International Journal of Biological Macromolecules 51 (2012) 244–249

Contents lists available at SciVerse ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Improvement of antioxidant activity of peptides with molecular weights ranging from 1 to 10 kDa by PEF technology

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ARTICLE INFO

Article history:
Received 23 April 2012
Accepted 14 May 2012
Available online 19 May 2012

Keywords:
Pulsed electric field (PEF)
Peptides with molecular weights ranging from 1 to 10 kDa
Antioxidant activity
Response surface methodology (RSM)

ABSTRACT

Egg white protein powder was hydrolyzed with Alcalase to produce antioxidant peptides. Then, the peptides were fractionated with ultrafiltration membranes. The peptides (1–10 kDa) were further treated by pulsed electric field (PEF) to investigate its effect on the antioxidant activity of the peptides. Antioxidant activity was evaluated using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical inhibition assay. The results indicated that optimal electric field intensity and standing times of PEF can enhance the antioxidant activity of the peptides. Therefore, a Box-Behnken design (BBD) with three independent variables including concentration, electric field intensity and pulse frequency was used to establish the regression equation of second-order response surface. The optimal conditions were as follows: concentration 8 mg/ml, electric field intensity 10 kV/cm and pulse frequency 2400 Hz. Under these conditions, the peptides antioxidant activity was 62.64% ± 0.98%. The present study demonstrated that the antioxidant activity of peptides (1–10 kDa) could be improved using PEF.

1. Introduction

Recently, emerging interest has been focused on identification and characterization of antioxidant peptides from plant and animal protein sources. These peptides are considered specific protein fragments that are inactive within the sequence of the parent protein. They can exert radical scavenging, lipid peroxidation inhibition and metal ion chelation properties [1]. The hydrolysates, derived from egg white protein (EWP), also showed excellent antioxidant activity. Huang et al. [2] used thermolysin and pepsin to hydrolyze the EWP ovotransferrin, and a hydrolysate fraction was obtained that possessed stronger oxygen radical absorbance capacity than the nonhydrolyzed protein. You et al. [3] hydrolyzed lysozyme, separated from EWP, with Alcalase and the peptides had higher oxygen radical absorbance capacity-fluorescein value and 2,2′-Azino-bis(3-ethyl benzo thiazoline-6-sulfonic acid) diammonium salt radical cation scavenging activity. A recent report indicated that the peptides produced by hydrolyzing EWP powder with Alcalase possessed strong reducing power [4]. Many studies have investigated methods to improve antioxidant activities of peptides, however, most of them focused on optimizing the hydrolysis conditions, such as the degree of hydrolysis, the type of protease, and the peptide concentration [5–7].

Pulsed electric field (PEF) is a nonthermal food-processing technology which uses short bursts of electricity, providing fresh-like and safe foods with less detrimental quality changes compared to other techniques [8]. Until now, most of the studies in PEF have concentrated on the several aspects of food science including: (1) extracting bioactive compounds from raw material [9,10]; (2) extending food storage shelf life with food sterilization and enzyme inactivation [11]; and (3) maintaining physical–chemical properties and nutritional values of foods [12]. Moreover, PEF has been used to degrade the behavior of two pesticides, methamidophos and chlorpyrifos in apple juice [13]. There are some reports on the effects of PEF on antioxidant compounds in recent years. Compared to thermally treated fruit juice–soymilk beverages during refrigerated storage, a previous report indicated that PEF was a feasible technology to obtain extended shelf-life of fruit juice–soymilk beverages with similar antioxidant characteristics as freshly made beverages [14]. Odriozola-Serrano et al. [15] compared the carotenoid and phenolic profiles of tomato

Abbreviations: EWP, egg white protein; PEF, pulsed electric field; MW, molecular weight; DPPH, 2,2-diphenyl-1-picrylhydrazyl; UF, ultrafiltration equipment; RSM, response surface methodology; BBD, Box-Behnken design; SD, standard deviation.

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http://dx.doi.org/10.1016/j.ijbiomac.2012.05.017

juices processed by PEF and conventional thermal treatments, and found that PEF-processed tomato juices maintained higher carotenoid (lycopene, neurosporene and γ-carotene) contents than the thermally treated and untreated juices during storage. Similarly, PEF-treated tomato juice maintained higher lycopene and vitamin C content than the thermally treated juices during storage [16]. All of these studies indicated that PEF affects the antioxidant ability of various compounds in a variety of matrices.

Therefore, the effect of PEF processing on improving antioxidant activity of the hydrolysates derived from EWP was evaluated. The aims of the experiment were: (1) optimize the PEF processing to obtain the best parameters; and (2) establish a technical foundation for further studies on the mechanism of structure alterations of antioxidant peptides with molecular weights (MW) ranging from 1 to 10 kDa.

2. Materials and methods

2.1. Materials and instruments

EWP powder (protein content of 80.96%) was obtained from Jinjianli Co. (China). Alcalase was purchased from Fanfuer International Chem (China). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma Chemicals Co. (USA). The ethanol, methanol and sodium hydroxide were purchased from Beijing Chemical Plant (China) and were analytical grade purity. Ultrafiltration equipment was obtained from Millipore (USA). The μQuant Bio-Tek microplate reader was purchased from BioTek Instruments (USA). PEF system was self-designed by Yongguang Yin and described in previous report [17].

2.2. Preparation of antioxidant peptides

The antioxidant peptides were prepared from EWP as described in Lin et al. [4]. Briefly, EWP powder was dissolved with deionized water to a final concentration of 5% (W/W) followed by a heat treatment at 90 °C in a water bath for 10 min to denature the protein. The pH value of EWP solution was adjusted to 11 and incubated in 50 °C water bath. Then, 3% of Alcalse (W/W) was added to EWP solution. The solution was incubated for 3 h in a continuous stirred vessel by controlling the pH at 11 ± 0.05 by adding 1 M NaOH. After the reaction, enzyme activity was terminated by heating at 90 °C for 10 min in a water bath. Then, the hydrolysates were centrifuged at 2100 × *g* for 10 min at 4 °C. Ultrafiltration equipment (UF) was used to fractionate enzymatic hydrolysates to obtain the antioxidant peptides with MW ranging from 1 to 10 kDa. The supernatant was subjected to UF first, through the 10 kDa membrane. The permeated fraction was further treated using a 1 kDa UF membrane. The retentate was freeze-dried, placed in sealed bags, and stored in desiccators until use.

2.3. Determination of antioxidant activity

Antioxidant activity was determined by using the stable DPPH radical following the method described by Yu et al. [18] with modifications. An aliquot of 100 μl of the peptides solution was mixed with 100 μl of 0.6 mM of freshly prepared DPPH and 100 μl of methanol in 96-well microplate, shaken vigorously, and incubated in 37 °C water bath for 1.5 h. The absorbance was measured by a μQuant Bio-Tek microplate reader at 520 nm. The blank substituted 100 μl of methanol instead of the sample. The radical scavenging capacity of the tested sample was measured as a decrease in the absorbance of DPPH radical and was calculated by using the following equation [19]:

$$\text{DPPH radical inhibition}\,(\%) = \frac{A_B - A_S}{A_B} \times 100$$

where A_B is the absorbance of the blank at 520 nm and A_S is the absorbance of the sample at 520 nm. The DPPH test was conducted in triplicate.

Table 1
Independent variables and their levels used for Box-Behnken rotatable design.

Independent variable	Level		
	−1	0	1
Concentration (X_1)	6	8	10
Electric field intensity (X_2)	5	10	15
Electric field frequency (X_3)	2000	2400	2700

2.4. Effects of electric field strength and standing times on antioxidant activity

The freeze-dried antioxidant peptides powder was weighed and dissolved in distilled water. The pump and circuit were washed with distilled water and ethanol 2–3 times. Subsequently, the solution of peptides was pumped into the PEF system at a flow velocity of 1.6 ml/min. Then, the high-voltage pulse was turned on, and the charge voltage and pulse frequency were adjusted to the desired level. After processing for the desired number of minutes, the high-voltage pulse was turned off. The effect of electric field strength on antioxidant activity was optimized first. A solution whose concentration was 10 mg/ml was treated by the PEF at frequency of 2000 Hz with the electric field intensity of 0, 10, 20, and 30 kV/cm respectively. The antioxidant activity was assayed 2 h later after the PEF treatment. Hereafter, in order to evaluate the effect of standing times on improving peptides antioxidant activity, the peptides concentration, pulse frequency and electric field intensity were fixed at 8 mg/ml, 2400 Hz and 10 kV/cm, respectively. The antioxidant activity was assayed with standing times of 0, 2, 4, and 6 h respectively after the PEF treatment.

2.5. Experimental design of response surface methodology

Response surface methodology (RSM) was applied to identify optimum levels of three variables: concentration, electric field intensity and pulse frequency. RSM was used to model the peptide antioxidant activity improvement treated using PEF and to optimize the influence of these three variables. A Box-Behnken design (BBD) with three variables including three replicates at the centre point was used for fitting the second-order response surface. These three independent variables were the concentration (Z_1), electric field intensity (Z_2), and pulse frequency (Z_3), which were labeled as X_1, X_2 and X_3, respectively, as presented in Table 1. Each variable was coded at three levels: −1, 0 and +1. Triplicates at the center (0, 0 and 0) of the design were conducted to allow the estimation of the pure error sum of squares. The measured variable response was antioxidant activity. The order of the experiments had been fully randomized to minimize the effects of unexplained variability in the observed responses, which might be caused by extraneous independent variables. Analytical determinations were performed in triplicate. Data from the BBD were analyzed by multiple regressions to fit the following quadratic polynomial model:

$$Y = \beta_0 + \sum_{i=3}^{3}\beta_i X_i + \sum\sum_{i/j=1}^{3}\beta_{ij}X_iX_j + \sum_{i=3}^{3}\beta_{ii}X_i^2$$