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Effect of Enzyme Type on the Antioxidant Activities and Functional Properties of Enzymatic Hydrolysates from Sea Cucumber (*Cucumaria frondosa*) Viscera

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ABSTRACT

Effects of various proteases were obvious on the amino acid composition, antioxidant activities, and functional properties of enzymatic hydrolysates prepared from sea cucumber viscera (SCV). Alcalase, trypsin, and flavourzyme conferred better antioxidant activities on hydrolysates and endowed them with a higher degree of hydrolysis, coming to 19.08, 32.38, and 15.94%, respectively. For functional properties, neutrase- and flavourzyme-derived hydrolysates showed better foaming properties. Amino acid composition suggested that papain, neutrase, alcalase, and bromelain granted the hydrolysate's high nutritional quality. The results indicated that SCV hydrolysates could be used in food systems to impart desirable characteristics to products.

KEYWORDS

Sea cucumber; viscera; hydrolysate; antioxidant activity; functional property

Introduction

Sea cucumber is one of the important aquatic species and enjoyed by people as a traditional healthy food and medicinal resource in China, Japan, Korea, and some southeastern Asian countries for thousands of years (Zhao et al., 2007; Fu et al., 2005). It has a high commercial value with increasing global production and world trade. For convenient storage and transportation, dried sea cucumbers, promptly prepared after caught from the sea, are the most popular final products on the market (Wu et al., 2009). In the processing of dried sea cucumber, viscera are usually discarded as industrial wastes without any attempt at recovery, which induces environmental pollution and resources waste. Research shows that sea cucumber viscera (SCV) are a rich source of protein, but they have low storage stability if not frozen or otherwise preserved. If the protein is recovered and utilized, it can represent significant economic and social benefits.

One way to utilize such sea food processing by-products is to convert them to protein hydrolysates. Enzymatic hydrolysis is an effective approach, by which a broad spectrum of food ingredients or industrial products for a wide range of applications may be produced (Ovissipour et al., 2009), and the resulting hydrolysates normally reveal an increased nutritional quality and improved intestinal absorption (Kristinsson and Rasco, 2000a). Enzymes are specific for peptide bonds adjacent to certain amino acid residues (Khantaphant and Benjakul, 2008); different proteases have different action sites to produce peptides with various properties. Previous studies showed that functional properties and antioxidant activities of protein hydrolysates are correlated with enzyme specificity (Klompong et al., 2007; Zhao et al., 2012; Huang et al., 2011). Therefore, the

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selection of enzyme type was of vital importance, and it was also the first step in enzymolysis technology, by which, the hydrolysates could be obtained with different functions.

Past studies of sea cucumber viscera have focused on characterization of endogenous proteases (Fu et al., 2005) and nutritional composition and antioxidant properties of protein hydrolysates prepared by Alcalase 2.4L (Mamelona et al., 2010). However, little is known about the effect of enzyme type on the antioxidant activities and functional properties of protein hydrolysates from sea cucumber viscera. This study is mainly to produce several protein hydrolysates from sea cucumber viscera using commercial proteinases, and their molecular weight distribution, amino acid composition, antioxidant activities, and functionalities were evaluated with the goal of recovering peptides with ideal functional properties and antioxidant activities. The results will provide the basis and reference for the enzymatic production and application of SCV hydrolysates.

Material and methods

Raw material

Sea cucumber (*Cucumaria frondosa*) viscera used in the study was provided by Yantai Leike Foods Inc. in Shandong, P.R. China. After sea cucumbers were caught from the sea, viscera were taken out immediately. They were taken to the laboratory, sediment removed, and kept at −20°C until used. Prior to the hydrolysis process, viscera were thawed overnight in a refrigerator at 4°C.

Enzymes and chemicals

Neutrase, alcalase, flavourzyme, papain, and bromelain were obtained from Guangxi Nanning Pangbo Biological Engineering Co., Ltd., P.R. China, and stored at 4°C until used. Trypsin and pepsin were provided by Hubei Kang Baotai Fine Chemical Co., Ltd., P.R. China, and stored at 4°C until used. The enzyme activities, shown in Table 1, were determined according to Chinese standard (SB/T 10317-1999), casein as substrate.

All other chemical reagents used for the experiments were of analytical grade.

Production of SCV hydrolysates

The SCV was dried at 60°C and ground, then defatted at 25°C for 60 min using anhydrous alcohol at a ratio of 1:5 (w/v), repeated three times. The supernatant was removed, and the precipitate was collected and dried at 60°C.

Defatted SCV (3 g) was suspended in 300 mL of distilled water. The mixtures were hydrolyzed with commercially available proteases at their respective optimum hydrolysis conditions at 4,000 U/g-substrate. The reactions were terminated by boiling at 100°C for 10 min, and the resulting hydrolysates were cooled on ice to room temperature and centrifuged at 8,000 × *g* for 20 min to separate any impurities and unhydrolyzed material. The supernatant was collected, concentrated, lyophilized, and stored in a desiccator for further use.

Table 1. Hydrolysis conditions of different proteases.

	Neutrase	Alcalase	Trypsin	Flavourzyme	Papain	Bromelain	Pepsin
pH	7.0	10.0	10.0	7.5	6.5	6.8	2.0
Temperature (°C)	50	50	50	50	50	45	40
Activity ($\times 10^4$ U/g)	12	10.9	11.4	7.54	5.4	5.2	5.05

Determination of the degree of hydrolysis

Degree of hydrolysis (DH), defined as the percent ratio of the number of the peptide bonds broken to the total number of peptide bonds in the substrate studied, was determined by ninhydrin colorimetric method with a slight modification (Hou et al., 2011). L-glycine (2–20 μg/mL) was used as standard. Each determination was performed in triplicate. DH was calculated as follows:

$$DH(\%) = \frac{h(mmol/g)}{h_{tot}(mmol/g)} \times 100 = \left[\frac{A(\mu mol/mL)}{6.25 \cdot N(mg/mL)} - B(mmol/g)\right] \div h_{tot}(mmol/g) \times 100,$$

where h is the number of broken peptide bonds per unit weight; h_{tot} is the total number of peptide bonds per unit weight, equal to 8.2 mmol/g protein; A is the concentration of $-NH_2$ amino groups in SCV hydrolysates, obtained by standard curve; B is the concentration of $-NH_2$ amino groups in SCV; and N is the total nitrogen in hydrolysates, determined by the Kjeldah method.

Determination of nitrogen recovery

The nitrogen masses of substrate and hydrolysate were determined using the Kjeldahl method. The nitrogen recovery (NR) was calculated as below:

$$NR(\%) = \frac{N_H}{N_S} \times 100,$$

where N_H is the nitrogen mass of hydrolysate (g), and N_S is the nitrogen mass of substrate (g).

Determination of the molecular weight distribution

Molecular weight distributions of hydrolysates were determined by gel permeation chromatography on a TSK gel 3000 PWXL column (Tosoh, Tokyo, Japan) with a UV detector at 220 nm, using a high-performance liquid chromatography system (Agilent 1100, Santa Clara, CA, USA). The mobile phase used was acetonitrile/water (1:1, v/v) in the presence of 1 mL/L trifluoroacetic acid at a flow rate of 0.5 mL/min. Five protein standards—bovine serum albumin (66,000 Da), cytochrome C (12,384 Da), bacitracin (1423 Da), glutathione (307 Da), and Gly-Gly-Gly (189 Da)—were used as the molecular mass standards.

Amino acid composition analysis

Samples were hydrolyzed with 6 mol/L HCl at 110°C for 24 h in a sealed tube, and the hydrolysates were analyzed on a Hitachi-835-50 amino acid analyzer (Hitachi, Tokyo, Japan).

Determination of the antioxidant activities

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was carried out by the method described by Zhou et al. (2012) with slight modifications. Briefly, the reaction fluid was prepared by mixing 0.8 mL of acetic acid buffer (pH 5.0), 1.0 mL of brilliant green (1×10^{-4} mol/L), 2.2 mL of EDTA ferrous (2×10^{-2} mol/L), 1.4 mL of hydrogen peroxide (0.3%), and 1.0 mL of hydrolysate (2 mg/mL) previously dissolved in distilled water. After the mixture was diluted to 10 mL and shaken up, it was incubated at 25°C for 45 min, and the absorbance was measured at 617 nm. The hydroxyl radical scavenging rate was calculated according to the following equation:

$$Hydroxyl\ \ radical\ \ scavenging\ \ rate(\%) = \frac{A_s - A_b}{A_0 - A_b} \times 100,$$

where A_s is the absorbance of the reaction mixture with hydrolysate, A_b is the absorbance of the reaction mixture with hydrolysate replaced by equivalent volume of deionized water, and A_0 is the absorbance of the reaction mixture with hydrolysate but hydrogen peroxide replaced by equivalent volume of deionized water.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

DPPH radical scavenging activity was measured using the method described by Zhao et al. (2012) with some modifications. Briefly, 1 mL of sample (2 mg/mL) previously dissolved in distilled water was mixed with 3 mL of 120 μmol/L DPPH solution in 95% ethanol. After the mixture was shaken and left for 30 min in dark at room temperature, the absorbance was measured at 517 nm. The ability of the hydrolysate to scavenge DPPH radical was calculated according to the following equation:

$$DPPH\ \ radical\ scavenging\ \ rate(\%) = \frac{A_c - A_s}{A_c} \times 100,$$

where A_s and A_c represent the absorbance of the sample and the control, respectively. Control contains everything except the hydrolysate.

Reducing power

The reducing power of protein hydrolysates from sea cucumber viscera was assayed according to the method of Klompong et al. (2007). One milliliter of hydrolysate (5 mg/mL) was mixed with 2.5 mL of 0.2 mol/L phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min. Then, 2.5 mL of 10% trichloroacetic acid was added to stop the reaction. After centrifugation at 3,000 × *g* for 10 min, 2.5 mL of the supernatant was mixed with 2.5 mL of distilled water and 2.5 mL of 0.1% ferric chloride. After a 10 min reaction, the absorbance of the resulting solution was read at 700 nm. A high absorbance indicates a strong reducing power.

Determination of functional properties

Foaming properties

Foaming capacity and stability of protein hydrolysates from SCV were determined according to the procedure of Sathe and Salunkhe (1981). Sample solutions (5 mg/mL) were prepared by dispersing accurate weights of hydrolysates in distilled water. After homogenization at speed of 13,000 × *g* for 1 min, the sample solution was immediately transferred into a 50 mL cylinder. The total volumes were recorded before and after whipping. The whipped solutions were allowed to stand at room temperature for 20 min, and the volume was then recorded. Foaming capacity (FC) and foaming stability (FS) were calculated using the following equations:

$$FC(\%) = \frac{volume\ of\ foam}{original\ volume\ of\ liquid} \times 100,$$

$$FS(\%) = \frac{volume\ of\ foam\ after\ s\tan ding}{initial\ volume\ of\ foam} \times 100.$$

Emulsifying properties

Emulsifying activity index (EAI) and emulsifying stability index (ESI) of the protein hydrolysates from SCV were determined according to the method described by Zhang et al. (2013). A mixture of 45 mL of 5 mg/mL hydrolysate solution previously prepared in distilled water and 15 mL of soybean

oil was homogenized at 10,000 rpm for 1 min at room temperature. An aliquot of the emulsion (200 μL) was pipetted from the bottom of the container and dispersed into 5 mL of 0.1% sodium dodecyl sulphate solution at 0 and 10 min after homogenization. The absorbance values of the diluted solutions measured at 500 nm were used to calculate the EAI and ESI, as follows:

$$EAI(m^2/g) = \frac{2 \times 2.303 \times dil \times A_0}{c \times \varphi \times 10,000},$$

$$ESI(\text{min}) = \frac{10 \times A_0}{A_0 - A_{10}},$$

where A_{10} and A_0 represent the absorbance at 500 nm after 10 min and at time zero, respectively; dil is dilution factor; c is protein concentration (g/mL); and φ represents the oil volume fraction of the emulsion (φ = 0.25).

Statistical analysis

All experiments were carried out in triplicate. Statistical data were analyzed using Microsoft Excel 2003 (Microsoft Corp., Redmond, WA, USA) and Origin 7.5 (OriginLab Corp., Northampton, MA, USA). Student's *t*-test was applied to compare the averages of properties with a level of 95% confidence interval.

Results and discussion

Enzyme hydrolysis

Enzyme type is an important factor influencing the product's quality, because different enzymes have different cutting sites (Hou et al., 2011). In this study, optimal hydrolysis enzyme for producing peptides with good performance was selected by manipulating enzyme type. The proteases were used under the optimal hydrolysis conditions according to the manufacturers. Equivalent units of enzyme activities were used for hydrolysate production so as to objectively compare the ability of the different protease products to generate antioxidant hydrolysates. Figure 1 shows the enzymolysis

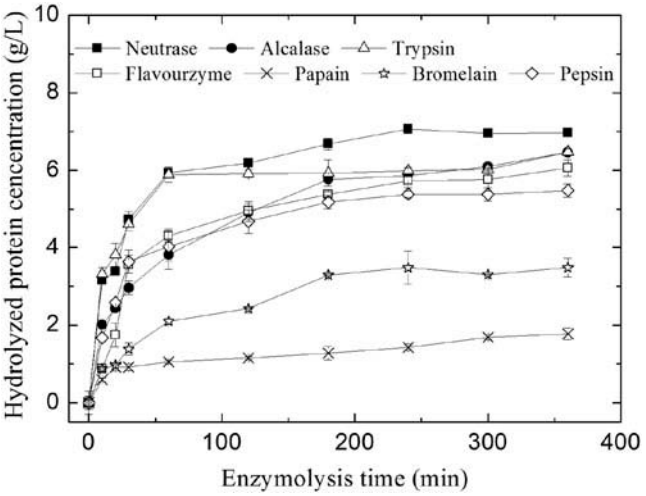

Figure 1. Enzymolysis process of protein from sea cucumber viscera.

process of SCV protein. The concentration of hydrolyzed protein increased sharply during the initial 30 min and then displayed a slow increase to stabilization after SCV protein hydrolyzed by the seven different enzymes, respectively, indicating that large numbers of peptide bonds were hydrolyzed within the first 30 min. This typical shape of the hydrolysis curve was previously found in hydrolysis of wheat germ (Qu et al., 2012) and cheese whey protein (Galvão et al., 2009). The initial reaction rate, expressed by the increase of hydrolyzed protein concentration per min in 10 min, was usually applied to evaluate the starting hydrolysis. In the conditions of the same protein substrate and same activity of enzyme, neutrase and trypsin showed the highest initial reaction rate in the production of hydrolysates, and the lowest was observed in the hydrolysates prepared by papain and bromelain, which might be related to the concentration of peptide bonds available for hydrolysis (Guerard et al., 2002), further confirming that enzyme type could influence the hydrolysis. From Figure 1, it could also be found that after 240 min of incubation, the hydrolysis of SCV protein using seven proteases all reached equilibrium, thus the hydrolysates prepared by hydrolysis for 240 min were applied for the next study. To sum up, from the entire enzymolysis process, neutrase and trypsin were found to be the most efficient, while papain has the least hydrolytic efficiency.

Effect of enzyme type on the properties of hydrolysates

Degree of hydrolysis and nitrogen recovery

Degree of hydrolysis and nitrogen recovery (NR) of hydrolysates prepared from sea cucumber viscera using seven different enzymes are displayed in Figure 2. The extent of protein degradation by proteolytic enzymes was usually estimated by assessing the degree of hydrolysis (Hou et al., 2011; Qian et al., 2008). As shown in Figure 2(A), there were significant differences in DH among hydrolysates obtained with various proteases, in decreasing order of trypsin > alcalase > flavourzyme > papain > neutrase > pepsin > bromelain ($p < 0.05$). Trypsin clearly performed best, DH of the hydrolysate reaching 32.38%. The amounts of proteases added were same in enzyme activity in the experiment, so the differences observed in DH were attributed to the differences in the cutting sites of enzymes (Zhou et al., 2012). Protein recovery, expressed by nitrogen recovery, is also an important measurement for the hydrolysis of enzyme, as a maximum recovery is desirable for the production of hydrolyzed food protein (Zhao et al., 2012). Neutrase gave the highest NR, coming to 83.88%, and those of the hydrolysates prepared by alcalase, trypsin, and flavourzyme were all above 70%, as displayed in Figure 2(B), while other enzymes showed lower NR. Antioxidant activities and functional properties of protein hydrolysates are closely related to DH (Khantaphant and Benjakul, 2008); therefore, it was presumed that distinct antioxidant activities and functional properties could be observed in the hydrolysates prepared from SCV by different enzymes.

Molecular weight distribution

During the proteolytic process, peptide bonds were broken and lower molecular weight peptides were released, which means the products may have different molecular weight distribution under different hydrolysis condition (He et al., 2015). As shown in Figure 3, the components with molecular mass of about 1,000 Da were dominant in the hydrolysates except those prepared by pepsin and papain, pepsin mainly generating the peptides with the molecular weight of 2,000 Da, and papain producing the peptides with molecular weights between 1,000 and 2,000 Da. This outcome, in combination with the data from DH and NR, suggested that alkaline enzymes were much more effective in producing smaller peptides from SCV protein, similar results found in rice dreg protein (Zhao et al., 2012).

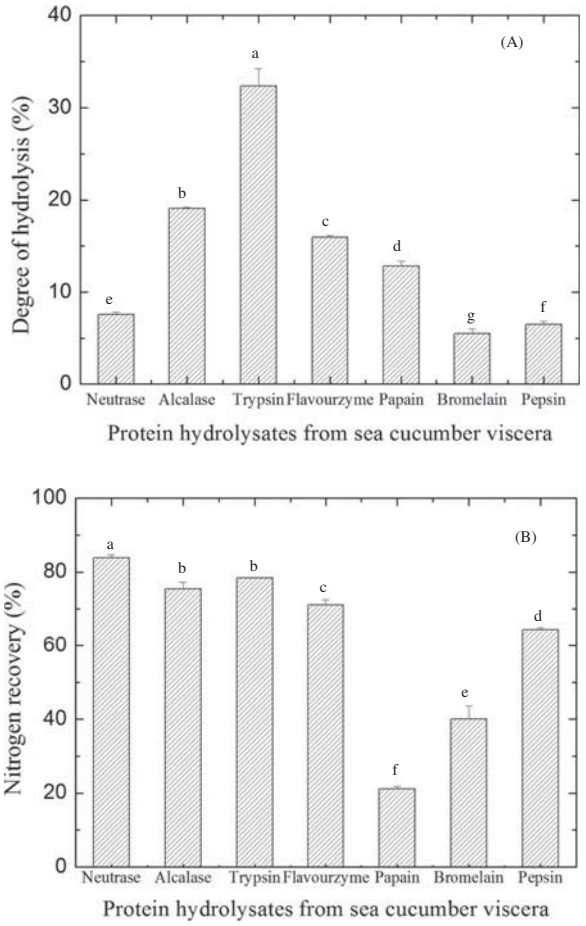

Figure 2. Degree of hydrolysis (A) and nitrogen recovery (B) of protein hydrolysates from sea cucumber viscera prepared using seven different enzymes. For the same parameter, different letters indicate significant differences ($p < 0.05$).

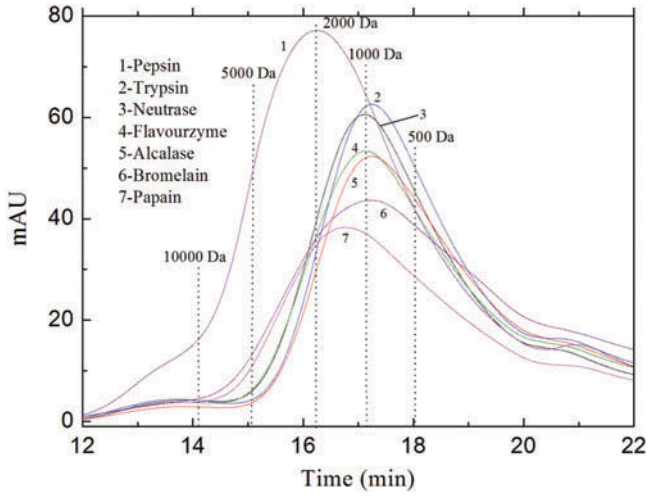

Figure 3. Gel filtration chromatograms showing the molecular weight distribution of protein hydrolysates from sea cucumber viscera prepared using seven different enzymes.

Amino acid composition

Amino acid composition of protein hydrolysate has been shown to play an important role in their antioxidant activities (Alashi et al., 2014). Asp and Glu were reported to exhibit strong antioxidant activities, and several other amino acids—such as Pro, Arg, His, Met, Leu, Ala, Tyr, and Vla—were accepted as having antioxidant properties (Zhao et al., 2012; Zhou et al., 2012). From Table 2, it could be found that the main amino acids in hydrolysates were Asp and Glu, so it was deduced that the hydrolysates should display good antioxidant activities.

Amino acid composition is also one of the most important nutritional qualities of protein, and the chemical score of amino acid is usually used to evaluate protein quality (Hou et al., 2011). In the current study, chemical score is computed based on the reference protein (FAO/WHO, 1991), expressed by a ratio of amino acid content in hydrolysate to that in reference protein. As shown in Table 2, His, Thr, Tyr, Val, and Ile contents were all higher than the FAO/WHO recommendations for animal proteins; and by evaluation of leucine, lysine, and phenylalanine + tyrosine, it was found that the chemical scores in most hydrolysates were more than 0.80. In particular, chemical scores of amino acid exhibited that contents of essential amino acids in papain-derived hydrolysate were higher than in reference protein given by FAO/WHO (1991), and those in hydrolysates prepared by neutrase, alcalase, and bromelain were close to those in reference protein. The results revealed that the hydrolysates prepared by papain, neutrase, alcalase, and bromelain are of high nutritional quality and may be used as a protein source in the human diet.

Effect of enzyme type on antioxidant activities

Previous study indicated that antioxidant activities of protein hydrolysates depend on the protease and the hydrolysis conditions employed (Jun et al., 2004). In order to select the desirable protease to hydrolyze SCV for antioxidant peptides, the material was independently hydrolyzed by neutrase, alcalase, trypsin, flavourzyme, papain, bromelain, and pepsin. The antioxidant activities of different hydrolysates were evaluated by the hydroxyl radical and DPPH radical scavenging activity and reducing power. Results are shown in Figure 4.

Table 2. Percentage amino acid profile of hydrolysates prepared from sea cucumber viscera.

	Hydrolysates prepared by seven enzymes							
Amino acids	Neutrase	Alcalase	Trypsin	Flavourzyme	Papain	Bromelain	Pepsin	Reference protein*
Asp	11.82	13.60	14.35	12.84	12.20	12.43	10.19	
Glu	14.25	14.36	16.56	15.25	15.12	15.43	13.21	
Ser	5.89	5.92	6.63	5.99	5.40	5.51	6.36	
Gly	7.07	7.27	8.92	8.42	7.28	10.07	10.57	
Trp				Not determined				
His	3.33	3.14	3.49	2.62	2.79	2.65	4.40	1.9
Arg	7.92	7.60	4.30	3.92	6.41	6.92	7.40	
Thr	5.19	5.41	5.85	5.91	5.20	5.02	5.28	3.4
Ala	4.07	4.11	5.18	5.20	5.31	5.63	6.05	
Pro	6.48	7.02	8.26	6.57	5.67	6.22	6.95	
Tyr	2.78	2.86	1.84	2.11	3.18	2.91	2.60	1.1
Val	4.33	4.87	4.84	5.71	5.39	4.86	4.74	3.5
Met	1.66	1.96	1.77	2.07	1.94	1.63	1.79	
Cys	2.71	1.51	1.99	3.35	2.19	1.92	2.47	
Ile	3.79	4.34	3.96	4.88	4.82	4.04	3.81	2.8
Leu	5.93	6.28	4.86	6.12	6.98	6.11	5.50	6.6
Phe	3.02	3.55	2.56	3.24	3.91	3.36	2.64	
Lys	9.76	6.22	4.67	5.86	6.17	5.34	6.07	5.8
Phe+Tyr	5.80	6.40	4.40	5.35	7.09	6.27	5.24	6.3

*Essential amino acids of reference protein according to FAO/WHO (1991).

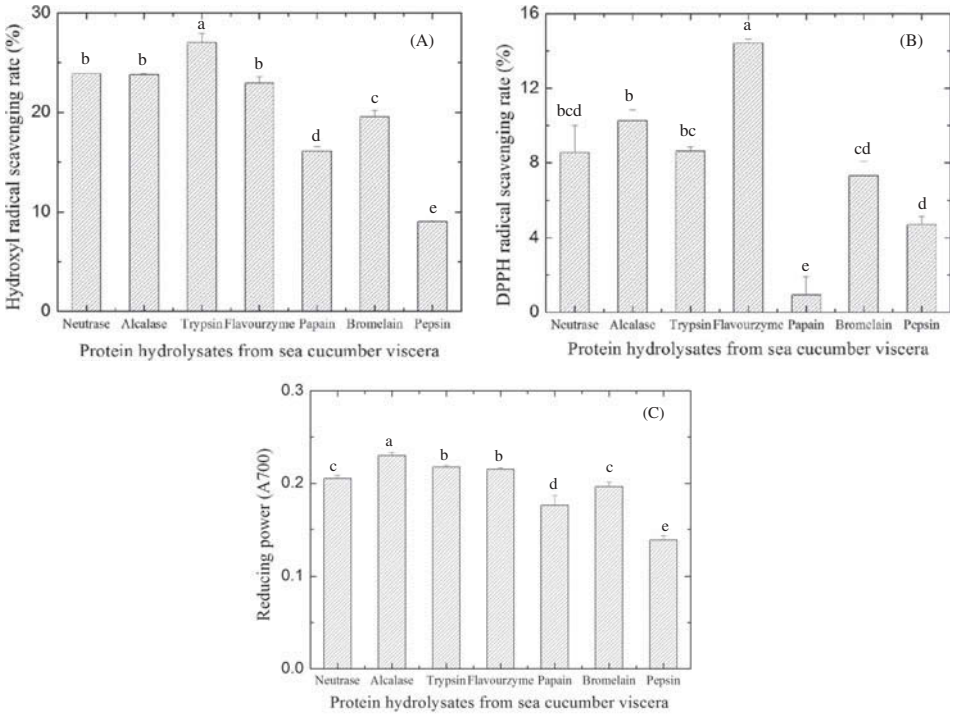

Figure 4. Hydroxyl radical (A) DPPH radical (B) and scavenging activity and reducing power (C) of protein hydrolysates from sea cucumber viscera prepared using seven different enzymes. For the same parameter, different letters indicate significant differences ($p < 0.05$).

The hydroxyl radical is the most biologically active free radical. It can react with biomolecules such as amino acids, proteins, and DNA, as well as trigger lipid peroxidation (Xie et al., 2008). Therefore, removal of the hydroxyl radical is probably one of the most effective defenses of a system against oxidation (Xia et al., 2012). Previous study showed that protein hydrolysates may scavenge hydroxyl radical by electron/hydrogen donation and radical quenching (Kong and Xiong, 2006). As shown in Figure 4(A), at the same concentration (2 mg/mL), trypsin conferred the strongest ability to scavenge hydroxyl radical on the hydrolysate ($p < 0.05$), the scavenging rate reaching 27.04%; and neutrase, alcalase, and flavourzyme were the second efficient enzyme, the rate being 23.86, 23.76, and 22.96%, respectively. DPPH is a stable free radical and accepts an electron of hydrogen radical to become a stable diamagnetic molecule (Xia et al., 2012). The model of scavenging this radical has been widely applied to evaluate the free radical scavenging activity of various antioxidants (Zhao et al., 2012; Klompong et al., 2007; Farvin et al., 2014). As depicted in Figure 4(B), the highest DPPH radical scavenging activity was observed in the hydrolysate prepared by flavourzyme ($p < 0.05$), the rate coming to 14.42% at the concentration of 2 mg/mL, followed by alcalase, trypsin, and neutrase. Rajapakse et al. (2005) reported that the high level of DPPH radical scavenging activity of protein hydrolysates is associated with a high amount of hydrophobic amino acids or peptides. It was deduced that the hydrolysate prepared by flavourzyme should display higher surface hydrophobicity. However, DPPH radical scavenging rate was poor in papain-derived hydrolysate, although amino acid composition showed that there was also higher content of hydrophobic amino acid, which was probably attributed to the conformation of peptides. Besides, it could be found that DPPH radical scavenging rate was lower than hydroxyl radical scavenging rate for the same hydrolysate at the same concentration, which should be correlated with the ratio of hydrophilic and hydrophobic amino acids. The

reducing power assay is often used to evaluate the ability of natural antioxidant to donate an electron or hydrogen (Xia et al., 2012). As described in Figure 4(C), alcalase, trypsin and flavourzyme gave a much higher reducing power to the hydrolysates than the other four proteases ($p < 0.05$), revealing that the three hydrolysates have more active amino acids or peptides to react with free radicals to form more stable products.

The DPPH/OH· radical scavenging and reducing power assay suggested that SCV hydrolysates potentially contained substances that could react with free radicals to convert them to more stable products and terminate the radical chain reaction, and trypsin, alcalase, and flavourzyme endowed the hydrolysates with higher antioxidant activities than other enzymes—such as neutrase, papain, bromelain, and pepsin. It could be concluded that alkaline proteases were more suitable to produce hydrolysates with desirable antioxidant activities, in good accordance with the conclusion obtained by degree of hydrolysis and molecular weight distribution of hydrolysates. Our results demonstrated that enzyme type employed is a key factor in determining antioxidant activities of SCV hydrolysates. The differences in antioxidant activity may be attributed to the differences in the amino acid compositions and their sequences, as well as the peptide level resulting from the specificity of the enzymes used (Zhao et al., 2012). From the amino acid composition in Table 2, total contents of amino acid exhibiting antioxidant activities were similar in the hydrolysates, so it was thought that amino acid sequence, conformation, and peptide level should play an important role in the antioxidant activities of SCV hydrolysates. Thus, the study currently underway will discuss the relationship between antioxidant activities and peptide structure.

Effect of enzyme type on functional properties

Foaming properties, expressed by FC and FS of SCV hydrolysates, are shown in Figure 5(A). A significant effect of enzyme type on the foaming properties of the hydrolysates was observed. Neutrase, flavourzyme, and bromelain gave higher FC to the hydrolysates than the other enzymes ($p < 0.05$), and neutrase-, alcalase-, and flavourzyme-derived hydrolysates showed better FS ($p < 0.05$). It could be found that neutrase and flavourzyme were ideal enzymes to produce hydrolysates with good foaming properties. FC and FS are influenced by two different sets of molecular properties of protein/peptides. Mutilangi et al. (1996) reported that FC of protein could be enhanced by making it more flexible, exposing more hydrophobic residues and increasing capacity to reduce interfacial tension. FS mainly depends on the nature of the viscoelastic film formed between air bubbles and aqueous phase (Mutilangi et al., 1996; Klompong et al., 2007). Therefore, the hydrolysate prepared by bromelain showed good FC, but poor FS, while that by alcalase showed poor FC, but good FS. Neutrase- and flavourzyme-derived hydrolysates had superior foaming capacity, presumably attributed to the flexibility and hydrophobicity of peptide, and their good foaming stability was principally related to the flexible interfacial membrane.

Emulsifying activity and stability are two indices used to evaluate the emulsifying properties of protein. Emulsifying activity measures the amount of oil that can be emulsified per unit of protein, whereas emulsifying stability measures the ability of the emulsion to resist changes to its structure over a defined time period. As illustrated in Figure 5(B), the EAIs of the hydrolysates prepared by papain and bromelain were higher than those by other proteases; however, their ESIs were lower ($p < 0.05$). Meanwhile the EAI of trypsin-derived hydrolysate was relatively low, but with the highest ESI ($p < 0.05$). The results suggested that EAI and ESI did not correlate directly, and they were also influenced by different factors.

The foaming and emulsifying attributes have vital applications in food products. Foaming properties are important in food systems such as confectionery, whipped toppings, and frozen desserts, while emulsions are important in meat products, frozen desserts, and baked goods (Toews and Wang, 2013). The results in the study suggested that the foaming and emulsifying properties of hydrolysates are influenced by specificity of enzyme, in good agreement with previous reports (Klompong et al., 2007; Zhao et al., 2012). Therefore, protease should be selected to prepare

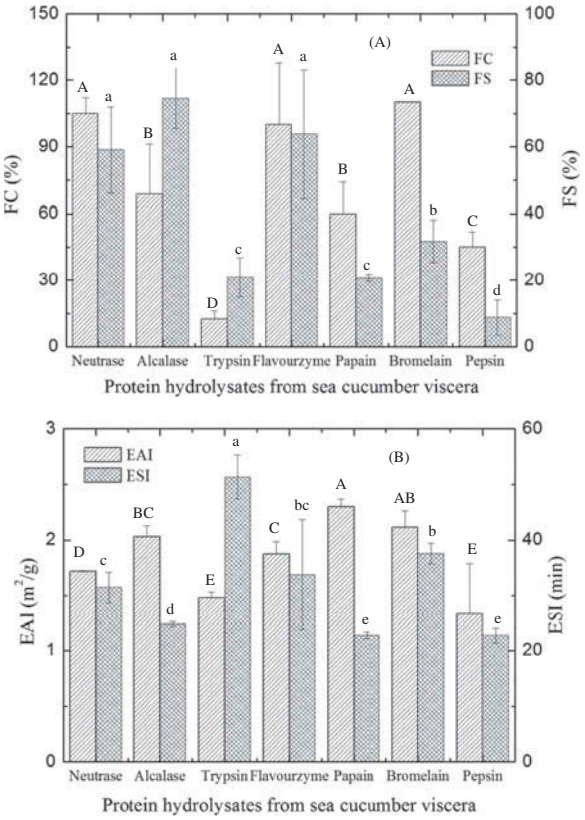

Figure 5. Foaming properties (A) and emulsifying properties (B) of protein hydrolysates from sea cucumber viscera prepared using seven different enzymes. For the same parameter, different letters indicate significant differences ($p < .05$).

the hydrolysate according to the production requirement. Enzymatic hydrolysis was beneficial to improve the functional properties of protein, but extensive hydrolysis could adversely affect functional properties of peptides (Kristinsson and Rasco, 2000b). Further studies are being planned in which the functional properties of hydrolysates under different DH.

It is known that most seafood hydrolysates were bitter, and this limits their application. In this study, the hydrolysates with the concentration of 1 mg/mL were evaluated for their bitter taste. Eight volunteers said that the taste could be accepted. Therefore, it was thought that the hydrolysates from sea cucumber viscera could be applied as food additives.

Conclusions

Here, seven commercial enzymes—including neutrase, alcalase, trypsin, flavourzyme, papain, bromelain, and pepsin—were used for the enzymatic hydrolysis of SCV protein. It was noticeable that the molecular weight distribution, amino acid composition, antioxidant activities, and functional properties of hydrolysates were affected by enzyme type. Alcalase, trypsin, and flavourzyme were more effective in producing peptides with higher antioxidant activities, and they were also effective in generating smaller peptides. Neutrase- and flavourzyme-derived hydrolysates showed relatively good foaming properties. Papain, neutrase, alcalase, and bromelain conferred high nutritional quality on the hydrolysates. Results showed that protease could be selected according to the production requirement for preparing SCV hydrolysate that might be used in food systems to impart desirable characteristics to products.

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