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# Analysis of protein composition and antioxidant activity of hydrolysates from *Paphia undulate*

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**Abstract** Three types of proteins including sarcoplasmic protein, myofibrillar protein and stromal protein were isolated and from *Paphia undulate* muscle tissue. Myofibrillar protein had the highest content (45.42%), followed by sarcoplasmic protein (31.20%) and stromal protein (20.69%). Analysis of DSC found that the denaturation temperatures of sarcoplasmic protein, myofibrillar protein and stromal protein were 53.4°C, 47.23°C and 55.73°C, respectively. The three protein fractions contained higher contents essential amino acids, alkaline, hydrophobic and branched chain amino acid. Protein hydrolysates prepared with trypsin revealed a good radical scavenging activity. Moreover, the hydrolysates of sarcoplasmic protein exhibited the strongest radical scavenging activities including DPPH radical, hydroxyl radical and superoxide anion radical, with the $IC_{50}$ values being 4.82, 4.70 and 3.75 mg/mL, respectively. In addition, the hydrolysates of sarcoplasmic protein and myofibrillar protein showed a similar reducing power and their reducing power were 0.58 and 0.57 at 10 mg/mL, respectively. While the hydrolysates of stromal protein displayed lower reducing power.

***Keywords:*** *protein isolation, DSC, protein hydrolysate, free radical scavenging activity, reducing power*

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## 1. Introduction

*Paphia undulate* is an abundant marine shellfish resources, which widely cultured in China. The yield of *Paphia undulate* was estimated to be 40,000 tons in Fujian province in 2007 [1]. However, the products are mainly sold at a very low price, which caused low efficiency of resource utilization and low economic benefit. Aquatic proteins especially essential amino acids are an essential source of nutrients for many people, especially in developing countries. In our previous researches indicated that *Paphia undulate* is a good source of protein, consisting of approximately 68.77% crude protein (dry weight basis). As a consequence, in order to increase the added value, fundamental researches and effective utilisation methods are required.

Reactive radicals play a very important role in signal transduction [2]. However, excess free radicals can give rise to some diseases. Lipid peroxidation during processing and storage of foods can cause unacceptable flavour and taste and decrease consumer acceptability for foods. Antioxidants are now increasingly added to foods to reduce oxidation. Synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), t-butylhydroquinone (TBHQ) and propyl gallate (PG) have been widely added to foods to retard the deterioration caused by oxidation. However, synthetic antioxidants are restricted in some countries for their potential health risks [3,4] which caused an increasing demand for safe and natural antioxidants.

The antioxidant activity of peptides obtained from the hydrolysates of various proteins has caused much attention in recent years. Many studies have reported that plant- and animal-derived protein hydrolysates showed significant antioxidant ability. The peptides from chickpea [5], corn [6], soybean [7], oat bran [8], eggs [9], milk [10] and porcine plasma [11] have all been displayed great antioxidant activity. Additionally, aquatic products including fishes [12], *Sphyrna* [13], squids [14], shrimps [15], echinoderms [16] and bivalve mollusks [17] have also been proven to be good sources of antioxidant active peptides.

However, little information is yet available on antioxidant activity from *Paphia undulate*. Therefore, the purpose of this study was to separate the *Paphia undulate* proteins into different fractions based on their solubility. Nitrogen content, amino acid composition and protein denaturation temperature were subsequently determined. The antioxidant activity of hydrolysates of the three protein fractions as well as preliminary discussion on the relationship between amino acid composition and the antioxidant activity were also investigated

# 2. Materials and Methods

## 2.1. Materials

Fresh *Paphia undulate* were bought from Zhanjiang aquatic products wholesale market (Zhanjiang, Guangdong, China), in December 2012. After decladding, the *Paphia undulate* muscle was collected, rinsed, frozen and stored at -20°C for further use. Trypsin was purchased from NovoCo. (Novo Nordisk, Bagsvaerd, Denmark). 1,1-diphenyl-2-pycrylhydrazyl (DPPH) and reduced glutathione (GSH) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents were analytical-grade.

## 2.2. Separation of Hard Clam Proteins

After thawing, *Paphia undulate* meat was homogenized with a homogenizer (AM-6; Nissei Co., Ltd., Tokyo, Japan). The admixture was extracted with three times volume of PBS (phosphate buffer solution, 0.05 mol/L, pH 7.0) at 4°C for 90 min and the solution was continuously stirred, according to the method of Saito et al. [18] with slight modifications. After centrifugation (15 min, 10000g, 4°C), the supernatant fluid $S_1$ was first collected. The precipitation $P_1$ was further extracted by repeating the above operation. Subsequently, supernatant fluid $S_2$ and precipitation $P_2$ were obtained. The mixture of $S_1$ and $S_2$ was sarcoplasmic protein. In addition, $P_2$ was extracted with three times volume of PBS (0.1 mol/L, pH 7.0, containing 1.0 mol/L NaCl), at 4°C for 18h and the solution was continuously stirred. The supernatant fluid $S_3$ was collected after centrifugation (15 min, 10000g, 4°C ). The precipitation $P_3$ was extracted one more time with the same procedure. Supernatant fluid $S_4$ and precipitation $P_4$ were obtained. $S_3$ and $S_4$ were myofibrillar protein component. $P_4$ was stromal protein. Finally, to remove residual salt, myofibril protein was dialyzed with a 14,000 Da molecular weight (MW) membrane over 48 h against distilled water. It was then lyophilized and stored at -20°C.

## 2.3. Differential Scanning Calorimetry (DSC)

Thermal measurements were carried out using a Perkin Elmer DSC-7 (Perkin Elmer Corp., Massachusetts, USA) differential scanning calorimeter, conducted as described previously by Guo and Xiong [19] with slight modification. Approximately 3 mg protein powders were carefully weighed into an aluminium pan and a sealed empty aluminium pan was used as the reference. The run was conducted in the temperature range 10°C -90°C in a nitrogen stream (flow rate at 20 mL/min, heating rate 10°C /min). The peak temperature (Tp) and heat flow were determined

## 2.4. Amino Acid Composition

Amino acids were estimated with a high-speed amino acid analyzer (L-8500A, Hitachi Co., Tokyo, Japan) according to the method modified by Cao et al. [20]. Amino acids except for tryptophan and cystine were analyzed by hydrolyzing samples with 6 M HCl in tubes sealed under nitrogen at 110°C for 22h.

## 2.5. Preparation of *Paphia undulate* Protein Fractions Hydrolysates

*Paphia undulate* protein hydrolysates were prepared with trypsin at temperature of 37°C , pH of 8.0, enzyme to substrate ratio of 2500:1(U/g) and substrate concentration of 4% for 180 min. After hydrolysis, the hydrolysate was boiled for 10 min to inactivate the protease and subsequently centrifuged at 3000g for 10 min. Then the supernatant was collected, lyophilised and stored at -20°C before use.

## 2.6. DPPH radical Scavenging Activity

DPPH radical scavenging activity was measured according to the method described by Zhu et al. [21] with slight modifications. Briefly, DPPH-ethanol solution (1.5 mL, 100uM) was mixed with equivalent volume of sample solution with various concentrations. After vortex, the solution was kept in dark at room temperature for 30 min. Then the absorbance was measured at 517 nm. The DPPH radical scavenging activity ($Y_1$) was expressed as:

$$Y_1(\%) = \frac{A-(A_S-A_0)}{A} \times 100 \quad (1)$$

where $A_s$ is the absorbance of the reaction solution, $A_0$ is the absorbance of the reaction solution including 1.5 mL of 95% ethanol and 1.5 mL of sample, $A$ is the absorbance of the solution including 1.5 mL of DPPH (100uM) and 1.5 mL of deionised water. GSH was used as a positive control.

## 2.7. Hydroxyl Radical Scavenging Activity

Hydroxyl radical scavenging assay was modified on the basis of a method described by Wang et al. [22]. In this system, hydroxyl radicals can oxidise $Fe^{2+}$ into $Fe^{3+}$, and $Fe^{2+}$ can combine with 1,10-phenanthroline forming a red compound (1,10-phenanthroline-$Fe^{2+}$) with the maximum absorbance at 536 nm. Briefly, 1.0 mL of 1,10-phenanthroline (750uM) was mixed with 1.0 mL of sample solution of various concentrations, 1 mL of sodium phosphate buffer (0.2 M, pH 7.4) and 1 mL of ferrous sulphate (750 uM). Then the reaction was initiated by adding 1.0 mL $H_2O_2$ (1mol/L). After being incubated at 37°C for 1 h in a water bath, the absorbance of the reaction mixture was read at 536 nm. GSH was used as a positive control. The hydroxyl radical scavenging activity ($Y_2$) was calculated by the following formula:

$$Y_2(\%) = \frac{A_S-A_0}{A_C-A_0} \times 100 \quad (2)$$

where $A_s$ was the absorbance of the reaction solution with sample, $A_0$ was the absorbance of reaction solution with sample replaced by equal volume of deionised water, $A_c$ was the absorbance of the reaction solution with sample and hydrogen peroxide replaced by equal volume of deionised water.

## 2.8. Superoxide Anion Radical Scavenging Activity

The superoxide anion scavenging activity assay was determined by Yang et al. [23] with minor modifications. The reaction mixture was consisted of 2.5 mL of Tris-HCl buffer (50 mM, pH 8.2) and 2 mL of sample with different concentrations. The mixture solutions were pre-incubated at 25°C for 20 min. The reaction was initiated by the addition of 0.15 mL of 3 mM 1,2,3-trihydroxybenzene (dissolved in 10 mM HCl). The absorbance at 325 nm was recorded every 30s for 4min. GSH was used as positive control. The scavenging activity on superoxide anion radical was expressed as follows:

$$Y_3(\%) = \frac{V_0 - V_1}{V_0} \times 100 \quad (3)$$

where $V_0$ and $V_1$ are the autoxidation rate of 1,2,3-trihydroxybenzene ($\Delta$OD $min^{-1}$) without and with sample, respectively.

## 2.9. Reducing Power

The reducing power was based on a method developed by Wu et al. [12] with some modifications. Briefly, 1.0mL of sample with various concentrations or deionized water (control) was added to 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 10 mg/mL potassium ferricyanide. The mixtures were incubated at 50°C for 20 min, followed by addition of 2.5mL of 10% trichloroacetic acid. Then the solution was centrifuged (2000g, 10min), and the supernatant (2.5mL) was mixed with 2.5 mL of deionized water and 0.5 mL of 0.1% ferric chloride. After incubation at room temperature for 10 min, the absorbance of the mixture was measured at 700 nm. GSH was used as a positive control. A higher absorbance indicated higher reducing power. GSH was used as a positive control

## 2.10. Statistical Analysis

All the tests were done with three replicates. Data were presented as averaged, and standard deviation was also calculated.

# 3. Results and Discussion

## 3.1. Measurement of Protein Compositions Content

Proteins of *Paphia undulate* were classified into three fractions based on solubility (Table 1). Myofibrillar protein was found as a major protein constituent in *Paphia undulate* (45.42%, accounted for crude protein content of raw materials). It was consistent with the result reported by Zheng et al. [24] that the major protein of pearl oyster meat was salt-soluble protein (66.3%). Myofibrillar protein was the dominant protein, which is not only involved in muscle contraction [25], but also the rheological properties of meat products such as elasticity, bondability, water retention and so on. The organization structure of *Paphia undulate* was destroyed seriously and mouthfeel has been changed in the process of freezing storage. This might be due to its higher proportion of myofibril protein which is prone to denaturation. The content of sarcoplasmic protein (31.20%) was generally lower than that of pelagic fish, such as sardine [26]. The higher level of stromal protein content (20.69%) could be related to the high mechanical strength of muscle [27]. Regarding *Paphia undulate* muscle protein composition, it is suitable to product some meat products with shellfish meat flavor by adding hard clam muscle

**Table 1 Nitrogenous constituents in *Paphia undulate* muscles (%)**

| Sarcoplasmic protein | Myofibrillar protein | Stromal protein |
|---|---|---|
| 31.20±0.65 | 45.42±0.73 | 20.69±0.36 |

Values are shown as means ± SD of triplicate determinations

## 3.2. Measurement of Thermal Denaturation Temperature

The denaturation temperature ($T_{max}$) is used to describe the thermal stability of protein and it is one of the most important factors in food processing. Because protein denaturation can cause protein senior structure unfold and sequentially various food features reduced or lost significantly. Therefore, determination of food protein denaturation temperature has important guiding significance for application of protein. Differential Scanning Calorimetry (DSC), a heat analysis method developed in the 1960s, has become an important tool in detection of protein denaturation temperature. Therefore, in this study, DSC technology was used to determinate the thermal denaturation temperature of *Paphia undulate* three protein fractions. As presented in Figure 1, all the three protein fractions displayed a prominent endothermic peak. This is because the protein senior structural changed during heating process. In other words, it caused by protein denaturation. Peak temperature is the denaturation temperature. Therefore, the denaturation temperatures of sarcoplasmic protein, myofibrillar protein and stromal protein were 53.4°C, 47.23°C and 55.73°C, respectively. The denaturation temperature of stromal proteins was the highest, while myofibrillar protein denaturation temperature was minimal. Chen et al. [28] have reported that the denaturation temperature of myofibrillar protein from oyster adductor muscle was about 46°C, which was similar to the denaturation temperature of myofibrillar protein in this paper.

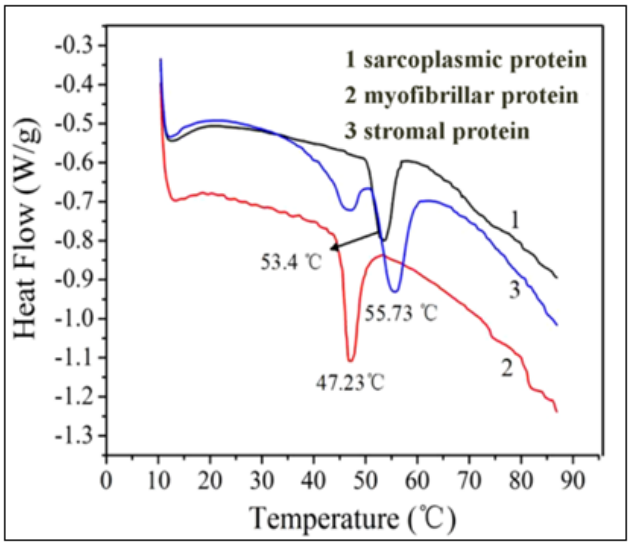


**Figure 1.** DSC thermograms of sarcoplasmic protein, myofibrillar protein and stromal protein from *Paphia undulate*

## 3.3. Amino Acid Composition

The amino acid composition of any food proteins has significant role in various physiological activities of human body and affects either directly or indirectly in maintaining good health. Amino acid composition of *Paphia undulate* three protein fractions was analysed in our preliminary experiments (Table 2). The results indicated clearly that essential amino acid contents in sarcoplasmic protein, myofibrillar protein and stromal protein were 35.21%, 38.92%, 32.78%, respectively. Moreover, the three protein fractions contained higher contents alkaline amino acids (histidine, arginine, lysine), hydrophobic amino acids (phenylalanine, valine, leucine, isoleucine, proline, alanine, glycine) and branched chain amino acid (valine, isoleucine, leucine). It existed a certain differences in amino acids content of each protein component. Sarcoplasmic protein exhibited the highest percentage of alkaline amino acids, accounting for 21.42% of the total amino acids, followed by myofibrillar protein (18.29%) and stromal protein (14.70%). The percentages of hydrophobic amino acids in total amino acids of the three protein fractions were 40.19%, 35.32%, 43.85%, respectively. However, the contents of branched chain amino acid were relatively lower than alkaline amino acids and hydrophobic amino acids, and the percentages of the three protein fractions were 16.05%, 19.34%, 16.19%, respectively. It is believed that peptides with high contents of histidine, proline, alanine, valine, methionine, and leucine can enhance the activities of antioxidant peptides [29]. *Paphia undulate* three protein fractions contained all these antioxidant-related amino acids.

**Table 2. Amino acid composition of protein components from *Paphia undulate* (%)**

| Amino acid | Sarcoplasmic protein | Myofibrillar protein | Stromal protein |
|---|---|---|---|
| Aspartic acid | 9.56 | 11.98 | 10.70 |
| Threonine* | 4.87 | 4.59 | 4.59 |
| Serine | 4.08 | 4.17 | 4.37 |
| Glutamic acid | 14.08 | 19.34 | 15.56 |
| Glycine | 8.95 | 3.34 | 11.56 |
| Alanine | 7.64 | 6.87 | 6.78 |
| Valine* | 4.98 | 4.60 | 4.59 |
| Methionine* | 2.43 | 2.82 | 2.85 |
| Isoleucine* | 4.60 | 5.09 | 4.37 |
| Leucine* | 6.57 | 9.66 | 7.22 |
| Tyrosine | 3.39 | 3.53 | 3.22 |
| Phenylalanine* | 3.93 | 3.32 | 3.63 |
| Histidine | 1.90 | 1.30 | 1.59 |
| Lysine* | 7.82 | 8.85 | 5.52 |
| Arginine | 11.69 | 8.14 | 7.59 |
| Proline | 3.51 | 2.45 | 5.70 |
| ∑AA | 47.8 | 81.7 | 27 |
| ∑EAA /∑AA | 35.21 | 38.92 | 32.78 |
| ∑AAA /∑AA | 21.42 | 18.29 | 14.70 |
| ∑HAA /∑AA | 40.19 | 35.32 | 43.85 |
| ∑BCAA/∑AA | 16.05 | 19.34 | 16.19 |

The data were the proportion of individual amino acid to total amino acids (%). * Essential amino acid (EAA, for short). Branched chain amino acid (BCAA, for short) including Val, Ile, Leu; Alkaline amino acids (AAA, for short) including His, Arg, Lys; Hydrophobic amino acid (HAA, for short) including Phe, Val, Leu, Ile, Pro, Ala, Gly. Trp and Cys content were not determined.

## 3.4. DPPH radical Scavenging Activity

DPPH free radical (DPPH$^{\bullet}$) is widely used to assess the antioxidant activity of natural compounds, although it is not a biologically relevant radical [30]. The DPPH$^{\bullet}$ scavenging activity shows the ability of the antioxidant compounds to donate hydrogen or electrons, thus converting the radical to more stable species [31]. Moreover, DPPH$^{\bullet}$ has a characteristic absorbance at 517 nm, which decreases significantly on exposure to proton radical scavengers [32].

DPPH$^{\bullet}$ scavenging activities of hydrolysates of the three protein fractions from *Paphia undulate* are depicted in Figure 2. The DPPH$^{\bullet}$ scavenging activity was increased as the concentration of hydrolysates was increased. Our results were similar to those reported for tuna liver protein hydrolysates [33]. The effective concentration for 50% scavenging activity ($IC_{50}$) was determined by the regression equation, and the $IC_{50}$ of sarcoplasmic protein, myofibrillar protein and stromal protein was 4.82, 9.19 and 9.31mg/mL, respectively. Thus, the hydrolysates of sarcoplasmic protein showed the strongest DPPH$^{\bullet}$ scavenging activity in comparison to myofibrillar protein and stromal protein. While hydrolysates of myofibrillar protein and stromal protein have similar DPPH$^{\bullet}$ scavenging activity. In addition, GSH, the positive control, performed better DPPH$^{\bullet}$ scavenging ability than the hydrolysates of three protein fractions, and the $IC_{50}$ was 0.03 mg/mL. This was in agreement with [34] who reported that the DPPH$^{\bullet}$ scavenging activity of the hydrolysate from Alaska Pollack skin ($IC_{50}$= 2.5 mg/mL) was lower than that of GSH ($IC_{50}$= 0.025 mg/mL). Amino acid analysis of the three protein fractions showed that sarcoplasmic protein was rich in alkaline amino acids including histidine, arginine and lysine compared to myofibrillar protein and stromal protein. Alkaline amino acids have been reported to perform a strong antioxidant activity [35,36]. It is also widely accepted that peptides with high contents of hydrophobic amino acids (leucine, phenylalanine, valine, and tryptophan) may have contributed to its DPPH$^{\bullet}$ scavenging activity. However, different proteases have different cleavage sites. The hydrolysate produced using trypsin in the present study contained high concentrations of arginine and lysine.

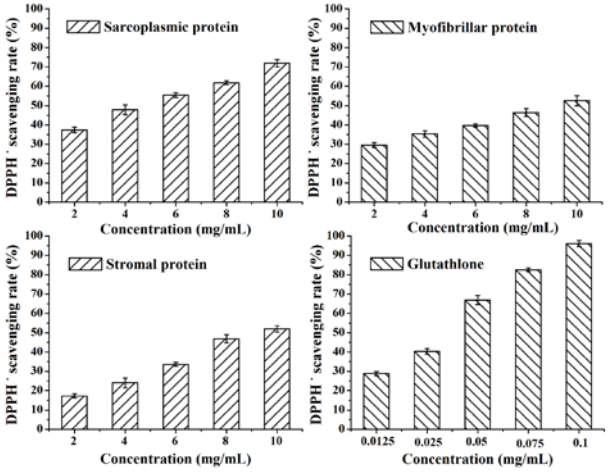


**Figure 2.** DPPH radical scavenging activities of glutathione and the hydrolysates of *Paphia undulate* three protein fractions

## 3.5. Hydroxyl Radical Scavenging Activity

As the most reactive free radical, the hydroxyl radical ($OH^{\cdot}$) can be formed from a superoxide anion and hydrogen peroxide. $OH^{\cdot}$ can easily react with biomolecules, such as amino acids, proteins and DNA [37]. Thereby, removal of $OH^{\cdot}$ is important to protect humans against some diseases. In this paper, $OH^{\cdot}$ scavenging activity of hydrolysates of the three protein fractions at varying concentrations was measured. As shown in Figure 3, the hydrolysates of sarcoplasmic protein displayed the strongest $OH^{\cdot}$ scavenging activity ($IC_{50}$= 4.7mg/mL), followed by myofibrillar protein hydrolysates ($IC_{50}$= 6.34mg/mL). While the hydrolysates of stromal protein displayed weaker hydroxyl radical scavenging activity ($IC_{50}$= 8.42mg/mL). Sarcoplasmic protein and myofibrillar protein were both contained higher concentrations of arginine and lysine in comparison to stromal protein. Phenylalanine may also contribute to enhancing the antioxidant activity of peptides. This is because the aromatic ring of phenylalanine can react with OH• to form stable compound [38].

However, it was observed that the activity of the hydrolysates of sarcoplasmic protein and myofibrillar protein were higher than that of GSH ($IC_{50}$= 7.98mg/mL). The similar phenomenon was also observed in the hydrolysates from loach [39].

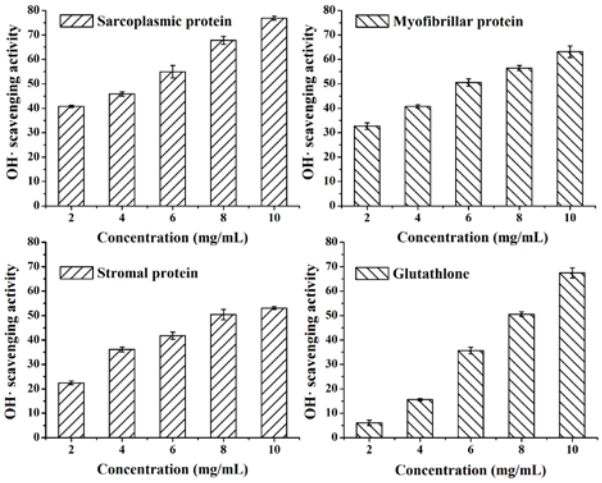


**Figure 3.** OH• radical scavenging activities of glutathione and the hydrolysates of *Paphia undulate* three protein fractions

## 3.6. Superoxide Anion Radical Scavenging Activity

Superoxide anion radical ($O_2^{\cdot}$) can cause the generation of dangerous hydroxyl radicals and singlet oxygen, both of which can give rise to damage to membrane as well as DNA of cell, although they cannot directly initiate lipid oxidation. Therefore, learning the scavenging activity of antioxidant on superoxide radicals is one of the most important ways of clarifying the mechanism of antioxidant activity [40].

As shown in Figure 4, the hydrolysates of sarcoplasmic protein showed the highest $O_2^{\cdot}$ scavenging activity, and the $IC_{50}$ was 3.75 mg/mL, whereas the hydrolysates of myofibrillar protein and stromal protein revealed slightly lower $O_2^{\cdot}$ scavenging activity ($IC_{50}$ were 7.03, 6.11mg/mL, respectively). It was also suggested that the $O_2^{\cdot}$ scavenging activity was related to hydrophobic amino acids [5]. Stromal protein contained higher concentrations of proline and hydrophobic amino acids. However, it did not display strong $O_2^{\cdot}$ scavenging activity. This may because the hydrolysate prepared by trypsin contained high concentrations of arginine and lysine, not hydrophobic amino acids in the present study. Therefore, the hydrolysates of sarcoplasmic protein containing more alkaline amino acids (histidine, arginine and lysine) showed the highest $O_2^{\cdot}$ scavenging activity compared with these of myofibrillar protein and stromal protein.

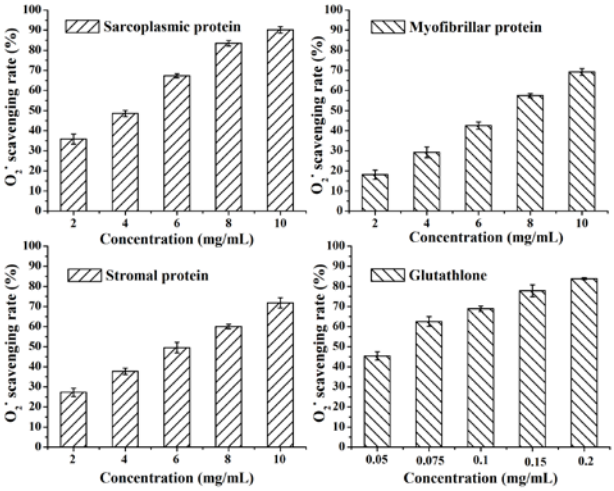


**Figure 4.** $O_2^{\cdot}$ scavenging activities of glutathione and the hydrolysates of *Paphia undulate* three protein fractions.

## 3.7. Reducing Power

Many studies have indicated that there was a direct correlation between reducing power and antioxidant activity of some bioactive compounds. The reducing power assay is often used to assess the ability of antioxidant to donate a hydrogen or electron. And this method measures the ability of antioxidant to reduce $Fe^{3+}$-ferricyanide complex to ferrous form ($Fe^{2+}$) [30]. In addtion, the $Fe^{2+}$ complex can be detected by measuring the formation of Perl' s Prussian blue at 700 nm. It has been widely accepted that the stronger the absorbance at 700 nm, the better the reducing power. Table 3 presents the reducing power of hydrolysates of the three protein fractions. All of the hydrolysates showed some degree of electron donating capacity. However, the hydrolysates revealed very weak reducing power when in comparison with glutathione. In this study, the reducing power of hydrolysates of sarcoplasmic protein, myofibrillar protein and stromal protein reached 0.25±0.01, 0.27±0.04 and 0.14±0.01 at 2 mg/mL, respectively. Li et al. [5] reported that the reducing power of fraction IV of chickpea protein hydrolysates reached about 0.30 at 2.5 mg/mL. Je et al. [33] found that the tuna liver protein hydrolysates reached about 0.36 at 2.0 mg/mL. Moreover, the reducing power was concentration dependent, increased with the increasing concentration of the hydrolysates, which was in accordance with those reported for horse mackerel skin and croaker skin protein hydrolysates [41].

Furthermore, the reducing power of peptides is associated with the specific amino acids and peptide

sequences. You et al. [39] reported that loach peptides had stronger reducing power and the hydrolysate of loach contained histidine, methionine, tryptophan, lysine, and tyrosine. Rapeseed fractions performed strongest reducing power also had abundant hydrophobic amino acids, which were considered to contribute to enhancing the reducing power of peptides [42]. In this study, glutathione performed very strong reducing power, which shows that the sulfhydryl group of cysteine is an important reducing agent. The three protein fractions contained low amounts of sulfur-containing amino acids. On the basis of these studies, the hydrolysates of the three protein fractions do not have strong reducing power.

**Table 3 Reducing power of *Paphia undulate* protein hydrolysate**

| Concentration (mg/mL) | Sarcoplasmic protein | Myofibrillar protein | Stromal protein | Concentration (mg/mL) | Glutathlone |
|---|---|---|---|---|---|
| 2 | 0.25±0.01 | 0.27±0.04 | 0.14±0.01 | 0.025 | 0.30±0.03 |
| 4 | 0.29±0.01 | 0.31±0.06 | 0.16±0.03 | 0.05 | 0.49±0.01 |
| 6 | 0.41±0.03 | 0.42±0.02 | 0.28±0.01 | 0.075 | 0.62±0.01 |
| 8 | 0.46±0.01 | 0.51±0.07 | 0.39±0.06 | 0.1 | 0.74±0.02 |
| 10 | 0.58±0.05 | 0.57±0.01 | 0.43±0.01 | 0.125 | 0.83±0.05 |

Values are shown as means ± SD of triplicate determinations.

# 4. Conclusions

*Paphia undulate* contains higher protein contents, especially sarcoplasmic protein and myofibril protein. The denaturation temperature of myofibril protein is lower, while stromal protein has higher denaturation temperature, which may contribute to the utilization of *Paphia undulate* protein resources. *Paphia undulate* three protein fractions especially sarcoplasmic protein are good natural sources of antioxidants as well as the special amino acids such as alkaline and hydrophobic amino acids that may enhance the antioxidant activity. However, further more researches on the structure-function relationship between amino acids composition and antioxidant activity are needed.

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