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Composition Analysis and Antioxidant Activity Evaluation of Oyster Enzymatic Hydrolysates

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Abstract

The focus of the study was to investigate composition and in vitro antioxidant activity from oyster enzymatic hydrolysates. Oyster enzymatic hydrolysates from oyster muscle were hydrolyzed using flavourzyme and ficin. Amino acid contents, volatile flavor components and molecular weight distribution of oyster enzymatic hydrolysates were determined by reverse phase high-performance liquid chromatography (RP-HPLC), gas chromatography-mass selective detector (GC-MS) and gel permeation chromatography (GPC), respectively. The RP-HPLC result indicated that 17 kinds of amino acids were detected in oyster enzymatic hydrolysates and the hydrolysates were rich in glutamic acid, histidine, glycine and cysteine. Totally 34 volatile compounds were described by mass spectrum (MS). Moreover, aldehydes were the most abundant, which accounted for 31.48% of the total components, followed by esters, which rank the second in the volatile flavor components identified. The number average molecular weight (Mn) of oyster enzymatic hydrolysates were mainly 10134Da, 324 Da and 109Da. The oyster enzymatic hydrolysates exhibited the higher scavenging activity on 2,2-azino-bis(3-ethylbenzthiazoline)-6-sulfonic acid (ABTS) radicals (IC_{50}=0.86 mg/mL), 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radicals (IC_{50} = 4.24 mg/mL), hydroxyl radicals (IC_{50}= 6.43 mg/mL) and reducing power (0.698 at 2.5 mg/mL). These results had shown that oyster enzymatic hydrolysates can be used as a tremendous source of antioxidant peptides, and of high interest for food and pharmaceutical industries to develop new nutraceuticals and functional foods.

Keywords: *Oyster muscle; Antioxidant activity; Oyster enzymatic hydrolysates; Volatile flavor components; Amino acid*

Abbreviations

RP-HPLC: Reverse Phase High-Performance Liquid Chromatography; GPC: Gel Permeation Chromatography; GC-MS: Gas Chromatography-Mass Spectrometer; MS: Mass Spectrum; DPPH: 1, 1-Diphenyl-2-Picrylhydrazyl; ABTS: 2, 2-Azino-Bis (3-ethylbenzthiazoline)-6-ulfonic acid

Introduction

Reactive oxygen species (ROS) are highly reactive due to their single and unbalanced electrons, which can attack membrane lipids, protein and DNA. This in turn can be a causative factor in many diseases such as cardiovascular disease, diabetes, cancer and Alzheimer's disease [1]. In order to act against these deleterious oxidative induced reactions in food and biological system, many synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butylhydroquinone (TBHQ) and propyl gallate (PG) are usually added to food products to retard oxidation. However, the use of synthetic antioxidants in food products is concern about their safety, so it is under strict regulation [2]. Therefore, the negative consumer perception of synthetic antioxidants has led to an increasing demand for natural antioxidants. In order to expand the source of natural antioxidants further, researchers have begun to focus on bio macromolecules, such as polysaccharide, protein and peptides. A number of studies have shown that peptides and protein hydrolysates of plant and animal origins possess significant antioxidant activity, for example, the protein hydrolysates of corn gluten meal [3] and the pro-

tein hydrolysates of bluefin leather jacket heads [4]. These antioxidant peptides are inactive within the sequence of the precursor protein molecules but can be released after enzymatic hydrolysis. Antioxidants not only improve the stability of lipids and lipid-containing foods, but are also used to preserve food products by retarding discoloration and deterioration resulting from oxidation, which also results in enhancing shelf life. What is more, protein hydrolysates can also be applied to food products to extend their shelf life.

Oysters are not only the biggest farming shellfish in the world, but also one of four major economic farming shellfishes in China. With the development of economy and the changes in dietary habit, the output of oysters has shown a trend of increasing demand in China since 2009. Several recent studies have shown that oyster protein hydrolysates or peptides with antioxidant activities can be released from oyster proteins after enzymatic hydrolysis. Various methods have been utilized to release bioactive peptides from food proteins, among which enzymatic hydrolysis is the most widely used technique.

In our previous study, ficin [5] and ficin combined with flavourzyme had been used to prepare oyster enzymatic hydrolysates. At present, there are less reports about composition and *in vitro* antioxidant activity of oyster enzymatic hydrolysates. The flavor and antioxidant activity of oyster emzumatic hydrolysates were affected by the amino acid compositions and flavor components. In this study, the oyster enzymatic hydrolysates were prepared by two proteases (ficin and flavourzyme). Amino acid contents, volatile flavor components, molecular weight distribution and antioxidant activities of the oyster enzymatic hydrolysates were investigated.

Materials and Methods

Materials and chemicals

Oyster muscle (*Crassostrea talienwhanensis*) was purchased from a local market (Hainan, China). They were kept in cold seawater and transported to the laboratory. After rinsed, the whole fresh muscle was homogenized by speed tissue homogenizer. The homogenates were transferred into beakers, sealed and stored at -20^{0}C until used for enzymatic hydrolysis. Ficin was supplied by Henan Biological Engineering Technology Co. (Henan, China). Flavorzyme was provided by Jiangsu Biotechnology Co. (Jiangsu, China). 2,2-azino-bis(3-ethylbenzthiazoline)-6-sulfonicacid (ABTS) and 1,1-Diphenyl-2-picrylhydrazyl (DPPH) were obtained from Shanghai Yuanye Biotechnology Co. (Shanghai, China). All other chemicals and solvents used in the experiments were of analytical grade.

Preparation of enzymatic hydrolysates from oyster muscle

According to our previous study, the oyster muscle was hydrolyzed by double proteases including ficin and flavourzyme. Briefly, a certain amount of homogenate was taken into a beaker and mixed with distilled water. The proportion of homogenate and distilled water was1:2(g/mL) and the amount of proteases was 12% (the proportion of ficin and flavourzyme was 3:1). After adjusting to a required pH 6.0 by addition of 1 M NaOH or 1 M HCl, the mixture was incubated at temperature of 50^{0}Cfor 3 h, with continuously stirring. After hydrolysis, the homogenate was boiled for 10 min to inactivate proteases, and then cooling it immediately in a water bath to room temperature. The homogenate was centrifuged at 4000 g for 20 min to remove any insoluble residues. The supernatants containing water-soluble hydrolysates were collected. The oyster enzymatic hydrolysates were lyophilized and stored at −20^{0}C for further use.

Determination of amino acid composition

The oyster muscle (1g) was digested with 10mL 6 M HCl at 110±1^{0}C under a vacuum for 22 h, using qualitative filter paper to filter the hydrolyzed oyster muscle, and then adjusted to pH 8.0. While the oyster enzymatic hydrolysates (20 mL) were mixed with 20mL of 5-sulphosalicylic acid solution (10%, w/w) at 4^{0}C for 17 h. After that, the solution was filtered by qualitative filter paper and then adjusted pH to 8.0. Amino acids of oyster enzymatic hydrolysates and oyster muscle were quantified by reverse phase high-performance liquid chromatography (RP-HPLC) on a AccQ-Tag amino acid analysis column (15cm length × 3.9mm i.d., Waters) coupled with a 2695-2475 system (Waters Corporation, Milford, MA) were detected at 248nm, 37^{0}C. The samples were then eluted with two solvents (A: 10% acetate- phosphate buffer solution in ultrapure water, B: 60% aceto-nitrile in ultrapure water) at a flow rate of 1.0mL/min. Amino acids were detected by a UV detector at 220nm. The amounts of amino acids were calculated by comparing the peak area with standards [6].

Identification and quantification of the volatile content

The ether soluble volatile flavor components in oyster enzymatic hydrolysates (10ml) were extracted with 15ml of aether by intermittent extraction 60min (each ultrasonic 15min, 5min), using ultrasonic oscillator. The organic extracts were dehydrated over anhydrous magnesium sulphate for 12h, and then dried the aether to obtain a final sample of 0.2ml. Qualitative and quantitative identification of volatile flavor components were analyzed by a HP 6890N gas chromatography-5973 mass selective detector (GC-MS) (Hewleft-Packard, Palo Alto, CA, USA) equipped with a DB-5MS capillary column (30m length × 0.25mm i.d., Agilent). Helium was the carrier gas at settled flow rate of 0.3ml/min, no split. The GC oven temperature was initially at 40^{O}C for 2min, and ramped at 5^{O}C /min to 60^{O}C, then raised to 100^{O}C at a rate of 3^{O}C /min, post run at 18^{O}C /min, 240^{O}C for 6min at last. Injector and detection temperature were 250^{O}C and 240^{O}C, respectively. The electron-impact (EI) mass spectra were generated at 70eV, an ion source temperature of 200^{O}C with m/z scanning range from 20 to 500 amu. For mass spectrum (MS), tentative identifications were compared with those in the NIST98 MS database.

Determination of *in vitro* antioxidant activity

Scavenging activity on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical

The DPPH radical scavenging activity was measured according to the procedure described by Wu [7] with a slight modification. Briefly, 2.0mL DPPH-ethanol solution (0.2mM) was mixed with 2.0mL of the oyster enzymatic hydrolysates. After vortex, the reaction mixture was kept in dark at room temperature for 30 min and then the absorbance of reaction mixture was measured at 517nm. The DPPH radical scavenging activity was expressed as: scavenging rate (%) = $[1-(A_i-A_j)/A_0]\times100$, where A_i is the absorbance of the reaction solution, A_j is the absorbance of the solution including 2.0mL of ethanol and 2.0mL of the oyster enzymatic hydrolysates, A_0 is the absorbance of the solution including 2.0mL of DPPH-ethanol solution (0.2mM) and 2.0mL of distilled water. And 2.0mL of ethanol with equal volume distilled water as blank zero. The IC_{50} value was defined as the concentration of sample (mg/mL) required to scavenge 50% of DPPH radical. Ascorbic acid was used as a positive control. All experiments were carried out in triplicate.

Scavenging effect on hydroxyl radical

The scavenging effect of the oyster enzymatic hydrolysates on hydroxyl radical was conducted in accordance with the method of Wang [8] with a slight modification. Briefly, the reaction fluid mixed with 1mL of 2-hydroxybenzoic acid (9mM), 1mL of ferrous sulphate (9mM), 1mL of hydrogen peroxide (10mM) and 1mL of oyster enzymatic hydrolysates with various concentrations. Following incubation at 37^{O}C for 30 min, the absorbance of the mixture was measured at 510nm. The hydroxyl radical scavenging activity was expressed as: scavenging rate(%)= $[1-(A_1-A_2) / A_0]\times100$, where A_1 is the absorbance of the reaction solution, A_2 is the absorbance of the solution including 2.0mL of ethanol and 2.0mL of oyster enzymatic hydrolysates A_0 is the absorbance of reaction solution mixture with oyster enzymatic hydrolysates replaced by equivalent volume of distilled water. Ascorbic acid was used as a positive control. All experiments were carried out in triplicate. The IC_{50} value was defined as the concentration of sample (mg/mL) required to scavenge 50% of hydroxyl radical.

ABTS radical scavenging activity

ABTS radical scavenging activity was determined according to the modified method of Roberta [9]. $ABTS^{+\bullet}$was produced by reacting 5.0mL of 7.0mM aqueous $ABTS^{+\bullet}$ solution with 88.0 µL of 140 mM potassium persulfate in the dark at room temperature for 12 to 16 h and was used within 2 days. The $ABTS^{+\bullet}$ solution was diluted with phosphate buffer (0.01mol / L, pH7.4) to an absorbance of 0.70 ± 0.02 at 734nm. 0.1mL of diluted sample was mixed with 3.9mL of diluted $ABTS^{\bullet+}$ solution. The mixture was then allowed to stand for 6 min at room temperature, and the absorbance was immediately recorded at 734nm. The percentage of scavenged $ABTS^{\bullet+}$ was calculated as: scavenging rate (%) = $[1- A_1/A_0]\times100$, where A_1 is the absorbance of the solution including 0.1mL of diluted sample and 3.9mL of diluted $ABTS^{\bullet+}$ solution, A_0 is the absorbance of the reaction solution with sample replaced by equal volume of phosphate buffer. The IC_{50} value was defined as the concentration of sample (mg/mL) required to scavenge 50% of ABTS radical. Ascorbic acid was used as a positive control. All experiments were carried out in triplicate.

Reducing power

The reducing power was determined according to the procedure described by Gu [10] with minor modifications. Briefly, the sample with various concentrations (1.0mL) was mixed with 1.0mL of phosphate buffer (0.2 M, pH 6.6) and 1.0mL of potassium ferricyanide (1%, w/v). After incubating at 50^{0}C for 20 min, 1.0mL of trichloroacetic acid (10%, w/v) was added to the mixture. After vortex, the fluid was centrifuged at 3000 g for 10 min. 2.0mL of upper layer of solution were collected and mixed with 2.0mL of distilled water and 0.4mL of ferric chloride (0.1%, w/v). After storage at room temperature for 10 min, the absorbance was measured at 700nm. Blank sample included neither sample nor ferric chloride. Higher absorbance indicated greater reductive potential. The synthetic antioxidant ascorbic acid was used as a positive control. All experiments were carried out in triplicate.

Statistical Analysis

All the tests were carried out in triplicate. The experimental data were presented as means ± standard deviations (SD). The statistical analysis was performed using one-way analysis of variance (ANOVA) and Origin_8.0. The significant difference was determined with 95% confidence interval ($P < 0.05$).

Results and Discussion

The analysis of amino acid compositions in oyster enzymatic hydrolysates and oyster muscle

Protein hydrolysates obtained after hydrolysis of proteins are composed of free amino acids and short chain peptides exhibiting many advantages as nutraceuticals or functional foods because of their amino acid profile. The amino acid composition of any food proteins plays a significant role in various physiological activities of human body and affects good health directly or indirectly. Amino acids are essential for synthesis of a wide variety of proteins with important functions including carriers of oxygen, vitamins, CO_2, enzymes and structural proteins [11]. The amino acid compositions of fish protein hydrolysates are important because of the nutritional value and the influence on the functional properties [12]. The amino acid compositions of oyster enzymatic hydrolysates and oyster muscle are shown in Table 1.

Amino Acid	Quality composition (mg/g)	
	Oyster Muscle	Enzymatic Hydrolysate
Aspartic acid (Asp)	67.70	41.52
Serine (Ser)	42.72	79.80
Glutamic acid (Glu)	100.12	93.53
Glycine (Gly)	62.45	41.94
Histidine (His)	143.83	95.14
Arginine (Arg)	22.20	27.16
Threonine (Thr)*	74.87	118.27
Alanine (Ala)	28.34	42.14
Proline (Pro)	49.65	39.93
Cysteine (Cys)	70.40	20.93
Tyrosine (Tyr)	53.40	60.51
Valine (Val)*	48.35	62.49
Methionine (Met)*	26.93	29.52
Lysine (Lys)*	49.00	76.47
Isoleucine (Ile)*	37.50	59.91
Leucine (Leu)*	67.82	89.17
Phenylalanine (Phe)	53.48	21.10

Table 1: *Amino acid composition of the oyster muscle and oyster enzymatic hydrolysates (mg/g).*

**Essential amino acids*

It's easy to see that, 17 kinds of amino acid were detected in oyster enzymatic hydrolysates and oyster muscle. Amino acid compositions of the oyster enzymatic hydrolysates and oyster muscle showed the presence of both essential and non-essential amino acids.

Histidine and glutamic acid were the most abundant amino acids in the oyster muscle, whereas the oyster enzymatic hydrolysates were rich in threonine, histidine, glutamic acid and leucine, which accounted for 118.27, 95.14, 93.53 and 89.17 mg per gram of protein respectively. Additionally, the oyster enzymatic hydrolysates contained high contents of hydrophobic amino acids, such as leucine (89.17 mg), glycine (41.94 mg), valine (62.49 mg), methionine (29.52 mg), proline (39.93 mg), tyrosine (60.51 mg) and alanine (42.14 mg). From the results, oyster protein hydrolysates had a high nutritional value. Therefore, the obtained protein hydrolysates could possibly be a supplementary protein to the poorly balanced diet. It has been reported by Zhu [13] that oyster enzymatic hydrolysates containing histidine, valine, leucine, methionine and alanine possessed strong antioxidant activity.

Analysis of volatile flavor components in oyster enzymatic Hydrolysates

The volatile flavor components of oyster enzymatic hydrolysates extracted by ether were detected using GC-MS and analyzed both qualitatively and quantitatively. Compounds were identified by comparison with the mass spectra in the electron impact mode. Table 2

Number	Retention time/min	Compound	Relative content/%
1	4.56	Isoamyl butyrate	0.91
2	5.38	Lauric acid	0.50
3	5.87	3-Ethyl-3-methylheptane	0.52
4	6.39	Pentadecanoate	0.38
5	6.72	Hexaldehyde	1.27
6	6.85	2-Octanone	0.34
7	7.38	Paraxylene	1.79
8	7.53	2,2-Dimethyl-1-octanol	0.61
9	7.72	Benzaldehyde	1.45
10	7.87	Methyl pentadecanoate	0.62
11	8.21	Benzyl alcohol	0.90
12	9.65	Hexylenic aldehyde	0.30
13	9.81	Methyl-cyclohexanol	0.37
14	10.15	4-Hydroxy-benzeneethanol	0.46
15	11.45	Methyl hexadecanoate	1.55
16	11.90	1-Octene-3-ol	0.61
17	12.78	(E, E) -2,4- Heptadienal	1.53
18	13.45	Cyclohexyl methyl sulphide	0.19
19	14.80	Trans -2- decent aldehyde	0.99
20	15.89	2,3- Pentanedione	0.17
21	16.54	Nonaldehyde	2.97
22	16.81	4-Ethyl-4-heptanol	0.19
23	19.57	Trans-2, cis -6- nonadienal	0.93
24	25.35	2-Undecanone	0.07
25	25.78	Hexadecanoic acid methyl ester	2.37

26	26.82	n-Hexadecanoic acid	1.21
27	27.68	Phthalate	0.84
28	28.39	Glycinamide	0.97
29	33.50	Phenol,2,2'-Methylenebis[6-(1,1-dimethyl-ethyl)-4-methyl-	2.93
30	35.48	Stearic Acid	0.62
31	39.82	4,4-Dimethyl-undecane	0.11
32	40.12	Diisobutyl phthalate	0.45
33	40.27	Isopropyl palmitate	1.03
34	44.31	Cyclohexylamine	0.46

Table 2: *Identification and contents of volatile flavour components in oyster enzymatic hydrolysates by GC-MS.*

lists the volatile flavor components determined, their relative peak areas, and retention time identified on the DB-5MS column. There are 34 volatile components mainly assorted into 7 chemical categories:

(1) 8 esters presented with fragrant and sweet aroma [14]. (2) 5 alcohols were described as delicious smooth flavor formed with carbonyl compound [15]. (3) 7 aldehydes. Aldehydes play an important role in food odor. Hexanal and octanal with fruit smell are polyunsaturated fatty acid metabolites. (4) 4 ketones, (5) 4 acids, (6) 2 amines, (7) 4 other compositions like 3-ethyl-3-methylheptane, 4,4-dimethyl-undecane, paraxylene and 2, 2'-Methylenebis [6-(1, 1-dimethyl-ethyl)-4-methyl- phenol.

Among the 34 volatile compounds identified, aldehydes contents were as high as 31.48% of total components. Esters were the second most abundant class in the volatile flavor components identified. These are important for the particular flavor formation. Esters have ester group in the ring structure, which come from dehydration by carboxylic acid and alcohol, the flavor generated by the esters volatile is similar to the alcohols more or less. Among the volatile alcohol components, these aromas are beneficial to dispel the stench leading to a better sensory evaluation. According to others' finding, the generation of ketones is the result of degradation and thermal oxidation by the unsaturated fatty acid (UFA). 1-Octene-3-ol is a kind of linoleic acid hydrogen peroxide degradation products that shows a similar mushroom aroma [16]. These volatile flavor components indicated important sensory characteristic. In general, peculiar seafood flavor of oyster enzymatic hydrolysates consist of the whole volatile components.

Effect of scavenging DPPH radicals

Radicals DPPH is a stable free radical that shows maximum absorption at 517nm in ethanol. When DPPH encounters a proton-donating substance (H^+), the radical is scavenged by changing color from purple to yellow and the absorbance is reduced [17].

DPPH accepts an electron or hydrogen to become a stable molecule. Therefore, DPPH is often used as a substrate to evaluate the antioxidant activity of the oyster enzymatic hydrolysates [18]. Figure1 shows the DPPH radical scavenging activity of oyster enzymatic hydrolysates at various concentrations. As shown in Figure 1, the oyster enzymatic hydrolysates were found to have the ability of scavenging DPPH radicals in a dose-dependent manner. The IC_{50} for the oyster enzymatic hydrolysates was 4.24 mg/mL. It was higher than frog protein hydrolysates reported by Gu [10]. But the scavenging activity of the oyster enzymatic hydrolysates was significantly lower than that of ascorbic acid at each concentration point (Figure 2), which IC_{50} value was 21.86 μg/mL.

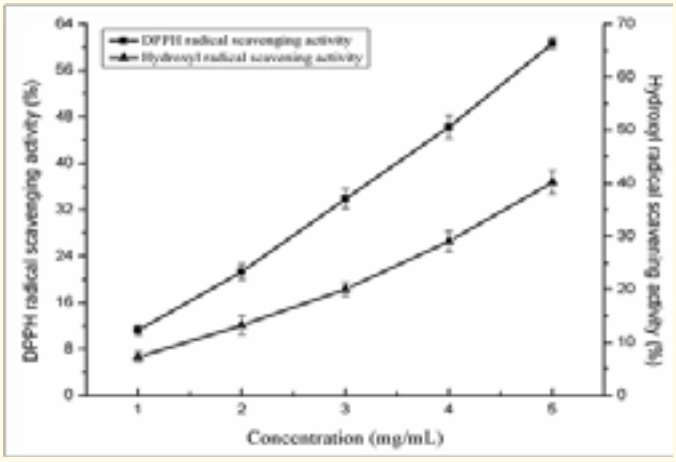

Figure 1: *DPPH radical scavenging activity and hydroxyl radical scavenging activity of oyster hydrolysates at different concentrations.*

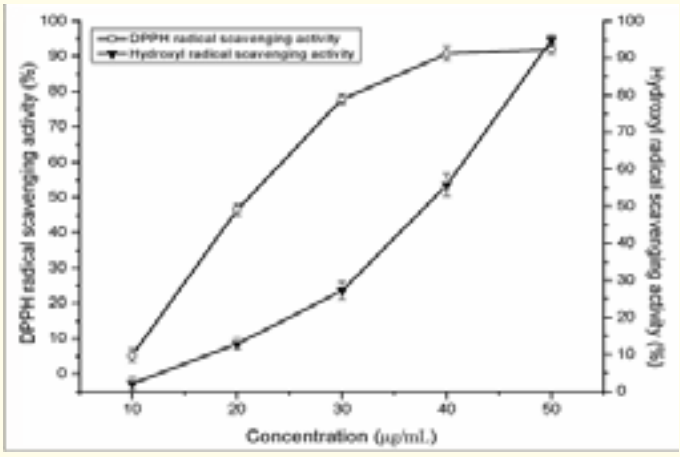

Figure 2: *DPPH radical scavenging activity and hydroxyl radical scavenging activity of ascorbic acid at different concentrations.*

Effect of scavenging hydroxyl radicals

It is well known that hydroxyl radical is extremely reactive and damages adjacent biomolecules such as protein, fatty acids, DNA and nucleic acids [19]. Therefore, hydroxyl free radical is deemed to be the typical representative among all free radicals, and is widely used to monitor and evaluate the antioxidant ability of protein hydrolysates [20]. The hydroxyl radical scavenging activities of oyster enzymatic hydrolysates and ascorbic acid are presented in Figure 1 and Figure 2, respectively. The oyster enzymatic hydrolysates were found to have the ability of scavenging hydroxyl radicals. The scavenging effects of oyster enzymatic hydrolysates were gradually increased with increasing concentration. The oyster enzymatic hydrolysates may scavenge hydroxyl radicals by electron/hydrogen donation and radical quenching [21]. The IC_{50} value for the oyster enzymatic hydrolysates was 6.43 mg/mL, which is lower than ascorbic acid (IC_{50}= 38.90 μg/

Effect of scavenging ABTS radicals

The scavenging effect of oyster enzymatic hydrolysates on ABTS radicals was measured, and the results are shown in Figure 3. The scavenging activity of enzymatic hydrolysates toward ABTS radicals was related to oyster enzymatic hydrolysates concentrations, the results indicated that oyster enzymatic hydrolysates could effectively scavenge ABTS radicals in a dose-dependent way at the concentra-

tions increasing from 0.5 to 2.5 mg/mL. The IC_{50} value of the oyster enzymatic hydrolysates was 0.86 mg/mL for ABTS radical scavenging activity. It was higher than those of other protein hydrolysates reported [22]. As shown in Figure 4, the positive control as ascorbic acid showed more potent scavenging activity and its IC_{50} value was 26.95 μg/ML.

Reducing power of oyster enzymatic Hydrolysates

The reducing power assay is often used to evaluate the ability of an antioxidant to donate electrons [23]. The reducing power of oyster enzymatic hydrolysates was measured by transformation of Fe^{3+} to Fe^{2+}. Figure3 shows the reducing power, expressed as absorbance at 700nm, of oyster enzymatic hydrolysates. The oyster enzymatic hydrolysates showed reductive capabilities in a dose dependent manner and the maximum reducing power was found at 0.698 ± 0.02 in 2.5 mg/mL and the minimum reducing power was found at 0.165 ± 0.017 in 0.5 mg/mL. The oyster enzymatic hydrolysates had the strongest reducing power with an IC_{50} value of 1.52 mg/mL. Although the reducing power of oyster enzymatic hydrolysates was lower than that of ascorbic acid (IC_{50}= 13.84 μg/mL), it was higher than the oyster protein hydrolysates reported by Dong [24]. Hence, from the results, it appears that the oyster enzymatic hydrolysates could be excellent electron donors to free radicals.

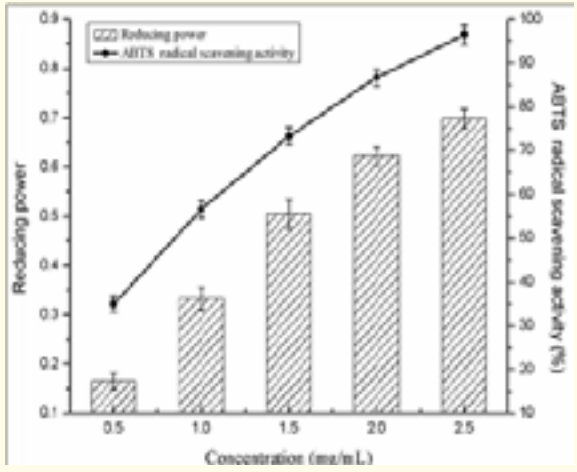

Figure 3: *ABTS radical scavenging activity and reducing power of oyster enzymatic hydrolysates at different concentrations.*

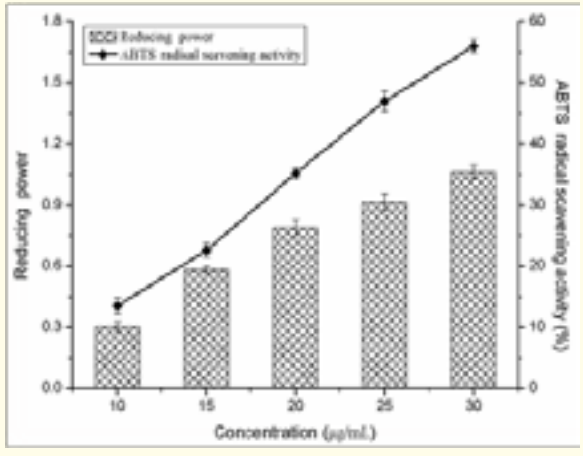

Figure 4: *ABTS radical scavenging activity and reducing power of ascorbic acid at different concentrations.*

The molecular weight of oyster enzymatic hydrolysates

Using gel permeation chromatography (GPC) to determinate the number average molecular weight (Mn) of oyster enzymatic hydrolysates. The results are shown in Table 3 and Figure 5.

Peak	Mn	Mw	Mp	Mz	Mz+1
1	10134	12978	9022	17788	23944
2	324	338	327	354	372
3	109	112	110	114	116

Table 3: *GPC results of oyster enzymatic hydrolysates.*

The molecular weight distribution of the antioxidant peptides from oyster enzymatic hydrolysates mainly had three peaks, which showed that the antioxidant peptides were mainly concentrated in the three molecular weights. The number average molecular weights (Mn) were 10134Da, 324Da and 109Da, respectively. Meanwhile, the weight average molecular weight (Mw) were 12978 Da, 338 Da and 112Da, As shown in Figure5, the peak molecular weight (Mp) were 9022Da, 327Da and 110Da.

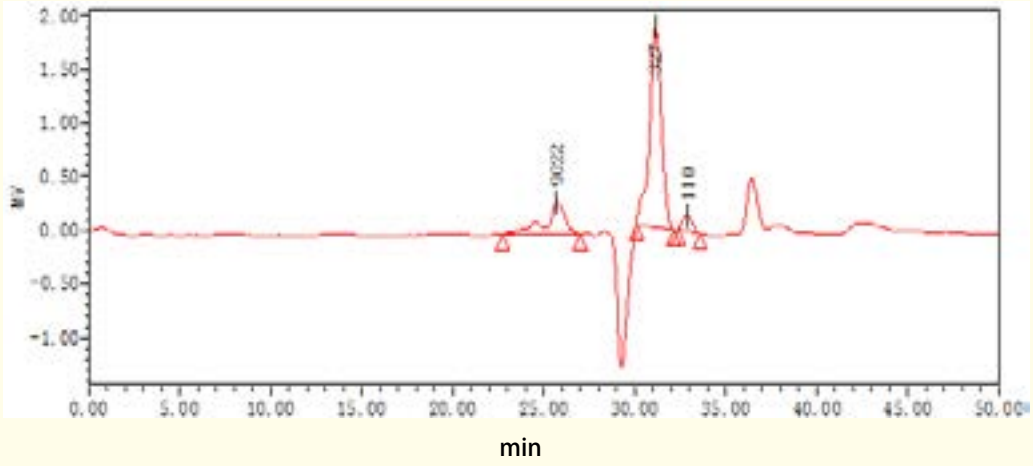

Figure 5: *GPC chromatogram of oyster enzymatic hydrolysates.*

Conclusion

In the present study, oyster enzymatic hydrolysates derived from ficin and flavourzyme showed good antioxidant activity. It exhibited antioxidant activities by evaluation of reducing power, DPPH, ABTS and hydroxyl radical scavenging assay and reducing power. The antioxidant activity of protein hydrolysates is related to the amino acid compositions. The oyster enzymatic hydrolysates containing histidine, valine, leucine, methionine and alanine possessed strong antioxidant activity. The molecular weight of the oyster enzymatic hydrolysates was mainly distributed in less than 500Da. The whole volatile components formed peculiar seafood flavor of oyster enzymatic hydrolysates. The results of our study suggest that oyster enzymatic hydrolysates could be utilized to develop new nutraceuticals and functional foods. A number of studies have demonstrated that foods with high in vitro antioxidant capacity can produce an antioxidant activity response *in vivo* [25][26], so the further research is required to in-depth study in vivo antioxidant activity of oyster enzymatic hydrolysates.

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Conflict of Interest

We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

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