



Optimization of Maillard reaction with ribose for enhancing anti-allergy effect of fish protein hydrolysates using response surface methodology



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ABSTRACT

Halibut is served on sushi and as sliced raw fish fillets. We investigated the optimal conditions of the Maillard reaction (MR) with ribose using response surface methodology to reduce the allergenicity of its protein. A 3-factored and 5-leveled central composite design was used, where the independent variables were substrate (ribose) concentration (X_1 , %), reaction time (X_2 , min), and pH (X_3), while the dependent variables were browning index (Y_1 , absorbance at 420 nm), DPPH[•] scavenging (Y_2 , EC₅₀ mg/mL), FRAP (Y_3 , mM FeSO₄/mg extract) and β -hexosaminidase release (Y_4 , %). The optimal conditions were obtained as follows: X_1 , 28.36%; X_2 , 38.09 min; X_3 , 8.26. Maillard reaction products of fish protein hydrolysate (MFPH) reduced the amount of nitric oxide synthesis compared to the untreated FPH, and had a significant anti-allergy effect on β -hexosaminidase and histamine release, compared with that of the FPH control. We concluded that MFPH, which had better antioxidant and anti-allergy activities than untreated FPH, can be used as an improved dietary source.

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1. Introduction

Halibut (*Hippoglossus hippoglossus*) is a popular food material for use in sushi and as sliced raw fish fillets in many countries. Although fish provides proteins with high nutritional values (Pascual, Esteban, & Crespo, 1992), fish protein is known as a food allergen causing an immunoglobulin E (IgE)-mediated allergy response, called type I food hypersensitivity (Akiyama, Imai, & Ebisawa, 2011). In addition, many studies have reported that IgE-mediated food allergy was associated with the sensitivity to fish protein in children (Dannaeus & Inganäs, 1981; Saarinen & Kajosaari, 1980; Sampson, 2001). Type I hypersensitivity is known

for the specific crosslinking of antigens to IgE molecules, facilitating the binding to IgE receptors on the surface of mast cells (Cruse & Lewis, 2010). The concentration of IgE is closely associated with allergic diseases such as asthma and allergic rhinitis (Karli, Balbaloglu, Uzun, Çinar, & Uğur, 2012). The reactions in the IgE-stimulated signaling pathway lead to the synthesis or release of cytokines related to allergy and antigen-mediated degranulation (Abbas, Lichtman, & Pillai, 1994; Ohguchi et al., 2010).

The Maillard reaction (MR), which was discovered by Louis-Camille Maillard, is a non-enzymatic browning reaction with amino acid and sugars which occurs during the roasting, cooking and baking of foods. The initial products are called Schiff bases. They form Amadori products via rearrangement, which undergo further reactions to form irreversible advanced glycation end products (AGEs) (Ahmed & Thornalley, 2003). The AGEs from Maillard reaction products (MRPs) are known to be involved in diabetes, atherosclerosis and inflammation (Helliwig & Henle, 2014; Ikeda

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et al., 1996; Reddy & Beyaz, 2006). However, recent studies demonstrated that some MRPs have functional activities, such as anti-oxidative or anti-inflammatory activities (Kitts, Chen, & Jing, 2012; Yilmaz & Toledo, 2005). Many factors such as the sugar, substrate concentration, time, pH and temperature can influence the yields and properties of resulting MRPs. Several studies have reported the optimum conditions of MR which lead to antioxidant activity (Echavarria, Pagan, & Ibarz, 2014); however, little study has been performed on the interaction of factors involved in MR.

Response surface methodology (RSM) was introduced as a convenient method for the optimization of complex processes by reducing the number of experimental trials needed to evaluate various parameters and interactions (Box & Wilson, 1951). Due to the development and improvement in the statistical and mathematical techniques, RSM can be used to evaluate more experimental conditions for the optimization of multiple factors and their interaction with respect to response variables (Zhu, Gao, Li, Zhao, & Deng, 2010).

In this study, RSM was employed to obtain optimum conditions for MR with fish protein hydrolysate (FPH) and ribose, measuring the index of the Maillard reaction and bioactivities. In addition, the anti-allergy effects of MRPs of FPH (MFPH) were confirmed by examining the cell degranulation and secretion of histamine *in vitro*.

2. Materials and methods

2.1. Materials

Dulbecco's Modified Eagle Medium (DMEM), Eagle's Minimum Essential Medium (MEM) cell culture media, penicillin, streptomycin, trypsin–EDTA and fetal bovine serum (FBS) were purchased from GIBCO (Grand Island, NY, USA). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), dimethyl sulfoxide (DMSO), 2,4,6-tripyridyl-S-triazine (TPTZ), 2,2-diphenyl-1-picrylhydrazyl (DPPH[•]), N-(1-naphthyl)ethylene diamine dihydrochloride (NEAD), sodium nitrite, lipopolysaccharide (LPS), 4-nitrophenyl N-acetyl- β -D-glucosaminide, anti-dinitrophenol-IgE (anti-DNP-IgE) and DNP-BSA were purchased from Sigma–Aldrich (St. Louis, MO, USA). A bicinchoninic acid (BCA) protein assay kit was purchased from Thermo Scientific (Rockford, IL, USA).

2.2. Cell culture

Rat basophilic leukemia RBL-2H3 cells were obtained from the Korean Cell Line Bank (KCLB, Seoul, South Korea), and murine macrophage RAW 264.7 cells were purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA). RBL-2H3 cells were cultured in MEM medium containing 10% (v/v) FBS, 2.2 g/L sodium bicarbonate, 100 U/mL penicillin and 100 U/mL streptomycin, while RAW 264.7 cells were cultured in high glucose DMEM with 10% (v/v) FBS, 100 U/mL penicillin and 100 U/mL streptomycin. Cells were maintained at 37 °C in a humidified atmosphere of 5% CO₂. RBL-2H3 and RAW 264.7 were split into suitable ratios every 3 d, and were routinely grown in medium containing 10% FBS; however, the medium was changed to serum-free medium 20 h before assay performance.

2.3. β -Hexosaminidase release and histamine release assay

RBL-2H3 cells in 96-well plates were stimulated with 0.5 μ g/mL mouse anti-DNP-IgE at 37 °C for 24 h. The cells were then washed twice with modified Tyrode's buffer (25 mM Hepes, 116.9 NaCl, 5.4 mM KCl, 0.8 mM MgSO₄, 5.6 mM Glucose, 2 mM CaCl₂ and 1 mg/mL BSA), after which they were incubated with 45 μ L of Tyrode's buffer containing 1000 μ g/mL of the samples at 37 °C for 10 min. After being washed twice more, the cells were stimulated

for 30 min with 10 μ g/mL of DNP-BSA diluted in modified Tyrode's buffer. The culture supernatants were used for the β -hexosaminidase release and histamine release assays.

To determine the β -hexosaminidase release of the mast cells, 20 μ L of supernatant was mixed with 80 μ L of substrate solution (5 mM 4-nitrophenyl-N-acetyl- β -D-glucosaminide in 0.5 M citrate buffer, pH 4.5), and the mixture was incubated at 37 °C for 30 min. The reaction was terminated by the addition of 100 μ L of stop solution (0.1 M sodium carbohydrate buffer, pH 10). The absorbance was then measured at 405 nm using a multiple plate reader.

Histamine release was measured by a spectrofluorometric assay using supernatant from the RBL-2H3 cell culture. For the reaction, 40 μ L of 0.5 M NaOH and 20 μ L of 2.5 mg/mL *o*-phthalaldehyde were added to 100 μ L of the supernatant, and incubated at 37 °C for 30 min. The reaction was terminated by the addition of 10 μ L of 3 M HCl. Fluorescence intensity was then measured at an excitation of 365 nm and an emission of 465 nm. Both β -hexosaminidase release and histamine release levels were calculated as the percentage compared to the control.

2.4. Antioxidant activity and browning index measurements

The FRAP value was measured using the previously reported method with a few modifications (Wang, Zhang, & Yang, 2005). FRAP reagent was produced by mixing 2.5 mL of 10 mM TPTZ solution in 40 mM HCl, 2.5 mL of 120 mM FeCl₃·6H₂O and 25 mL of 0.3 M acetate buffer (pH 3.6). Next, 100 μ L of extract were added to 3 mL of the working FRAP reagent, after which the samples were incubated for 5 min. The absorbance was then recorded at 593 nm.

DPPH[•] was dissolved in ethanol to a final concentration of 700 μ M, after which 100 μ L of the samples were mixed with 100 μ L of DPPH[•] reagent. After incubation in the dark for 30 min, the absorbance was measured at 515 nm using a multiple plate reader.

The characteristic yellow and brown pigments of the MRPs were measured using a spectrophotometer. The samples were diluted to an optical density between 0.2 and 0.8 then measured at 420 nm on a UV–visible spectrophotometer (DU650 spectrophotometer, Beckman, USA).

2.5. Nitric oxide (NO) synthesis assay

The concentration of NO produced by RAW 264.7 cells was measured with a slightly modified Griess assay (Schulz, Kerber, & Kelm, 1999). RAW 264.7 cells were plated in 96 well plates at a density of 5×10^5 cells/mL for 18 h, and then starved in serum-free DMEM for 6 h. The cells were then incubated with LPS or sample for 24 h, after which 50 μ L of the supernatant was mixed with 100 μ L of Griess reagent (1:1 mixture of 1% sulfanilamide in 5% phosphoric acid and 0.1% NEAD in D.W.) and incubated for 10 min at room temperature. The optical density was then measured by a multiplate reader at 540 nm. The concentration of NO production was quantified with comparison to a standard curve generated with sodium nitrite in the range of 0–100 μ M.

2.6. Preparation of FPH

FPH was kindly provided by Dr. Kim from the Korea Food Research Institute (KFRI, Seongnam, Korea). Halibut, excluding internal organs, was homogenized and then hydrolyzed using Protamax protease (Novo Korea, Seoul, Korea). The mass ratio of protein substrate to protease was 50:1 at 56 °C (pH 6.8). The FPH was fractionated by ultracentrifuge membranes (Millipore Co., Billerica, MA, USA) with a molecular weight cut-off of 3 kDa.

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