Detection of EPA and DHA in the Viscera of Marine Organisms

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Abstract. The viscera of 6 kinds of non-fish marine organisms were collected for detection of DHA/EPA using gas chromatography. The oil extraction ratio varied from 0.72% to 6.23%(wt%). Scylla serrata presented the highest oil yield, followed by Architeuthis dux and Panopea abrupta. The EPA concentration (in methyl ester form) in the oil varied from 0.56 to 7.75(mg/g). Scylla serrata presented the highest EPA concentration(p<0.05), followed by Architeuthis dux and Panopea abrupta. The DHA concentration(in methyl ester form) varied from 0.15 to 4.71(mg/g). Architeuthis dux and Scylla serrata presented higher DHA concentration than other species(p<0.05).

Introduction

EPA(5, 8, 11, 14, 17-eicosapentaenoic acid) and DHA(4, 7, 10, 13, 16, 19- docosahexaenoic acid) and are two important omega-3 polyunsaturated fatty acids. Both of them belong to essential fatty acids that human body needs but can hardly produce by itself. Therefore, they depend on consumption through food or supplements[1]. EPA and DHA were confirmed to benefit the functions of various systems in human body, including cardiovascular health, brain health, eyesight health, etc. For cardiovascular health, their functions include inhibiting platelet condensing, performing antithrombosis, helping vasorelaxation, raising HDL level, decreasing LDL and cholesterol level, and so on[2-4]. For brain health, their functions include improving brain cell development, improving brain function, improving memory and learning ability, and preventing senile dementia, and so on[5-6]. For eyesight, they could strengthen retinal reflection ability. They also benefit the therapy of diabetes inflammation, kidney disease and various cancers[7-10]. Polyunsaturated fatty acids are recommended for patients with wide-ranging chronic diseases, including coronary heart disease, rheumatoid arthritis, dementia, and depression1. Health authorities in many countries recommend increased intake of EPA/DHA. For example, European health authorities recommend at least 0.45-0.50g/day EPA+DHA to maintain good health. The mean daily intake of EPA+DHA and ALA suggested in Australia is 0.175g and 1.07g, respectively. A sharp increasing of consumption of EPA/DHA is occurring worldwide recently.

Most attentions on EPA and DHA source were paid to marine fishes, which are rich in polyunsaturated fatty acids, and a lot of other non-fish organisms have long been ignored. In addition to fish, there are a huge number of other marine organisms in the ocean. For example, over 1580 kinds of marine organisms have been confirmed in the beach in China. Mollusks(513 species) accounts for the largest proportionate, followed by seaweed(358 species) and...
crustaceans (308 species). This study aims to establish a gas chromatography for detection of the EPA and DHA in the viscera of some marine organisms, and to analyze some typical samples.

Materials and Methods

Marine Organisms

Marine organisms used in this study were summarized in Table 1.

<table>
<thead>
<tr>
<th>Species</th>
<th>Living conditions</th>
<th>Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sepioidea</td>
<td>Generally moving to the coastal shallow sea in the spring, and moving to the offshore deep water in winter</td>
<td>Distributed extensively in most oceans, especially in the tropical and temperate district</td>
</tr>
<tr>
<td>Portunus trituberculatus</td>
<td>Lurking underwater in the day, and foraging in the night with obvious phototaxis. Adaptive water temperature of 8-31°C; Adaptive salinity of 13-38‰</td>
<td>Mainly distributed in Japan, Korea, Malaysia, the Red Sea and Guangxi province of China.</td>
</tr>
<tr>
<td>Panopea abrupta</td>
<td>Embedded type shellfish; Perched substrate of sand and mud, with 3-18m of water depth; Buried depth of about 50-80cm; Adapted to water temperature of 3-23°C</td>
<td>Living originally in the north Pacific coast of USA and Canada. Introduced to many areas later, including the southeast coast of China</td>
</tr>
<tr>
<td>Abalone</td>
<td>Attached to the rocks; Crawling in reef tents and holes.</td>
<td>Distributed worldwide, along the coastal waters of every continent(except the Atlantic coast, the Caribbean, and the USA East Coast)</td>
</tr>
<tr>
<td>Scylla serrata</td>
<td>Beach-habitating swimming type crabs; living in intertidal mudflats or silt on the tidal flats; Staying in the puddles on the beach or rock seams.</td>
<td>Distributed in the west Pacific tropical/ subtropical waters in India, the Southeast Africa and the Red Sea.</td>
</tr>
<tr>
<td>Architeuthis dux</td>
<td>Generally living in the upper/middle layer of shallow sea; Moving vertically in a range over 100m.</td>
<td>Mainly distributed in the shallow sea in tropical and temperate zones</td>
</tr>
</tbody>
</table>

Treatment of Viscus Oil Samples

The viscera were steamed at 90°C for 45 min for oil extraction. After a centrifugation at 6000g for 15 min, the oil phase was collected as viscus oil sample for analysis. Viscus oil then reacted with methanol in alkaline conditions for formation of fatty acid methyl ester. For each kind of fish, 0.05g viscus oil was dissolved with 5mL n-hexane in 10mL tube with lid, and was added with 2mL 0.5mol/L KOH-methanol solution(2.8g KOH, 1.6g methanol, diluted using water to 100mL final total volume). After 1min of violent shaking, some ultrapure water was added for washing. Discarding the water layer, and washing the n-hexane 3 more times using...
water. After a centrifugation for 5 min at 4000g, the n-hexane layer was collected and was diluted to 10 mL final volume. After a 5× dilution, 1.0 μL of the final sample was loaded for detection.

**Gas Chromatography**

Chromatographic column: Agilent technologies, inc. 19091N-133 (30m×0.250mm, 0.5 μm); column oven temperature: initial temperature 180℃, rise to 220℃ at 10℃/min speed, rise to 250℃ at 8℃/min speed, maintain for 13 min; Injection port temperature: 250℃; Detector temperature: 270℃; carrier gas and flow rate: N₂(≥99.99%) 1.0 mL/min; air 450 mL/min; H₂ 40 mL/min; sample size: 1.0 μL; split rate: 20:1. For each sample, 1.0 μL of sample was loaded precisely for detection. Standard curves were established in our previous study for EPA/DHA quantification. The equations between the peak area (Y) and the concentration (X) in the loaded samples were established as Eq.1 and Eq.2, respectively. The concentration of EPA or DHA (mg/g) in the viscous oil could be calculated according to Eq.3 and Eq.4, respectively.

\[
\begin{align*}
YEPA & = 5971.3 \times \text{EPA}(μg/mL) + 3.53; \quad R^2 = 0.9991 \\
YDHA & = 5119.5 \times \text{DHA}(μg/mL) + 3.95; \quad R^2 = 0.9993
\end{align*}
\]

\[
\begin{align*}
\text{CEPA(mg/g)} & = \text{XEPA(μg/mL)} \times 50(\text{mL}) \times 10^{-3} / 0.05g \\
\text{CDHA(mg/g)} & = \text{XDHA(μg/mL)} \times 50(\text{mL}) \times 10^{-3} / 0.05g
\end{align*}
\]

**Evaluation of the Precision and the Recovery Ratio of the Gas Chromatography**

Fish oil samples extracted from *Pseudosciaena crocea* were chosen for precision and the recovery ratio evaluation. For precision, a sample was detected repeatedly for 5 times. The peak areas were recorded and the relative standard deviation (RSD) was calculated for precision evaluation. For recovery ratio, 9 samples containing three concentrations of EPA methyl ester and DHA methyl ester were prepared. The low dose group was prepared with 0.15 g fish oil, 900 μL of 10 mg/mL EPA methyl ester and 1500 μL of 10 mg/mL DHA methyl ester. The middle dose group was prepared with 0.15 g fish oil, 1125 μL of 10 mg/mL EPA methyl ester and 1875 μL of 10 mg/mL DHA methyl ester. The high dose group was prepared with 0.15 g fish oil, 1350 μL of 10 mg/mL EPA methyl ester and 2250 μL of 10 mg/mL DHA methyl ester. The solutions above were treated as described for samples for detection and recovery ratio evaluation.

**Statistical Analysis**

The data was analyzed using One-way ANOVA in SPSS (V11.5). Statistical significance was determined with α=0.05.
Result and Discussion

Viscus oil yields and the concentration of EPA and DHA was shown in Table 2. The viscus oil extraction ratio varied from 0.72% to 6.23% (wt%). *Scylla serrata* presented the highest oil yield, followed by *Architeuthis dux* and *Panopea abrupta*. The EPA concentration (in methyl ester form) in the oil varied from 0.56 to 7.75(mg/g). *Scylla serrata* presented the highest EPA concentration(p<0.05), followed by *Architeuthis dux* and *Panopea abrupta*. The DHA concentration (in methyl ester form) varied from 0.15 to 4.71(mg/g). *Architeuthis dux* and *Scylla serrata* presented obviously higher DHA concentration than other species(p<0.05).

Table 2. Viscus oil yields and the concentration of EPA and DHA in the oil.

<table>
<thead>
<tr>
<th>Species</th>
<th>Oil yield(wt%)</th>
<th>EPA(mg/g)</th>
<th>DHA(mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Sepioidea</em></td>
<td>2.23±0.35</td>
<td>0.96±0.28</td>
<td>1.23±0.34</td>
</tr>
<tr>
<td><em>Portunus trituberculatus</em></td>
<td>0.80±0.25</td>
<td>0.56±0.29</td>
<td>0.51±0.18</td>
</tr>
<tr>
<td><em>Panopea abrupta</em></td>
<td>3.78±0.62</td>
<td>2.50±0.85</td>
<td>0.15±0.05</td>
</tr>
<tr>
<td><em>Abalone</em></td>
<td>0.72±0.26</td>
<td>1.95±0.46</td>
<td>3.34±0.76</td>
</tr>
<tr>
<td><em>Scylla serrata</em></td>
<td>6.23±1.02</td>
<td>7.75±1.04</td>
<td>4.30±0.59</td>
</tr>
<tr>
<td><em>Architeuthis dux</em></td>
<td>3.86±0.93</td>
<td>5.97±1.19</td>
<td>4.71±1.01</td>
</tr>
</tbody>
</table>

Acknowledgement

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References


