polylysine 0.2% (w/w). After complete dissolution, the pH was adjusted back to 8.5, using 1 M HCl or NaOH, if necessary.

Experiments were performed in the subcritical water equipment (CWYF, Huaan, China). Reaction temperature and pressure were designed as variable parameters to investigate the effect of reaction parameters in subcritical water on the Maillard reaction. For the impact of the temperature, the Mailard reactions were conducted under 5 MPa for 60 min at 90, 100, 110, 120 and 130 °C. In order to evaluate the influence of the pressure, the Mailard reactions were performed at 110 °C for 60 min under 0, 2.5, 5, 7.5 and 10 MPa. At the end of each reaction process, the final solution was cooled down in ice water bath to terminate the reaction. And then the solutions were freeze-dried and stored at 4 °C until analysis. The freeze-dried powder was termed as the conjugates of ε-polylysine and dextran.

2.3. Analysis for the degree of graft

Degree of graft (DG) was indirectly determined from analysis of free amino groups, using OPA method according to the literature (Vigo, Malec, Gomez, & Liosa, 1992) with slight modifications. The OPA reagent was prepared freshly before use by mixing the following reagents: 40 mg of OPA dissolved in 1 mL of methanol, 25 mL of 0.1 mol sodium borate buffer (pH 9.85), 100 μL β-mercaptoethanol, 2.5 mL of 20% (w/v) SDS in water. The mixture was brought to 50 mL with deionized water. A 4 mL of OPA reagent and 200 μL of sample solution were mixed uniformly in tubes, and then reacted in 35 °C water bath for 2 min. The absorbance was measured immediately at 340 nm using a Shimadzu UV-1800 spectrophotometer. A calibration curve was obtained by using 0.5–3 mmol/L lysine as a standard.

Degree of graft (DG) was calculated from the loss in free amino groups compared to the mixture of ε-polylysine and dextran as follows:

$$DG = (C_0 - C_t)/C_0 \times 100\%$$

C_0: content of free amino groups of the mixture of ε-polylysine and dextran,
C_t: content of free amino groups of the conjugates.

2.4. Absorbance measurements of the solution

The absorbance of the conjugate solutions at 420 nm was measured as an index of browning using a Shimadzu UV-1800 spectrophotometer. Appropriate dilutions were made in order to make absorbance value under suitable range, if necessary.

2.5. Wavelength spectrum of the solution

Wavelength spectrums of the solutions were scanned by a UV–vis spectrophotometer with the wavelength ranging from 200 nm to 400 nm.

2.6. SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was carried out according to the method of Laemmli (1970), using 12% acrylamide separating gel and 5% acrylamide stacking gel containing 0.1% SDS. A 20 μL samples (protein concentration was 2 mg/mL) were prepared with equal volume of double sample buffer. Electrophoresis was performed at a current of 160 mA for 90 min in tris–glycine electrophoretic butter containing 0.1% SDS. After electrophoresis, gel sheets were stained with Coomassie brilliant blue R-250. The stained gel sheets were destained with 7.5% acetic acid containing 5% methanol.

2.7. Emulsifying activity

The emulsifying activity of conjugates was determined according to the method of Pearce and Kinsella (1978). To prepare the emulsions, the freeze-dried conjugate powder was dissolved in water at a concentration of 5% (w/v), and pH was adjusted to 7.0 with 1 M HCl, if necessary. After complete dissolution, oil-in-water emulsions, which were prepared by adding 25% (w/w) soy oil into the conjugate solution at room temperature, were shaken and stirred by high-speed blender at a speed of 10 000 rpm/min for 3 min. A 100 μL was taken from the bottom of the emulsion and diluted with 10 mL of 0.1% SDS solution. The emulsifying activity was determined on the basis of the absorbance at 500 nm measured immediately with a Shimadzu UV-1800 spectrophotometer after the formation of emulsion.

2.8. Antimicrobial activity

The minimum inhibitory concentrations (MIC, μg/mL) of conjugates against six strains of spoilage and poisoning microorganisms were determined by the broth dilution method referring to the National Committee for Clinical Laboratory Standards (NCCLS,