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Research Article

Preparation and Characterization of Antioxidant Peptides from Carrot Seed Protein

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Carrot is a very popular vegetable and used for culinary and cosmetic purposes. Carrot seeds can be used for treatment of hangovers and stimulating menstruation. In the present study, the carrot seed protein (CSP) extracted from carrot seed (*Daucus carota* L.) was hydrolysed by four proteases (papain, trypsin, neutrase, and alcalase). Alcalase hydrolysate exhibited the strongest DPPH radical-scavenging activity (DRSA). The optimum hydrolysis condition for the antioxidant peptide production from CSP was obtained using response surface methodology (RSM). The optimum condition was as follows: hydrolysis time of 3.50 h, substrate concentration of 52.8 g/L, and protease dosage of 419.36 U/g, under which DRSA of 82.46% at 2 mg/mL was obtained. The carrot seed protein hydrolysates (CSPHs) were separated using size exclusion chromatography in order to obtain peptides with stronger antioxidant activity. The hydrolysates were fractionated into four peaks, and fractions F3 and F4 with smaller molecular weight showed stronger antioxidant activity. These findings indicated that the success of RSM in optimizing the hydrolysis conditions and the further work in separation of antioxidant peptides in CSPH is feasible. The CSPH exhibites good antioxidant properties and can be used as useful ingredient in foods.

1. Introduction

Reactive oxygen species can damage cells or tissues and lead to aging, inflammation, coronary heart disease, diabetes mellitus, stroke, and cancer [1, 2]. Although endogenous antioxidants can prevent oxidative damage, exogenous antioxidants are necessary to maintain the oxidative and antioxidative balance in healthy biological systems. According to the published research, commercial synthetic antioxidants, such as butylated hydroxytoluene (BHT), butylated hydroxylanisole (BHA), and propyl gallate, showed potential risks *in vivo*, and their usage should be strictly limited [3]. Compared with chemical antioxidants, antioxidant peptides can offer potential health benefits with high activity and easy absorption and have no side effects [4]. The search for antioxidant peptides from natural sources and food has gained growing interests among researchers. The enzymatic hydrolysis is mostly used in the preparation of antioxidant peptide in food industries. Lots of antioxidant peptides were obtained by proteolysis of various natural sources such as corn protein [5], sandfish [6], and *Octopus vulgaris* protein [7].

Carrot, scientifically *Daucus carota* L. (Umbelliferae), is a very popular vegetable cultivated world-wide and popularly used for culinary and cosmetic purposes [8]. The carrot seeds are collected after maturation and then dried. The extraction of carrot seeds showed healthy benefits, such as cardio- and hepatoprotective, cholesterol-lowering, antibacterial, antifungal, anti-inflammatory, and wound-healing benefits [9]. So far, there have been research on the small chemical elements and essential oil [8] and little reports on the protein and their hydrolysate from carrot seeds. Carrot seed protein hydrolysates with antioxidant properties could be applied in food and increase its value in the market.

Response surface methodology (RSM) is a very useful method to optimize the factors that influence the hydrolysis processes and has been widely applied in the preparation of

antioxidant peptides [10, 11]. The objective of this work was to obtain peptides with potent antioxidant activity from carrot seed protein extraction by proteolysis. To achieve this goal, response surface methodology (RSM) was used to obtain the optimum hydrolysis conditions including hydrolysis time, substrate concentration, and protease dosage. In addition, the peptides with stronger activity were further purified by Sephadex G25 gel filtration chromatography.

2. Materials and Methods

2.1. Materials. Carrot seeds were obtained from a local market (Fuzhou, Fujian, China). Pepsin, trypsin, neutrase and alcalase were purchased from Notlas biotechnology Co., Ltd (Beijing, China). 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was a product of Sigma-Aldrich Co. (Pasadena, Texas, USA). All other chemicals and reagents were of analytical grade from Sinopharm Chemical Reagent Co., Ltd (Fuzhou, Fujian, China).

2.2. Extraction of Carrot Seed Protein. The carrot seed protein (CSP) was extracted on the basis of the published method [12] with some modifications. The carrot seed powder was suspended in borax sodium hydroxide buffer (pH 12.0) and then incubated at 55°C for 4 hours with constant agitation, and the buffer-to-sample ratio was 20 mL/g. The extract was centrifuged at 14,300 ×g for 20 min at 4°C using a XZ-21K refrigerated centrifuge (Xiangzhi Centrifuger Instrument Co., Ltd., Changsha, Hunan, China) after incubation. Then, the collected supernatant pH was adjusted to 4.5. After maintaining at 4°C for 30 min to allow protein aggregation, the suspension was centrifuged at 14,300 ×g (15 min, 4°C). The protein precipitate was collected and lyophilized (FD-1C-50, Boyikang Experimental Instrument Co., Ltd., Beijing, China) after separation and stored under −20°C before further use.

2.3. Preparation of Protein Hydrolysis. To choose the optimum enzyme to hydrolysate carrot seed protein (CSP), four representative proteases (papain, trypsin, neutrase, and alcalase) were used in the preliminary hydrolysis. Briefly, the protein powder was dissolved in buffer and homogenized before hydrolysis and then placed in water bath to the optimum temperatures for four enzymes before the addition of a proper amount of protease with the same activity. The detailed conditions for four proteases are list in Table S1. Each solution was incubated with stirring (SKY-110WX, Sukun Industry & Commerce Co., Ltd., Shanghai, China) and then heated in a boiling water bath for 10 min to inactivate the enzyme. The pH of hydrolysate was adjusted to 4.5 with 1.0 mol/L HCl solution to precipitate the unhydrolysed CSP and then centrifuged at 14,300 ×g (30 min, 4°C), and the supernatant was lyophilized and stored under −20°C before further use.

2.4. Degree of Hydrolysis. Degree of hydrolysis can be used as a remarkable parameter (DH) in the hydrolytic process. It is defined as the ratio of free residues cleaved from proteins. It was evaluated in this study as the ratio of α-amino nitrogen (AN) to total protein nitrogen (TPN). AN was determined with the formaldehyde titration method described by Adler-Nissen [13], while TPN was calculated according to the Kjeldahl method [14].

Table 1: Independent variables and their coded and actual values used for optimization.

Independent variables	Units	Symbols	Coded levels		
			−1	0	+1
Hydrolysis time	h	X_1	0.5	2.0	3.5
Substrate concentration	% (g/100 mL)	X_2	1.0	3.5	6.0
Protease dosage	U/g	X_3	100	300	500

2.5. Single Factor Experiment. The effect of hydrolysis parameters for alcalase (i.e., hydrolysis time, substrate concentration, and protease dosage) was studied according to the method mentioned in Section 2.3. Different combinations of hydrolysis conditions were conducted as follows:

Effect of hydrolysis time: The hydrolysis of CSP was carried out at different times (0.5, 1, 2, 4, 6, and 8 h), while the extraction substrate concentration and protease dosage were fixed at 20 g/L and 4000 U/g, respectively.

Effect of substrate concentration: The hydrolysis of CSP was carried out at varying substrate concentrations (5, 10, 20, 40, 60, and 80 g/L), while the hydrolysis time and protease dosage were set at 2 h and 4000 U/g, respectively.

Effect of protease dosage: The gradients for dosage of protease used in the experiment were set as 100, 200, 300, 400, 500, and 600 U/g, while the hydrolysis time and substrate concentration were fixed at 2 h and 20 g/L, respectively.

2.6. Experimental Design and Optimization. Response surface methodology was applied to identify the optimum hydrolysis conditions of alcalase. A Box–Behnken design (BBD) was applied [15]. As shown in Table 1, the factors used in the design were chosen based on the results of single factor experiments using software Design-Expert 8.0 (Stat-Ease Inc., USA). A total of 17 runs (i.e., twelve factorial points and five central points) were carried out (Table 2). The antioxidant activity of the hydrolysates, evaluated by DRSA (Y), was taken as the response. BBD was applied with three factors and three levels, containing three replicates at the center point. The second order polynomial regression equation was given as

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j, \quad (1)$$

where Y was the dependent variable (i.e., DRSA); β_0 was the model constant; β_i, β_{ii}, and β_{ij} were the model coefficients; and X_i and X_j are levels of the independent variables. They represented the linear, quadratic, and interaction effects of the variables, respectively.

Table 2: The Box–Behnken design for optimizing hydrolysis conditions with DPPH radical-scavenging activity.

Run number	Coded levels of variable			Experimental values			Response value Y (%)
	X_1	X_2	X_3	Hydrolysis time (h)	Substrate concentration (%)	Protease dosage (U/g)	
1	0	1	1	2.0	6.0	500	77.58
2	−1	0	−1	0.5	3.5	100	60.55
3	−1	1	0	0.5	6.0	300	73.08
4	0	0	0	2.0	3.5	300	73.52
5	−1	0	1	0.5	3.5	500	73.61
6	1	1	0	3.5	6.0	300	78.04
7	0	−1	1	2.0	1.0	500	35.13
8	0	−1	−1	2.0	1.0	100	33.98
9	1	0	1	3.5	3.5	500	70.64
10	0	0	0	2.0	3.5	300	70.26
11	0	0	0	2.0	3.5	300	73.96
12	1	0	−1	3.5	3.5	100	64.34
13	0	0	0	2.0	3.5	300	73.43
14	−1	−1	0	0.5	1.0	300	36.36
15	0	0	0	2.0	3.5	300	66.37
16	1	−1	0	3.5	1.0	300	38.55
17	0	1	−1	2.0	6.0	100	63.72

2.7. Purification of CSPH. The lyophilized CSPH (300 mg) was redissolved in the distilled water and then loaded onto a gel filtration column (Sephadex G-25, $\Phi 1.6 \times 100$ cm). All fractions were eluted with distilled water at a flow rate of 0.3 mL/min and pooled after spectrophotometric measurements at 220 nm, which is the typical absorbance of peptide bonds.

2.8. Antioxidant Activity

2.8.1. DPPH Radical-Scavenging Activity. The DPPH radical-scavenging activity (DRSA) was measured according to the method previously published [16]. An aliquot of 500 μL sample solution was added to 500 μL of 0.04 (mg/100 mL) DPPH which was dissolved in 95% ethanol. The mixture was shaken and incubated for 30 min in dark at room temperature. Then, the absorbance of the mixture was recorded at 517 nm. Ethanol was used for the blank, while distilled water and Vc were used for negative and positive controls, respectively. The DPPH radical-scavenging activity (DRSA) was expressed using the following equation:

$$\text{DRSA}\,(\%) = \left(1 - \frac{A_i - A_j}{A_0}\right) \times 100, \tag{2}$$

where A_0, A_i, and A_j are the absorbance of the control, sample, and blank, respectively.

2.8.2. ABTS Radical-Scavenging Activity. The ABTS radical-scavenging activity was determined according to our previous published methods [16]. The $ABTS^+$ radical was generated by adding ABTS stock solution (7 mM) into $K_2S_2O_8$ solution (2.45 mM), and the mixture was incubated in dark for 16 h at room temperature. The generated $ABTS^+$ radical was diluted with phosphate buffer (5.0 mM, pH 7.4) to an absorbance of 0.70 ± 0.02 at 734 nm, and then 0.5 mL sample was added to 0.5 mL of diluted $ABTS^+$ radical solution. The mixture was kept in the dark for 10 min and detected at 734 nm. Distilled water and Vc were used as the blank and positive control, respectively. The $ABTS^+$ radical-scavenging activity was calculated as follows:

$$\text{ABTS radical} - \text{scavenging activity}\,(\%) = \frac{A_0 - A_s}{A_0} \times 100, \tag{3}$$

where A_0 is the absorbance of the blank control and A_s is the absorbance of the sample.

2.8.3. Chelating Activity. The ability of fractions from G25 gel filtration to chelate metal ions was obtained using the method published previously [17] with slight modification. Firstly, an aliquot of 200 μL of peptide was mixed with 10 μL 2 mM $FeCl_2$ and 600 μL deionized water. Secondly, an aliquot of 20 μL ferrozine solution was added to the mixture and homogenized. The mixture was kept at room temperature for 10 min, and the absorbance was recorded at 562 nm. The water and EDTA (ethylene diamine tetraacetic acid) were used as the blank and positive control, respectively. The chelating activity was calculated as follows:

$$\text{Chelating activity}\,(\%) = \frac{1 - A_{\text{sample}}}{A_{\text{blank}}} \times 100. \tag{4}$$

2.8.4. Superoxide Radical-Scavenging Activity. The superoxide radical-scavenging activity of all fractions was investigated with our published method [16]. Briefly, 0.4 mL sample solution was mixed with 0.4 mL of Tris-HCl buffer (50 mM, pH 8.3). The mixture was kept at 25°C for 10 min, and then 0.1 mL pyrogallol (1.5 mM, dispersed in 1.0 mM HCl) was added into the mixture and then left to react for 5.0 min at 25°C. The absorbance was detected at 320 nm from 0.5 min intervals to 5.0 min. Distilled water and Vc were

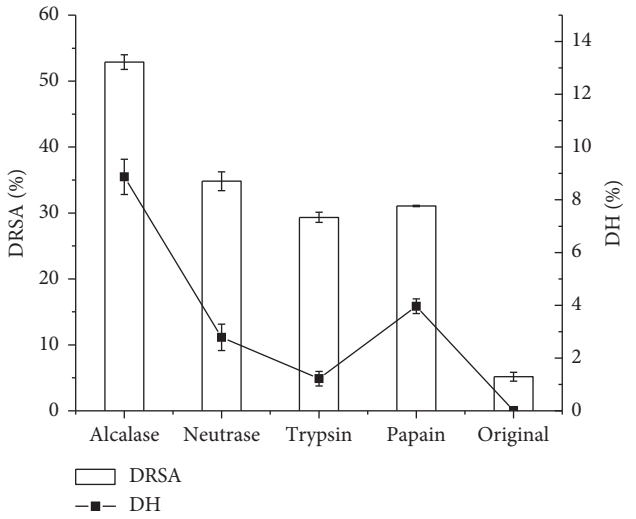

Figure 1: The DRSA and DH of CSPH prepared by different proteases. Error bars represent mean and SD from triplicate experiments.

used as the blank and positive control, respectively. The superoxide radical-scavenging activity was calculated as follows:

$$\text{Superoxide radical} - \text{scavenging activity}\,(\%) = \frac{(\Delta A_c/\text{min}) - (\Delta A_s/\text{min})}{\Delta A_c/\text{min}} \times 100, \quad (5)$$

where ΔA_c/min is the slope of the absorbance line of blank control and ΔA_s/min is the slope of the absorbance line of the sample mixture.

2.9. Statistical Analysis. Every DH and DRSA assay in this study was repeated three times, and the data were analyzed using SPSS 19.0 (SPSS, Chicago, IL, USA). The significance in differences was determined by Duncan's multiple range test ($P < 0.05$). The response values of the RMS model were analyzed using ANOVA.

3. Results and Discussion

3.1. Selection of Proteolytic Enzymes. Protease type could affect the DH and the antioxidant activity of protein hydrolysates [11]. Hence, it is important to choose an appropriate protease in order to derive the hydrolysates from protein with the highest antioxidant activity. DPPH radical-scavenging activity (DRSA) has been widely used in tracking the antioxidant activity of protein hydrolysates [18, 19] and was used in this study.

CSP was hydrolysed by papain, trypsin, neutrase, and alcalase, and the hydrolysates obtained possessed different DRSA (Figure 1). All these hydrolysates exhibited higher DRSA than CSP. In general, antioxidant peptides are composed of 2–20 amino acid residues [20, 21]. The antioxidant activity of peptide was higher than protein, and effected by both amino acid composition and molecular weight of the peptide [20]. The hydrolysate digested by alcalase exhibited the highest DRSA (52.90%), followed by neutrase, papain, and trypsin, respectively (Figure 1). A study on the specificity of alcalase revealed a preference for sites containing hydrophobic residues [22]. Specifically, the higher exposure of hydrophobic residues to peptides is associated with stronger antioxidant activities. The peptides with more hydrophobic amino acid residues are accessible to the water-lipid interface and can scavenge free radicals generated at the lipid phase [23]. The result implied that the peptides derived by alcalase may contain more hydrophobic amino acid residues.

The DH value of CSP was observed as 8.87% for alcalase, which is higher than that (<5%) detected for papain, trypsin, and neutrase, respectively. More low-molecular peptides could be obtained if proteins have a higher DH [24]. It is generally believed that smaller peptides possess higher radical-scavenging capacity than larger ones [25, 26]. Therefore, alcalase with the highest DRSA and DH was chosen as the optimum proteolytic enzyme.

Table 3: ANOVA for quadratic model.

Source	SS[a]	DF[b]	MS[c]	F-value	P value
Model	3994.9	9	443.88	60.78	<0.0001***
X_1	7.95	1	7.95	1.09	0.3315
X_1^2	0.11	1	0.11	0.015	0.907
X_2	2752.96	1	2752.96	376.98	<0.0001***
X_2^2	927.24	1	927.24	126.97	<0.0001***
X_3	147.62	1	147.62	20.21	0.0028**
X_3^2	69.60	1	69.60	9.53	0.0176*
$X_1 \cdot X_2$	1.92	1	1.92	0.26	0.6237
$X_1 \cdot X_3$	11.44	1	11.44	1.57	0.2509
$X_2 \cdot X_3$	40.38	1	40.38	5.53	0.051
Residual	51.12	7	7.30		
Lack of fit	9.39	3	3.13	0.30	0.8249
Pure error	41.73	4	10.43		
Cor total	4046.02	16			
R-Squared	0.9874				
Adj R-Squared	0.9711				
Pred R-Squared	0.9468				
Adeq Precision	22.046				

[a]SS, sum of square; [b]DF, degree of freedom; [c]MS, mean square; ***significant within a 99.9% confidence interval; **significant within a 99% confidence interval; *significant within a 95% confidence interval.

3.2. Optimization of Hydrolysis Conditions. The influence of different hydrolysis parameters on antioxidant activity of hydrolysates has been studied using RSM [14, 27]. Three factors including hydrolysis time (X_1), substrate concentration (X_2), and protease dosage (X_3) were selected in RSM based on the results of single factor experiments (Figure S1). The results of 17 treatments designed by the BBD method are presented in Table 2, which includes the design and response values. It was observed that values for DRSA ranged from 33.98 to 78.04% under different tested conditions. In addition, the statistical analysis for linear, quadratic, and interaction of the three variables (X_1, X_2, and X_3) on the DRSA (Y) was derived (Table 3). The results of ANOVA

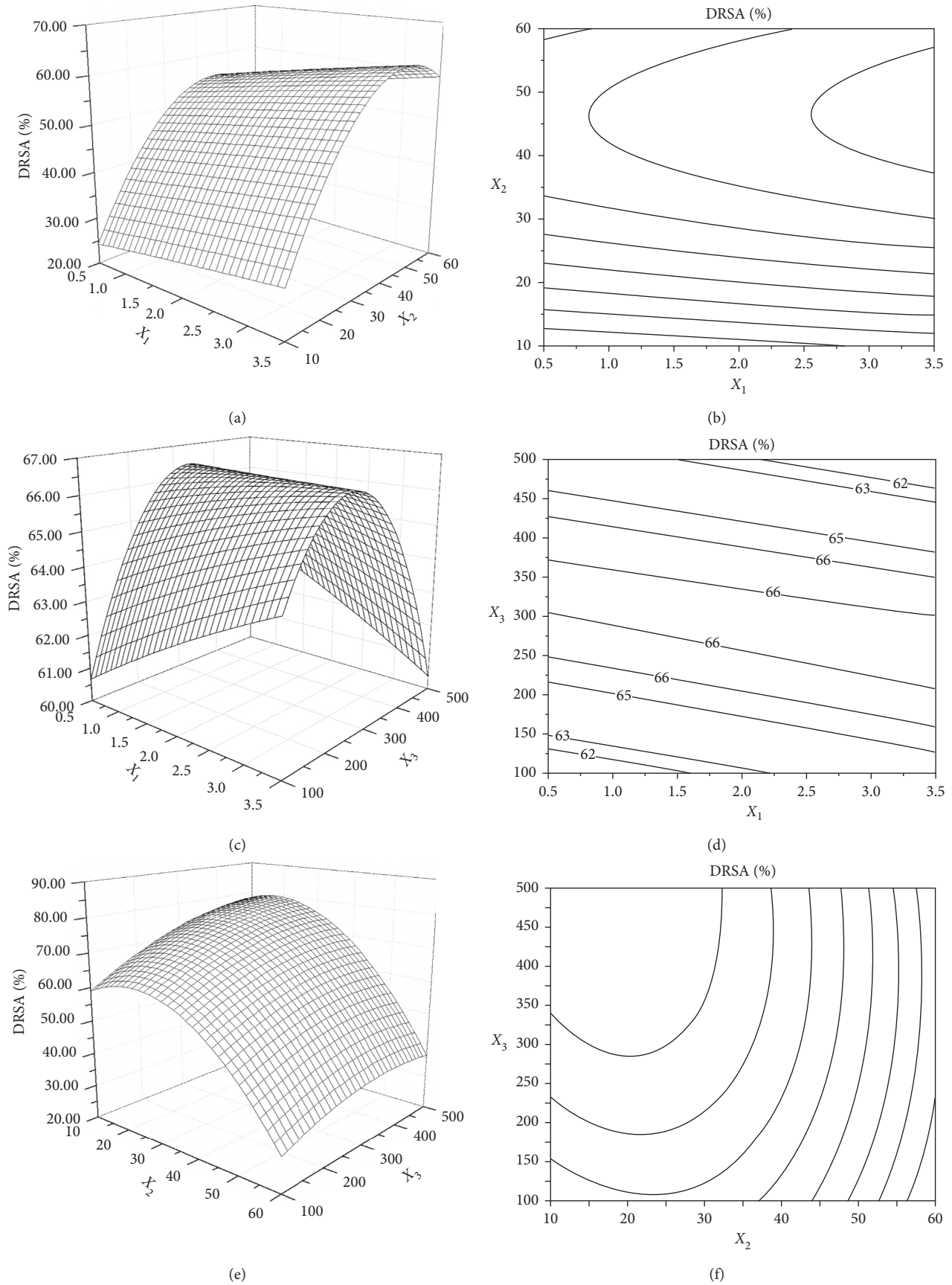

Figure 2: Response surface plots (a, c, and e) and contour plots (b, d, and f) for the effect of hydrolysis time (X_1), substrate concentration (X_2), and protease dosage (X_3) on the DRSA. (a and b indicate the effect of hydrolysis time, c and d indicate the effect of substrate concentration, and e and f indicate the effect of protease dosage).

indicated that the model was significant, demonstrated by that probability (P) value of the regression model was less than 0.0001. Among the three variables, substrate concentration (X_2) was the largest effect on the DRSA, followed by protease dosage (X_3). In contrast, the linear terms of hydrolysis time (X_1) were insignificant ($P > 0.05$). The result indicated that the substrate concentration and protease dosage were two primary factors in CSP hydrolysis and should be set strictly under optimum conditions, while the hydrolysis time was of no significance and did not affect DRSA within the experimental range.

The values of "R-Squared" (0.9874) (Table 3) and "Adj R-Squared" (0.9711) indicated that the model explains 97.11% of the variation in the data, and the experiment error was small. The "Pred R-Squared" of 0.9468 is in reasonable agreement with the "Adj R-Squared" of 0.9711. These results implied that the fitted model was significant and reliable. "Adeq Precision" evaluates the signal to noise ratio. A value higher than 4 is desirable [28]. This model had a high ratio of 22.046, which indicated an acceptable signal. In conclusion, this model expressed by the following equation was powerful for navigating the design space.

$$Y = 71.51 + 4.30X_1 + 18.55X_2 + 1.00X_3 + 3.18X_1X_2 - 1.69X_1X_3 + 0.69X_2X_3 - 4.07X_1^2 - 14.84X_2^2 - 0.16X_3^2. \quad (6)$$

The 3D response surface plots are convex in shape (Figures 2(a), 2(c), and 2(e)), indicating that there was a maximum predicted value of DRSA, and the optimum points were within the design limits. The two-dimensional contour plots were shown in Figures 2(b), 2(d), and 2(f), implying the significance of interaction between variables. Figures 2(a) and 2(b) showed that increase in hydrolysis time increased DRSA slightly. This may be because increase in hydrolysis time increases degree of hydrolysis and releases more antioxidant peptides. The DRSA increased steadily and reached to the highest when the substrate concentration was 50 g/L. Higher substrate concentration can increase chances of contact between substrate and enzyme and lead to higher release of antioxidant peptides. When substrate concentration was higher than 50 g/L, DRSA decreased because of the dilution of enzyme concentration. As shown in Figures 2(c) and 2(d), DRSA increased as the enzyme concentration was increased from 100 U/g to 400 U/g because higher enzyme concentration provides more chances for the hydrolysis to occur and releases more antioxidant peptides. However, DRSA decreased when more enzymes were added, and the excess enzyme might increase the concentration of the system and limit the enzyme activity [29]. At lower enzyme concentrations, DRSA increased when hydrolysis time increased; on the contrary, DRSA decreased when hydrolysis time increased at higher enzyme concentrations. This was likely because increased hydrolysis time at lower enzyme concentration will increase the hydrolysis and produce more antioxidant peptides from proteins. However, at higher enzyme concentration, increased hydrolysis time might cause more antioxidant peptide to hydrolyse into peptides or amino acids without antioxidant activities [14]. As shown in Figures 2(e) and 2(f), the result was the same as Figures 2(a) and 2(e), DRSA increased at first and decreased afterwards due to the increase of substrate concentrations or enzyme concentrations. The interaction between protease dosage and substrate concentration is significant, demonstrated by the elliptical shape of the contour plots.

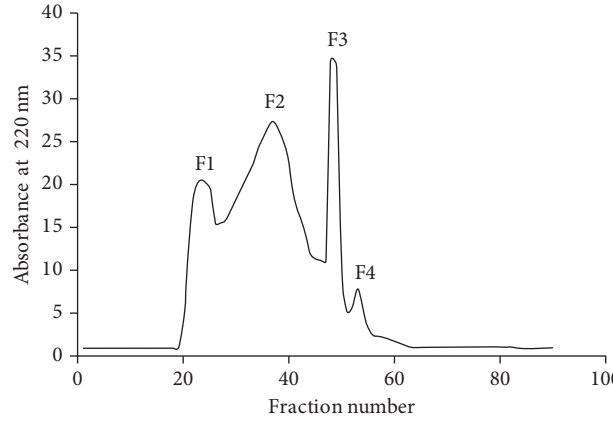

Figure 3: Elution profiles of CSPH separated by gel filtration chromatography on a Sephadex G-25 column. (Column size: $\Phi 1.6 \times 100$ cm; flow rate: 0.3 mL/min; elution solution: deionized water).

The optimum level of DRSA occurred with 79.98% by Design-Expert software at hydrolysis time (X_1) of 3.50 h, substrate concentration (X_2) of 52.8 g/L, and protease dosage (X_3) of 419.36 U/g, respectively. To verify the efficacy of the model, we performed three assays under the optimum condition. The average DRSA of 82.46% agreed with the predicted result by the model within a 95% confidence interval. In conclusion, the results certified that the model was valid and can be used in the optimizing process of hydrolysis. The carrot seed powder was redissolved in distilled water and hydrolysed by alkaline protease under the optimum conditions: hydrolysis time of 3.50 h, substrate concentration of 52.8 g/L, protease dosage of 420 U/g, temperature of 45°C, and pH of 10.0. The carrot seed protein hydrolysate (CSPH) derived was lyophilized and stored in −20°C for further analysis.

3.3. Peptide Purification and Antioxidant Assay. The size of peptide is important for the antioxidant activity, and smaller molecular weight has excellent antioxidant activity and is easy to be absorbed [30]. To obtain the peptides with higher antioxidant activity, CSPH was purified by gel filtration chromatography on Sephadex G-25 column, and four fractions (F1, F2, F3 and F4) were eluted (Figure 3). The antioxidant activities of the purified fractions were assayed by DPPH radical-scavenging activity, $ABTS^+$ radical-scavenging activity, superoxide radical-scavenging activity, and reducing power. Although fraction F1 possessed the highest molecular weight, it exhibited the strongest antioxidant activity (Figure 4). The reason is that F1 may include pigment that contributed to its high activity considering the deep color of it. The fraction F2 and F4 showed higher $ABTS^+$ radical-scavenging activity than F3, whereas they showed lower DPPH radical-scavenging

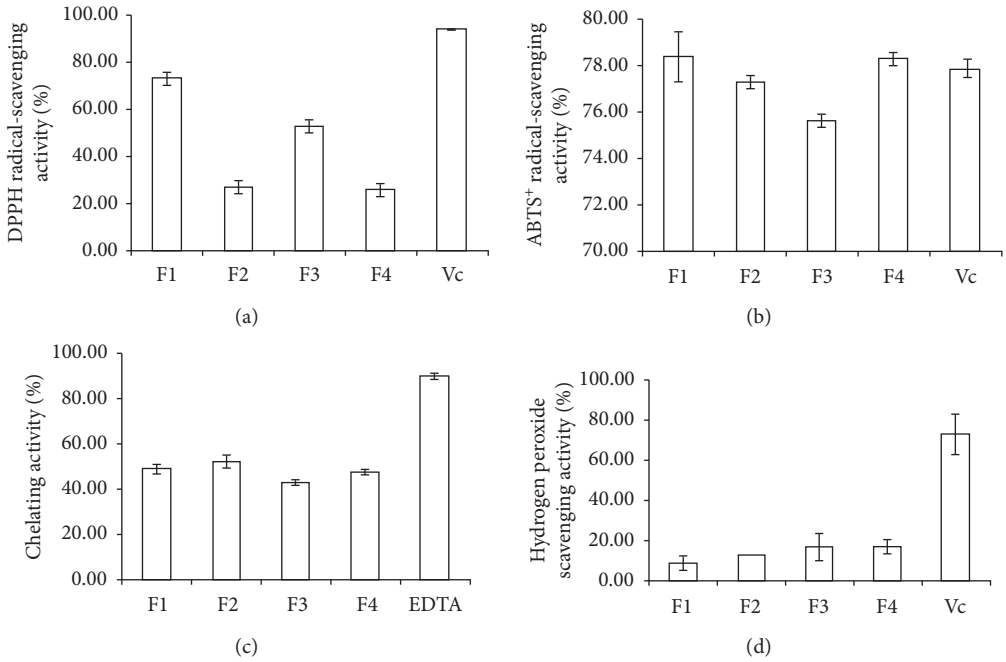

Figure 4: The DRSA of the eluted peaks from Sephadex G-25 column. All the results are triplicates of mean ± SD.

activity than F3. Although both DPPH and $ABTS^+$ radicals are eliminated by antioxidants in electron-transfer way [31], the two radicals with different property showed different selectivity in antioxidants. $ABTS^+$ radicals dissolve in aqueous media, but DPPH radicals resolve in alcoholic media; different solvent environment may affect the interaction between antioxidant peptides and radicals [16]. The different peptide compositions in fraction F2 and F4 may lead to different scavenging activity for $ABTS^+$ and DPPH radicals. It was reported that high Gly and His content provided higher iron-chelating activity [32]. Both F2 and F4 exhibited potent chelating activity, implying that they have high content of Gly and His in the amino acid composition. All fractions showed weak superoxide radical-scavenging activity (Figure 4(d)), which could be due to limited solubility of the oxygen species in water or different structure of peptides [33]. It is also reported that most antioxidant peptides hydrolysed from food protein by proteolytic enzyme were short peptides with residues less than 20 [4]. In the same case, the fractions F3 and F4 possessed higher antioxidant activities than hydrolyses and other fractions. The antioxidant activity of peptides was also related with the amino acid composition and structure. Therefore, further work has been planned to separate and characterize the antioxidant peptides in order to elucidate the antioxidant activity more clearly.

4. Conclusions

The hydrolysate with antioxidant activity from protein in various kinds of food and its by-products has been investigated widely. The optimum hydrolysis condition of carrot seed protein (CSP), which obtained hydrolysates with excellent DRSA, was studied using RSM in the present study. The results of response surface methodology indicated that the optimum hydrolysis conditions to derive antioxidant peptides were as follows: hydrolysis time of 3.50 h, substrate concentration of 52.8 g/L, and protease dosage of 419.36 U/g. The predicted maximum DRSA of 79.98% obtained was in consonance with the experimental value (82.46%) within a 95% confidence interval, indicating a good fit between the model and the experimental data. The results indicated that RSM was successfully applied in the optimization of hydrolysis condition to obtain the best DRSA. The hydrolysate of CSP which is a rich and safe source could be exploited as a promising addictive to prevent food oxidative damage. In addition, the peptides with stronger antioxidant activity were obtained using the size exclusion chromatography. Although more work should be done, the results could supply basic data for further research on the purification of antioxidant peptides.

Abbreviations

CSP: Carrot seed protein
DPPH: 1,1-Diphenyl-2-picrylhydrazyl
DRSA: DPPH radical-scavenging activity
RSM: Response surface methodology
BBD: Box–Behnken design
ANOVA: Analysis of variance
CSPH: Carrot seed protein hydrolysate
DH: Degree of hydrolysis
Vc: Vitamin C (L-ascorbic acid).

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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Supplementary Materials

Figure S1: single factor experiment. (a) Effect of hydrolysis time on the DRSA and DH; (b) Effect of substrate concentration on the DRSA and DH; (c) Effect of protease dosage ratio on the DRSA and DH. Table S1: the hydrolysis conditions for different proteases. (*Supplementary Materials*)

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