Journal of Food Composition and Analysis 88 (2020) 103435

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

journal homepage: www.elsevier.com/locate/jfca

Original Research Article

Ingredient label claim compliance and oxidative quality of EPA/DHA omega-3 retail products in the U.S.

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ARTICLE INFO

Keywords:
Dietary supplement
Docosahexaenoic acid
Eicosapentaenoic acid
Fatty acids
Omega-3
Food analysis
Food composition
Fish oil
Food quality
n-3 polyunsaturated fatty acid
Regulation

ABSTRACT

The 48 most widely sold retail EPA/DHA omega-3 fatty acid dietary supplements on the U.S. market were tested for EPA + DHA label claim compliance and for oxidative quality. Each product was tested by at least three laboratories using validated methods. Most EPA/DHA products have a nutrient content consistent with the label declaration and contain levels of oxidation in accordance with industry and pharmacopeial quality requirements. It is challenging to evaluate the compliance for products sold in the U.S. given the lack of government regulations on oxidative quality specific to dietary supplements and content labeling requirements that are currently not clear. 48 % of the products contained less than the EPA + DHA amount declared on the label, although they are still within the current legal range. Adequate product storage conditions are suggested based on absence of correlation between the chemical markers and product expiration. Some limitations exist in the use of current methods to evaluate oxidative stability. Marked inter-laboratory variability was found when the same product is analyzed. Room for improvement in quality of EPA/DHA finished products in the U.S. is suggested since nearly half of 17 tested products for which all quality parameters could be tested did not meet at least one.

1. Introduction

Adequate dietary intake of omega-3 long-chain polyunsaturated fatty acids (omega-3 LCPUFA) is important for achieving and maintaining good health. Foods that contain significant amounts of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), the main omega-3 LCPUFA, are limited to fish and seafood. Dietary supplements rich in EPA and DHA are an alternative source of these fatty acids for consumers that do not consume sufficient fatty fish. These supplements contain EPA- and DHA-rich oils that originate from fish, such as anchovy, tuna and cod liver, as well as other sources such as krill and purposefully cultivated microalgae and protists. Furthermore, EPA/

Abbreviations: DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; Omega-3 LCPUFA, Omega-3 long-chain polyunsaturated fatty acids; GOED, Global Organization for EPA and DHA Omega-3s; p-AV, *para*-Anisidine Value; PV, Peroxide Value; rTG, re-esterified triglyceride

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https://doi.org/10.1016/j.jfca.2020.103435
Received 11 April 2019; Received in revised form 21 November 2019; Accepted 26 January 2020
Available online 28 January 2020
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DHA-containing dietary supplements can be found in different chemical forms, most commonly as triglycerides (refined fish oils and re-esterified triglyceride concentrates), as ethyl esters (concentrated forms of EPA and/or DHA), as mixtures of oils, as well as oils with a significant content of phospholipids in addition to triglycerides (such as krill oils) (Kutzner et al., 2017; Sprague et al., 2018). These products provide a portion of the global human dietary intake of EPA and DHA, while showing enormous scope for future growth since only less than 5 % of the human population is estimated to achieve sufficient EPA/DHA intake (Stark et al., 2016).

A large portion of global producers and marketers of EPA/DHA dietary supplements are members of the Global Organization for EPA and DHA Omega-3 s (GOED), a trade organization that, among other things, establishes criteria on the quality of EPA and DHA products for its members in the GOED Voluntary Monograph. Two aspects of omega-3 LCPUFA supplement quality have received considerable attention in recent years, namely the content labeling of EPA and DHA, and secondly, their oxidative stability. Incorrect determination of EPA and DHA concentrations readily leads to an incorrect product label, and because omega-3 LCPUFA are inherently sensitive to oxidation, their proper manufacturing, handling, formulation and storage is critical to avoid the development of rancidity (Bannenberg et al., 2017; Oterhals and Vogt, 2013; Song et al., 1997).

Content label declarations and oxidative status are aspects of product quality that need to comply with the applicable regulation(s) where the product is marketed. A number of studies published over the past three decades have reported that a substantial portion of dietary supplements containing EPA and DHA on the market contain lower amounts of those components than stated on the label (Albert et al., 2015; Galuch et al., 2018; Madsen and Dyerberg, 1990; Opperman and Benadé, 2013; Opperman et al., 2011; Ritter et al., 2012; Shim et al., 2003) or have levels of oxidation that suggest rancidity and/or are higher than industry or pharmacopeial standards (Albert et al., 2015; Chee et al., 1990; Fantoni et al., 1996; Fierens and Corthout, 2007; Jackowski et al., 2015; Kolanowski, 2010; Kragballe and Shukla, 1990; Kutzner et al., 2017; Lee et al., 2016; Madsen and Dyerberg, 1990; Mason and Sherratt, 2017; Opperman and Benadé, 2013; Opperman et al., 2011; Ritter et al., 2012; Rupp et al., 2013; Turner et al., 2006). In contrast, other studies report that EPA/DHA label claim non-compliance is low (Ackman et al., 1989; Bannenberg et al., 2017; Bengtson Nash et al., 2014; Fantoni et al., 1996; Fierens and Corthout, 2007; Hamilton et al., 2010; Kleiner et al., 2015; Kolanowski, 2010; Merkle et al., 2017; Nichols et al., 2016; Sprague et al., 2018; Srigley and Rader, 2014; Forbrugerradet Taenk, 2017; Tatarczyk et al., 2007), and non-compliance with respect to oxidation is not a major problem for the majority of EPA/DHA ingredients and finished products (Bannenberg et al., 2017; De Boer et al., 2018; Halvorsen and Blomhoff, 2011; Heller et al., 2019; Kolanowski, 2010; Nichols et al., 2016; Sprague et al., 2018; Forbrugerradet Taenk, 2017). This disparity in assessment has the potential to create consumer confusion and erode consumer confidence.

Non-compliance of products with regard to omega-3 LCPUFA content and oxidative quality may reflect a product formulation that is inappropriate for the storage and shelf-life conditions of the market where the product will be sold. Accurate testing of LCPUFA-rich oils is challenging, and the reporting of incorrect results can also be the consequence of poor sample handling during sample preparation, the use of analytical methodology that is inappropriate for LCPUFA-rich oils, or the testing by an inexperienced and non-certified laboratory. Fatty acid concentration and oxidative quality of encapsulated EPA/DHA oils need to be reported for the ingredient oil, which can only be determined accurately if the actual oil capsule content is verified (Sprague et al., 2018). In addition, specific limitations in the suitability of testing methodologies exist and should be taken into account when assessing the quality of EPA/DHA dietary supplements. For example, a false-positive non-compliance in secondary oxidation occurs with *para*-anisidine value (p-AV) testing of many flavored and colored oils. Furthermore, global voluntary quality guidelines developed by industry should not be confused with the regulations applicable in individual markets (Heller et al., 2019). Global voluntary guidelines may be established by industry that are more strict than local regulations in order to facilitate global trade or improve consumer compliance. The applicable regulations need to be understood in order to make lawful statements about product compliance for a specific country. For instance, products may contain EPA + DHA contents that are below a certain level of the labeled content, but they can still be compliant with local regulations depending on the legally permitted minimum content and/or the applicable tolerance (Bannenberg et al., 2017; Sprague et al., 2018).

To gain a better understanding if marked deviations from expected product compliance are occurring, GOED has undertaken and supported several studies to assess omega-3 LCPUFA dietary supplement quality. First, a recent study demonstrated that a large set of fish oil products available to consumers in New Zealand comply with local regulatory guidelines (Bannenberg et al., 2017). This conclusion was reached after having multiple laboratories analyze the same products using methods that are suitable for the analysis of fish oils. Next, the examination of a multi-year third-party database has shown that the majority of over 672 globally-sourced fish oils, fifteen krill oils, and nine algal oils are compliant with maximum oxidation limits stipulated by industry, pharmacopeial monographs and international regulations (De Boer et al., 2018). Recently, near complete label compliance and high oxidative quality of fish oils on the UK market was documented (Sprague et al., 2018).

Though these recent studies (Bannenberg et al., 2017; De Boer et al., 2018; Sprague et al., 2018) indicate that high rates of quality non-compliance do not seem to be a serious problem, a complete picture cannot be obtained from each individual study. Conclusions on product quality in one country cannot be used to represent the global market. Results from an off-the-shelf situation of supplement products, from various producers, give an instant description of the market but may not be generalized to all available products unless all products are tested or at least those that are most widely consumed. The results from the global third-party database indicated that compliance rates for products sourced from different parts of the world are high, but the tested products were supplied by the manufacturers and predominantly comprised of recently produced oils (bulk, encapsulated or formulated products). Thus, these results are not a reflection of products that may have been on retail shelves or in warehouse storage (a typical product shelf-life can be three years).

To expand our understanding if EPA/DHA-containing dietary supplements that are directly available to consumers are of acceptable product quality, the current study addressed the oxidation status and compliance against the amount of EPA + DHA specified in their supplement facts panel for finished products in a very large consumer market. For this study, the 50 dietary EPA/DHA supplements with the highest sales in the U.S. in the year 2016, and with shelf-lives reaching up to the end of 2020, were analyzed using multiple experienced laboratories for each product.

2. Materials and methods

2.1. Sample description

Fifty of the highest selling retail EPA and DHA products in triglyceride, phospholipid, or ethyl ester forms in the U.S. were identified from U.S. sales data obtained from commercial retail measurement services, Nielsen (http://www.nielsen.com/us/en.html) and SPINS (http://www.spins.com). The top 25 products in the Food, Drug and Mass (FDM) channel were identified from Nielsen retail scan data, and the top 25 Natural channel products were identified from SPINS retail scan data. The 25 products from both consumer product types

accounted for 50 % and 49 % of consumption (measured by portion of total sales) in each channel, respectively. Products were initially listed by their stock-keeping unit (SKU) or Universal Product Code (UPC) number. Thereafter, unique products were identified by their brand, form (softgel or liquid), serving size, and product composition. Six private label products, for which brands were not identifiable from the retail sales data, were present in the Nielsen data set, but could be identified by industry analysts based on a comparison of the product description provided by Nielsen and the description on the product label. No private label products were present among the 25 Natural channel products. The 50 products were purchased in November and December 2016. Three purchased products with different UPC codes resulted in being the same product (Nature's Bounty Fish Oil 1200 mg) in various pack sizes and with retailer-specific UPC codes, of which only one was tested in the present study. A final set of 48 products, described in Supplementary Table 1, was therefore organized for analysis.

To perform a comprehensive analysis of product quality, the 48 products were each tested by 4–5 laboratories that are experienced in the handling and analysis of omega-3 oils and that participate in annual inter-laboratory proficiency testing through the American Oil Chemists Society (AOCS) Laboratory Proficiency Program (LPP), GOED Nutraceutical Oils (AOCS-GOED, 2019). The analytical laboratories were located at DSM Nutritional Products (Columbia, MD, USA), Golden Omega (Arica, Chile), Nature's Way of Canada (Dartmouth, Nova Scotia, Canada), TASA (Callao, Peru), Eurofins Scientific Inc. (Des Moines, IA, USA), Organic Technologies (Coshocton, Ohio, USA) and the Department of Food Science and Technology at the University of California, Davis (Davis, CA, USA).

Each participating laboratory was asked to indicate the amount of sample material required to analyze the samples for EPA and DHA content, Peroxide Value (PV) and *para*-Anisidine Value (p-AV). The University of California, Davis, only analyzed PV and p-AV, while the other laboratories also analyzed EPA and DHA content. Products were procured by either visiting retail stores, wherever possible, or from the internet, when not found in stores. If multiple packages of each product were required to procure enough sample volume, they were bought from the same store and were part of the same manufacturing batch to control for variability as much as possible. In the case of liquid and emulsion products, an entire bottle of material was acquired for each laboratory to reduce the influence of sampling procedures on oxidation before the products were shipped to each laboratory. Products were stored at room temperature, out of direct light.

Once all sample material was acquired, a numeric code was assigned to each product and a letter to each package of the same product, affixing a sticker to the packages and recording the information in a table and in a spreadsheet. In addition to identifying the product number, the spreadsheet recorded the following product aspects: brand owner, brand, product name, country of manufacture, date of manufacture (where available), expiration date, date of purchase, batch number, UPC Code, labeled EPA and DHA content, labeled form of EPA and DHA (where available), labeled serving size (mg), portions per serving (*i.e.* capsules per serving), flavorings or other additives, price paid per package, and packaging form. In cases where information was not identifiable from the label, the company was contacted *via* their customer service line for additional detail.

Each softgel product was then divided into one numbered amber glass bottle per participating laboratory with no other identifying marks on the bottle, ensuring that each bottle had sufficient sample material for the respective laboratory. In the case of liquid or emulsion products, the sample material was not divided, but the labels were photographed and then removed along with any other identifying information from the container and affixed with its appropriate assigned number. The laboratories receiving each numbered sample were recorded by hand on a table and in a spreadsheet. The only information about the samples provided to the laboratories were the chemical form (triglyceride, ethyl esters, phospholipid or mixture), and whether the oil was flavored (according to the product label). This information was disclosed to the participating laboratories in order to ensure that the correct analytical methodology was applied. The products were then shipped in cold storage boxes (with cold packs) to each laboratory with instructions to chill the samples until they were tested. Upon arrival, all samples were stored refrigerated, away from heat and light, until analysis.

2.2. Testing of EPA and DHA content, and oxidative quality

Each analytical laboratory was instructed to measure the EPA and DHA content, PV and p-AV of each sample. All participating laboratories were asked to use one of the following methods for measuring fatty acid content: European Pharmacopoeia method Ph.Eur. 2.4.29, United States Pharmacopeia (USP) method USP 401"Fats and Fixed Oils", AOCS Official Method Ce 1i-07, GOED analytical method "Assay for EPA and DHA", or a quantitative method equivalent to the latter. The laboratories were asked to express EPA and DHA concentration as mg free fatty acid per gram oil, which is the industry standard for content expression, rather than chromatographic area % (a less commonly used and incorrect way to express fatty acid content).

In order to perform label claim calculations, instructions to determine capsule weight were also provided to the laboratories. Laboratories determined average fill weight by cutting open the capsules using a clean dry scalpel, rinsing out the oil with several portions of hexane (or heptane), and drying the capsule shell under vacuum. The fill weight is the difference between the capsule weight and the dry shell weight. Three laboratories specifically followed USP <2091>, 'Weight Variation of Dietary Supplements' (section on soft capsules), to determine average fill weight. The average of a number of replications, which ranged by individual laboratories from 3 to 10 capsules per encapsulated product, was used to determining the values of capsule fill weight and was used for label claim calculations.

The EPA and DHA content were not specified on the label of Nature's Bounty® Fish Oil 1200 mg, Spring Valley® Fish Oil 1000 mg 200 CT, and the four Sundown Naturals® products, precluding the calculation of EPA + DHA content as % of label claim for these products.

PV was determined according to AOCS Official Method Cd 8b-90 (AOCS, 2011a) or European Pharmacopeia method 2.5.5. p-AV was determined according to AOCS Official Method Cd 18-90 (AOCS, 2011b). Total oxidation number (TOTOX) was calculated from PV and p-AV following the formula TOTOX = (2 x PV) + p-AV.

Advice on proper sample handling was provided to the analytical laboratories, which is important to avoid inadvertent oxidation. Samples had to be handled quickly and under nitrogen. For gelatin capsules, advice was given to puncture capsules with a syringe and transfer the needed volume of oil to a glass recipient, while minimizing the exposed surface area. Material for PV and p-AV measurements had to be analyzed immediately after isolation from capsules. It was advised that the required amount of oil was collected separately for each analysis. No specific instructions were provided for the analysis of emulsions or phospholipid-rich oils. All measurements were carried out in duplicate or triplicate by the participating laboratories.

2.3. Data analysis and statistics

The blind sample results, from each participating laboratory, were sent to one of the study coordinators, where the results were recorded into a spreadsheet for analysis. A separate person checked the spreadsheet for accuracy. Once all results had been received, any required conversions for proper comparison between laboratories were made. For instance, if laboratories reported EPA and DHA content as free fatty acids while others reported it as triglycerides, the results were converted to be equivalent. Mean values and standard error of the mean (S.E.M.) were calculated for EPA content, DHA content, EPA + DHA content, PV, p-AV, and TOTOX from the results obtained by the

laboratories for each sample tested. The EPA and DHA concentrations were converted to labeled serving sizes for comparison to label claims.

Graphs were made using SigmaPlot v.13 (Systat Software). For the evaluation of relationships between time to product expiry and oxidative quality, best fits were determined by simple linear regression. Inter-laboratory variability for each tested parameter was summarized in box and whisker plots using the Cleveland method, with whiskers (error bars) above and below the box indicating the 95th and 5th percentiles, respectively. To evaluate over- or under-reporting by individual laboratories, linear modeling was carried out using the lm (linear modeling) function from the base package of the R statistical computing language (version 5.3.1) to estimate the values as a function of the tested products and laboratory (R Foundation for Statistical Computing, 2018). Missing values, reflecting products not tested by any individual laboratory, were eliminated from the dataframe input to the linear model. Statistically significant ($P < 0.05$) deviations from the estimated model for each measured dependent variable (% EPA + DHA label claim, PV and p-AV) were reported as under- or over-reporting, employing one of the laboratories' results as a reference.

3. Results

3.1. Product characteristics

Of the 48 tested products (Supplementary Table 1), 18 were indicated in the product description or label to be made from marine oils produced in the U.S., or to be produced in the U.S. without further indication of the country of origin. Twelve of the 48 products originated from Norway and twelve from Peru. Twenty-nine products were triglyceride oils, five were phospholipid products (*i.e.* krill oils, which have a high proportion of the oil mass as phospholipids), ten were ethyl esters, and one was a mixture of oils (likely as triglycerides). For three products, the chemical form of the oil was not given on the label or product information. Three of the products were re-esterified triglyceride (rTG) products (labeled separately in Supplementary Table 1). Forty products were in encapsulated form (*i.e.* softgel), and eight products were liquid oils in bottles. Among the encapsulated products, seven had an enteric coating. Twenty-five of the 48 tested products were flavored with one of various types of flavors. Eleven of the Norwegian products, and eight of the U.S. products, contained added flavors. None of the products from Peru were flavored. Three products were emulsions (Barlean's; labeled separately in Supplementary Table 1). The expiration dates of the tested finished products ranged from November 2016 to October 2020.

3.2. EPA and DHA label claim compliance

The EPA + DHA as a percentage of the content declared on the product label for the tested products is shown in Table 1. The 42 products that could be evaluated for label claim compliance contained 102 % ± 15.9 % (mean ± S.E.M.) of the label declaration for EPA + DHA (Table 2). These products all complied with the requirement in the U.S. that the ingredient constitutes at least 80 % of the labeled content (Fig. 1A). This ingredient content minimum applies to products that are considered Class II Nutrients (see Discussion). Of the tested products with a content declaration, 40.5 % (17 of 42 products) had an EPA + DHA content between 80 % and 100 % of the labeled content, while 57.1 % had a content between 100 % and 110.4 %, and one product contained 138.7 % of labeled content (Fig. 1A). No difference in compliance rates were found when median values were calculated instead of mean values (Supplementary Table 5). The twenty-five products containing an added flavor or consisting of a colored oil contained 100.3 % ± 20.5 % of the claimed content, which was similar to that of all tested products, and 44.0 % had an EPA + DHA content that fell between 80 % and 100 % of the label declaration. Three rTG products contained 103.9 %, 98.6 %, and 101.6 % of labeled content of EPA + DHA, respectively. Five of nine ethyl ester products with a content declaration had EPA + DHA levels ranging from 1 to 8% below the declared value (Fig. 2A).

Products that were closer in time to their expiration date showed no difference in EPA + DHA content as a percent of labeled content compared to products with longer times to expiration (Fig. 1A; n = 42). No remarkable differences were observed in EPA + DHA label claim compliance between products (with a content declaration on the label) containing EPA/DHA oils as triglycerides (n = 29 products), ethyl esters (n = 9) or rich in phospholipids (n = 4) (Fig. 2A). There was no major difference between EPA + DHA as percent of labeled content in encapsulated products (n = 34) or liquid products (n = 8) (Fig. 3A). EPA + DHA content was close to 100 % of labeled content for all encapsulated products, whether enterically-coated (n = 6) or encapsulated in regular softgels (n = 35) (Fig. 4A). No major differences in the average EPA + DHA content as percent of labeled content between the country of origin or manufacturing were found, as shown for products from the U.S. (99.5 ± 8.6 %, n = 16), Norway (103.1 ± 4.2 %, n = 12) and Peru (107.0 ± 3.0 %, n = 6) were observed (data not shown graphically).

3.3. Peroxide Value of finished products

The PV of the individual products is shown in Table 1. Of all tested products, the average PV was 3.6 ± 0.53 meq O_2/kg (Table 2) and 85.4 % (41 products) (Fig. 1B) of the products complied with the maximum limit of 5 meq O_2/kg provided in the GOED Voluntary Monograph and the USP monographs for fish oil and krill oil. No difference in compliance rates were found when median values were calculated instead of mean values (Supplementary Table 5). No regulatory limits for PV exist in the U.S. For flavored and colored oils, the average PV was 2.2 ± 0.46 meq O_2/kg, with 96 % compliance to industry and USP limits (data not shown graphically). Products that were closer in time to their expiration date showed no clear, nor easily distinguishable, difference in PV compared to products with longer times to expiration (Fig. 1B; n = 48). Two of the twenty-nine triglyceride products and five of the ten ethyl ester products had PVs higher than 5 meq O_2/kg (Fig. 2B). Although most finished products had a PV below 5 meq O_2/kg, the ethyl ester products (n = 10) usually had higher PVs than TG products (n = 29). Four of the phospholipid products for which PVs could be determined showed values close to zero (Fig. 2B). The PV of the eight liquid products were lower than 5 meq O_2/kg. Encapsulated products (n = 40) showed a wider distribution in PV, ranging between 0–8 meq O_2/kg while two products exceeded 15 meq O_2/kg (Fig. 3B). The latter corresponded to two enteric coated products (Fig. 4B). The range of PVs of the other five enteric coated encapsulated products for which PV were measured was similar to the 36 uncoated softgel products (Fig. 4B). Out of 33 uncoated softgel products, four (12.1 %) had a PV greater than 5 meq O_2/kg (Fig. 4B). No marked differences in the PV between products incorporating oils from the U.S. (3.91 ± 1.17 meq O_2/kg), Norway (3.17 ± 0.31 meq O_2/kg) and Peru (3.86 ± 0.67 meq O_2/kg) were observed (data not shown graphically).

3.4. Para-Anisidine Value of finished products

The p-AV of the tested products is shown in Table 1. Of the 23 unflavored products, the average p-AV was 8.97 ± 1.91 (Table 2), and 95.7 % of the products complied with the maximum limit of 20 stipulated in the GOED Voluntary Monograph, and according to the USP monograph for fish oil. No regulatory limits for p-AV exist in the U.S. No difference in compliance rates were found when median values were calculated instead of mean values (Supplementary Table 5). Products that were closer in time to their expiration date showed a tendency for a higher p-AV value, although all of the tested products, except one, were within maximum industry limits (Fig. 1C; n = 23). Among the unflavored products, one ethyl ester product had a p-AV above 20

Table 1
Tested products in alphabetical order with content of EPA + DHA as % of label claim, PV, p-AV and TOTOX. Values are expressed as the mean value of the individual results from four or five laboratories (see Supplementary tables 2, 3 and 4 for the individual laboratories' measured values). Results are shown for the unflavored products (first part of Table 1), and the flavored and colored oils (bottom part of the Table 1).

Product	EPA + DHA (% label claim)	PV (mEq O_2/kg)	p-AV	TOTOX
Unflavored oils				
Bayer Pro Vitamin D_3	106.7	7.8	10.5	26.1
Carlson Fish Oil O3 Gems 500mg	106	7	2.8	16.8
Natural Factors Rx Omega 3 900mg	92.2	4.7	6.1	15.5
Natural Factors Rx Omega-3 Fish 400EPA/200DHA	106.2	3.2	5	11.4
Natural Factors Wild Salmon Oil	138.7	2.7	6.8	12.2
Nature Made Burpless Fish Oil 1200 mg	107.7	6	13.8	25.8
Nature Made Fish Oil 1200 mg	110.1	2.9	8.5	14.3
Nature Made Ultra Omega 3 Fish Oil	100.6	4.2	4.4	12.8
Nature's Bounty Fish Oil 1200 mg		4.1	15.3	23.5
Nature's Bounty Odor-Less Max Strength Fish Oil 1400mg	95.9	14.9	10.1	39.9
New Chapter Wholemega 1000 mg	99	2.4	9.3	14.1
Now Foods Fish Oil 1000 mg	106.4	1.9	6.2	10
Pure Alaska Omega	98.3	3.7	5	12.4
Spectrum Fish Oil	101.5	4.6	8.5	17.7
Spring Valley Fish Oil 1000 mg 200 CT		4.1	8.2	16.4
Spring Valley Fish Oil 1000 mg 300 CT	109.1	2.6	6.9	12.1
Spring Valley Fish Oil 1200 mg	106.9	2	12.1	16.1
Spring Valley Fish Oil 1400mg	93.5	3.9	10	17.8
Sundown Naturals Fish Oil 1000 mg		5	10.4	20.4
Sundown Naturals Fish Oil 1200 mg		5.4	13.7	24.5
Sundown Naturals Omega 3 1290mg		16.8	26.1	59.7
Sundown Naturals Omega 3 6 1200 mg		4.7	4.8	14.2
Wileys Finest Peak EPA	95.3	2.8	1.9	7.5
Flavored and colored oils				
Barleans Fish Oil	94.2	2.4	14	18.8
Barleans Omega Swirl Key Lime	87.1	1.1	34.45	36.65
Barleans Omega Swirl Lemon Zest	89.5	1.3	49.7	52.3
Barleans Omega Swirl Mango Peach	86.3	0.9	5.95	7.75
Bausch + Lomb Ocuvite Eye Health 50+	109	0.6	7.8	9
Carlson Cod Liver Oil Lemon	106.1	1.7	242.7	246.1
Carlson Elite Gems Lemon 1250mg	97.4	5.3	50.9	61.5
Carlson Fish Oil Lemon Kids	97.9	2.2	120.9	125.3
Carlson Fish Oil Liquid	97.2	4.4	121.2	130
Country Life Omega-3 1000 mg	88.7	3.4	8.1	14.9
Kirkland Omega 3 Krill Oil 500mg	107.2	0.2	94.48	94.88
Members Mark Alaska Fish Oil D3	110.4	4.5	4.3	13.3
Nordic Naturals Arctic CLO Orange	110.4	2.8	5.2	10.8
Nordic Naturals DHA Extra	103.9	2.9	22.48	28.28
Nordic Naturals Omega 3 6 9	104.1	3.2	44.8	51.2
Nordic Naturals Omega-3	109.1	3.2	45.7	52.1
Nordic Naturals Omega-3 6 9 Junior Lemon	103.4	3.1	44.4	50.6
Nordic Naturals Omega 3 Formula Lemon Liquid	104.2	3.6	48.4	55.6
Nordic Naturals Ultimate Omega	98.6	3.6	35.9	43.1
Nordic Naturals Ultimate Omega W Vit D3	101.6	3.1	36.3	42.5
Ocean Blue Omega 3 Fish Oil 2100mg	93.4	1.7	44.4	47.8
Schiff Mega Red 1000 mg	99.2	0.2	119.6	120
Schiff Mega Red Krill Oil 500mg	100.4	0.1	65.6	65.8
Schiff Mega Red Omega 3 Krill Oil 350	105.1	0.2	107.8	108.2
Schiff Mega Red Omega 3 Krill Oil 750	104.2	0.1	205.2	205.4

(Fig. 2C). Flavored and colored oils cannot be reliably tested for secondary oxidation using the p-AV test due to method interference (see Discussion). Unflavored products were all in softgel form, so no comparison between products in liquid or softgel dosage form could be made for p-AV.

3.5. TOTOX of finished products

For unflavored products, the average TOTOX number was 19.2 ± 4.1 (Table 2) and 87 % of the products complied with the maximum industry limit of 26 provided in the GOED Voluntary Monograph and the USP monograph for fish oil. TOTOX numbers cannot be reliably determined for flavored and colored oils, because it is a calculated value that utilizes p-AV as one of its addends. No regulatory limits for TOTOX exist in the U.S. Three of the eight ethyl ester products had a TOTOX number greater than 26, and three of the triglyceride products had TOTOX numbers that were close to this maximum limit (Fig. 2D). No change in TOTOX was observed as products were closer in time to their expiration date (Fig. 1D; n = 23). Unflavored products were all in softgel form, and no comparison between products in liquid or softgel form could be made for TOTOX.

4. Discussion

4.1. The most widely sold EPA/DHA omega-3 dietary supplements in the U.S.

The current study evaluated the EPA + DHA content and oxidative status of the 48 most widely sold retail EPA/DHA omega-3 dietary supplements in the U.S. Currently (2019), the U.S. is the country with the highest sales of EPA/DHA omega-3 dietary supplements to consumers. The U.S. market for EPA/DHA omega-3 dietary supplements in 2015 was 1200 MM$ (unpublished GOED market data). This study focused on the dietary supplements that were sold in 2016 to consumers

Table 2
Product quality compliance with GOED and U.S. Pharmacopoeial limits. N; number of products tested for a particular parameter, Percent compliance with GOED Voluntary Monograph, U.S. Pharmacopeia monograph for fish oil, and U.S. Pharmacopeia monograph for krill oil.

Quality parameter	Mean ± SEM	N	Percent compliance
EPA + DHA (% label claim)	102.0 ± 15.9	42	100[1] / 59.5[2]
PV (meq O_2/kg)	3.6 ± 0.5	48	85.4[3]
p-AV	9.0 ± 1.9	23*	95.7[4]
TOTOX	19.2 ± 4.1	23*	87.0[4]

[1]Products with more than 80 % of label claim (applicable to Class II nutrients that are naturally occurring).
[2]Products with at least 100 % of label claim.
Standards of reference:
[3]GOED Voluntary Monograph, USP Fish Oil monograph, USP Krill Oil monograph.
[4]GOED Voluntary Monograph, USP Fish Oil monograph.
*Unflavored oils only.

predominantly through one of two retail channels, the Food, Drug and Mass retail segment, which corresponds to groceries, and the Natural channel, which is the retail segment selling products of natural and organic origin. Out of the 48 most widely sold EPA/DHA omega-3 supplements, the product with the highest sales was a fish oil (Nature Made Fish Oil 1200 mg). In the Natural channel, the most popular product was a lemon-flavored re-esterified triglyceride concentrate (Nordic Natural Ultimate Omega). Due to a lack of data, the most popular products in other channels, such as multilevel marketing, healthcare practitioners or internet sales, could not be determined. Also, some stores do not provide data to Nielsen and SPINS, a limitation of the current study. Although most of the tested products were triglyceride and ethyl ester products, six of the most widely sold EPA/DHA products in the US contained oils rich in phospholipids (krill oils).

4.2. EPA and DHA label claim compliance

The present study shows that the U.S. nutrient classification of EPA/DHA determined the compliance of the products for level of EPA/DHA. 21 C.F.R. §101.9 on Nutrition labeling of food, which is inclusive of dietary supplements, describes two classes of nutrients for purposes of compliance (FDA, 2018). For Class I nutrients, the nutrient content must be equal to at least 100 % of the declared value. For Class II nutrients the nutrient content must be equal to at least 80 % of the declared value. The limited description of the two nutrient classes makes it difficult to differentiate them. Class I nutrients are described as "Added nutrients in fortified or fabricated foods", while Class II nutrients are described as "Naturally occurring (indigenous) nutrients". Given that EPA and DHA exist in nature, it has been industry practice to classify the EPA and DHA in omega-3 rich oils as "naturally occurring". This is likely to continue as best practice until such time that the FDA provides guidance on the definition of "natural," an issue that the FDA has been hesitant to weigh in on historically. Indeed, when the products are all defined to be of natural origin (Class II Nutrients), all tested products were compliant (also shown in Fig. 2A for triglyceride, ethyl ester and phospholipid-rich products). Of all tested products, 40.5 % had an EPA + DHA content between 80 % and 100 % of the labeled content (and the other 59.5 % of the products between 100 % and 138.7 %). This finding may reflect the intention of finished product manufacturers to use ingredient EPA and DHA oils that will provide at least 80 % of the claimed content, which would be in accordance with the applicable FDA regulation in the US.

In the case of certain concentrates (for example ethyl esters which contain EPA and DHA in a form and concentration not found naturally),

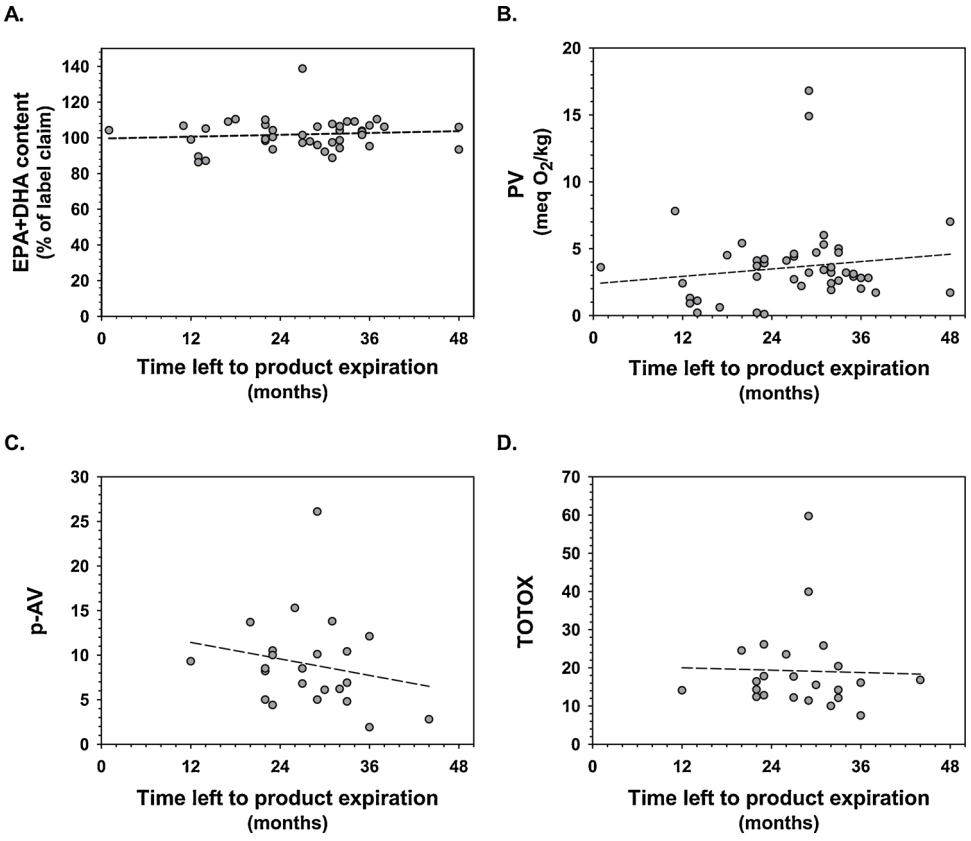

Fig. 1. Relationship between the time left to product expiration (months) to EPA + DHA as percent of labeled content (panel A; n = 42), PV (panel B; n = 48), p-AV (panel C; n = 23), or TOTOX (panel D; n = 23). Dashed lines: Best fit of data by simple linear regression.

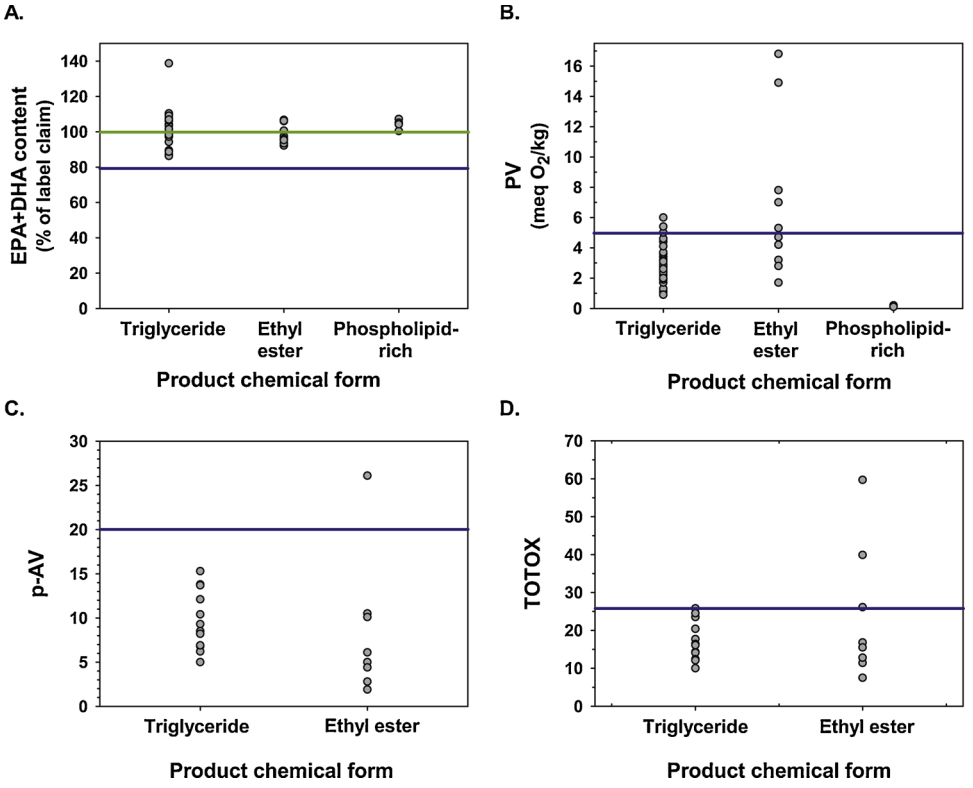

Fig. 2. EPA + DHA (percent of labeled content) (panel A), PV (panel B), p-AV (panel C) and TOTOX (panel D) as function of chemical form. Panel A: *Left*, triglyceride products (n = 25); *center*, ethyl ester products (n = 9); and *right*, phospholipid products (n = 4); Blue line: 80 % of labeled content. Green line: 100 % of labeled content. Panel B: *Left*, triglyceride products (n = 29); *center*, ethyl ester products (n = 10); and *right*, phospholipid products (n = 4); Blue line: maximum limit of 5 meq O_2/kg. Panel C: Unflavored products only, *Left*, triglyceride products (n = 13); *right*, ethyl ester products (n = 8); Blue line: maximum limit of 20. Panel D: Unflavored products only, *Left*, triglyceride products (n = 13); *right*, ethyl ester products (n = 8); Blue line: maximum limit of 26.

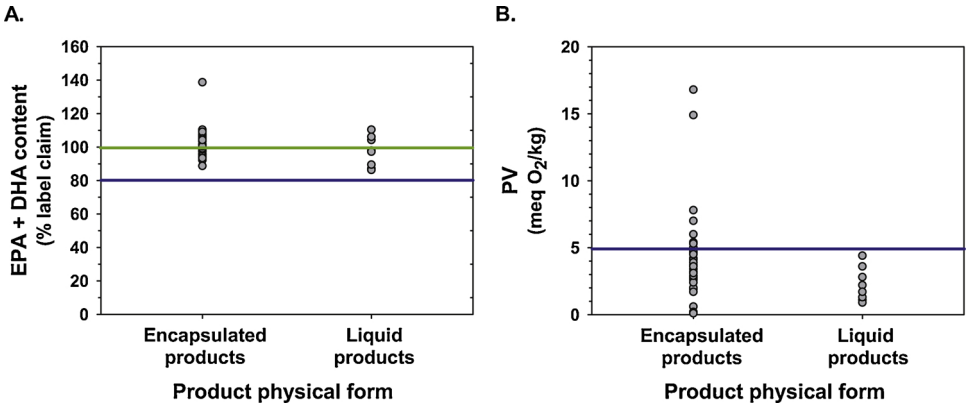

Fig. 3. EPA + DHA as percent of labeled content (panel A), and PV (panel B) as function of physical form. *Left*, encapsulated products (n = 34); *right*, liquid products (n = 8). Panel A: Blue line: 80 % of labeled content; Green line: 100 % of labeled content. Panel B: Blue line: maximum limit of 5 meq O_2/kg.

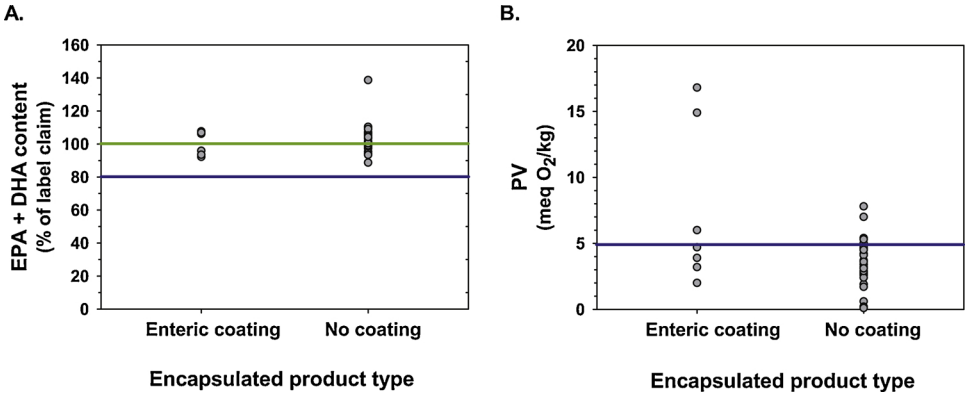

Fig. 4. EPA + DHA as percent of labeled content (panel A), and PV (panel B) of encapsulated products as function of enteric coating. Panel A: *Left*, encapsulated products with enteric coating (n = 6); *right*, encapsulated products without enteric coating (n = 28). Blue line: 80 % of labeled content; Green line: 100 % of labeled content. Panel B: *Left*, encapsulated products with enteric coating (n = 7); *right*, encapsulated products without enteric coating (n = 33). Blue line: maximum limit of 5 meq O_2/kg.

there is a possibility that the EPA and DHA could be interpreted as Class I nutrients, in which case they would need to be present in the amount of at least 100 % of the label claim. Several of the widely sold products that were tested in this study were ethyl esters (n = 10) and re-esterified triglycerides (rTG) (n = 3), which in turn could be considered Class I Nutrients. Of nine ethyl ester products with a content declaration, five had EPA + DHA levels ranging from 1 to 8% below the declared value (Fig. 2A). Re-esterification of concentrated EPA- and DHA-ethyl esters with glycerol allows for the production of enriched EPA and/or DHA triglyceride oils. One of the three rTG products contained 98.6 % of the labeled content. In all products in this category whose EPA and DHA were below the label claim, the content was close (95.0–99.3 %) to the label. Applicable analytical and regulatory tolerances would decide if part of the products that could currently be evaluated as Class I nutrients would be out of compliance.

In addition, the U.S. FDA Dietary Supplement Labeling Guide: Chapter IV on Nutrition Labeling, provides indications on the correct expression of content (FDA, 2005). The constituents of a dietary ingredient, such as EPA and DHA in a fish oil, may be listed voluntarily on a product label in the supplement facts panel along with their quantitative weights per serving. If listed, dietary supplements found to contain less than this amount (or 80 % of it) will be deemed to be misbranded and in violation of the law. Whereas the majority of the tested products chose to list EPA and DHA amounts, six products did not provide this information.

The first published study on the quality of fish oil supplements in the U.S. reported that the label claims of the majority of fish oils and concentrates, purchased during the period 1984–1988, were presented with reasonable accuracy (Ackman et al., 1989). Of the 17 tested products, three products had EPA + DHA contents that were at least 100 % of the label claim, 11 products (65 %) had levels between 80 % and 100 %, and three products (18 %) had contents below 80 % of the label claim. There is some uncertainty in this estimate because a large proportion of products were, at that time, merely indicating the contents of EPA and DHA in a standardized format, such as “180″ and “120″ for anchovy-derived refined fish oils (reflecting a typical or average seasonal area % profile of these two fatty acids in anchovy oil), or for example “300″ and “200″, respectively, for some concentrated forms. Chee et al. (1990) reported similar findings on eight fish oil products sold in the U.S.: five products contained between 80 and 100 % of the EPA + DHA label claim, and three products contained inferior amounts (38 %).

Shim et al. (2003) reported that of 21 US fish oil and algal oil products with a stated EPA + DHA content on the product label, only five products (23.8 %) contained more than 80 % of the claimed content of EPA + DHA, and only one product had at least 100 %. Ten years later, the same research group noted a marked improvement in label claim compliance of U.S. fish oil and algal oil supplements (Kleiner et al., 2015); 84 % of 47 tested products contained at least 80 % of the label claim, and 26 % at least 100 %.

Three further recent studies reported on label claim compliance of U.S. fish oil supplements. Ritter et al. (2012) showed that among 16 of the most widely sold fish oils, seven contained more than 100 % of the claimed EPA + DHA content, and the other nine products contained between 80 % and 100 %. Srigley and Rader (2014) reported a high label claim compliance for 48 U.S. marine oil omega-3 supplements purchased in 2012: more than 80 % of the analyzed products were described to have EPA + DHA contents that were within ±20 % of their label declarations, and six of the products failed to meet label declarations. Both studies are in large agreement with the current study, showing that the majority of marine oil omega-3 supplements in the U.S. market are adhering to locally-applicable regulations. One algal and two fish oil products from the U.S. were reported in 2017 to contain 150 %, and 102.4 % and 98.6 %, respectively, of the claimed EPA + DHA content (Kutzner et al., 2017). Together with the results of the current study, it appears that the relatively elevated rates of non-compliance with EPA + DHA label claims observed until the beginning of the 2000′s have decreased significantly in the last ~15 years for U.S. products. It also emerges that producers of EPA and DHA-containing dietary supplements primarily aim at meeting the FDA requirement that at least 80 % of the claimed content is indeed present (Kleiner et al., 2015). The present study conducted on the top 48 selling retail products extends these conclusions to the products that consumers are actually consuming.

Still today, one factor that hampers making precise external assessments of label claim compliance is the continued practice of listing the same EPA and DHA ingredient levels on products in a standardized manner. As explained above, a significant portion of fish oil products are labeled as “180/120″, to denote a seasonal average or typical product composition, but do not provide a precise label claim about the content of these fatty acids. For example, Yi et al. (2014) reported that ten fish oil products sold in Hong Kong (of which seven with U.S. provenance) with the same labeled EPA and DHA ingredient levels of 180 and 120 mg/g, had EPA + DHA contents that ranged from 25 % to 355 % of the claimed content. In the current study, six products were of the “180/120″-labeled type fish oils. The assessment of label claim compliance can only be correctly made if manufacturers make precise statements on the true ingredient contents. In addition, nearly all products stated the content of “total omega-3″ on the label, but it is seldomly clear to which omega-3 LCPUFA species the content refers. GOED recommends expression of “total omega-3″ as the combined weight of seven omega-3 fatty acids: alpha-linolenic acid, stearidonic acid, eicosatetraenoic acid omega-3, EPA, heneicosapentaenoic acid, docosapentaenoic acid omega-3 and DHA.

4.3. Peroxide Value

No regulatory limits for primary or secondary oxidation quality exist in the U.S. for EPA/DHA dietary supplements, but a large majority (89.5 %) of the 48 tested products was found to comply with strict industry and relevant pharmacopeial quality standards. There was no evidence for higher levels of primary oxidation products, *i.e.* fatty acid hydroperoxides, in the products that were closer to their expiration date. It was not possible to draw conclusions about possible differences in the average PV of liquid and encapsulated products, between triglyceride and ethyl ester forms, or that there is a possibility that (manufacturing of) enteric coated softgels are more sensitive to oxidation, given the limited number of tested products.

Only a limited number of studies, all published in the last few years, have addressed primary oxidation levels of U.S. EPA/DHA products. Ritter et al. (2012) reported that among 16 of the top-selling brands, 5 products (31 %) had PV levels higher than 5 meq O_2/kg. In 2015, a study on the PV of 139 EPA/DHA supplements purchased in Canada, and considered to reflect product quality in North-America, found that 17 % exceeded the 5 meq O_2/kg limit (Jackowski et al., 2015). Kutzner et al. (2017) showed that one of two tested fish oils and one algal oil product from the U.S. exceeded 5 meq O_2/kg. Broadly, the results of the current study are in line with these previous reports, suggesting that approximately up to one-third of products of the U.S. may exceed recommended quality limits on primary oxidation. Although most products appear to be of good quality, in the absence of clear regulation in the U.S., only industry-driven voluntary guidelines and pharmacopeial guidelines currently provide stimuli towards improving this situation.

One interesting observation was that encapsulated products in the U.S. showed a very wide distribution in PV. This confirms that it is certainly possible for finished products to achieve good oxidative stability, but also that significant improvements in product quality can still be attained for specific products. Improvements can likely be achieved by manufacturers and retailers by, for example, evaluating the risk for increases in primary oxidation during the encapsulation process and using an adequate formulation for the anticipated storage and shelf-life conditions (*e.g.* use of adequate antioxidant type and concentration, and

improvements in packaging). PVs were comparatively low for the krill oil products, in line with the high oxidative stability reported for this phospholipid-rich oil type (Ryckebosch et al., 2013).

4.4. para-Anisidine Value and TOTOX

Flavored and colored oils cannot be reliably tested for secondary oxidation using the colorimetric p-AV test due to method interference, involving the generation of false-positive signals from aldehydes present in added flavors or directly from the color of the oil itself. Measurement of p-AV as a product quality parameter can only be performed reliably on non-flavored oils, and on those oils for which a laboratory is certain that a specific additive or color does not contribute to the p-AV signal. To illustrate the very high false-positive signals measured for flavored and colored oils, the 25 tested flavored and colored oils had an average p-AV of 63.2 ± 12.9. The p-AV assay was developed for animal and vegetable fats and oils and is a suitable method for the measurement of secondary oxidation in refined marine triglyceride oils or ethyl ester concentrates but is not robust to many additives. This aspect has been overlooked in a number of previous reports and has contributed to the inadvertent over-reporting of non-compliance rates (Albert et al., 2015). Recent publications have started to acknowledge this methodological limitation for the assessment of finished product quality (Heller et al., 2019; Sprague et al., 2018). In the present study, only 23 of the 48 products were not flavored (or had a strong color), reflecting the high penetration of flavored products in the U.S. market and their popularity with consumers. Nearly all of these non-flavored products (95.7 %), complied with voluntary industry limits and the USP quality standard on secondary oxidation quality.

Only one study has previously addressed the non-compliance of US products with guidelines for secondary oxidation levels and TOTOX number (Jackowski et al., 2015). In that study, non-flavored softgel products had significantly lower p-AV values than flavored softgel products, with the mean values falling below and above the limit of 20, respectively. A similar behavior was observed for TOTOX number, although the difference was not statistically significant.

4.5. Specific method limitations

Though the chemical analysis of oxidative quality and omega-3 fatty acid content of triglyceride fish oils, ethyl ester concentrates, and re-esterified triglyceride oils is routine for the participating laboratories, the accurate measurement of specific parameters for a few specific products proved to be challenging. The following methodological challenges and solutions proposed by individual laboratories, are reported here. For p-AV, four laboratories reported on three of the phospholipid products, and three were able to report on the other two products (Supplementary Table 4). A very large variation was apparent in the reported p-AV for the same products by different laboratories. Such variation was greatest for one krill oil product, with a lowest reported value of 0.1 and the highest of 223 (Supplementary Table 4). This indicates that significant improvements and method standardization needs to be achieved to reliably test secondary oxidation in phospholipid-rich products. The main challenge was that the solubility of these products was found to be insufficient in the organic solvents specified by the methods. Use of alternative solvents instead of iso-octane, such as dichloromethane or chloroform, provided some solubility improvements. One sample was impossible to dissolve in dichloromethane even at half the amount (one laboratory), or in chloroform (one laboratory). An alternative solution by one laboratory for the measurement of p-AV for these products was filtration of the cloudy samples. One laboratory reported that addition of the *para*-anisidine reagent caused layer separation in two of these products. Inadequate dissolution of three liquid products in the assay for p-AV was also reported, by one laboratory (corresponding to the three emulsion products; Supplementary Table 1).

One laboratory observed for all phospholipid samples the formation of an additional emulsion-like layer in between the upper iso-octane and the bottom aqueous layers after the water addition step in the method for fatty acid quantification. The upper iso-octane layer was transferred after an additional centrifugation at 1000 rpm for 10 min, which minimized the additional layer.

Awareness of potential interference by pigments present in colored oils with the iodometric assay for PV is also important. Four laboratories reported PV values for five phospholipid products, and five laboratories on a sixth product of unknown composition (Supplementary Table 3). One laboratory that reported two phospholipid products with PV levels of zero, tested if aliquots of 300 mg instead of 2000 mg would change the value. No change in PV values could be detected, leading the laboratory to conclude that PV was zero in these samples.

A methodological aspect that became apparent during the study was the difference in sample preparation of the three emulsion products (Supplementary Table 1) by the participating laboratories. Two laboratories pre-extracted the oil from these products prior to analysis, whereas the other laboratories did not. One laboratory followed method AOAC 996.06 “Fat (Total, Saturated, and Unsaturated) in Foods.“ for the extraction step. A second laboratory employed a method developed in-house, modified from previous approaches by Hu et al. (2003) and Sørensen et al. (2013), using a solvent extraction with 3:1 hexane/isopropanol (with 0.05 g/L butylated hydroxytoluene) that preserves fatty hydroperoxides and is suitable for both fatty acid quantification and PV measurements in EPA/DHA dietary supplements.

Details of such methodological limitations and laboratory-specific approaches are provided here merely to indicate that even among proficient laboratories differences in execution exist, particularly when challenged with the analysis of specific omega-3 LCPUFA products that are different from neat triglyceride and ethyl ester oils. Future studies assessing EPA/DHA dietary supplement quality will thus need to address the need for suitable methods for specific oil types and complex formulations. This is of relevance given the high proportion of EPA/DHA dietary supplements available to consumers today that are flavored, strongly colored, or correspond to formulation formats that are very different from neat refined oils.

4.6. Product shelf-life

No strong relationships between the level of primary oxidation and secondary oxidation products as a function of time remaining to the end of shelf-life could be determined. It has been argued that oxidation could consume sufficient omega-3 LCPUFA leading to a measurable decrease in their content (Albert et al., 2015). It has previously been indicated that at the low levels of oxidation that typically occur in fish oil supplements, this does not occur (Bannenberg et al., 2017). In the present study, no further evidence to support this hypothesis was found, as there was no evidence for higher non-compliance with EPA/DHA label claims in products that were closer to the end of shelf-life. It was observed that primary oxidation levels were higher in a portion of ethyl ester products than in triglyceride products, suggesting that ethyl ester products might be more sensitive to oxidation (Indrasena and Barrow, 2010). No evaluations of any relationships between the content of naturally-present or added antioxidants and product oxidative status could be made given the few products for which the concentrations of antioxidants was provided on the label. Low PV does not mean an oil is not oxidized, and secondary oxidation should be measured in parallel to PV for EPA/DHA omega-3 products. The voluntary industry requirements provided by GOED generally include PV, p-AV and TOTOX to assess oxidative quality because PV levels can furthermore decline over time as primary oxidation products are transformed into secondary oxidation products. The current limitation in being able to assess p-AV and TOTOX in flavored or colored oils hampers the determination of the oxidative status of such products.

4.7. General considerations

This study highlights that accurate statements on the quality of dietary supplements should first be made in view of the regulatory framework that is applicable to the respective geography where the tested products are being marketed. This point is nicely illustrated taking the U.S. as an example where no regulatory guidelines exist with respect to oxidative quality of omega-3 supplements, and there is room for interpretation around label claim compliance of the active ingredients EPA and DHA. If strict industry guidelines (which are voluntarily set) for oxidative quality are followed, 85.4 % of the products with the highest sales in the U.S. complied with limits on primary oxidation, and 95.7 % of the products that could be tested complied with limits on secondary oxidation. This high rate of product compliance is in agreement with recent reports on the status of fish oils supplements in Australia, New Zealand and the UK (Bannenberg et al., 2017; Nichols et al., 2016; Sprague et al., 2018). Other studies have reported mixed findings, including reports of massive non-compliance. GOED replicated one such study and identified several methodological and reporting errors that contributed to the reporting of low compliance, in addition to over-reporting of non-compliance as a result of not taking the applicable regulations into account (Bannenberg et al., 2017). These include the potential for mistakes in sample handling, the use of analytical methods that are not suitable for omega-3 LCPUFA-rich marine oils or EPA and DHA concentrates, the inadvertent reporting of false-positive non-compliance consequent to method interference, and the incorrect reporting of units. In order to minimize the risk of incorrect product compliance testing and reporting, analyses should ideally be made by accredited laboratories with experience in the handling of omega-3 LCPUFA-rich oils, and which regularly participate in laboratory proficiency testing for the applicable methods. The increased adoption by laboratories of Certified Reference Materials for omega-3 oils and concentrates (Schantz et al., 2013) may also promote achieving increased accuracy by individual laboratories, and over time lower inter-laboratory variability.

In the present study, as well as in a previous study (Bannenberg et al., 2017), the value of having the same product analyzed by multiple laboratories is demonstrated. Average results calculated from multiple independent and blinded measurements provide improved insight about the “true” value of their quality. A comparison of the mean values and distribution of analyzed products for EPA + DHA content, PV, and p-AV suggests that there is some variability even between the qualified laboratories (Supplementary Figs. 1A–C). For the determination of EPA + DHA label claims, one laboratory measured statistically significantly higher (5.1 %) median levels of EPA + DHA combined. For PV, four laboratories measured lower levels than the reference laboratory chosen for the linear model. For p-AV, one laboratory under-reported. Although product quality testing is usually not performed by multiple laboratories in parallel on a routine basis, the present study shows that relying on the results of a single laboratory can skew the conclusions about the compliance of an entire set of tested products, as a consequence of possible systematic under- or over-reporting by any particular laboratory. It is common industry practice to have an independent third laboratory adjudicate product quality disputes between suppliers and customers. Such replication and verification of a negative result by an independent source is often missed in scholarly publications.

Some divergence between the number of capsules used for the determination of EPA and DHA content, PV and p-AV, and average fill weight of the encapsulated products, was also noted (Supplementary information on methods). This shows that each laboratory was organized in its own manner to optimally use the available sample. One laboratory reported that the relative standard deviation of replicate determinations of capsule fill weight was less than 3 %, and less than 7 % for very viscous products.

The effect of this inter-laboratory variability on compliance reporting was shown by evaluating the rates of compliance based on the results from each individual laboratory (summarized in Supplementary Table 5). All the products tested by all laboratories complied with an EPA + DHA content of at least 80 % of the label claim. However, depending on the laboratory, the reported compliance with at least 100 % of claimed EPA + DHA content ranged from 54.1 % of products to 73.3 % of products. For PV, compliance (PV max of 5) ranged from 73.8%–94.7 %. For secondary oxidation, compliance (p-AV max of 20) ranged from 90 % to 100 % (of the non-flavored products). Although not all laboratories evaluated all products, the compliance rates varied substantially, up to approximately 20 %, depending on which laboratory would have been chosen for testing. In the present study, results were obtained by experienced laboratories that use validated methods, recommended by GOED (GOED, 2019). Inexperienced laboratories will likely produce results that have a higher probability to systematically under- or overreport. The selection of a proficient laboratory can be made based on their testing proficiency in a recent laboratory proficiency program. Taking inter-laboratory variability into account underscores the importance of verifying results before making declarations about compliance rates.

Product compliance was understood in a more detailed manner when the number of products that were compliant with all tested parameters was evaluated, *i.e.* for the present study with the label claim, primary and secondary oxidation. Of the 17 products that could be evaluated for all tested parameters, 13 products (77 %) complied with an EPA + DHA label claim of at least 80 % and the industry limits on primary and secondary oxidation. Compliance with at least 100 % of the label claim and both oxidation limits was achieved by eight products (47.1 %). Taking the limited number of products that could be evaluated into account, significant room for improvement exists for a major portion of the most widely sold omega-3 supplements on the U.S. market. This result does not mean that a significant portion of products available to American consumers do not comply with regulatory requirements in the U.S. or would be of poor quality. Furthermore, for a variety of reasons, many products cannot be tested for all their quality attributes, for example because the content of EPA and DHA is not provided on the product label, or because secondary oxidation cannot be reliably measured in a significant portion of products, such as those that are flavored or in phospholipid-rich oils.

Reports of low label claim compliance due to poor sample handling, inaccurate testing methodology and uncertified or inexperienced testing laboratories, can reduce consumer confidence and potentially lead to lower omega-3 intake. People in most countries currently already have low to very low blood levels of omega-3 LCPUFA, which are associated with an increased risk in cardiovascular related mortality (Stark et al., 2016). Omega-3 deficiencies can also be identified in specific life-stages when the demand for omega-3 LCPUFA intake is increased, for example during pregnancy (Zhang et al., 2018). Not meeting dietary recommendations for seafood consumption leads to low intakes and, consequently, low tissue levels of EPA and DHA, which is expected to have a long-term negative impact on a person’s risk for chronic disease, and may jeopardize optimal infant development (Carlson et al., 2018). The supplemental intake of omega-3 LCPUFA-rich dietary supplements can help to overcome the EPA and DHA intake gap, and a portion of U.S. adults increasingly value the consumption of such products on a regular basis. In order to present consumers with accurate information on dietary supplements, it is important that the assessment of, and reporting on, the quality of finished products is carried out in the best way possible.

5. Conclusion

This study has found that the 48 EPA/DHA omega-3 dietary supplements with the largest market penetration in the U.S. largely complied with the industry limits for oxidative quality of EPA/DHA oils set voluntarily by producers and finished products manufacturers. Most products adhere to the FDA requirement that natural ingredients should contain at least 80 % of the labeled content. It is nevertheless challenging to evaluate the compliance for products sold in the U.S. given the lack of government regulations on oxidative quality specific to dietary supplements, and content labeling requirements that are currently not clear. This study highlights the variety of omega-3 LCPUFA products available to consumers, some methodological limitations associated with currently available products and formulations, and the importance of taking into account analytical variability when reporting on compliance. Good product storage conditions are suggested based on absence of correlation between the chemical markers and the product expiration. Room for continued improvement in quality of EPA/DHA finished products in the U.S. is however suggested since nearly half of 17 tested products for which all quality parameters could be tested did not meet at least one of the oxidative quality criteria or the label claim for EPA + DHA content.

Funding

The purchase of products and shipping costs were financed by GOED. Laboratory services and materials were provided by each of the participating laboratories. G.B., H.R. and A.B. are employees of GOED. A.F., C.M., L.N., R.H., J.I., K.P., L.L. and A.I. are employees of companies that are members of GOED. A.I. was an employee of GOED at the time the study was conducted. S.W. is an employee of UC Davis, and A.P. was a M.Sc. student at UC Davis supported by funding from the Department of Chemistry at UC Davis and GOED.

Author contributions

A.B. and A.F. analyzed U.S. market data. A.I. collected finished products and organized sample distribution to the different laboratories. A.I. and G.B. coordinated the study. C.M., L.N., R.H., J.I., K.P., L.L., A.P. and S.W. performed chemical analysis and data analysis. G.B. and A.I. analyzed results and wrote the manuscript. H.R. provided input on regulatory aspects. A.B. provided input on statistical analysis. All authors revised the manuscript.

Declaration of Competing Interest

G.B., A.B. and H.R. are employees of the Global Organization for EPA and DHA Omega-3s (GOED), a 501(c)6 not-for profit trade association. A.F, C.M., L.N., R.H., J.I., L.P., L.L., and A.I. are employees of manufacturers of ingredients and supplements, and are members of GOED.

Acknowledgements

The authors thank the analytical laboratories for their collaboration and independent analyses. Vangie Lund is acknowledged for helping with sample organization.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jfca.2020.103435.

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