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Investigation of Gelatin Polypeptides of Jellyfish (*Rhopilema esculentum)* for Their Antioxidant Activity *in vitro*

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Summary

Jellyfish gelatin was hydrolyzed by different proteases to obtain antioxidative polypeptides. The gelatin hydrolysate obtained by progressive hydrolysis using trypsin and Properase E exhibited the highest hydrolysis degree and antioxidant activity. Three series of gelatin polypeptides (SCP1, SCP2 and SCP3) were obtained by ultrafiltrating the gelatin hydrolysate through molecular mass cut-off membranes of 10, 6 and 2 kDa, respectively. Amino acid composition analysis showed that SCP3 had the highest total hydrophobic amino acid content. The *in vitro* antioxidant tests demonstrated that SCP2 had the strongest hydroxyl radical and hydrogen peroxide scavenging activities and metal chelating ability, while SCP3 showed the highest reducing power, antioxidant activity in linoleic acid emulsion system and superoxide anion radical scavenging activity. The results support the feasibility of jellyfish gelatin as a natural antioxidant polypeptide provider, and enzymatic hydrolysis and ultrafiltration could be potent future processing technologies to utilize the abundant jellyfish resource.

Key words: jellyfish, gelatin polypeptide, antioxidant activity, copper(II)-binding ability

Introduction

Jellyfish, *Rhopilema esculentum*, is widely distributed in the South China Sea, the Yellow Sea and the Bohai Sea, where it is abundant from the late summer to early autumn. With the development of aquaculture, the yield of jellyfish increases year by year. Jellyfish has been used as Chinese food for thousands of years because of its high nutritive value and pharmacological activity, and it has also become popular in other countries recently. However, salted jellyfish is the only process and preservation technology at present, seriously limiting the utilization of jellyfish resource. Therefore, it is significant to develop further processing technologies and high-value products to adequately utilize the abundant jellyfish resource. Jellyfish umbrella is rich in collagenous protein (*1*), which contains above 50 % of the dry matter. When heated above 40 °C, collagen is converted into gelatin, so jellyfish could be an abundant source of gelatin. However, the application of gelatin in food and biomaterial industries is limited for its poor solubility, negative sensory properties and weak gel strength.

Enzymatic hydrolysis is a simple and inexpensive method to digest a protein into short-chain peptides and free amino acids. Protein enzymatic hydrolysates from many animals and plants, such as milk casein (*2*), fish protein (*3*), herring (*4*), mackerel (*5*), porcine myobrillar protein (*6*), yellowfin sole frame (*7*), soy protein (*8,9*), wheat protein (*10*) and yellow stripe trevally (*11*), have been found to possess antioxidant activities. The antioxidant properties of hydrolysates are highly influenced

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by several parameters, such as the amino acid composition, molecular structure and mass, which are determined by hydrolyzing conditions (*12*). Previous studies had shown that enzymatic hydrolysis modified the function of gelatin without altering its overall amino acid composition (*13*). Gelatin peptides contain mainly hydrophobic amino acids and the majority of these are more soluble in oil and have better emulsifying ability. Therefore, gelatin is expected to provide natural antioxidant peptides and have higher antioxidant effects than other peptides. Enzymatic hydrolysis has also been considered as effective means for developing proteins that would be more easily marketable. So, enzymatic hydrolysis could be a potent future processing technology to adequately utilize jellyfish gelatin.

However, little work has been carried out on the enzymatic hydrolysis of jellyfish gelatin. In this work, gelatin was extracted from jellyfish and its enzymatic hydrolysates were prepared by single protease and double-enzyme hydrolysis. The antioxidant activities of the obtained hydrolysates were evaluated *via* superoxide anion and hydroxyl radical scavenging assays. The hydrolysate showing the strongest antioxidant activity was fractioned using three different molecular mass cut-off (MMCO) ultrafiltration membranes to obtain three series of gelatin polypeptides. The antioxidant activities of three series of polypeptides are investigated and compared *via* six *in vitro* assays. The relationship between the antioxidant effects and molecular mass (M_r) and amino acid composition is discussed.

Materials and Methods

Materials

Jellyfish (*Rhopilema esculentum*) was obtained from Aoshan Bay in Qingdao, PR China. Proteases Multifect® neutral, GC106, trypsin, Flavourzyme® and Properase® E were purchased from Genencor International Co, Jiangsu, PR China. Papain and pepsin were purchased from Sigma Chemical Co, St Louis, MO, USA. All other reagents used in this study were of analytical grade.

Preparation of jellyfish gelatin

Jellyfish gelatin was extracted using the method of Grossman and Bergman (*14*) with slight modifications. Jellyfish umbrella was rinsed, cleaned and treated with 0.2 g per 100 mL of sodium hydroxide solution (1:8, by mass per volume) for 30 min, followed by rinsing with water to pH=7.0, and then treated with 0.2 g per 100 mL of sulphuric acid solution (1:8, by mass per volume) for 30 min with subsequent rinsing with water to pH=7.0. Finally, jellyfish umbrella was homogenized with Waring blender (DS-1, BIAO, Beijing, PR China) and extracted with distilled water (1:10, by mass per volume) for 8 h at 60 °C with continuous stirring. The resulting viscous solution was clarified by centrifugation at 5200×*g* for 20 min (BR4i, Jouan, France) and then lyophilized.

Preparation of gelatin enzymatic hydrolysates and three series of gelatin polypeptides

A mass of 1 g of gelatin was dissolved with 100 mL of sodium phosphate (0.02 M, pH=7.5) and the hydrolyzing process was adjusted to the optimum (based on the manufacturer's recommendations) of the respective enzyme used (Table 1). The hydrolysis processed without pH control and the enzyme inactivation was accomplished by heating for 3 min in boiling water. The double-enzyme hydrolysis includes the progressive and mixed hydrolysis of two selected enzymes. The progressive hydrolysis is a single enzyme hydrolysis at its optimum, following the other enzyme hydrolysis at its optimum; the mixed hydrolysis is a mixture of double-enzyme hydrolysis at either optimum. The obtained hydrolysates were centrifuged at 5200×*g* for 20 min. The degree of hydrolysis (DH) was determined using the pH-stat method (*13*), and the supernatants were evaluated for the antioxidant activities *via* superoxide anion and hydroxyl radical scavenging assays.

The supernatant of hydrolysate showing the strongest scavenging activity was fractioned using three different MMCO (10, 6 and 2 kDa) membranes (Yadong Hitech Co. Ltd, Shanghai, PR China). The fractioned temperature was kept at 4 °C and the pressure was adjusted to 0.15 MPa. Three series of gelatin polypeptides were obtained, namely SCP1 with M_r=6–10 kDa, SCP2 with M_r=2–6 kDa and SCP3 with M_r<2 kDa. The polypeptides were lyophilized and stored at –20 °C until future use.

Amino acid composition analysis

Three series of gelatin polypeptides were hydrolyzed under reduced pressure in 6 M HCl at 110 °C for 24 h and the major amino acid composition was analyzed using a Hitachi amino acid analyzer 835-50 (Hitachi, Tokyo, Japan). Total hydrophobic amino acids (THAA) were calculated as the total content of Ala, Val, Leu, Ile, Phe, Pro, Cys and Met.

Table 1. The optimum conditions (based on the manufacturer's recommendations) for seven proteases hydrolyzing jellyfish gelatin

Enzyme	Activity/(U/g)	pH	Temperature/°C	Time/h	(*m*(E)/*m*(S))/(g/g)
Multifect neutral	$2.0 \cdot 10^5$	7.0–7.2	45	4	1/50
Papain	$8.0 \cdot 10^4$	6.0–6.2	37	3	1/100
Pepsin	$4.5 \cdot 10^4$	2.0–2.2	37	6	1/50
Properase E	$7.0 \cdot 10^4$	9.0–9.2	50	4	1/50
Flavourzyme	$5.5 \cdot 10^4$	7.0–7.2	45	4	1/100
Trypsin	$9.5 \cdot 10^4$	7.5–7.7	45	3	1/100
GC106	$5.0 \cdot 10^4$	4.3–4.5	45	6	1/33

Reducing power assay

The reducing power of three series of gelatin polypeptides was determined by the method of Yen and Duh (*15*). Different concentrations of peptide samples in 3.5 mL of phosphate buffer (0.2 M, pH=6.6) were mixed with 2.5 mL of 1 % potassium ferricyanide in 10-mL test tubes. The mixtures were incubated for 20 min at 50 °C. At the end of the incubation, 2.5 mL of 10 % trichloroacetic acid were added to the mixtures, followed by centrifugation at 650×*g* for 10 min. A volume of 2.5 mL of supernatant fluid was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1 % ferric chloride, and the absorbance was measured at 700 nm. The increase in absorbance of the reaction mixture indicated the reducing power of the polypeptide samples.

The assay of antioxidant activity in linoleic acid emulsion

The antioxidant activity in linoleic acid emulsion of peptide samples was determined using the thiocyanide method (*16*) with slight modifications. Each sample in 0.5 mL of phosphate buffer (0.05 M, pH=7.0) was mixed with 0.5 mL of linoleic acid (0.05 M) in absolute ethanol, and the final volume was adjusted to 1.25 mL with distilled water. A volume of 0.5 mL of phosphate buffer (0.05 M, pH=7.0) was used instead of gelatin polypeptide as a control. The reaction mixture was incubated in the dark at 40 °C in an oven. Aliquots of 0.1 mL were taken 48 h after incubation. The degree of oxidation was measured by adding 4.7 mL of 75 % ethanol, 0.1 mL of 30 g per 100 mL of ammonium thiocyanate and 0.1 mL of ferrous chloride (0.02 M, in 3.5 % HCl) to the aliquots. The mixture was left to settle for 3 min, and then the peroxide value was determined by monitoring absorbance at 500 nm until the absorbance of the control reached the maximum. The antioxidant activity was calculated as percentage of inhibition relative to the control:

$$\text{Inhibition}=\{[(A'_{48}-A'_0)-(A_{48}-A_0)]/(A'_{48}-A'_0)\}\times 100 \quad /1/$$

where A_{48} is sample absorbance at 48 h, A_0 is sample absorbance at 0 h, A'_{48} is control absorbance at 48 h and A'_0 is control absorbance at 0 h.

Superoxide anion radical scavenging assay

Superoxide radical scavenging activity was assessed by chemiluminescence analysis of a pyrogallol luminol system (*17*) with a slight modification. A volume of 50 μL of freshly prepared pyrogallol solution (0.625 mM), 100 μL of gelatin polypeptides in distilled water and 850 μL of luminal solution (1 mM, in 0.1 M $NaCO_3$) were mixed in a reaction tube. Luminol chemiluminescence of the system was measured using an ultraweak luminescence analyzer (BPCL, Beinjing, PR China). Phosphate buffered saline (50 mM, pH=7.8) was used instead of gelatin polypeptides as a blank. The superoxide anion radical scavenging activity was calculated according to the following equation:

$$\text{Inhibition}=[(C_0-C_1)/C_0]\times 100 \quad /2/$$

where C_1 is sample chemiluminescence and C_0 is blank chemiluminescence.

Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity was assessed using an ascorbic acid-Cu^{2+}-hydrogen superoxide-yeast suspension system (*17*) with a slight modification. A volume of 0.2 mL of freshly prepared ascorbic acid solution (2 mM), 0.4 mL of $CuSO_4$ (2 mM), 50 μL of luminal (1 mM, in 0.1 M $NaCO_3$), 0.2 mL of yeast suspension (7.5 g per 100 mL) and 0.6 mL of gelatin polypeptides in distilled water were injected into the reaction tube and kept in a water bath at 37 °C for 30 min. Then 0.6 mL of H_2O_2 solution (6.8 mM) were added to start the reaction. Luminol chemiluminescence of the system was measured using an ultraweak luminescence analyzer. Phosphate buffered saline (50 mM, pH=7.8) was used instead of gelatin polypeptides as a blank. The scavenging activity of hydroxyl radical was calculated using Eq. 2.

Hydrogen peroxide scavenging assay

Hydrogen peroxide scavenging ability of gelatin polypeptides was determined according to the method described by Ruch *et al.* (*18*) with a few modifications. Solution of hydrogen peroxide (4 mM) was prepared in phosphate buffered saline (0.1 M, pH=7.4). Volumes of 0.2 mL of peptide samples in distilled water were mixed with 2.8 mL of hydrogen peroxide solution. Absorbance of the mixture was measured at 230 nm after 10 min against the blank solution in phosphate buffer without hydrogen peroxide. Phosphate buffered saline was used instead of gelatin polypeptides as control. The percentage of hydrogen peroxide scavenging of gelatin polypeptides was calculated according to the following equation:

$$\text{Inhibition}=[(A_0-A_1)/A_0]\times 100 \quad /3/$$

where A_1 was the absorbance of the sample and A_0 was the absorbance of control.

Metal chelating ability assay

Copper(II)-binding ability of gelatin polypeptides was determined using the dual-wavelength spectrophotometric tetramethylmurexide (TMM) method described by Wijewickreme and Kitts (*19*) with some modifications. A volume of 0.5 mL of gelatin polypeptides in Millipore grade water was mixed with 1.5 mL of hexamine buffer (10 mM, pH=6.8) containing 10 mM KCl. The mixture was then treated with 0.5 mL of $CuSO_4$ (2 mM) and incubated for 30 min at room temperature. Blanks without samples were treated under the same conditions. A volume of 0.1 mL of the reaction mixture was mixed with 0.1 mL of TMM (1 mM) in 2.4 mL of hexamine buffer. The amount of free copper(II) in the solutions was obtained from a standard curve, where the absorbance ratio A_{460}/A_{530} in a solution of 0.5 mL of $CuSO_4$ (0.02–0.1 mM), 2.0 mL of hexamine buffer and 0.1 mL of TMM was plotted against the amount of $CuSO_4$ (0.02–0.1 mM). The amount of copper(II) bound to gelatin peptide samples was calculated as the difference between the amount of added copper(II) and free copper(II) present in the solution.

Statistical analysis

All the tests were done in triplicate and the data were averaged. Standard deviation was also calculated. Student's *t*-test was used to evaluate significant differences ($p<0.05$) between the means for each sample.

Results and Discussions

Preparation of jellyfish gelatin enzymatic hydrolysates

Jellyfish gelatin was separately hydrolyzed by Multifect neutral, papain, pepsin, Properase E, Flavourzyme, trypsin and GC106, and the seven hydrolysates were evaluated for their antioxidant activities using superoxide anion and hydroxyl radical scavenging assays. As shown in Fig. 1, seven enzymes hydrolyzed jellyfish gelatin effectively, and enzymatic hydrolysates could retard free radicals significantly. The hydrolysates from Properase E and trypsin showed higher DH and stronger antioxidant activities. Based on the results, Properase E and trypsin were chosen to hydrolyse jellyfish gelatin by double-enzyme hydrolysis with different strategies.

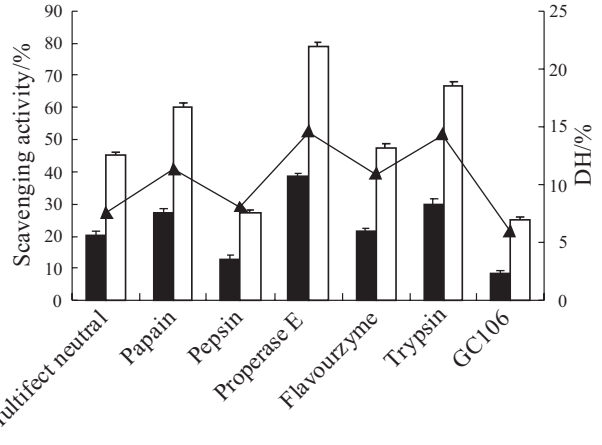

Fig. 1. Radical scavenging activity and hydrolysis degree of the jellyfish gelatin hydrolysates from single protease treatments. Black column – superoxide anion radical scavenging activity, white column – hydroxyl radical scavenging activity, (▲) degree of hydrolysis (DH)

Double-enzyme hydrolysis could improve DH (Table 2), with C1 and C3 being 26.1 and 27.8 %, respectively. The hydrolysates with higher DH showed stronger antioxidative activities. C3 with the highest DH exhibited the strongest scavenging ability among all hydrolysates, with superoxide anion and hydroxyl radical scavenging percentage being 52.8 and 94.2 %, respectively. Considering both DH and antioxidative effect, C3 was fractioned into three series of gelatin polypeptides, namely SCP1, SCP2 and SCP3 (M_r=6–10, 2–6 and <2 kDa, respectively). The amino acid compositions and antioxidant activities of three series of gelatin polypeptides were assayed.

Amino acid composition analysis

The analysis of amino acid composition (Table 3) revealed that three series of gelatin polypeptides were rich in Gly, Glu, Pro, Asp, Ala, Arg and THAA. The result was similar to that of silver carp protein hydrolysates (*20*). The THAA content of SCP3 was higher than of SCP1 and SCP2. For peptides, high content of hydrophobic amino acids could increase their solubility in lipids and therefore enhance their antioxidative activity (*3*). Rajapakse *et al*. (*21*) found that fish skin gelatin peptides possessed higher antioxidant activity than peptides from meat protein because of the high percentage of Gly and Pro. His and Pro played important roles in the antioxidant activity of synthetic peptides (*22*). Therefore, the antioxidant activities of the three series of gelatin polypeptides were expected because of the characteristic amino acid compositions.

Table 2. The radical scavenging activity and degree of hydrolysis (DH) of the hydrolysates from different protease treatments

Enzyme	Superoxide radical scavenging	Hydroxyl radical scavenging	DH
	%	%	%
Properase E	38.3±1.1	78.9±0.6	14.5±0.1
Trypsin	30.3±0.9	66.5±0.2	14.3±0.1
C1	47.6±1.0	90.4±0.9	26.1±0.2
C2	43.5±1.3	84.3±0.5	22.3±0.2
C3	52.8±0.9	94.2±1.2	27.8±0.1
C4	40.3±0.9	81.9±0.7	20.2±0.2

C1 was hydrolyzed with the mixture of Properase E and trypsin at the optimum of Properase E, C2 was hydrolyzed with the mixture of Properase E and trypsin at the optimum of trypsin, C3 was hydrolyzed with trypsin at its optimum then Properase E at its optimum, C4 was hydrolyzed with Properase E at its optimum then trypsin at its optimum. Values represent mean± S.E. (*N*=3)

Table 3. Amino acid composition of three series of gelatin polypeptides (No. of residues per 1000 residues)

Amino acid	SCP1	SCP2	SCP3
Asp	78.82	86.66	83.22
Thr	35.72	28.18	24.15
Ser	42.97	45.22	43.03
Glu	102.94	97.94	92.64
Pro	116.64	115.45	156.70
Gly	256.06	259.89	189.71
Ala	78.43	75.48	69.69
Val	41.33	45.59	44.81
Met	12.36	13.33	15.17
Ile	21.59	22.51	32.00
Leu	28.55	32.09	46.00
Tyr	8.86	10.44	29.32
Phe	13.29	16.04	32.55
Lys	26.70	32.59	35.18
His	3.45	12.18	5.06
Arg	53.26	65.79	57.98
Cys	21.57	19.23	39.67
Hyp	57.46	20.41	3.13
Total	1000	1000	1000
THAA	333.76	340.72	436.59

Reducing power

For the measurement of the reducing power, transformation of Fe^{3+} to Fe^{2+} in the presence of gelatin polypeptides was investigated. The reducing power of three series of gelatin polypeptides as a function of their concentration is shown in Fig. 2. At a low concentration (<1 mg/mL), three series of polypeptides showed similar reducing power, but at a high concentration (>2 mg/mL), SCP3 exhibited much higher reducing power than SCP1 and SCP2. The reducing power of three series of gelatin polypeptides increased with the use of increased concentration, suggesting that the reducing power of jellyfish gelatin peptides was dependent on the concentration. Similar observation has been reported on wheat germ protein hydrolysates and chickpea protein hydrolysates (*10,23*). The strongest reducing power shown by SCP3 might depend on the highest THAA content and the lowest M_r. The results show that SCP3 is an effective electron donor.

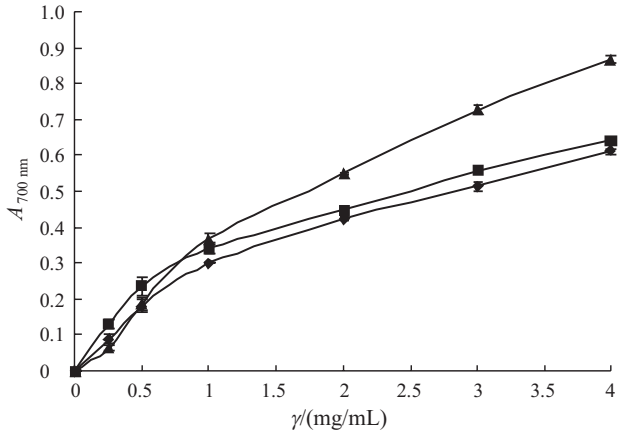

Fig. 2. Reducing power of three series of gelatin polypeptides as a function of the used concentrations. (◆) SCP1, (■) SCP2, (▲) SCP3. Bars indicate the standard deviation of triplicate determinations

Antioxidant activity in linoleic acid emulsion

Lipid peroxidation was thought to proceed *via* radical-mediated abstraction of hydrogen atoms from methylene carbons in polyunsaturated fatty acids (*21*). The inhibition of lipid peroxidation *in vitro* of three series of peptides was determined by assaying their abilities to inhibit oxidation of linoleic acid in an emulsified model system and compared with butylated hydroxytoluene (BHT) and α-tocopherol (Fig. 3). Results suggest that gelatin polypeptides could effectively hinder the self-oxidation of linoleic acid, and the antioxidant activity increased with the use of increased concentration. SCP3 exhibited stronger antioxidant ability than α-tocopherol, indicating that SCP3 had a noticeable inhibition of linoleic acid peroxidation. The results were in agreement with those claiming that lower molecular mass peptides had higher antioxidant activity (*21,24*). The highest THAA content of SCP3 might also favour its solubility in lipid system and thus increase its antioxidant effect.

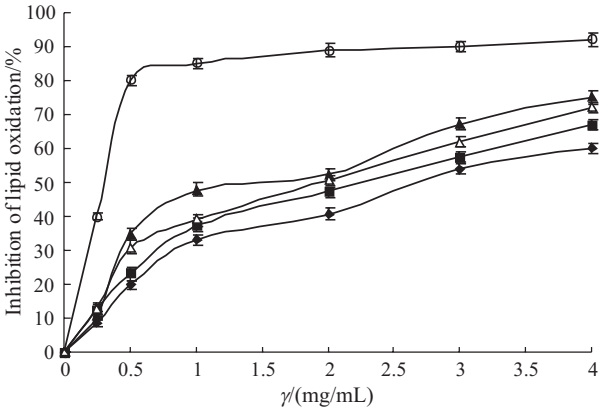

Fig. 3. Antioxidant activity of three series of gelatin polypeptides, butylated hydroxytoluene (BHT) and α-tocopherol as a function of concentrations used. (◆) SCP1, (■) SCP2, (▲) SCP3, (○) BHT, (△) α-tocopherol. Bars indicate the standard deviation of triplicate determinations

Superoxide anion radical scavenging activity

Superoxide anion radical cannot directly initiate lipid oxidation, but it is a potential precursor of stronger reactive oxygen species such as hydroxyl radical (*25*), so it is significant to scavenge this radical. Fig. 4a shows superoxide anion radical scavenging abilities of three series of gelatin polypeptides as a function of the used concentration. The scavenging activities increased as the concentration of all peptides increased. The correlation was linear and R^2 was 0.9894, 0.9892 and 0.9884 ($p<0.01$, Table 4), respectively. Similar linear relationship was also reported by Li *et al.* (*26*) in their study of corn gluten meal hydrolysates. Similar to reducing power and antioxidant activity, SCP3 exhibited the strongest scavenging effect at whatever concentration, being the highest for THAA content and the lowest for M_r.

Hydroxyl radical scavenging activity

Hydroxyl radical is the most reactive radical and can be formed from superoxide anion and hydrogen peroxide in the presence of metal ions, such as copper or iron. The hydroxyl radical has been demonstrated to be highly damaging species in free radical pathology, attacking almost every molecule in living cells. Therefore, scavenging or inhibiting the formation of hydroxyl radical is significant for organisms.

Hydroxyl radical was scavenged or inhibited by gelatin polypeptides in this study. Fig. 4b shows the scavenging effects of three series of peptides as a function of the used concentration. SCP2 exhibited the strongest scavenging activity, about 55.4 %, even at a lower concentration (0.5 mg/mL); SCP1 and SCP3 exhibited 50.2 and 40.5 %, respectively.

Scavenging activity of hydrogen peroxide

Hydrogen peroxide itself is not very reactive, but it is toxic to cells when decomposed to hydroxyl radical. Thus, the removal of H_2O_2 is very important for antioxidant defense in cells or food systems.

The hydrogen peroxide scavenging ability of three series of gelatin polypeptides is shown in Fig. 4c. The scavenging activity was also dependent on the concentration used. However, SCP2 exhibited the rapidly increasing scavenging effect as the concentration increased. The scavenging effects of peptides followed the sequence of SCP2>SCP3>SCP1. The results are similar to the hydroxyl radical scavenging activity. These results prove that the gelatin polypeptides have strong hydrogen peroxide scavenging activity and effective inhibition of the formation of hydroxyl radical.

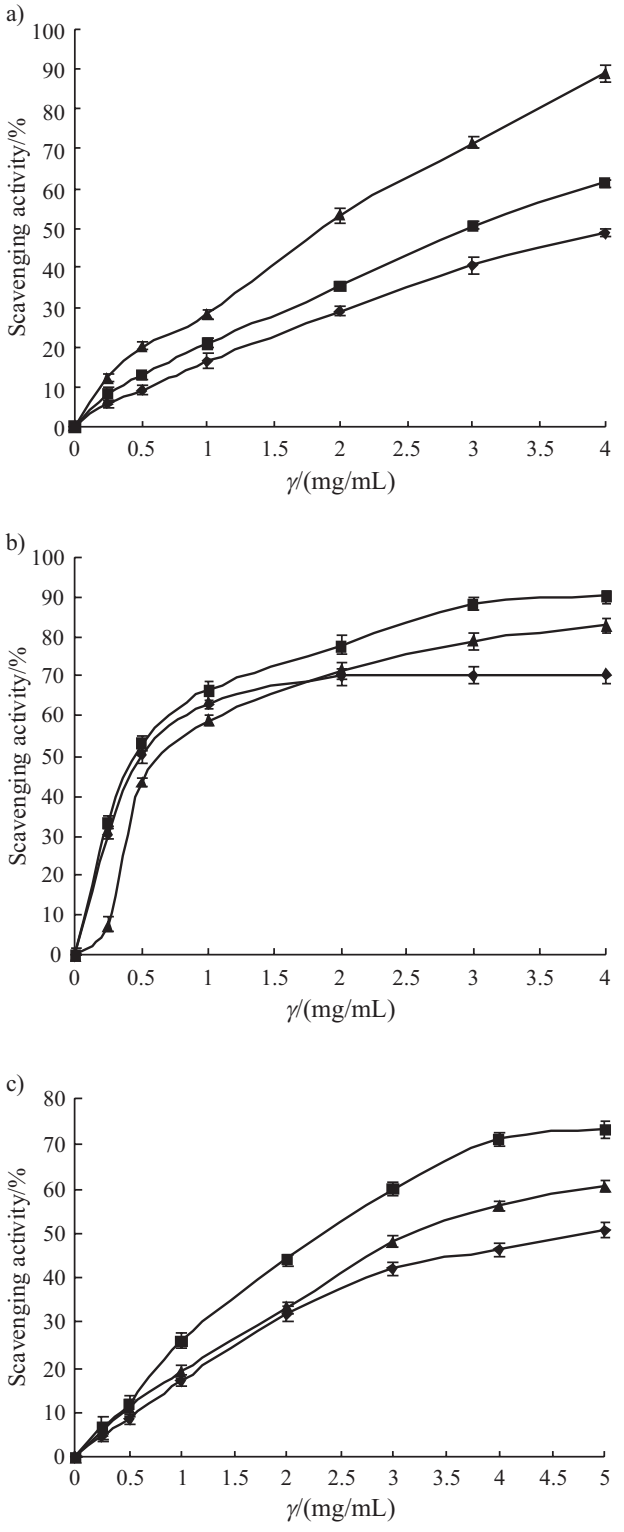

Fig. 4. Superoxide anion radical (a), hydroxyl radical (b) and hydrogen peroxide (c) scavenging activities of three series of gelatin polypeptides as a function of the used concentrations. (◆) SCP1, (■) SCP2, (▲) SCP3. Bars indicate the standard deviation of triplicate determinations

Table 4. Linear regression equations and correlation coefficients between the superoxide anion radical scavenging abilities and concentrations of three series of gelatin polypeptides

Samples	Regression equation	Correlation coefficient (R^2)
SCP1	Y=12.162x+2.7471	0.9894
SCP2	Y=14.962x+4.0900	0.9892
SCP3	Y=21.494x+1.1648	0.9884

Metal chelating activity

As shown in Fig. 5, three series of gelatin polypeptides demonstrated effective copper(II)-binding abilities. The copper-chelating ability increased with the increase of the concentration of used peptides. SCP2 exhibited the highest copper-chelating ability, and the chelating ability of the three series of gelatin peptides followed the sequence of SCP2>SCP3>SCP1. This was similar to the scavenging effect of gelatin polypeptides on hydroxyl radical (Fig. 4b). In the hydroxyl radical scavenging assay in this study, transition metal Cu^{2+} catalyzed the generation of hydroxyl radical. Meanwhile, in the reaction mixture of metal chelating activity, the same metal ion was chosen. The results indicate that jellyfish gelatin polypeptides might inhibit the formation of hydroxyl radical *via* chelating Cu^{2+}. Megías *et al.* (*27*) also suggested that polypeptides used as peroxidation protector might be related to their ion binding capacity.

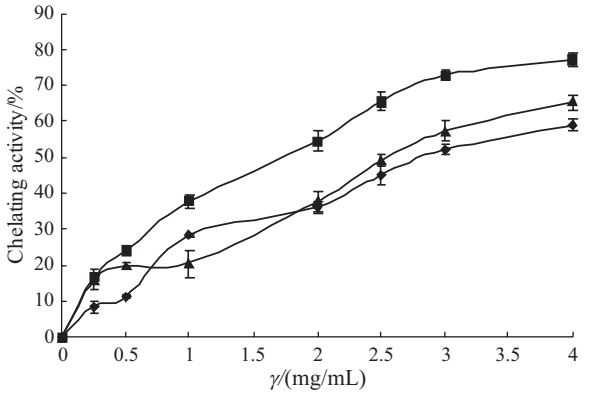

Fig. 5. Copper-binding ability of three series of gelatin polypeptides as a function of the used concentrations. (◆) SCP1, (■) SCP2, (▲) SCP3. Bars indicate the standard deviation of triplicate determinations

It was reported that the structure of peptides played an important role in the chelation of Cu^{2+} (*27*). The difference in amino acid composition exhibited different metal-chelating ability, and THAA could increase the chelating activity of peptides (*28*). The M_r of peptides plays a key role in chelating metals. If the length of pep-

tides is too short, the chelation is unstable; otherwise, the utilization of peptides is inadequate. Therefore, the high THAA content and appropriate M_r of SCP2 might favour its strongest copper-chelating ability.

Conclusions

Seven selected proteases hydrolyzed jellyfish gelatin effectively. The progressive hydrolysis of trypsin and Properase® E showed the highest DH and the hydrolysate exhibited the strongest superoxide anion and hydroxyl radical scavenging abilities. This hydrolysate was ultrafiltered through different MMCO membranes to enrich polypeptide fractions with a definite molecular mass range. Three series of gelatin polypeptides (SCP1, SCP2 and SCP3) were obtained. Each fraction exhibited significant antioxidant effects and free radical scavenging activities. The results supported the enzymatic hydrolysis and ultrafiltration as future processing technologies to adequately utilize the abundant jellyfish resources. Experiments studying the *in vivo* bioactivity of jellyfish gelatin peptides will be carried out in further research.

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