THE FATTY ACIDS COMPOSITION OF SELECTED FISH OILS USED AS DIETARY SUPPLEMENTS

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Abstract

The paper presents the qualitative composition of the fatty acids in the selected fish oil available on the Polish pharmaceutical market. Determination of the fatty acids composition was performed by gas chromatography (GC-FID). The results showed that all the tested fish oil composition contained essential fatty acids (EFA). All tested fish oils were characterized by high content of fatty acids from the group of n-3 and n-6. Differences in the composition of fatty acids in the tested fish oil were observed. The Norwegian fish oil was the richest in n-3 acids, i.e. eicosapentaenoic acid (34.43%) compared to the other samples tested: Icelandic fish oil (7.95%); Scandinavian fish oil (7.01%); Norwegian lemon fish oil (8.34%); kaps fish oil (7.95%); Norwegian fish oil forte (10.90%) and Scandinavian multi-tabs fish oil (6.11%). The highest share of acids from the group EFAs ie. linoleic acid n-6 (6.07%) and α-linolenic acid, n-3 (1.02%) was found in Scandinavian multi-tabs fish oil.

Introduction

Until the end of the 19th century, it was thought that the only and the most important role of fats in the body is the supplying of energy. Fats also have many other important functions, i.e. facilitate the perception of taste and food swallowing; inhibit stomach cramps and secretion of acidic gastric juice; they build cell membranes in the white mass of the brain; protect against excessive heat loss (as subcutaneous fat); stabilize the kidneys and other organs inside the body (as organ fat); provide essential fatty acids, from which tissue hormones regulating processes in the cells of
various parts of the body are produced; determine the efficiency of the cardiovascular system by increasing the blood flow through the coronary vessels of the heart; affect the condition of the skin and hair and are a carrier of some vitamins (A, D, E, K), facilitating their absorption from food (MARCIJAN-LUKASIK and KRYGIER 2004).

An important role in the proper development and functioning of the human body act essential fatty acids (EFAs) from the series n-3 and n-6. They should be provided in the diet because they are not produced by the human body. Among them, the following are considered to be basic: α-linolenic acid (C18:3) from the n-3 family, which is a precursor of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and linoleic acid (C18:2) from the n-6 family as a precursor of arachidonic acid (AA) (MATERAC et al., 2013). The rich source of these acids are fish products, seeds and nuts and fats obtained from them.

**n-3 fatty acids**

Polyunsaturated fatty acids (PUFA) from the group of n-3 and n-6 are part of the phospholipids of cell membranes and their quantitative relation depends on the proportion in the diet. These acids are released from the phospholipid material for the synthesis of eicosanoids: prostaglandin (PG), prostacyclin (PGI), tromboksants (TXA), leukotrienes (LT). The health effects of EFAs are due in large part, to the effects of the activity of eicosanoids – tissue hormones. EPA and DHA influence on various metabolic effects in the human body. EPA affects the cardiovascular system by the synthesis of eicosanoids. DHA is an important structural component of the nervous tissue, especially the brain and retina cortices. Also it has an important role during development of the nervous system that occurs in early childhood and foetal life. Too low level of DHA in the diet of women may shorten the period of pregnancy, and may lead to low birth weight of the child. Mother’s milk is the source of EPA and DHA for young children, and their content depends mainly on diet and ranges from 0.05% to 0.7% and even 1.9% at women which consume large amounts of fish. Scientific reports show that low intake of DHA affects the lower intelligence rate. Declining with age D4 desaturase enzyme activity leads to the inhibition of the synthesis of DHA and of disorders of the central nervous system of the elderly. Therefore, an adequate level of intake of n-3 DHA is especially important for the elderly people. In addition, the beneficial effects of DHA is the prevention of stress, depression and aggression (THIES et al. 2001, COREY et al. 2015, SZPONAR et al. 2007).
Significant action in the human body show the long-chain fatty acid forms (LC PUFA). The beneficial effect of n-3 LC PUFA on the functioning of the human body were observed for the first time in the 1970s. Dyerberg et al. (1975) conducted experiments among Greenland Eskimos have observed a very low incidence of cardiac diseases, psoriasis, cancer, allergy and lack of atherosclerosis in this population compared to the population of Denmark. An important fact is that the Eskimo diet is rich in cholesterol due to the high consumption of fish and marine mammals, and includes few fruits and vegetables, which were considered the main dietary risk factors for cardiovascular disease. Analysis of the results confirmed that the health effects are combined with a high level of n-3 LC PUFA in the diet. The fat fish and marine mammals are particularly rich in n-3 LC PUFA. Despite the health benefits of n-3 acids, their excessive consumption may lead to the development of certain health disorders such as: lengthening the bleeding time and the formation of bruising, blood clotting disorders, and the development of type II diabetes and most of all severity of lipid peroxidation within the body, especially the LDL-cholesterol fraction.

Fortification of food with n-3 acids

N-3 LC PUFA are obtained from fishes in the form of oil. The term “fish oil” is quite extensive, and its composition may vary depending on: the species of fish, their age, time and place of fishing and area of life. It is assumed that all marine fish, in particular fatty fish, have a significant content of n-3 LC PUFA. This includes some predatory species of freshwater fish (eg. from the salmonid family). The most important raw materials for the production of fish oil are: cod (langoustine), herring (menhaden), anchovies, mackerel, tuna and salmon. Two types of fish oil are obtained on an industrial scale. The first one are tissue oils obtained by extrusion of overcooked fish raw material, and the second are obtained from fish livers such as fish cod liver oil. They are the main source of vitamin A and D. Fish oil is used for the production of dietary supplements and is a rich source of squalene and alkylglycerols. Formerly, fish oil was considered as a by-product in the production of fishmeal. In industrial conditions, fish oil is subjected to hydrogenation. This process extends durability and changes its state from liquid to solid. Hydrogenation saturates the double bonds and causes the loss of health-promoting properties of LC PUFA. Fish oil used for the production of supplements and food additives shouldn’t be subjected to a hydrogenation process.
International Society for the Study of Fatty Acids and Lipids recommends the consumption of n-3 fatty acids up to 0.65g DHA and EPA acid per day (at least 0.22 g per day). The ratio of n-6 to n-3 in the diet should be 4: 1 (MARCINIAK-ŁUKASIK 2011). Low fish consumption is observed in highly developed countries. For this reason, fish oil rich in long-chain n-3 PUFA is added to food products. Alternative sources are currently being sought of n-3 acids, e.g. from Cryptecodiniumcohnii microalgal cultures, which oil contains 40% DHA (with a small amount of other unsaturated fatty acids). This DHA source is called DHASCO. Food and Drug Administration qualified these products as GRAS (Generally Recognized as Safe) (MATERAC et al. 2013).

Food enriched in n-3-acids are produced in South Korea, Japan, European Union USA, Australia and Canada on industrial scale. In 1995 the first products with the addition of EPA and DHA appeared in Europe. The Spain offered a wide range of such products. Mainly used are preparations of n-3 acids to enrich fats for spreading bread, pastas, breakfast cereals, milk desserts, meats, confectionery, cottage cheese, eggs, milk, yogurt, bread, salad dressings, food concentrates, mayonnaise and instant products. In highly developed countries food with n-3 and n-6 acids appeared for pregnant women in the form of bars and for school-aged children (milk and bars). Research is still ongoing on the use of fish oil preparations for production: milk products, fruit juices, oils, sausages, smoked and seasoned products (MASZEWSKA and GANKO 2010, COLDER 2001).

Inadequate diet and pace of life causes deficiencies of appropriate nutrients in the human body. An alternative to supplement such deficiencies is supplementation, which of course can’t to replace a well-balanced diet. Fish oils contains large amounts of n-3 and n-6 fatty acids as well as fat-soluble vitamins. It has a positive effect on the body, especially on the teeth, eyes, cardiovascular system, immune system and improves concentration. That is why fish oils are recommended by dieticians or doctors in order to supplement everyday menu with valuable active ingredients. Introducing new products rich in n-3 fatty acids may contribute to increasing the share of these acids in the diet (COREY et al. 2015, SZPONAR et al. 2007).

The aim of this study was to evaluate the qualitative composition of fatty acids in selected fish oils available on the Polish pharmaceutical market.
Materials and Methods

Determination of fatty acids

The material for research were 7 selected fish oils available on the Polish market. The characteristics of the trans containing the manufacturer’s code (indicated by symbols A to G), the trade name and declared composition of the most important fatty acids shown in Table 1.

Table 1

<table>
<thead>
<tr>
<th>Producent code</th>
<th>The name of fish oils</th>
<th>Declared composition in one capsule (ca. 0.69 g)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Icelandic fish oil</td>
<td>28</td>
<td>21</td>
</tr>
<tr>
<td>B</td>
<td>Scandinavian fish oil</td>
<td>57.5</td>
<td>46.5</td>
</tr>
<tr>
<td>C</td>
<td>Norwegian lemon fish oil</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td>D</td>
<td>kaps fish oil</td>
<td>110</td>
<td>90</td>
</tr>
<tr>
<td>E</td>
<td>Norwegian fish oil</td>
<td>38.5</td>
<td>31.5</td>
</tr>
<tr>
<td>F</td>
<td>Norwegian fish oil forte</td>
<td>108</td>
<td>146.5</td>
</tr>
<tr>
<td>G</td>
<td>multi-tabs Scandinavian fish oil</td>
<td>70</td>
<td>50</td>
</tr>
</tbody>
</table>

About 100 mg of fat samples were weighed into glass ampules (20 ml capacity). A volume of 0.1 ml of hexane solution of internal standard (heptadecanoic acid – 10 mg ml⁻¹) was added to the extract (KOWALSKI 2007). Fat saponification and fatty acid esterification (with a 14% solution of BF3 in methanol) were performed in accordance to previously described procedures (Animal and vegetable... PN-EN ISO 12966-1:2015-01, Animal and vegetable... PN-EN ISO 12966-2:2011, KOWALSKI 2009).

GC was performed with Varian GC 450 gas chromatograph equipped with a flame-ionization detector FID. The fatty acids methyl esters were separated on 30 m × 0.32 mm × 0.25 μm film of Select™ Biodiesel for FAME. A temperature gradient was applied (200°C for 10 min, then incremented by 3°C/min to 240°C, 240°C for 5 min) The injection port and detector temperatures were 250°C and 300°C; split ratio 1:50; Flow: carrier gas (helium) – 28 ml min⁻¹, detector supply (hydrogen) – 30 ml min⁻¹, detector supply (synthetic air) – 300 ml min⁻¹; flow rates have been adjusted so that the ratio of gas flows (column + carrier gas): (detector supply): (air) was 1:1:10.
Quantitative analysis was performed with the method of internal normalisation, assuming that the sum of surface areas of peaks was 100%.

All tests were performed in triplicate.

Data were analyzed by analysis of variance (Duncan’s test) at 5% significance level using the SAS statistical system (SAS Version 9.1, SAS Inst., Cary, N.C., U.S.A.).

Results and Discussion

Table 2 presents the results of the content of selected fatty acids in the tested fish oils.

The Figure 1 shows the percentage of n-3 and n-6 acids in the tested fish oils.

It has been shown that all of the tested fish oils in the composition contained a significant proportion of EFA with linoleic acid LA and α-linolenic ALA. The highest content of these fatty acids as compared to other characterized Scandinavian fish oil “G”, respectively: LA acid – 6.07% and ALA – 1.02% acid. In contrast, the lowest content of these fatty acids was observed in Norwegian fish oil “F” i.e. LA – 1.28% and ALA – 1.11%. The remaining fish oils were demonstrated the following concentrations of LA and ALA respectively:

- Icelandic fish oil (A) – 2.57% and 1.06%;
- Scandinavian fish oil (B) – 1.6% and 1.03%;
- Norwegian lemon fish oil (C) – 2% and 1.01%;
- kaps fish oil (D) – 3.45% and 1.41%;
- Norwegian fish oil (E) – 3.18% and 1.37%.

GRELA and DUDEK (2007), confirmed that LA was dominant in the group of n-6 fatty acids in the meat of selected marine fish species. BAKES and NICHOLS (1995) showed in fish oil from shark liver oil the presence of LA in the share of 1.4%, while there no ALA acid was found. In fish oil, the percentage of LA was 1.43% and ALA 0.17% (GUILLERRERO and BELARBI 2001). In other studies, no ALA was found and LA concentration was 1.6% (THORSTEINN et al. 2016). For comparison, in vegetable oils, ie., soybean, corn, sunflower, grape seed is from 55.07 to 65.90% linoleic acid (CICHOSZ and CZECZOT 2011). The above results show that oils obtained from fish are not a good source of these acids in comparison to vegetable oils.

The test fish oils are characterized by a high proportion of oleic acid, i.e. from 13.84% for F to “26.67” for A. The fishes are a source of oleic acid, for example 9.11% was found in carp fat and 8.89% in salmon fat, 6.23% in salmon fat, 11.6% in herring oil (ŁUCZYŃSKA et al. 2011). GRELA et al. (2010)
Table 2

Profile of selected fatty acids in the tested fish oils

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Participation [%] (±SD)</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>C14:0 myristic acid</td>
<td>4.98 ± 0.44b</td>
<td>4.61 ± 0.07bc</td>
<td>4.49 ± 0.31bc</td>
<td>5.68 ± 0.64a</td>
<td>4.67 ± 0.18bc</td>
<td>3.52 ± 0.27d</td>
<td>4.20 ± 0.42c</td>
<td></td>
</tr>
<tr>
<td>C16:0 palmitic acid</td>
<td>12.99 ± 1.15b</td>
<td>11.72 ± 0.13cd</td>
<td>10.95 ± 0.59d</td>
<td>15.39 ± 1.18a</td>
<td>13.92 ± 0.38b</td>
<td>8.32 ± 0.31e</td>
<td>12.91 ± 0.18bc</td>
<td></td>
</tr>
<tr>
<td>C16:1 palmitoleic acid</td>
<td>9.72 ± 0.76b</td>
<td>8.63 ± 0.08c</td>
<td>10.70 ± 0.65a</td>
<td>8.59 ± 0.72c</td>
<td>7.56 ± 0.19d</td>
<td>4.63 ± 0.43e</td>
<td>8.52 ± 0.47c</td>
<td></td>
</tr>
<tr>
<td>C18:0 stearic acid</td>
<td>2.74 ± 1.39cd</td>
<td>1.75 ± 0.11de</td>
<td>1.65 ± 0.09e</td>
<td>2.28 ± 0.06cde</td>
<td>3.00 ± 0.19bc</td>
<td>3.79±0.14ab</td>
<td>4.57 ± 0.50a</td>
<td></td>
</tr>
<tr>
<td>C18:1 n9c oleic acid</td>
<td>26.67 ± 1.71a</td>
<td>25.35 ± 0.24ab</td>
<td>25.72 ± 1.22ab</td>
<td>21.94 ± 1.55c</td>
<td>24.59 ± 0.56b</td>
<td>13.84 ± 0.07d</td>
<td>25.54 ± 0.40ab</td>
<td></td>
</tr>
<tr>
<td>C18:2 n6c linoleic acid</td>
<td>2.57 ± 1.63bcd</td>
<td>1.60 ± 0.06d</td>
<td>2.00 ± 0.10cd</td>
<td>3.45 ± 0.24b</td>
<td>3.18 ± 0.12bc</td>
<td>1.28 ± 0.09d</td>
<td>6.07 ± 0.91a</td>
<td></td>
</tr>
<tr>
<td>C18:3 n3 (alpha) alpha-linoleic acid</td>
<td>1.06±0.07b</td>
<td>1.03±0.01b</td>
<td>1.01±0.05b</td>
<td>1.41±0.09a</td>
<td>1.37±0.06a</td>
<td>1.11±0.08b</td>
<td>1.02±0.07b</td>
<td></td>
</tr>
<tr>
<td>C18:3 n6 (gamma) gamma-linolenic acid</td>
<td>2.38±0.15bc</td>
<td>1.99±0.05d</td>
<td>2.51±0.16b</td>
<td>2.41±0.14bc</td>
<td>2.61±0.09b</td>
<td>2.09±0.28cd</td>
<td>3.55±0.35a</td>
<td></td>
</tr>
<tr>
<td>C20:1 eicosenoic acid</td>
<td>14.79 ± 0.57a</td>
<td>13.92 ± 0.25b</td>
<td>14.38 ± 0.51ab</td>
<td>11.36 ± 0.70c</td>
<td>8.98 ± 0.20d</td>
<td>2.83 ± 0.02e</td>
<td>14.42 ± 0.45ab</td>
<td></td>
</tr>
<tr>
<td>C20:5 eicosapentaenoic acid (EPA)</td>
<td>7.95 ± 0.47c</td>
<td>7.01 ± 0.27d</td>
<td>8.34 ± 0.56c</td>
<td>7.95 ± 0.35c</td>
<td>10.90 ± 0.34b</td>
<td>34.43 ± 0.29a</td>
<td>6.11 ± 0.11e</td>
<td></td>
</tr>
<tr>
<td>C22:1 n9 erucic acid</td>
<td>0.97 ± 0.02ab</td>
<td>1.06 ± 0.02a</td>
<td>0.84 ± 0.04c</td>
<td>1.04 ± 0.13a</td>
<td>0.07 ± 0.02e</td>
<td>0.33±0.02d</td>
<td>0.92±0.05bc</td>
<td></td>
</tr>
<tr>
<td>C22:2 cis-13,16-docosadienoic acid</td>
<td>0.26±0.18a</td>
<td>0.30±0.08a</td>
<td>0.35±0.03a</td>
<td>0.27±0.10a</td>
<td>0.07±0.05b</td>
<td>0.23±0.12ab</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>C22:6 docosahexaenoic acid (DHA)</td>
<td>8.32 ± 0.71c</td>
<td>8.83 ± 0.61c</td>
<td>6.88 ± 0.73d</td>
<td>7.99 ± 0.42c</td>
<td>11.88 ± 0.41b</td>
<td>17.90 ± 0.20a</td>
<td>8.05 ± 0.87c</td>
<td></td>
</tr>
</tbody>
</table>

* The designations are given in accordance with Table 1
a, b, c, d ... – values designated with the same letters do not significantly differ at 5% error (Duncan’s test)
confirmed that the lipids of carp and bream were dominated by oleic acid 32.63% and 20.9% respectively, while in pike fat 17.4%. For shark liver oil, BAKES and NICHOLS (1995) have demonstrated its presence in the amount of 48.8%, whereas in fish oil, GUIL-GUERRERO and BELARBI (2001) showed in the composition of 14.5% oleic acid content. This result are similar to the obtained value for F. Higher content of oleic acid in fish oil was given in the work of THORSTEINN et al. (2016) at the level of 16.3%. Oleic acid is a fatty acid common in our diet and no deficiency is observed. A good source of oleic acid are vegetable oils such as olive oil (68.76%), rapeseed oil (57.14%) and animal fat – lard (43.20%).

Figure 2 shows percentage of n-3 and n-6 acids in the tested fish oils in relation to the composition declared by the manufacturers. The com-
sition of the tested fish oils and the content of n-3 acids (DHA and EPA) declared by the producers was mostly consistent with the results of the analysis (Figure 2). Fish oil A according to the quantitative declaration for DHA and EPA acid should contain respectively 8.32% and 7.95%. In the present work were obtained for slightly higher levels of DHA – 10.18% and a similar concentration of EPA – 7.64%. In this study was obtained similar levels to the declared by the manufacturers contents of DHA and EPA acid for fish oils B, C, D and G. However, in fish oils E and F, lower levels than those reported by the manufacturers were found. According to the manufacturer fish oils E and F should contain respectively 24% and 45%. The obtained results of the analysis are at the level of: 10.9% E and 34.43% F. The variable acid content may be due to the raw material from which the fish oils were obtained. According to KRIS-ETHERTON et. al. (2003) fat content in tissues of various fish species depends not only on the sex, but also on the season and age of the individual.

Fish oil is the main source of long-chain n-3 PUFAs in the diet. It was found that the PUFAs: EPA and DHA acid have the better beneficial effect on the health due to the prevention of cardiovascular disease than a short-ALA acid (RUXTON et al. 2003, STEPHENSEN 2004). KOLANOWSKI (2007) and KOŁODZIEJCZYK (2007) report that fish, including fish oils, are the best source of polyunsaturated fatty acids n-3. The studies showed a high content of EPA acid. The highest content of EPA acid in respect of the others was found in the Norwegian fish oil forte F – 34.43%. In other fish oils observed comparable amount of the acid in the range of 7.01% to 10.90%. CICHOSZ and CZECZOT (2011), indicated the presence of EPA and DHA acids only in fish oil. LUCZYŃSKA et al. (2011) showed the highest content of DHA acid in trout muscles (14.5%), in salmon muscle tissues and carp, this acid was present in the amounts respectively of 9.93 and 3.39%. According to HALIŁOĞLU et al. (2002) trout are the best sources of DHA (19.17%) and EPA acid (3.07%) compared to other fish species. According to GRELA and DUDEK (2007), the group of n-3 in the meat of marine and freshwater fish are dominated by DHA and EPA acids. GRELA et al. (2010) showed the presence of DHA and EPA acids in poultry meat respectively (16.01%) and (13.64%) and in pike meat (15.73%) and (13.64%). BAKES and NICHOLS (1995) for fish oil, showed the presence of DHA and EPA acids respectively in 7% and 0.7%. GUIL-GUERRERO and BELARBI (2001) in fish oil showed that the amount of DHA acid in the study was 10.7% and EPA acid 8.89%. These values are similar to those obtained during the above analysis. THORSTEINN et al. (2016) observed a slightly higher content of these acids, in the amount of 9.6% – for EPA acid and 12.5% for DHA acid.
The analysis of the average diet of Europeans and Americans, too low intake of fatty acids from the n-3 family is observed, mainly DHA and EPA acids (Maszewska and Gańko 2010). In many developed countries, the consumption of these acids is on average 0.15 g per day and is below the recommended level (Thautwein 2001, Kolanowski et al. 2004). The use of a daily diet of foods enriched with fish fats or using supplementation prevents the deficiency of nutrient DHA and EPA in the human body. The source of ALA from the n-3 group are leafy vegetables and oils, i.e. linseed, rapeseed and soybean, which are commonly found in our menu. However, the source of EPA and DHA acids are greasy sea fish, which can be replaced with encapsulated fish oils.

The obtained results confirm that acids in the n-3 family dominate in the fish oils. This is particularly evident for example, Norwegian fish oil forte “F”, which contained fatty acids from the group n-3 in the amount of 53.44% (Figure 1). According to the FAO/WHO, the recommended dose of polyene acids (PUFA) in a healthy diet in daily nutrition is (5–10): 1 (n-6: n-3) (Marciniak-Łukasik 2011). Although there is no established reference relationship, it is recommended to aim for the maximum consumption of omega-3s in relation to omega-6 (Kościel et al. 2017). Appropriate amount of DHA acid in the diet can be provided by eating fatty fish such as herring, sprat and salmon 1-2 times a week. Recent research results show that early fish consumption promotes the development of immune tolerance and reduces the risk of allergies (Szajewska et al. 2014).

The fatty acid composition of all the analyzed fish oils were similar. However, individual deviations from the declared composition were reported, where the percentage of EPA and DHA acids differed from the others.

**Summary and Conclusions**

It was observed that manufacturers declare higher levels of fatty acid content compared to the results obtained during the analysis. The composition of the selected fish oil declared by the producers didn’t differ slightly in the results obtained. The studies showed that all the fish oil are characterized a high content of acids from the group of n-3 and n-6. Fish oil had a higher content of n-3 acids than n-6. The average value was higher 4 times compared to the other tested fish oils. The content of other acid were at a similar level.
References


