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Full Length Research Paper

Physicochemical properties of silkworm larvae protein isolate and gastrointestinal hydrolysate bioactivities

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The objectives of this study were to investigate the amino acid composition and thermal properties of silkworm larvae protein isolate (SLPI) and to evaluate the *in vitro* angiotensin-converting enzyme (ACE) inhibitory and antioxidant activities of its hydrolysate prepared with gastrointestinal enzymes. The results showed that, SLPI was a high quality protein source with a well-balanced composition of essential amino acids, which was especially rich in glutamic acid (13.79 g/100 g protein), aspartic acid (10.44 g/100 g protein), leucine (8.68 g/100 g protein), lysine (8.01 g/100 g protein) and arginine (6.59 g/100 g protein). In additon, three endothermic denaturation transitions were observed in DSC thermograms of SLPI. The maximum transition peak occurred in the third thermal transition, which denaturation temperature (T_d), peak temperature of denaturation (T_p) and enthalpy change (ΔH) were 76.95°C, 80.42°C and 783.75 J/g, respectively. SLPI hydrolysate exhibited strong ACE-inhibitory activity (IC_{50}=8.3 µg/ml) and relatively higher 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity (IC_{50}=57.91 µg/ml) and ferrous ions chelating capacity (IC_{50}=2.03 mg/ml). Moreover, the hydrolysate showed notable reducing power. It was concluded that, SLPI might be considered as a multifunctional ingredients for functional foods with protein supplements, ACE-inhibitory and antioxidant activity.

Key words: Silkworm larvae protein isolates (SLPI), amino acid composition, thermal properties, gastrointestinal enzymes, hydrolysis, ACE inhibition, antioxidant.

INTRODUCTION

Protein is a fundamental nutrient for human; its inadequate supplies cause malnutrition. Overcoming deficiencies in protein diet quantity and quality are major nutritional challenges globally, especially in the developing countries where a population explosion is prevailing. Animal food source is an excellent source of good-quality and readily digested protein. However, animal proteins are expensive and not available in many countries and regions. Thus, the search for new alternatives and cheap sources of high-quality proteins has become an important research trend. Insect as a protein source has been reported, which has good quality and high digestibility to meet the nutritional requirements for human (Raksakantong et al., 2010). Silkworm protein is an important protein source, which is consumed in China, Japan, Korea, India and Thailand (Zhou and Han, 2006). Many studies have shown that silkworm pupae, which contain 45 to 55% protein (18 amino acids, including 8 essential amino acids) on a dry matter basis, is a good-quality protein source (Yang et al., 2009; Zhou et al., 2006). Until now, there has been no information about the nutritional quality and physicochemical properties of silkworm larvae protein isolate (SLPI).

In addition, animal proteins may have health benefits other than energetic and nutritional functions, such as angiotensin I-converting enzyme (ACE) inhibitory activities, antioxidant and free radical-scavenging activities. ACE plays a key physiological role in the control of blood pressure, by virtue of the rennin-angiotensin system

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Abbreviations: SLPI, Silkworm larvae protein isolate; **ACE,** angiotensin I-converting enzyme; **HHL**, hippuryl-His-Leu; **DPPH,** 1,1-diphenyl-2-picrylhydrazyl; **DSC,** differential scanning calorimetry.

(Ganten et al., 1984). Its Inhibitors have been used in therapy to reduce morbidity and mortality of patients with hypertension. Recently, many peptides with potential ACE-inhibitory activities have been isolated from animal proteins, such as oyster proteins (Wang et al., 2008), cotton leafworm (Vercruysse et al., 2008) and egg white protein (Liu et al., 2010). Free radicals are involved in the occurrence of many chronic diseases such as cancer, coronary heart disease and Alzheimers diseases (Diaz et al., 1997). To provide protection against serious diseases, it is very important to scavenge free radical in the living body. Hence, there is great interest in the use of natural antioxidants, which may have less potential health hazard compared with synthetic antioxidants, for treatment or prophylaxis of various oxidative stress-related diseases. The hydrolysates obtained from animal protein sources have been found to possess strong antioxidant activities, such as loach (You et al., 2010), casein (Suetsuna et al., 2000) and hoki frame (Kim et al., 2007). However, there is little information about the ACE-inhibitory, antioxidant and free radical-scavenging activities of the hydrolysate from silkworm larvae protein isolate (SLPI) until now.

In this work, the SLPI from the fifth larval instar of the silkworm was extracted. The amino acid compositions as well as thermal properties of SLPI were evaluated. In addition, the *in vitro* ACE-inhibitory, antioxidant and free radical-scavenging activities of the hydrolysate obtained from SLPI using gastrointestinal proteases was investigated in detail.

MATERIALS AND METHODS

Materials and chemicals

The fifth larval instar of silkworm was donated by Sericultural Research Institute, Chinese Academy of Agricultural Sciences (Zhenjiang, China). ACE (from rabbit lung), Hippuryl-His-Leu (HHL) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich Trading Co. (Shanghai, China). Pepsin, trypsin and α-chymotrypsin were purchased from Sangon Biotechnology Co. (Shanghai, China). All other chemicals and solvents were of analytical grade.

SLPI preparation

The 5th larval instar of silkworm (50 g) was homogenized in a waring-type blender with 500 ml of deionized water. The homogenate was adjusted to pH 8.0 with 1.0 mol/l NaOH. After stirring for 30 min, the homogenate was centrifuged at 5,000 × *g* and 4°C for 15 min. The supernatant was adjusted to pH 4.0 with 1.0 mol/l HCl to precipitate the proteins and was centrifuged again at 5,000 × *g* at 4°C for 15 min. The precipitates were washed several times with distilled water (pH 4.0), dispersed in a small amount of distilled water and adjusted to pH 7.0 using 0.1 mol/l NaOH. The dispersed product was lyophilized.

Hydrolysis of SLPI using gastrointestinal enzymes

The hydrolysis of SLPI was designed to simulate the human gastrointestinal digestion process. The hydrolysis process was carried out according to the method of Vercruysse et al. (2005) with some modifications. 2 g of freeze-dried SLPI were dispersed in 100 ml of deionised water at room temperature. The dispersions were first hydrolyzed with pepsin (enzyme-substrate ratio, 1,750 U/mg protein) for 90 min at 37°C (pH 2.0), followed by hydrolysis with trypsin (enzyme-substrate ratio, 2,500 U/mg protein) and α-chymotrypsin (enzyme-substrate ratio, 1,000 U/mg protein) at 37°C and pH 6.5 for 2.5 h. The enzymatic hydrolysis was stopped by boiling for 10 min in a water bath and then, the mixture of protein and enzyme was centrifuged at 5000 × *g* for 15 min at 4°C. The supernatant (hydrolysate) was collected, lyophilized and stored at -20°C before further analysis.

Amino acid analysis

The freeze-dried samples were hydrolysed with the 6 mol/l HCl for 24 h at 110°C in a glass tube under nitrogen. The hydrolysed amino acids were then subjected to RP-HPLC analysis (Agilent 1100, USA) after precolumn derivatization with O-phthalaldehyde (Jarrett et al., 1986) and with 9-fluorenylmethyl chloroformate (for Pro) (Näsholm et al., 1987). Methionine and cysteine were determined after performic acid oxidation prior to hydrolysis in 6 mol/l HCl and measured as methionine sulphone and cysteic acid, respectively (Moore, 1963). Tryptophan was determined by the method of Ravindran and Bryden (2005) after alkaline hydrolysis of each sample. Amino acid composition was reported as g of amino acid/100 g of protein.

Amino acid score

The FAO/WHO reference pattern of essential amino acid requirements for the preschool child (2 to 5 years) was used as the standard. The amino acid score was calculated as:

$$\text{Amino acid score} = \frac{\text{Sample amino acid}}{\text{Reference amino acid}} \times 100 \quad (1)$$

Differential scanning calorimetry (DSC)

The thermal properties of SLPI were analyzed with a Pyris-1 DSC (Perkin-Elmer, Australia). Lyophilized samples (2 mg) were directly weighed into the aluminum pan, then, 20 µl phosphate buffer (pH 8.0) was added. The DSC analyzer was calibrated using indium and an empty aluminium pan was used as a reference. Sample pans were heated at a rate of 10°C/min from 30 to 120°C. The denaturation temperature (T_d), peak temperature of denaturation (T_p) and enthalpy change (ΔH) were calculated from thermograms.

Measurement of ACE-inhibitory activity

ACE-inhibitory activity was measured by the method of Cushman and Cheung (1971), with slight modifications as described in a previous publication (Jia et al., 2010). An aliquot (45 µl) of hippuryl-His-Leu (HHL) sodium borate buffer (6.5 mmol/l HHL in 0.1 mol/l borate buffer containing 0.3 mol/l NaCl, pH 8.3) were mixed with 10 µl of sample solution (sample in 0.1 mol/l borate buffer containing 0.3 mol/l NaCl, pH 8.3) and pre-incubated for 5 min at 37°C. The reaction was initiated by adding 10 µl ACE (ACE in 0.1 mol/L borate buffers containing the 0.3 mol/L NaCl, pH 8.3) and the reaction was carried out at 37°C for 30 min. The reaction was stopped by adding 85 µl of 1 mol/l HCl to the samples except for the blank (85 µl of 1 mol/l HCl were added before the pre-incubation). The hippuric acid formed was extracted by adding ethyl acetate

(1000 µl) to the mixture with vigorous shaking for 2 min. After centrifugation at 4000 × *g* for 10 min, 800 µl of the ethyl acetate layer was collected and then, dried at 100°C for 20 min. The hippuric acid was re-dissolved in distilled water (800 µl) and determined in a spectrophotometer at 228 nm. The ACE inhibition activity was calculated using the following equation:

$$\text{ACE - inhibition activity } (\%) = \frac{C-S}{C-B} \times 100 \quad (2)$$

Where C, is the optical density without sample (buffer for samples) S, is the optical density in the presence of both ACE and sample and B is the optical density of the blank (hydrochloric acid was added before ACE). ACE inhibition was also expressed in terms of IC_{50}, defined as the concentration of inhibitor required to inhibit 50% of the ACE activity.

Antioxidant activity

DPPH radical-scavenging assay

DPPH radical scavenging activity was determined according to the procedure described by Cumby et al. (2008) with minor modify-cations. 2 ml of the sample solution (0 to 50 mg/ml) was added to 2 ml of 0.1 mmol/l DPPH that was dissolved in 95% ethanol. Then, the mixture was shaken vigorously using a WH-2 mixer (Huxi Analytical Instruments Co., Shanghai, China) and kept for 30 min in the dark. The absorbance of the resulting solution was recorded at 517 nm. Ascorbic acid was used as a positive control. The scavenging activity was calculated using the following equation:

$$\text{Scavenging activity } (\%) = (1 - \frac{S-B}{E}) \times 100 \quad (3)$$

Where, *S* is the absorbance for 2 ml of the sample solution mixed with DPPH solution, *B* is the absorbance for 2 ml of the sample solution mixed with 2 ml of 95% ethanol; and *E* is the absorbance for 2 ml of 95% ethanol mixed with 0.1 mmol/l DPPH solution.

Ferrous ions chelating activity

Ferrous ions chelating activity was measured according to the method described by Zhu et al. (2006b). All samples were dissolved in distilled water at series of concentrations (62.5, 125.0, 250.0, 500.0 and 1000.0 µg/ml). An aliquot (3 ml) of the sample solution was added to 0.05 ml of iron dichloride solution (2 mmol/l). The reaction was initiated by the addition of 5 mmol/l ferrozine (0.2 ml) and the mixture was shaken vigorously and left standing at room temperature for 10 min. The absorbance was determined spectrophotometrically at 562 nm. EDTA was used as a positive control. Analyses of all the samples were run in triplicate and averaged. The chelating effect was calculated by using the following equation:

$$\text{Ferrous ions chelating activity}(\%) = [(A_0 - A_1)/A_0] \times 100 \quad (4)$$

Where, A_0 is the absorbance of the control (distilled water) and A_1 is the absorbance in the presence of samples.

Reducing power activity assay

Reducing power activities were determined according to the method of Zhu et al. (2006b). All samples were dissolved in 0.2 mol/l phosphate buffer (pH 6.6) at series of concentrations (62.5, 125.0, 250.0, 500.0 and 1000.0 µg/ml). An aliquot (2.5 ml) of the sample solution was mixed with 2.5 ml of 10 mg/ml potassium ferricyanide and the mixture was incubated at 50°C for 20 min. Then, 2.5 ml of 100 mg/ml trichloroacetic acid was added. After centrifugation at 1500 g for 10 min, 2.5 ml of the supernatant was collected and mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% (w/v) ferric chloride. After standing at room temperature for 10 min, the absorbance of the resulting solution was measured at 700 nm. Ascorbic acid was used as a positive control. Analyses of all samples were run in triplicate and averaged.

Statistical analysis

The results were expressed as mean values ± standard deviation (n = 3). Analysis of variance (ANOVA), followed by the Tukey test, were used for statistical comparisons among groups, with a value of $P < 0.05$ indicating significance. All calculations and comparisons were done using SAS statistical software package (V.8.3, SAS Institute Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

Amino acid composition

Amino acid composition and score of the SLPI obtained from the 5th larval instar of silkworm are shown in Table 1. Aspartic acid and glutamic acid were the most abundant amino acids found in the SLPI, making up about 10.44 and 13.79 g/100 g of the protein, respectively. The percentage of all of the essential amino acids or amino acid pairs of the SLPI fulfilled or exceeded their respective percentages stated in the reference pattern for 2 to 5 year old child (FAO/WHO/UNU, 1985), except for tryptophan. Specifically, isoleucine, leucine, lysine, methionine + cysteine, phenylalanine + tyrosine, threonine, valine and histidine of the SLPI were 161.4, 131.5, 138.1, 113.6, 174.0, 139.1, 152.6 and 121.6% of their counterparts stated by FAO/WHO in the reference pattern for children, respectively. The ratio of essential to total amino acids in SLPI was 42.5%, which was apparently higher than the pattern recommended (at least 36%) by WHO (1985). Among the essential amino acids, phenylalanine + tyrosine had the highest amino acid score (174.0), while the amino acid score of tryptophan was the lowest (80.9) in SLPI. The lower content of tryptophan is probably due to mild acid treatment in the preparation process of SLPI. Many food proteins that have lower content of tryptophan have been reported, such as fish bone protein (Toppe et al., 2007) and pomfret muscle (Zhao et al., 2010). Histidine, serine and arginine are semi-essential amino acids which are not normally required in the diet, but which must be exogenously supplied to specific populations under special conditions, such as intensive growth, stress or in some disease status (Usydus et al., 2009). The contents of histidine, serine and arginine in SLPI were 2.31, 5.04 and 6.59 g/100 g of protein, respectively, which were greater than the values (histidine,

Table 1. Amino acid composition and scores of SLPI obtained from 5th larval instar of silkworm.

Amino acid	Content [a](g/100 g of protein)	Reference [b](g/100 g of protein)	Amino acid Score
Essential amino acids			
Isoleucine (Ile)	4.52 ± 0.04	2.80	161.4
Leucine (Leu)	8.68 ± 0.09	6.60	131.5
Lysine (Lys)	8.01 ± 0.08	5.80	138.1
Methionine (Met)	2.24 ± 0.01		
Met + Cys	2.84	2.50	113.6
Phenylalanine (Phe)	5.79 ± 0.06		
Phe + Tyr	10.96	6.30	174.0
Threonine (Thr)	4.73 ± 0.05	3.40	139.1
Valine (Val)	5.34 ± 0.06	3.50	152.6
Histidine (His)	2.31 ± 0.01	1.90	121.6
Tryptophan (Trp)	0.89 ± 0.01	1.10	80.9
Nonessential amino acids			
Aspartic acid (Asp)[c]	10.44 ± 0.12		
Glutamic acid (Glu)[d]	13.79 ± 0.15		
Serine (Ser)	5.04 ± 0.05		
Glycine (Gly)	4.90 ± 0.04		
Arginine (Arg)	6.59 ± 0.07		
Alanine (Ala)	5.83 ± 0.06		
Tyrosine (Tyr)	5.17 ± 0.05		
Cysteine (Cys)[e]	0.60 ± 0.01		
Proline (Pro)	5.15 ± 0.05		

[a] Values are expressed as means ± standard deviation based on three replications. [b]Reference amino acid pattern of preschool children (2–5 years) (FAO/WHO/UNU, 1985); [C]aspartic acid + asparagine; [d] glutamic acid + glutamine; [E]cysteine + cystine.

serine and arginine were 2.00, 3.72 and 5.56 g of protein) reported by Zhao et al. (2010) for pomfret muscle. SLPI was also rich in lysine (amino acid score, 138.1), which is the limiting amino acid in cereal-based diets of children in developing countries (Zhao et al., 2010). Therefore, SLPI has a well-balanced amino acid composition and is an excellent food protein resource.

Differential scanning calorimetry (DSC) analysis of SLPI

Differential scanning calorimetry (DSC) is a sensitive technique for studying thermal denaturation and conformational transitions of proteins and has been used extensively in various food systems (Zhu et al., 2006a). The DSC thermogram of SLPI is presented in Figure 1. Three endothermic denaturation transitions were observed in the DSC thermograms of SLPI (not shown), probably because the SLPI was a crude protein and contained multiple protein fractions. Similar reports have been presented for the DSC thermogram of soybean protein isolates (Liu et al., 2008), which shows two peaks (thermal denaturation peaks of 7S and 11S fractions). Therefore, the result seems to suggest that, the SLPI had at least three protein fractions in this research. Data (denaturation temperature T_d, peak temperature of denaturation T_p and enthalpy change ΔH) from the DSC thermograms of SLPI are summarised in Table 2. As shown in Table 2, among the three thermal transitions of SLPI, the lowest denaturation temperature (T_d) was observed at 50.46°C (T_p 54.09°C), whereas, the highest denaturation temperature (T_d) was obtained at 76.95°C (T_p 80.42°C). The third transition peak had the highest enthalpy change (ΔH=783.75 J/g), followed by the second transition peak (ΔH=44.87 J/g) and first transition peak (ΔH=10.22 J/g). Ibanoglu (2005) reported that, big transition peak and ΔH in the thermograms of whey protein isolate (WPI) represented the denaturation of the high percentage of fraction (β-lactoglobulin). It could, therefore, be assumed that the 3rd transition peak in the DSC thermograms was caused by denaturation of the fraction with the highest content in the SLPI.

ACE-inhibitory activity of enzymatic hydrolysate

The *in vitro* ACE-inhibitory activity was expressed in terms of IC_{50} value, defined as the concentration of inhibitor required to inhibit 50% of the ACE activity. The IC_{50} was determined using graphical extrapolation by plotting ACE inhibition as a function of different hydrolysate concen-

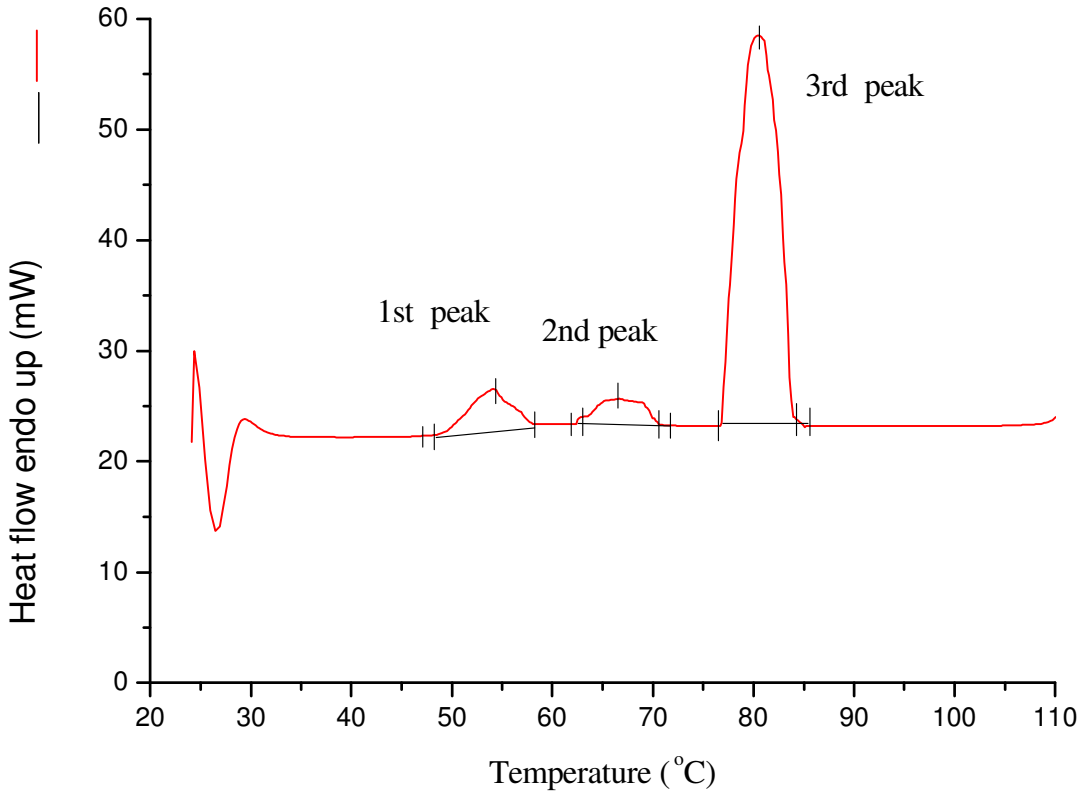

Figure 1. Differential scanning calorimetry thermogram of SLPI.

Table 2. Summary of the prominent endothermic denaturation transitions observed during differential scanning calorimetry of SLPI.

Transition	Thermal property		
	T_d (℃) [a]	T_p (℃) [b]	ΔH (J/g) [c]
1st transition peak	50.46	54.09	44.87
2nd transition peak	62.34	66.64	10.22
3rd transition peak	76.95	80.42	783.75

[a] Denaturation temperature; [b] peak temperature of denaturation; [c] enthalpy change.

trations. Captopril, the most widely used antihypertensive drug at the present time, was used in this study as a positive control. ACE-inhibitory activities of captopril and the gastrointestinal hydrolysate of SLPI are shown in Figure 2. The IC_{50} values of captopril and SLPI hydrolysate were 5.3 and 8.3 µg/ml, respectively. The IC_{50} value of captopril in this study is in accordance with Hayes et al. (2007) who reported that, the IC_{50} value for captopril was 0.005 mg/ml. The IC_{50} value of captopril was only about 1.6 times lower than that of the SLPI hydrolysate. That is to say, the ACE-inhibitory activity of the SLPI hydrolysate was close to that of captopril. This result showed that, gastrointestinal digest of SLPI exhibited strong *in vitro* ACE-inhibitory activity. Under the same measuring method for ACE-inhibitory activity, the gastrointestinal digest of SLPI also showed markedly higher ACE-inhibitory activity than those of cotton leafworm protein (IC_{50}=125 µg/ml) (Vercruysse et al., 2008) and royal jelly protein (IC_{50}=99 µg/ml) (Matsui et al., 2002). These results indicated that, the SLPI were more suitable for production of ACE-inhibitory peptides.

In addition, the type of enzyme used has a marked effect on ACE inhibitory activity of the hydrolysate (Vercruysse et al., 2009). Studies have also showed that, the ACE-inhibitory activity of a peptide depends on its composition. For instance, the preferred ACE inhibitors are those peptides that contain aromatic amino acid on the C-terminal (Jia et al., 2010; Lourenço da Costa et al., 2007). As well known, α-chymotrypsin acts as an endo-peptidase and preferentially cleaves the C-terminal aromatic amino acid (phenylalanine, tyrosine and tryptophan) residues (Lourenço da Costa et al., 2007). It

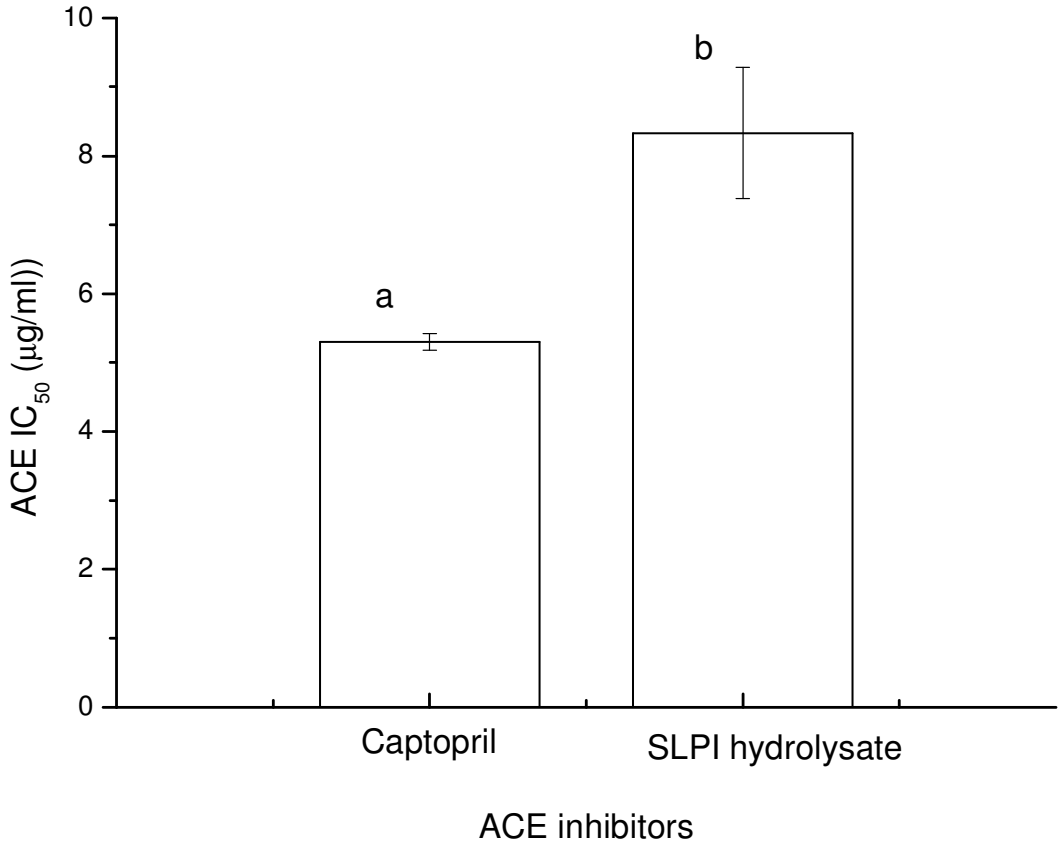

Figure 2. ACE-inhibitory activities of Captopril and the SLPI hydrolysate prepared with gastrointestinal enzymes. Results represent the mean of three determinations ± standard deviation. Means with different superscripts are significantly different ($P < 0.05$).

could therefore, be presumed that the hydrolysate obtained from SLPI with gastrointestinal proteases had much higher ACE-inhibitory activity due to more peptides with a C-terminal aromatic amino acid residue, which were released from SLPI with α-chymotrypsin.

Antioxidant activity

Different methods used for measuring antioxidant activity based on different mechanisms may lead to different observations (Sun and Ho, 2005). Hence, in order to better understand the antioxidant properties of SLPI hydrolysate, three different chemical *in vitro* assays (DPPH radical scavenging assay, reducing power assay and ferrous ions chelating assay) were used in this research.

In this study, DPPH radical scavenging properties were evaluated at six different concentrations for each sample and repeating experiments in triplicate. Figure 3 shows the results of the scavenging DPPH radical capability for SLPI hydrolysate at various concentrations. A sharp increase (from about 27.6 to 41.6%) of DPPH radical scavenging activity for SLPI hydrolysate was observed, as its concentration increased from 33.0 to 50.0 µg/ml. The linear regression analysis showed that, the concentration of SLPI hydrolysate had a good linear (R^2=0.9942) relationship to its DPPH radical scavenging activity. The IC_{50} value of SLPI hydrolysate for scavenging DPPH radical, which was calculated from the linear regression equation, was 57.91 µg/ml. As shown in Figure 3, ascorbic acid, used as a positive control, showed excellent scavenging ability of 93.84% for DPPH radical at a concentration as low as 33.0 µg/ml. Compared with the scavenging DPPH radical activity of ascorbic acid, that of SLPI hydrolysate was lower. The results of this study are in agreement with those of Zhu et al. (2006b) which reported higher scavenging DPPH radical activity of ascorbic acid than the wheat germ protein hydrolysates (IC_{50}= 1.30 mg/ml). Under the same measuring method for scavenging DPPH radical activity, SLPI hydrolysate showed markedly higher scavenging activity for DPPH radical than canola protein hydrolysates (IC_{50}= about 5.0 mg/ml) (Cumby et al., 2008). The result showed that, SLPI hydrolysate was a free radical inhibitor, which contained the bio-peptides with strong free radical scavenging.

The *in vitro* reducing power of the test samples was measured using the potassium ferricyanide reduction

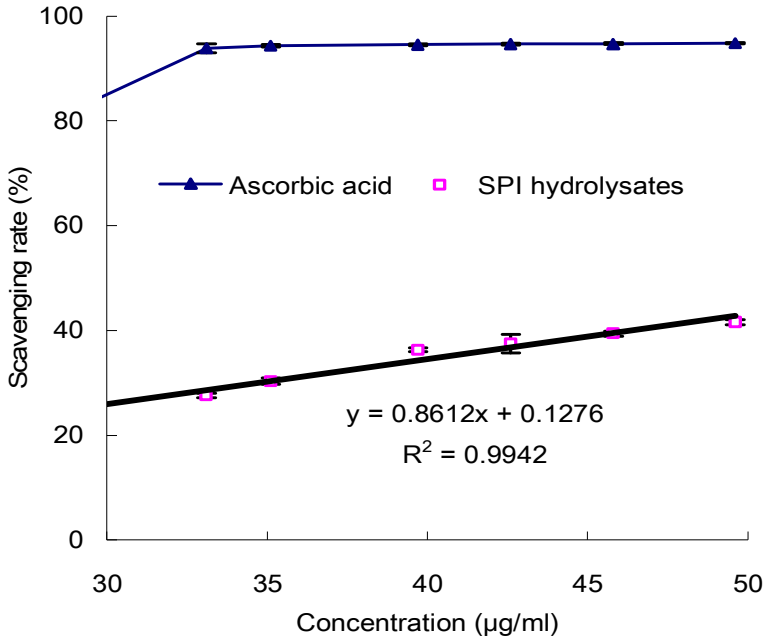

Figure 3. Scavenging DPPH radical activities of the SLPI hydrolysate prepared with gastrointestinal enzymes. Ascorbic acid was used as the positive controls. Values were expressed as mean ± standard deviation of three replicate determinations.

method (Zhu et al., 2006b). The *in vitro* reducing power was expressed in terms of absorbance at 700 nm. The plot of the SLPI hydrolysate concentration versus the reducing power gave a straight line (R^2=0.9919) with a positive slope (Figure 4), indicating that SLPI hydrolysate exhibited a dose-dependent manner for reducing power. Its reducing power increased with increasing concentration (62.5 to 1000 μg/ml). As presented in Figure 4, ascorbic acid exhibited strong *in vitro* reducing power (as concentration increased from 62.5 to 1000 μg/ml, its reducing power increased rapidly from 1.64 to 2.50) and was stronger than that of SLPI hydrolysate. However, under the same measuring method for reducing power, the reducing power of SLPI hydrolysate (absorbance at 700 nm was about 0.83) was found higher than that of wheat germ protein hydrolysates (absorbance at 700 nm was about 0.19) at a dosage of 1000 μg /ml (Zhu et al., 2006b). These results indicated that, the SLPI hydrolysate had a notable effect on reducing power and seemed to contain some antioxidant peptides.

Ferrous ions (Fe^{2+}) chelation may render important antioxidative effects by retarding metal-catalyzed oxidation and has been applied to measure a compound's potential antioxidant activity (Gülçin et al., 2010). The chelating effects of SLPI hydrolysate and EDTA for ferrous ions are shown in Figure 5. As the concentration increased from 62.5 to 1000 μg/ml, the ferrous ions chelating capability of SLPI hydrolysate increased slightly from 0.30 to 23.36%. Since correlation of linear regression (R^2) was 0.9503 for SLPI hydrolysate concentration versus ferrous ions chelating capacity, the linearity was good. The IC_{50} value of SLPI hydrolysate for ferrous ions chelating capacity was calculated from the linear regression equation to be 2.03 mg/ml. EDTA was a known metal ion chelator and was used as a positive control in this study, showing good chelating ability of 98.72% at 1000 μg/ml. Under the same measuring method for ferrous ions chelating capacity, Zhu et al. (2006b) reported that, the ferrous ions chelating capability of EDTA at 1000 μg/ml was about 100%; this result is in accordance with those of this study. As shown in Figure 5, although the ferrous ions chelating capability of SLPI hydrolysate was lower than that of EDTA, SLPI hydrolysate had an effective capacity for chelating ferrous ions

Though the stated assays were based on different principles and represented different aspects of the antioxidant potential, the data reported in this study indicated that, the SLPI hydrolysates prepared with gastrointestinal enzymes were effective antioxidants. Therefore, these results also showed that, SLPI was a potential protein resource for preparation of bio-peptides with antioxidant activity.

Conclusions

This research revealed that, the SLPI obtained from the 5th larval instar is a high quality protein source with a well-balanced composition of essential amino acids. Three endothermic denaturation transitions were

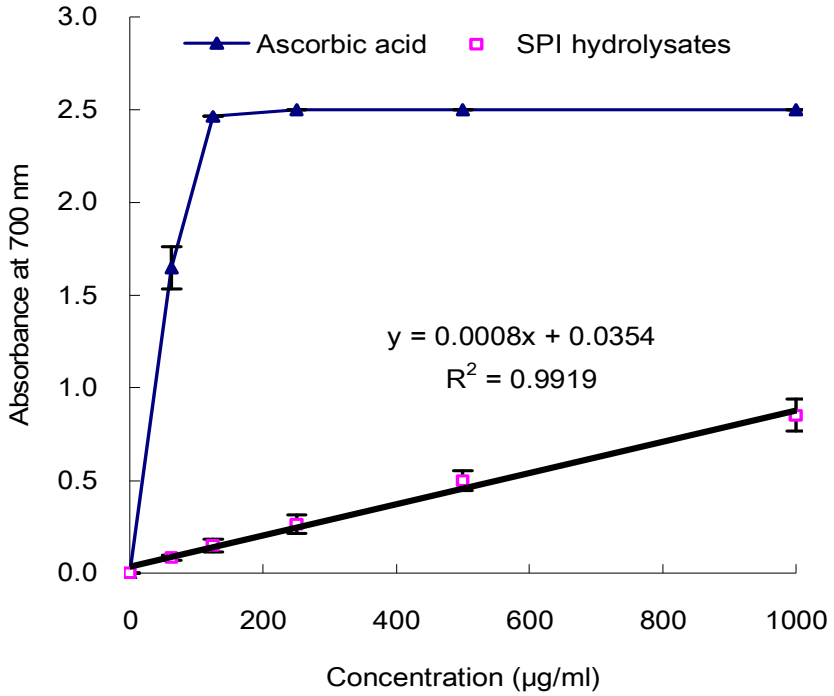

Figure 4. Reducing powers (absorbance at 700 nm) of the SLPI hydrolysate prepared with gastrointestinal enzymes. Ascorbic acid was used as positive controls. Values were expressed as mean ± standard deviation of three replicate determinations.

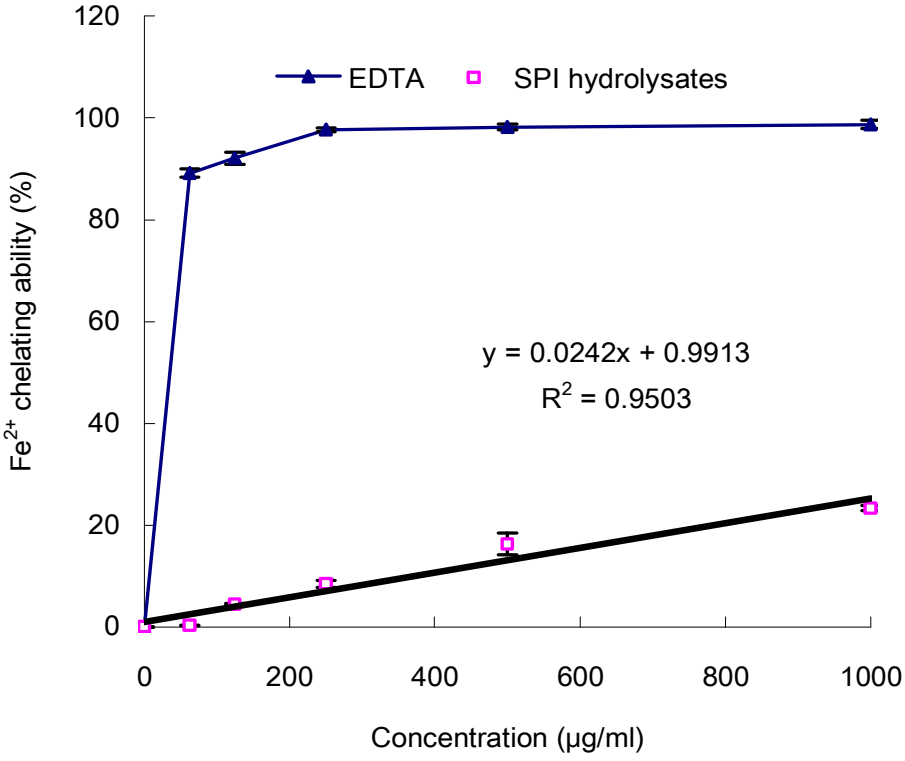

Figure 5. Ferrous ions chelating capacities of the SLPI hydrolysate prepared with gastrointestinal enzymes. EDTA was used as the positive controls. Values were expressed as mean ± standard deviation of three replicate determinations.

observed in the DSC thermograms of SLPI. The maximum transition peak occurred in the third thermal transition, its denaturation temperature (T_d), peak temperature of denaturation (T_p) and enthalpy change (ΔH) were 76.95°C, 80.42°C and 783.75 J/g, respectively. SLPI hydrolysate prepared with gastrointestinal enzymes exhibited strong *in vitro* ACE-inhibitory activity (IC_{50}=8.3 µg/ml) and a relatively higher DPPH radical scavenging activity (IC_{50}=57.91 µg/ml), reducing power and ferrous ions chelating capacity (IC_{50}=2.03 mg/ml). As a result, SLPI can be considered as not only a potential ingredient for future feed supplements and/or formulation of infant foods, but also a promising protein resource for preparation of the bio-peptides with ACE-inhibitory and antioxidant activities.

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