



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 (Hellwig & 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-triphenyl-1,3,5-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.

Table 1

Experimental range and values of the independent variables in the central composite design.

Independent variables	Symbol	Levels				
		−1.682	−1	0	1	1.682
Substrate concentration (%)	X_1	1.182	8	18	28	34.818
Reaction time (min)	X_2	1.182	30	60	90	110.46
pH	X_3	3.636	5	7	9	10.364

2.7. Experimental design

After mixing the mixture of ribose and FPH in glass test tube, its pH was adjusted. The reaction mixture was incubated at 121 °C for different times, then rapidly cooled, lyophilized, and stored at −70 °C until further use. A three-factored (X_1 , X_2 and X_3) and five-leveled (−2, −1, 0, 1 and 2) central composite design (CCD) was used to achieve the optimal condition for MR (Table 1). 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 420 nm), DPPH· scavenging (Y_2 , IC₅₀, mg/mL), FRAP (Y_3 , mM FeSO₄/mg extract) and β-hexosaminidase release (Y_4 , %).

Replicates were performed for each experiment, and the average values were recorded as the response. Each experimental Y value was fitted to the following quadratic equation:

$$Y = A_0 + \sum_{i=1}^3 A_i X_i + \sum_{i=1}^3 A_{ii} X_i^2 + \sum_{i=1}^2 \sum_{j=i+1}^3 A_{ij} X_i X_j$$

Where Y is the dependent variable, A_0 is a constant, A_i , A_{ii} and A_{ij} are the coefficients estimated by the model, and X_i and X_j are the coded values of the independent variables.

To perform the experimental and calculate the predicted data, Minitab version 16.1 (Minitab Inc., State College, PA, USA) software was implemented for the analysis of statistical data. The statistical significance of the experimental design was examined by analysis of variance (ANOVA).

2.8. Statistical analysis

Statistical analyses were conducted using SAS version 9.4.0 (SAS institute Inc. Cary, NC, USA). Differences among the groups were evaluated by one-way analysis of variance and Tukey's studentized range tests. Values were expressed as the mean ± standard deviation (S.D.).

3. Results

3.1. Optimization of the MR condition

3.1.1. Statistical analysis and the model fitting

RSM was applied to monitor the parameters of antioxidant activity and allergen reducing effect, and to determine the optimum conditions of MR with FPH using the data obtained in the central composite experiment. A total of 17 runs were performed to optimize the three independent variables. The experimental data are shown in detailed in Table 2, and were analyzed by multiple regression analysis using the Minitab software. Second-order polynomial equations and coefficient of determination (R^2) were also obtained (Table 3). As seen in Table 3, the R^2 values of the dependent variables were 0.8735 (Y_1), 0.8607 (Y_2), 0.7973 (Y_3) and 0.8958 (Y_4). The F values of the dependent variables were 5.37, 4.81, 3.06 and 6.69, respectively. This statistical data indicates the significance of the model for MR. ANOVA was used to analyze the significance of the experimental model, suitability, and to provide a statistical summary (data not shown). In the case of browning index (Y_1), the linear variables X_1 and X_3 showed statistically significant influences ($p < 0.05$); however, X_2 had no statistically significant influence ($p > 0.1$). The independent variables, X_1 and X_3 , along with the quadratic variable, X_3^2 also significantly influenced DPPH· radical scavenging; however, only X_3 had a significant influence ($p < 0.05$) on FRAP, while X_1 significantly influenced ($p < 0.001$) β-hexosaminidase release activity. By observing the linear and quadratic

Table 2

Factors and levels in the response surface central composite design arrangement and experimental results.

No.	Coded levels of variable			Browning Index	DPPH·EC ₅₀ ^a	FRAP ^b	β-Hexosaminidase release
	X_1 (%)	X_2 (min)	X_3 (pH)				
1	4	30	5	103.38	1.08	1.89	126.29
2	12	30	5	170.60	0.98	1.97	85.37
3	4	90	5	71.87	1.31	1.80	126.55
4	12	90	5	160.57	1.23	1.34	84.22
5	4	30	9	86.98	0.8	2.32	114.51
6	12	30	9	342.13	0.65	2.5	88.40
7	4	90	9	124.57	0.72	2.23	158.90
8	12	90	9	402.85	0.69	2.70	105.91
9	1.272	60	7	3.74	1.68	1.22	157.13
10	14.728	60	7	612.73	0.55	3.04	94.88
11	8	9.54	7	219.56	0.48	2.71	107.20
12	8	110.46	7	259.02	0.64	3.09	100.47
13	8	60	3.636	108.00	1.98	0.99	128.82
14	8	60	10.364	268.78	0.83	2.96	123.02
15	8	60	7	246.52	0.65	2.74	116.44
16	8	60	7	250.42	0.74	2.79	110.28
17	8	60	7	246.32	0.67	2.67	114.88

^a Amount of sample necessary to decrease the initial 2,2-diphenyl-1-picrylhydrazyl radical (DPPH·) concentration by 50%.

^b Ferric-reducing antioxidant power.

Table 3

Second order polynomial equations and coefficients of determinations of the response variables (X_1 , substrate concentration; X_2 , reaction time; X_3 , pH).

Responses	Second order polynomial equations	R^2
Browning index (Y_1)	$Y_1 = 51.767 + 125.473X_1 + 9.018X_2 + 52.758X_3 + 7.556X_1^2 - 16.821X_2^2 - 34.815X_3^2 + 5.574X_1X_2 + 47.189X_1X_3 + 17.480X_2X_3$	0.8735
DPPH· (Y_2)	$Y_2 = 0.694 - 0.166X_1 + 0.051X_2 - 0.269X_3 + 0.126X_1^2 - 0.070X_2^2 + 0.229X_3^2 + 0.017X_1X_2 - 0.000X_1X_3 - 0.064X_2X_3$	0.8607
FRAP (Y_3)	$Y_3 = 22.749 + 0.245X_1 + 0.001X_2 + 0.445X_3 - 0.261X_1^2 + 0.010X_2^2 - 0.316X_3^2 - 0.034X_1X_2 + 0.132X_1X_3 + 0.102X_2X_3$	0.7973
β-Hexosaminidase release (Y_4)	$Y_4 = 114.359 - 19.554X_1 + 3.64X_2 + 2.601X_3 + 2.604X_1^2 - 5.235X_2^2 + 2.572X_3^2 - 3.535X_1X_2 + 0.518X_1X_3 + 7.849X_2X_3$	0.8958

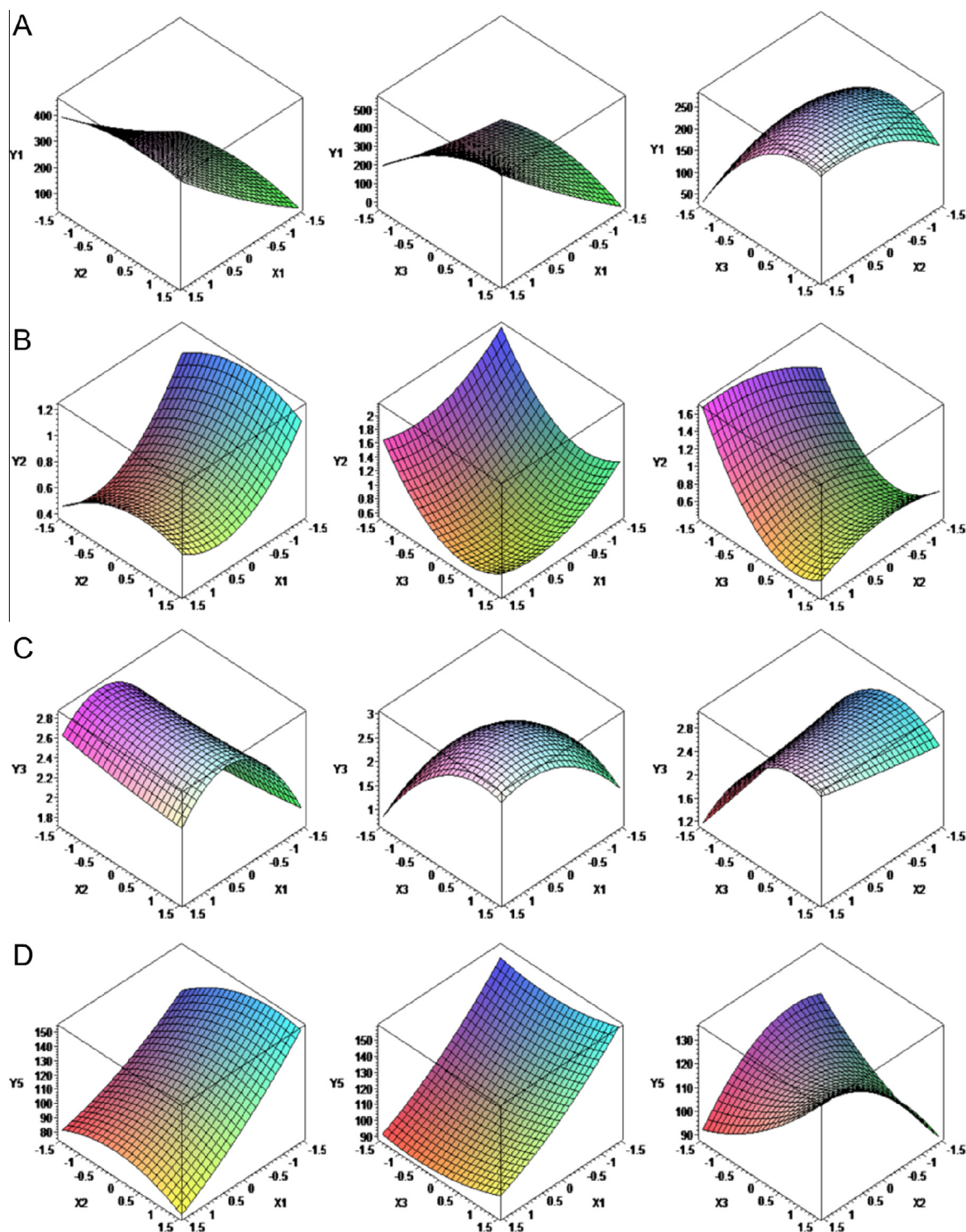


Fig. 1. Response surface plots for the effect of independent variables (X_1 , substrate (ribose) concentration; X_2 , reaction time; X_3 , pH) on response variables. (A) Browning index (Y_1); (B) DPPH radical scavenging (Y_2); (C) FRAP (Y_3); (D) β -hexosaminidase release (Y_4).

coefficients, it was concluded that the antioxidant activity and anti-allergy effect of MRPs were more influenced by the substrate (ribose) concentration and pH than by the reaction time.

3.1.2. Response surface analysis

Three-dimensional response surface plots were made with the dependent variables of browning index (Y_1), DPPH $^{\cdot}$ (Y_2), FRAP (Y_3) and β -hexosaminidase release (Y_4), related to two independent

Table 4

Predicted and experimental values of response variables.

Response variables	Predicted values	Experimental values
Y_1 (Browning index, absorbance 420 nm)	412.24	369.43
Y_2 (DPPH $^{\cdot}$, IC ₅₀ , mg/mL)	0.52	0.57
Y_3 (FPAP, mM FeSO ₄ /mg DM)	2.95	3.92
Y_4 (β -hexosaminidase release, %)	93.50	114.99

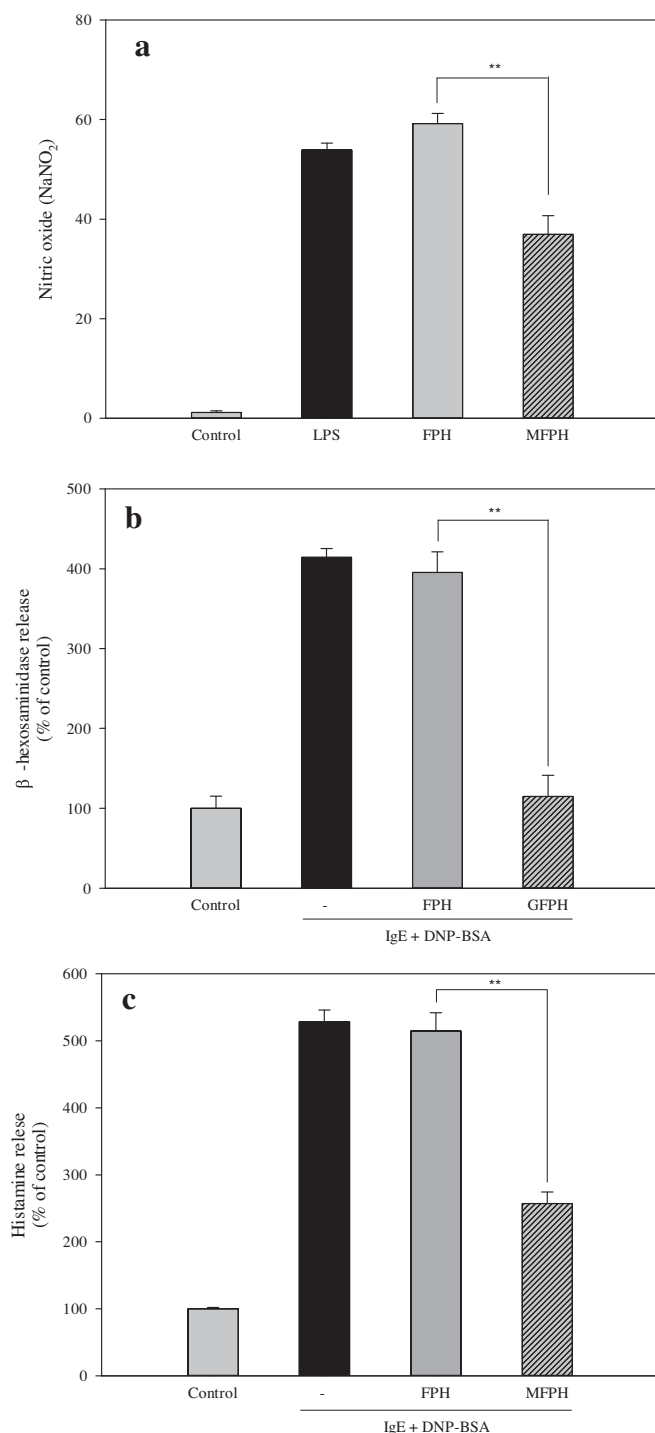


Fig. 2. Effects of fish protein hydrolysate (FPH) and Maillard reaction products of fish protein hydrolysate (MFPH) on nitric oxide synthesis by RAW 264.7 cells, and on β-hexosaminidase and histamine release in IgE-antigen complex-stimulated RBL-2H3 cells. (a) Macrophage RAW 264.7 cells (2×10^5 cell/well in 96-well plate) were cultured for 24 h with LPS (1.0 μg/mL), FPH or MFPH (1000 μg/mL). Values are expressed as the mean $\pm$ SD ($n = 5$). Significant differences (* $p < 0.05$, ** $p < 0.01$) were determined by Tukey's studentized range tests. (b and c) The IgE-stimulated cells were pretreated with the samples for 10 min, followed by treatment with DNP-BSA (10 μg/mL) for 30 min. The optical density was measured by a microplate reader at 405 nm for β-hexosaminidase release, and fluorescence intensity was measured at an excitation of 365 nm and an emission of 465 nm for histamine release.

variables among substrate (ribose) concentration (X_1), reaction time (X_2) and pH (X_3). As seen in Fig. 1A, increase of the substrate concentration drastically escalated the browning index, which was also

increased by increase of reaction time and enhancement of the pH. The reaction time had no influence on the antioxidant activity variables, DPPH $\cdot$ and FRAP (Fig. 1B and C); however, high substrate concentration and pH remarkably increased the browning index (Fig. 1A). For β-hexosaminidase release, indicating anti-allergy effect, the reaction time and pH enhanced the anti-allergenicity (Fig. 1D).

3.1.3. Optimization and verification of the model

The optimal conditions for MR of FPH suggested by the model to enhance the antioxidant activity and anti-allergy effect were as follows: substrate concentration, 28.36%; reaction time, 38.09 min; and pH, 8.26. These reaction conditions determined by the RSM model predicted maximum response of the dependent variables (Table 4). As seen in Table 4, the experimental values were slightly different from the predicted data; however, they were not significantly different. The results of analysis clearly indicated RSM to be adequate for the optimization of MR.

3.2. Effects of FPH and MFPH on nitric oxide synthesis and anti-allergy effect

NO synthesis assay was determined by analyzing the amount of MFPH produced by the optimal conditions for MR. Raw 264.7 cells were treated with LPS or samples for 24 h. In the presence of LPS (0–10 μg/mL) or samples (0–1000 μg/mL), the cell viabilities were greater than 95% (data not shown). Therefore, neither LPS nor the samples exhibited cytotoxicity during 24 h to RAW 264.7 cells. The amount of NO synthesis induced by 1 μg/mL of LPS and 1000 μg/mL of FPH and MFPH was analyzed. The NO production induced by FPH was similar to that of LPS; however, the treatment with MFPH showed significantly reduced NO production by about 30% compared with FPH treatment ($p < 0.01$) (Fig. 2a), suggesting that MR of FPH contributes to reduction in the NO synthesis.

To confirm the reduction in allergenic effect induced by MFPH, the amounts of histamine and β-hexosaminidase release were measured in RBL-2H3 cells. The group treated with Anti-IgE plus DNP and BSA had significantly increased secretion of histamine (Fig. 2b) and β-hexosaminidase (Fig. 2c) compared with the untreated control group. Treatment with anti-IgE plus DNP and BSA in the presence of FPH resulted in no changes to the release of histamine and β-hexosaminidase compared to the cells treated with only Anti-IgE plus DNP and BSA; however, the release of histamine and β-hexosaminidase induced by anti-IgE plus DNP and BSA was significantly inhibited by MFPH treatment ($p < 0.01$), to 51.4% and 38.4% of the levels, respectively, compared to FPH treatment.

4. Discussion

Type I hypersensitivity is a reaction mediated by IgE antibodies on mast cells or basophils, targeted against specific allergens (Cruse & Lewis, 2010). Mast cells release allergenic mediators such as β-hexosaminidase, histamine and cytokines during the hypersensitive immune reaction (Barnes, 2008). Rat basophilic RBL-2H3 cells are routinely used to measure cell degranulation and cytokine secretion related to allergenic reactions (Ahn et al., 2014; Chung, Sohng, Choi, & Park, 2013). Fish proteins have the potential to act as a food allergen, while also being popular food sources and food ingredients (Pascual et al., 1992). Several studies demonstrated the immediate intake of fish leads to allergic reactions such as asthma and diarrhea (Demartino et al., 1993; Oneil, Helbling, & Lehrer, 1993). In this study, we hypothesized that performing MR on FPH may modify the functional properties of the original FPH to increase the antioxidant activity and confer an anti-allergy effect. When many interacting factors affect

experimental systems, RSM is a useful experimental method (Box & Wilson, 1951). We first prepared MFPH with optimum conditions to confer the above properties by employing RSM. As seen in Table 2, the experimental system was designed to consist of three independent variables, including substrate concentration, reaction time and pH, along with four dependent variables, including browning index, DPPH, FRAP and β -hexosaminidase release. A previous study reported that RSM was acceptable when the R^2 values were larger than 0.75 (Le Man, Behera, & Park, 2010). Therefore, the R^2 values of the dependent variables in this study were suitable. As can be seen in Table 4, the predicted values calculated from the RSM model were highly matched with those of the experiments, suggesting that the RSM model is adequate for optimizing the conditions of MR. In addition, MR using FPH was also confirmed by measuring the fluorescence intensity, an MRP, furosine and SDS-PAGE (data not shown).

It is well known that inflammation can contribute to allergic disorders (Cruse & Lewis, 2010). Cell damage by NO plays a role in allergic inflammation (Lamas, 2010), which tends to develop a sustained IgE response to common environmental antigens or food ingredients (Guzik, Korbust, & Adamek-Guzik, 2003). Th2-like TCD4+ cells are the major regulator of IgE synthesis, while CD8+ T cells such as Th1 type T helper cells can suppress IgE synthesis via the inhibitory effects of IFN- γ (Chung, 2001; Mosmann & Sad, 1996; Thomas, Noble, Sawicka, Askenase, & Kemeny, 2002). NO has been reported to inhibit Th1 cell proliferation and the production of IFN- γ (Sternberg & Mabbott, 1996), while Th2 cells are influenced by NO to decrease cell extension (Taylor-Robinson et al., 1994). The results of these previous studies suggest that the increase of NO production contributed to the IgE generation in allergy and asthma. Our study showed that MFPH reduced the amount of NO synthesis and the release of histamine and β -hexosaminidase in IgE-stimulated RBL-2H3 cell compared to FPH only. Therefore, MR of FPH contributed to an anti-allergy effect via reducing the NO production.

Finally, we conclude that MR provided FPH with anti-allergy activity. Based on this study, the signaling pathways related to the allergenic reaction and *in vivo* anti-allergy effects with MFPH should be studied further.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.foodchem.2014.12.090>.

References

- Abbas, A. K., Lichtman, A. H., & Pillai, S. (1994). *Cellular and molecular immunology*. Elsevier Health Sciences.
- Ahmed, N., & Thornalley, P. (2003). Quantitative screening of protein biomarkers of early glycation, advanced glycation, oxidation and nitrosation in cellular and extracellular proteins by tandem mass spectrometry multiple reaction monitoring. *Biochemical Society Transactions*, 31(6), 1417–1422.
- Ahn, K. B., Jeon, J. H., Kang, S. S., Chung, D. K., Yun, C. H., & Han, S. H. (2014). IgE in the absence of allergen induces the expression of monocyte chemoattractant protein-1 in the rat basophilic cell-line RBL-2H3. *Molecular Immunology*, 62(1), 114–121.
- Akiyama, H., Imai, T., & Ebisawa, M. (2011). Japan food allergen labeling regulation—history and evaluation. *Advances in Food and Nutrition Research*, 62, 139–171.
- Barnes, P. J. (2008). The cytokine network in asthma and chronic obstructive pulmonary disease. *Journal of Clinical Investigation*, 118(11), 3546–3556.
- Box, G. E. P., & Wilson, K. B. (1951). On the experimental attainment of optimum conditions. *Journal of the Royal Statistical Society Series B-Statistical Methodology*, 13(1), 1–45.
- Chung, F. (2001). Anti-inflammatory cytokines in asthma and allergy: Interleukin-10, interleukin-12, interferon-gamma. *Mediators of Inflammation*, 10(2), 51–59.
- Chung, M. J., Sohng, J. K., Choi, D. J., & Park, Y. I. (2013). Inhibitory effect of phloretin and biochanin A on IgE-mediated allergic responses in rat basophilic leukemia RBL-2H3 cells. *Life Science*, 93(9–11), 401–408.
- Cruse, J. M., & Lewis, R. E. (2010). *Atlas of immunology*. CRC Press.
- Dannaeus, A., & Inganäs, M. (1981). A follow-up study of children with food allergy. Clinical course in relation to serum IgE- and IgG-antibody levels to milk, egg and fish. *Clinical & Experimental Allergy*, 11(6), 533–539.
- Demartino, M., Peruzzi, M., Deluca, M., Amato, A. G., Galli, L., Lega, L., et al. (1993). Fish allergy in children. *Annals of Allergy*, 71(2), 159–165.
- Echavarria, A., Pagan, J., & Ibarz, A. (2014). Optimization of Maillard reaction products isolated from sugar-amino acid model system and their antioxidant activity. *AFINIDAD*, 70(562).
- Guzik, T. J., Korbust, R., & Adamek-Guzik, T. (2003). Nitric oxide and superoxide in inflammation and immune regulation. *Journal of Physiology and Pharmacology*, 54(4), 469–487.
- Hellwig, M., & Henle, T. (2014). Baking, ageing, diabetes: A short history of the Maillard reaction. *Angewandte Chemie International Edition*.
- Ikedo, K., Higashi, T., Sano, H., Jinnouchi, Y., Yoshida, M., Araki, T., et al. (1996). N ϵ -(carboxymethyl) lysine protein adduct is a major immunological epitope in proteins modified with advanced glycation end products of the Maillard reaction. *Biochemistry*, 35(24), 8075–8083.
- Karli, R., Balbaloglu, E., Uzun, L., Çınar, F., & Uğur, M. B. (2012). Correlation of symptoms with total IgE and specific IgE levels in patients presenting with allergic rhinitis. *Therapeutic advances in respiratory disease*, 1753465812468500.
- Kitts, D. D., Chen, X.-M., & Jing, H. (2012). Demonstration of antioxidant and anti-inflammatory bioactivities from sugar-amino acid Maillard reaction products. *Journal of Agricultural and Food Chemistry*, 60(27), 6718–6727.
- Lamas, S. (2010). *Nitric oxide, cell signaling and gene expression*. CRC Press.
- Le Man, H., Behera, S., & Park, H. (2010). Optimization of operational parameters for ethanol production from Korean food waste leachate. *International Journal of Environmental Science & Technology*, 7(1), 157–164.
- Mosmann, T. R., & Sad, S. (1996). The expanding universe of T-cell subsets: Th1, Th2 and more. *Immunology Today*, 17(3), 138–146.
- Ohguchi, K., Nakajima, C., Oyama, M., Iinuma, M., Itoh, T., Akao, Y., et al. (2010). Inhibitory effects of flavonoid glycosides isolated from the peel of Japanese persimmon (*Diospyros kaki* 'Fuyu') on melanin biosynthesis. *Biological and Pharmaceutical Bulletin*, 33(1), 122–124.
- Oneil, C., Helbling, A. A., & Lehrer, S. B. (1993). Allergic reactions to fish. *Clinical Reviews in Allergy*, 11(2), 183–200.
- Pascual, C., Esteban, M. M., & Crespo, J. F. (1992). Fish allergy - evaluation of the importance of cross-reactivity. *Journal of Pediatrics*, 121(5), S29–S34.
- Reddy, V. P., & Beyaz, A. (2006). Inhibitors of the Maillard reaction and AGE breakers as therapeutics for multiple diseases. *Drug Discovery Today*, 11(13), 646–654.
- Saarinen, U., & Kajosaari, M. (1980). Does dietary elimination in infancy prevent or only postpone a food allergy? A study of fish and citrus allergy in 375 children. *The Lancet*, 315(8161), 166–167.
- Sampson, H. A. (2001). Utility of food-specific IgE concentrations in predicting symptomatic food allergy. *Journal of Allergy and Clinical Immunology*, 107(5), 891–896.
- Schulz, K., Kerber, S., & Kelm, M. (1999). Reevaluation of the Griess method for determining NO/NO₂ in aqueous and protein-containing samples. *Nitric Oxide-Biology and Chemistry*, 3(3), 225–234.
- Sternberg, M. J., & Mabbott, N. A. (1996). Nitric oxide-mediated suppression of T cell responses during Trypanosoma brucei infection: Soluble trypanosome products and interferon-gamma are synergistic inducers of nitric oxide synthase. *European Journal of Immunology*, 26(3), 539–543.
- Taylor-Robinson, A. W., Liew, F. Y., Severn, A., Xu, D., McSorley, S. J., Garside, P., et al. (1994). Regulation of the immune response by nitric oxide differentially produced by T helper type 1 and T helper type 2 cells. *European Journal of Immunology*, 24(4), 980–984.
- Thomas, M. J., Noble, A., Sawicka, E., Askenase, P. W., & Kemeny, D. M. (2002). CD8 T cells inhibit IgE via dendritic cell IL-12 induction that promotes Th1 T cell counter-regulation. *Journal of Immunology*, 168(1), 216–223.
- Wang, K. J., Zhang, Y. J., & Yang, C. R. (2005). Antioxidant phenolic compounds from rhizomes of *Polygonum paleaceum*. *Journal of Ethnopharmacology*, 96(3), 483–487.
- Yilmaz, Y., & Toledo, R. (2005). Antioxidant activity of water-soluble Maillard reaction products. *Food Chemistry*, 93(2), 273–278.
- Zhu, C.-P., Gao, G.-T., Li, J.-K., Zhao, Y.-H., & Deng, D.-D. (2010). Response surface analysis for optimizing microwave-assisted extraction of *Pleurotus ostreatus* polysaccharide [J]. *Food Science*, 4, 016.