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Optimization of hydrolysis conditions for bovine plasma protein using response surface methodology

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ABSTRACT

The purpose of this study was to establish optimal conditions for the hydrolysis of bovine plasma protein. Response surface methodology was used to model and optimize responses [degree of hydrolysis (DH), 2,2-diphenyl-1-picrydrazyl (DPPH) radical-scavenging activity and Fe^{2+}-chelating activity]. Hydrolysis conditions, such as hydrolysis temperature (46.6–63.4 °C), hydrolysis time (98–502 min), and hydrolysis pH (6.32–9.68) were selected as the main processing conditions in the hydrolysis of bovine plasma protein. Optimal conditions for maximum DH (%), DPPH radical-scavenging activity (%) and Fe^{2+}-chelating activity (%) of the hydrolyzed bovine plasma protein, were respectively established. We discovered the following three conditions for optimal hydrolysis of bovine plasma: pH of 7.82–8.32, temperature of 54.1 °C, and time of 338.4–398.4 min. We consequently succeeded in hydrolyzing bovine plasma protein under these conditions and confirmed the various desirable properties of optimal hydrolysis.

1. Introduction

The nutritional and functional properties of food proteins have been investigated for years. The nutritional value of protein depends on the essential amino acid content and on the utilization of specific amino acids after digestion and absorption (Friedman, 1996). In terms of their functional properties, proteins contribute to the physicochemical and sensory properties of various protein-rich foods (Korhonen & Pihlanto, 2003).

The process of harvesting animals produces a considerable amount of by-products with high biological value, with one of the most important being blood proteins. These plasma proteins can be used in both the feed and food industries owing to their good nutritional value and excellent functional properties (Tybor, Dill, & Landmann, 1975). Animal blood contains 60–70% plasma and 30–40% suspended red blood cells by weight (Ockerman & Hansen, 2000). Blood protein has been used in limited quantities for direct human consumption because of its intense color and taste. The meat industry also needs to develop new products to satisfy emerging consumer demands.

Recently, protein hydrolysates have been shown to have more dietary uses due to their high nutritional and therapeutic values (Bhaskar, Modi, Govindaraju, Radha, & Lalitha, 2007). Much research has focused on the generation of bioactive peptides from food sources including meat and meat by-products (Daoud et al., 2005). Protein hydrolysates have been shown to have more dietary uses due to their high nutritional value and they are known to have specific functional activities, such as inhibitory activity on angiotensin I converting enzyme (ACE), immunological regulatory activity and antioxidant activity (Amarowicz & Shahidi, 1997; Cross & Gill, 2000; Hyun & Shin, 2000; Kong & Xiong, 2006; Pan, Luo, & Tanokura, 2005).

Protein hydrolysates of animal muscles and their by-products also possess antioxidant activity and improved functional activity (Liu, Kong, Xiong, & Xia, 2010; Xu, Cao, He, & Yang, 2009). Blood plasma protein hydrolysates, such as porcine plasma protein (Liu, Kong, Jiang, Cui, & Liu, 2009) and bovine plasma (Salgado, Fernandez, Drago, & Mauri, 2011), have been shown to possess antioxidant activities. However, there is little information relating to the functional peptides generated and how to establish optimal conditions in meat-based by-products such as bovine plasma protein.

Response surface methodology (RSM) is a statistical procedure frequently used to model and optimize a response that is affected by one or more factors, such as ingredients or process variables (Dean & Voss, 1999). Multivariate equations describe the effects of test factors on any given response and determine interrelationships among factors and the combined effects of all test factors on

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the response (Madamba, 2002). For this reason, RSM is widely used in food research (Paseephol, Small, & Sherkat, 2007).

Therefore, the present study was undertaken to establish the optimal conditions for the hydrolysis of bovine plasma protein (pH, temperature and time), and these conditions were established on the basis of obtaining high values of degree of hydrolysis (%), DPPH radical-scavenging activity (%) and Fe^{2+}-chelating activity (%).

2. Materials and methods

2.1. Materials

Blood of bovine species was obtained from a local slaughterhouse. Cattle blood was anticoagulated by adding 0.5 N ethylenediaminetetraacetic acid (EDTA) in a 1:9 (v/v) proportion. This blood was immediately placed on ice and transported to the laboratory within 30 min. Samples were centrifuged (SUPRA 25K, Hanil Science, Korea) at 14,000*g* for 15 min at 4 °C. Alcalase 2.4 L (6×10^4 U/g) was obtained from Novozymes (Bagsvaerd, Denmark). Testing chemicals, including 3-(2-pyridyl)-5,6-bis (4-phenyl-sulphonic acid)-1,2,4-triazine (Ferrozine), 2,4,6-trinitrobenzenesulfonic acid (TNBS), 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals and reagents used were of analytical grade.

2.2. Bovine plasma protein hydrolysates

The plasma protein hydrolysates were prepared according to the method described by Liu et al. (2009). The mixture of bovine plasma protein solution [5% w/v 10 mM sodium phosphate buffer (pH 7.0)] was heat pretreated (90 °C, 5 min) to inactivate the bovine plasma protein and then hydrolyzed with Alcalase. The enzyme-to-substrate ratio (E/S) was 2:100 (g/g). The pH of the bovine plasma protein solution was adjusted to the optimal values for Alcalase (pH 6.32, 7.00, 8.00, 9.00 and 9.68) before hydrolysis was initiated, and it was readjusted to the optimal value every 15 min during hydrolysis using 1 M NaOH. The hydrolysates were produced by varying the hydrolysis time (98, 180, 300, 480 and 502 min) and hydrolysis temperature (46.4, 50.0, 55.0, 60.0 and 63.4 °C). After hydrolysis, the pH of the solution was brought to 7.0 and the solution was then heated to 95 °C for 5 min to inactivate the enzyme.

After the optimum hydrolysis conditions were obtained, four bovine plasma protein hydrolysates (T1, T2, T3 and T4) were prepared under these conditions (hydrolyzed pH, 8.32, 8.32, 7.82 and 7.82; hydrolyzed temperature, 54.1 °C; and hydrolyzed time, 338.4, 394.4, 338.4 and 394.4 min, respectively). The hydrolysates were investigated for DH, DPPH radical-scavenging activity and Fe^{2+}-chelating activity. Experiments were done in triplicates.

2.3. Degree of hydrolysis (DH)

DH was determined by assaying free amino groups with 2,4,6-trinitrobenzenesulfonic acid (TNBS) according to Adler-Nissen (1986). The content of free amino groups in samples was expressed as leucine amino equivalents, based on the equation of leucine standard curve generated. The DH of hydrolyzed plasma protein was calculated by using the following equations:

$$\text{DH}\ (\%) = [(h_s - h_o)/(h_t - h_o)] \times 100$$

Here h_s and h_0 represent, respectively, the amino concentrations of hydrolyzed and non-hydrolyzed plasma protein and h_s represents the total amino concentration of plasma protein as measured by completely hydrolyzing the plasma protein with 6 N HCl. The non-hydrolyzed protein solution containing no enzyme was set as 0% DH.

2.4. 2,2-Diphenyl-1-picryhydrazyl (DPPH) radical scavenging activity

The DPPH radical scavenging activity was measured based on the method of Bersuder, Hole, and Smith (1998). Test samples in 4 ml of water were mixed with 1 ml of 99.5% ethanol containing 0.02% DPPH. This mixture was shaken, kept at room temperature for 30 min, and then the absorbance of the mixture was measured at 517 nm. The residual radicals were calculated as follows:

$$\text{Inhibition}\ (\%) = \frac{\text{DPPH Blank} - (\text{Control Sample} - \text{DPPH Sample})}{\text{DHHP Blank}} \times 100$$

where the DPPH blank is the value of 4 ml of water/1 ml of ethanol including 0.02% DPPH, the DPPH sample is the value of 4 ml of sample solution/1 ml of ethanol including 0.02% DPPH and the control sample is the value of 4 ml of sample solution/1 ml of ethanol.

2.5. Ion (Fe^{2+})-chelating activity

The ability of plasma protein hydrolysate to chelate the pro-oxidative transitional metal ions Fe^{2+} was investigated according to Kong and Xiong (2006) with slight modifications. For the Fe^{2+} chelation experiment, 1 ml of 20 μM $FeCl_2$ was mixed with 2 ml of 50 μM Ferrozine, which produces a chromaphore that absorbs strongly at 562 nm. After the addition of 0.5 ml of bovine plasma protein hydrolysates, the color change, due to dissociation of Fe^{2+}, was measured spectrophotometrically at 562 nm. The Fe^{2+} chelating activity by bovine plasma protein or hydrolysates was calculated as follows:

$$\text{Metal Ion-Chelating Activity}\ (\%) = \left[1 - \frac{\text{Sample solution absorbance}}{\text{Blank solution absorbance}}\right] \times 100.$$

2.6. Experimental design and statistical analysis

Statistical analysis was performed based on the following modifications reported by Kim et al. (2013). The main effects of process variables (hydrolysis pH, X_1; hydrolysis temperature, X_2; hydrolysis time, X_3) on DH (Y_1), DPPH radical-scavenging activity (Y_2) and Fe^{2+}-chelating activity (Y_3) were investigated using response surface methodology (RSM). Replicates were performed for each experiment and the average values were recorded as the response. A central composite rotatable design (CCRD) was used and 20 different formulations with 6 central points and 6 axial points and 2^3 full factorial designs were produced. The least square regression methodology using SAS 9.0 (2009) for Windows was used to fit the data to the following second-order equation:

$$Y = b_0 + \sum_{i=1}^{3} b_i X_i + \sum_{i=1}^{3} b_{ii} X_i^2 + \sum_{i<j=1}^{3} b_{ij} X_i X_j$$

where Y is the dependent or response variable: b_0, b_i, b_{ij} and b_{ij} are the intercept, linear, quadratic and interaction coefficients, respectively; and X_i and X_j are independent variables (Myers, 1976). The ANOVA test was employed to evaluate the statistical significance of the regression coefficients. Non-significant terms ($P > 0.05$) were deleted from the second-order polynomial and a new polynomial was recalculated to obtain a predictive model for each dependent variable (Faveri, Torre, Perego, & Converti, 2004). Once the fitted regression equations were determined, the response surface plots were drawn using the SAS 9.0 (2009) program. One independent