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Research on the preparation of antioxidant peptides derived from egg white with assisting of high-intensity pulsed electric field

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ABSTRACT

Egg white protein powder, one of the main egg products, was hydrolysed by Alcalase, Trypsin, and Pepsin respectively to prepare antioxidant peptides. All hydrolysates were assayed by determination of reducing power (RP) ability. Three kinds of hydrolysates were prepared under optimal enzymatic parameters that were obtained from the preliminary one-factor-at-a-time (OFAT) and response surface methodology (RSM) experiments. The results showed that the Alcalase hydrolysates exerted the best RP ability. Thereafter, the Alcalase hydrolysates were sequentially fractionated by ultra filtration membranes in cut-off molecular weight (MW) of 30, 10, and 1 kDa, and tested their antioxidant activities in terms of RP ability, DPPH radical scavenging ability, ABTS radical scavenging ability, and FRAP assay. Effects of high intensity pulsed electric field treatment were further investigated on antioxidant peptides to improve their activities. The results showed that Alcalase hydrolysates possessed the strongest antioxidant ability compared with the other two hydrolysates, particularly for the Fraction-3 with MW <1 kDa. After PEF treatment, this fraction showed a significant improvement of RP ability within 5 h ($P < 0.05$).

1. Introduction

Nowadays, many researchers have focused on the bio-active function of polypeptides. Some peptides have shown the ability to scavenge superfluous free radicals and restrain lipid peroxidation in vivo. They can also help the human body to resist various kinds of diseases, such as atherosclerosis, cardiovascular disease, cancer, neurological degenerative diseases, and others (Wei & Lee, 2002). Recently, myriad types of antioxidant peptides derived from animals and plants have been studied. Saiga, Tanabe, and Nishimura (2003) made use of enzymatic hydrolysis to produce pork myofibril protein, the hydrolysates showed strong antioxidant ability in the iron-catalysed peroxidant linoleic acid system. Je, Qian, and Kim (2007) had studied the antioxidant ability of bullfrog hydrolysates, which were purified by consecutive chromatography. The results showed that the antioxidant compound has the following amino acid sequence: Leu-Glu-Gln-Gln-Val-Asp-Asp-Leu-Glu-Gly-Ser-Leu-Glu-Gln-Glu-Lys-Lys. The peptide whose molecular weight is 1.988 kDa has similar scavenging ability of DPPH, hydroxyl radical, and superoxide radical as vitamin C. Xu et al. (2006) hydrolysed soybean protein by Pepsin with the hydrolysis temperature of 50 °C at pH 8 and a Sephadex G-25 column was used to separate the hydrolysates, and obtain the active soybean peptide. Pedroche et al. (2007) underutilised an immobilised enzyme method to process the protein of *Brassica carinata* seeds, and obtained two kinds of peptides whose molecular weights were 1.4 and 1.8 kDa, these two kinds of peptides both showed strong antioxidant ability.

High-intensity pulsed electric field (PEF) processing is a potential complement to, or replacement of traditional thermal pasteurisation. PEF processing has been shown to effectively inactivate microorganisms and various kinds of enzymes without affecting the antioxidative potential of peptides in foods (Elez-Martínez & Martín-Belloso, 2007). Results from several studies indicate that field strength, pulse duration, number of pulses and pulse shape are the main variables affecting activity of proteins (Peña, Salvia-Trujillo, Rojas-Graü, & Martín-Belloso, 2010). Li, Chen, and Mo (2007) found that short pulse duration had little effects on the soybean protein isolates (SPI), while longer pulse duration caused a structure change of SPI. Elez-Martínez and Martín-Belloso (2007) compared the content of vitamin C retention and DPPH inhibition of orange juice processed by PEF and thermal pasteurisation, and found that the content of vitamin C retention could reach to 87.5–98.2% in PEF-treated sample, and the DPPH radical inhibition showed little difference compared with control, while the

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DPPH radical inhibition of the sample processed by thermal treatment significantly decreased. All these studies indicate that PEF processing has effects on the antioxidant ability of various components.

Egg proteins are important components for the development of functional foods and nutraceuticals. Many previous studies have already reported that egg protein hydrolysates or peptides could exert different biological activities, such as antibacterial, antifungal, antiviral, anticarcinogenic, antimutagenic, antiinflammatory, antihypertensive, as well as antioxidant activities (Erdmann, Cheung, & Schröder, 2008). This paper used the egg white protein powder as raw material to produce antioxidant peptides. Then the hydrolysates were separated by ultra filtration membranes in cut-off MW of 30, 10, and 1 kDa to obtain the fraction with different molecular weights (MW). The fractions were further assayed for their antioxidant ability. Finally, PEF was used to process the fraction which showed the best antioxidant activity, and evaluated its effects on these fractions.

2. Materials and methods

2.1. Materials and reagents

Egg white protein powder (protein content in 80.96%) was purchased from Jinjianli Co. (Peking, China). Alcalase were purchased from Fanfuer International Chem. Co. (Tianjin, China). 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2′-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), potassium persulfate, fluorescein, 2,2′-azobis (2-methylpropionamidine) dihydrochloride (AAPH) were purchased from Sigma–Aldrich Company (St. Louis, Missouri, USA). All other chemical reagents required in the experiments were purchased from Peking Chemical Plant (Beijing, China). All of the chemicals and reagents were of analytical grade.

2.2. Instruments and equipments

The PEF system was homemade and has been described in our previous report (Jin et al., 2011; Yin, Han, & Han, 2006). It includes a high-voltage repetitive pulse generator, a coaxial liquid materials temperature sensing instrument, and a pump. It can generate exponentially decaying bipolar triangle pulse waveforms with pulse duration of 2 μs. The frequency is adjustable, ranging from 1000–3000 Hz. The system can process samples continuously. The pulse number (C) can be calculated by the equation of $C = 2L\pi r^2 f/Q$, while the electric field intensity is expressed in $E = Vpp/2L$, where f is the frequency (Hz), L is the length of electrode (cm), r is the radius of electrode (cm), Q is the flow velocity (mL/s) of sample, E is the electric field intensity (kV/cm), and Vpp is the input voltage shown on the oscillograph. In this paper, L is 0.1 cm; r is 0.05 cm; Q is 4 mL/s, other parameters were changed with the experimental condition.

2.3. Enzymatic hydrolysis

one-factor-at-a-time variable experiments (OFAT) and response surface methodology (RSM) were used to optimise the enzymatic hydrolysis parameters for preparing antioxidant peptides by Alcalase, Trypsin and Pepsin, respectively. The substrate was dissolved in water, and then heated in 90 °C water bath for 10 min to denature the protein. After that, the substrate was cooled to room temperature, and then the pH was regulated to the desired level. Then, the desired concentration of enzyme was added to the solution. The mixture was incubated in a constant temperature bath at each optimal temperature with stirring and then heated in 90 °C water for 10 min to inactivate the enzyme. The concentration of egg white solution [S] (1%, 3%, 5%, and 7%), ratio of enzyme/egg white powder ([E/S]) (1%, 3%, 5%, and 7%), incubation time (1, 2, 3, and 4 h), temperature at 40, 45, 50, 55, and 60 °C for the Alcalase treatment, and at 34, 37, 40, 43, and 46 °C for the Pepsin and Trypsin, the pH value designed for Alcalase (8, 9, 10, 11, 12 and 13), Pepsin (1.0, 1.5, 2.0, 2.5, and 3.0) and Trypsin (7.0, 7.5, 8.0, 8.5, and 9.0), respectively. During the hydrolysis, the pH value was adjusted with 1 N NaOH for the Alcalase and Trypsin treatments and 1 N HCl for the Pepsin treatment, respectively, to keep the pH value change within ±0.05. The OFAT experiment design was shown in Tables 1a–c. From the results obtained from OFAT experiments, [S], [E/S], and pH value at three levels (3^3) were adopted for the response surface methodology (RSM) using the Box-Behnken design (BBD) by the Design Expert Software (Trial Version 7.0.0, Stat-Ease Inc., Minneapolis, MN, USA). The three independent variables were labelled as X_1, X_2 and X_3, respectively, as shown in Table 2.

Table 1a
The design proposal and results of OFAT experiments.

Factor		Enzymes		
		Alcalase	Pepsin	Trypsin
[S] (%)	1	0.758 ± 0.002$^{a\#*}$	0.586 ± 0.052^{a}	0.116 ± 0.043^{a}
	3	1.187 ± 0.059$^{b\#*}$	0.907 ± 0.022^{b}	0.285 ± 0.056^{b}
	5	1.498 ± 0.006$^{c\#*}$	0.970 ± 0.072^{c}	0.330 ± 0.002^{c}
	7	1.474 ± 0.002$^{c\#*}$	0.964 ± 0.002^{c}	0.356 ± 0.022^{d}
[E/S] (%)	1	1.467 ± 0.023$^{a\#*}$	0.858 ± 0.002^{a}	0.401 ± 0.034^{a}
	3	1.486 ± 0.034$^{b\#*}$	0.839 ± 0.054^{a}	0.796 ± 0.006^{b}
	5	1.470 ± 0.057$^{a\#*}$	0.776 ± 0.002^{b}	0.525 ± 0.056^{c}
	7	1.452 ± 0.002$^{c\#*}$	0.743 ± 0.002^{b}	0.523 ± 0.065^{c}
Hydrolysis time (h)	1	1.300 ± 0.020$^{a\#*}$	0.852 ± 0.000^{a}	0.688 ± 0.056^{a}
	2	1.326 ± 0.054$^{b\#*}$	0.780 ± 0.060^{b}	0473 ± 0.055^{b}
	3	1.457 ± 0.050$^{c\#*}$	0.808 ± 0.070^{c}	0.527 ± 0.089^{c}
	4	1.344 ± 0.087$^{b\#*}$	0.800 ± 0.030^{d}	0.482 ± 0.002^{d}

2.4. Separation and purification methods

Ultra filtration (UF) equipment (Millipore Minitan system, Millipore, Bedford, MA) was used to separate the Alcalase hydrolysates (AH) under the optimal hydrolysis parameters. The AH were separated by UF membranes in the cut-off MW of 1, 10, 30 kDa subsequently. The hydrolysates were first UF treated trough the 30 kDa membrane. The retentate (Fraction-1) and permeate fractions were collected separately and then the permeates were further UF treated trough the 10 kDa membrane. The retantates (Fraction-2) and permeates were once more collected and a final UF was applied to permeates by using a 1 kDa membrane to obtain the other fraction (Fraction-3). Each fraction was freeze-dried and stored in a desiccator. Thereafter, each fraction was tested their antioxidant activity in term of RP, DPPH radicals scavenging, ABTS radical scavenging, and FRAP assay.

2.5. Determination of antioxidant activities of enzymatic hydrolysates

2.5.1. Reducing power method

The reducing power (RP) method was measured using the method described by Oyaizu (1986) with a little modification. 1 mL hydrolysate (5 mg/mL) was mixed with 2.5 mL phosphate buffer (0.2 mol/L, pH 6.6) and 2.5 mL 1% potassium ferricyanide. The mixture was incubated in the 50 °C water bath for 20 min, and then added 2.5 mL 10% trichloroacetic acid solution. After mixing vigorously, the mixture was centrifuged for 10 min under the rotate rate of 12,000g. 2.5 mL supernatant was mixed with 0.1 mL 0.1% ferric chloride solution, and then mixed well. The