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Composition and quality of marine Omega-3 supplements on the Russian and Norwegian markets: a comparative study

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Abstract (English)

The possible health effects of the long-chain Omega-3 fatty acids, eicosapentaenoic (EPA; 20:5 n-3) and docosahexaenoic (DHA; 22:6 n-3) have been studied for the last 40 years. The outcome of these studies has shown that intake of these Omega-3 may reduce risk of death from cardiovascular disease and possibly also have anticancer effects, improve brain development, reduce depression and delay the beginning of Alzheimer's disease. The reference daily intake for EPA+DHA is 500 mg and it is advised to obtain this quantity from fish. Omega-3 supplements or functional foods with added Omega-3 fatty acids could be an alternative for people with low consumption of fish. Supplements containing long-chain Omega-3 fatty acids reached $33 billion in global sales in 2012 with an annual growth rate for the period of 2007-2012 equal to 12%.

An increase in reliability of producers within quality and safety issues is essential in a way to obtain fisheries management goals, sustainable use of marine resources and maximizing of benefits for all involved. Composition of a product claimed on the label is the one of the main criteria for consumers in a product choice and evaluation of benefits.

The overall aim of the thesis was to compare the composition and quality of marine Omega-3 supplements on Russian and Norwegian markets by accomplishment of the following tasks:

1. Determination of fatty acid composition of the products by gas chromatography and comparison with the specifications given on the products labels;
2. Analysis of lipid classes by high performance thin layer chromatography and comparison with the specification given on the products labels.

Thirteen products (7 Russian and 6 Norwegian) were analyzed.

The Russian products contained triacylglycerols and generally had a low concentration of long-chain Omega-3 fatty acids. All the supplements were declared as fish oils, but, in fact, 4 of them contained plant oils resulting in an increased n-6/n-3 ratio. The Russian product descriptions had no information about the amount of oil in capsules or quantity of EPA and DHA. The recommended intake for 5 of the 6 capsule products was high, from 9 to 24 per day.

The Norwegian capsule products (5) were mostly concentrated supplements containing up to 71% of long-chain Omega-3 fatty acids, either in the form of ethyl esters or triacylglycerols. Information about content of Omega-3 fatty acids and EPA and DHA was included on the labels. However, information about oil amount in capsule was lacking for one product. The analysis suggested that the specification with regard to the lipid class form was not always precise. Two products were described as native marine oils, but the analysis showed that they contained ethyl esters. Claimed and found content of Omega-3, EPA and DHA, were slightly different for one product, up to 39% lower for 3 products and higher for one supplement. Daily intake of 2 capsules on the average met the recommended daily intake of 500mg of EPA+DHA.

The average price per gram of Omega-3 was approximately the same for the Russian and Norwegian products.

Abstract (Norwegian)

Mulige helseeffekter av de lang-kjedede omega-3 fettsyrene, eicosapentaensyre (EPA; 20:5n-3) og docosahexaensyre (22:6n-3) har blitt studert i 40 år. Resultatene av disse studiene har vist inntak av disse omega-3 fettsyrene kan redusere antallet hjerte-kar sykdommer og kanskje også bidra til å minke antall krefttilfeller, forbedre hjerneutvikling, redusere depresjoner og forsinke utvikling av Alzheimers sykdom. The anbefalte daglige inntak av EPA + DHA blir ofte satt til 500 mg og det anbefales at fettsyrene kommer fra fisk. For folk som spiser lite fisk kan kosttilskudd være et alternativ. På verdensbasis ble det i 2012 solgt kosttilskudd med omega-3 fettsyrer for 33 milliarder US $. I perioden 2007-2012 var den årlige veksten i salget på 12 %.

Økt tillit til produsenter både når det gjelder kvalitet og sikkerhet er helt sentralt både I forbindelse med fiskeriforvaltning, bærekraftig bruk av de marine ressurser og for å gjøre tilfredse.

Hovedmålet med oppgaven var å sammenligne innholdet og kvalitet av marine omega-3 kosttilskuddsprodukter på det russiske og norske markedet ved å undersøke:

1. Fettsyresammensetningen i produktene ved hjelp av gasskromatografi og å sammenligne med de oppgitte verdier.
2. Analyse av fettklassesammensetning med tynnskiktskromatografi og å sammenligne med produktspesifikasjon.

Tretten produkter (7 russiske og 6 norske) ble analysert.

De russiske produktene inneholdt triacylglycerol og hadde generelt en lav konsentrasjon av langkjedede omega-3 fettsyrer. Alle disse kosttilskuddene var deklarert å inneholde fiskeoljer, men 4 av produktene inneholdt planteoljer som bidro til et forhøyet n-6/n-3 forhold. Varedeklarasjonene hadde ikke noe informasjon om mengden olje i kapslene eller om innholdet av omega-3 fettsyrer. Det anbefalte inntaket for 5 av 6 kapselprodukter var fra 9 til 24 kapsler per dag.

De norske kapselproduktene (5) var i hovedsak konsentrerte produkter som inneholdt opptil 71 % lang-kjedede omega-3 fettsyrer enten i form av etylestere eller triacyglycerol. Informasjon om innholdet av omega-3 fettsyrer og EPA og DHA var oppgitt på produktene. Et av produktene manglet opplysning om mengden olje i kapslene. Analysene av fettklasseinnholdet i produktene viste at opplysningene gitt for de norske produktene alltid var presise. To av produktene var beskrevet å inneholde marine oljer, men undersøkelsen viste at de inneholdt etylestere. Oppgitt og funnet innhold av omega-3 fettsyrer, EPA og DHA, var noe forskjellig for et produkt, opptil 39% lavere for tre produkter og høyere for ett produkt. Daglig inntak av 2 kapsler i gjennomsnitt bidro til cirka anbefalt dose av EPA + DHA på 500 mg.

Gjennomsnittlig pris per gram omega-3 fettsyrer var omtrent likt for de russiske og norske produktene.

Abstract (Russian)

Возможное влияние эйкозопентаеновой (ЭПК; 20:5 n-3)и докозогексаеновой (ДГК; 22:6 n-3) омега-3 жирных кислот на здоровье человека изучается последние 40 лет. Различные исследования показали, что потребление этих жирных кислот может предотвратить смерть от сердечно-сосудистых заболеваний, сократить вероятность развития раковых заболеваний, стимулировать работу мозга, снижать нервное напряжение, задерживать наступление болезни Альцгеймера. Рекомендуемая суточная норма потребления ЭПК + ДГК составляет 500 мг, при этом желательно получать эти кислоты из рыбы. Пищевые добавки, содержащие длинноцепочечные Омега-3 жирные кислоты, могут быть альтернативным источником ЭПК и ДГК для людей, потребляющих малое количество рыбы. Мировой объем продаж таких добавок в 2012 году составил 33 миллиарда $ с годовым темпом роста 12% за период 2007 – 2012 гг.

Рост доверия к производителям, в рамках качества и безопасности продуктов, является важной составляющей достижения таких целей управления рыбным хозяйством, как устойчивое использования морских биоресурсов и максимизация выгоды для всех заинтересованных сторон. Состав продукта, указываемый на упаковке, является одним из главных критериев для покупателей при выборе продукта и оценке его возможной пользы.

Цель данной дипломной работы – сравнить состав и качество норвежских и российских пищевых добавок с морскими омега-3 жирными кислотами. Следующие задачи были выполнены для достижения цели:

1. Определение композиции жирных кислот в продуктах с помощью газовой хроматографии и сравнение результатов со спецификацией добавок;
2. Анализ классов липидов с помощью высокоэффективной тонкослойной хроматографии и сравнение результатов со спецификацией добавок.

Было проанализировано 7 российских и 6 норвежских пищевых добавок, содержащих морские омега-3 жирные кислоты.

Большая часть проанализированных российских добавок имела невысокую концентрацию омега-3 жирных кислот, представленных в качестве триглицеридов. При этом все добавки были описаны как рыбий жир, однако, 4 из 7 добавок содержали растительное масло, что повлияло на увеличение n-6/n-3 соотношения в продуктах. Спецификации российских добавок не содержали информацию о количестве вещества в капсулах и концентрации ЭПК и ДГК. Рекоммендуемая суточная доза для 5 из 6 капсулированных продуктов была достаточно велика и составляла от 9 до 24 капсул.

Норвежские капсулированные продукты содержали, в основном, большое количество длинноцепочных омега-3 жирных кислот (до 71%) в форме триглицеридов или этиловых эфиров. Информация о содержании ЭПК и ДГК присутствовала на всех упаковках. Тем не менее, количество вещества в капсулированных продуктах не было указано для одного товара. Анализ классов липидов показал, что информация на упаковках норвежских продуктов не всегда точна. Так в двух продуктах, описанных как натуральный рыбий жир, содержались этиловые эфиры. Содержание омега-3 жирных кислот, ЭПК и ДГК, указанное на упаковке было практически одниковым по сравнению с результатами анализа для одного продукта, до 39% ниже для 3 продуктов и выше для одной добавки. Рекомендуемое в спецификации суточное потребление капсульных норвежских добавок составляло, в среднем, 2 капуслы и содержало рекомендуемую суточную дозу ЭПК + ДГК.

Средняя цена за грамм омега-3 жирных кислот была примерно одинаковой для российсикх и норовежских добавок.

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Abbreviations

AA	Arachidonic acid
ALA	α-linoleic acid
COX	Cyclooxygenases
DAG	Diacylglycerol
DGLA	Dihomo-γ-linolenic acid
DHA	Docosahexaenoic acid
EE	Ethyl esters
EPA	Eicosapentaenoic acid
FA	Fatty acid
FID	Flame ionization detector
GC	Gas chromatography
GLA	γ-linolenic acid
HPTLC	High performance thin layer chromatography
HUFA	Highly unsaturated fatty acids
LA	Linoleic acid
Lc-PUFA	Long-chain polyunsaturated acids
Lc n-3	Long-chain Omega-3 fatty acids
LOX	Lipoxygenases
MAG	Monoacylglycerol
MUFA	Monounsaturated fatty acids
p.a.	pro analysis
PUFA	Polyunsaturated fatty acids
RDI	Recommended daily intake
SFA	Saturated fatty acids
TAG	Triacylglycerol

Introduction

The possible health benefits of the long-chain Omega-3 fatty acids, eicosapentanoic (EPA; 20:5 n-3) and docosahexanoic acid (DHA; 22:6 n-3) found in seafood were discovered about 40 years ago by Danish scientists studying health and nutrition of Escimos in Greenland (Dyeberg et al., 1978). Since then a huge number of scientific studies on Omega-3 fatty acids and health have been carried out. A search on the bibliographic database “Web of science” using the term “Omega-3 fatty acids” results in more than 12 000 units in scientific publications.

It is generally agreed that intake of long-chain Omega-3 fatty acids may reduce the number of deaths from cardiovascular disease (Harris et al., 2008; Mozzafarian & Wu, 2011).

Some studies have also reported that Omega-3 fatty acids are necessary for optimal brain development of babies and for reducing the possibilities of developing mental disturbances like depression (Innis, 2008; Grosso et al., 2014).

Health authorities in many countries recommend that people should eat fish twice a week, one of which should be a fatty species like Atlantic salmon, mackerel or herring to achieve the reference dietary intake of 400 – 500 mg EPA+DHA per day. The World Health Organization (WHO) also recommends fish twice per week providing 400 – 1000 mg of EPA+DHA daily (Kris-Etherton et al., 2009). It is usually recommended that the long-chain Omega-3 fatty acids should come from fish. For those who do not eat enough fish, Omega-3 supplements or functional foods with added Omega-3 fatty acids could be an alternative (Mozzafarian & Wu, 2011).

Supplements containing long-chain Omega-3 fatty acids reached $33 billion in global sales in 2012 and for the period of 2007-2012 had an annual growth rate equal to 12% (Schofield, 2013).

There are different types of marine Omega-3 supplements that are available on the market. It can be sold in form of original fish liver oil or in form of oil in capsules. It could be also produced from krill or consist of mixed oils. The capsules may contain native oils where the long-chain Omega-3 fatty acids constitute 20 – 30% of the total fatty acids. Alternatively, the Omega-3 fatty acids may be presented in concentrated form, composing even as much as 90% of the total fatty acids in the products. In such products the fatty acids are esterified with ethanol forming so-called ethyl esters or with glycerol to traicylglycerols (Dyeberg et al., 2010; Sears et al., 2010). Krill oil, which now has become a popular supplement, has most of the long-chain Omega-3 fatty acids in the form of phospholipids.

An increase in reliability of producers within quality and safety issues is essential in a way to obtain such fisheries management goals as optimizing of marine resources utilization and maximizing of benefits for all involved. Apart of other tools, quality control should be used to maintain fishery sector to reach and/or keep a quality level that would satisfy the customer. Therefore, from one hand, quality control helps producers to maximize benefits by avoiding consumers' complaints about products and, from other hand, helps consumers to have influence on the industry and to decide if desired benefits would be obtained by them.

Composition of a product claimed on a package is the one of the main criteria for consumers in a product choice and benefits evaluation. Traditionally, creation of specification is the responsibility of a producer and this is not always a case when regulations provide clear and full guidelines of what must be written in a product description.

At the same time mislabeling in fishery sector has become a big problem and was observed and studied mostly in sales of fresh fish and fillets. Some producers rename fish, hide their origin, and sell cheaper species as species with higher value (Jacquet and Pauly, 2007). This is obvious that for other fishery products the problem could also be significant.

Thus, the overall aim of the thesis is to compare the quality of marine Omega-3 supplements on Russian and Norwegian markets by accomplishment of the following tasks:

1. Determination of fatty acid composition of the products by gas chromatography and comparison with the specifications given on the products labels;
2. Analysis of lipid classes by high performance thin layer chromatography and comparison with the specification given on the products labels.

1. Basic Background

1.1 Fatty acids and lipid classes

Fatty acids (FA) are aliphatic carboxylic acids, common chemical formula is R – COOH, where R is hydrocarbon chain and COOH is carboxylic group. The biological properties of fatty acids are defined by the length of the carbon chain and by both the number and position of any double bonds present. Most FAs in plants and animals have 12 – 22 atoms of carbon and, typically, quantity of the carbon atoms is even number. Fats and oils from plants and terrestrial animals have only small amount of fatty acids with carbon chain longer than C18 while fish and marine mammals also have substantial amount of fatty acids with 20 and 22 carbon atoms.

Fatty acids which do not contain double bonds between the carbons are named saturated and the fatty acids which contain, at least, one double bond are called unsaturated. Unsaturated fatty acids having one double bond are named monounsaturated acids (MUFA) while those with 2 or more double bonds are named polyunsaturated fatty acids (PUFA). Fatty acids with 4 – 6 double bonds are also commonly named highly unsaturated acids (HUFA) or long-chain polyunsaturated acids (Lc-PUFA) because usually there are 20 or 22 carbon atoms in them.There are two groups of PUFA: omega-3 (n-3 or ω-3) and omega-6 (n-6 or ω-6). This classification is based on a position of first double bond between the carbons in relation to methyl end (omega) of an acid.

Lipids can have one or more fatty acids in their composition. Such lipids can be saponified, i.e. produce soaps in reaction with lye. Triacylglycerol (TAG), phospholipids, waxes, glycolipid are examples of these lipids. Lipids which do not contain fatty acids are non-saponifiable and represented by cholesterol, squalene, steroid hormone, bile salts, carotenoid, fat soluble vitamins. Under certain conditions some of these lipids, like cholesterol and the carotenoid astaxanthin, may be esterified with fatty acids. Herewith, according to its function in organism, TAGs are classified as depot fat; phospholipids, glycolipids and cholesterol are membrane lipids; other lipids, like vitamin D and E, have special biological functions.

1.2 Omega-3 supplements

The traditional Omega-3 supplements consist of native fish body oils or fish, often cod, liver oil. The Omega-3 fatty acids are esterified in triacylglycerol. Nowadays the most part of the supplements available on the market is in concentrated form with as much as 85% of EPA and DHA presented in them as ethyl esters (EE) (Figure 1.1) or triacyglycerols (Figure 1.2) (Dyeberg et al., 2010; Sears et al., 2010). In krill oils most the Omega-3 fatty acids are esterified in phospholipids (Figure 1.3).

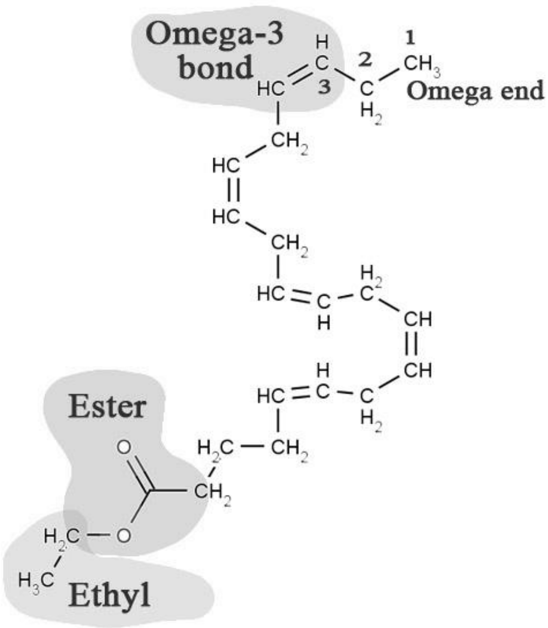

Figure 1.1 – Ethyl ester of EPA. The ethyl group from ethanol, the ester bond and the Omega-3 bond are shaded in grey.

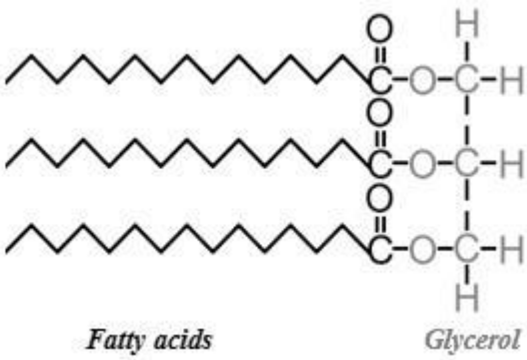

Figure 1.2 – Molecule of triacyglycerol where the 3 fatty acids are esterified with glycerol.

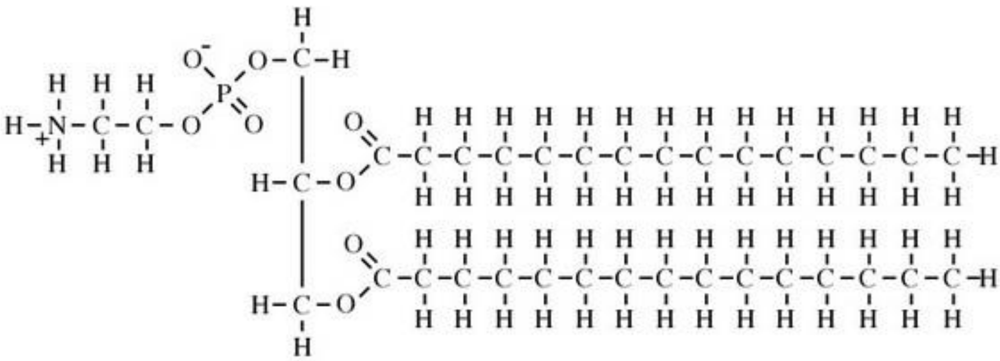

Figure 1.3 – Phospholipid structure: example of lecithin. In phospholipids (membrane lipid) 2 fatty acids are esterified in the glycerol molecule. The B.hydroxyl groups are bond to a phosphate group and making up the polar part of the molecule.

A transesterification process is used to isolate EPA and DHA from fish oil. So, all the fatty acids are split from the glycerol molecules and reesterified with ethanol to ethyl esters. Then long-chain Omega-3 esters are concentrated with use of molecular distillation or urea complexation. The concentrated LC-PUFA esters may be, if needed, converted back to TAG rich in EPA and DHA by transesterification with glycerol (Kravolec et al., 2012). The absorption of EPA and DHA is equally effective from triacyglycerols or ethyl esters (Nordøy et al. 1991).

The main source of raw materials for the production of LC-PUFA concentrates is fish oil from South American waters, especially fish oil from anchovy (*Engraulis ringens*), because it is present on the market in large quantities and has a high content of EPA and DHA, approximately 30% (Tacon & Metian, 2009).

1.3 Essential fatty acids and their health implications

Linoleic acid (LA; 18:2 n-6) and α-linoleic acid (ALA; 18:3 n-3), the two simplest acids representing each PUFA group, are precursors to the longer chain n-6 and n-3 series of fatty acids, respectively, and are essential for humans because they cannot be synthesized in human organism. However, these both acids are not the most important vital acids but arachidonic acid (AA; 20:4 n-6), eicosapentaenoic acid (EPA; 20:5 n-3) and docosahexaenoic (DHA; 22:6 n-3) which could be synthesized from them. So, by the same enzymes, LA is converted to AA (20:4n-6) via γ-linolenic acid (GLA, 18:3n-6) and dihomo-γ-linolenic acid (DGLA, 20:3n-6) and ALA can be converted to EPA (20:5n-3) and DHA (22:6n-3) (Figure 1.4).

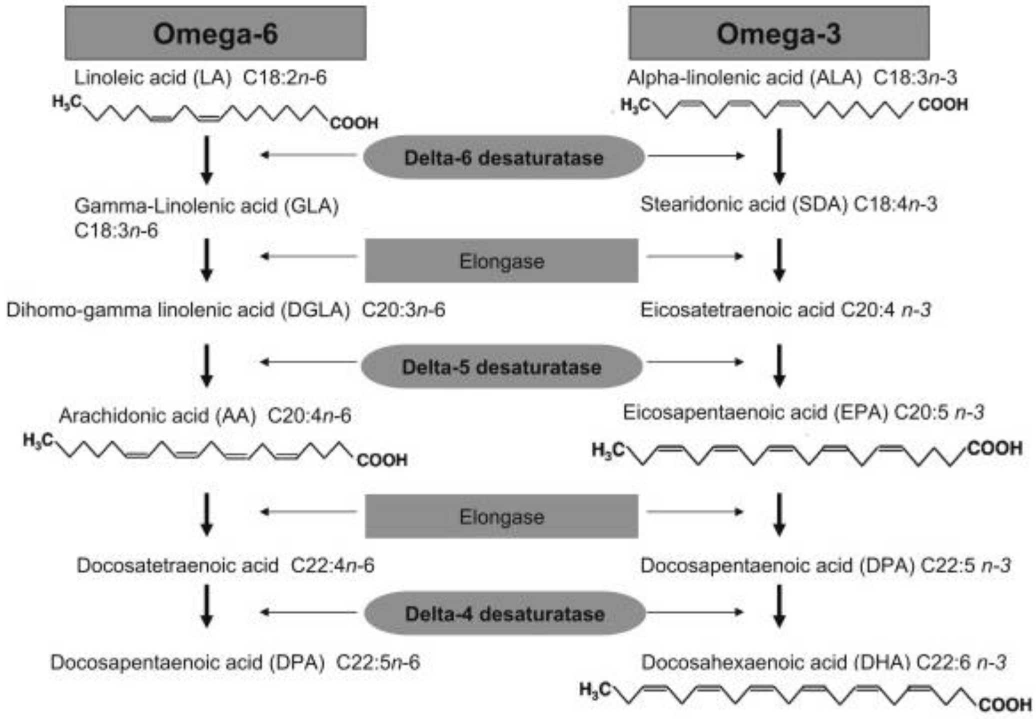

Figure 1.4 – Linoleic acid and α-linoleic acid conversion by several enzymes.

Nevertheless, conversion of LA to AA is more efficient than that of ALA to EPA and DHA, and only 0,5% of dietary ALA is converted to DHA in humans (Plourde & Cunnane, 2007; Brenna et al., 2009).

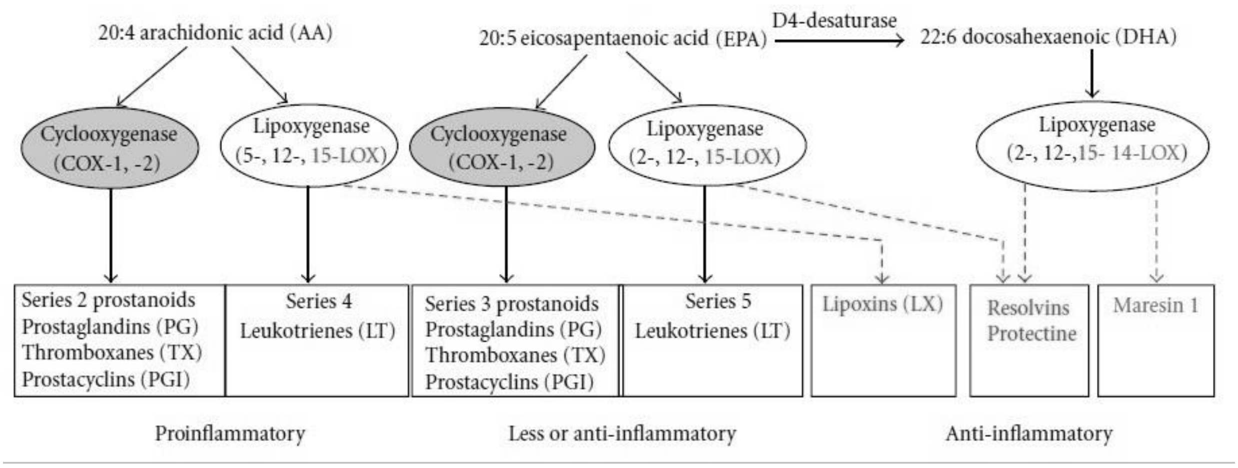

Figure 1.5 – Eicosanoids produced from AA, EPA and DHA; adapted from Molendi-Coste et al., 2010.

In general, PUFA are significant for the composition of cell membranes in which they maintain correct membrane protein function and influence membrane fluidity regulating cell signaling processes, cellular functions and gene expression by this (Das, 2006). But other functions of PUFA require their metabolism to more highly unsaturated PUFA (as in example with AA, DHA and EPA synthesis from LA and ALA) and production of hormones.

So, cyclooxygenases (COX) and lipoxygenases (LOX) can convert AA, EPA and DHA to short-lived hormones, eicosanoids (prostaglandins, thromboxanes, prostacyclins). Depending on series, the eicosanoids can have proinflammatory, less proinflammatory or anti-flammatory properties. Eicosanoids produced from EPA and DHA are generally less pro-inflammatory than if they produced from AA (omega-6) (Figure 1.5).

There are a number of diseases considered to involve inflammation (Table 1.1). Having inflammatory properties, eicosanoids generated from n-6 and n-3 LC-PUFA can be suggested to contribute or prevent such diseases. Herewith, different studies have confirmed that intake of long-chain Omega-3 may reduce risk of death from cardiovascular disease, have possible anticancer effects and positive influence on brain functioning, reduce depression development, delay the beginning of Alzheimer's disease, be a promising treatment for non-alcoholic fatty liver disease (Masterton, 2009; Riediger et al., 2009).

Table 1.1 – Diseases and conditions with a recognized inflammatory component (Calder, 2009).

Disease or condition	
Rheumatoid arthritis	Allergic disease
Chron's disease	Chronic obstructive pulmonary disease
Ulcerative colitis	Psoriasis
Lupus	Multiple sclerosis
Type-1 diabetes	Neurodegenerative disease of aging
Type-2 diabetes	Atherosclerosis
Cystic fibrosis	Acute cardiovascular events
Childhood asthma	Obesity
Adult asthma	Response to surgery, injury, trauma and critical illness
Cancer cachexia	Acute respiratory distress syndrome

2. Materials and Methods

2.1 Omega-3 supplements

The 13 products were analyzed with the information provided to the buyers translated from Russian and Norwegian into English. The Russian and Norwegian products were marked 1R to 7R and 8N to 13N, respectively.

Russian Omega-3 supplements

Product: 1R
Name: Pischevoi rubii jir 'BioKontur'"/"Food fish oil 'BioContour".
Producer: Polaris Ltd., Russia, Vladimirskaya oblast, Aleksandrov city.
Components: food fish oil.
Subcomponents: gelatin, glycerol, water
Product form: oil in capsules.
Other information: recommended daily intake dose is 5 capsules 3 times during food consumption.
This dose would provide consumption of 1.1 g of PUFA, including 0.315 g of Omega-3.
Weight of one capsule is 0.3 g.
Package size: 100 capsules.
Expiry date: 05.09.2015.
Price per gram of Omega-3: 3.86 NOK (currency exchange rate NOK/rubles is 0.18).

Product: 2R
Name: "Detskii rubii jir"/"Fish oil for children".
Producer: "Realkaps" company, Russia, Moscow region.
Components: cod liver fish oil, vitamins: E-acetate, A-palmitate, D.
Subcomponents: gelatin.
Product form: oil in capsules.
Other information: recommended daily intake for children from 3 to 7 years old is 5 capsules during food consumption, from 7 to 14 years – 10 capsules.
One capsule has 50 mg of Omega-3, 0.2 mg of vitamin E, 21 mcg of vitamin A, 0.8 mcg of D3.
Weight of one capsule is 0.2 g.
Package size: 100 capsules.
Expiry date: 02.10.2015.
Price per gram of Omega-3: 4.04 NOK.

Product: 3R
Name: Biologically active food supplement "Okeanol".
Producer: "KorolevPharm" LLC, Moscow region.
Components: natural concentrated fish oil of oceanic fish with content of Omega-3 not less than 35%.
Product form: oil in capsules.
Other information: recommended daily intake dose is 2 capsules for people older than 14.

The dose provides 95 % of recommended daily intake of Omega-3.
Package size: 30 capsules.
Expiry date: 02.10.2015.
Price per gram of Omega-3: could not be obtained from product description. Price for product is 26.1 NOK.

Product: 4R
Name: "Biafishenol".
Producer: "BioPharm" Ltd., Moscow region.
Components: 100% fish oil.
Product form: oil in capsules.
Other information: recommended daily intake dose for adults is 5 capsules 2 times during food consumption.
Recommended dose has 0.48 g of Omega-3 PUFA, 0.14 mg of vitamin A, 5.65 mcg of vitamin D.
Package size: 100 capsules.
Expiry date: 01.11.2015.
Price per gram of Omega-3: 1.05 NOK.

Product: 5R
Name: "Rubka"/"Small fish".
Producer: "Bagira" Ltd., Orenburg region, Orsk city.
Components: fish oil.
Subcomponents: distillated water, gelatin, glycerol, sodium benzoate.
Product form: oil in capsules.
Other information: recommended daily intake dose for people older than 14 years is 6 capsules 4 times during food consumption.
Recommended dose provides 1.2 – 1.25 g of Omega-3.
Weight of one capsule is 0.2 g.
Package size: 100 capsules.
Expiry date: 05.08.2015.
Price per gram of Omega-3: 3.06 – 3.18 NOK.

Product: 6R
Name: "Rubii jir"/"Fish oil".
Producer: Polaris Ltd, Murmansk.
Components: food fish oil, natural flavoring 'Lemon', vitamin mix ADE.
Subcomponents: gelatin, glycerol, citric acid.
Product form: oil in soft chewing capsules.
Other information: recommended daily intake dose for people older than 14 years is 3 capsules 3 times during food consumption.
Recommended dose provides 0.765 g of Omega-3, 3.15 mcg of vitamin D, 12.6 mg of vitamin E, 1.35 mg of vitamin A.
Weight of one capsule is 0.47 g.
Package: 50 capsules.
Expiry date: 07.03.2015.
Price per gram of Omega-3: 4.38 NOK.

Product: 7R
Name: "Jir rubii ochischenui"/"Purified fish oil".

Producer: “Tula pharmaceutical fabric” Ltd., Russia, Tula city.
Components: cod liver fish oil.
Product form: oil.
Other information: 1 g of oil contents 350-1000 ME of vitamin A, 50 – 100 ME of vitamin D.
Volume: 50 ml.
Expiry date: 01.08.2014.
Price per gram of Omega-3: could not be obtained from description. Product price is 17.2 NOK.

<u>Norwegian Omega-3 supplements</u>

Product: 8N
Name: “Moller’s tran Omega-3”/ “Moller’s cod liver oil Omega-3”.
Producer: Axellus AS, Oslo.
Components: cod liver oil, vitamin E, tocopherols.
Product form: oil.
Other information: recommended daily intake dose is 5 ml.
The recommended dose contains 1.2 g of Omega-3, 0.6 mg of which is DHA and 0.4 mg is EPA, 10 mcg of vitamin E, 250 mcg of vitamin A, 10 mcg of vitamin E..
Volume: 500 ml.
Expiry date: 01.05.2015.
Price per gram of Omega-3: 0.97 NOK.

Product: 9N
Name: “Moller’s Omega-3 Ekstra Sterk”/“Moller’s Omega-3 Extra Strong”.
Producer: Axellus AS, Oslo.
Components: fish oil concentrate (triglycerides), gelatin, glycerol, glazing agent (shellac, carboxymethylcellulose, talc, ammonium bicarbonate, coconut oil), antioxidant (tocopherols), aroma of peppermint.
Product form: oil in capsules.
Other information: recommended daily intake dose is 2 capsules.
The recommended dose provides with 1.0 g of Omega-3, 0.5 g of which is EPA and 0.4 g is DHA.
Package size: 80 capsules.
Expiry date: 01.10.2014.
Price per gram of Omega-3: 3.04 NOK.

Product: 10N
Name: “Trippel Omega-3 med vitamin A –D og E”/“Triple Omega-3 with vitamin A – D and E”.
Producer: Biopharma AS, Rolvsøy.
Components: fish oil, gelatin, glycerol, dl-alpha-tocopherols, retynil palmitate, cholecalciferol.
Product form: oil in capsules.
Other information: recommended daily intake dose is 2 capsules which contain 1000 mg of fish oil.
The recommended dose contains 650 mg of Omega-3, 330 mg of which is EPA and 220 mg is DHA, 10 mg of vitamin E, 250 mcg of vitamin A, 10 mcg of vitamin D.
Package size: 144 capsules.
Expiry date: 06.09.2015.

Price per gram of Omega-3: 1.50 NOK.

Product: 11N
Name: “Ruis Omega-3 Høykonsentrert Selolje”/“Ruis Omega-3 Highly concentrated Seal Oil”.
Producer: Medica Clinical Nord AS, Moss.
Components: seal oil concentrate MCN-369, gelatin, glycerol, E-vitamin.
Product form: oil in capsules.
Other information: recommended daily intake dose is 1-2 capsules.
1 capsule contains 1000 mg of fish oil 360 mg of which are Omega-3, 150 mg of which is DHA, 80 mg – EPA, 70 mg – DPA; DHA, EPA and DPA are represented as ethylesters.
Package size: 60 capsules.
Expiry date: 15.11.2014.
Price per gram of Omega-3: 9.21 NOK.

Product: 12N
Name: “Moller’s Omega-3 Hjerte”/“ Moller’s Omega-3 heart”.
Producer: Axellus AS, Oslo.
Components: fish oil concentrate (triglycerides), linseed oil (cold-pressed oil), gelatin, glycerol, caramel colouring, vitamin E.
Product form: oil in capsules.
Other information: recommended daily intake dose is 2 capsules.
The recommended dose contains 1046 mg of fish oil concentrate (content of EPA/DHA is 464 mg), 666 mg of linseed oil (308 mg of which is ALA), 869 mg of Omega-3 in total, 6 mg of vitamin E.
Package size: 76 capsules.
Expiry date: 01.01.2015.
Price per gram of Omega-3: 3.62 NOK.

Product: 13N
Name: “Ekstra sterk Omega-3 med krill antioksidant”/ “ Extra strong Omega-3 with krill antioxidant ”.
Producer: Biopharma AS, Rolvsøy.
Components: fish oil, krill oil, glycerol, d-alpha-tocopherols.
Product form: oil in capsules.
Other information: recommended daily intake dose is 2 capsules.
The recommended dose contains 900 mg of fish oil and 200 mg of krill oil, 635 mg of which are Omega-3 (DHA – 394 mg, EPA – 200 mg, DPA – 36mg).
Package size: 60 capsules.
Expiry date: 03.06.2015.
Price per gram of Omega-3: 5.21 NOK.

2.2 Chemicals

The following list of chemicals used during all the experiments was obtained from Sigma-Aldrich, Steinheim, Germany: Heptan (32287), Dichlormethan (32222N), Methanol(32213), Aecetic acid (33209), Copper sulphate, Phosphoric acid (345245), Sulfuric acid (30743), Sodium chloride (41380), Diethyl ether (32203N).

Copper sulphate (1.02790) was obtained from Merk, KGaA, Darmstad, Germany.

All the chemicals have p.a. quality.

The internal standard used in the gas chromatography (GC) analysis was heptadecanoic acid; C 17:0 (Sigma-Aldrich, Steinheim, Germany).

The standards used to identify the fatty acids by GC were PUFA no1, PUFA no2, PUFA no3 (Supelco Analytical, Bellefonte, PA, USA) and GLC 68 D (Nu-Chek Prep, Inc., Elysan, MN, USA).

The standards used in the high performance thin layer chromatography were 18-5A (cholesterol, cholesterol oleate, triolein, oleic acid, lecithin (L-A-distearil) and 18-1A (monoolein, doilein, triolein, methyl oleate) both from Nu-Chek Prep. Inc., Elysian, MN, USA.

2.3 Fatty acid composition

The fatty acid composition in the products was determined by gas chromatograph, Agilient 6890N, equipped with a 7683B auto injector and a flame ionization detector (FID) (Agilent Technologies Inc., Santa Clara, CA, United States). The column used was a Varian CP7419 capillary column (50 m x 250 µm x 0,25 µm nominal) (Varian Inc., Middelburg, The Netherlands).

Oil from capsules was taken with syringes. Before the analysis in the gas chromatograph every sample of oil in products was methylated by method described by Stoffel et al. (1959) with some modifications:

1. A sample was dissolved in dichlormethan:methanol (2:1, v/v) to a concentration of 10 mg/ml;
2. Internal standard (IS) was dissolved in dichlormethan:methanol (2:1, v/v) to a concentration of 10 mg/ml;
3. IS was added to the dissolved sample in quantity of 10% and mixed well;
4. 100 µl of the sample were transferred to a new glass tube;
5. 0.9 ml of DCM and 2 ml of 2% H2SO4 in methanol were added to the sample and mixed;
6. Glass tube was heated at 100 ^{o}C for one hour in heating cabinet, incubator type TS4115 (Termaks AS, Bergen, Norway).

7. 3.5 ml heptane and 3.5 ml 5%NaCl was added to glass tube and mixed well;
8. The upper phase, consisted of heptane and lipids, was transferred into new small glass tube then solvents were removed by evaporating under nitrogen gas.
9. The sample was re-dissolved in 100 µl heptane and transferred into GC-vials.

GC-vials were put in gas chromatograph which took 1 µl injection of every sample. During the analysis helium gas carried fatty acids throughout the column to the flame ionization detector were the acids were burned. The time during which fatty acid stays in the column before being burned is called the retention time. It is used to identify fatty acids represented in samples by comparison this with retention time of the known fatty acids in external standards.

A temperature program with the following characteristics was used: initial temperature 125 °C, increase by 8°C/ min to 145 °C, hold at 145°C for 26 minutes, increase by 2°C/ min to 220 °C, hold at 220°C for 5 minutes.

Calculation of the amount of particular fatty acid in a sample (g of FA in 100 g of oil in a product) is done using the following formula [2.1]:

$$\text{FA amount} = \left(\frac{AP\ FA}{AP\ IS} \times \frac{WIS}{WS}\right) \times 10, \qquad [2.1]$$

where AP FA – area of particular fatty acid peak;

AP IS – area of IS peak;

WIS – weight of internal standard added to a sample, g;

WS – weight of sample, g;

10 – multiplier to obtain amount of FA in mg per g of oil in product.

Fatty acid composition in a sample also can be represented in percentage form where percent particular fatty acid in total amount of FAs is represented. It is done through divining of an area of an individual peak by the total area of all peaks.

Peaks which are less than 0.5% of the total area of the peaks are not included in the results of the analysis.

2.4 Lipid classes

High performance thin layer chromatography was conducted the way described by Vaghela & Kilara (1995) with some modifications and had the following steps:

1. Samples were dissolved in dichlormethane to a concentration of 25mg/ml;
2. 1 µl of dissolved samples and standards were applied to the HPTLC plates (Silica gel 60, 10 cm x 10 cm, Merck);
3. The plates were put in glass chambers saturated with solution of heptane:diethyl ether:acetic acid (80:40:2) and taken out when the mobile phase was approximately 1 cm from the top;
4. The plates were left to dry for a few minutes and then sprayed with 10% (w/v) copper sulphate in 8% phosphoric acid (v/v);
5. The plates were left to dry and then placed in heating cabinet where it was for approximately 15-20 minutes unless temperature of 180 °C is reached;
6. The plates were scanned.

To define lipid classes comparison of patterns with patterns of the two known HPTLC.

3. Results

3.1 Fatty acid composition of marine Omega-3 supplements from Russian and Norwegian markets

The fatty acid composition (% of total fatty acids) of the Russian and Norwegian products is shown in Table 3.1 and Table 3.2, respectively. This is a result of 2 independent experiments with 3 analytical parallels each.

Table 3.1 – Fatty acid composition (% of total FAs) of marine Omega-3 supplement from Russian market.

	Percentage of fatty acid in total quantity of acids						
Fatty acid Product:	**1R**	**2R**	**3R**	**4R**	**5R**	**6R**	**7R**
C14:0	3.0	6.8	7.2	2.8	5.6	7.5	4.2
C16:0	9.3	16.9	17.4	9.8	15.2	17.5	12.4
C18:0	2.5	3.5	3.6	3.0	3.7	3.5	2.9
C16:1 n-7	2.9	9.8	9.6	4.2	7.6	9.8	5.3
C18:1 n-9	36.8	8.1	8.3	21.4	12.6	10.6	29.3
C18:1 n-7	2.9	3.0	3.1	2.4	2.8	3.2	3.3
C20:1 n-9	5.1	1.2	1.2	5.4	1.1	0.9	3.8
C22:1 n-11	4.8*	-	0.7	5.5*	0.9	0.6	4.2*
C18:2 n-6	15.9	1.7	0.7	27.8	13.7	2.0	9.2
C18:3 n-3	4.8	0.8	0.4	1.3	0.7	0.8	3.5
C18:4 n-3	0.8	3.8	3.5	1.5	2.4	2.7	1.5
C20:4 n-6	-	0.9	1.1	-	0.9	1.1	-
C20:4 n-3	-	1.1	1.0	-	0.7	0.8	-
C20:5 n-3	3.2	20.7	20.7	5.5	15.9	19.1	8.5
C22:5 n-3	1.5	1.9	2.1	0.8	1.8	2.1	2.0
C22:6 n-3	4.1	12.6	13.1	6.4	10.1	12.6	7.6
NIA**	2.5	7.5	6.2	2.2	4.3	5.2	2.3
∑ SFA	14.9*	27.8	28.2	15.5*	24.5	28.5	19.4*
∑ MUFA	53.0	22.0	22.9	38.9	25.0	25.0	46.5
∑ PUFA	32.1	46.5	45.6	43.4	48.3	43.7	33.2
– **n-6**	17.8	2.5	1.8	27.8	14.7	3.1	10.1
– **n-3**	14.4	40.8	41.0	15.6	31.6	38.2	23.2
Lc n-3* **	8.8	35.0	36.0	12.7	27.8	33.8	18.0
n-6/n-3	1.24	0.06	0.04	1.78	0.47	0.08	0.44

*** -** may contain small amount of 20:4 n-3; ** - non-identified acids and acids represented in few samples in relatively small amounts (less than 1.5 % of total FAs); *** - Long chain Omega-3 fatty acids, i.e. summed content of EPA, DPA, DHA.
SFA=saturated fatty acids; MUFA=monounsaturated fatty acids; PUFA=polyunsaturated fatty acids.

The Russian products can be grouped in 2 sets according to content of saturated (SFA) and monosaturated fatty acids (MUFA). The Russian products 1R, 4R and 7R had a relatively low content of SFA (14.9 – 19.4%) and a high content of MUFA (38.9 – 53.0%) while the reverse was true for 2R, 3R, 5R and 6R. The SFA and MUFA in these products were 24.5 – 28.5% and 22.0 – 25.0%, respectively. The first group of products also had a relatively low content of long-chain Omega-3 fatty acids, 8.8 – 18.0%. A higher content of Lc n-3 was found in the second group (2R, 3R, 5R, 6R), namely 27.8 – 36.0%. Linoleic acid (18:2 n-6), the signature fatty acid of plant oils, was found to be abundantly present in products 1R, 4R, 5R and 7R (9.0 – 27.8%) while in the other products it was presented in 2% or less. The high content of 18:2 n-6 in 4 of the products contributes strongly to the high n-6/n-3 ratio in some products (0.44 – 1.78%). In other products this ratio was 0.04 – 0.08.

Some distinct differences in fatty acid composition may also be seen for the Norwegian products. Four of the products (9N, 10N, 12N and 13N) have a very high content of Lc n-3. More than 60% of the fatty acids are this type PUFA. In products 8N and 11N content of Lc n-3 was 28.8 and 43.3%, respectively. The concentration of linoleic acid was low in all the Norwegian products (<2.1%) except for product 12N which contained 7.2%. This product also had a high content of α-linoleic acid (18:3 n-3), almost 24%. The amount of SFA was relatively low and variable in the products (2.1 – 15.2%). The amount of MUFA was high in 8N and 11N, 50.2 and 41.9%, respectively. The other products had less than 20% MUFA. The high content of Lc n-3 and low content of 18:2 n-6 in 5 of the Norwegian products led to low n-6/n-3 ratio of less than 0.07. Product 12N had ratio of 0.14.

Table 3.2 – Fatty acid composition (% of total FAs) of marine Omega-3 supplement from Norwegian market.

	Percentage of fatty acid in total quantity of acids					
Fatty acid **Product:**	**8N**	**9N**	**10N**	**11N**	**12N**	**13N**
C14:0	3.4	-	-	-	1.6	3.1
C16:0	9.8	1.5	2.3	1.0	6.4	5.1
C18:0	2.0	3.6	4.2	1.1	3.9	2.0
C16:1 n-7	9.9	0.3	0.8	1.6	2.2	2.4
C18:1 n-9	14.5	6.2	9.1	12.5	12.9	6.1
C18:1 n-7	4.9	2.4	3.0	3.2	2.0	2.1
C20:1 n-9	12.9	2.8	1.8	16.8	1.0	2.6
C22:1 n-11	7.1*	1.8	1.1	6.4*	0.6	4.9*
C18:2 n-6	2.1	1.1	1.2	1.4	7.2	0.9
C18:3 n-3	0.9	-	0.7	-	23.9	-
C18:4 n-3	2.8	1.4	2.0	1.2	1.2	1.0
C20:4 n-6	-	2.3	2.1	0.7	1.0	1.8
C20:4 n-3	-	1.7	1.7	-	0.9	-
C20:5 n-3	10.0	37.0	35.7	11.0	19.1	21.7
C22:5 n-3	1.3	5.6	4.0	9.9	2.7	4.3
C22:6 n-3	13.9	28.2	26.9	21.2	12.4	37.0
NIA**	4.6	3.3	3.3	12.0	0.9	4.3
∑ **SFA**	15.2*	5.1	6.5	2.1*	11.9	10.9*
∑ **MUFA**	50.2	14.6	15.8	41.9	18.8	19.7
∑ **PUFA**	30.9	77.3	74.8	45.8	68.4	66.6
– **n-6**	2.1	3.3	3.7	2.5	8.3	2.7
– **n-3**	28.8	74.0	71.1	43.3	60.1	63.9
Lc n-3* **	25.2	70.8	66.6	42.1	34.2	63.0
n-6/n-3	0.07	0.04	0.05	0.06	0.14	0.04

*** -** may contain small amount of 20:4 n-3; ** - non-identified acids and acids represented in few samples in relatively small amounts (less than 1.5 % of total FAs); *** - Long chain Omega-3 fatty acids, i.e. summed content of EPA, DHA, DPA.
SFA=saturated fatty acids; MUFA=monounsaturated fatty acids; PUFA=polyunsaturated fatty acids.

The results from the analysis of the fatty acids in the commercial products were also expressed as milligrams of the individual fatty acid per gram of oil. This was done to be able to calculate the amount oil/products necessary to consume on a daily basis to obtain a recommended intake of 0.5g Lc n-3.

In the Russian products content of fatty acids varied from 792.8 to 967.8 with the average equal to 859.3 mg/g of oil.

Table 3.3 – Content of fatty acids in mg per g of oil in marine Omega-3 supplements from Russian market.

		Quantity of fatty acid, mg/g of oil in product						
Fatty acid	**Product:**	**1R**	**2R**	**3R**	**4R**	**5R**	**6R**	**7R**
C14:0		27.3	57.0	57.3	26.3	44.1	58.0	33.1
C16:0		86.9	140.4	137.9	92.7	119.8	135.6	97.9
C18:0		23.6	28.9	28.4	28.2	28.9	27.4	22.6
C16:1 n-7		27.4	81.5	75.9	40.2	60.0	75.8	42.0
C18:1 n-9		348.4	67.2	66.0	202.7	99.2	82.0	231.7
C18:1 n-7		27.6	24.7	24.4	22.9	22.0	24.5	25.9
C20:1 n-9		48.1	9.7	9.7	51.3	8.7	6.6	30.3
C22:1 n-11		45.9*	-	5.6	52.0*	7.3	4.8	33.1*
C18:2 n-6		150.3	13.8	5.4	264.2	108.3	15.3	73.0
C18:3 n-3		45.8	6.8	3.1	12.4	5.7	6.2	27.8
C18:4 n-3		7.3	31.6	27.9	14.6	18,8	21.2	11.6
C20:4 n-6		-	7.4	8.5	-	7.3	8.6	-
C20:4 n-3		-	8.8	8.0	-	5.4	6.5	-
C20:5 n-3		29.9	172.4	164.5	52.4	125.5	148.0	67.0
C22:5 n-3		14.8	15.9	17.0	7.8	13.9	16.3	16.1
C22:6 n-3		38.5	104.7	104.3	60.5	79.3	97.7	60.4
NIA**		24.6	77.7	53.1	20.5	33.8	40.0	18.4
∑ **SFA**		137.8*	231.4	223.6	147.3*	192.7	220.9	153.6*
∑ **MUFA**		503.9	183.0	181.5	369.0	197.1	193.7	367.8
∑ **PUFA**		301.6	387.2	361.9	411.9	380.3	338.2	262.6
	– **n-6**	165.3	21.2	14.0	264.2	115.6	23.9	79.6
	– **n-3**	136.3	340.2	324.8	147.7	248.7	295.9	183.0
Lc n-3* **		83.3	293.1	285.8	120.7	218.7	262.0	143.6
n-6/n-3		1.21	0.06	0.04	1.79	0.46	0.08	0.43
∑ **FA**		967.8	879.3	820.1	948.7	803.9	792.8	802.4

*** -** may contain small amount of 20:4 n-3; ** - non-identified acids and acids represented in few samples in relatively small amounts (less than 1.20 g of acid per 100 g of oil in sample); *** - Long chain Omega-3 fatty acids, i.e. summed content of EPA, DHA, DPA.
SFA=saturated fatty acids; MUFA=monounsaturated fatty acids; PUFA=polyunsaturated fatty acids.

In the Norwegian products content of fatty acids varied from 780.3 to 907.3 with the average equal to 844.3 mg/g of oil.

Table 3.4 – Content of fatty acids in mg per g of oil in marine Omega-3 supplements from Norwegian market.

		Quantity of fatty acid, mg/g of oil in product					
Fatty acid	**Product:**	**8N**	**9N**	**10N**	**11N**	**12N**	**13N**
C14:0		27.9	-	-	-	13.8	23.9
C16:0		80.2	13.9	18.6	8.6	54.8	38.7
C18:0		16.6	32.3	33.5	9.7	33.2	15.0
C16:1 n-7		80.6	2.6	6.6	13.7	18.7	18.1
C18:1 n-9		118.9	55.6	73.1	110.3	110.7	46.8
C18:1 n-7		39.9	21.6	23.9	28.6	17.4	16.3
C20:1 n-9		105.8	25.6	14.4	148.0	9.0	20.0
C22:1 n-11		58.2*	15.9	8.6	56.1*	5.3	37.6*
C18:2 n-6		17.1	9.6	9.8	12.4	61.9	7.0
C18:3 n-3		6.9	-	5.8	-	203.8	-
C18:4 n-3		22.8	12.8	16.3	10.8	10.1	7.4
C20:4 n-6		-	20.2	17.1	5.8	8.8	13.4
C20:4 n-3		-	15.3	13.6	-	7.4	-
C20:5 n-3		81.6	332.1	286.0	96.9	163.4	165.8
C22:5 n-3		10.4	50.0	32.4	86.7	23.1	32.5
C22:6 n-3		113.6	253.3	215.8	186.6	106.0	282.3
NIA**		37.7	36.5	24.3	105.0	7.5	38.4
∑ **SFA**		124.7*	46.2	52.2	18.2*	101.7	83.4*
∑ **MUFA**		411.1	131.3	126.6	368.5	161.0	150.0
∑ **PUFA**		252.5	693.3	599.6	402.9	584.5	508.5
	– **n-6**	17.1	29.8	29.8	21.8	70.6	20.4
	– **n-3**	235.4	663.5	569.9	381.1	513.8	488.0
Lc n-3***		205.6	635.4	534.2	370.3	292.5	480.6
n-6/n-3		0.07	0.04	0.05	0.06	0.14	0.04
∑ **FA**		825.9	907.3	802.7	894.7	854.7	780.3

*** -** may contain small amount of 20:4 n-3; ** - non-identified acids and acids represented in few samples in relatively small amounts (less than 1.20 g of acid per 100 g of oil in sample); *** - Long chain Omega-3 fatty acids, i.e. summed content of EPA, DHA, DPA.
SFA=saturated fatty acids; MUFA=monounsaturated fatty acids; PUFA=polyunsaturated fatty acids.

3.2 Lipid classes

The lipid classes in the marine Omega-3 supplements were determined by high performance thin layer chromatography. Two independent experiments gave the similar results, which are presented in Figures 3.1 – 3.3.

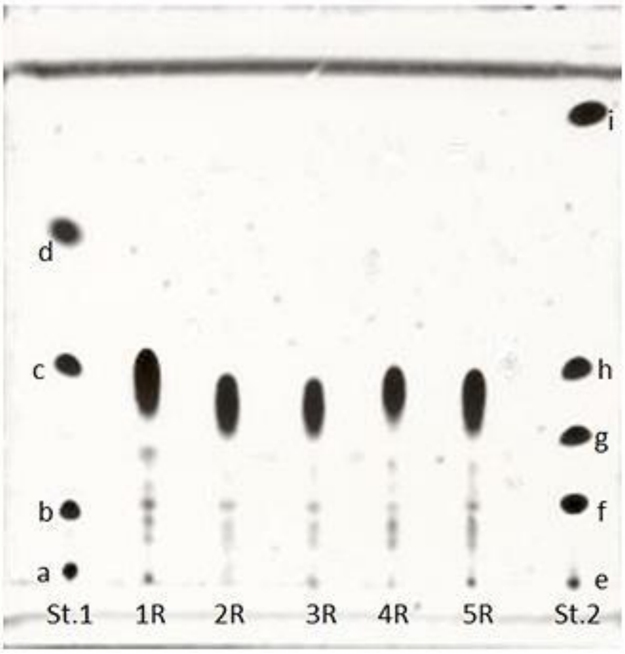

Figure 3.1 – TLC of lipids in samples 1R – 5R. St.1: fatty acid standard 18-1A contained monoolein (a); diolein (b); TAG, triolein (c); methyl oleate (d). St.2: fatty acid standard 18 – 5A contained lecithin (e), cholesterol (f); oleic acid (g); TAG, triolein (h); and cholesteryl oleate (i).

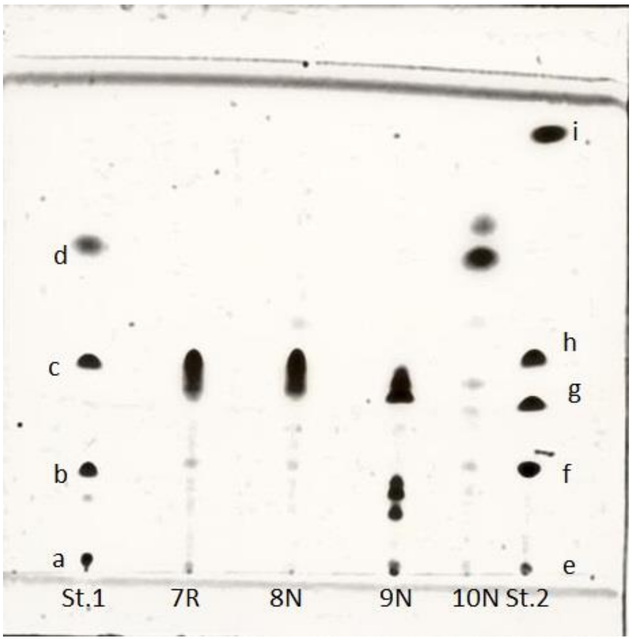

Figure 3.2 – TLC of lipids in samples 7R, 8N, 9N, 10N. St.1: fatty acid standard 18-1A contained monoolein (a); diolein (b); TAG, triolein (c); methyl oleate (d). St.2: fatty acid standard 18 – 5A contained lecithin (e), cholesterol (f); oleic acid (g); TAG, triolein (h); and cholesteryl oleate (i).

All Russian products (1R-7R) contain lipids mostly in form of TAG (Figures 3.1, 3.2 and 3.3). Other lipid classes with low mobility like phospholipids, diacylglycerol (DAG) and probably also cholesterol are present in all the products in very small amounts.

The Norwegian products (Figure 3.2 and 3.3) can be divided into 3 main types with reragd to lipid class composition. Supplement 8N consists almost exclusively of triacylglycerol. Supplements 9N and 12N have TAG as the dominating class. In 9N the other lipid classes present have lower mobility than TAG and can be identified as diacylglycerol and 2 other unidentified lipid classes with slightly lower mobility. Using the standards as references the presence of phospholipids and monoacylglycerol (MAG) can be seen.

Product 12N has lipid classes with both lower and higher mobility than TAG present. Of the 4 spots with higher mobility only ehtyl esters may tentatively be identified. In addition, DAG is identified in appriable amounts. Monoacylglycerol may also be present in minor ammount.

In the third type of Norwegian supplements (10N, 11N and 13N) the dominating lipid class was identified as ethyl esters. Product 10N contains very small amount of other lipid classes while in 11N MAG or phospholipids are apparently present in small amount. In addition to ethyl esters, product 13N also contains TAG, free fatty acids, DAG and/or cholesterol and phospholipids.

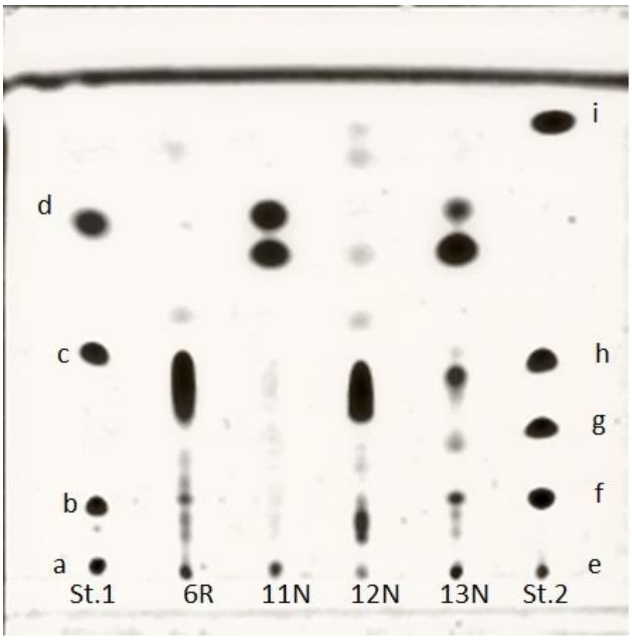

Figure 3.3 – TLC of lipids in samples 6R, 11N, 12N, 13N. St.1: fatty acid standard 18-1A contained monoolein (a); diolein (b); TAG, triolein (c); methyl oleate (d). St.2: fatty acid standard 18 – 5A contained lecithin (e), cholesterol (f); oleic acid (g); TAG, triolein (h); and cholesteryl oleate (i).

4. Discussion

According to the product information all Russian products were described as fish oils containing no plant components. However, in 4 of 7 products (2R, 3R, 5R and 6R) linoleic acid (LA;18:2 n-6) was present in 9.2% or more. This fatty acid is the signature fatty acid in plant oils (Gunstone, 2004). Wild fish oils normally contain less than 2% 18:2 n-6 (McGill & Moffat, 1992; Jensen et al., 2013). This suggested that these 4 products are mixes of fish oil and plant oil, and this is not declared on the packages.

The n-6/n-3 ratio in the Russian products was reflected by the presence of plant oils. It has been estimated that this ratio in the current Western diet is 15 – 17/1 (Simopoulos, 2008). A reduction of this ratio has been suggested to benefit human health (Harris and von Schacky, 2004; Simopoulos, 2008; Stanley et al., 2007). Although plant oils with n-6 fatty acids were included in 4 of the products, all the Russian supplements would contribute to improve the ratio.

There were no claimed quantities of EPA and DHA for all Russian products. Observed content of EPA and DHA in the Russian products and the amount of oil needed to be consumed to meet the daily recommended intake (RDI) of 500 mg EPA and DHA are summarized in Table 4.1. The quantity of Omega-3 fatty acids was on the labels of supplements 1R, 2R, 4R – 6R. However, there was no information how much oil one capsule includes in these products. It is, therefore, difficult to make a comparison between observed and claimed values. Two products, 3R and 7R, did not give information about content of any fatty acid. For 3R it was specified that 2 capsules provide 95% of the recommended daily intake. However, the RDI was not mentioned. 7R description had only information about vitamins present.

For 5 of the Russian products the recommended daily dose was from 9 to 24 capsules. Most people would probably find it difficult to take so many capsules on a daily basis. Product 3R recommended only 2 capsules while 7R is a liquid oil product (cod liver oil). It was, however, not specified how much oil one should take.

Table 4.1 – Amount of oil from Russian products needed to obtain recommended daily intake of DHA+EPA (500 mg).

	Product						
	1R	**2R**	**3R**	**4R**	**5R**	**6R**	**7R**
Observed DHA+EPA amount, mg/g of oil	68.4	277.1	268.8	112.9	204.8	245.7	127.4
Needed amount of oil in product*, g	7.3	1.8	1.9	4.4	2.4	2.0	3.9**
Observed needed amount of product*, capsules	-	-	-	-	-	-	-

* To consume obtaining recommended daily intake; ** ml.

All the Russian supplements contained oil in form of triacylglycerol. Three of the Russian products had a long-chain Omega-3 fatty acids (Lc n-3) content of more than 30% (33.8 – 36%) suggesting that fatty acid in them may have been concentrated in chemical/biochemical process. This is, however, not certain since some fish may contain 32 – 33% of Lc-n-3 (McGill & Moffat, 1992).

The Norwegian product 8N was described as cod liver oil, while 9N, 10N and 12N were said to contain fish oil or fish oil concentrate (triglyceride). Product 12N was also declared to contain linseed oil which explains the high content of 18:2 n-6 (7.2%) and 18:3 n-3 (23.9%). In other products no elevated level of 18:2 n-6 could be detected confirming the marine nature of the products.

Product 11N was declared to contain highly concentrated seal oil and 13N to contain a combination of fish oil and krill oil. The results from the analysis of the fatty acid composition showed that the product 8N is native cod liver oil. The high amount of Lc n-3 suggested the other Norwegian supplements are concentrated products.

The HPTLC analysis suggested that the information given on the Norwegian products sometimes was misleading. For example, 11N which was declared to contain “highly concentrated seal oil” contains ethyl esters, not triacylglycerols. Ethyl esters are not found in nature but made by processing as described in the background chapter. Product 13N was declared to be fish and krill oil. The HPTLC showed that the dominating lipid class is ethyl esters, not TAG, which are found in fish oil.

All Norwegian products had values of EPA, DHA and Omega-3 content in description. Only product 9N did not contain information of oil amount in capsules. Comparison of claimed and observed values of Omega-3, EPA and DHA for all Norwegian products, except 9N, is present in Table 4.2. Product 8N had claimed values of Omega-3 and DHA slightly lower than observed ones with difference of 2.0 – 5.3%. Product 11N had observed values higher than values claimed on pack with difference of 5.9 – 24.4%. Products 10N, 12N and13N had claimed values lower than observed with difference from 12.0 to

39.3%. Herewith, difference between observed and claimed DHA content in product 10N was 1.9%.

Table 4.2 – Comparison of claimed and observed content of fatty acids in oil in product, mg/g.

	Fatty acids mg/g of oil in product					
	Omega-3		**EPA**		**DHA**	
Product	Claimed	Observed	Claimed	Observed	Claimed	Observed
8N	240	235.4	80	81.6	120.0	113.6
10N	650	569.9	330	286.0	220	215.8
11N	360	381.1	80	96.9	150	186.6
12N	831	513.8	444*	163.4	444*	106.0
13N	577	488.0	182	165.8	358	282.3

*EPA+DHA.

N-6/n-3 ratio in all the Norwegian products was comparatively low. Five of the Norwegian products had the ratio of less than 0.07 and product 12N had ratio of 0.14. Therefore, these supplements will contribute to improve current Western diet ratio of n-6/n-3, benefiting human health.

Most of the Norwegian products had RDI of 2 capsules on their description what meets observed RDI (Table 4.3).

Table 4.3 – Amount of oil from Norwegian products needed to obtain recommended daily intake of DHA+EPA (500 mg).

	Product					
	8N	**9N**	**10N**	**11N**	**12N**	**13N**
Observed DHA+EPA amount, mg/g of oil	195.2	585.4	501.8	283.5	269.4	448.1
Needed amount of oil in product*, g	2.6	0.9	1.0	1.8	1.9	1.1
Needed amount of product*, capsules	2.6**	-	2	2	2	2
On-pack RDI, capsules	5**	2	2	1-2	2	2

* To consume obtaining recommended daily intake; ** ml

Price of the Omega-3 supplements in Russia and Norway varied from 0.97 to 9.21 NOK per gram of Omega-3 declared on package, with average price of Russian product equal to 3.28 NOK and Norwegian product – 3.93 NOK. However, for 2 Russian products, 3R and 7R, price per g of Omega-3 cannot be obtained from product label.

Conclusion

The Russian products contained triacylglycerols and generally had a low concentration of long-chain Omega-3 fatty acids. All the supplements were declared as fish oils, but, in fact, 4 of them contained plant oils resulting in an increased n-6/n-3 ratio. The Russian product descriptions had no information about the amount of oil in capsules or quantity of EPA and DHA. The recommended intake for 5 of the 6 capsule products was high, from 9 to 24 per day.

The Norwegian capsule products (5) were mostly concentrated supplements containing up to 71% of long-chain Omega-3 fatty acids, either in the form of ethyl esters or triacylglycerols. Information about content of Omega-3 fatty acids and EPA and DHA was included on the labels. However, information about oil amount in capsule was lacking for one product. The analysis suggested that the specification with regard to the lipid class form was not always precise. Two products were described as native marine oils, but the analysis showed that they contained ethyl esters. Claimed and found content of Omega-3, EPA and DHA, were slightly different for one product, up to 39% lower for 3 products and higher for one supplement. Daily intake of 2 capsules on the average met the recommended daily intake of 500mg of EPA+DHA.

The average price per gram of Omega-3 was approximately the same for the Russian and Norwegian products.

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