EPA and DHA content in fish oil supplements on the Italian market: A preliminary study

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INTRODUCTION

While a good fish consumption is generally accepted to have beneficial effects on health mainly due to Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) omega-3 supplements are not universally recognized to exert the same positive action. Some authors suggest that the easy oxidation such supplements go through invalidates the goodness of the experiments giving conflicting results.

In any case, the market of supplements has grown a lot in recent years with a turnover of billions of euros. Italy ranks first in Europe in the consumption of food supplements with a 23% share of the European market. Analytical controls on omega-3 supplements are rarely carried out but they are needed since from the few available papers some frauds were detected with the addition of large amounts of soybean oil, for example [1].

The present work investigated the fatty acid (FA) composition of omega-3 supplements available on the Italian market. Measured FA concentrations were compared with the labels to assess the product compliance. Method reliability has been particularly taken care of, with the total amount of fatty acids determined experimentally by saponification, according to the Kinsella method [2]. Gas Chromatography - Mass Spectrometry was subsequently used by performing a peak-by-peak identification and integration until the signal to noise ratio of 3 in order to assign each fatty acid to its exact quantity. About 50 different fatty acids were detected in each sample.

MATERIALS AND METHODS

Three Omega3 supplements of the most popular brands were purchased from retailers. Four capsules for each brand were opened and approximately 10 mg of the pooled oil were methyl derivatized. Derivatization was carried out with BF_3 methanol solution 14% and methanol (1:1). Individual analytical standards of the main important 27 FAs were purchased as methyl esters either from Merck KGaA® or from Larodan® (Solna, Sweden). After the purchase, the pure FA standards were dissolved in n-hexane and stored at a temperature of -30°C by using the Certan® capillary bottles, i.e. vials specially designed for optimum storage available from Merck KGaA®. Instrument used was a Varian 3900 gas chromatograph connected to the Mass Spectrometer Saturn 2100T (GC-MS) equipped with an Ion Trap analyzer. Injections were made in split mode (15:1) with an injection volume of 1 µL. Capillary column installed was a CP-WAX 52 CB (60 m × 0.32 mm I.D., 0.50 µm film thickness) from Chrompack®, the Netherlands. Mass spectra were obtained in EI (Electron Ionization) mode at 70 eV. Ion trap temperature was 180°C. The selected Mass to Charge Ratio to acquire was in the 40-440 m/z range. Analyses were carried out in full scan mode. Fatty acids detected in the samples were identified by multiple confirmation criteria, mainly the coincidence of mass spectrum and the retention time with the mass spectrum and the retention time of the pure analytical standard injected in the same conditions. Checking with the mass spectrum NIST® database completed the validation. The limit of detection was 0.005 mg FA / 100 mg oil. An aliquot of oil from each brand was used to derive the total quantity of fatty acids per 100 mg of oil. To such aim it was used the Kinsella method. Briefly said about 100 mg of oil were subjected to saponification by means of 10% alcoholic KOH, the nonsaponifiable material was extracted with hexane and the residual soaps were acidified to pH 1.5. The free fatty acids were extracted with hexane, dried in a tared vial and the weight of fatty acids was determined. Total FAs for each one of the three supplements are shown in Table 1. As reported by Kinsella: "*These data are used to calculate the weights of individual fatty acids separated by gas chromatography*".

RESULTS

Table1. Fatty acid composition of the three omega-3 supplements analyzed (mg/100 mg oil)

Fatty acids	Supplement n.1	Supplement n.2	Supplement n.3
12:0 (Lauric)	<LOD	<LOD	0.14
13:0	<LOD	<LOD	0.03
14:0 (Myristic)	0.05	0.13	6.29
14:1 ω-5 (Myristoleic)	<LOD	<LOD	0.05
15:0	0.01	0.06	0.54
16:0 (Palmitic)	0.16	1.32	13.80
16:1 ω-7 (Palmitoleic)	0.11	0.44	7.71
16:2 ω-4 (Palmitolinoleic)	<LOD	0.08	1.21
17:0 (Margaric)	0.06	0.10	0.35
18:0 (Stearic)	2.94	4.27	2.77
18:1 ω-9 (Oleic)	2.87	6.17	9.55
18:1 ω-7 (Vaccenic)	1.29	2.48	2.72
18:2 ω-6 (Linoleic)	0.52	1.33	0.99
19:0	0.39	0.52	0.37
18:3 ω-3 (Linolenic)	0.34	0.70	0.72
18:4 ω-3 (Stearidonic)	0.47	1.54	2.75
20:0 (Arachidic)	0.93	1.02	0.38
20:1 ω-11 (Gadoleic)	0.73	0.26	<LOD
20:1 ω-9 (Gondoic)	3.58	1.61	0.93
20:2 ω-6	0.39	0.13	<LOD
20:4 ω-6 (Arachidonic)	2.56	1.59	0.78
20:4 ω-3 (ω3-Arachidonic)	1.58	1.32	0.69
20:5 ω-3 (EPA)	32.41	25.76	16.68
22:1 ω-11 (Cetoleic)	2.60	1.42	<LOD
22:1 ω-9 (Erucic)	0.18	0.44	<LOD
22:5 ω-3 (DPA)	2.71	2.91	1.37
22:6 ω-3 (DHA)	19.68	20.06	12.89
Others	13.39	12.32	8.26
Sum (total FAs)	90	88	92

LOD: Limit Of Detection

Table 1 shows the fatty acid composition of the three supplements analyzed. In the supplement n.1 they were detected a total of 46 FAs while in the supplements n.2 and n.3 they were detected 55 and 47 FAs, respectively. 27 fatty acids represented the majority of the total being 85% in the supplement n.1, 86% in the supplement n.2 and 91% in the supplement n.3. These 27 FAs, from Lauric to DHA, are listed in Table 1. EPA and DHA compliance with the labels was as follows (mg/100 mg oil):

Supplement n.1 - EPA declared 40, EPA measured 32.41 (81%); DHA declared 20, DHA measured 19.68 (98%).

Supplement n.2 - EPA declared 33, EPA measured 25.76 (78%); DHA declared 22, DHA measured 20.06 (91%).

Supplement n.3 - EPA declared 15, EPA measured 16.68 (111%); DHA declared 10, DHA measured 12.89 (129%).

It may be noted that in supplements n.1 and 2 EPA is about 80% of what was declared on the label. Also Chee et al. [3] in their study of marine oil capsules observed about 80% of labeled content as regards EPA. The other measured values (DHA in all supplements, EPA in supplement n.3) are essentially in agreement with the label being the supplement 3 slightly in excess of what declared but this does not necessarily mean a higher quality, as exposed below.

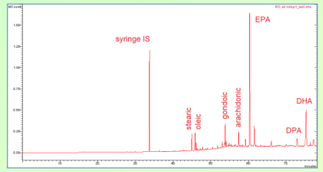
By considering the 27 main fatty acids, the ratio ω-6/ω-3 is equal to 0.06, 0.06 and 0.05 for supplement n.1, n.2 and n.3 respectively. These values are well in compliance with the current guidelines which recommend not to exceed the value of 1 in the diet for the ratio ω-6/ω-3. Such values were to be expected for products derived from fish oil which are in fact used also to rebalance the diet.

As regards the distribution between saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) a noticeable difference can be observed between the supplement n.3 and the other two. The series SFA-MUFA-PUFA as mg/100 mg oil is equal to 5-11-61 for supplement n.1, 7-13-55 for supplement n.2 and 25-21-38 for supplement n.3. This may indicate that an effective purification step has probably been carried out for supplements n.1 and n.2 in order to eliminate the less valuable saturated fatty acids, in fact the above values, especially for SFA, are not typical of fish oil, while the values for supplement n.3 are.

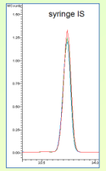
In this regard we can consider, for example, the value of 16:0 (Palmitic) that is generally one of the most concentrated fatty acids in fish oils. In supplements n.1 and 2 16:0 is instead one of the less concentrated FAs (0.16 and 1.32 mg/100 mg oil) while in the supplement n.3 it is one of the most concentrated (13.80 mg/100 mg oil). A similar situation can be observed for 14:0. Furthermore the two saturated FAs 12:0 and 13:0 are completely absent in supplement 1 and 2. On the contrary they are present in supplement 3 even if in low quantity (the same low quantity generally observed in fish oils). We must emphasize that the production of good omega-3 supplements involves accurate purification steps by means of which free fatty acids, heavy metals, colored compounds and other impurities are removed from raw fish oil, making the oil much purer. Generally the Short Path Distillation technique is used. During this process there is also the concentration of fish oils which leads to a higher total omega-3 content and a higher concentration of EPA and DHA. A fractionation step is used to remove saturated fatty acids. Supplement n.3 appears to have undergone incomplete purification process. in addition to the significant presence of saturated fatty acids, supplement no. 3 has in fact a total omega-3 content of only 35 mg /100 mg oil compared to 57 and 52 mg/100 mg oil of supplements 1 and 2 respectively.

For EPA and DHA there is a noticeable difference between the supplement n.3 and the other two. The EPA content in n.3 is half of n.1 and two thirds of n.2, while the DHA content in n.3 is two thirds of n.1 and n.2. This forces the consumer to take more oil from supplement n.3 to obtain the same quantity of bioactive molecules. In terms of percentages the sum EPA+DHA represents 58 and 51% in supplements n.1 and 2, while in supplement n.3 is 33%.

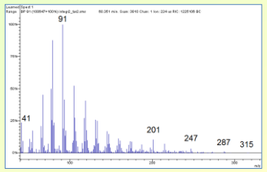
CONCLUSIONS. Apart from a slight difference in the measured and declared content of EPA, supplements n.1 and 2 have a higher quality than the supplement n.3, the cheapest. This last in fact has a high content of saturated fatty acids, a lower content of total omega-3 and a lower content of EPA + DHA. The lower content of EPA + DHA per 100 mg of oil (with respect the supplements n.1 and 2) forces the consumer to take more oil through the supplement n.3 with possible side effects. This indicates that the compliance with the EPA and DHA labeled contents is not the only quality parameter to consider for fish oil supplements.



GC-MS chromatogram of the omega-3 supplement n.1. The "syringe IS" was added in the final vial prior to injecting in order to monitor possible variations in the retention time and in the injection volume



GC-MS chromatograms of the three supplements. Overlap. Peak of the "syringe IS". Note the good injection repeatibility both for the retention time and for the injected volume. IS used was 15:1 ω-5



Mass spectrum of EPA. Supplement n.2

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