Comparison of ω-3 Fatty Acids by GC-MS in Frequently Consumed Fish and Fish Oil Preparations on the Turkish Market


INTRODUCTION

Fish is considered to be a food with health advantages. Besides its nutritive value, the lipid fraction shows an interesting fatty acid profile with a significant presence of ω-3 polyunsaturated fatty acids (PUFA), the health benefits of which have been widely studied. The ratio of ω-6 / ω-3 fatty acids in cell membrane phospholipids and plasma phospholipids plays a pivotal role in determining membrane fluidity, gene expression, cytokine formation, lipid levels and immune responses, all of which may prevent or contribute to coronary heart disease, hypertension, diabetes, cancer, arthritis, psoriasis, ulcerative colitis, multiple sclerosis and other autoimmune disorders (1).

Previous researches about diets including a high quantity of fish have demonstrated its advantages with regard to health (2, 3). According to the results of experimental, clinical and epidemiological studies, ω-3 fatty acids including high amounts of double bonds in the long chain can prevent atherosclerotic heart disease and acute coronary disease. The

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consumption of fish in meals two or three times during a week provides enough quantities of double-bonded ω-3 fatty acids and especially plays a protective role for people at risk of cardiovascular heart disease (4).

The fatty acid composition of fish oils is dominated by two members of the ω-3 PUFA family: 20:5, ω-3 eicosapentaenoic acid (EPA) and 22:6, ω-3 docosahexaenoic acid (DHA). In epidemiological, clinical and experimental studies, the concentration of EPA has been related to the risk of cardiovascular disease, thrombotic events, regulation of plasma lipids and lipoproteins, arterial wall activity and regulation of blood pressure, as well as to many other biological activities (5).

There are several forms of fish oil supplements on the turkish market. The most common form is natural fish oil, usually produced from the body of cold-water fish. These fish oils typically contain 30% EPA and DHA with a ratio of EPA to DHA of 1.5. A typical 1 soft gel capsule of fish oil contains 180 mg EPA and 120 mg DHA. Natural EPA and DHA are chemically triacylglycerols. Natural fish oil capsules containing 50% EPA and DHA in a 1.5 ratio are now available (6).

In this study, fish oils from anchovy (Engraulis encrasicolus L.) and horse mackerel (Trachurus trachurus L.) as well as three fish oil products sold on the Turkish market were analyzed by gas chromatography-mass spectrometry (GC-MS). Fatty acid composition of the fish oils from anchovy, horse mackerel and the commercial products was determined.

2. Materials and methods

2.1. Materials

Anchovy (Engraulis encrasicolus L.) and horse mackerel (Trachurus trachurus L.) originating from the sea of Marmara were purchased from the local market. Three fish oil preparations were obtained from a pharmacy.

2.2. Chemicals

Boron trifluoride-methanol complex (Merck-801663), sodium hydroxide (Merck-106482) and sodium chloride (Merck-106400) of analytical-reagent quality were used for sample esterification. Analytical grade chloroform (Merck-102431), petroleum ether (Merck-159542), hexane (Merck-104368), and methanol (Merck-106008) were used for extraction of the samples. Chromatographic grade hexane (Merck-104391) was used for dissolving and diluting samples before GC-MS analysis.

2.3. Chromatographic Conditions

Gas chromatography-mass spectrometry was carried out on a Varian-Chrompack CP-3800 coupled to a Varian-Chrompack Saturn 2000 under electron impact ionization (70 eV). The interface temperature was 230 °C and the MS scan range was 40-650 atomic mass units (AMU). The chromatographic column was fused silica (WCOT-Fused Silica) capillary column (30m x 0.25mm i.d.; CP-Sil 5CP, 0.25 mm). Helium was used as carrier gas at a flow rate of 1 ml/min. Samples were analyzed with the column held initially at 150°C for 1 min. It was then increased to 180°C, with a 3°C/min heating ramp, and then kept there for 15 mins and then increased to 230°C with a 3°C/min heating ramp and kept there for 10 mins. Finally, temperature was increased to 250°C with a 5°C/min heating ramp and the temperature was kept there for 10 mins. The injection was performed in split mode (20:100) at 250°C.

2.4. Extraction

After removing the internal organs, the fish samples were washed. 464.07 g of anchovy and 382.81 g of horse mackerel were homogenized and extracted for 5 mins with 750 ml chloroform/methanol (2:1) mixture and then the extracts were filtered. Chloroform layers were separated by a separation funnel and were condensed at a rotary evaporator to obtain 26.73 g (5.76% w/w) fish oil from anchovy and 27.86 g (7.28% w/w) from horse mackerel.
2.5. Preparation of Methyl Esters of Fatty Acids

Fish oils from anchovy and horse mackerel as well as commercial products prepared as soft gelatin capsules were weighed (450 mg) in a 50 ml of volumetric flask, then 12 ml 0.5 N methanolic NaOH was added to each mixture. The mixtures were then heated on a steam bath until the fat globules entered the solution, an approximately five-minute step. 20 ml of BF₃/MeOH was added to each flask and the mixtures were boiled for 2 mins. After cooling, saturated NaCl solution, was added to each solution to reach 50 ml. The mixtures were then transferred to a separation funnel individually and each extracted with 30 ml petroleum ether. The ether phase of each sample was then evaporated on a water bath at 60°C (7). The obtained methyl esters of the fatty acid fractions were dissolved in 5 ml of hexane, and 1 µl of samples were injected to GC-MS.

2.6. Identification of Lipid Components

Identification of each peak was done using Wiley and Nist Library, and by a comparison of its retention time (RI), retention index (RI), and mass spectrum with those of the standard compounds. Relative content % of fatty acids was determined by calculating the area under peaks using Varian software. The results are expressed as an average of three determinations in all cases.

3. Results and Discussion

The following fatty acids were identified in the two species and three commercial preparations: myristic acid (14:0), palmitoleic acid (16:1, ω-7), palmitic acid (16:0), linoleic acid (18:2, ω-6), oleic acid (18:1, ω-9), elaidic acid (18:1, ω-9), stearic acid (18:0), EPA (20:5, ω-3), gondoic acid (20:1, ω-9), DHA (22:6, ω-3), docosapentaenoic acid (22:5, ω-6), and 11-docosenoic acid (22:1, ω-11). In general, peaks were identified by computer searches in commercial reference libraries. Good spectral matches were obtained in the Wiley and Nist mass spectral library.

Table 1 summarizes the fatty acid composition of the anchovy (Engraulis encrasicolus L.), horse mackerel (Trachurus trachurus L.) and the three commercial fish oil preparations sold in Turkey.

Table 1. Relative content (% of total ion current) of fatty acids in anchovy, horse mackerel and three commercial fish oil preparations sold in Turkey.

<table>
<thead>
<tr>
<th>Peak No</th>
<th>Fatty Acids</th>
<th>Kowats Index</th>
<th>Anchovy oil</th>
<th>Horse mackerel oil</th>
<th>Commercial Product 1</th>
<th>Commercial Product 2</th>
<th>Commercial Product 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Myristic acid (14:0)</td>
<td>1695</td>
<td>5.400</td>
<td>2.982</td>
<td>3.938</td>
<td>5.576</td>
<td>1.484</td>
</tr>
<tr>
<td>2</td>
<td>Palmitoleic acid (16:1, ω-7)</td>
<td>1883</td>
<td>8.630</td>
<td>6.671</td>
<td>6.297</td>
<td>8.348</td>
<td>2.626</td>
</tr>
<tr>
<td>3</td>
<td>Palmitic acid (16:0)</td>
<td>1907</td>
<td>11.920</td>
<td>12.715</td>
<td>10.814</td>
<td>12.449</td>
<td>6.801</td>
</tr>
<tr>
<td>4</td>
<td>Linoleic acid (18:2, ω-6)</td>
<td>2072</td>
<td>1.903</td>
<td>1.180</td>
<td>11.760</td>
<td>3.471</td>
<td>19.608</td>
</tr>
<tr>
<td>5</td>
<td>Oleic acid (18:1, ω-9)</td>
<td>2083</td>
<td>7.429</td>
<td>17.598</td>
<td>12.170</td>
<td>11.113</td>
<td>20.551</td>
</tr>
<tr>
<td>6</td>
<td>Elaidic acid (18:1, ω-9)</td>
<td>2086</td>
<td>2.755</td>
<td>3.162</td>
<td>3.374</td>
<td>3.656</td>
<td>2.261</td>
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<tr>
<td>7</td>
<td>Stearic acid (18:0)</td>
<td>2111</td>
<td>3.519</td>
<td>6.927</td>
<td>3.362</td>
<td>3.943</td>
<td>3.359</td>
</tr>
<tr>
<td>8</td>
<td>Eicosapentaenoic acid (EPA) (20:5, ω-3)</td>
<td>2240</td>
<td>8.842</td>
<td>6.264</td>
<td>12.304</td>
<td>15.448</td>
<td>2.743</td>
</tr>
<tr>
<td>9</td>
<td>Gondoic acid (20:1, ω-9)</td>
<td>2289</td>
<td>7.641</td>
<td>7.641</td>
<td>1.922</td>
<td>2.205</td>
<td>4.054</td>
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<tr>
<td>10</td>
<td>Docosahexaenoic acid (DHA) (22:6, ω-3)</td>
<td>2428</td>
<td>15.243</td>
<td>11.513</td>
<td>8.440</td>
<td>10.717</td>
<td>3.758</td>
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<td>11</td>
<td>Docosapentaenoic acid (22:5, ω-6)</td>
<td>2436</td>
<td>1.030</td>
<td>0.138</td>
<td>1.980</td>
<td>2.439</td>
<td>1.353</td>
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<td>12</td>
<td>11-Docosenoic acid (22:1, ω-11)</td>
<td>2483</td>
<td>10.928</td>
<td>12.263</td>
<td>1.987</td>
<td>1.650</td>
<td>3.952</td>
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</tbody>
</table>

Because minor fatty acids are not included, values do not total to %100.
Table 2. Concentrations of SAFA, MUFA, PUFA, ω-3 and ω-6 fatty acids in oil samples (g/100 g)

<table>
<thead>
<tr>
<th>Sample</th>
<th>ΣSAFAs</th>
<th>ΣMUFAs</th>
<th>ΣPUFAs</th>
<th>ω-3</th>
<th>ω-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anchovy Oil</td>
<td>20.839</td>
<td>26.455</td>
<td>37.943</td>
<td>25.115</td>
<td>1.903</td>
</tr>
<tr>
<td>Horse Mackerel Oil</td>
<td>22.624</td>
<td>35.072</td>
<td>31.220</td>
<td>17.915</td>
<td>1.180</td>
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<tr>
<td>Commercial 1</td>
<td>18.104</td>
<td>23.763</td>
<td>36.382</td>
<td>22.724</td>
<td>11.760</td>
</tr>
<tr>
<td>Commercial 3</td>
<td>11.644</td>
<td>29.492</td>
<td>32.138</td>
<td>7.854</td>
<td>19.608</td>
</tr>
</tbody>
</table>

SAFA: Saturated fatty acid, MUFA: Monounsaturated fatty acid, PUFA: Polyunsaturated fatty acid

Saturated fatty acid content in obtained fish oils (anchovy and horse mackerel) and fish oil products ranged between 20.839 - 22.624% and 11.644 – 21.968%, respectively. Palmitic acid was noted as the predominant SAFA in all samples, and its composition ranged from 6.801% to 12.715%. Generally, the MUFA content (25.750 – 47.335%) was higher than the polyunsaturated fatty acids (19.095 – 34.484%) in all samples analyzed.

EPA and DHA content of the commercial fish oil products ranged between 2.743 – 15.448% and 3.758 – 10.717%, respectively. EPA content was found to be 8.842% in anchovy and 6.264% in horse mackerel oils. DHA contents were found to be 15.243% in anchovy and 11.513% in horse mackerel oils. It was reported in the previous studies that the concentrations of ω-3 fatty acids varied between 26.7 and 43.32% in anchovy oil and 20.43 and 39.70% in horse mackerel oil. Anchovy and horse mackerel oils were also reported to be rich in long-chained double-bonded (ω-3) fatty acids (5, 8-11).

All fish oils contain EPA and DHA; however, the quantities may vary among species and within a species, variations may occur due to the environmental factor. Whether the fish are wild or farm-raised can also be important. Most fish oil supplements contain 18% EPA and 12% DHA, or a total of 30% ω-3 fatty acids (12).

In our study, the fish oil isolated from two different fish species provided interesting data regarding fatty acid composition. Medicinally important fatty acids like PUFA and ω-3 are abundant, especially in the anchovy oil, when compared with the three fish oil products sold on the Turkish market. The present study shows that the anchovy oil can be used in the form of gelatin capsules as dietary supplements, which may have a commercial importance in the Turkish pharmaceutical industry.

References
9. İmre S, Sağlık S. Engraulis encrasicolus (Hamsi) ve...

