Research Article

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Quality analysis of commercial fish oil preparations

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Abstract

BACKGROUND: Fish oil supplements have grown in popularity in recent years owing to their multiple health benefits, leading to rapid growth in the number of fish oil supplements available for consumers. When choosing a product, it is important that label claims for eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are met, especially when a specific dosage is required. In this study the amounts of EPA and DHA in 16 of the top-selling liquid fish oil products from the American marketplace were analysed and compared with their label claims. Peroxide value, a measure of oxidation, was also determined, along with lipid class.

RESULTS: This study found that over half of the supplements did not meet their label claims for EPA and DHA, and a quarter exceeded recommended limits for peroxide value.

CONCLUSION: These results suggest that more stringent regulation is required for fish oil products.

Keywords: fish oil; EPA; DHA; peroxide value; dietary supplement

INTRODUCTION

The long-chain omega-3 fatty acids found in fish have been shown to be beneficial for human health. Numerous clinical studies have shown that these fatty acids, specifically eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are especially beneficial for the cardiovascular system (see e.g. Refs 1–3). EPA and DHA can be obtained by consuming fatty fish; however, most North American consumers do not ingest the two servings of fatty fish per week as recommended by the United States Department of Agriculture (http://www.choosemyplate.gov) and Health Canada (http://www.hc-sc.gc.ca/fn-an/food-guide-aliment/index-eng.php). Because of this, there has been a dramatic increase in the consumption of fish oil by the general public in recent years as an alternative to eating fish. This has resulted in a rapid increase in the number of fish oil supplements on the market, making it difficult for consumers to differentiate between products. Fish oil supplements are typically composed of either cod liver oil (CLO) or a blend of anchovy, sardine and mackerel oils referred to as fish body oil (FBO). Although there are benefits to the consumption of both types of oil, FBO tends to have higher levels of EPA and DHA, while CLO usually contains vitamins A and D but lower levels of the omega-3 fatty acids.

Regardless of the species of fish used in a supplement, it is important that the dosage of EPA and DHA in the product is accurate, especially if it is being used to treat a specific condition. For example, a daily dose of 1000 mg of EPA and DHA is recommended by the American Heart Association (http://www.heart.org/HEARTORG/) for people with coronary heart disease. Oxidative degradation is also a concern in fish oil supplements, because the large number of double bonds in EPA and DHA makes these products prone to rapid oxidation. Hydroperoxides are primary oxidation products and their concentration is the most commonly used indicator of oxidative quality. The levels of these compounds are assessed by measuring peroxide value (PV). The Global Organization for EPA and DHA (GOED) has a Voluntary Monograph that many reputable FBO supplement producers adhere to, which stipulates that the maximum PV of a fish oil product should be <5 meq kg^{-1}.[4] Although this monograph is not applicable to CLO, the European Pharmacopeia Monograph for farmed CLO also specifies a PV maximum of <5 meq kg^{-1} for that oil.[5] Fish oils are available in two forms, triacylglycerol (TG) and ethyl ester (EE), and the structure of the fatty acids can also affect product quality, with EE products generally considered to be inferior to TG ones. Numerous studies have demonstrated that EPA and DHA have better bioavailability in the TG form than as EE.[6–9] TG is also more resistant to oxidation than EE,[10] potentially resulting in products with better sensory properties. Often the form of the product is not stated on the label.

The two most popular dosage forms for fish oils are capsules and liquids, although chews and emulsions are also available. Numerous studies have been conducted to assess oxidative stability and EPA and DHA levels in encapsulated products (see e.g. Refs 11–13), but no studies on liquid fish oil products were found. The purpose of this work was to assess the quality of the 16 most popular fish oil supplements in the American market and to determine if they meet their label claims for EPA and DHA. The

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amounts of EPA and DHA in these products were measured and compared with their label claims. PV was measured as an indicator of oxidation. The lipid class of each sample, either TG or EE, was also determined.

EXPERIMENTAL

Materials

Methyl tricosanoate (C23), methyl eicosapentaenoate, methyl docosahexaenoate and 1,2-dipalmitin were purchased from Nu-Chek Prep (Elysian, MN, USA). Tripalmitin was obtained from Sigma Aldrich (Oakville, ON, Canada). Pure EE was purchased from Ocean Nutrition Canada Ltd (Dartmouth, NS, Canada). Optima chloroform was obtained from VWR (Mississauga, ON, Canada). Thin layer chromatography (TLC) plates (Absorbil Plus 1 Prekotes-soft layer 20 × 20 preadsorbent/prechannel) were purchased from Mandel Scientific Company (Guelph, ON, Canada). All other chemicals and glassware were obtained from Fisher Scientific (Ottawa, ON, Canada).

Sixteen liquid omega-3 dietary fish oil supplements from nine manufacturers, representing the top-selling brands in the US market, were selected using market sales data for 2010 provided by SPINS (Schaumburg, IL, USA), a natural health product market research company (Table 1). Of the samples analysed, eight were FBO and eight were CLO. All samples had a serving size of 5 mL. The eight FBO products included six different manufacturers, four of which are members of GOED (samples 1, 2, 4, 5 and 8), meaning that they comply with the GOED Voluntary Monograph. The CLO included seven different brands. Three bottles of each sample were purchased from supermarkets and pharmacies. All samples were within their stated shelf lives.

Fatty acid analysis

Fatty acid analysis was performed by the Canadian Institute of Fisheries Technology (CIFT, Halifax, NS, Canada). All fish oil samples were analysed for EPA and DHA contents and compared with their label claims for these fatty acids. Fatty acids were converted to methyl esters following the modified GOED Voluntary Monograph[4] and analysed using a gas chromatograph with a flame ionisation detector (GC-FID). C23 was used as an internal standard. An external standard C23 and methyl esters of EPA and DHA were also used, allowing EPA and DHA contents to be calculated in mg g^{-1} TG. Methyl esters were separated using a column coated with 50:50 (w/w) cyanopropyl-methylpolysiloxane (30 m × 0.25 mm, 0.25 µm film thickness). Helium was used as carrier gas at a flow rate of 1 mL min^{-1}. The oven temperature was initially held for 2 min at 153 °C, then increased at 2.3 °C min^{-1} to 205 °C and held for 8.3 min. The total run time was approximately 32 min. The FID was maintained at 270 °C and the injector (split mode 1:100, 4 mm liner) at 250 °C. To determine if products met their label claims for EPA and DHA, the density of samples was measured and used to calculate the mass of each serving.

Peroxide value

PV analysis was performed by CIFT. PV was measured as an indicator of oxidative stability following AOCS method Cd 8–53.[14] The maximum acceptable PV according to the GOED Voluntary Monograph[4] and the European Pharmacopeia Monograph for farmed CLO[5] is 5 meq kg^{-1}. Because it was not specified whether the CLO samples tested were from wild or farmed sources, for the purposes of this study, PV ≥ 5 meq kg^{-1} was considered to be unacceptable for all samples.

Thin layer chromatography

TLC was performed to determine whether the fish oil samples were in the form of TG or EE. In this procedure, samples were dissolved in hexane to a final concentration of 0.3 mg mL^{-1}, then 20 µL aliquots of these solutions were applied to TLC plates. TG and EE standards were prepared in the same way and also applied to TLC plates. All plates were developed in a solvent system consisting of 85:15:1 (v/v/v) hexane/ethyl ether/acetic acid, then stained with 2,7-dichlorofluorescein solution (2 g L^{-1} in ethanol) and viewed under UV light.

Table 1. Oil type and label claims for EPA and DHA for each sample tested

Sample	Oil type	Serving (g)	EPA label claim (mg per serving)	DHA label claim (mg per serving)	EPA + DHA label claim (mg per serving)
1[a]	Anchovies, sardines and/or mackerel	4.65	750	500	1250
2[a]	Anchovies, sardines and/or mackerel	4.65	750	500	1250
3	Anchovies, sardines and/or mackerel	4.50	1500	750	2250
4	Anchovies, sardines and/or mackerel	4.60	800	500	1300
5	Anchovies, sardines and mackerel	4.60	850	550	1400
6	Anchovies, sardines and/or mackerel	4.55	100	20	120
7	Sardines and anchovies	4.65	740	475	1215
8	Anchovies and sardines	4.62	825	550	1375
9[a]	Cod liver oil	4.50	540	360	900
10	Cod liver oil	4.60	365	550	865
11	Cod liver oil	4.60	320–510	460–640	830–1100
12	Cod liver oil	4.65	554	369	923
13	Cod liver oil	4.60	554	369	923
14	Cod liver oil	4.60	400	500	900
15	Cod liver oil	4.60	400	550	950
16	Cod liver oil	4.60	453	320	773

[a] Product weight given on label. All other masses were measured.

Table 2. Label claims and measured (actual) values for EPA and DHA (mg per serving, mean ± standard deviation, $n = 3$) for each fish oil sample

Sample	Actual EPA	EPA claim − actual	Actual DHA	DHA claim − actual	Actual EPA + DHA	EPA + DHA claim − actual
1	794 ± 18	44	521 ± 12	21	1315 ± 28	65
2	844 ± 36	94	478 ± 41	−22	1322 ± 8	72
3	1545 ± 10	45	892 ± 2	142	2437 ± 12	187
4	770 ± 5	−30	488 ± 3	−12	1258 ± 8	−42
5	781 ± 4	−69	526 ± 3	−24	1308 ± 7	−92
6	27 ± 2	−73	99 ± 3	79	126 ± 5	6
7	732 ± 2	−8	477 ± 2	2	1209 ± 4	−6
8	803 ± 2	−22	488 ± 1	−62	1291 ± 3	−84
9	345 ± 3	−195	456 ± 2	96	800 ± 4	−100
10	481 ± 2	28	417 ± 2	97	897 ± 3	124
11	321 ± 6	0 to −189	448 ± 8	−14 to −193	769 ± 13	−14 to −381
12	541 ± 5	−13	427 ± 3	58	968 ± 8	45
13	488 ± 2	−66	463 ± 2	94	950 ± 3	27
14	378 ± 2	−22	516 ± 1	16	894 ± 1	−6
15	357 ± 7	−43	472 ± 15	−78	829 ± 21	−121
16	347 ± 5	−18	457 ± 4	−93	804 ± 8	−111

RESULTS

Only nine of the 16 products tested met their stated label claims for total EPA + DHA; four were FBO and five were CLO (Table 2). The label claims for FBO were generally higher than those for CLO, with the exception of sample 6, which had very low levels of EPA and DHA. The serving size for all products was 1 teaspoon (5 mL); however, the mass of a single serving varied among products from 4.50 to 4.65 g, likely owing to differences in the amounts and types of flavour used. It is worthwhile noting that samples 6, 12 and 13 met their claims for EPA + DHA but were below label claims for EPA alone. For example, sample 6 met its label claim of 120 mg per serving for total EPA + DHA but was 73% below its EPA claim, while its DHA content was almost four times the amount stated on the label. These results suggest that manufacturers may be more concerned with meeting label claims for total EPA + DHA rather than for the individual fatty acids. Samples 4, 5 and 8 were manufactured by GOED members and failed to meet label claims for total EPA + DHA. In contrast to the results for EPA and DHA, of the 16 products tested, only five exceeded the PV maximum of 5 meq kg^{-1} specified by GOED and European Pharmacopoeia (Table 3). Sample 6 had a high standard deviation for PV, which can be attributed to one of the three bottles tested having a PV of 26.8 meq kg^{-1} while the others had values of 8.7 and 8.9 meq kg^{-1}. Retesting the bottle with the high PV resulted in the same value. Sample 5 was the only product manufactured by a GOED member that failed the PV test. Only one product was found to be composed entirely of EE, though one (sample 6) contained both EE and TG (Table 3). The remaining 14 products were TG.

Table 3. Peroxide value (mean ± standard deviation, $n = 3$) and lipid class for each fish oil sample

Sample	Peroxide value (meq kg^{-1})	Triacylglycerol (TG) or ethyl ester (EE)
1	2.7 ± 0.8	TG
2	1.6 ± 0.4	TG
3	11.2 ± 0.2	EE
4	1.9 ± 0.1	TG
5	8.4 ± 0.4	TG
6	14.8 ± 10.4	TG and EE
7	1.4 ± 0.1	TG
8	2.1 ± 0.1	TG
9	4.2 ± 0.1	TG
10	1.0 ± 0.7	TG
11	2.1 ± 0.1	TG
12	3.5 ± 0.1	TG
13	5.1 ± 0.2	TG
14	1.4 ± 0.0	TG
15	7.8 ± 3.3	TG
16	4.0 ± 0.1	TG

DISCUSSION

In North America, consumers have a large variety of omega-3 fatty acid supplements to choose from; however, the results of this study show that there are often differences between the claimed and measured EPA and DHA levels. The findings of this study are similar to those of other researchers measuring encapsulated omega-3 supplement content in various other countries. Opperman *et al.*[15] tested 45 commercially available omega-3 supplements sold in South Africa and found that 56% contained <90% of the EPA and DHA amounts stated on the label. The authors attributed these results to the fact that there is no formal regulatory structure for dietary supplements in South Africa, resulting in the dietary supplement industry being self-regulating. Fierens and Corthout[12] examined 16 commercially available fish oil products in Europe and obtained similar results to this study, with seven products failing to meet label claims for EPA and/or DHA. Additionally, the authors found that five products had PV > 5 meq kg^{-1}. Fantoni *et al.*[11] examined 16 different encapsulated CLO products in Brazil. Of these samples, only five stated EPA and DHA contents on the label, and two were found to be below the stated amount. When PV was examined, six samples had values exceeding 5 meq kg^{-1}. Kolanowski[16] analysed 19 different encapsulated fish oil products for sale in Poland to determine whether they met their label claims and found only one sample that did not meet its label claim. When the oxidative stability of these products was measured, the author found that only two products had PV > 5 meq kg^{-1} at the initial

testing point. Tatarczyk *et al*.[17] analysed the levels of EPA and DHA in nine commercially available fish oil supplements in Austria. They found that all products met their label claims, with four products having significantly higher levels of EPA and DHA than stated on the label. The study did not assess oxidation markers. These studies all suggest that adherence to EPA and DHA label claims varies from country to country. With most countries having no governing body for natural health products, some manufacturers of fish oil supplements may not see the need to test each batch of product for active ingredients. This could lead to supplements with lower levels of EPA and DHA and high levels of oxidation products being sold to unsuspecting customers.

There are a number of reasons that might explain the failure of products to meet label claims. For instance, the fatty acid profiles of fish tissues are known to vary with season,[18–20] so those of FBO and CLO can also be expected to exhibit some seasonal differences in EPA and DHA levels. When FBO supplements are produced, the levels of these fatty acids can be manipulated by blending oils containing higher levels of EPA and DHA, referred to as concentrates, with unconcentrated, or natural, fish oil to obtain desired levels of EPA and DHA. The availability of fish oil concentrates should ensure that all manufacturers are able to meet their label claims for FBO products; however, concentrates are more expensive than natural fish oils, which may make their use unattractive to manufacturers. CLO concentrates are not readily available, meaning that manufacturers of these supplements are more susceptible to natural variations in fatty acid profile. This would explain why five CLO samples failed to meet their total EPA + DHA label claims, and only one product met label claims for EPA and DHA individually (sample 10).

The way in which theoretical EPA and DHA values were calculated by the manufacturer could also result in a failure to meet label claims. If amounts were calculated assuming that one 5 mL serving of product is equivalent to 5 g of fish oil, then EPA and DHA values will be inflated, as the density of oil is less than 1 g mL^{-1}. In this study the actual mass of 5 mL of product varied from 4.50 to 4.65 g. Additionally, the majority of these products contain a certain amount of flavouring and antioxidants whose mass must be taken into account by the manufacturer when calculating the theoretical EPA and DHA levels of a product. When the EPA and DHA levels of the samples that did not meet label claims were recalculated using the measured EPA and DHA values and a serving size of 5 g rather than the measured mass of 5 mL, the four failing FBO samples then exceeded their claims. Unfortunately, these results are not representative of what is actually in the product. When the same procedures were followed for the CLO samples, one sample (sample 11) met its label claim but the other three still failed.

Of the five samples that exceed a PV of 5 meq kg^{-1}, three were FBO and two were CLO. Elevated PV can be caused by a number of factors. For example, if air was introduced at some point during manufacturing or if the manufacturer failed to purge oxygen from the headspace of the finished product, the product would likely oxidise over time. Packaging defects such as a poorly fitting cap could result in elevated PV as well. This is likely the cause of the large standard deviation seen in PV of sample 6. If the ingredients used in the supplement were very close to the end of their shelf life or were not handled properly during their shelf life, oxidation could also occur, causing elevated PV.

The form in which EPA and DHA are present, either TG or EE, can also be indicative of product quality. One FBO product in this study (sample 3) was found to be in the form of EE (Table 3). Another FBO product (sample 6) contained both TG and EE, with EE possibly being added to the product to cheaply and easily increase amounts of EPA and DHA. These samples had PV of 11.16 ± 0.17 and 14.81 ± 10.36 meq kg^{-1} respectively, the highest values seen in this study. These results suggest that FBO products containing EE may in fact be less resistant to oxidation than TG products. Fish oil manufacturers are not required to state the form that a supplement is in, making it difficult, if not impossible, for consumers to determine whether products are EE or TG. This information should be required on the labels of fish oil supplements so that consumers are able to make educated choices about what form of fish oil they are choosing to consume.

CONCLUSIONS

This study evaluated 16 of the most popular FBO and CLO supplements for adherence to label claims for EPA and DHA. Three FBO and five CLO products were found to have EPA + DHA levels below the values stated on the product labels. Additionally, three FBO and two CLO had PV exceeding 5 meq kg^{-1}, indicating that oxidation of the products had occurred. Two FBO oils contained EE, a fact not stated on the product labels. The results of this study demonstrate that not all fish oil products meet their label claims for active ingredients, and may be oxidised at the time of purchase.

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