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Research Article

Optimization of Enzymatic Conditions of Sturgeon Muscles and Their Anti-Inflammatory Potential

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The objective of this study was to investigate the effects of different enzymolysis conditions on the NO inhibition rate and DH (degree of hydrolysis) of sturgeon hydrolysates (SH) prepared by Alcalase. The NO inhibition rate of 60.23% was attained under the optimum enzymolysis conditions as follows: pH 9.0, enzymolysis time of 4.92 h, enzymolysis temperature of 55°C, solid/liquid ratio of 1 : 20, and enzyme additive amount of 7674.22 U/g protein, which was well matched with the predicted value 61.44% of the Box–Behnken design model. After the ultrafiltration of SH, SH-3 (SH < 3 kDa) could significantly decrease the levels of NO and proinflammatory cytokine level IL-6. Also, we found that the obtained SH-3 contained good properties of emulsification and possessed good WHC and OHC. SH-3 demonstrated appreciable antioxidant potential on DPPH and ABTS radical scavenging activities. These results suggested that SH-3 derived from sturgeon muscles could potentially be used as a promising ingredient against inflammatory and oxidative stress-associated diseases.

1. Introduction

Fish and fishery products provide not only essential micronutrients, but also high-value protein. However, approximately 50% of protein-rich fish byproducts are used as fishmeal and animal feed or discarded, causing a lot of waste. Enzymatic hydrolysis is the most efficient method for recovering value-added proteins from fish byproducts and keeping the value of nutrition. According to previous studies, fish protein-derived hydrolysates possess different bioactivities, such as antioxidative, antitumor, anti-inflammatory, neuroprotective, and antidiabetic effects [1–3].

Inflammation is a defense mechanism triggered by pathogen invasion or tissue damage caused by biological, physical, or chemical damage [4, 5]. The activation of macrophages is an important part of initiating defensive responses, and the release by macrophages of inflammatory mediators such as nitric oxide (NO) and proinflammatory cytokines such as interleukin-6 (IL-6), interleukin-1β (IL-1β), and tumor necrosis factor-α (TNF-α) can enhance the defense ability [6, 7]. NO is an important signaling molecule in neurotransmission, vasodilation, and host immune defense [8]. However, overproduction of NO and inflammatory cytokines is related to various diseases, such as atherosclerosis, rheumatoid arthritis, and endotoxin-induced organ injury [9, 10]. Some investigators found that the anti-inflammatory effects were mainly related to the antioxidant activities [11, 12]. Besides, plenty of research studies have reported that anti-inflammatory hydrolysates can be obtained from fish protein hydrolysates [13, 14]. For example, salmon byproduct protein hydrolysates and fish scale collagen peptides possess anti-inflammatory activity [14, 15].

In China, sturgeon caviars are expensive and in short supply; sturgeon muscles are found in large quantities and most of them are discarded as waste. Sturgeon muscles have been used in traditional Chinese medicine to help people

maintain overall wellness for thousands of years. However, in recent years, sturgeon muscles have received limited attention as a potential resource of bioactive hydrolysates. Thus, exploring the anti-inflammatory activity of sturgeon muscles hydrolysates is significant to expand the application in food industries.

The objectives of this study were to optimize the enzymatic hydrolysis conditions of sturgeon muscles and investigate its anti-inflammatory and anti-oxidant effects to verify the possibility for application of functional food materials.

2. Materials and Methods

2.1. Materials. Alcalase were purchased from Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azino-bis(3-ethylbenzthiazoline)-6-sulfonic acid (ABTS) were obtained from Aladdin Industrial Corporation (Ontario, CA). Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were from Biological Industries (Kibbutz Beit Haemek, Israel). 3-(4,5-Dimethylthizaol-2-yl)-2,5-diphenylterazolium bromide (MTT) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Penicillin, streptomycin, lipopolysaccharide (LPS), and Griess Reagent were from Beyotime Biotechnology (Shanghai, China). Interleukin-6 (IL-6) enzyme-linked immunosorbent assay (ELISA) kits were obtained from Fcmacs (Nanjing, China). All other chemicals were of reagent grade.

2.2. Single-Factor Experiments. In order to obtain the optimal enzymatic hydrolysis conditions of sturgeon muscles by using Alcalase, the effect of pH, time, temperature, solid/liquid ratio, and enzyme additive amount was tested by single-factor experiment. The chosen pH is 7.5, 8.0, 8.5, 9.0, and 9.5. The chosen hydrolysis times are 2, 3, 4, 5, and 6 h. The chosen reaction temperatures are 45, 50, 55, 60°C, and 65°C. The chosen solid/liquid ratios are 1 : 25, 1 : 20, 1 : 15, 1 : 10, and 1 : 5. The selected enzyme additive amounts are 1000, 2000, 4000, 6000, 8000, and 10,000 U/g protein. SH was prepared according to previous reports with slight modification [16, 17]. Sturgeon muscles were initially homogenized with distilled water. The suspension was added with Alcalase and the pH was adjusted to hydrolyze. Afterwards, the reaction was terminated by heating the mixture in a water bath at 95°C for 20 min. The supernatant was collected and freeze-dried for further analysis.

2.3. Response Surface Method. According to the single-factor experimental results, the range of level values of each factor was determined. The response surface method with three-level and four test variables (pH (X_1), time (X_2), temperature (X_3), and enzyme additive amount (X_4)) was employed in optimization procedure. The NO inhibition rate was chosen as the response variable of the experiments. The range of independent variables and levels are shown in Table 1. A second-order polynomial model [18] was used to describe the optimal hydrolysis conditions of sturgeon muscles.

Table 1: Independent variables and levels used in response surface design.

Independent variables	Units	Levels		
		−1	0	1
pH (X_1)		8.5	9.0	9.5
Time (X_2)	h	4	5	6
Temperature (X_3)	°C	45	50	55
Enzyme additive amount (X_4)	U/g	6000	8000	10000

Design Expert (8.0.6) software was used for the design and statistical analysis.

2.4. Ultrafiltration. SH under the optimal enzymatic conditions was filtered through ultrafiltration membranes with a molecular weight cut-off (MWCO) of 10 and 3 kDa (Millipore Co., USA). All fractions including SH-1 (>10 kDa), SH-2 (3–10 kDa), and SH-3 (<3 kDa) were freeze-dried for further experiment.

2.5. Determination of the Degree of Hydrolysis. The DH was estimated by the o-phthaldialdehyde (OPA) method [19].

2.5.1. Cell Culture. The murine macrophage RAW264.7 cells were cultured in DMEM supplemented with 10% fetal bovine serum and 1% penicillin and streptomycin. Cells were grown in a humidified incubator with 5% CO_2 at 37°C.

2.5.2. Cell Viability. RAW264.7 cells were seeded into a 96-well plate overnight and pretreated with different concentrations (0.125, 0.25, 0.5, 1.0, and 2.0 mg/mL) of SH for 24 h. MTT solution was added to each well and incubated for another 4 h at 37°C. Then, the supernatant was discarded, 150 μL DMSO was added to each well, and the absorbance was detected at 570 nm using a microplate reader (Tecan Infinite PRO TWIN 200, Switzerland). Viability was expressed as a percentage of the control.

2.6. Determination of Nitric Oxide (NO) and IL-6. RAW264.7 cells were cultured in 24-well plates overnight and pretreated with SH for 12 h. Then, cells were stimulated with LPS (2 μg/mL) for 24 h. The nitrite concentration was measured with Griess reagent. Inflammatory cytokine IL-6 was assessed by ELISA kit following the manufacturer's instructions.

2.7. Functional Properties of SH

2.7.1. Emulsifying Properties. Emulsifying activity index (EAI) and the emulsion stability index (ESI) were measured according to previous reports with slight modifications [20]. 30 mL of 1% SH solution with different pH (2, 4, 6, 8, and 10) was mixed with 10 mL soybean oil and homogenized at 15,000 rpm for a minute. 50 μL of the emulsion was pipetted from the bottom of the container at 0 and 10 min after homogenization and diluted with 5 mL of 0.1% sodium

dodecyl sulfate solution. The absorbance of the solution was measured at 500 nm using a spectrophotometer. EAI and ESI were calculated as follows:

$$\mathrm{EAI}\left(\mathrm{m}^2/\mathrm{g}\right) = \frac{2 \times 2.303 \times \mathrm{dil} \times A}{(C \times \Phi \times 10000)}, \quad (1)$$

$$\mathrm{ESI}\,(\mathrm{min}) = \frac{(A_0 \times 10)}{\Delta A}, \quad (2)$$

where A is absorption at 500 nm; dil is dilution factor (100); C is protein concentration in aqueous phase (g/mL); Φ is oil volume fraction (0.25); and $\Delta A = A_0 - A_{10}$.

2.7.2. Foaming Capacity and Foam Stability. Foaming capacity (FC) and foam stability (FS) were determined according to the previous methods with some modifications [21]. 0.4 g of the sample was placed in 40 mL distilled water and stirred at room temperature for 30 min. The foam was prepared using a homogenizer at 15,000 rpm for 2 min. The foam volume was noted immediately after 2 min. FS was calculated by measuring the fall of the foam volume after every 1 min. FC and FS were calculated as follows:

$$\text{Foaming capacity} = \left[\frac{V_2 - V_1}{V_1}\right] \times 100, \quad (3)$$

where V_1 is volume before whipping and V_2 is volume after whipping.

$$\text{Foam stability} = \left[\frac{\text{Foam volume after time}}{\text{initial foam volume}}\right] \times 100. \quad (4)$$

2.7.3. Water Holding Capacity. WHC was estimated by the centrifuge method according to the previous study with some modifications [22]. 0.5 g of SH was dissolved in 10 mL of distilled water and vortexed for 30 s. The solution was allowed to stand for 6 h at room temperature and then centrifuged at 5,000 g for 30 min. The supernatant was then filtered and the volume collected was noted. The difference between the initial volume of distilled water added to the sample and the volume of the supernatant was measured, and the results were calculated as milliliters of water absorbed per gram of SH.

2.7.4. Oil Holding Capacity. OHC was determined by the centrifuge method according to the reported method with slight modifications [22]. 0.5 g of SH was added to 10 mL of soybean oil and vortexed for 30 s. The oil dispersion was then centrifuged at 2,800 g for 30 min. The volume of oil separated from the hydrolysate was measured, and OHC was calculated as the grams of oil absorbed per gram of SH.

2.8. Determination of Antioxidant Activity

2.8.1. DPPH Radical Scavenging Activity. DPPH free radical-scavenging activity of SH was measured according to the method described by previous studies with some modifications [23, 24]. 2 mL of each sample with different concentrations was added to 2 mL of 0.1 mM DPPH in 99.5% ethanol and incubated for 30 min at 37°C in dark conditions; the absorbance was determined at 517 nm with spectrophotometer. In addition, a control sample containing DPPH solution without sample was prepared. In blank sample, DPPH solution was substituted with ethanol. The DPPH radical scavenging activity was evaluated by the inhibition percentage of DPPH radical as in the following equation:

$$\text{DPPH radical scavenging activity}\,(\%) = \left(1 - \frac{A_1 - A_2}{A_0}\right) \times 100\%, \quad (5)$$

where A_1 was the absorbance of the sample; A_0 was the absorbance of the control group; and A_2 was blank absorbance.

2.8.2. ABTS Radical Scavenging Activity. Radical scavenging ability was measured as described previously with slight modifications [25]. The ABTS radical cation was generated by mixing 7.4 mM ABTS with 2.45 mM $K_2S_2O_8$ and exposing the resultant mixture to the dark at room temperature for at least 12 h. The ABTS radical solution was diluted in phosphate buffered saline (PBS) (pH 7.0) to an absorbance of 0.70 ± 0.02 at 734 nm. 1 mL of diluted ABTS free radical solution was mixed with 1 mL of the sample at different concentrations. After 10 minutes, the absorbance was measured at 734 nm against the corresponding blank. The ABTS scavenging activity was calculated as in the following equation:

$$\text{ABTS scavenging activity}\,(\%) = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100\%, \quad (6)$$

where A_{control} was the absorbance without sample and A_{sample} was the absorbance with sample.

2.9. Statistical Analysis. Data are expressed as means ± standard deviation of more than two replicates. The statistical difference among groups was evaluated by one-way ANOVA (SPSS for win 8.0, SPSS Inc., Chicago, IL). The results were considered significant when $p < 0.05$.

3. Results and Discussion

3.1. Cell Viability. In order to evaluate cytotoxic effects of SH on RAW264.7 cells, MTT assay was performed. Figure 1(a) showed that SH did not have cytotoxic effects up to 2.0 mg/mL. Therefore, the concentration of 0.5 mg/mL of SH was selected for further study.

3.2. The Analysis of Single-Factor Experiments

3.2.1. The Effect of pH on DH and NO Inhibition Rate. As shown in Figure 2(a), the effect of enzymolysis pH on DH and NO inhibition rate of SH was investigated. The pH was

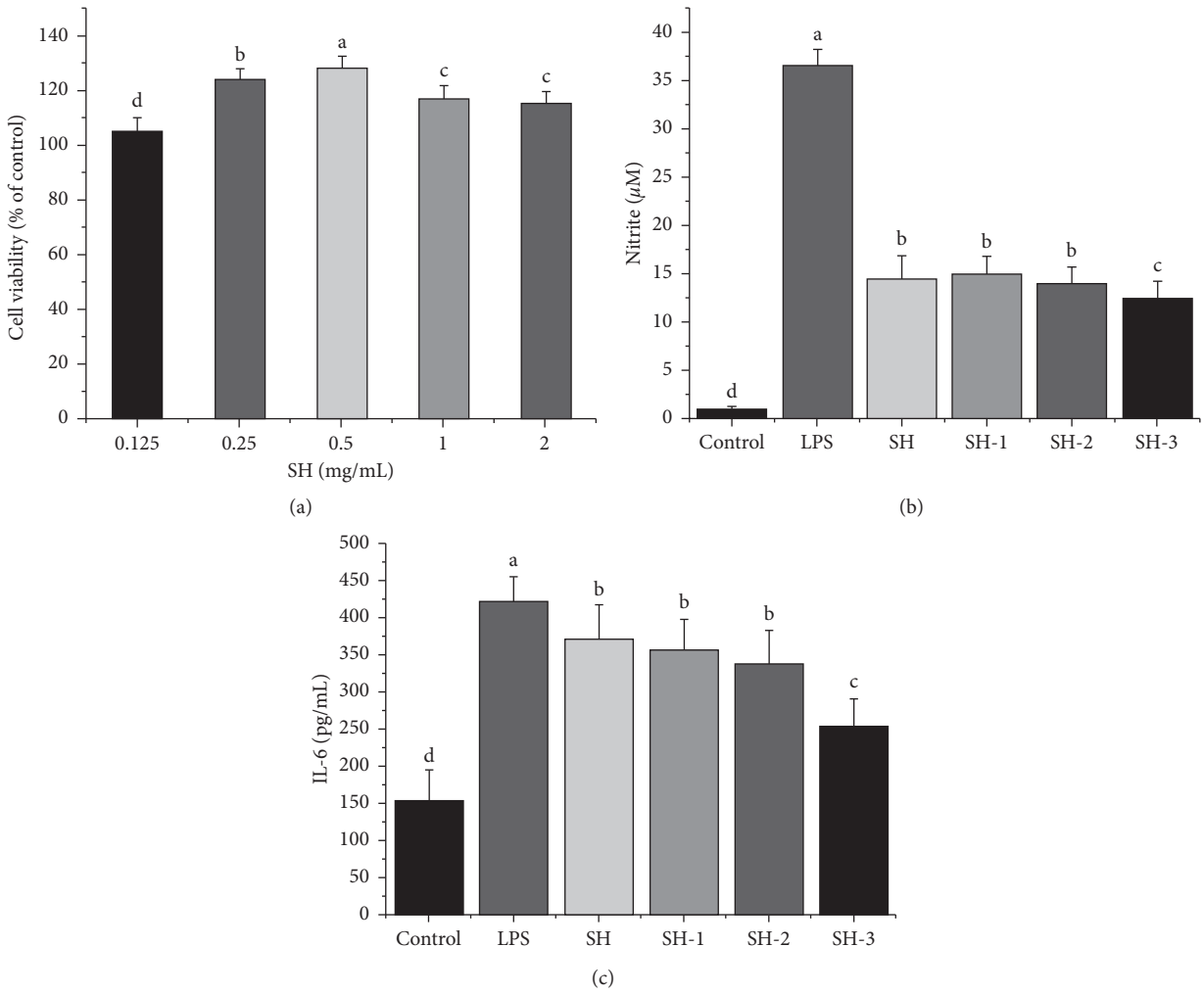


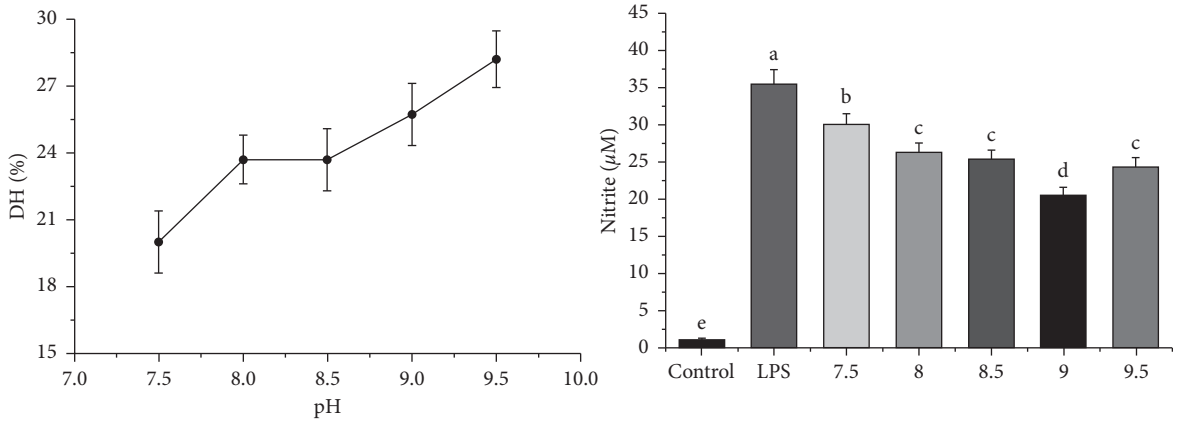
Figure 1: SH supplementation inhibited LPS-stimulated inflammation in macrophages: cell viability (a), nitrite level (b), and IL-6 level (c) in RAW 264.7 cells. Data are expressed as means ± SEM. Values without a common letter are significantly different at $P < 0.05$.

changed from 7.5 to 9.5, while other variables were set as follows: enzymolysis temperature 50°C, enzymolysis time 6 h, solid/liquid ratio 1 : 25, and enzyme additive amounts 2000 U/g protein. The DH was increased from 20.0% to 28.2% as the pH increased from 7.5 to 9.5. However, the NO inhibition rate of SH increased with pH until it was up to 9.0 and then began to decrease. The maximum NO inhibition rate was 42.36% at the pH of 9.0. Such a decline in the NO inhibition rate might be due to the change of spatial structure of the enzyme or the interference of the ionic properties of the substrate, which could reduce the ability of the substrate to bind the enzyme, resulting in a decrease or even loss of enzyme activity [20].

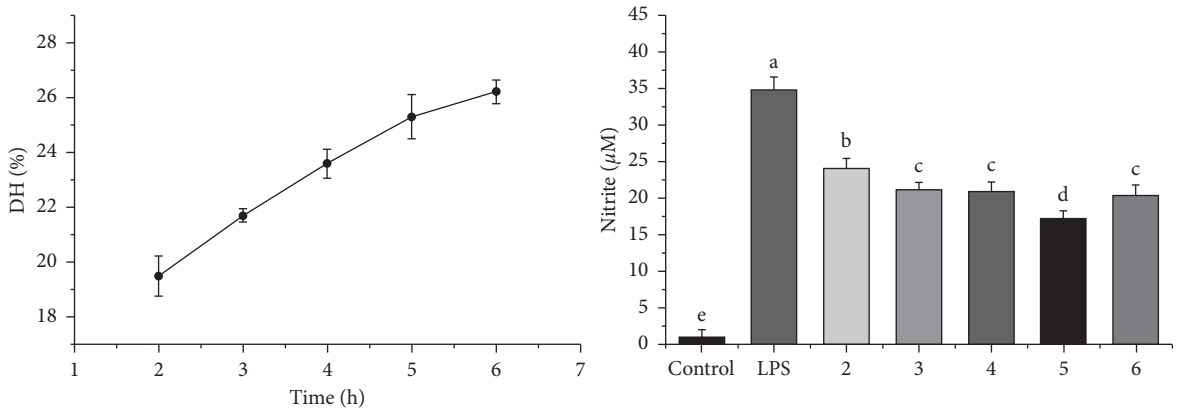
3.2.2. The Effect of Enzymolysis Time on DH and NO Inhibition Rate. Figure 2(b) showed the effects of different enzymolysis times on the DH and NO inhibition rate of SH when the pH was 9.0 and other three factors remained unchanged. The NO inhibition rate increased when the enzymolysis time varied from 2 h to 5 h peaked at 50.66% and then decreased. While DH kept increasing with the increase of time. This might be attributed to the fact that longer enzymolysis time could lead to excessive hydrolysis of SH.

3.2.3. The Effect of Enzymolysis Temperature on DH and NO Inhibition Rate. The reaction temperature of SH was set with the range of 45 to 65°C. Enzymolysis time was 5 h while the other three conditions were unchanged. As shown in Figure 2(c), the NO inhibition rate of SH peaked when the temperature was 50°C. When the temperature exceeded 50°C, the rate decreased. This phenomenon could be attributed to the possibility that the protease might be denatured and inactivated under high temperature conditions, the stability of the enzyme will decline, and its role of cutting the peptide chain will be lost [26].

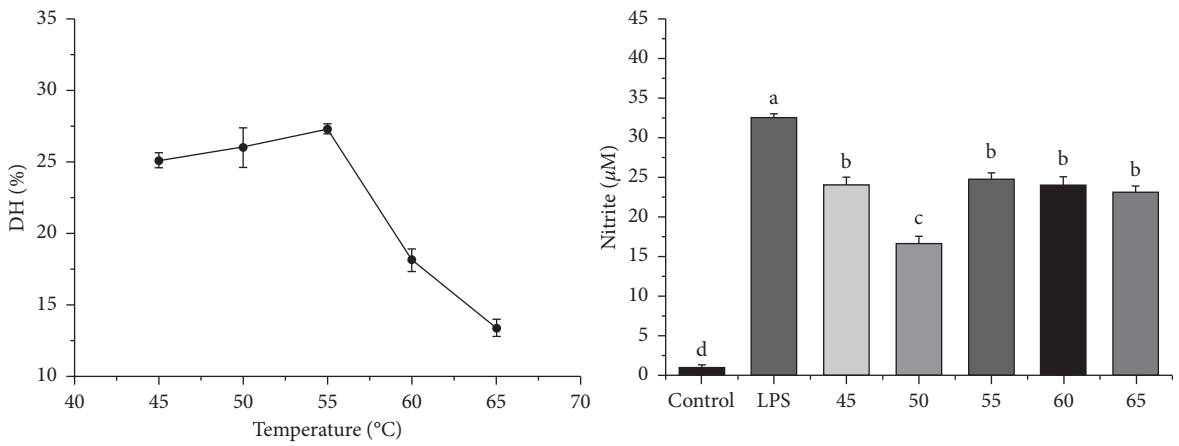
3.2.4. Effect of Solid/Liquid Ratio on DH and NO Inhibition Rate. The solid/liquid ratio of SH was assessed within the range of 1 : 5 to 1 : 25. Enzymolysis temperature was 50°C while the other three conditions were unchanged. The results



(a)



(b)



(c)

Figure 2: Continued.

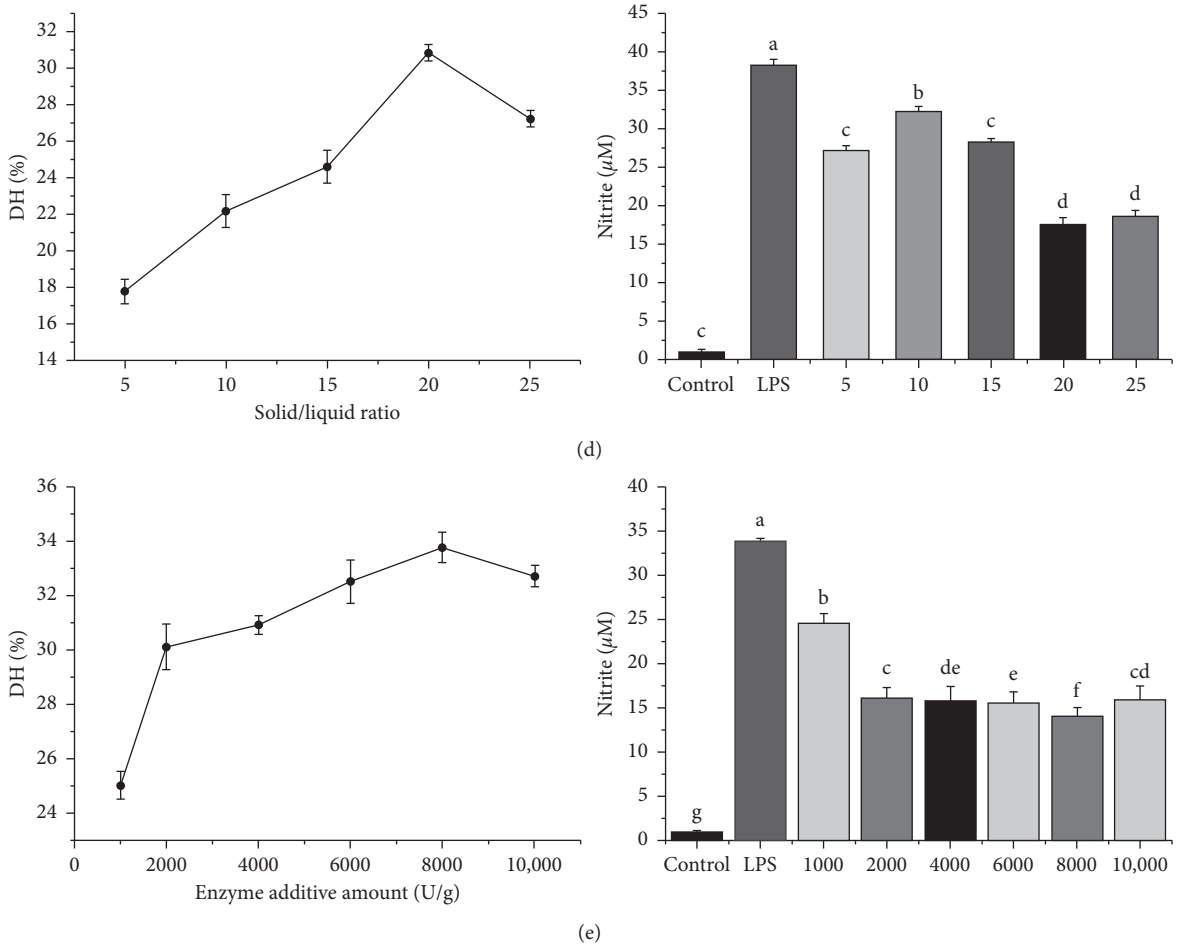


Figure 2: Effects of enzymolysis conditions on the DH and NO inhibition rate of SH: (a) pH; (b) enzymolysis time; (c) enzymolysis temperature; (d) solid/liquid ratio; and (e) enzyme additive amount. Data are expressed as means ± SEM. Values without a common letter are significantly different at $P < 0.05$.

were displayed in Figure 2(d). When the S/L ratio is 1 : 20, the NO inhibition rate is relative higher, which was 53.94% and the DH reached the peak. Considering the subsequent mass production, 1 : 20 of the S/L ratio was selected for further optimization.

3.2.5. Effect of Enzyme Additive Amounts on DH and NO Inhibition Rate. The effect of enzyme additive amounts on DH and NO inhibition rate was shown in Figure 2(e). It was set at 1000, 2000, 4000, 6000, 8000, and 10,000 U/g protein, respectively. Solid/liquid ratio was 1 : 20 and the other three conditions were unchanged. When the enzyme additive amount was 8000 U/g protein, NO inhibition rate and DH reached 58.73% and 33.76%, respectively. However, there was a decrease when the enzyme additive amount continued to rise. This finding is probably attributed to the increase of enzyme molecular collision and aggregation, leading to the enzyme autolysis, which is not conducive to the enzymatic reaction. This result indicated that the enzyme additive amount of 8000 U/g protein was sufficient to obtain a high NO inhibition rate.

3.3. Response Surface Analysis. The response surface analysis of the experimental data was shown in Tables 2 and 3. The response variable and the test variables were related by the following a second-order regression equation:

$$\begin{aligned}\text{NO inhibition rate} = {} & 58.53 - 2.39X_1 + 1.20X_2 + 2.46X_3 \\ & - 0.59X_4 + 3.30X_1X_2 + 2.98X_1X_3 \\ & + 2.42X_1X_4 - 1.33X_2X_3 + 2.18X_2X_4 \\ & - 0.14X_3X_4 - 4.05{X_1}^2 - 3.24{X_2}^2 + 0.39X_3^2 \\ & - 2.82{X_4}^2,\end{aligned} \tag{7}$$

where Y is the NO inhibition rate of SH, X_1 is pH, X_2 is enzymolysis time, X_3 is enzymolysis temperature, and X_4 is enzyme additive amount. As presented in Table 3, a high F-value (10.31) and a low p-value (<0.0001) indicated that the regression model was significant. In addition, the value of the determination coefficient ($R^2 = 0.9116$), the adjusted coefficient of determination ($R^2_{\text{adj}} = 0.8332 > 0.8$), and the coefficient of variation (C.V. = 3.35%) indicated a high

Table 2: Box–Behnken experimental design with independent variables.

Run	Coded variable levels				NO inhibition rate (%)
	X_1	X_2	X_3	X_4	
1	−1	−1	0	0	56.51
2	1	−1	0	0	45.24
3	−1	1	0	0	52.24
4	1	1	0	0	54.17
5	0	0	−1	−1	54.37
6	0	0	1	−1	60.24
7	0	0	−1	1	53.84
8	0	0	1	1	59.17
9	−1	0	0	−1	57.57
10	1	0	0	−1	49.51
11	−1	0	0	1	49.04
12	1	0	0	1	50.64
13	0	−1	−1	0	49.04
14	0	1	−1	0	56.51
15	0	−1	1	0	57.57
16	0	1	1	0	59.7
17	−1	0	−1	0	58.64
18	1	0	−1	0	46.2
19	−1	0	1	0	55.94
20	1	0	1	0	55.44
21	0	−1	0	−1	53.31
22	0	1	0	−1	49.04
23	0	−1	0	1	49.91
24	0	1	0	1	54.37
25	0	0	0	0	58.64
26	0	0	0	0	59.7
27	0	0	0	0	57.44
28	0	0	0	0	59.44
29	0	0	0	0	57.44

degree of correlation between the NO inhibition rate and the four variables. Furthermore, the p-value of the lack of fit was 0.1113 ($p > 0.05$), which indicated that the factors other than these four factors had less interference with the test results and the differences were not significant, suggesting good reliability of the regression model. Moreover, the linear coefficients and quadratic term coefficients (X_1, X_2, and X_3) (X_1^2, X_2^2, and X_4^2) (X_1X_2, X_1X_3, X_1X_4, and X_2X_4) were significant ($p < 0.05$).

Response surfaces were plotted using Design-Expert to study the effects of variables and their interactions on NO inhibition rate [27]. The 3D response surface plots and 2D contour plots were shown in Figure 3. It provided a method to visualize the relationship between the responses and experimental levels of each variable and the type of interactions between the two test variables. The shapes of the contour plots indicate the significance of the interactions between two tested variables, where a circular contour plot indicates no significant interaction, and an elliptical or saddle contour plot suggests a significant interaction. As shown in Figure 3, the interactions of the variables (pH and enzymolysis time, pH and enzymolysis temperature, pH and enzyme additive amount, and enzymolysis time and enzyme additive amount) were significant ($p < 0.05$).

According to the regression model, the optimal enzymolysis conditions were as follows: pH = 9.0, enzymolysis time = 4.92 h, enzymolysis temperature = 55°C, and enzyme additive amount = 7674.22 U/g protein. Under these conditions, the theoretical NO inhibition rate of SH was 61.44%. In order to confirm the validity of the regression model, an additional experiment was conducted under the optimal enzymolysis conditions. The NO inhibition rate of SH was 60.23%, almost approaching the predicted value 61.44%, which meant that the regression model was valid.

Table 3: ANOVA for the response surface quadratic model of NO inhibition rate.

Source	SS	Df	MS	F value	p-value	
Model	480.18	14	34.3	10.31	<0.0001	Significant
X_1	68.83	1	68.83	20.7	0.0005*	
X_2	17.4	1	17.4	5.23	0.0382*	
X_3	72.32	1	72.32	21.75	0.0004*	
X_4	4.17	1	4.17	1.25	0.2819	
X_1X_2	43.56	1	43.56	13.1	0.0028*	
X_1X_3	35.64	1	35.64	10.72	0.0055*	
X_1X_4	23.33	1	23.33	7.02	0.0191*	
X_2X_3	7.13	1	7.13	2.14	0.1652	
X_2X_4	19.05	1	19.05	5.73	0.0312*	
X_3X_4	0.073	1	0.073	0.022	0.8844	
X_1^2	106.34	1	106.34	31.98	<0.0001*	
X_2^2	68.1	1	68.1	20.48	0.0005*	
X_3^2	0.99	1	0.99	0.3	0.5935	
X_4^2	51.41	1	51.41	15.46	0.0015*	
Residual	46.55	14	3.33			
Lack of fit	41.97	10	4.2	3.66	0.1113	Not significant
Pure error	4.59	4	1.15			
Total variation	526.73	28				
R^2	0.9116					
Adj R^2	0.8332					
C.V. (%)	3.35					

3.4. LPS-Stimulated Inflammation Was Inhibited by SH in Macrophages. In RAW264.7 cells, LPS-stimulation increased the secretion of NO and IL-6 (Figures 1(b) and 1(c)). SH dramatically inhibited the secretion of NO and IL-6, with SH-3 (SH < 3 kDa) showing the best inhibitory effect by 66.24% and 39.54%, respectively. This result indicated that SH could inhibit the inflammatory response in macrophage cells, and thus SH-3 was used for further investigation.

3.5. Functional Properties

3.5.1. Emulsifying Properties. SH is a surface-active material that contains both hydrophilic and hydrophobic groups which could promote the formation of an oil-in-water emulsion. Protein ability can be determined through the formation and stabilization of the emulsion [21, 28]. As shown in Figures 4(a) and 4(b), the EAI and ESI of SH-3 were affected by pH, where the EAI and ESI were the worst near the isoelectric point (pH = 4) and better under acidic and alkaline conditions that deviated from the isoelectric point. Several studies have shown that a range of pH at 6–10

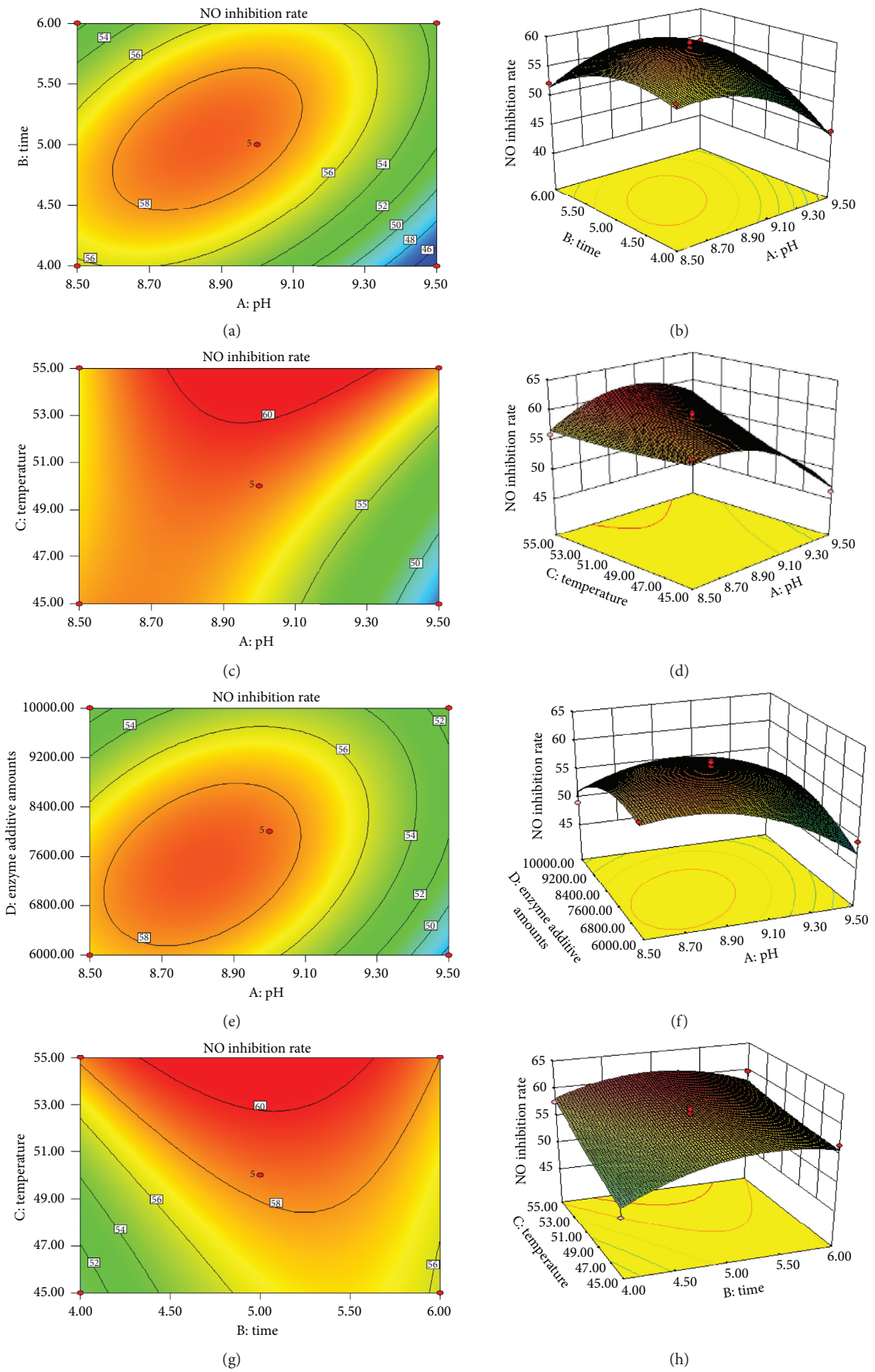


Figure 3: Continued.

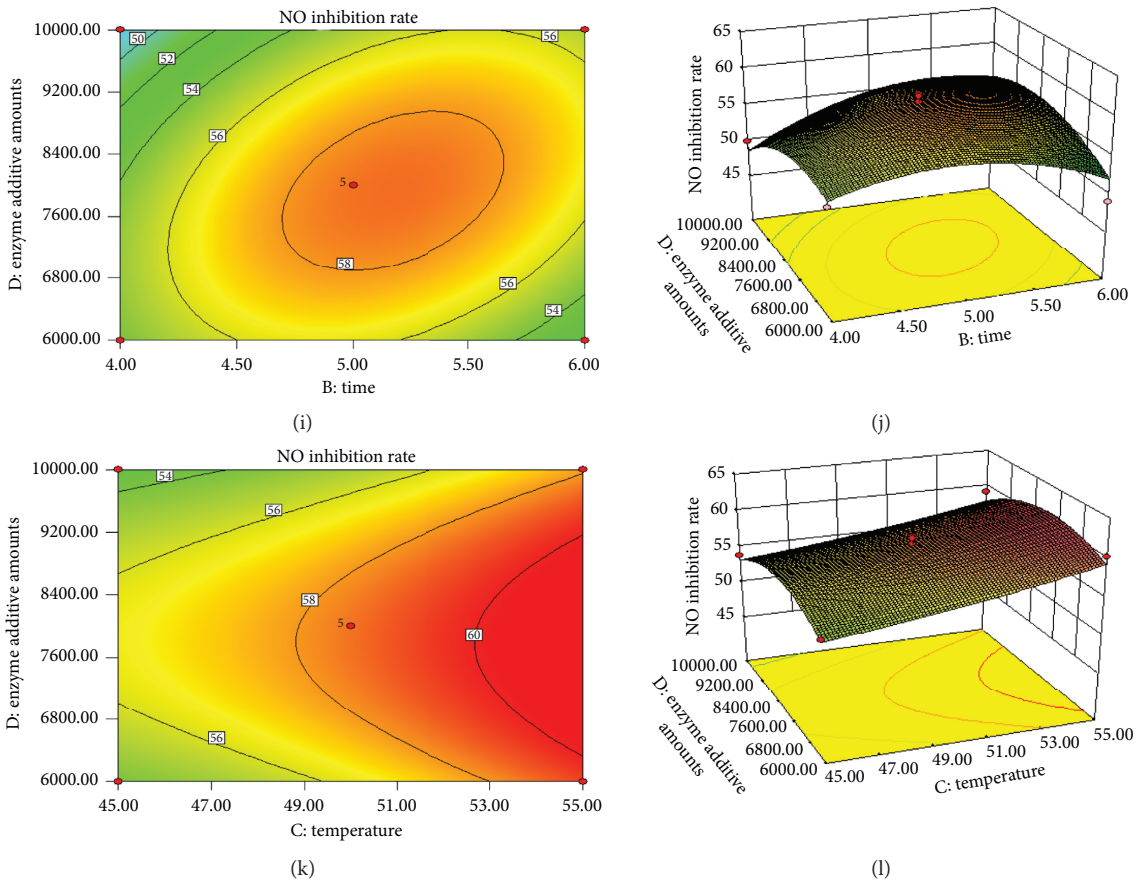


Figure 3: Response surface (3D) and contour plots (2D) showing interaction effect of (a) pH, (b) enzymolysis time, (c) enzymolysis temperature, and (d) enzyme additive amount on NO inhibition rate.

produced the highest EAI, with the lowest EAI at pH 4 [29, 30]. According to Taheri et al. [29], polypeptides unfold at highly alkaline pH due to their negative charges. Repulsion caused by this change allows a better orientation at the interface. Therefore, this condition makes the exposure of hydrophilic and hydrophobic peptide residues more effective and promotes significant interactions at the oil–water interface.

3.5.2. Foaming Capacity and Foam Stability. Foaming capacity is one of the important functional properties of proteins and peptides. The surface tension at the air–water interface is lower by the proteins or peptides which leads to the formation of a stable foam [31]. Generally, foam formation follows three major steps, transportation, penetration, and restructuring of the molecules at the air–water interface [32]. FC and FS of SH-3 are shown in Table 4 and Figure 4(c), respectively. FC of SH-3 was 73.75 ± 2.58%. Regarding FS, it was 17.50% after 1 min and 0.63% after 4 min at pH 6, and the foam faded afterwards. Low FS possibly arises from the formation of free amino acids during hydrolysis [33]. Nalinanon et al. [34] reported that low-molecular-weight (~1 kDa) peptides were unable to maintain a well-ordered, interface orientation of the molecule. Besides, water has fluidity under gravity; gas diffusion between foams will cause gas imbalance, which is the main factor of foam instability [35].

3.5.3. Water and Oil Holding Capacity. The solubility of protein affects both WHC and OHC because high solubility means smaller molecular size, which influences the absorption of water and oil [28]. As shown in Table 4, the values of WHC and OHC in SH-3 were 1.90 ± 0.12 and 2.35 ± 0.05 g/g, respectively.

3.5.4. Antioxidant Activity. Oxidative stress is an imbalance between reactive oxygen species (ROS) generation and scavenging by antioxidative agents [36, 37]. In addition to natural antioxidants (carotenoids, phenolic compounds, vitamin C, vitamin E, and so on), the hydrolysates and peptides have also been recognized for their antioxidative activity [38, 39]. As free radical compounds, DPPH and ABTS are extensively employed to estimate the antioxidant capacity of various samples [40]. As shown in Table 5, the IC_{50} values of DPPH and ABTS of SH were 1.215 ± 0.081 mg/mL and 1.563 ± 0.080 mg/mL. After ultrafiltration of SH, DPPH radical scavenging activity and ABTS radical scavenging activity increased with the decrease of molecular weight, indicating that low molecular weight possessed higher antioxidant activity, which were in agreement with

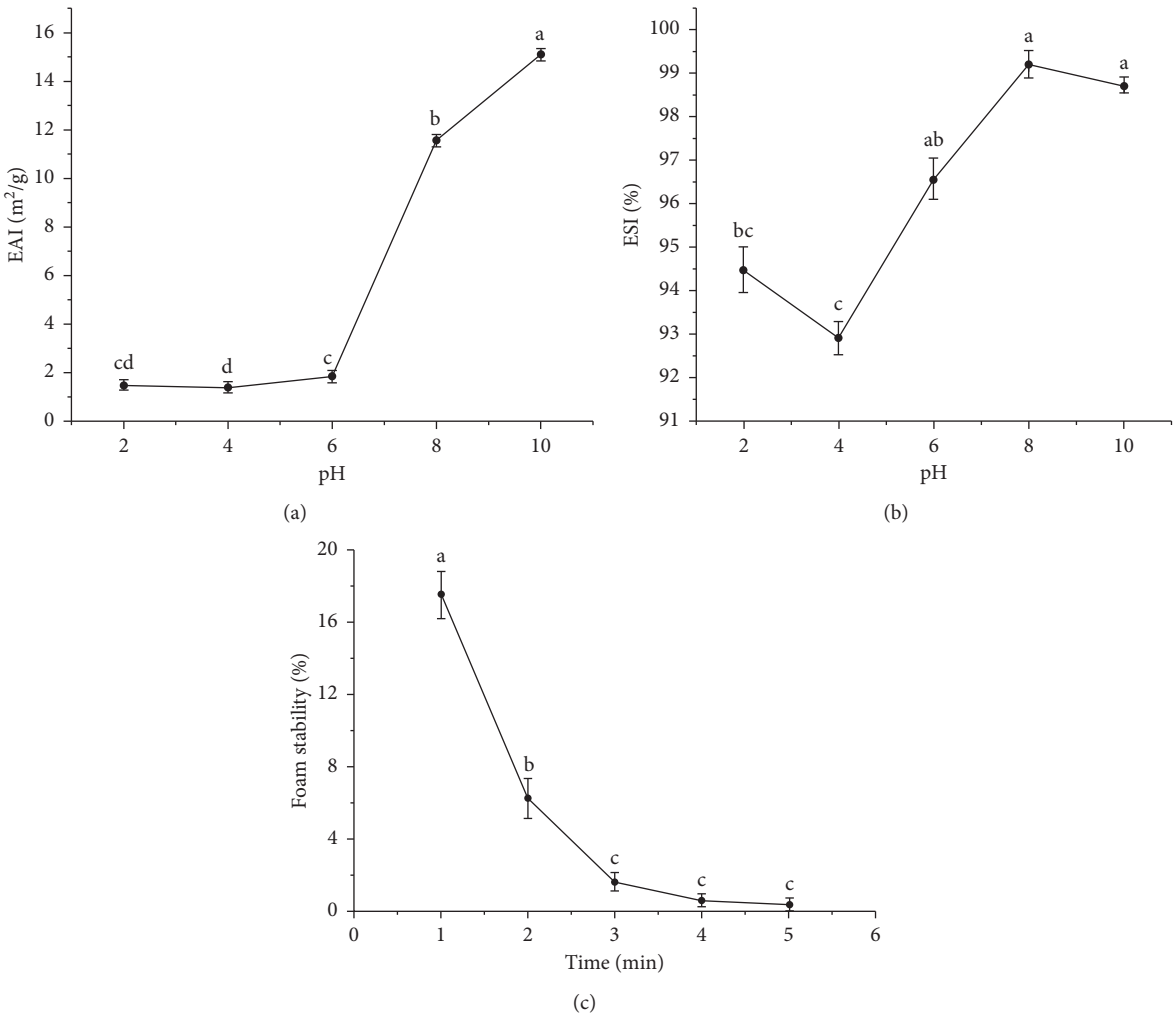


Figure 4: Functional properties of SH-3 at different pH: (a) emulsion activity index; (b) emulsifying stability index; and (c) foam stability at different time. Data are expressed as means ± SEM. Values without a common letter are significantly different at $P < 0.05$.

Table 4: Functional properties of SH-3 obtained under optimal enzymolysis conditions.

Functional property	Capacity
WHC (g water/g protein)	1.90 ± 0.12
OHC (g oil/g protein)	2.35 ± 0.05
Foam capacity (%)	73.75 ± 2.58

Table 5: IC_{50} value of SH and its fractions by ultrafiltration on DPPH radical scavenging activity and ABTS radical scavenging activity.

Fraction	IC_{50} DPPH radical scavenging activity (mg/mL)	IC_{50} ABTS radical scavenging activity (mg/mL)
SH	1.215 ± 0.081^{b}	1.563 ± 0.080^{B}
>10 kDa	1.968 ± 0.095^{a}	2.215 ± 0.109^{A}
3–10 kDa	1.264 ± 0.105^{b}	1.603 ± 0.063^{B}
<3 kDa	1.043 ± 0.090^{c}	1.396 ± 0.074^{C}
GSH	0.007 ± 0.001^{d}	0.009 ± 0.002^{D}

GSH was used as positive control. Data are expressed as means ± SEM. Values without a common letter are significantly different at $P < 0.05$.

the published reports on antioxidant peptides [41]. However, GSH showed higher antioxidative ability compared to SH.

4. Conclusion

In this study, underutilized sturgeon muscles were successfully transformed to bioactive hydrolysates by enzymatic hydrolysis. Based on the single-factor experiments, RSM was used to optimize the experimental variables. The optimum enzymolysis conditions were as follows: pH = 9.0, enzymolysis time = 4.92 h, enzymolysis temperature = 55°C, solid/liquid ratio = 1 : 20, and enzyme additive amount = 7674.22 U/g protein. Under these conditions, the NO inhibition rate of SH was 60.23%. After ultrafiltration of SH, SH-3 (SH < 3 kDa) showed the best inhibitory effect of NO and proinflammatory cytokine IL-6 by 66.24% and 39.54%, respectively. In addition, we found that SH-3 had good properties of emulsification and possessed good WHC and OHC. SH-3 was also found to exhibit stronger antioxidant capacity in DPPH and ABTS radical scavenging activity. These results suggest that SH-3 might be the potential safer alternative ingredient possessed of anti-inflammatory and antioxidative activities. However, the structure characterization and anti-inflammatory mechanism need to be further investigated.

Data Availability

All data generated or used during the study appear in the submitted article.

Conflicts of Interest

There are no conflicts of interest associated with the authors of this manuscript.

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References

[1] S.-K. Kim and I. Wijesekara, "Development and biological activities of marine-derived bioactive peptides: a review," *Journal of Functional Foods*, vol. 2, no. 1, pp. 1–9, 2010.

[2] E. Kati, G. Nina, and S. Katrin, "The ACE inhibitory dipeptide met-tyr diminishes free radical formation in human endothelial cells via induction of heme oxygenase-1 and ferritin," *Journal of Nutrition*.vol. 136, no. 8, pp. 2148–2152, 2006.

[3] H. G. Kristinsson and B. A. Rasco, "Fish protein hydrolysates: production, biochemical, and functional properties," *Critical Reviews in Food Science and Nutrition*, vol. 40, no. 1, pp. 43–81, 2000.

[4] Y. Z. Hong, E. M. Shin, L. Y. Guo et al., "Anti-inflammatory activity of 4-methoxyhonokiol is a function of the inhibition of iNOS and COX-2 expression in RAW 264.7 macrophages via NF-κB, JNK and p38 MAPK inactivation," *Planta Medica*, vol. 74, no. 9, pp. 340–349, 2008.

[5] D. B. Reddy and P. Reddanna, "Chebulagic acid (CA) attenuates LPS-induced inflammation by suppressing NF-κB and MAPK activation in RAW 264.7 macrophages," *Biochemical and Biophysical Research Communications*, vol. 381, no. 1, pp. 112–117, 2009.

[6] J.-Y. Je and S.-K. Kim, "Chitosan as potential marine nutraceutical," *Marine Medicinal Foods-Implications and Applications-Animals and Microbes*, vol. 65, no. 65, pp. 121–135, 2012.

[7] Y. Peng, R. Gan, H. Li et al., "Absorption, metabolism, and bioactivity of vitexin: recent advances in understanding the efficacy of an important nutraceutical," *Critical Reviews in Food Science and Nutrition*, pp. 1–16, 2020.

[8] P. Pacher, J. S. Beckman, and L. Liaudet, "Nitric oxide and peroxynitrite in health and disease," *Physiological Reviews*, vol. 87, no. 1, pp. 315–424, 2007.

[9] S. S. Rabinowitz, "Nitric oxide and inflammatory bowel disease," *Journal of Pediatric Gastroenterology & Nutrition*, vol. 27, no. 3, pp. 372-373, 1998.

[10] C. T. Ritchlin, S. A. Haas-Smith, P. Li, D. G. Hicks, and E. M. Schwarz, "Mechanisms of TNF-α- and RANKL-mediated osteoclastogenesis and bone resorption in psoriatic arthritis," *Journal of Clinical Investigation*, vol. 111, no. 6, pp. 821–831, 2003.

[11] Q. Zhao, J. F. Kennedy, X. Wang et al., "Optimization of ultrasonic circulating extraction of polysaccharides from Asparagus officinalis using response surface methodology," *International Journal of Biological Macromolecules*, vol. 49, no. 2, pp. 181–187, 2011.

[12] Y. Lin, M. Jiang, W. Chen, and Y. Wei, "Cancer and ER stress: mutual crosstalk between autophagy, oxidative stress and inflammatory response," *Biomedicine and Pharmacotherapy*, vol. 118, Article ID 109249, 2019.

[13] P. A. Harnedy and R. J. FitzGerald, "Bioactive peptides from marine processing waste and shellfish: a review," *Journal of Functional Foods*, vol. 4, no. 1, pp. 6–24, 2012.

[14] C.-B. Ahn, Y.-S. Cho, and J.-Y. Je, "Purification and anti-inflammatory action of tripeptide from salmon pectoral fin byproduct protein hydrolysate," *Food Chemistry*, vol. 168, pp. 151–156, 2015.

[15] F. Subhan, H. Y. Kang, Y. Lim, M. Ikram, S. J. O. M. Yoon, and C. Longevity, "Fish scale collagen peptides protect against $CoCl_2$/TNF- α-induced cytotoxicity and inflammation via inhibition of ROS, MAPK, and NF-κB pathways in HaCaT cells," *Oxidative Medicine and Cellular Longevity*, vol. 2017, Article ID 9703609, 17 pages, 2017.

[16] M. Ovissipour, A. Abedian, A. Motamedzadegan, B. Rasco, R. Safari, and H. Shahiri, "The effect of enzymatic hydrolysis time and temperature on the properties of protein hydrolysates from Persian sturgeon (Acipenser persicus) viscera," *Food Chemistry*, vol. 115, no. 1, pp. 238–242, 2009.

[17] S. Saidi, A. Deratani, M.-P. Belleville, and R. Ben Amar, "Antioxidant properties of peptide fractions from tuna dark muscle protein by-product hydrolysate produced by membrane fractionation process," *Food Research International*, vol. 65, pp. 329–336, 2014.

[18] H. Hu, Q. Zhao, J. Xie, and D. Sun, "Polysaccharides from pineapple pomace: new insight into ultrasonic-cellulase synergistic extraction and hypoglycemic activities," *International Journal of Biological Macromolecules*, vol. 121, pp. 1213–1226, 2019.

[19] P. W. M. L. H. K. Marambe, P. J. Shand, and J. P. D. Wanasundara, "An in-vitro investigation of selected biological activities of hydrolysed flaxseed (*Linum*

usitatissimum L.) proteins," *Journal of the American Oil Chemists' Society*, vol. 85, no. 12, pp. 1155–1164, 2008.

[20] A. Noman, Y. Xu, W. Q. AL-Bukhaiti et al., "Influence of enzymatic hydrolysis conditions on the degree of hydrolysis and functional properties of protein hydrolysate obtained from Chinese sturgeon (*Acipenser sinensis*) by using papain enzyme," *Process Biochemistry*, vol. 67, pp. 19–28, 2018.

[21] R. Pacheco-Aguilar, M. A. Mazorra-Manzano, and J. C. Ramírez-Suárez, "Functional properties of fish protein hydrolysates from Pacific whiting (*Merluccius productus*) muscle produced by a commercial protease," *Food Chemistry*, vol. 109, no. 4, pp. 782–789, 2008.

[22] J. Wasswa, J. Tang, X. Gu, and X. Yuan, "Influence of the extent of enzymatic hydrolysis on the functional properties of protein hydrolysate from grass carp (*Ctenopharyngodon idella*) skin," *Food Chemistry*, vol. 104, no. 4, pp. 1698–1704, 2007.

[23] C. Juliana, M. Adriana, M. Rosso, J. Tellechea, and J. García-Pardo, "Adding value to the chia (*Salvia hispanica* L.) expeller: production of bioactive peptides with antioxidant properties by enzymatic hydrolysis with Papain," *Food Chemistry*, vol. 274, pp. 848–856, 2019.

[24] H. Y. Luo, B. Wang, C. G. Yu, Y. Qu, and C. Su, "Evaluation of antioxidant activities of five selected brown seaweeds from China," *Journal of Medicinal Plants Research*.vol. 4, no. 23, pp. 2557–2565, 2010.

[25] B. Hernández-Ledesma, A. Quirós, L. Amigo, and I. Recio, "Identification of bioactive peptides after digestion of human milk and infant formula with pepsin and pancreatin," *International Dairy Journal*, vol. 17, no. 1, pp. 42–49, 2007.

[26] F. Guerard, L. Guimas, and A. Binet, "Production of tuna waste hydrolysates by a commercial neutral protease preparation," *Journal of Molecular Catalysis B: Enzymatic*, vol. 19-20, pp. 489–498, 2002.

[27] Y. Li, C.-p. Zhu, X.-c. Zhai et al., "Optimization of enzyme assisted extraction of polysaccharides from pomegranate peel by response surface methodology and their anti-oxidant potential," *Chinese Herbal Medicines*, vol. 10, no. 4, pp. 416–423, 2018.

[28] S. D. A. D. Santos, V. G. e. Martins, M. Salas-Mellado, and C. Prentice, "Evaluation of functional properties in protein hydrolysates from bluewing searobin (Prionotus punctatus) obtained with different microbial enzymes," *Food and Bioprocess Technology*, vol. 4, no. 8, pp. 1399–1406, 2011.

[29] A. Taheri, S. A. A. Anvar, H. Ahari, and V. Fogliano, "Comparison the functional properties of protein Hydrolysates from poultry byproducts and rainbow trout viscera," *Iranian Journal of Fisheries Sciences*, vol. 12, no. 1, pp. 154–169, 2013.

[30] N. R. A. Halim, H. M. Yusof, and N. M. Sarbon, "Functional and bioactive properties of fish protein hydolysates and peptides: a comprehensive review," *Trends in Food Science & Technology*, vol. 51, pp. 24–33, 2016.

[31] K. Surówka and M. J. I. J. o.F. S. Fik, "Studies on the recovery of proteinaceous substances from chicken heads I An application of neutrase to the production of protein hydrolysate," *International Journal of Food Science & Technology*, vol. 27, no. 1, pp. 9–20, 1992.

[32] Y. Thiansilakul, *Antioxidative Activity and Functional Properties of Protein Hydrolysate from Round Sead (Decapterus Maruadsi) Muscle*, Prince of Songkla University, Hat Yai, Thailand, 2006.

[33] N. R. Galla, P. R. Pamidighantam, S. Akula, and B. Karakala, "Functional properties and in vitro antioxidant activity of roe protein hydrolysates of Channa striatus and Labeo rohita," *Food Chemistry*, vol. 135, no. 3, pp. 1479–1484, 2012.

[34] C.-F. Chi, Z.-H. Cao, B. Wang, F.-Y. Hu, Z.-R. Li, and B. Zhang, "Antioxidant and functional properties of collagen hydrolysates from Spanish mackerel skin as influenced by average molecular weight," *Molecules*, vol. 19, no. 8, pp. 11211–11230, 2014.

[35] A. Moro, G. D. Báez, G. A. Ballerini, P. A. Busti, and N. J. Delorenzi, "Emulsifying and foaming properties of β-lactoglobulin modified by heat treatment," *Food Research International*, vol. 51, no. 1, pp. 1–7, 2013.

[36] Y.-Z. Fang, S. Yang, and G. Wu, "Free radicals, antioxidants, and nutrition," *Nutrition*, vol. 18, no. 10, pp. 872–879, 2002.

[37] Y. Peng, Q. Sun, and Y. Park, "The bioactive effects of chicoric acid as a functional food ingredient," *Journal of Medicinal Food*, vol. 22, no. 7, pp. 645–652, 2019.

[38] N. M. Sarbon, "Antioxidant activity and functional properties of fractionated cobia skin gelatin hydrolysate at different molecular weight," *International Food Research Journal*, vol. 22, no. 2, pp. 651–660, 2015.

[39] I. Lassoued, L. Mora, A. Barkia, M.-C. Aristoy, M. Nasri, and F. Toldrá, "Bioactive peptides identified in thornback ray skin's gelatin hydrolysates by proteases from Bacillus subtilis and Bacillus amyloliquefaciens," *Journal of Proteomics*, vol. 128, pp. 8–17, 2015.

[40] Z. Li, B. Wang, C. Chi, Y. Gong, H. Luo, and G. Ding, "Influence of average molecular weight on antioxidant and functional properties of cartilage collagen hydrolysates from *Sphyrna lewini*, Dasyatis akjei and Raja porosa," *Food Research International*, vol. 51, no. 1, pp. 283–293, 2013.

[41] J. M. Lorenzo, P. E. S. Munekata, B. Gómez, F. J. Barba, M. Leticia, and P.-S. Cristina, "Bioactive peptides as natural antioxidants in food products–A review," *Trends in Foods Science & Technology*.vol. 79, pp. 136–147, 2018.