In Vitro Antioxidant Activity and In Vivo Anti-fatigue Effects of Oyster (Ostrea plicatula Gmelin) Peptides Prepared Using Neutral Proteinase

Gengxin HAO1,2,3, Wenhong CAO4,5, Jiming HAO4,5 and Chaohua ZHANG4,5*

1 South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, China
2 Graduate School of the Chinese Academy of Sciences, Beijing 100049, China
3 College of Biological Engineering, Jimei University, Xiamen 361021, China
4 College of Food Science and Technology, Guangdong Ocean University, Zhanjiang 524088, China
5 Guangdong Provincial Key Laboratory of Aquatic Products Processing and Safety, Zhanjiang 524088, China

Received December 16, 2012; Accepted March 12, 2013

The amino acid profile of oyster (Ostrea plicatula Gmelin) peptides (OP) was assayed. Its in vitro antioxidant activity was determined, and its in vivo anti-fatigue activity was compared with those of oyster meat and oyster protein. Seven amino acids that may play an important role in enhancing antioxidant activity account for 25.8% of total OP content. These amino acids are histidine, proline, methionine, cysteine, tyrosine, tryptophan, and phenylalanine. The F-value of OP was 4.89, and the concentration of taurine was 45.43 mmol/mL. OP acted as a scavenger for hydroxyl radicals, 1,1-diphenyl-2-picrylhydrazyl, and superoxide anion radicals. It also inhibited lipid peroxidation. The intragastric administration of OP prolonged the swimming time to exhaustion of mice by 78% over that of the control. It decreased the levels of serum lactic acid and blood urea nitrogen by 24.8% and 11.2%, respectively. Finally, it increased the levels of liver glycogen (1.0-fold) and gastrocnemius muscle glycogen (55.6%).

Keywords: oyster peptides, antioxidant activity, anti-fatigue effect, endurance exercise

Introduction

Acute and/or chronic exercises are associated with increases in free radicals and ROS production, which are due primarily to the increase in oxygen uptake by active tissues (Ji, 1995; Powers and Jackson, 2008). The imbalance between free radical/ROS production and its removal by antioxidant defense systems causes oxidative stress (Chalamaiah et al., 2012). Oxidative stress may lead to potential damage and consequently diminish athletic performance. Thus, increased free radical/ROS production has been associated with altered muscle function and elevated rates of muscle fatigue (Deaton and Marlin, 2003).

Research consensus reflects support for the idea that antioxidative peptides can reduce the damage induced by ROS and free radicals. The antioxidative peptides from aquatic products have become of recent concern because they are considered safe and healthy molecules with low molecular weight, easy absorption, low cost, and high activity (Sarmadi and Ismail, 2010). The precursor protein in these peptides is mostly inactive. These active peptides can also be released from the precursor protein by hydrolysis techniques, such as enzymatic hydrolysis, autolytic process using endogenous enzymes, and microbial fermentation (Chalamaiah et al., 2012; Je et al., 2007; Rajapakse et al., 2005; Kim et al., 2007).

In this study, we assayed the amino acid profile of oyster peptides (OP), and then investigated its in vitro antioxidant activities and the in vivo anti-fatigue effects of OP previously prepared by neutral proteinase digestion. The in vitro antioxidative activities were studied by determining the activities of hydroxyl radicals, 1,1-diphenyl-2-picrylhydrazyl (DPPH), and superoxide anion radical scavengers, as well as lipid peroxidation inhibitory activity in a linoleic acid emulsion system. In the determination, glutathione (GSH) which is a well known peptide antioxidant (Novelli et al., 1991) was used as control. In the subsequent in vivo study, mice were fed with OP for 14 d. Then, the exhaustive swimming time was recorded and several biochemical parameters related to

*To whom correspondence should be addressed.
E-mail: zhangch2@139.com
fatigue [serum lactic acid (LA), blood urea nitrogen (BUN), and liver and gastrocnemius muscle glycogen] were determined to explore the anti-fatigue activity of OP.

Materials and Methods

Materials  
Edible parts of the flesh oyster (Ostrea pli- cattula Gmelin) were purchased from a local market in Xiamen, China in February, 2011, and minced using a chopper (Braun Co., Germany).

Methods  
**Oyster peptides preparation**  
Minced oyster meat was mixed with a 3-fold volume of deionized water and homogenized for 1 min at 10,000 rpm using a basic homogenizer. The homogenate obtained was then adjusted to pH 6.7 with 0.01 mol/L HCl and hydrolyzed with neutral proteinase (China Pangbo Biological Engineering Co., China) (1500 U/g) at 44°C (China Pangbo Biological Engineering Co., China) (1500 U/g) at 44°C (China Pangbo Biological Engineering Co., China) (1500 U/g) at 44°C (China Pangbo Biological Engineering Co., China) (1500 U/g) at 44°C (China Pangbo Biological Engineering Co., China) (1500 U/g) at 44°C (China Pangbo Biological Engineering Co., China) (1500 U/g) at 44°C (China Pangbo Biological Engineering Co., China) (1500 U/g) at 44°C (China Pangbo Biological Engineering Co., China) (1500 U/g) at 44°C (China Pangbo Biological Engineering Co., China) (1500 U/g) at 44°C. The hydrolysate reaction was performed in a shaking incubator. At the end of the hydrolysis period, the mixture was heated in boiling water for 15 min to inactivate the enzyme. The hydrolysate was centrifuged at 4,000 rpm for 10 min. Then the supernatant was collected and subjected to ultrafiltration using a membrane bioreactor (Braun Co., Germany). The mixture was made to a certain volume. Tryptophan content of samples was determined by colorimetric analysis (UV-1700, Shimadzu Co., Japan) at 400 nm under the condition of pH 5.0 − 5.5, column oven temperature 55°C, reactor temperature 100°C, and reaction time 10 − 15 min. Free amino acid content of samples was directly determined by using an amino acid analyzer. Free tryptophan content was determined by colorimetric analysis at 440 nm under the conditions of pH 5.0 − 5.5, column oven temperature 55°C, reactor temperature 100°C, reaction time 10 − 15 min.

**Hydroxyl radical scavenging activity assay of OP**  
The hydroxyl radical scavenging activity was assayed according to the method of Li et al. (2008) with some modifications. A mix of 600 μL of 1,10-phenanthroline (5.0 mM), 600 μL of FeSO₄ (5.0 mM) and 600 μL of ethylenediaminetetraacetic acid (EDTA) (15 mM) were mixed with 400 μL of sodium phosphate buffer (0.2 M, pH 7.4). Then, 600 μL of OP (dried OP dissolved in deionized water and the concentration was adjusted to 0 − 25 mg/mL) and 800 μL of H₂O₂ (0.01%) were added. The mixture was incubated at 37°C for 60 min, and the absorbance was measured at 536 nm. GSH (Sigma-Aldrich, St. Louis, MO, USA) at 0 − 10 mg/mL was used as a control. The following equation was used:

\[
\text{Hydroxy radical scavenging activity (\%)} = \frac{A_s - A_0}{A_i - A_0} \times 100
\]

where \(A_s\) is the absorbance of the sample; \(A_0\) is the absorbance of the blank solution using distilled water instead of sample; and \(A_i\) is the absorbance of a control solution in the absence of \(H_2O_2\). The plot of scavenging activity against the concentration of the hydrolysate was prepared, and the IC₅₀ (concentration of samples to decrease the scavenging activity by 50%) obtained.

**DPPH radical scavenging activity assay of OP**  
The DPPH radical scavenging activity was determined by the method of Wu et al. (2003), with a slight modification. Two milliliters of OP (0 − 5 mg/mL) were mixed with 2.0 mL of 0.15 mM DPPH that was dissolved in 95% ethanol. The mixture was then shaken vigorously using a mixer, and was kept for 30 min in the dark. The absorbance of the resulting solution was recorded at 517 nm. GSH at 0 − 5 mg/mL was used as a control. The scavenging activity was calculated using the following equation:

\[
\text{DPPH radical scavenging activity (\%)} = \frac{A_s - A_0}{A_i - A_0} \times 100
\]

where \(A_s\) is the absorbance of the sample; \(A_0\) is the absorbance of the blank solution using distilled water instead of sample; and \(A_i\) is the absorbance of a control solution in the absence of \(H_2O_2\). The plot of scavenging activity against the concentration of the hydrolysate was prepared, and the IC₅₀ (concentration of samples to decrease the scavenging activity by 50%) obtained.
DPPH radical scavenging activity (\%) = \frac{A_s - A_t}{A_s} \times 100

where \( A_s \) is the value for the 2 mL of sample solution mixed with the DPPH solution; \( A_t \) is the value for the 2 mL of sample solution mixed with the 2 mL of 95% ethanol; and \( A_b \) is the value for the 2 mL of 95% ethanol mixed with the DPPH solution. The plot of scavenging activity against the concentration of the hydrolysate was prepared, and the \( IC_{50} \) obtained.

**Antioxidant activity of OP by superoxide anion radical**

The superoxide anion radical scavenging capability was assayed according to Zhang & Yang (2008). Briefly, reaction mixtures containing Tris-HCl buffer (4.5 mL, 50 mmol/L, pH 8.2) and test compounds (2.0 mg/mL) was incubated immediately at 30 s intervals. The autooxidation rate constant of pyrogallic acid was calculated from the curve of \( A_{325\text{nm}} \) vs time. The negative control did not contain test compound. The inhibitory actions of test compounds against the autooxidation rate indicate their superoxide anion radical scavenging abilities.

**Lipid peroxidation inhibition activity of OP in a linoleic acid emulsion system**

The lipid peroxidation inhibition activity of OP was measured in a linoleic acid emulsion system according to the methods of You et al. (2011). Briefly, 2.0 mL of OP (0 – 20 mg/mL) was mixed with 2 mL of 2.5% linoleic acid dissolved in 95% ethanol. Then, 4 mL of 50 mM sodium phosphate buffer (pH 7.0) and 2 mL of distilled water were added. The mixture was incubated in a 50 mL conical flask with a screw cap at 40 ± 1°C in dark, and the degree of oxidation was evaluated by measuring the FeSCN values described below. The reaction solution (100 μL), incubated in the linoleic acid model system, was placed in a swimming tank (50 cm × 50 cm × 40 cm) with 30 cm deep water and subjected to swim-training at 30°C water after the sample administration. During the exercise protocol, the mice in the control group were kept in a plastic cage containing approximately 3 cm of water maintained at the same temperature to exclude potential stress and other potential confounding effects (Kumar et al., 2011). The mice were acclimatized to their environment for 3 d before starting the experiment. They had free access to water and a balanced murine diet. After adaptation, the mice were randomly assigned to four groups with 20 mice each (Table 1). Samples were administered to the mice by intragastric administration 0.5 h before swimming training every day at 9:00 am.

A special swim-training model was used. Mice were placed in a swimming tank (50 cm × 50 cm × 40 cm) with 30 cm deep water and subjected to swim-training at 30°C water after the sample administration. During the exercise protocol, the mice in the control group were kept in a plastic cage containing approximately 3 cm of water maintained at the same temperature to exclude potential stress and other potential confounding effects (Kumar et al., 2011). The training lasted for 14 d. During the first 2 d, the mice swam for 20 min. Then, the swimming time was extended by 10 min every 2 d. On the 15th day, the final body weight was measured. The 20 mice housed in each group were randomly divided into two subgroups with 10 mice each. One subgroup was loaded with an equivalent volume of distilled water was used as blank. GSH at 0 – 20 mg/mL was used as a control.

Lipid peroxidation inhibition activity (\%) = \frac{A_{k=144\text{h}} - A_{k=0\text{h}}}{A_{k=144\text{h}} - A_{k=0\text{h}}} \times 100

where \( A_{k=144\text{h}} \) and \( A_{k=0\text{h}} \) are the absorbances for the sample at 144 h and 0 h, respectively. \( A_{k=144\text{h}} \) and \( A_{k=0\text{h}} \) are the absorbances for the blank at 144 h and 0 h, respectively. The plot of the scavenging activity against the concentration of sample was prepared, and the \( IC_{50} \) obtained.

**Animals and in vivo test**

All procedures with animal subjects have been approved by The Ethics Committee of the Jimei University (SYXXK Min 2012-0005). A total of 80 male Kunming mice [specific pathogen-free grade, Approval No. SCXK-(Min) 2008-B001], with average weight of 20 g ± 2 g, were used in the experiment. The mice (five per cage) were housed in an air-conditioned specific pathogen-free grade level laboratory (25°C ± 2°C). The mice were grown under moderate humidity (50% ± 10%) with a 12 h/12 h light/dark cycle. Noise was less than 60 dB. The mice were acclimatized to their environment for 3 d before starting the experiments. They had free access to water and a balanced murine diet. After adaptation, the mice were randomly assigned to four groups with 20 mice each (Table 1). Samples were administered to the mice by intragastric administration 0.5 h before swimming training every day at 9:00 am.

A special swim-training model was used. Mice were placed in a swimming tank (50 cm × 50 cm × 40 cm) with 30 cm deep water and subjected to swim-training at 30°C water after the sample administration. During the exercise protocol, the mice in the control group were kept in a plastic cage containing approximately 3 cm of water maintained at the same temperature to exclude potential stress and other potential confounding effects (Kumar et al., 2011). The training lasted for 14 d. During the first 2 d, the mice swam for 20 min. Then, the swimming time was extended by 10 min every 2 d. On the 15th day, the final body weight was measured. The 20 mice housed in each group were randomly divided into two subgroups with 10 mice each. One subgroup was loaded with an equivalent volume of distilled water was used as blank. GSH at 0 – 20 mg/mL was used as a control.

A special swim-training model was used. Mice were placed in a swimming tank (50 cm × 50 cm × 40 cm) with 30 cm deep water and subjected to swim-training at 30°C water after the sample administration. During the exercise protocol, the mice in the control group were kept in a plastic cage containing approximately 3 cm of water maintained at the same temperature to exclude potential stress and other potential confounding effects (Kumar et al., 2011). The training lasted for 14 d. During the first 2 d, the mice swam for 20 min. Then, the swimming time was extended by 10 min every 2 d. On the 15th day, the final body weight was measured. The 20 mice housed in each group were randomly divided into two subgroups with 10 mice each. One subgroup was loaded with an equivalent volume of distilled water was used as blank. GSH at 0 – 20 mg/mL was used as a control.

**Table 1.** Assigned mice group.

<table>
<thead>
<tr>
<th>Group name</th>
<th>No. of mice</th>
<th>Diets</th>
</tr>
</thead>
<tbody>
<tr>
<td>C (Control group)</td>
<td>20</td>
<td>Basal diet + distilled water</td>
</tr>
<tr>
<td>OP (Oyster Peptides group)</td>
<td>20</td>
<td>Basal diet + 0.8 mg/(g·d) protein</td>
</tr>
<tr>
<td>O (Oyster group)</td>
<td>20</td>
<td>Basal diet + 0.8 mg/(g·d) protein</td>
</tr>
<tr>
<td>P (Protein group)</td>
<td>20</td>
<td>Basal diet + 0.8 mg/(g·d) protein</td>
</tr>
</tbody>
</table>

a. A balanced murine basal diet can be obtained freely at any time.
b. According crude protein content of each sample, 0.8 mg/(g·d) means one mouse can get 0.8 mg crude protein supplementation per g body weight daily.
c. The mice of protein group were intragastrically administrated with oyster protein.
a lead block weighing approximately 2% of their bodyweight attached to the tail (Huang et al., 2011). Then, the mice were subjected to the exhaustive swimming test (time to exhaustion was defined as the time when the mouse failed to rise to the surface to breathe after 7 s). After the exhaustive swimming test, blood was collected from the mice for further research. The other subgroup was used for collecting liver and gastrocnemius muscle after forced unloading swimming for 80 min.

At the end of the exhaustive swimming test, blood was collected from the orbital sinus to determine LA and BUN levels (Jung et al., 2007). The assay of these biochemical parameters was performed using commercially available kits (Product nos. A019 and C013-2) from the Institute of Biological Engineering of Nanjing Jiancheng (Nanjing, China).

Mouse livers were collected to determine the liver glycogen content. The liver was dissected immediately after it was removed, washed with 0.9% saline, blotted dry with filter papers, quick-frozen in liquid nitrogen, and stored at −80°C (Giesel et al., 2009; Pederson et al., 2005). Liver samples were accurately weighed and homogenized in 8 mL of homogenization buffer for liver glycogen analysis. The gastrocnemius muscle was also removed and weighed for muscle glycogen analysis. The liver/muscle glycogen content analysis was performed using the Assay Kit A043 (Institute of Biological Engineering of Nanjing Jiancheng). All the assays were conducted by strictly following the recommended procedures.

**Statistical analysis**  All the tests were conducted in triplicate. The experimental data were expressed as mean ± SD. The results were subjected to one-way analysis of variance. Duncan's and Dunnett's T3 tests were performed to determine the significant difference between samples within the 95% confidence interval, using SPSS 12.0 software (SPSS Inc., Chicago, IL, USA).

**Results**

**Amino acid analysis of samples**  Table 2 shows that OP, oyster meat, and oyster protein contain 21.2%, 13.3%, and 16.3%, respectively, of key amino acids, namely, histidine, proline, methionine, cysteine, tyrosine, tryptophan, and phenylalanine (Chalamaiah et al., 2012). The mole ratio of branched-chain amino acids (BCAA) to aromatic amino acids is defined as the F-value. The amino acid analysis indicates that the F-value of OP was 4.89, higher than those of oyster meat and oyster protein (2.88 and 2.68, respectively).

Among the samples, the highest taurine content was exhibited by oyster meat, followed by OP and oyster protein.

**In vitro antioxidant activities of OP**  Several methods for determining the total antioxidant capacity of OP were selected. Table 3 shows that OP had a hydroxyl IC$_{50}$ value of 17.29 ± 0.73 mg/mL, which is 2.4-fold higher than that of GSH. It had a DPPH IC$_{50}$ value of 2.82 ± 0.44 mg/mL, a value 1.6-fold higher than that of GSH. The IC$_{50}$ value for the inhibition activity of superoxide anion radical was 7.02 ± 0.48 mg/mL, which is 2.0-fold higher than that of GSH. The lipid peroxidation inhibitory activity of OP yielded an IC$_{50}$ value of 14.40 ± 1.30 mg/mL, a value 0.4-fold higher than that of GSH.

**Effect on body weight**  Body weight was measured at the beginning of the experiment (day 0) and after the mice had been fed for 15 d. The results are shown in Table 4. Over the long term, body weight is controlled by the balance between energy (food) intake and energy expenditure (Kretschmer et al., 2011). Then, the mice were subjected to the exhaustive swimming test (time to exhaustion was defined as the time when the mouse failed to rise to the surface to breathe after 7 s). After the exhaustive swimming test, blood was collected from the mice for further research. The other subgroup was used for collecting liver and gastrocnemius muscle after forced unloading swimming for 80 min.

![Table 2. Amino acid composition (mmol/mL).](image)

<table>
<thead>
<tr>
<th>Samples</th>
<th>Asp</th>
<th>Thr</th>
<th>Ser</th>
<th>Glu</th>
<th>Pro</th>
<th>Gly</th>
<th>Ala</th>
<th>Cys</th>
<th>Val</th>
<th>Met</th>
<th>Ile</th>
<th>Leu</th>
<th>Tyr</th>
<th>Phe</th>
<th>Lys</th>
<th>His</th>
<th>Arg</th>
<th>Try</th>
<th>Tau</th>
</tr>
</thead>
<tbody>
<tr>
<td>OP</td>
<td>28.95</td>
<td>34.52</td>
<td>14.28</td>
<td>73.38</td>
<td>48.44</td>
<td>64.76</td>
<td>58.99</td>
<td>4.72</td>
<td>41.21</td>
<td>20.61</td>
<td>33.26</td>
<td>48.52</td>
<td>30.08</td>
<td>13.21</td>
<td>10.83</td>
<td>29.35</td>
<td>34.47</td>
<td>45.43</td>
<td></td>
</tr>
<tr>
<td>Oyster meat</td>
<td>51.51</td>
<td>29.24</td>
<td>31.53</td>
<td>67.80</td>
<td>24.52</td>
<td>68.00</td>
<td>53.71</td>
<td>1.24</td>
<td>29.58</td>
<td>14.49</td>
<td>25.57</td>
<td>36.34</td>
<td>17.88</td>
<td>33.01</td>
<td>8.51</td>
<td>28.34</td>
<td>33.20</td>
<td>62.40</td>
<td></td>
</tr>
<tr>
<td>Oyster protein</td>
<td>63.24</td>
<td>32.82</td>
<td>35.99</td>
<td>56.74</td>
<td>26.82</td>
<td>54.18</td>
<td>48.97</td>
<td>0.00</td>
<td>35.00</td>
<td>15.33</td>
<td>29.81</td>
<td>46.77</td>
<td>23.41</td>
<td>41.24</td>
<td>11.07</td>
<td>34.06</td>
<td>31.97</td>
<td>1.85</td>
<td></td>
</tr>
</tbody>
</table>
Antioxidative and Anti-fatigue Effects of Oyster Peptides

creased the BUN levels of fatigue mice \((p < 0.05)\) by 11.2%, 16.1%, and 10.6%, respectively. These results indicate that the treatments increase body adaptability for exercise tolerance.

**Effect on liver and gastrocnemius muscle glycogen** Compared with the control group \((p < 0.05)\), the intervention groups showed a significant increase in liver glycogen content with the administration of OP, oyster meat, and oyster protein. In this study, body weight did not differ across groups at day 0 or 15 \((p > 0.05)\), indicating the balanced use of energy in vivo; this finding also demonstrates that the intragastric administration of the samples did not affect body weight. These results verify that the fatigue model of mice is credible.

**Swimming time** The swimming capacities of the test mice are depicted in Figure 1. The intragastric administration of OP, oyster meat, and oyster protein prolonged the swimming time to exhaustion of mice by 78%, 72%, and 44%, respectively, compared to that of the control. The OP value was 6% higher than that of oyster meat and 34% higher than that of oyster protein.

**Effect on LA content** No significant difference in LA levels was found between the intervention and control groups before the exhaustive swimming test \((p > 0.05); \text{Table 5}\). Because of the administration of OP and oyster meat, the level of LA significantly decreased compared with that of the control group \((p < 0.05)\). Oyster protein also decreased the LA level compared with that of the control, but this decline is nonsignificant \((p > 0.05)\). OP, oyster meat, and oyster protein decreased the LA levels by 24.8%, 24.8%, and 9.1%, respectively.

**Effect on BUN content** No significant difference in BUN levels was observed between the intervention and control groups before the exhaustive swimming test \((p > 0.05)\). Table 6 illustrates that all the treatments significantly decreased the BUN levels of fatigue mice \((p < 0.05)\) by 11.2%, 16.1%, and 10.6%, respectively. These results indicate that the treatments increase body adaptability for exercise tolerance.

**Table 4.** Effects of intragastric administration on mice body weight.

<table>
<thead>
<tr>
<th>Group name</th>
<th>No. of mice</th>
<th>Bodyweight (g)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial (day 0)</td>
</tr>
<tr>
<td>C (Control group)</td>
<td>20</td>
<td>19.4 ± 1.3a</td>
</tr>
<tr>
<td>OP (Oyster peptides group)</td>
<td>20</td>
<td>19.2 ± 1.3a</td>
</tr>
<tr>
<td>O (Oyster group)</td>
<td>20</td>
<td>19.2 ± 1.0a</td>
</tr>
<tr>
<td>P (Protein group)</td>
<td>20</td>
<td>19.3 ± 1.3a</td>
</tr>
</tbody>
</table>

* Values were expressed as means ± SD. Values among groups had no significant difference based on Duncan’s multiple range test \((p < 0.05, n = 20)\).

**Table 5.** Effect on the contents of serum lactic acid of mice.

<table>
<thead>
<tr>
<th>Group name</th>
<th>No. of mice</th>
<th>Serum lactic acid (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before exhaustive swimming test*</td>
</tr>
<tr>
<td>C (Control group)</td>
<td>10</td>
<td>5.8 ± 0.8a</td>
</tr>
<tr>
<td>OP (Oyster peptides group)</td>
<td>10</td>
<td>4.7 ± 0.6a</td>
</tr>
<tr>
<td>O (Oyster group)</td>
<td>10</td>
<td>5.0 ± 1.7a</td>
</tr>
<tr>
<td>P (Protein group)</td>
<td>10</td>
<td>5.8 ± 1.0a</td>
</tr>
</tbody>
</table>

* Values were expressed as means ± SD of mice per group. Values among groups had no significant difference based on Duncan’s multiple range test \((p < 0.05, n = 10)\).

† Values with different superscripts indicate a significant difference among groups based on Duncan’s multiple range test \((p < 0.05, n = 10)\).

Fig. 1. Effects of administration of samples on swimming time of mice.

Values were expressed as means ± SD of mice per group. Values with different superscripts indicate a significant difference among groups based on Duncan’s multiple range test \((p < 0.05, n = 10)\).
protein (Table 7). The treatments increased the levels of liver glycogen by 1.00-fold, 0.96-fold, and 0.67-fold, respectively. They also enhanced gastrocnemius muscle glycogen content by 55.6%, 63.0%, and 3.7%, respectively. Unlike the control group ($p > 0.05$), however, the oyster protein group did not exhibit a statistical significance.

**Discussion**

The amino acid constituent and sequence of peptides are crucial to their bioactivities. The amount of amino acids, especially histidine, proline, methionine, cysteine, tyrosine, tryptophan, and phenylalanine, can enhance the activities of antioxidative peptides (Chalamaiah et al., 2012; Je et al., 2007; Ren et al., 2008; You et al., 2010). The results of the current study suggest that enzymic hydrolysis can enhance the content of the seven amino acids. That is, OP exhibits strong antioxidant activity. As previously stated, a relationship exists between oxidative stress and fatigue. Accordingly, exogenous dietary antioxidants are candidate anti-fatigue substances for physical fatigue (Mizuno et al., 2008). Thus, OP may enhance exercise capability.

BCAA contributes to energy metabolism during exercise, and sustained exercise can increase BCAA uptake by muscles (Monteiro et al., 2009). However, the aromatic amino acids and BCAA in plasma compete against each other in binding to carriers (Acworth et al., 1986). Therefore, the mole ratio of BCAA to aromatic amino acids (F-value) is vital to the development of fatigue. Our results suggest that OP imposes an anti-fatigue effect.

Some reports indicate that peripheral fatigue results from decreasing resting membrane potential or dysfunction of the calcium pump in the sarcoplasmic reticulum in skeletal muscles (Mizuno et al., 2008). The increase in plasma taurine concentration may decrease damaged muscle cells by modulating intracellular Ca$^{2+}$ levels and reducing free radicals (El Zahraa Z El Ashry et al., 2011; Westermann et al., 2011). Plasma taurine concentrations can therefore be used as biochemical indicators of early muscle damage (Dunnett et al., 2002). That is, taurine supplementation can play an important role in eliminating fatigue in vivo.

The antioxidant capacity of OP is an important indicator of its in vitro potential as a health promoter. Several methods were selected in determining the total antioxidant capacity of OP. Hydroxyl radicals, formed from superoxide radicals and hydrogen peroxide, are short-lived, but are the most reactive free radicals in the body; they oxidize biological macromolecules, including lipids, proteins, and nucleic acids (Siddhuraju and Becker, 2007; Sasipriya and Siddhuraju, 2012). Such oxidation can cause physiological disorders (You et al., 2011). DPPH is one of the few free radicals that can remain stable at room temperature (Xie et al., 2008). It can accept an electron or hydrogen radical to become a stable molecule (O’Sullivan et al., 2011). Superoxide anion radicals are the most common types of in vivo-generated free radicals (Zhang and Yang,

<table>
<thead>
<tr>
<th><strong>Table 6.</strong> Effect on the contents of serum BUN of mice.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group name</strong></td>
</tr>
<tr>
<td><strong>Before exhaustive swimming test</strong></td>
</tr>
<tr>
<td>--------------------------------------------</td>
</tr>
<tr>
<td>C (Control group)</td>
</tr>
<tr>
<td>OP (Oyster peptides group)</td>
</tr>
<tr>
<td>O (Oyster group)</td>
</tr>
<tr>
<td>P (Protein group)</td>
</tr>
</tbody>
</table>

* Values were expressed as means ± SD of mice per group. Values among groups had no significant difference based on Duncan’s multiple range test ($p < 0.05, n = 10$).

§ Values with different superscripts indicate a significant difference among groups based on Duncan’s multiple range test ($p < 0.05, n = 10$).

<table>
<thead>
<tr>
<th><strong>Table 7.</strong> Effect on the contents of liver and gastrocnemius muscle glycogen of mice.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group name</strong></td>
</tr>
<tr>
<td><strong>Liver</strong></td>
</tr>
<tr>
<td>--------------------------------------------</td>
</tr>
<tr>
<td>C (Control group)</td>
</tr>
<tr>
<td>OP (Oyster peptides group)</td>
</tr>
<tr>
<td>O (Oyster group)</td>
</tr>
<tr>
<td>P (Protein group)</td>
</tr>
</tbody>
</table>

Values were expressed as means ± SD of mice per group. Values with different superscripts indicate a significant difference among groups based on Duncan’s multiple range test ($p < 0.05, n = 10$).
They can produce hydrogen peroxide and hydroxyl radicals through dismutation and other types of reactions; they are also a source of in vivo-formed free radicals (Xie et al., 2008). Lipid peroxidation in biological membranes is considered one of the major mechanisms of cell injury in aerobic organisms subjected to oxidative stress. The chain reaction of lipid peroxidation ensures the continuous supply of free radicals, which initiate further peroxidation (Adefegha and Oboh, 2012). The antioxidative assay conducted in this study indicates that OP has in vitro antioxidative activities.

To date, many antioxidative peptides have been confirmed as having anti-fatigue effects, making them potentially suitable ingredients for health-promoting foods. Jellyfish collagen hydrolysate significantly alleviates fatigue in mice and exerts antioxidative effects on aging mice (Ding et al., 2011). Loach peptide has in vitro antioxidative activities; it also increases endurance and facilitates recovery from fatigue (You et al., 2011). Grass carp peptide improves the endurance of mice, an effect attributed primarily to the enhancement of antioxidant enzyme levels [CAT and superoxide dismutase (SOD)] (Ren et al., 2011). Decapeptide CMS001 (Pro-Thr-Thr-Lys-Thr-Tyr-Phe-Pro-His-Phe) isolated from pig spleen is confirmed as having anti-fatigue effects, making them potentially suitable ingredients for health-promoting foods. Jellyfish collagen hydrolysate significantly alleviates fatigue in mice and exerts antioxidative effects on aging mice (Ding et al., 2011). Decapeptide CMS001 (Pro-Thr-Thr-Lys-Thr-Tyr-Phe-Pro-His-Phe) isolated from pig spleen decreases malondialdehyde and increases SOD levels, as well as exerts anti-fatigue effects (Wang et al., 2008).

Some biochemical parameters related to fatigue can be widely used to evaluate anti-fatigue drugs; these parameters are LA, BUN, and liver and gastrocnemius muscle glycogen. In vivo changes of these parameters also have some relationship with oxidant stress. LA increases during exercise because it can reduce oxidized species by acting as a hydrogen donor that prevents oxidation (Deaton and Marlin, 2003). BUN levels significantly increase because the renal system suffers from exercise-induced oxidative stress (Feng et al., 2011). Of particular concern is the fact that excess reactive oxygen can induce lipid peroxidation (Wang et al., 2008), which diminishes the integrity of biological membranes and ultimately decreases glycogen content (Borges-Silva et al., 2007). Body depletion often occurs with the exhaustion of glycogen during strenuous exercise (Wang et al., 2008). Thus, glycogen content illustrates the speed and degree of fatigue development. In the present study, the administration of OP significantly decreased LA and BUN levels, and increased liver and gastrocnemius muscle glycogen content. Our data suggests that OP has higher anti-fatigue effects than do oyster meat and oyster protein.

Antioxidative peptides could inhibit lipid peroxidation, scavenge free radicals, chelate metal ions (Sarmadi et al., 2010). These merits are vital to eliminate fatigue. In vivo tests, some antioxidant peptides were reported to reduce metabolic products of oxidative stress reaction (Yu et al., 2011), or to modulate the levels of antioxidant enzymes (Ren et al., 2008). In addition, some antioxidant peptides such as dipeptide Met-Tyr derived from sardine muscle (Erdmann et al., 2006) protects endothelial cells from oxidative stress through the induction of genes. In this study, OP performed in vitro antioxidant activity and in vivo anti-fatigue effects. However, further research should be carried out on evaluating its anti-fatigue mechanisms at the cellular and molecular levels.

Conclusion

This study demonstrated that OP prepared by neutral proteinase digestion has not only in vitro antioxidative activities, but also in vivo anti-fatigue effects in mice. OP supplementation may benefit athletes in terms of improving performance, which in turn, elevates endurance and facilitates recovery from fatigue. The experiment shows that OP is better than oyster meat and oyster protein on endurance performance. Our results serve as important bases for developing OP as a novel antioxidant and anti-fatigue compound.

Acknowledgements

This project was supported by the Earmarked Fund for Modern Agro-industry Technology Research System of China (CARS-48) and the Li Shangda Fund of Jimei University (ZC2011016).

References


Antioxidative and Anti-fatigue Effects of Oyster Peptides


