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Lipid Class Specific Quantitative Analysis of n-3 Polyunsaturated Fatty Acids in Food Supplements

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Supporting Information

ABSTRACT: Supplementation products containing n-3 PUFA from marine sources serve a large market. Although the amount of eicosapentaenoic acid and docosahexaenoic acid in the products is provided by the manufacturer, no or little information is available on their lipid pattern. Therefore, we quantitatively analyzed the fatty acid pattern in the lipid fractions triglycerides, phospholipids, ethyl esters, and free fatty acids in supplementation products by means of solid phase extraction and gas chromatography. Twelve products from the European and U.S. markets containing fish, krill, algal, or plant oil were analyzed. Total n-3 PUFA content ranged from 68 g/100 g fat (fish oil) to 42 g/100 g fat (algal oil) to 17 g/100 g fat (krill oil). On the basis of the n-3 PUFA containing lipid class, the supplements can be separated dominantly in ethyl ester, re-esterified triglyceride, triglyceride, and phospholipid containing products. Algae-based products contained natural triglycerides, krill oils a complex mixture of phospholipids, triglycerides, and free fatty acids, and fish oil products either ethyl esters, re-esterified triglycerides, or triglycerides. Even products of the same class and source showed distinct differences in their lipid pattern. A specification of the lipid composition of n-3 PUFA products would allow distinguishing the different (qualities of) supplements.

KEYWORDS: *lipid class, supplements, n-3 PUFA, fish oil, krill oil, algal oil, plant oil*

INTRODUCTION

The intake of polyunsaturated fatty acids (PUFA) affects human health. In the Western diet, large amounts of n-6 PUFA, particularly linoleic acid (18:2 n-6 from soy, safflower, and corn oil) are consumed. On the contrary, the intake of n-3 PUFA, particularly of the long-chain n-3 PUFA eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3), is low in nonfish eaters.[1−3] However, a growing body of evidence suggests beneficial effects of the intake of EPA and DHA on human health. For instance, these n-3 PUFA lower blood triglyceride levels and are incorporated into phospholipids in the membrane of cells, thereby affecting fluidity and lipid rafts.[1,4] Furthermore, EPA and DHA intake shows anti-inflammatory effects and reduces the risk for cardiovascular diseases.[1,4,5] It is believed that these effects are at least partly mediated by a modulation of the endogenous pattern of eicosanoids and other oxylipins.[1] Aside from a reduction of the formation of pro-inflammatory oxylipins such as series 2 prostaglandins (PG; e.g., PGE_2), EPA and DHA can give rise to anti-inflammatory, pro-resolving cardioprotective lipid mediators such as resolvins or epoxy-PUFA.[6,7]

Both n-6 and n-3 PUFA are essential nutrients, and mammals cannot transform one into the other. However, the long-chain n-3 PUFA EPA and DHA can be synthesized endogenously from α-linolenic acid (ALA, 18:3 n-3), found in land plants such as flaxseed,[2,4] although conversion rates are low in healthy subjects without nutritional deficiencies.[8] Therefore, the main source of EPA and DHA is the diet. Especially, fatty cold-water fish such as salmon or mackerel are rich in these FA.[1,2,9] Other nutritional sources contribute to the intake of EPA and DHA with only minor importance.[3] However, fish and consequently n-3 PUFA intake is low in Western countries such as the United States and Germany.[1,10,11] Several n-3 PUFA containing products are available as food supplements in the European and U.S. markets. Most products are oil-filled capsules with a high content of DHA and EPA. Additionally, other products such as emulsions or gums can also be found in supermarkets and pharmacies. The main natural sources for DHA and EPA comprise marine products, such as krill, algae, and fish.[12] Raw material for the production of fish oil is fish specifically caught for this purpose, but also includes by-catch and byproducts from fishery and fish meal production.[12] Among other methods, fish oil is mostly produced from the raw material by the wet rendering process, which includes cooking and pressing of the raw fish. The raw oil is dewatered and refined, yielding purified fish oil.[13] Usually, the lipids are transesterified to fatty acid ethyl esters (EE), allowing further purification and concentration by distillation or urea complexation.[14,15] The resulting DHA- and EPA-EE are directly used in supplements and pharmaceuticals. To generate the "more natural" binding form of the FA, several manufactures (trans-) re-esterify the EE to triglycerides (TG).[14,15]

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Table 1. Fat Content and Fatty Acid Pattern of n-3 PUFA Products[a]

			fatty acid profile (g fatty acids/100 g fat content)													
	fat content (g/100 g capsule)		Σ SFA	Σ MUFA	Σ PUFA	Σ n-9	Σ n-6	Σ n-3		EPA		DHA		DPA	ALA
product		ms							ms		ms		ms		
AO1	71.7 ± 0.3	66	19.9 ± 0.1	23.4 ± 0.8	44 ± 2	22.9 ± 0.8	3.5 ± 0.1	41 ± 1	na	12.1 ± 0.4	12	25.9 ± 0.9	21	2.87 ± 0.04	nd
AO2	68.5 ± 0.1	85[b]	20.3 ± 0.1	25.1 ± 0.1	46.4 ± 0.2	24.7 ± 0.2	3.552 ± 0.004	42.9 ± 0.2	30	13.2 ± 0.1	9.0	27.3 ± 0.1	18	2.190 ± 0.003	0.057 ± 0.003
PO	75.5 ± 0.5	73[b]	9.59 ± 0.03	19.6 ± 0.2	70.8 ± 0.6	18.8 ± 0.2	21.1 ± 0.2	49.8 ± 0.4	50	nd	na	0.1 ± 0.1	na	nd	49.6 ± 0.4
FO1	72.3 ± 0.0	74	6.7 ± 0.2	12.6 ± 0.1	54.6 ± 0.1	9.18 ± 0.03	3.44 ± 0.06	51.17 ± 0.05	60	27.18 ± 0.06	30	19.88 ± 0.02	20	3.43 ± 0.01	0.536 ± 0.000
FO2	70.2 ± 0.9	67[b]	5.3 ± 0.3	9.4 ± 0.1	58 ± 1	7.2 ± 0.1	3.51 ± 0.03	54 ± 1	60	28.2 ± 0.5	33	21.5 ± 0.4	22	4.18 ± 0.03	0.420 ± 0.007
KO1[c]	71.3 ± 0.2	69	19.6 ± 0.4	15.3 ± 0.5	19.0 ± 0.7	7.6 ± 0.3	1.80 ± 0.05	17.2 ± 0.6	30	10.3 ± 0.4	15	5.8 ± 0.2	9.0	0.29 ± 0.01	0.97 ± 0.02
KO2[c]	71.0 ± 0.1	66[b]	18.8 ± 0.6	12.9 ± 0.4	19.9 ± 0.5	6.6 ± 0.2	1.22 ± 0.05	18.7 ± 0.5	22	11.9 ± 0.3	14	5.2 ± 0.1	5.5	0.26 ± 0.01	1.83 ± 0.04
ES	70.0 ± 0.5	69[b]	0.74 ± 0.06	0.40 ± 0.03	84 ± 2	0.18 ± 0.01	3.3 ± 0.1	81 ± 2	84	43 ± 1	46	34.5 ± 0.9	38	3.1 ± 0.1	0.134 ± 0.001
FO3	68.8 ± 0.8	66[b]	1.78 ± 0.02	1.84 ± 0.05	63 ± 2	1.37 ± 0.05	2.14 ± 0.04	61 ± 1	58	4.7 ± 0.1	4.5	46 ± 1	45	9.8 ± 0.2	0.089 ± 0.001
FO4	65.4 ± 0.2	84[b]	1.01 ± 0.01	4.46 ± 0.04	70.8 ± 0.3	3.25 ± 0.02	3.34 ± 0.06	67.5 ± 0.3	70	45.2 ± 0.2	45	18.9 ± 0.1	20	2.78 ± 0.01	0.526 ± 0.001
Gum	4.2 ± 0.3	4.2[b]	27.0 ± 0.4	18.7 ± 0.2	31.5 ± 0.5	12.9 ± 0.2	3.72 ± 0.05	27.7 ± 0.5	25	5.5 ± 0.1	4.2	20.6 ± 0.3	21	0.93 ± 0.02	0.54 ± 0.02
Emul	80.19 ± 0.05	80[b]	26.7 ± 0.2	22.2 ± 0.3	32.5 ± 0.3	11.0 ± 0.1	3.25 ± 0.04	29.2 ± 0.2	29	16.2 ± 0.1	16	10.3 ± 0.1	9.8	1.85 ± 0.03	0.75 ± 0.01

[a]The products were based on algal oil (AO), plant oil (PO), fish oil (FO), krill oil (KO), or ethyl esters (ES) as well as n-3 PUFA gums (Gum) and a n-3 PUFA enriched emulsion (Emul). Fat content was determined gravimetrically from the total lipid extract, and fatty acids were quantified by means of GC-FID. For total n-3 PUFA, EPA, and DHA contents, the manufacturer's specification (ms) are given. na: no specifications were provided on the label; nd: fatty acid(s) were not detected. [b]Fat content was calculated on the basis of capsule weight. [c]Total lipid extraction was performed according to Twisselmann because polar lipids contained are not stable during Weibull−Stoldt extraction.

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However, it should be noted that these TG contain several n-3 PUFA per molecule, which rarely occurs naturally. Apart from these highly processed and concentrated products, purified oils from marine sources other than fish are used directly for food supplements, for example, krill oils. Krill oils contain n-3 PUFA in TG and a major portion as polar phospholipids (PL).[16,17] In addition, vegetarian alternatives for food supplements containing n-3 PUFA EPA and DHA are algae.[12] It should be noted that several oils from land plants such as flaxseed are sold alongside the marine n-3 PUFA products, which may serve as sources for ALA,[2,4,18] but do not contain relevant amounts of EPA and DHA.[19]

The total amount of n-3 PUFA and the content of other relevant PUFA are declared on the label of the supplements as requested by European and U.S. law. Despite the relevance of n-3 PUFA for nutrition and their economic importance (annual world market volume of about $U.S. 25 billion in 2011[12]), no or only little information about the lipid composition of the food supplements is available or provided by the manufacturer. New studies suggest that the bioavailability of n-3 PUFA differs between the lipid classes in which the n-3 PUFA are bound.[20] Therefore, we evaluated the quantitative pattern of n-3 PUFA in the major lipid classes in food supplements, that is, EE, TG, PL, and free fatty acids (FFA). We not only show the results from 12 exemplary products from both the European and U.S. markets but also provide an optimized method for the determination of the quantitative distribution of n-3 PUFA in the lipid classes. This will readily enable other researchers and institutions to expand this analysis to a larger, more representative set of n-3 PUFA supplements.

MATERIALS AND METHODS

Chemicals. Chloroform (uvasolv grade), ethanol (uvasolv grade), and ammonium acetate (p.a.) were obtained from Merck (Darmstadt, Germany). *n*-Hexane (HPLC grade) and methyl *tert*-butyl ether (HPLC grade) were purchased from Carl Roth (Karlsruhe, Germany). Methanol (HPLC grade) was purchased from J. T. Baker (Deventer, The Netherlands). Acetic acid (Acros Organics) was obtained from Fisher Scientific (Nidderau, Germany). Methyl tricosanoate (FAME 23:0, >98%) and cholesteryl heptadecanoate (CE 17:0, >98%) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Dihenarachidoyl-*sn*-glycero-3-phosphocholine (PL 21:0, >99%) was purchased from Avanti Polar Lipids (Alabaster, AL, USA). Ethyl tricosanoate (EE 23:0, >98%) was obtained from Cayman Chemicals (Ann Arbor, MI, USA). Pentadecanoic acid (FFA 15:0, >98%), methyl pentacosanoate (FAME 25:0, >99%, used as internal standard (IS) 2), and all other chemicals were purchased from Sigma-Aldrich (Schnelldorf, Germany).

n-3 PUFA Supplementation Products. Six n-3 PUFA products were obtained on the European market: algal oil Ovega3 Life Vegetarian (Energybalance AG, Muralto, Switzerland; AO1; purchased via Amazon Germany), fish oil Omega3 Loges (Dr. Loges + Co. GmbH, Winsen (Luhe), Germany; FO1; purchased at a local pharmacy, Hannover, Germany), Doppelherz Omega3 fish oil concentrate (Queisser Pharma, Flensburg, Germany; FO2; purchased via Meddox mail-order pharmacy, Limburg, Germany), pure NKO krill oil (Neptune Technologies & Bioressources Inc., Quebéc, Canada; KO1; purchased at Tresorix, Ruggell, Liechtenstein), Superba krill oil (KALA Health BV, Den Haag, The Netherlands; KO2; purchased via the KALA Health online shop), and ethyl ester oil Omacor (Abbott GmbH & Co. KG, Wiesbaden, Germany; ES; purchased at a local pharmacy, Hannover, Germany). Six n-3 PUFA products were obtained on the U.S. market (purchased at CVS Pharmacy, Davis, CA, USA): Super DPA menhaden fish oil (Swanson Health Products, Fargo, ND, USA; FO3), OceanBlue Omega 3 superconcentrated fish oil (Sancilio & Co., Inc., Riviera Beach, FL, USA; FO4), plant oil Vegetarian Omega 3-6-9 (Rexall Sundown Inc., Boca Raton, FL, USA; PO), algal oil Vegetarian Omega 3 (Rexall Sundown Inc.; AO2), Adult Gummies Fish Oil (Nature Made Nutritional Products, Mission Hills, CA, USA; Gum), and fish oil containing Omega Squeeze'n'Go (Rexall Sundown Inc.; Emul).

Determination of Total Fat Content, Cholesterol, Overall Fatty Acid Pattern, and Peroxide Value. The total fat content of all supplementation products was determined by gravimetry after lipid extraction according to the Weibull–Stoldt method performed as rapid microextraction.[21] For products KO1 and KO2 the total fat content was determined after lipid extraction according to the method of Twisselmann using a mixture of cyclohexane and ethanol (1:1) as extraction solvent.[22] The cholesterol content was determined after alkaline hydrolysis of the lipid extract (Twisselmann) by means of gas chromatography with flame ionization detection (GC-FID) as described[23] following derivatization with 1-(trimethylsilyl)imidazole. The overall FA patterns in the extracts were determined by GC-FID after transesterification of the lipids to fatty acid methyl esters (FAME) calculated as g FA/100 g fat (lipid extract). For each product, two independent extractions were carried out. The peroxide value (PV) was determined by iodometry as described.[24] In brief, the capsule content (~0.5 g) was dissolved in acidic potassium iodide, and a starch solution was added. The formed iodine was titrated with sodium thiosulfate.

Separation of Lipid Classes by Solid Phase Extraction (SPE). The capsule content or the extracts from methyl *tert*-butyl ether (MTBE)/methanol extraction[25] of the products Gum and Emul was fractionated by SPE into the lipid classes: ethyl and cholesterol esters (EE/CE), triglycerides (TG), free fatty acids (FFA), and phospholipids (PL). Separation was carried out according to the method of Kaluzny et al.[26] with modifications as described in detail in the Supporting Information. In brief, the lipid extract was dissolved in chloroform and separated using two Supelclean LC-NH_2 columns (100 mg, 1 mL, Sigma-Aldrich), which were washed with 2 column volumes of each elution solvent prior to sample loading. The neutral lipid fraction, containing EE/CE/TG, was eluted with 1.5 mL of chloroform/isopropanol (2:1 (v/v)). Free fatty acids and the polar lipid fraction were eluted with 2 mL of diethyl ether/acetic acid (98:2 (v/v)) and 3 mL of methanol, respectively. The neutral lipid fraction was reconstituted in hexane and subjected to a second column; the CE/EE fraction was eluted with 4 mL of hexane and the TG fraction with 4 mL of hexane/diethyl ether/dichloromethane (89:1:10 (v/v/v)).

For quantification, an IS was added for each lipid class (10 μL containing 0.75 mM FA equivalent concentration) prior to SPE: ethyl tricosanoate (EE 23:0), glyceryl trinonadecanoate (TG 19:0), pentadecanoic acid (FFA 15:0), 1,2-dihenarachidoyl-*sn*-glycero-3-phosphocholine (PL 21:0). FAME 25:0 served as IS 2 (added after SPE separation) and was utilized to calculate the recovery rates of IS 1 as described.[27,28] All separations were carried out in triplicate, and results are calculated as the mean ± standard deviation.

Gas Chromatographic Quantification of Fatty Acids as Fatty Acid Methyl Esters. FA were quantified by means of GC-FID on a 6890 series instrument (Agilent, Waldbronn, Germany) as described.[25] In brief, lipid extracts/fractions were transesterified utilizing acetyl chloride in methanol (1:9) at a temperature of 95 °C for 1 h.[29] The resulting FAME were separated on a 30 m × 0.25 mm FAMEWAX column (0.25 μm film, Restek, Bad Homburg, Germany) within 24 min. The carrier gas was helium (1.5 mL/min, constant flow), and the oven temperature was ramped from 140 to 210 °C within 7 min and to 230 °C within 10 min and held at 230 °C for 7 min. Absolute FA content and relative FA pattern were calculated using odd-chain IS (see above) based on response factors.[25,30]

RESULTS

The total fat content of the products is shown in Table 1 and ranged for the capsules between 65.4 ± 0.2 g/100 g (FO4) and 75.5 ± 0.5 g/100 g (PO). The lowest fat content was observed for the product Gum (4.2 ± 0.3 g/100 g); the highest fat content was observed for the product Emul (80.19 ± 0.05 g/100 g).

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The cholesterol content was 1.4 ± 0.1 and 0.99 ± 0.01 g/100 g fat for products KO1 and KO2, respectively. Table 1 also shows the overall FA profile of the products. The pattern of saturated (SFA), monounsaturated (MUFA), n-3 and n-6 PUFA varied considerably between products from different sources, whereas similar patterns were observed for products of the same source (Figure 1; Table 1).

The amount of SFA was low in fish oil based products. The lowest amount was found in the pharmaceutical product ES with 0.74 ± 0.06 g/100 g fat and the highest in the product Gum with 27.0 ± 0.4 g/100 g fat. MUFA were also lowest in product ES (0.40 ± 0.03 g/100 g fat) and highest in the algal oil product AO2 with 25.1 ± 0.1 g/100 g fat. In most of the marine products only low amounts of n-6 PUFA were found (1.22 ± 0.05−3.72 ± 0.05 g/100 g fat), whereas the plant-based product PO contained 21.1 ± 0.2 g/100 g fat.

The content of n-3 PUFA varied considerably between the products from 17.2 ± 0.6 g/100 g fat (KO1) to 81 ± 2 g/100 g fat (ES; Table 1; Figure 1). The lowest amount of n-3 PUFA in

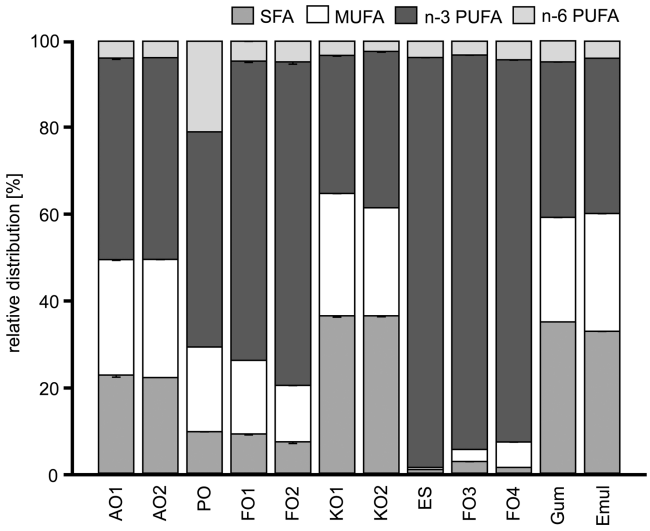

Figure 1. Relative distribution of saturated (SFA), monounsaturated (MUFA), n-3 and n-6 polyunsaturated fatty acids (PUFA) in supplements. The products were based on algal oil (AO), plant oil (PO), fish oil (FO), krill oil (KO), or ethyl esters (ES) as well as n-3 PUFA gums (Gum) and a n-3 PUFA enriched emulsion (Emul). The fatty acids were quantified in the lipid extracts by means of GC-FID as fatty acid methyl esters (FAME).

fat was found in the krill oils (KO1 and KO2) and the products Gum and Emul (from 17.2 ± 0.6 to 29.2 ± 0.2 g/100 g fat). The other fish oil based products showed higher n-3 PUFA content between 51.17 ± 0.05 and 67.5 ± 0.3 g/100 g fat. The vegetarian products showed intermediate n-3 PUFA amounts: in algal oil based products from 41 ± 1 to 42.9 ± 0.2 g/100 g fat was determined; in the plant oil based product the n-3 PUFA amount was 49.8 ± 0.4 g/100 g fat.

The main n-3 PUFA in the supplements were EPA and DHA, which make up >90% of the n-3 PUFA in most of the products (Table 1). The absolute amount of EPA and DHA in grams per capsule also differed between the products (Figure 2), varying from 0.03 g/gum (Gum) to 0.79 g/capsule (ES). Only the product PO did not contain significant amounts of EPA or DHA, as the main n-3 PUFA in this product is ALA (49.6 ± 0.4 g/100 g fat). The product FO3 contained, aside from EPA and DHA, 9.8 ± 0.2 g/100 g fat docosapentaenoic

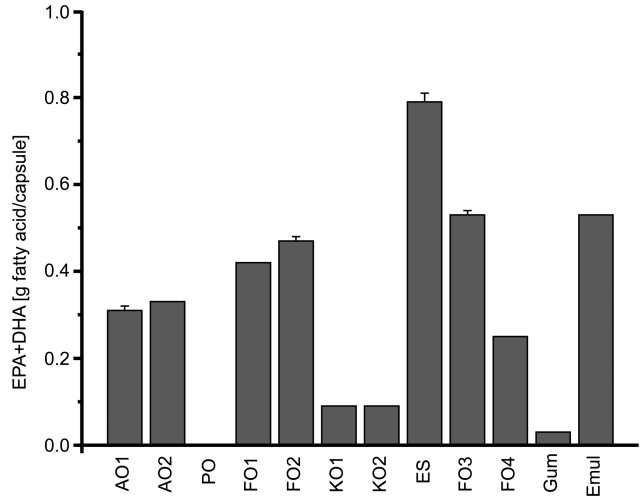

Figure 2. Determined EPA and DHA contents in n-3 PUFA supplementation products. Products were based on algal oil (AO), plant oil (PO), fish oil (FO), krill oil (KO), or ethyl esters (ES) as well as n-3 PUFA gums (Gum) and a n-3 PUFA enriched emulsion (Emul). The fatty acids were quantified in the lipid extracts by means of GC-FID as fatty acid methyl esters (FAME). For products FO1, KO2, and Gum the daily intake of two capsules/gums is recommended by the manufacturer.

acid (DPA, 22:5 n-3). The amount of DPA in all other products was lower (between 0.26 ± 0.01 and 4.18 ± 0.03 g/100 g fat, Table 1).

If the lipid content and FA pattern were provided by the manufacturer, it was mostly in good agreement with the obtained results (Table 1). Discrepancies were observed only for the krill oil based product KO1 and for the fat content of the products AO2 and FO4.

The lipid pattern of the products is shown in Figure 3 and in detail in the Supporting Information (Table S1). The relative distribution of the lipid classes is shown separately for total FA (Figure 3A), n-3 PUFA (Figure 3B), and n-6 PUFA (Figure 3C) as well as EPA, DHA, and ALA (Figure 3D−F).

FA in algal oil (AO1 and AO2) were only found in the TG fraction. In the other vegetarian product containing n-3 PUFA obtained from flaxseed and sunflower oil (PO), FA were also found solely in the TG fraction (Figure 3A). Similarly, FO1 and FO2 and the oil extract of Gum and Emul contained mainly TG. It should be noted that products FO1 and FO2 contained a portion of FA bound in mono- and diglycerides, which were not taken into account for the calculation of the relative lipid class pattern. Minor amounts (1−5%) of total FA in the TG containing fish oils FO1 and FO2 and of the krill oils were found in the EE/CE fraction. To differentiate between EE and CE, a direct GC analysis (without derivatization) was performed of the mixed EE/CE fraction. In this way the main FA EPA and DHA were identified to be EE (>98% of EPA and DHA for products FO1 and FO2, >90% of EPA and DHA for products KO1 and KO2) (Figure S4). By contrast, the fish oils FO3 and FO4 as well as ES contained predominantly EE (>95%). For TG and EE containing oils, the distribution of n-3 PUFA in the lipid fraction correlated to the distribution of total FA (Figure 3A,B).

For all marine products the n-3 PUFA occurred dominantly as EPA and DHA (Table 1). Thus, the relative distribution of EPA and DHA correlated in general with that of n-3 PUFA (Figure 3B,D,E). Interestingly, the amount of n-3 PUFA in TG

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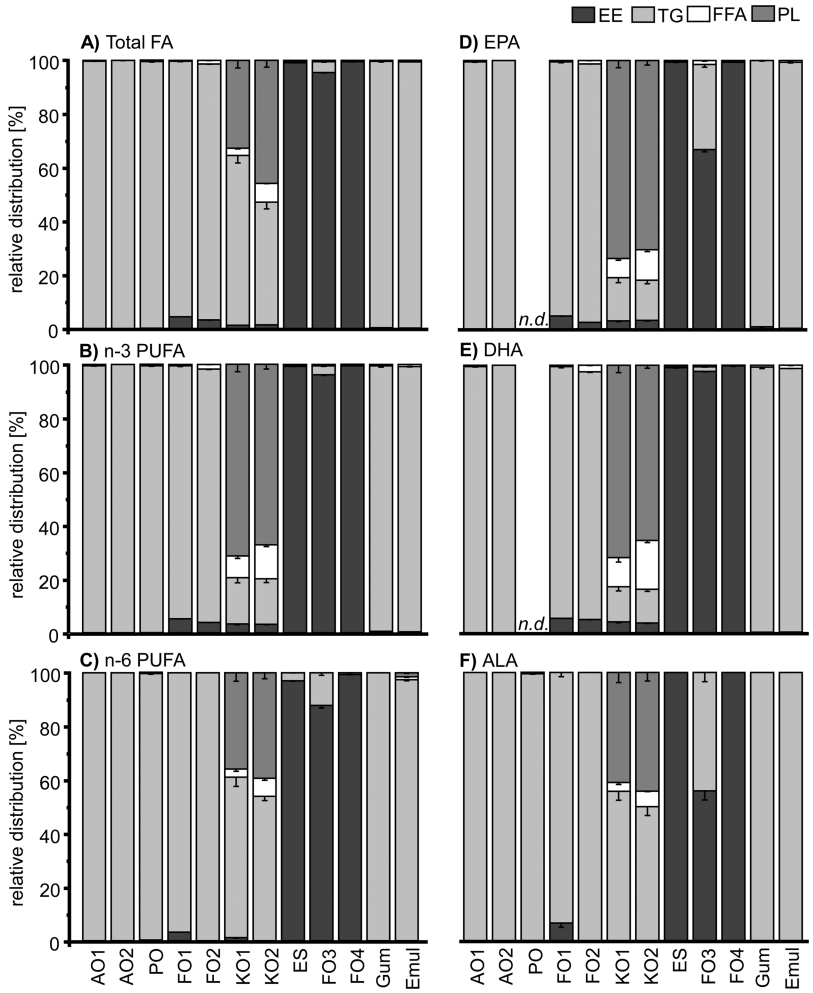

Figure 3. Lipid class pattern of n-3 PUFA supplements. The lipid classes were separated into ethyl esters (EE), triglycerides (TG), free fatty acids (FFA), and phospholipids (PL) in n-3 PUFA supplementation products based on algal oil (AO), plant oil (PO), fish oil (FO), krill oil (KO), or ethyl esters (ES) as well as n-3 PUFA gums (Gum) and a n-3 PUFA enriched emulsion (Emul). Shown is the relative distribution for (A) total fatty acids, (B) n-3 PUFA, (C) n-6 PUFA, (D) EPA, (E) DHA, and (F) ALA. The capsule content (MTBE extract in the cases of Gum and Emul) was separated by SPE, and the fatty acid concentrations in the fractions were determined by GC-FID as fatty acid methyl esters (FAME). The relative amount of fatty acids in each fraction was calculated as a percentage of the sum of total FA. n.d., fatty acid(s) were not detected (<0.1%). It should be noted that the re-esterified fish oils (FO1, FO2) contain also di- and monoglycerides, which were not taken into account for the calculation of the relative lipid class pattern.

is not representative for the EPA content of FO3: Only 3% of n-3 PUFA was found in the TG fraction, whereas 32% of EPA was detected as TG. With respect to the lipid binding form, there were remarkable differences between the EE containing fish oil products: In FO3 32% of EPA was found as TG, whereas FO4 contained EPA only as EE.

PL were not found in the fish, plant, and algal oil based products. However, the majority of n-3 PUFA (67−71%) in krill oil was bound in PL. Although the patterns of total FA and n-3 PUFA were similar in all products, the distribution of total FA in krill oil (Figure 3A) was different from that of n-3 PUFA (Figure 3B). Total FA (dominantly 14:0, 16:0, 16:1n-7, 18:1n-9) were mostly found as TG (46−63%), whereas only 33−46% were bound in PL. Consistently, the n-6 PUFA in krill oil were mainly bound in TG (54−60%), whereas only 36−39% were found in the PL fraction (Table S1).

The krill oils (KO1 and KO2) were the only products containing relevant amounts of FFA: 8−13% of n-3 PUFA and 3−7% of total FA. All other tested products contained <2% of n-3 PUFA and <1.5% of total FA as FFA.

Overall, the n-3 PUFA supplements showed considerable differences in n-3 PUFA content (Table 1; Figure 1) and in the pattern of lipids (Figure 3) depending on the origin of the contained oil, thereby reflecting the technology used for production.

DISCUSSION

Supplementation products containing n-3 PUFA serve a large economic market in Western countries. However, detailed information on the lipid composition of n-3 PUFA supplementation products is scarce. Particularly, information is lacking in which lipid classes n-3 PUFA occur. Therefore, we analyzed and characterized 12 n-3 PUFA food supplements from the European and U.S. markets with respect to the lipid composition as well as the FA profile in each fraction, the total FA profile, and fat content. The analyzed n-3 PUFA products are based on different sources. Most products are from marine origin, such as fish (FO), krill (KO), or algal oil (AO), and one of the products is based on plant oil (PO). In addition to the food supplements, one fish oil based pharmaceutical n-3 PUFA

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product (ES) was included in the analysis. The total n-3 PUFA content as well as the amount of EPA and DHA was generally in agreement with the manufacturer's information provided on the label (Table 1). The land plant product (PO) tested contained neither EPA nor DHA in relevant quantities and the main n-3 PUFA was, as expected, ALA.

For the products from marine sources, distinct differences in the FA pattern and absolute concentrations of FA were found. On the basis of the production process and source (fish, krill, and algae) the products can be separated in four groups. The pharmaceutical ES contained the highest amounts of EPA and DHA present solely as EE (>99%), consistent with the manufacturer's information. In the fish oil food supplement products FO3 and FO4, FA were dominantly found as EE (>96%), although at lower EPA and DHA concentrations than in the pharmaceutical product (Table 1). Both supplementation products showed distinct differences in the lipid pattern, as FO3 consisted dominantly of DHA (Table 1) and contained a relevant portion of EPA (32%) as TG and about 1.5% as FFA. In the dominantly EPA containing product FO4 >99% of all FA were present as EE.

The almost identically presented (in terms of labeling and advertisement) fish oil supplements FO1 and FO2 contained the major part of FA as TG. Because of the high content of n-3 PUFA in these TG, which is by far higher than that of fish-fat,[12] it is likely that these TG are so-called re-esterified TG (rTG) generated via EE by transesterification. This "more natural" binding form is a common n-3 PUFA enriched lipid product.[14,15] The assumption that FO1 and FO2 contain rTG is further supported by the finding that 3−4% of FA in these products were found in the EE fraction. The identity of at least 98% of both EPA and DHA as EE in this fraction was confirmed by direct GC analysis and quantification of EPA and DHA as EE (Figure S4). Because EE do not occur naturally, trace amounts of EE resulting from incomplete re-esterification may serve as an indicator for rTG in addition to the occurrence of mono- and diglycerides.

The analyzed n-3 PUFA enriched gummies and emulsions contain n-3 PUFA from fish oils. All FA, including n-3 PUFA, were found in the TG fraction. No residual EE were found, and the contents of n-3 PUFA, EPA, and DHA corresponded to those of nonconcentrated fish oil.[12] Thus, it can be assumed that these products contain natural TG made from purified and refined fish oil.

Algae are the primary producer of (marine) long-chain n-3 PUFA. Thus, algal products are the (only relevant) vegetarian source of EPA and DHA.[31] The tested products showed a fat content comparable to that of the fish oil products, with a lower concentration of n-3 PUFA (Table 1; Figure 1) because of higher contents of SFA, MUFA, and n-6 PUFA. Both tested products contained FA only as TG. Moreover, the observed pattern of SFA and MUFA, n-6 PUFA, and n-3 PUFA (EPA and DHA) was within the range of algal fat of different species, for example, *Schizochytrium*.[12] On the basis of this finding and the lack of EE it can be assumed that these products contain purified and refined algal oil containing natural TG.

The land plant product (PO) was the only product that contained relevant amounts of ALA (49.6 ± 0.4 g/100 g fat), whereas the ALA content in all other products was below 2 g/100 g fat. Moreover, the content of n-6 PUFA was clearly higher for PO (21.1 ± 0.2 g/100 g fat) compared to the products from marine sources (from 1.22 ± 0.05 to 3.72 ± 0.05 g/100 g fat) (Table 1). Due to the FA composition of PO and the presence of all FA in the TG fraction, it contains most likely natural TG. However, as conversion rates of ALA to long-chain n-3 PUFA are low in healthy subjects on a Western diet, the product PO cannot be considered a relevant source for EPA and DHA.[8]

The krill oil based supplements differed considerably from fish and algal oil based products. The n-3 PUFA content in fat was with 17−19% lowest among the supplements tested (Table 1). Whereas the other products consisted mainly of one lipid class, the lipid pattern of the krill products was diverse, comprising PL (33−46% of total FA), TG (46−63% of total FA), and FFA (3−7% of total FA). This indicates that the content is only little processed natural krill fat, which has not been refined, enriched, and/or chemically modified. Consistently, the oil was cloudy and had a significant odor. A minor portion of total FA (1%) was found in the EE/CE fraction. In earlier studies from 2 ± 3 to 27 ± 9% of lipids are described as steroid esters for *Euphausia crystallorophias*.[32] In other krill species, such as *Euphausia superba*, one of the krill species mainly used for human consumption,[33] no or little steroid esters were found.[17,32] With a cholesterol content for the krill oil based products of 1.4 ± 0.1 g/100 g fat (KO1) and 0.99 ± 0.01 g/100 g fat (KO2), one could assume that a significant portion of n-3 PUFA-CE is present in the krill oil products. However, >90% of both EPA and DHA in the EE/CE fraction could be identified as EE by GC-FID analysis of the underivatized EE/CE fraction (Figure S4). A contamination of the krill oil products with (non-natural) EE during the production process is the most likely explanation for this finding.

It is highly interesting that the dominant portions of EPA and DHA in krill products were bound in polar lipids (Figure 3B,D,E), whereas—as expected for a natural oil—the majority of FA was bound in TG (Figure 3A). These findings are in line with a previous study of krill oil, in which n-3 PUFA were mainly found in PL but the majority of SFA was bound to TG.[34] With regard to the total FA, we detected 46−63% as TG and 33−46% as PL in the krill oil products. Castro-Gómez found about equal amounts of FA in TG and PL (49 and 44%),[35] whereas Gigliotti found 1−3% in TG and 20−33% of FA in PL,[34] Araujo 12−30% in TG and 19−81% in PL,[16] Bottino 8−36% in TG and 53−57% in PL,[32] and Phleger 2−54% in TG and 36−96% in PL.[17] The large range of variation between the levels can be explained by differences between krill species and gender as well as variations during season, diet, or maturity.[17,36] In contrast to the other products, in which FFA are removed during refining/purification, krill oil products contained a relevant amount (3−7%) of FFA (Table S1). The concentration of FFA can be regarded as a quality marker for these oils, as the content increases rapidly between the harvest and freezing/processing and with the storage temperature of the raw material.[13] With respect to the shelf life, the high FFA content of up to 13% of n-3 PUFA for KO2 could be highly problematic for the stability of the krill oil product because free PUFA are prone to (aut)oxidation, leading to rancidity. A common parameter for the determination of the degree of oxidation is the peroxide value (PV), giving an estimation of the primary lipid oxidation products (hydroperoxides).[37,38] The PV threshold for food oils is suggested as 5 for refined oils and 10 for native oils.[39] However, for the krill oil based products the PV was found to be <1 mequiv O_2/kg (see Table S2).

The 11 EPA and DHA containing supplements from three different marine sources (algae, fish, and krill) contained n-3 PUFA as: PL and TG and, naturally not occurring, EE and rTG formed by transesterification. It is beyond doubt that all

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different classes of lipids can be cleaved and absorbed in the human gastrointestinal tract and are incorporated or catabolized. TG with long-chain FA are cleaved at the *sn*-1 and *sn*-3 positions by the action of lipases to form 2-monoglycerides (2-MG) and FFA.[40] EE can also be hydrolyzed by the action of pancreatic lipases and are absorbed by the cells as FFA.[41] In contrast, PL are cleaved by phospholipase A_2, yielding 1-lysophospholipids and FFA.[40] All hydrolysis products of TG, EE, and PL are absorbed by enterocytes, re-esterified, and incorporated in chylomicrons. Differences in bioavailability of FA in the different lipid binding forms could result from differences in velocity of the involved lipase or differences in absorption of the cleavage products.[20] With regard to the absorption of long-chain n-3 PUFA, the position in the lipid molecule is relevant. For example, in natural krill oil the majority of n-3 PUFA are bound at the *sn*-2 position of the PL molecule.[42] By cleavage of the PL by phospholipase A_2, this FA will most likely be absorbed as FFA. However, an increased incorporation of DHA into lymph PL and HDL was shown when DHA was bound to the *sn*-1 position of PL and absorbed as lyso-PL compared to the FFA form.[43] In natural fish oil the long-chain n-3 PUFA are mostly bound to the *sn*-2 position of the TG molecule. Therefore, cleavage by lipase action results in a long-chain n-3 PUFA MG,[20] which may be absorbed differently from the cleavage products of PL. Furthermore, the binding form of the FA influences the structure of mixed micelles and chylomicrons, which in turn may affect the accessibility for lipases and the absorption of the micelles.[44,45] Several studies have investigated differences in the absorption rate and thus bioavailability of n-3 PUFA bound in PL, TG, rTG, and EE. Overall, the results are conflicting.[20] Thus, absolute n-3 PUFA content seems to be much more relevant for the effective dose absorbed per gram fat of the product than the observed differences in bioavailability. For example, for PL, a slightly (but not significantly) higher bioavailability was found in comparison to EE, which is 1.7 for EPA and DHA.[44] With the n-3 PUFA content in krill oil products taken into account (2−3-fold lower than in the fish oil based products (Table 1)), the effective dose, when ingesting the same amount of total fat, is by far lower for the krill oil based products in comparison to EE based products.

The different marine sources raise questions regarding their production and sustainability. Answering this question is clearly beyond the scope of this work; however, it has to be kept in mind that fish oil is produced on a large scale as a byproduct from the fishing industry (annual catches 91 million tons/year in 2012[46]) and as a byproduct from fish meal production (about 20 million tons/year in 2012[46]). Krill is caught at a comparably low scale (188.000 tons/year in 2012[46]), and krill oil products have only a small market share. If one considers krill production, it has to be kept in mind that krill is the base of the trophic chain in the sea and enhanced catch may massively disturb the marine ecosystem.[47] By contrast, algae may be grown on a large scale; however, their current use is limited because of high production costs.[19] Although they currently cannot replace fish oil products because of the limited production capacities, algal products may be a promising future source of n-3 PUFA.

In the supplements n-3 PUFA are clearly the value-determining content. The consumer should not only be informed about the total n-3 PUFA, EPA, and DHA contents (as it is currently the case) but also get information on the natural source and detailed data on the lipid composition. In the present work, we provide data on the lipid composition of a set of 12 products from the European and U.S. markets. It is demonstrated that the products can easily be separated in different classes by their lipid pattern, which may be used in the future to assess the correct labeling of the natural source and the quality of the products. Moreover, the described optimized SPE method in combination with established instrumental analysis can easily be used in other laboratories to expand the investigation to other products, to gain a representative data set on the lipid composition of n-3 PUFA products on the world market.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.6b03745.

Protocol S1, Figures S1−S4, Tables S1 and S2 (PDF)

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Notes

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■ ABBREVIATIONS USED

ALA, alpha-linolenic acid; AO, algal oil; CE, cholesterol ester; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid (n3); EE, (fatty acid) ethyl ester; ES, pharmaceutical ethyl ester product; EPA, eicosapentaenoic acid; FFA, free fatty acid; FA, fatty acid; FAME, fatty acid methyl ester; FO, fish oil; GC-FID, gas chromatography-flame ionization detection; IS, internal standard; KO, krill oil; MG, monoglyceride; MUFA, monounsaturated fatty acid; MTBE, methyl *tert*-butyl ether; PL, phospholipid; PO, plant oil; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; rTG, re-esterified triglyceride; TG, triglyceride

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Supporting Information

Lipid Class Specific Quantitative Analysis of n-3 Polyunsaturated Fatty Acids in Food Supplements

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Protocol S1: Detailed protocol for the fractionation of lipid extracts

The method describes an optimized solid phase extraction procedure on an amino-propyl column according to Kaluzny et al. [1] for the separation of CE/EE, TG, FFA and PL fractions. The overall separation efficacy is shown in Fig. S1.

- **Dissolve** the lipid(extract) in chloroform ($CHCl_3$) yielding a concentration of ~1.5 mg/mL
- **Transfer** 100 µL in a glasstube
- **Add**10 µL of antioxidant BHT (0.2 mg/mL in methanol)
- **Add**10 µL of internal standard solution in $CHCl_3$ containing
  - IS-EE: Ethyl tricosanoate (EE 23:0), 750 µM[1]
  - IS-TG: Glyceryl trinonadecanoate (TG 19:0), 250 µM
  - IS-FFA: Pentadecanoic acid (FFA 15:0), 750 µM
  - IS-PL: 1,2-dihenarachidoyl-sn-glycero-3-phosphocholine (PL 21:0), 375 µM
- **Vortex** sample

- **Wash SPE column 1** (Supelclean LC-NH_2 SPE Tube,100 mg,1 mL, Sigma-Aldrich, Schnelldorf, Germany)
  - 2 mL methanol
  - 2 mL diethyl ether/acetic acid, 98/2 (*v/v*)
  - 2 mL chloroform/isopropanol, 2/1 (*v/v*)
  - 2 mL *n*-hexane
- **Equilibrate SPE column 1** with 400 µL *n*-hexane without allowing the sorbent to dry
- **Load lipid** sample on column and wash vial with 200 µL $CHCl_3$
  - Let sample completely soak into the column bed
  - Collect eluate from sample loading in neutral lipid (TG/CE/EE) fraction
- **Elute neutral lipid (TG/CE/EE) fraction** with 1.5 mL chloroform/isopropanol, 2/1 (*v/v*)
- **Elute free fatty acid fraction (FFA)** with 2 mL diethyl ether/acetic acid, 98/2 (*v/v*)
  - CAVE: The FFA fraction must be neutralized before evaporation (Fig. S2). For this purpose add 2 mL aqueous $NaHCO_3$ (1 M) in the vial prior elution, shake sample and collect organic (upper) layer.
- **Elute polar lipid fraction (PL)** with 3 mL methanol

- **Wash SPE column 2** (Supelclean LC-NH_2 SPE Tube, 100 mg,1 mL, Sigma-Aldrich, Schnelldorf, Germany)
  - 2 mL *n*-hexane/diethyl ether/dichloromethane, 89/1/10 (*v/v/v*)
  - 2 mL *n*-hexane
- **Equilibrate SPE 2 column** with 400 µL *n*-hexane without allowing the sorbent to dry

- **Evaporate neutral lipid fraction** to dryness and reconstitute in 200 µL *n*-hexane
- **Load reconstituted neutral lipid fraction** on column and wash vial with 200 µL *n*-hexane
- **Elute cholesterol ester / ethyl ester (CE/EE) fraction** with 4 mL *n*-hexane

    - CAVE: Lower elution volume for CE/EE fraction does not fully elute EE (Fig. S3)
- **Elute triglyceride (TG) fraction** with 4 mL *n*-hexane/diethyl ether/dichloromethane, 89/1/10 (*v/v/v*)
- (*optional*) **Elute DG and MG** in one fraction with chloroform/methanol, 2/1 (*v/v*)

- **Add internal standard 2** to all four lipid fractions: 10 µL FAME 25:0, 750 µM in ethanol/chloroform (9/1)
- **Evaporate all lipid fractions to dryness**
    - Vacuum centrifuge (35 °C, 1 mbar, 20-40 min)
- **Reconstitute** fractions in 400 µL *n*-hexane and transesterify fatty acids with acetylchloride in methanol (1/9) to fatty acid methyl esters (FAME) as described by Ostermann et al. [2]
- **Quantify FAME by means of gas chromatography flame ionization detection (GC-FID) [2]**
    - Calculate recovery rate of IS using IS2 (Fig. S1)
    - Calculate lipid concentration in the fractions using the fraction specific IS

[1] For the analysis of extracts from biological samples containing cholesterol esters (CE), the IS-EE is replaced by IS-CE: Cholesteryl heptadecanoate (CE 17:0), 750 µM

[1] Kaluzny, M. A.; Duncan, L. A.; Merritt, M. V.; Epps, D. E., Rapid separation of lipid classes in high yield and purity using bonded phase columns. *J Lipid Res* **1985,** *26*, (1), 135-40.

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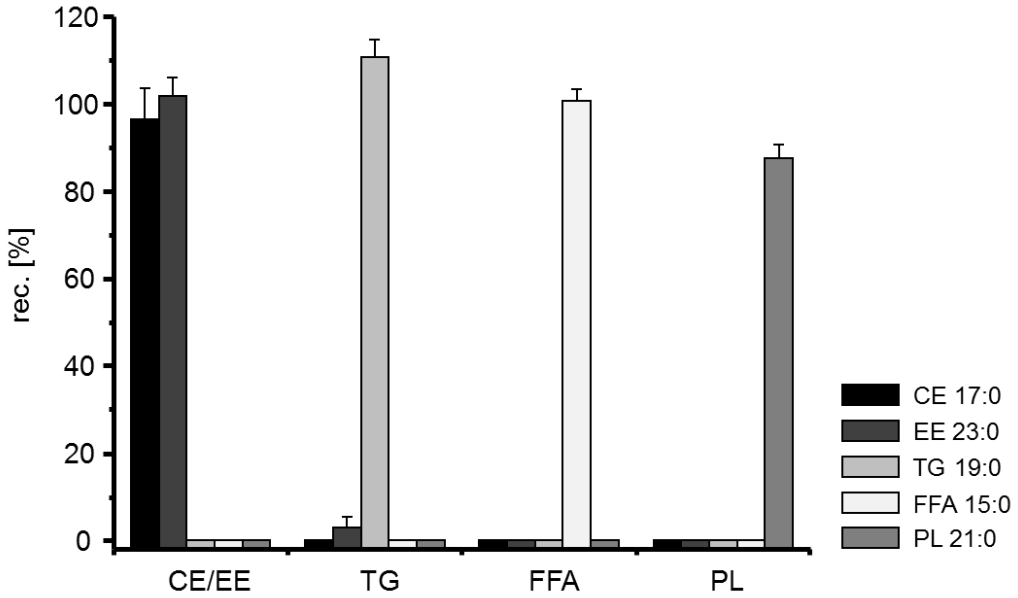

Fig. S1: Recovery rates of fraction specific internal standards.
Cholesterol esters (CE 17:0) and ethyl esters (EE 23:0), triglycerides (TG 19:0), free fatty acids (FFA 15:0) and phospholipids (PL 21:0) were separated by SPE with two aminopropyl columns. Recovery was calculated based on the concentration of the unfractionated standard.

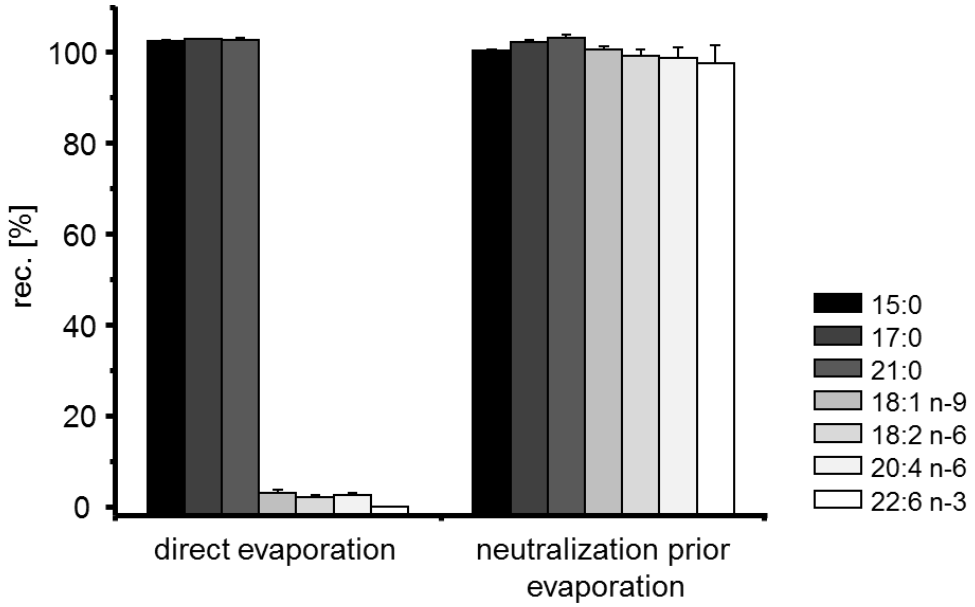

Fig. S2: Recovery rate of saturated and unsaturated fatty acids in the FFA fraction.
The elution solvent for the FFA fraction (2 mL diethyl ether/acetic acid, (98/2, *v/v*)) was spiked with different FFA (10 nmol). The solution was evaporated directly **(A)** and after neutralization of the organic phase by addition of 2 mL aqueous $NaHCO_3$ (1 M) **(B)**.

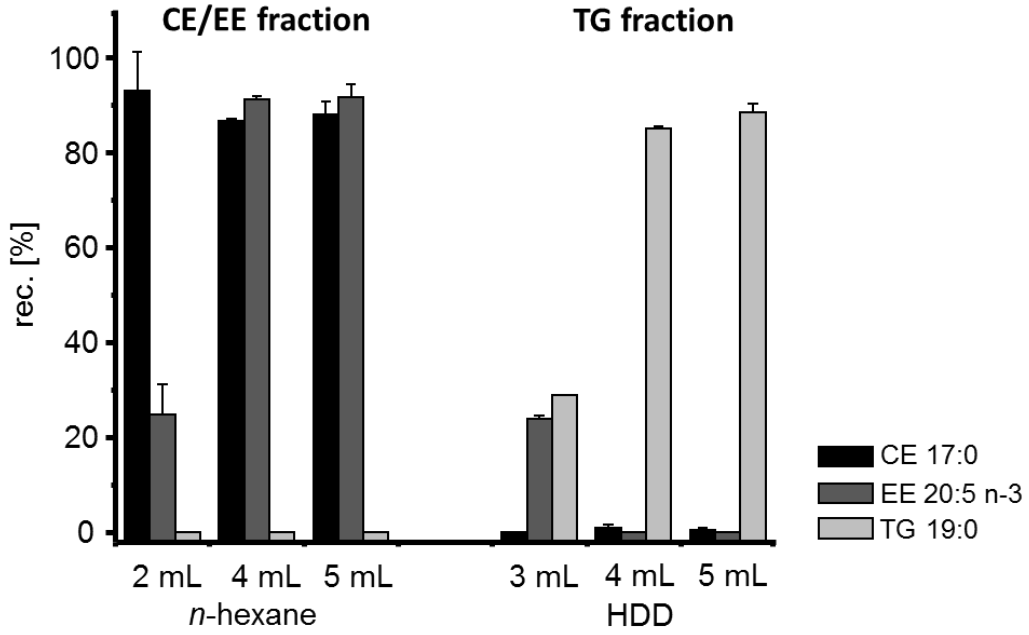

Fig. S3: Recovery rate of lipids CE 17:0, EE 20:5 n-3 and TG 19:0 in the CE/EE and TG fraction of the SPE.
As shown for both fractions an elution volume of at least 4 mL *n*-hexane and *n*-hexane/diethyl ether/dichloromethane (HDD, 89/1/10, *v/v/v*) is required to elute the fractions. Recovery was calculated based on the concentration of the unfractionated standard.

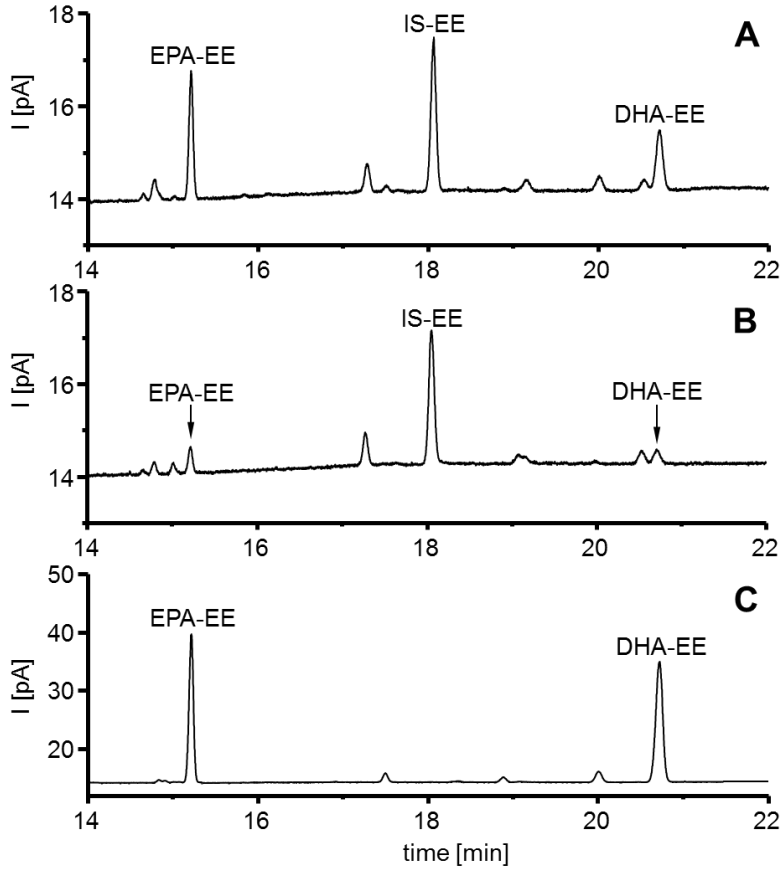

Fig. S4: GC-FID chromatogram showing signals for the ethyl esters (EE) of EPA and DHA in the CE/EE fraction of products FO1 (A) and KO1 (B).
EE were detected after direct injection of the underivatized CE/EE fraction and quantified using the fraction specific IS ethyl tricosanoate (EE 23:0). A standard (0.2 mg/mL) of EPA-EE and DHA-EE is exemplary shown in panel **(C)**.

Tab. S1: Relative distribution of fatty acids among the analyzed lipid fractions.

Relative distribution of total fatty acids (All FA), saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA), n-3 and n-6 polyunsaturated (n-3 and n-6 PUFA) fatty acids as well as EPA, DHA, DPA and ALA among ethyl esters (EE), triglycerides (TG), free fatty acids (FFA) and phospholipids (PL) as percentage of total fatty acids for twelve n-3 PUFA products based on algal oil (AO), plant oil (PO), fish oil (FO) or krill oil (KO) or pharmaceutical ethyl esters (ES) as well as n-3 PUFA gums (Gum) and a n-3 PUFA enriched emulsion (Emul).The diluted capsule content (MTBE extract in case of Gum and Emul) was separated by SPE followed by derivatization and FAME analysis by means of GC-FID.

Concentrations for sum parameters were calculated based on the sum of fatty acids that were >LOQ.

[a]100 % of the fatty acids were detected in one fraction, no detection in other lipid fractions.

Product	Fraction	% of total fatty acids										
		All FA	Σ SFA	Σ MUFA	Σ PUFA	Σ n-3 PUFA	Σ n-6 PUFA	Σ n-9 MUFA	EPA	DHA	DPA	ALA
AO1	EE	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*
	TG	99.7 ± 0.1	100[a]	99.7 ± 0.1	99.5 ± 0.1	99.5 ± 0.1	100[a]	99.7 ± 0.1	99.5 ± 0.1	99.5 ± 0.1	100[a]	100[a]
	FFA	0.3 ± 0.1	*n.d.*	0.3 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	*n.d.*	0.3 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	*n.d.*	*n.d.*
	PL	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*
AO2	EE	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*
	TG	99.95 ± 0.01	100[a]	99.8 ± 0.1	100[a]	100[a]	100[a]	99.82 ± 0.05	100[a]	100[a]	100[a]	100[a]
	FFA	0.05 ± 0.01	*n.d.*	0.2 ± 0.1	*n.d.*	*n.d.*	*n.d.*	0.18 ± 0.05	*n.d.*	*n.d.*	*n.d.*	*n.d.*
	PL	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*
PO	EE	0.10 ± 0.01	*n.d.*	0.10 ± 0.01	0.12 ± 0.01	0.066 ± 0.004	0.24 ± 0.03	0.11 ± 0.01	*n.d.*	*n.d.*	*n.d.*	0.066 ± 0.004
	TG	99.5 ± 0.2	100[a]	99.4 ± 0.3	99.4 ± 0.2	99.4 ± 0.2	99.5 ± 0.3	99.4 ± 0.3	*n.d.*	*n.d.*	*n.d.*	99.4 ± 0.2
	FFA	0.5 ± 0.2	*n.d.*	0.6 ± 0.2	0.5 ± 0.2	0.6 ± 0.2	0.5 ± 0.2	0.6 ± 0.2	*n.d.*	*n.d.*	*n.d.*	0.6 ± 0.2
	PL	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*
FO1	EE	4.38 ± 0.02	*n.d.*	3.6 ± 0.3	5.07 ± 0.05	5.20 ± 0.05	3.18 ± 0.02	3.9 ± 0.4	4.87 ± 0.06	5.72 ± 0.06	4.7 ± 0.5	7 ± 2
	TG	95.3 ± 0.2	100[a]	96.4 ± 0.3	94.5 ± 0.3	94.3 ± 0.3	96.82 ± 0.02	96.1 ± 0.4	94.6 ± 0.3	93.7 ± 0.4	95.3 ± 0.5	93 ± 2
	FFA	0.3 ± 0.2	*n.d.*	*n.d.*	0.5 ± 0.3	0.5 ± 0.3	*n.d.*	*n.d.*	0.5 ± 0.3	0.5 ± 0.4	*n.d.*	*n.d.*
	PL	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*
FO2	EE	3.20 ± 0.05	1.9 ± 0.2	1.6 ± 0.2	3.60 ± 0.04	3.84 ± 0.04	*n.d.*	1.6 ± 0.3	2.51 ± 0.04	5.3 ± 0.1	5.7 ± 0.1	*n.d.*
	TG	95.4 ± 0.1	98.1 ± 0.2	98.1 ± 0.2	94.7 ± 0.1	94.4 ± 0.1	100[a]	97.9 ± 0.4	96.25 ± 0.04	92.3 ± 0.2	92.2 ± 0.3	100[a]
	FFA	1.37 ± 0.06	*n.d.*	0.38 ± 0.04	1.7 ± 0.1	1.8 ± 0.1	*n.d.*	0.50 ± 0.05	1.25 ± 0.04	2.5 ± 0.2	2.1 ± 0.4	*n.d.*
	PL	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*
KO1	EE	1.23 ± 0.04	0.15 ± 0.01	1.1 ± 0.1	3.1 ± 0.3	3.3 ± 0.3	1.1 ± 0.2	0.8 ± 0.2	3.1 ± 0.4	4.4 ± 0.4	*n.d.*	*n.d.*
	TG	63 ± 3	76 ± 2	81 ± 2	22 ± 2	17 ± 2	60 ± 3	81 ± 2	16 ± 2	13 ± 2	33 ± 4	56 ± 3
	FFA	2.7 ± 0.3	0.38 ± 0.04	1.7 ± 0.4	7.5 ± 0.9	8.1 ± 0.9	3 ± 1	1.6 ± 0.8	7.2 ± 0.7	11 ± 2	*n.d.*	3.3 ± 0.7
	PL	33 ± 3	23 ± 2	16 ± 2	67 ± 3	71 ± 3	36 ± 3	17 ± 1	74 ± 3	72 ± 3	67 ± 4	41 ± 4
KO2	EE	1.36 ± 0.03	0.14 ± 0.01	1.0 ± 0.1	3.0 ± 0.1	3.2 ± 0.1	*n.d.*	0.6 ± 0.3	3.2 ± 0.2	4.0 ± 0.2	*n.d.*	*n.d.*

	TG	46 ± 2	56 ± 3	66 ± 2	19 ± 1	17 ± 1	54 ± 2	67 ± 2	15 ± 1	12.5 ± 0.8	32 ± 7	50 ± 3
	FFA	7.0 ± 0.1	4.4 ± 0.9	3.7 ± 0.2	12.3 ± 0.7	12.7 ± 0.7	6.8 ± 0.8	3.3 ± 0.3	11.3 ± 0.7	18.2 ± 0.7	*n.d.*	5.8 ± 0.2
	PL	46 ± 3	39 ± 3	29 ± 2	65 ± 2	67 ± 2	39 ± 2	29 ± 2	70 ± 2	65 ± 1	68 ± 7	44 ± 3
ES	**EE**	99.1 ± 0.1	100[a]	70 ± 5	99.2 ± 0.1	99.3 ± 0.1	97.0 ± 0.1	70 ± 5	99.4 ± 0.1	99.02 ± 0.05	100[a]	100[a]
	TG	0.4 ± 0.1	*n.d.*	30 ± 5	0.39 ± 0.05	0.28 ± 0.05	3.0 ± 0.1	30 ± 5	0.25 ± 0.04	0.35 ± 0.06	*n.d.*	*n.d.*
	FFA	0.4 ± 0.1	*n.d.*	*n.d.*	0.4 ± 0.1	0.4 ± 0.1	*n.d.*	*n.d.*	0.3 ± 0.1	0.58 ± 0.09	*n.d.*	*n.d.*
	PL	0.02 ± 0.03	*n.d.*	*n.d.*	0.02 ± 0.03	0.02 ± 0.03	*n.d.*	*n.d.*	*n.d.*	0.04 ± 0.08	*n.d.*	*n.d.*
FO3	**EE**	95.5 ± 0.2	94 ± 1	82.0 ± 0.5	95.8 ± 0.2	96.1 ± 0.2	88 ± 1	85.5 ± 0.2	66.8 ± 0.8	97.7 ± 0.2	98.3 ± 0.2	56 ± 3
	TG	4.0 ± 0.2	6 ± 1	18.0 ± 0.5	3.6 ± 0.1	3.3 ± 0.2	12 ± 1	14.5 ± 0.2	32 ± 1	1.7 ± 0.1	1.7 ± 0.2	44 ± 3
	FFA	0.51 ± 0.05	*n.d.*	*n.d.*	0.53 ± 0.05	0.55 ± 0.05	*n.d.*	*n.d.*	1.5 ± 0.2	0.61 ± 0.08	*n.d.*	*n.d.*
	PL	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*
FO4	**EE**	99.5 ± 0.1	100[a]	99.53 ± 0.04	99.50 ± 0.06	99.5 ± 0.1	99.32 ± 0.07	99.36 ± 0.05	99.37 ± 0.03	99.8 ± 0.2	100[a]	100[a]
	TG	0.2 ± 0.1	*n.d.*	*n.d.*	0.26 ± 0.08	0.2 ± 0.1	0.68 ± 0.07	*n.d.*	0.25 ± 0.06	0.2 ± 0.2	*n.d.*	*n.d.*
	FFA	0.25 ± 0.03	*n.d.*	0.46 ± 0.04	0.24 ± 0.03	0.26 ± 0.03	*n.d.*	0.64 ± 0.05	0.38 ± 0.05	*n.d.*	*n.d.*	*n.d.*
	PL	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*
Gum	**EE**	0.3 ± 0.1	*n.d.*	0.5 ± 0.2	0.5 ± 0.1	0.6 ± 0.1	*n.d.*	0.7 ± 0.3	0.8 ± 0.3	0.53 ± 0.09	*n.d.*	*n.d.*
	TG	99.3 ± 0.2	99.76 ± 0.03	99.1 ± 0.2	99.0 ± 0.4	98.9 ± 0.4	100[a]	98.8 ± 0.3	99.2 ± 0.2	98.8 ± 0.5	100[a]	100[a]
	FFA	0.4 ± 0.2	0.24 ± 0.03	0.4 ± 0.3	0.5 ± 0.4	0.5 ± 0.5	*n.d.*	0.6 ± 0.4	*n.d.*	0.71 ± 0.60	*n.d.*	*n.d.*
	PL	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*
Emul	**EE**	0.16 ± 0.01	*n.d.*	0.08 ± 0.01	0.33 ± 0.03	0.37 ± 0.04	*n.d.*	0.16 ± 0.03	0.30 ± 0.05	0.6 ± 0.2	*n.d.*	*n.d.*
	TG	99.2 ± 0.1	99.88 ± 0.02	99.19 ± 0.08	98.7 ± 0.1	98.9 ± 0.1	97.4 ± 0.4	98.6 ± 0.1	99.1 ± 0.2	98.20 ± 0.05	100[a]	100[a]
	FFA	0.5 ± 0.1	0.12 ± 0.02	0.48 ± 0.06	0.8 ± 0.2	0.8 ± 0.2	1.1 ± 0.2	0.8 ± 0.1	0.6 ± 0.2	1.2 ± 0.2	*n.d.*	*n.d.*
	PL	0.13 ± 0.02	*n.d.*	0.25 ± 0.02	0.15 ± 0.03	*n.d.*	1.5 ± 0.3	0.49 ± 0.03	*n.d.*	*n.d.*	*n.d.*	*n.d.*

Tab. S2: Peroxide value (PV) for different n-3 PUFA supplementation products from the European and US market.
Peroxide value (PV) for twelve n-3 PUFA products based on algal oil (AO), plant oil (PO), fish oil (FO) or krill oil (KO) or pharmaceutical ethyl esters (ES) as well as n-3 PUFA gums (Gum) and a n-3 PUFA enriched emulsion (Emul). For each product two independent measurements were carried out.

Product	PV [mEq O_2/kg]
AO1	2 ± 0.5
AO2	7 ± 0.5
PO	4.5 ± 0.3
FO1	11 ± 0.5
FO2	3 ± 0.3
KO1	<1
KO2	<1
ES	4 ± 0.4
FO3	5 ± 0.2
FO4	40 ± 1.6
Gum	*n.d.*[1]
Emul	<1

[1] Not determined because of low fat content of product (<5%)