Seasonal Variations of Fat and Fatty Acid Composition in Muscle Tissues of Mediterranean Octopuses

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Abstract
The effects of seasons on lipid and fatty acid profiles of muscle types (mantle and arm) of Mediterranean octopuses (common octopus- *Octopus vulgaris* and musky octopus-*Eledone moschata*) were investigated. The results showed that lipid levels ranged from 0.75% to 1.60% in both muscle types of octopuses which were considered as lean. Lipid levels in mantle tissues of both octopus species were higher than lipid levels in their arms. Although these two octopus species contain small amounts of lipid, they are good sources of *n*-3 PUFAs content (especially EPA and DHA) for all season regardless of muscle types. The highest levels of EPA were obtained from mantle and arms tissues (18.23%) of common octopus in spring. The highest levels of EPA were obtained from mantle (12.90%) and arms (12.67%) of musky octopus in autumn and winter. The levels of EPA in both of muscle tissues of common octopus were found to be higher than levels of EPA in both of muscle tissues of musky octopus. The highest DHA levels were obtained from mantle tissue (28.17%) of common octopus in summer. There were significant differences (p<0.05) in the levels of SFA, MUFA and PUFA in terms of species, season, and muscle types.

Keywords: Common octopus, Musky octopus, Season, Fatty acids, Lipid

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Introduction

Octopuses are important species of marine invertebrates consumed in the world. Total number of cephalopods caught were 4305482 and 4313510 tons were in 2007 and 2008, respectively (FAO, 2008).

Seafood contains high level of n-3 long chain polyunsaturated fatty acids (PUFA), especially eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) (Özogul et al., 2007; Ozogul et al., 2008; Nisa and Asadullah 2011). These fatty acids are of great importance to humans for the prevention of coronary heart disease (Mozaffarian et al., 2005; Özogul et al., 2007; Ozogul et al., 2008; Ayas and Ozogul, 2011). Even though humans can synthesize EPA and DHA from short-chain fatty acids, this synthesis is not generally sufficient (Burdge and Calder, 2005; Bradbury, 2011). As EPA and DHA are essential nutrients, dietary intake from seafood is necessary for adequate levels. However, levels of these long chain fatty acids may change among seafood species. Hence, dietary intake of EPA and DHA from seafood is dependent on the species. Also fatty acids compositions of seafood may be changed with season and muscle types. Therefore, the aim of this study was to investigate the effects of season and muscle type on the lipid and fatty acid compositions of the common octopus and musky octopus.

Materials and methods

Sample Preparation

Both of octopus species were caught by bottom trawling from the Mediterranean Sea in February, May, August, and November in 2011 consist of common octopus (Octopus vulgaris Cuvier 1797) and musky octopus (Eledone moschata Lamarck 1798). In every season, 10 individuals of each species were caught and kept in polystyrene boxes with ice and transferred to the laboratory of Faculty of Fisheries at University of Çukurova. The sex of octopus species was identified and their mantle length (mm) and total weight (g) were measured (Table 1). Meat of mantle and arms which is the main edible portion of octopuses was homogenized and chemical analyses were done on this part of fresh samples (10 g for each replication). The analyses were performed at least in triplicate.

Chemical analysis

Lipid analysis

Lipid content was determined according to the method of the Bligh and Dyer (1959) method.

Fatty acid analyses

Fatty acid profiles of lipid extracted from the octopus samples were determined by gas chromatography (GC). The fatty acid composition was analyzed by the GC Clarus 500 with autosampler (Perkin Elmer, Shelton, CT, USA) equipped with a flame ionization detector and a fused silica capillary SGE column (30 m 0.32 mm, ID 0.25 mm, BP20 0.25 UM; SGE Analitic Science Pty Ltd, Victoria, Australia). The oven temperature was 140 °C, held for 5 min, raised to 200 °C at a rate of 4 °C/min and to 220 °C at a rate of 1 °C/min while the injector and the detector temperature were set at 220 °C and 280 °C, respectively. The sample size was 1 µL.
and the carrier gas was controlled at 16 ps. The split used was 1:100. Fatty acids were identified by comparing the retention times of fatty acid methyl esters with a standard 37-component fatty acid methyl ester mixture (catalog no 18919; Supelco). Triplicate GC analyses were performed and the results were expressed in GC area % as the mean value±SD.

### Table 1: Measurements of the common octopus and musky octopus

<table>
<thead>
<tr>
<th>Season</th>
<th>Species</th>
<th>N</th>
<th>ML (mm) $\bar{X} \pm S_x$</th>
<th>weight (g) $\bar{X} \pm S_x$</th>
<th>Sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autumn</td>
<td><em>O. vulgaris</em></td>
<td>5</td>
<td>104.30±33.20$^a$</td>
<td>370.55±125.77$^a$</td>
<td>♀</td>
</tr>
<tr>
<td></td>
<td><em>O. vulgaris</em></td>
<td>5</td>
<td>99.65±28.64$^a$</td>
<td>350.11±115.00$^b$</td>
<td>♂</td>
</tr>
<tr>
<td></td>
<td><em>E. moschata</em></td>
<td>5</td>
<td>81.10±16.36$^a$</td>
<td>100.80±57.80$^a$</td>
<td>♀</td>
</tr>
<tr>
<td></td>
<td><em>E. moschata</em></td>
<td>5</td>
<td>80.68±14.31$^a$</td>
<td>105.48±32.09$^a$</td>
<td>♂</td>
</tr>
<tr>
<td>Winter</td>
<td><em>O. vulgaris</em></td>
<td>5</td>
<td>114.11±56.45$^a$</td>
<td>455.00±205.13$^a$</td>
<td>♀</td>
</tr>
<tr>
<td></td>
<td><em>O. vulgaris</em></td>
<td>5</td>
<td>100.22±19.19$^a$</td>
<td>375.49±55.33$^a$</td>
<td>♂</td>
</tr>
<tr>
<td></td>
<td><em>E. moschata</em></td>
<td>5</td>
<td>77.89±31.01$^a$</td>
<td>80.37±17.26$^a$</td>
<td>♀</td>
</tr>
<tr>
<td></td>
<td><em>E. moschata</em></td>
<td>5</td>
<td>79.90±18.67$^a$</td>
<td>95.11±10.10$^a$</td>
<td>♂</td>
</tr>
<tr>
<td>Spring</td>
<td><em>O. vulgaris</em></td>
<td>5</td>
<td>97.67±25.55$^a$</td>
<td>385.57±180.77$^a$</td>
<td>♀</td>
</tr>
<tr>
<td></td>
<td><em>O. vulgaris</em></td>
<td>5</td>
<td>119.37±44.04$^a$</td>
<td>501.01±118.92$^a$</td>
<td>♂</td>
</tr>
<tr>
<td></td>
<td><em>E. moschata</em></td>
<td>5</td>
<td>83.99±26.07$^a$</td>
<td>99.27±19.11$^a$</td>
<td>♀</td>
</tr>
<tr>
<td></td>
<td><em>E. moschata</em></td>
<td>5</td>
<td>80.07±27.44$^a$</td>
<td>101.07±30.78$^a$</td>
<td>♂</td>
</tr>
<tr>
<td>Summer</td>
<td><em>O. vulgaris</em></td>
<td>5</td>
<td>115.77±40.02$^a$</td>
<td>440.24±89.80$^a$</td>
<td>♀</td>
</tr>
<tr>
<td></td>
<td><em>O. vulgaris</em></td>
<td>5</td>
<td>110.31±33.61$^a$</td>
<td>444.89±101.73$^a$</td>
<td>♂</td>
</tr>
<tr>
<td></td>
<td><em>E. moschata</em></td>
<td>5</td>
<td>90.12±22.61$^a$</td>
<td>123.27±62.80$^a$</td>
<td>♀</td>
</tr>
<tr>
<td></td>
<td><em>E. moschata</em></td>
<td>5</td>
<td>86.45±23.23$^a$</td>
<td>110.22±47.90$^a$</td>
<td>♂</td>
</tr>
</tbody>
</table>

Values in same species with different letters are significantly different (p<0.05). ML: mantle length $\bar{X} \pm S_x$; SD

Methyl esters were prepared by transmethylation using 2 M KOH in methanol and n-heptane according to the method described by Ichibara et al. (1996) with minor modification. Extracted lipids (10 mg) were dissolved in 2 mL n-heptane followed by 4 mL of 2 M methanolic KOH. The tube was then vortexed for 2 min at room temperature. After centrifugation at 4,000 rpm for 10 min, the heptane layer was taken for GC analyses.

**Statistical analysis**

Prior to the analyses, all data were checked for outliers (Z values were checked) and homogeneity of variance (Duncan test was used) was also tested. One-way ANOVA (Analysis of Variance) was used to evaluate the effects of species, muscle tissues and season on the lipid and fatty acid composition. Statistical analysis of data was carried out with the SPSS 16.0.

**Results**

**Lipid content of muscle types**

In this study, lipid levels (0.90-1.60%) in mantle of both octopus species were higher than lipid levels (0.75-1.07%) found in their arms (Table 2) regardless of season.

**Fatty acids composition**

Saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs) of mantle and arms of both octopus species are presented in Tables 3, 4 and 5, respectively.
Table 2: The effect of season on lipid levels of common octopus and musky octopus (%)

<table>
<thead>
<tr>
<th>Latino name</th>
<th>Autumn</th>
<th>Winter</th>
<th>Spring</th>
<th>Summer</th>
<th>Tissue</th>
<th>Common name</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Octopus vulgaris</em></td>
<td>1.00±0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.90±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.11±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.19±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>mantle</td>
<td>Common octopus</td>
</tr>
<tr>
<td>0.90±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.75±0.01&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.89±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.92±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Eledone moschata</em></td>
<td>1.00±0.01&lt;sup&gt;*&lt;/sup&gt;</td>
<td>1.15±0.06&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.54±0.05&lt;sup&gt;*&lt;/sup&gt;</td>
<td>1.60±0.02&lt;sup&gt;e&lt;/sup&gt;</td>
<td>mantle</td>
<td>Musky octopus</td>
</tr>
<tr>
<td>0.89±0.01&lt;sup&gt;*a&lt;/sup&gt;</td>
<td>0.93±0.02&lt;sup&gt;g&lt;/sup&gt;</td>
<td>1.03±0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.07±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values in same lines with different letters are significantly different (p<0.05), <sup>X ± S</sup><sub>x</sub> (Average±SD)

Table 3: The effects of species and season on saturated fatty acids (SFA) of the Mediterranean octopuses (%)

<table>
<thead>
<tr>
<th>Season</th>
<th>C12:0</th>
<th>C14:0</th>
<th>C15:0</th>
<th>C16:0</th>
<th>C17:0</th>
<th>C18:0</th>
<th>C20:0</th>
<th>C22:0</th>
<th>ΣSFA</th>
<th>Species/Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autumn</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.86±0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.30±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>16.15x±0.27&lt;sup&gt;*&lt;/sup&gt;</td>
<td>1.92±0.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.71±0.38&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.32±0.21&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Winter</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>5.97±0.59&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.31±0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13.55±0.09&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.91±0.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.66±0.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.69±0.33&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Spring</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.72±0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.23±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>15.92±0.06&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.96±0.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.78±0.32&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.64±0.24&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Summer</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>4.28±0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.21±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12.45±0.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.02±0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.85±0.22&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>2.24±0.13&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values in same column with different letters are significantly different (p<0.05). <sup>X ± S</sup><sub>x</sub> : Average±SD, ND: not detected. OV-M, *Octopus vulgaris* mantle; OV-A, *Octopus vulgaris* arms; EM-M, *Eledone moschata* mantle; EM-A, *Eledone moschata* arms.
Table 4: The effects of species and season on monounsaturated fatty acids (MUFA) of the Mediterranean octopuses (%)

<table>
<thead>
<tr>
<th>Season</th>
<th>C14:1</th>
<th>C15:1</th>
<th>C16:1</th>
<th>C17:1</th>
<th>C18:1n9</th>
<th>C18:1n7</th>
<th>C20:1</th>
<th>C22:1n9</th>
<th>ΣMUFA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X ± Sx</td>
<td>X ± Sx</td>
<td>X ± Sx</td>
<td>X ± Sx</td>
<td>X ± Sx</td>
<td>X ± Sx</td>
<td>X ± Sx</td>
<td>X ± Sx</td>
<td></td>
</tr>
<tr>
<td>Autumn</td>
<td>1.14±0.02</td>
<td>0.67±0.25</td>
<td>0.41±0.04</td>
<td>0.21±0.01</td>
<td>1.38±0.06</td>
<td>0.17±0.01</td>
<td>4.06±0.00</td>
<td>2.76±0.03</td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Winter</td>
<td>0.52±0.02</td>
<td>0.30±0.01</td>
<td>0.77±0.00</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spring</td>
<td>0.92±0.01</td>
<td>0.62±0.05</td>
<td>0.61±0.02</td>
<td>0.42±0.01</td>
<td>0.60±0.02</td>
<td>0.29±0.01</td>
<td>3.87±0.02</td>
<td>1.97±0.05</td>
<td></td>
</tr>
</tbody>
</table>

Values in same column with different letters are significantly different (p<0.05). X ± Sx: Average±SD, ND: not detected, OV-M, Octopus vulgaris mantle; OV-A, Octopus vulgaris arms; EM-M, Eledone moschata mantle; EM-A, Eledone moschata arms.

Table 5: The effects of species and season on polysaturated fatty acids (PUFA) of the Mediterranean octopuses (%)

<table>
<thead>
<tr>
<th>Season</th>
<th>C18:2n6</th>
<th>C18:3n6</th>
<th>C18:3n3</th>
<th>C20:2n6</th>
<th>C20:4n6</th>
<th>C20:5n3</th>
<th>C22:2n6</th>
<th>C22:6n3</th>
<th>ΣPUFA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X ± Sx</td>
<td>X ± Sx</td>
<td>X ± Sx</td>
<td>X ± Sx</td>
<td>X ± Sx</td>
<td>X ± Sx</td>
<td>X ± Sx</td>
<td>X ± Sx</td>
<td></td>
</tr>
<tr>
<td>Autumn</td>
<td>0.49±0.07</td>
<td>0.16±0.01</td>
<td>0.14±0.00</td>
<td></td>
<td>0.09±0.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Winter</td>
<td>0.62±0.00</td>
<td>0.06±0.01</td>
<td>0.12±0.01</td>
<td></td>
<td>0.06±0.00</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spring</td>
<td>0.34±0.04</td>
<td>0.17±0.00</td>
<td>0.12±0.02</td>
<td>0.29±0.01</td>
<td>0.10±0.00</td>
<td></td>
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</tr>
</tbody>
</table>

Values in same column with different letters are significantly different (p<0.05). X ± Sx: Average±SD, ND: not detected, OV-M, Octopus vulgaris mantle; OV-A, Octopus vulgaris arms; EM-M, Eledone moschata mantle; EM-A, Eledone moschata arms.
Significant differences (p<0.05) in SFA contents were observed in terms of species, season and muscle types (Table 3). SFA levels of mantle of common octopus (O. vulgaris) were found to be 32.25% in autumn, 34.07% in winter, 32.21% in spring, and 30.05% in summer whereas its levels in arms were 31.61%, 33.69%, 32.63% and 32.90%, respectively. The highest levels of SFA were obtained from mantle and arms in winter. On the other hand, SFA levels of mantle of musky octopus were 29.69%, 30.89%, 31.00% and 32.09% while these levels in arms were obtained as 29.80%, 31.84%, 32.28% and 31.09% in autumn, winter, spring, and summer, respectively. Musky octopus had the highest SFA content in its mantle in winter and arms in autumn. The dominant SFAs were palmitic acid (16:0), ranging from 12.45% to 16.83% and stearic acid (18:0), ranging from 8.46% to 10.78% in both species. There were significant differences (p<0.05) in the level of MUFA between species, season, and also muscle types (Table 4). MUFA levels of mantle of O. vulgaris were found to be 9.73% in autumn, 7.10% in winter, 10.71% in spring and 9.11% in summer whereas these levels in arms were 10.31%, 7.48%, 9.91%, 9.07%, respectively. The highest MUFA levels of mantle and arms of common octopus were obtained in spring and autumn seasons. The major monounsaturated fatty acids (MUFA) were palmitoleic acid (C16:1, 0.95-2.78%), oleic acid (C18:1n-9, 2.94-6.06%), and octadecenoic acid (C18:1n-7, 1.95-2.97%) in both octopus species. MUFA levels in both muscle tissues of common octopus were lower than MUFA levels of musky octopus regardless of season.

PUFA contents were significantly different (p<0.05) in terms of species, seasons and muscle types (Table 5). PUFA levels of mantle in common octopus (O. vulgaris) were found to be 52.77% in autumn, 52.56% in winter, 51.25% in spring, and 53.59% in summer; however, PUFA levels in arms of this species were observed as 52.11%, 52.54%, 52.75% and 50.31%, respectively. The highest levels of PUFA were obtained from mantle (53.59%) and arms (52.75%) in summer and spring. On the other hand, PUFA levels in mantle of musky octopus were 47.09%, 46.44%, 47.06% and 46.30% while these levels in arms were determined as 46.25%, 46.84%, 45.69% and 46.38% in autumn, winter, spring and summer, respectively.

Significant differences were observed (p<0.05) in the level of EPA and DHA for species, season and also muscle types. The highest EPA level in mantle of common octopus was obtained in spring (18.23%), followed by autumn (17.31%) although arms gave the highest content of EPA in spring (18.23%). Similar to common octopus, mantle of musky octopus had the highest EPA content (12.90%) in autumn. However, the highest EPA content was obtained from arms of musky octopus in winter (12.67%).
Significant differences (p<0.05) in DHA contents were observed in terms of species, seasons and muscle types (Table 5). DHA levels found in mantle of common octopus were 26.50% in autumn, 27.29% in winter, 25.17% in spring, and 28.17% in summer whereas DHA levels in arms were 25.04%, 26.86%, 25.39% and 27.65%, respectively. The highest levels of DHA were obtained from mantle (28.17%) and arms (27.65%) in summer. Regarding DHA levels in mantle of musky octopus (E. moschata), the results were 25.49%, 25.09%, 24.99% and 25.31% whereas the levels in arms of this species were observed as 23.95%, 23.97%, 23.90% and 25.12% in autumn, winter, spring and summer, respectively.

**Discussion**

**Lipid content of muscle types**

The low lipid content of octopuses (lower than 2%) is a feature of cephalopods (Table 2), reported in several studies including Hayashi and Bower (2004), Özyurt and et al. (2006), Sieiro et al. (2006), Zlatanos et al. (2006), Ozogul et al. (2008). The variations of lipid content levels in both octopus species were found to be similar. They had higher lipid contents in spring and summer and lower lipid contents in autumn and winter in both of the muscle types, especially in mantle (Table 2). The lipid levels in mantle tissue of common octopus were reported as 1.2% in the study carried out by Zlatanos et al. (2006). This agrees with our observations in the current study.

**Fatty acids composition**

The major fatty acids found in both octopuses were palmitic acid (C16:0), heptadecanoic acid (C17:0), stearic acid (C18:0), palmitoleic acid (C16:1), oleic acid (C18:1n-9), octadecenoic acid (C18:1n-7), arachidonic acid (C20:4 n-6), cis-5,8,11,14,17-eicosapentaenoic acid (EPA, C20:5 n-3) and cis-4, 7, 10, 13, 16, 19-docosahexaenoic acid (DHA, C22:6 n-3). These results are in agreement with those in previous studies (Sieiro et al., 2006; Zlatanos et al., 2006; Ozogul et al., 2008).

The levels of DHA content of both octopus species were found to be similar. Also EPA and PUFA levels in both muscle tissues of common octopus were higher than EPA and PUFA levels of musky octopus regardless of season. These results correspond to those in previous study (Ozogul et al., 2008).

The results of fatty acid compositions indicated that mantle and arms of common octopus and musky octopus were very rich in n-3 fatty acids, especially EPA and DHA as reported in previous studies (Sieiro et al., 2006; Zlatanos et al., 2006; Ozogul et al., 2008).

The amount of longer-chain n-3 PUFAs differs among species and can be influenced by a number of factors (season, sex, age). Despite the fact that both octopus species contain small amounts of lipid, these organisms are good sources of n-3 PUFA content (especially EPA and DHA) for all seasons regardless of muscle types.

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References