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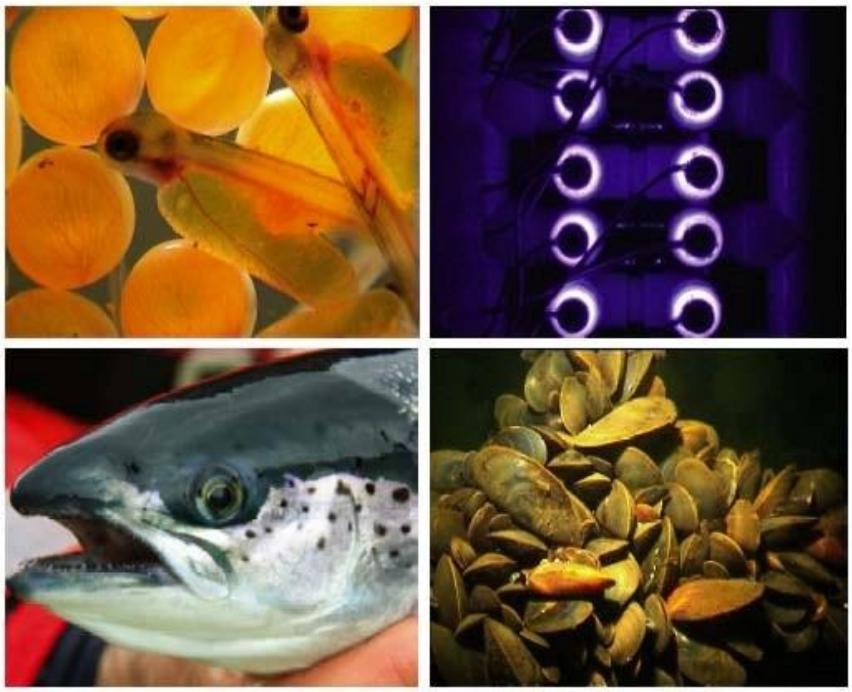
SARF091

Use of algal and other non-fish oils in Refined Edible Products



A REPORT COMMISSIONED BY SARF
AND PREPARED BY

Connel Marine Consultancy Services

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List of Abbreviations

AA	arachidonic acid
ALA	alpha-linolenic acid
DG	diglyceride
DHA	docosahexaenoic acid
DS	dietary supplement
EE	ethyl ester
EFA	essential fatty acids
EPA	eicosapentaenoic acid
FA	fatty acids
FAO	Food and Agriculture Organization
FODS	fish oil dietary supplement
FFA	free fatty acids
GHG	greenhouse gases
GLA	gamma-linolenic acid
HUFA	highly unsaturated fatty acids
LA	linoleic acid
LC	long chain
MG	monoglycerides
MMT	million metric tons
MO	micro-organism
MUFA	monounsaturated fatty acid
MT	metric ton
ORP	open raceway pond
PBR	photobioreactor
PL	phospholipid
PUFA	polyunsaturated fatty acid
SC	short chain
SDA	stearidonic acid
SFE	supercritical fluid extraction
TFA	total fatty acids
TG	triglyceride
rTG	re-esterified triglyceride
REP	refined edible product
UFA	unsaturated fatty acids
VLC	very long chain

1. Executive Summary

1.1. Background

1.1.1. Omega-3 fatty acids are a group of long chain, highly unsaturated fatty acids [HUFAs] commonly referred to as Essential Fatty Acids as they cannot be synthesized de novo by mammals and must be included in diet.

1.1.2. Omega-3 HUFA constitute a key element of carnivorous finfish diets with global aquaculture currently consuming >80% of the global resource annually.

1.1.3. High value salmonids such as Atlantic salmon and Rainbow trout are the largest user of these lipids consuming approximately 55% of global supply each year.

1.1.4. These species constitute the majority of UK aquaculture production which has one of the highest rates of inclusion of dietary Omega-3 lipids in the world.

1.1.5. The Scottish salmonid aquaculture industry in particular is one of the world's largest single consumers of Omega-3 lipids utilizing ~30,000 MT annually.

1.1.6. Approximately 85% of global Omega-3 resources are sold as crude fish oils derived from wild stocks of forage fish notably small inedible pelagic species such as anchovy, herring, menhaden and hoki.

1.1.7. The overwhelming majority of crude fish oil [>80%] comes from the Peruvian anchovy fishery however concerns over overfishing and climate change have resulted in tight quota restrictions, largely static oil production and soaring price increases over the past ten years.

1.1.8. As a result, levels of dietary Omega-3 lipids in farmed salmonid feeds are in decline with increasing substitution with short chain predominantly Omega-6 type PUFA from terrestrial plants.

1.1.9. The concomitant effect of lower LC Omega-3 levels in farmed fish products is a reduction in embedded levels in finished fish products and thus reduced appeal of farmed salmon as a healthy food choice for consumers.

1.1.10. In response, UK salmonid aquaculture is examining the effects on profitability of a separation of finished products into premium Omega-3 rich and lower quality, terrestrial oil fed categories however this strategy does not overtly address the human health and thus economic, costs that a widespread and well documented deficiency in these EFAs is known to have.

1.1.11. For example, long chain, Omega-3 HUFAs especially EPA+DHA are essential in maintaining healthy cell structure and serve as chemical precursors to a variety of anti-inflammatory, anti-thrombotic, anti-arrhythmic and vasodilatory compounds effective in the management of various chronic illnesses most notably cardiovascular disease.

1.1.12. Recently for example, the Omega-3 industry representative, the Global Organization for EPA and DHA [GOED], estimated the total cost of treating the numerous diseases and illnesses that EPA+DHA are believed to play a role in ameliorating at ~US$47 trillion over the next twenty years.

1.1.13. This equates to ~US$42,000 per adult if only the developed world assumes the cost.

1.1.2. The Refined Edible Products Sector

1.1.2.1. The production and commercialisation of highly refined marine-type lipids for direct human consumption has grown almost four fold over the past three decades driven primarily by increasing public awareness of the human health

benefits of Omega-3 intake, increasing consumer interest in functional and fortified foods and technical advancement in lipid extraction and refinement.

1.1.2.2. The Omega-3 REP market is comprised largely of refined EPA+ DHA concentrates encapsulated as dietary supplements or as functional ingredients in infant formula and myriad other foods such as cereal bars, yoghurt, milk, bread and cheese.

1.1.2.3. In terms of demand, the REP sector currently consumes approximately 25% of available crude Omega-3 lipid supplies with this expected to increase at a Compound Annual Growth Rate of ~15% over the next five years.

1.1.2.4. The great majority of these [>96%] came from wild fisheries with the remainder from farmed salmon, algae, yeasts and terrestrial oilseeds.

1.1.2.5. These new algal and other non-fish Omega-3 supply chains offer a more traceable, high purity source of Omega-3's for the tightly regulated REP sector and a number of large pharmaceutical companies currently supply microlalgal oils to the dietary supplements and infant formula markets.

1.1.2.6. This is particularly true for high-end clinical and pharmaceutical markets where high concentration, high quality Omega-3 products are required

1.1.2.7. Processing and refining of crude fish oil to produce the dietary supplements [DS] and other REP products however represents a major cost to manufacturers with up to 50% of total lipids [including some EPA+DHA] routinely lost during manufacture of standard pharmaceutical grade Omega-3 oil i.e. 30% - 40% EPA+DHA.

1.1.2.8. Moreover, production of clinical strength EPA+DHA concentrates [i.e. ≥ 80% EPA+DHA in total lipid] can result in the loss of up to 90% of crude fish oil and represents a highly inefficient use of these finite and highly sought after resources.

1.1.2.9. Industry representative GOED however recently stated that new extraction and refinement methods under development by major REP sector players should considerably increase EPA+DHA yields by as much as 70% for higher strength concentrates i.e. ≥80% EPA+DHA, thus reducing overall demand for crude fish oil from this sector in the near term.

1.1.2.10. Nevertheless, continued growth in consumer demand for Omega-3 REPs combined with the on-going expansion of global aquaculture production to feed a growing developing world population points to the emergence of a major "fish oil trap" within the next three years if cost-effective and sustainable alternative sources are not found soon.

1.1.3. Demand for Omega-3 Ingredients

1.1.3.1. The Food and Agriculture Organization [FAO] of the United Nations predicts overall global demand for Omega-3 HUFAs to increase by 45% to 50% of current production capacity by 2015.

1.1.3.2. While the majority of this increased demand is expected to come from net growth in total global aquaculture production, a significant proportion will come from the rapidly expanding Omega-3 REP sector.

1.1.3.3. Growth in the nascent Omega-3 REP sector has been driven primarily by an increasing body of evidence underlining the efficacy of EPA+DHA in the treatment of the myriad symptoms of cardiovascular disease in particular high

cholesterol, platelet aggregation and inflammation as well as the essential role they play in neonate and infant brain development.

1.1.3.4. As a result EPA+DHA have emerged as one of the fastest growing ingredients in the nutrition and drug marketplace with manufacturers of Omega-3-rich REPs paying up to 50% above current market price for crude fish oil and in so doing severely limiting supply share for less capital rich competitors such as the aquaculture sector.

1.1.3.5. Thus the Scottish Aquaculture Research Forum has commissioned this report to investigate the feasibility of increased Omega-3 production from novel alternative sources with the underlying rationale being that continued demand for Omega-3 oils particularly from a rapidly expanding REP sector could serve as a means of reducing prohibitively high production costs associated with algal lipids and in doing so improve access to an affordable supply share for the aquaculture sector.

1.1.4. Securing Future Supply

1.1.4.1. The current absence of commercially viable alternatives to forage fish or improved methods for extraction of greater proportions of EPA+DHA from crude fish oil is placing increasing pressure on Scottish aquaculture to compete with an ever growing Omega-3 REP sector to secure an affordable supply of these essential ingredients.

1.1.4.2. Intensive efforts are currently underway by several private and public bodies however to commercialise production of Omega-3 HUFAs from a variety of alternative sources.

1.1.4.3. Being the primary source of EPA+DHA in the marine environment, for example, a number of microalgal groups, including diatoms, crysophytes, cryptophytes and dinoflagellates for example, contain up to 80% of their cell dry weight in lipids with several species producing exceptionally high concentrations of LC Omega-3 HUFAs and low concentrations of Omega-6 HUFAs, MUFAs and SFAs.

1.1.4.4. Microalgae also produce an array of other potentially valuable proteins, carbohydrates, pigments, anti-oxidants and other metabolites while de-oiled biomass is also suitable for anaerobic digestion to produce biogas.

1.1.4.5. Nevertheless, the costs associated with algal Omega-3 production however remain prohibitively high and have thus far inhibited industrial scale production.

1.1.4.6. For example, compared to crude fish oils retailing at ~£1,600 per tonne, microalgal lipids, as currently produced, cost approximately £150,000 per tonne.

1.1.4.7. Some of the bottlenecks contributing to these high production costs include: 1) the need to dry algal biomass prior to lipid extraction when using traditional methods of oil extraction, 2) the large volumes of organic solvents commonly required to extract the algal oils, and 3) purification costs associated with generating usable pharmaceutical-grade oils.

1.1.4.8. Under a business as usual scenario however, the predicted growth in demand for Omega-3's creates a theoretical fish oil price threshold beyond which industrial scale production of non-fish oils becomes a commercially attractive proposition to the REP sector.

1.1.4.9. For example, forecasters predict fish oil prices will exceed £2,200 MT by 2020, this being the maximum aquafeed manufacturers can currently afford to pay for

Omega-3 oils. At this point algal oils, though still far outside the reach of aquaculture, will become attractive to the right buyer, increasing demand, reducing production costs and increasing their contribution to the global Omega-3 resource.

1.1.4.10. Other, more financially attractive, alternatives however are also under development by the pharmaceutical sector including the yeast, *Y. lipolytica* as well as a number of terrestrial oilseeds, notably soybean and rapeseed.

1.1.4.11. Neither yeasts nor terrestrial oilseeds however produce EPA or DHA naturally rather they produce the short chain Omega-3 precursor ALA.

1.1.4.12. ALA is only very inefficiently converted to EPA+DHA in the human body and although beneficial does not perform the same essential functions of longer chain varieties.

1.1.4.13. Combined with both traditional forage fish and new algal supply chains however these terrestrial short chain Omega-3 resources could combine to provide meaningful quantities of blended short [ALA] and long chain [EPA+DHA] Omega-3 oils to supply niche markets in both the aquaculture and REP sectors potentially reducing pressure on existing supplies and over time, helping reduce fish oil prices.

1.1.4.14. Moreover, the genetic modification of terrestrial oilseed plants to produce LC Omega-3 HUFAs is rapidly gaining traction as a cost-effective means of meeting future demand with a number of large pharmaceutical companies, including Monsanto, DOW Agrosciences and DSM currently working to enhance their Omega-3 content.

1.1.4.15. Successful transformations of both soybean and canola genomes through the insertion of algal genes responsible for elongation and desaturation of short chain fatty acids, have already been reported and while these have been shown to produce significant quantities Stearidonic Acid [SDA] and EPA, production of DHA has not yet been achieved.

1.1.4.16. Nevertheless while GM organisms present a tantalizingly cost effective source of industrial quantities LC Omega-3 oils for the future, fears regarding their safety may impede commercial scale cultivation and widespread consumer uptake.

1.2. Main Points

1.2.1. Lipids are the most costly and limiting ingredient for aquaculture finfish feed manufacture in particular Omega-3 HUFA rich lipids high in DHA+EPA.

1.2.2. The Scottish salmonid aquaculture industry's current requirement is for up to 30,000 MT of EPA+DHA ingredients annually.

1.2.3. Global supply of crude Omega-3 HUFA rich lipids is highly dependent on wild capture of forage fish especially the Peruvian anchovy.

1.2.4. Increased public awareness of the unique health benefits of Omega-3 lipids has seen substantial growth in demand over the past decade from a rapidly growing REP sector.

1.2.5. Static supply of crude fish oils and the superior purchasing power of the REP sector however have considerably reduced availability of adequate market share to meet the demands of an expanding aquaculture sector resulting in significant reduction in inclusion levels of these essential dietary lipids to compound aquafeeds and reduced health benefits of final fish products.

1.2.6. Continued strong demand from both the aquaculture and REP sectors has furthermore resulted in the emergence of a substantial gap in the global supply of fish oils stimulating intensive research and investment into the commercialisation of production from algal and other non-fish sources

1.2.7. These include algae, yeasts, fungi and genetically modified terrestrial plants and although small, commercial volumes of non-fish Omega-3 lipids are currently being produced by a number of large pharmaceutical companies using heterotrophic microalgae such as *Cryptothecodinium cohnii* and *Schizochytrium* and the yeast, *Y. lipolytica*, the costs associated with non-fish lipids production remain prohibitively high for the aquaculture sector.

1.2.8. The re-emergence of strong interest in photoautotrophic microalgae as a feedstock for biofuels however has seen the appearance of a multitude of publicly and privately funded research programmes examining production from a variety of other species including *Isochrysis, Tetraselmis, Chaetoceros, Thalassiosira, Nannochloropsis*.

1.2.9. Being the primary source of EPA+DHA in the marine environment, microalgal species produce exceptionally high concentrations of LC Omega-3 HUFAs, low concentrations of Omega-6 HUFAs, MUFAs and SFAs as well as an array of other potentially valuable proteins, carbohydrates, pigments, anti-oxidants and other metabolites.

1.2.10. De-oiled algal biomass is also suitable for anaerobic digestion to produce biogas however a major downfall of this is that lipid extraction of microalgae feedstock with present day commercial technology requires algae to be dried to approximately 90% (w/w)

1.2.11. This highly energy intensive process [seven times higher than biomass production] is one of the main hurdles currently constraining industrial scale production of microalgal lipids.

1.2.12. Other obstacles preventing commercial production of affordable algae lipids include: 1) the use of large volumes of organic solvents to extract algal oils and 2) purification and refinement costs associated with generating usable food-grade oils.

1.2.13. Given the predicted growth in demand from the Omega-3 REP sector however there does exist a fish oil price threshold beyond which industrial scale production of microalgal and other non-fish oils is an commercially attractive proposition. This is particularly true for the clinical and pharmaceutical markets where high concentration, high quality Omega-3 products are required.

1.2.14. For example, while currently in developmental stages, genetically modified terrestrial plant seeds are emerging as a cost effective source of industrial quantities of shorter chain precursor Omega-3 FAs including SDA and even EPA, however consumer fears regarding safety of GMO's may impede commercialisation and market uptake.

1.3. Recommendations

The following specific research is considered important to the protection of adequate share of affordable Omega-3 lipid resources for the UK salmonid aquaculture industry:

- A critical pharma-economic analysis of the impacts of increased use of ultra-refined high concentration Omega-3 oils as a standard treatment for prevention of the

symptoms of CVD and CHD on the costs of production from algal and other non-fish sources and the potential such an approach might have on extending global supply share for less economically robust end users i.e. the aquaculture sector.

2. Introduction

Incipient research into the commercial potential of microalgae for fatty acid [FA] production occurred during the 1970's oil crises with the U.S. Department of Energy's "Aquatic Species Program". This investigated the potential of microalgae as a source of short chain MUFAs for use as biodiesel fuel though proved commercially uncompetitive as oil prices re-stabilized. More recent volatility in hydrocarbon fuel supply and increased consumer demand for refined LC Omega-3 HUFAs for direct human consumption combined with concerns regarding both climate change and food supply has seen a second wave of research and development into the potential of algae and other non-fish organisms as alternative sources not only of lipids but an array of low and high value co-products including carbohydrates and proteins, metabolites and other biologically active compounds. Consequently many hundreds of commercial companies involved in the exploitation of these novel organisms for pharmaceutical, nutraceutical and cosmeceutical applications have emerged over the past decade

The strong growth in demand for LC HUFAs from a nascent Omega-3 REP sector in particular has, in the presence of appropriate government policy supports, created an environment conducive to the leveraging of cost-effective production of these highly sought after commodities from novel non-fish sources. For example, several promising microscopic organisms with high Omega-3 HUFA contents are currently being optimised by large industry stakeholders for commercial-scale production using both traditional heterotrophic culture infrastructure and rapidly developing two-step photoautotrophic methods. These include oleaginous microalgae and various GMO organisms primarily yeasts and terrestrial oilseeds such as flax, soya and hemp which, transcripted with algal genes, are capable of elongating and desaturating short-chain Omega-3 HUFA to LC HUFA products including SDA and EPA.

These new lipid resources are becoming an increasingly valuable resource for the REP sector which in addition to meeting increasing consumer demand, recognises the potential added value of non-fish Omega-3 resources in terms of their increased appeal to vegetarian and environmentally conscientious consumers. However due to the prohibitively high capital costs associated with production from non-fish sources [especially microalgae] ever increasing consumer demand from the REP sector is placing considerable pressure on static forage fish resources with large supply gaps in particular, for the aquaculture sector, forecast. Thus, the overarching aim of this report was a techno-economic evaluation of the potential for increased production of refined LC Omega-3 HUFAs from algal and other non-fish organisms, an assessment of efforts currently underway or in development to ensure future demand is met and the potential for scaled production from novel non-fish sources thereby reducing dependency on forage fish resources, narrowing supply gaps and reducing cost for all end-users.

2.1. Study Aims and Objectives

Specifically, this involved investigation, quantification and assessment of:

1. Current volumes of production of crude Omega-3 type marine lipids from both fish and non-fish sources for the REP sector.
2. Costs of extraction and refinement associated with the refinement of crude lipids and concentration of LC EPA+DHA fractions.

3. Value aspects of transformation through the Omega-3 lipids supply chain, taking into account aspects such as food safety and quality standards, process wastage during extraction, packaging; storage and shelf wastage due to oxidation, and the different profit requirement of different sectors in terms of perceived investment risk.
4. Marketing aspects of a change from forage fish oils to algal and other non-fish derived oils and the possible reaction of retailers towards a vegetarian supply of LC Omega -3 lipids in particular for use in dietary supplements [DS].

2.2. Approach and Methods

Research was undertaken in five ways:

- Extensive desk based research and analysis of both peer-reviewed and "grey" literature.
- Direct structured contact with organisations involved in marine-type lipid production from all commercially viable sources and ancillary services.
- Individual meetings, emails and telephone interviews with representatives of organisations at all stages of the marine-type lipids supply chain.
- Information gathering on techno-economic factors concerning the production and supply of refined Omega-3 PUFAs to the REP sector by means of an electronic survey with stakeholders from various supply chains including forage fish, krill, algal and GMO's
- Direct consultation with supermarket retailers to assess capital gains if any to providing consumers with alternative sustainable and/or vegetarian sources of Omega-3's and perceived attitudes to obtaining same from genetically modified organisms.

2.3 Refinements to Approach and Methods

The initial Steering Group meeting was held in Edinburgh on 22nd February 2013. The core original objectives and methods (Section 1. and 2.1) remain relevant, but it is important to note that the Steering Group recommended that efforts be focused on:

- Clear and accurate fact finding in relation to current scales of production of marine-type lipids from algal and other non-marine sources.
- A review of the most widely used methods of extraction of Omega-3 HUFA fractions from crude marine-type oils, associated yields and volumes of waste oils generated.
- An assessment of the fatty acid composition and fate of these waste streams and an evaluation of their potential as a supplementary supply for aquafeed manufacture. An evaluation of the scope for leveraging a transition to algal oils by the REP sector given the price premium currently paid for crude fish oil [+50% current market prices] and in light of projected market growth for Omega-3 enriched REPs.

  Thorough but discriminating analysis of the "grey" literature available not only through public domain databases but the extensive and rapidly evolving corpus of online information on REP market dynamics including press articles, commercial company, industry and non-governmental organisation websites.

2.4. Reliability of Data

While the review of current and historic literature was as wide-ranging as possible the specific foci of this study remained an accurate assessment of the cost-competitiveness of refined LC Omega-3 HUFA production from novel non-fish sources and the identification of commercially compelling levers for sustainable industrial-scale production specifically to supply the REP sector. The authors therefore focused on a number of recent academic review papers examining known or projected economics of oleaginous algal [and other non-fish] biomass and lipid production and the suitability in terms of lipid composition of the various species under consideration. This was augmented by commercial production statistics from key public databases notably the Fisheries and Agriculture Outlook of the United Nations, for quantification, characterisation and value of the extant global Omega-3 resource.

Furthermore, due to the rapid recent evolution of a manifold Omega-3 REP sector the necessity for thorough assessment of “grey” data resources listed in Section 2.3. was considered tantamount to peer reviewed evaluations of the non-fish Omega-3 supply chain. However, due to the commercially sensitive nature of private sector advancements in this area and the as yet putative nature of some of the human health benefits ascribed to Omega-3 HUFAs, “grey” literature findings were interpreted cautiously and where possible, against the backdrop of peer-reviewed bench or pilot scale productivity estimates and where available, results of human clinical trials. Furthermore in an effort to ground-truth literature review findings, the author initiated broad scale direct consultation with a range of organisations including academic researchers, industry representatives and numerous commercial companies. This included semi-structured, questionnaire-style email contact, *ad hoc* telephone and email dialogues to capture as accurately as possible developments at all stages of the Omega-3 supply chain including raw material [both fish and novel non-fish] production, crude oil refinement, REP manufacture and supermarket retailer. Full details of all communications initiated by the study team are provided in Annex 3.

3. Literature Review: Omega-3 Fatty Acids

3.1. Introduction

Fatty acids [FAs] are important for the proper functioning of all human body systems in particular skin, respiratory and circulatory systems, brain and vital organs. Humans acquire FAs largely through diet and most food borne FAs occur esterified either as Triglycerides [TGs] or Phospholipids [PLs] or occasionally due to a partial hydrolysis, as Free Fatty Acids [FFA]. Among these there are a number of Highly Unsaturated FAs [HUFAs] which humans cannot produce *de novo* and are thus termed Essential FAs [EFAs]. These include Linoleic Acid [LA] and Alpha-Linolenic Acid [ALA]. Both LA and ALA are found extensively in plants and serve as metabolic precursors to two important groups of Highly Unsaturated FAs [HUFAs] commonly termed Omega-6 and Omega-3 HUFAs respectively. Omega-6 and Omega-3 HUFA represent major structural components of all mammalian cell membranes where they carry out several vital biological functions. Due to their unique structural characteristics Omega-3 HUFAs are especially important in maintaining proper cell structure and function and play a key role in reducing inflammation. Among these the LC Omega-3 HUFAs Eicosapentaenoic Acid [EPA] and Docosahexaenoic Acid [DHA] in particular are vital in maintaining cell membrane fluidity and produce a variety of secondary metabolites essential in maintaining proper cognitive and cardiovascular function.

Due to their unique physiochemical properties Omega-3 EPA+DHA exhibit a high degree of biological activity in human systems both directly influencing cellular membrane fluidity and permeability and indirectly [through the production of a range of bioactive metabolites] affecting cell membrane protein and gene expression. Most notably, research has shown EPA+DHA to be crucial in neonatal brain and eye development and proper cognitive and cardiovascular function in adults while clinical trials in human subjects has highlighted their effectiveness in reducing inflammation, preventing breast and prostate cancers and in the amelioration of the symptoms of chronic disease such as arthritis, certain mental illness and autoimmune disorders such as lupus and nephropathy. One recent study by Hibbeln et al. [2006] found that the reported widespread dietary deficiency in EPA and/or DHA is a contributing factor in about 84,000 deaths from heart disease each year in the US alone. Moreover the Global Organisation for EPA+DHA [GOED] has recently estimated the cost, over twenty years, to treat the medical conditions that EPA+DHA are believed to play a role in preventing at US$47 trillion, equivalent to US$42,000 per person if this is assumed by the developed world only. Not surprisingly then EPA+DHA currently represent two of the most researched compounds in modern healthcare with annual consumer spending on Omega-3 lipids recently estimated at US$25.4 billion per annum.

Omega-3 HUFAs are produced exclusively by photosynthetic plants. ALA however is produced primarily by terrestrial plants such as rapeseed, soybean, walnut, flaxseed and perilla while LC Omega-3 EPA+DHA are produced exclusively in the marine environment. Here they are synthesized from ALA by photosynthetic microalgae and transferred through the food web to accumulate in the bodies of forage fish such as herring, mackerel, salmon and halibut. Their primary function here is to maintain membrane fluidity at low temperatures with the cold, upwelling system species *Engraulis ringens* commonly known as the Peruvian anchovy or Anchovetta, representing the world's richest sources of these essential fatty acids. Depending on environmental conditions, up to ~30% of an anchovetta's body weight occurs as EPA+DHA; making this fishery the world's largest

single source of Omega-3 lipids today. Concerns over population instability due to the combined effects of overfishing and climate change however have necessitated severe restrictions on anchovy catch quotas in recent decades resulting in largely static production of crude fish oil [~1 Million Metric Tons [MMT] per year], the world's single most important source of Omega-3 HUFAs. Resulting increases in the price of fish oil have moreover, severely limited access to an adequate share of fish oil for manufacture of finfish aquafeeds, currently the largest sink for these lipids, while steadily increasing demand from new, expanding markets is creating significant supply shortfalls for all end-users and underlines the urgent need for scaled production from cost-effective alternatives for example algae, yeasts, fungi and terrestrial oilseeds.

The Refined Edible Products [REP] sector for example currently represents the fastest growing consumer of Omega-3 rich lipids, increasing in market supply share almost 5 fold from 5% to 24% of available resources between 1990 and 2010. Independent industry analysts Frost & Sullivan [2009] forecast this growth to continue at an average Compound Annual Growth Rate [CAGR] of ~13% of REP sales over the next five years equating to an approximate doubling in demand for these lipids from ~250,000 Metric Tonnes [MT] per yr. in 2013 to ~>500,000 Mt.yr by 2018. Coupled with this, production of food fish from aquaculture to feed an expanding global population has increased almost 12 fold since 1990 growing at an average annual average rate of 7% [State of World Fisheries and Aquaculture 2012; FAO Report] with aquaculture now providing over 50% of all the fish and shellfish consumed globally. And while this sector currently represents by far the largest consumer of marine-type lipids [utilizing an average of ~80% of global supplies] recent soaring increases in the price of crude fish oil has resulted in the emergence of an aggressively competitive market place wherein aquafeed manufacturers are losing an ever increasing share of available resources to a "bullish" and capital-rich Omega-3 REP sector.

In response to such tight supply constraints, the aquaculture sector has significantly reduced inclusion levels of dietary fish oil in compound aquafeeds, most notably in Atlantic salmon feeds. Over the past three decades for example, this sector has opted instead to target fish oil use treating it as a specialty rather than a standard, ingredient for high value starter, finisher and broodstock feeds [Jackson, 2007]. However while rates of inclusion of dietary Omega-3 oils in aquafeeds are forecast to continue to fall [0.5% to 7% p.a.] overall, the total amount of crude fish oils used in compound aquafeeds is expected to increase ~16% from 782,000 MT [2.7% of total aquafeeds by weight] in 2008 to 908,000 MT [1.3%] by 2020 [source Seafish 2012]. Combined growth in demand from competing REP and aquaculture sectors therefore is not only placing enormous pressure on wild forage fish stocks, with >90% of key species currently classified as fully exploited or overfished [UNFAO Report 2005], but has also created a growing supply shortfall estimated to reach ~0.5 Million MT [MMT] crude fish oil by 2020. Moreover, demand from the REP sector is forecast to exceed the IFFO proposed typical sustainable cap for supply of Omega-3 lipids from extant forage fish stocks this year [2013] underlining the acute need for commercially viable alternative sources to satisfy the considerable demand projected for the coming decade

While by far the dominant source for Omega-type HUFA today is the Peruvian anchovetta fishery [~80% total supplies] several promising alternatives are currently being investigated for commercial scale production. These include marine algae and krill, fungi [in particular

the yeast *Y. lipolytica*] and Genetically Modified Organisms [GMOs] most notably the terrestrial oilseed plants soybean, canola and rape. In addition, currently under-exploited resources from capture fisheries by-catch, seafood processing waste and Omega-type wastes resulting from the EPA+DHA concentration process are also being economised to ensure maximum utilisation of available EPA+DHA fractions. For example, poor extraction efficiency resulting in losses of between 50% and 90% of crude oil during production of concentrated nutra- and pharmaceutical grade Omega-3 oils constitutes a substantial capital cost for the REP industry such that improving extraction technologies is now a primary focus for this sector. Additional capacity from wild fisheries other than the Peruvian anchovetta including menhaden, mackerel, tuna and wild salmon are also being examined while current research indicating that lower EPA+DHA inclusion levels in the diets of farmed fish [especially carps] results in higher retention and concentration of these in the flesh of fed fish and thus the potential for net production of these highly sought after commodities by the aquaculture sector[1].

3.2. Classification and Chemistry

3.2.1. Introduction

FA nomenclature systems are numerous and complex however all systems assign a trivial or common name in addition to two numbers representing the number of carbon atoms in the chain and the number of double bonds present. For example, oleic acid which contains 18 carbon atoms and 1 double bond could be written as 18:1. Trivial or common names have been in use for many years but because they provide no information on the chemical structure or biochemical role of FAs a number of systematic classification systems have been developed [see Appendix 1]. These include the Geneva, the Delta and the Omega systems. Briefly, the former describes the basic structure of the fat molecule while both the Delta and Omega systems provide more detailed information on both location and configuration of double bonds present. A complete list of Geneva names, formulas and physical properties of some common FAs are given in Table 1 however given it is the only system that refers to sites of bioactivity, the Omega system is most commonly used by both the professional and lay communities to describe nutritionally important fatty acid molecules including the LC Omega-3 FAs, EPA and DHA.

3.2.1.1. The Omega System

The Omega [n or ω] system is often used by biochemists to designate sites of enzyme reactivity or specificity on the FA molecule and appears in two very similar forms; the professional n-minus system and the lay omega [ω] system. Both systems identify carbon chain length and double bondedness numerically but in contrast to both the Geneva and Delta systems, the location of the last double bond is numbered according to its position relative to the methyl or N terminal, end and not the carboxyl end as with previous systems. Due to the reference made to molecular sites of bioactivity in nutritionally important UFAs these are commonly classified using the Omega system and five primary groups have been described. These include the saturated Omega-12 group the largely mono-unsaturated Omega-9 and Omega-7 groups and the more physiologically active polyunsaturated Omega-6 and Omega-3 groups. Unlike Omega-12s, -7 and -9 FAs, Omega-6 and Omega-3 FAs cannot be produced *de novo* in the human body and so are referred to as Essential FAs [EFAs] as they must be included in diet.

1. See: http://www.globefish.org/farmed-fish-a-major-provider-or-a-major-consumer-of-omega-3-oils.html

Table 1. List of Geneva names, trivial names, numbers of carbon atoms and double bonds and melting points for some common unsaturated, mono- and poly- unsaturated FAs

Geneva Name	Trivial Name	Carbon atoms	Double Bonds	Melting Point °C
Ethanoic	Acetic acid	2	0	-7.9
Butanoic	Butyric acid	4	0	-3.4
Hexanoic	Caproic acid	6	0	16.7
Octanoic	Caprylic acid	8	0	31.6
Decanoic	Capric acid	10	0	44.2
Dodecanoic	Lauric acid	12	0	54.4
Tetradecanoic	Myristic acid	14	0	62.9
Hexadecanoic	Palmitic acid	16	0	69.6
Octadecanoic	Stearic acid	18	0	75.4
Eicosnoic	Arachidic acid	20	0	80.0
Docosanoic	Behenic acid	22	0	16.3
9 Octadecanoic	Oleic acid	18:1	1	-6.5
9, 12 Octadecadienoic	Linoleic acid	18:2	2	-6.5
9,12,15 Octadecatrienoic	Linolenic acid	18:3	3	-12.8
5,8,11,14 Eicosatetraenoic	Arachidonic acid	20:4	4	-49

3.2.1.1.1. Omega-6 Fatty Acids

Omega-6 FAs are a family of PUFAs that have their final carbon–carbon double bond in the *n*−6 position, or on the sixth carbon-to-carbon bond when counting from the methyl end. The Omega-6 HUFA Arachidonic Acid [AA, 20:4n-6] in particular is essential to proper neural transmission and retinal development in humans and acts as a chemical precursor to a variety of important eicosanoids [short-lived hormone-like lipids including prostaglandins, thromboxanes, leukotrienes and other oxygenated derivatives].

Table 2. Some nutritionally important Omega-6 PUFAs

Common Name	Systematic Name	N-minus abbreviation	Common sources
Linoleic Acid	all *cis* 9,12-octadecadienoic	18:2n-6 [LA]	Most vegetable oils
y-linolenic Acid	all *cis* 6,9,12-octadecatrienoic	18:3n-6 [GLA]	Evening primrose, borage and blackcurrant seed oils
Dihomo-*y*-linolenic Acid	all *cis* 8,11,14-eicosatetrienoic	20:3n-6 [DHGLA]	Very minor component in animal tissues
Arachidonic Acid	all *cis* 5,8,11,14-eicosatetraenoic	20:4n-6 [AA]	Animal fats, liver, egg lipids, fish

3.2.1.1.2. Omega-3 Fatty Acids

Omega-3 FAs consist of a family of polyunsaturated FAs with their first double bond located three carbons from the methyl end of the FA molecule. Eicosapentaenoic Acid [EPA; 22:5 n-3], Docosapentaenoic Acid [DPA; 22:5n-3] and Docosahexaenoic Acid [DHA 22:6n-3] are three important Omega-3 FAs found in fish oils and due to their length and high degree of unsaturation are commonly referred to as VLC Omega-3 HUFAs. EPA+DHA appear particularly important in maintaining proper human health and serve as chemical precursors to a variety of anti-inflammatory, anti-thrombotic, anti-arrhythmic and

vasodilatory eicosanoids [i.e. biologically active hormone-like lipids]. In addition, through comparatively understudied, a single report published in 1996 suggests that DPA, an elongation metabolite of EPA and precursor to DHA, may in fact be 10-20 times more powerful in preventing arteriosclerosis[1] however the relatively low concentrations of DPA in fish oil appear to have limited further study into its function in human systems health.

Table 3. Some nutritionally important Omega-3 PUFAs

Common Name	Systematic Name	N-minus abbreviation	Common sources
α-linoleic Acid	all *cis* 9,12,15-octadecatrienoic	18:3n-3 [ALA]	Flaxseed oil, perilla oil, canola oil, soy oil
Stearidonic Acid	all *cis* 6,9,12,15-octadecatetraenoic	18:4n-3 [SDA]	Fish oils, genetically enhanced soy oil, blackcurrant seed oil, hemp oil
Eicosapentaenoic Acid	all *cis 5,*8,11,14,17-eicosapentaenoic	20:5 n-3 [EPA]	Fish, especially oily fish [anchovy, sardine, mackerel, herring, salmon]
Docosapentaenoic Acid	all *cis* 7,10,13,16,19-docosapentaenoic	22:5n-3 [DPA]	Fish, especially oily fish [anchovy, sardine, mackerel, herring, salmon]
Docosahexaenoic Acid	all *cis* 4,7,10,13,16,19-docosahexaenoic	22:6n-3 [DHA]	Fish, especially oily fish [anchovy, sardine, mackerel, herring, salmon]

3.2.1.1.2.1. Eicosapentaenoic acid [EPA]

Eicosapentaenoic acid [EPA] is an Omega-3 HUFA denoted by the numerical abbreviation 20:5n-3. In chemical structure, EPA consists of a 20-carbon chain and five *cis* double bonds; the first double bond is located at the third carbon from the omega or methyl end. EPA and its metabolites act in the body largely by their interactions with the metabolites of AA and serves as a precursor for three important groups of eicosanoids i.e. prostaglandin-3 [which inhibits platelet aggregation], thromboxane-3 and leukotriene-5 [involved in maintaining healthy circulation].

3.2.1.1.2.2. Docosahexaenoic acid [DHA]

Docosahexaenoic acid is given the name 22:6n-3 as it contains 22-carbons and 6 double bonds and is the extreme example of an Omega-3 HUFA. DHA [22:6*n*-3] is the most abundant *n*-3 PUFA in most human tissues and readily incorporates into membrane phospholipids. As with Omega-6 HUFA AA, DHA is an essential element of synaptic membrane phospholipids and is found in high concentrations in the retinal outer segment and cerebral grey matter of human neonates. Here it plays a vital role in signal transmission although details of a molecular mode of action remain unclear. At a fundamental level this is thought to relate to the high degree of conformational flexibility that the multiple double bonds confer which have been shown to significantly alter many of the basic physio-chemical properties of cell membranes.

1. Kanayasu-Toyoda et al. Prostaglandins Leukot Essent Fatty Acids. 1996 May; 54(5):319-25.

Through DHA's interaction with other membrane lipids, particularly cholesterol, it is also thought to play a prominent role in modulating the local structure and function of cell membranes and its presence in the body has been positively correlated to proper cardiovascular function and the prevention heart disease. DHA is also the predominant structural fatty acid in the central nervous system and retina and is essential to proper foetal brain development with incorporation into membrane lipids beginning in the last trimester of pregnancy and continuing into the first 2 years of life[1].

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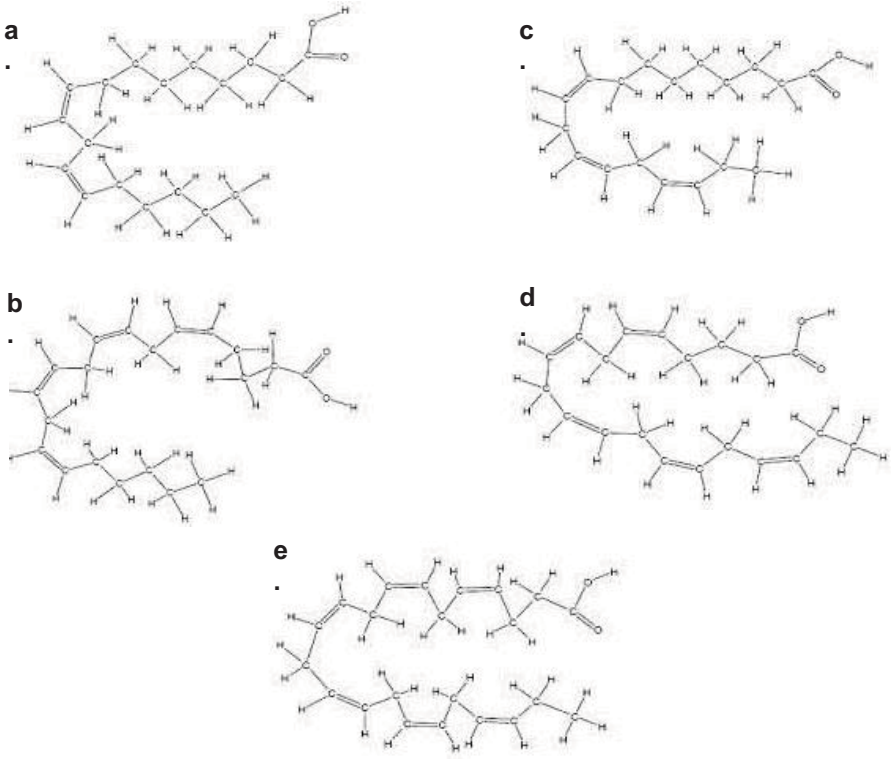


Fig. 3. Chemical structure of Omega-6 fatty acids a] Linoleic Acid [LA, 18:2n-6] and b] Arachidonic Acid [AA, 20:4n-6] and Omega-3 fatty acids c] Alpha-Linolenic Acid [ALA, 18:3n-3], d] Eicosapentaenoic Acid [EPA, 20:5n-3] and e] Docosahexaenoic Acid [DHA, 22:6n-3].

1. Connor, W.E.. *Am J Clin Nutr* 2001;74:415–6

Table 4. Typical Omega-3 FA composition of adult human tissues [Burdge & Calder 2006]

Lipid Fraction	Total Omega-3 Fatty Acids [%]		
	ALA	EPA	DHA
Plasma phosphatidylcholine	0.1	0.8	2.9
Plasma cholesterol ester	0.4	0.8	0.5
Plasma TG	0.8	0.8	0.5
Platelet phosphatidylcholine	0.3	0.2	1.1
Platelet phosphatidylethanolamine	0.2	0.6	6.3
Mononuclear cell phospholipid	0.1	0.3	2.3
Nutrophil phospholipid	-	0.6	1.3
RBC phospholipid	-	0.8	3.5
Liver phosphatidyethanolamine	0.2	1.6	7.7
Brain grey matter phosphatidylethanolamine	0.1	-	24.3
Brain grey matter phosphatidylserine	-	-	36.6
Brain grey matter phosphatidylcholine	-	-	3.1
Brain white matter phosphatidylethanolamine	-	-	3.4
Retina phosphatidylcholine	-	-	22.2
Retina phosphatidylethanolamine	-	-	18.5
Retina phosphatidylserine	-	-	4.6
Testis total lipid extract	-	-	8.5
Sperm phospholipid	-	-	35.2
White adipose tissue	0.7		0.1

3.3. Human Health Benefits

LC Omega-3 HUFAs (EFAs) are necessary for good health and a deficiency in them has been has been defined as an attributable risk from 13 morbidity and mortality outcomes including cardio vascular disease [CVD], coronary heart disease [CHD], stroke, homicide, bipolar disorder, major and postpartum depressions[1]. As mentioned LC Omega-3 HUFAs are essential components of all cell membrane phospholipid layers [Table 4] where due to their unique physio-chemical properties confer greater fluidity to cell membranes at low temperatures thereby reducing inflammation and improving joint mobility. As well as serving as structural components to cells, EPA+DHA in particular perform three key physiological functions:

i. They are oxidised to produce bioactive metabolites called eicosanoids [hormone-like signalling molecules] including thromboxanes, prostaglandins and leukotrienes which help regulate inflammation in the body.
ii. They modulate enzyme activity including inhibiting protein kinases and preventing increases in calcium and potassium in cells.
iii. They regulate the expression of genes involved in the control of inflammation, cell proliferation, cell death and oxidative stress.

1. Hibbeln JR et al. *Am J Clin Nutr* 2006;83(suppl):1483S–93S

Clinical studies indicate powerful anti-inflammatory and anti-thrombotic properties of LC Omega-3 HUFAs and the strongest evidence of a therapeutic effect is in the treatment of cardio vascular disease [CVD] and its many complications [Table 5]. CVD is the single largest cause of mortality in Europe, responsible for 4.3 million deaths in Europe every year and costing the EU economy €192 billion.yr^{-1}. Eicosanoid derivatives of EPA however have been shown to reduce blood cholesterol levels [a major contributing factor to CVD] by inhibiting synthesis of very low density lipoproteins [VLDL] and TGs in the liver. Similarly both eico- and doco-sanoid [signalling molecule] derivatives of DHA have been shown to reduce total blood TG levels and VLDL size [thereby reducing aggregation], decrease thrombosis, and prevent cardiac arrhythmias. Specifically, LC Omega-3 HUFAs inhibit synthesis of thromboxane A2 from the Omega-6 FA, AA in platelets which has the effect of reducing platelet aggregation, vasoconstriction and the risk of sudden death by heart attack[1].

LC Omega-3 HUFAs also play a key role in neural transmission in humans and as the predominant structural FA in the retina and central nervous system DHA is essential for early brain development and proper cognitive function in adulthood[2]. For example studies have shown infants of breast feeding mothers supplemented with DHA had higher mental processing scores, psychomotor development, eye-hand co-ordination and stereo-acuity at age 4[2,3,4,5]. Similarly, DHA supplementation is thought to enhance learning capabilities and academic performance as well as prevent attention deficit hyperactivity disorder in pre-school children. A compelling, but still emerging body of data also indicates efficacy in the treatment of psychiatric illnesses including depression, bipolar disorder and schizophrenia[6] with one study by Naylor et al [year] linking increased Omega-3 HUFA intake with reduced psychopathic, aggressive and impulsive behaviours in both recidivist and law-abiding populations[7,8].

Table 5. Actions of Omega-3 HUFA EPA+DHA in the prevention of Cardio Vascular Disease and sudden death [Connor 2001]

1. Prevent cardiac arrhythmias [ventricular tachycardia and fibrillation]
2. Act as antithrombotic agents
3. Inhibit the growth of atherosclerotic plaques
4. Act as anti-inflammatory agent [inhibit synthesis of cytokines and mitogens
5. Stimulate endothelial-derived nitric oxide
6. Lower plasma concentration of triacylglycerol and VLDL cholesterol and increase plasma concentrations of High Density Lipoprotein [HDL] cholesterol

1. Goodnight et al. Blood. 1981 Nov;58(5):880-5.
2. Haag M. *Canad J Psychiatry* 2003; 48: 195–203.
3. Makrides M et al. *Eur J Clin Nutr* 1996; 50: 352–357.
4. Williams C. et al. *Am J Clin Nutr* 2001; 73: 316–322.
5. Uauy R, Mena P, Rojas C.*Proceedings of the Nutrition Society* 2000; 59:3–15; Singh M. *I*ndian Pediatr 2003; 40: 213–220
7. For good review see: Hallahan and Garland Brit. J. Psych. 2 0 0 5 and Hibblen et al. 2004.
8. http://www.crimetimes.org/10c/w10cp12.htm; http://www.nytimes.com/2006/04/16/magazine/16wwln_idealab.html?pagewanted=print&_r=0

By contrast, excessive levels of Omega-6 HUFA especially AA are thought to promote inflammation, platelet aggregation and vasoconstriction both directly through the production of pro-inflammatory and pro-thrombotic eicosanoids and indirectly by inhibiting Omega-3 type HUFA production. For example, AA is known to produce series 2 prostanoids and series 4 leukotrienes[1,2] and the pro-thrombotic eicosanoid thromboxane A2 [Connor 2001]. EPA from fish oil however has been shown to inhibit thromboxane A2 synthesis, increase bleeding time and decrease platelet aggregation in CVD sufferers[3]. Furthermore, recent clinical trials indicate a relationship between high dietary intake of Omega-6 PUFAs and the occurrence of prostate and breast cancers resulting in recommendations for reduced intake of Omega-6 PUFA in particular AA relative to Omega-3 HUFA.

Perhaps more significant however is the recent estimation of the cost to global government of EPA+DHA deficiency in terms of managing those chronic diseases and illnesses LC Omega-3 HUFAs are believed to play a role in preventing. GOED recently put this at US$47 trillion over the next twenty years, or US$42,000 per person if only the developed world bears the cost. In more local terms, management of CVD and its many complications currently costs the EU economy €192 billion.yr^{-1}. A comprehensive analysis by Hibbeln et al. [2006] however established that dietary intake of 750 mg/d of LC Omega-3 HUFAs is sufficient to protect >98% of a given population from an increased risk of illness due to CHD, CVD, postpartum depression and other chronic diseases attributable to insufficient LC Omega-3 HUCFA intake [Table 6]. Similarly in the UK, consumption of EFAs is approximately 244 mg/d compared to the recommend 450mg/d, and in young females can be as low 109mg/d. Such deficiency is thought to have a major impact on population health with each 125mg/d increase EFAs associated with a 3% lower risk of heart failure.

1. Molende-Costi et al. Gastroenterol Res Pract. 2011; 2011: 364040.
2. Kapoor and Patil International Food Research Journal 18: 493-499 (2011)
3. Connor, W.E.. *Am J Clin Nutr* 2001;74:415–6

Table 6. Efficacy of 3 possible dietary intakes in reducing the risk of disease attributable to Omega-3 long-chain fatty acid (LCFA) deficiency[1] [Reproduced from Hibbeln et al. 2006]

Disease or disorder model	Disease burden potentially attributable to LC Omega-3 HUFA deficiency[3]	Worldwide protection from deficiency at 3 possible intakes of LC Omega-3 HUFA[2]		
		0.08% of energy (180 mg/d)[4]	0.22% of energy (500 mg/d)	0.34% of energy (750 mg/d)
	%		%	
CHD mortality, M	41.2	45.2	85.4	97.9
CHD mortality, F	42.5	52.4	89.7	98.6
Stroke mortality, M	32.9	97.7	99.9	>99.9
Stroke mortality, F	31.1	96.4	99.9	>99.9
CVD mortality, M	26.1	83.4	99.3	>99.9
CVD mortality, F	29.1	86.9	99.6	>99.9
Total mortality, M	20.8	73.6	97.7	99.8
Total mortality, F	31.5	48.3	87.3	98.2
Homicide mortality	28.4	95.6	>99.9	>99.9
Postpartum depression	65.5	55.7	91.3	98.9
Major depression[5]	98.5	38.5	83.2	99.2
Bipolar depression[5]	99.9	56.1	92.3	99.5

1 CHD, coronary heart disease; CVD, cardiovascular disease.
2 Protection from deficiency was calculated for each possible dietary intake and was repeated for each disease risk relation.
3 Percentage of the population disease potentially attributable to deficiency of LC Omega-3 HUFAs.
4 Based on a 2000-kcal/d diet.
5 These burdens of potentially attributable disease appear high.

3.4. Recommendations for Intake

Several authors have reported a widespread deficiency in LC Omega-3 HUFAs in industrialized countries[1] with the current ratio of Omega-6 to Omega-3 HUFAs [15:1 to 16:1] far lower than the recommended optimum of 2:1[2]. Thus a number of countries [Canada, Sweden, United Kingdom, Australia, and Japan] as well as the World Health Organization and North Atlantic Treaty Organization have made formal population-based dietary recommendations for Omega-3 HUFA intake including both ALA and preformed EPA+DHA [Table 7]. Typical recommendations range between 0.3 to 0.5 $g.day^{-1}$ of EPA+DHA which the American Heart Association [AHA] indicates can be met by consuming two servings of fish per week, with an emphasis on fatty fish richest in Omega-3 PUFAs [i.e. salmon, herring, and mackerel]. For individuals with CVD 1 g/d of EPA +DHA is recommended to reduce the risk of mortality[3] and equates to consumption of approx.170 g serving per week of fatty fish. However for patients with high cholesterol concentrated forms of Omega-3 HUFA devoid of saturated and monounsaturated fatty acids are a more appropriate than fatty fish as they keep daily intake of total lipids as low as possible. On the other hand, Gebauer *et al*. [2006] reported that consuming approximately two servings of fish per week [8 ounce/227g] may reduce the risk of mortality from coronary heart disease and that consuming EPA and DHA may reduce the risk of mortality from cardiovascular disease in people who have already experienced a cardiac event.

It is important to note however that these recommendations assume equivalence in terms of the health benefits conferred by whole fish and fish oil dietary supplement [FODS] consumption. Thus, for individuals that do not eat fish, have limited access to variety of fish, or cannot afford to purchase fish however Omega-3 supplements are a useful alternative however while providing a lower total lipid content most Omega-3 supplements do not provide the potentially beneficial protein, vitamin D and selenium that whole fish intake does. On a cautionary note however it has previously been indicated that consumption of fatty fish may result in increased exposure to a variety of environmental contaminants such as mercury, dioxins, polychlorinated biphenyls [PCBs] or polybrominated diphenyl ethers [PBDEs] with attendant potential health risks that potentially counteract the beneficial effects. However these tend to accumulate in higher predators such as swordfish and tuna and are relatively low in the small forage species used to produce fish oil and feed farmed finfish. Thus, based on strength of evidence the overall benefits of modest fish consumption [1- 2 serving per week] or daily fish oil dietary supplement usage greatly outweigh the risks[4].

Moreover a recently held global summit examining the human health impacts of Omega-3 deficiency concluded that due to high intakes of Omega-6 FAs in western diets [largely due to high intake of terrestrial plant oils] dietary intake of >1000mg LC-Omega-3 is needed to combat the excessively high ratios of Omega-6:Omega-3 so prevalent in the western-type diet. This summit also concluded that in vitro tissue concentrations, rather than dietary intake, are a much more accurate measure of Omega-3 status and should become not only a standard biomarker but a routine measurement of western health agencies in the battle against widespread Omega-3 deficiency[5].

1. Hibbeln JR et al. *Am J Clin Nutr* 2006;83(suppl):1483S–93S
2. Simopoulus, A.P. Biomedicine & Pharmacotherapy 60 (2006) 502–507
3. Kris-Atherton et al. doi: 10.1161/01.CIR.0000038493.65177.94
4. Mozzafarian and Rimm , JAMA 2006 Oct 18; 2969150: 1885-99
5. http://www.omega-3summit.org/pdf/ConsensusStatements.pdf

Table 7. Recommended Daily Intake [RDI] of Omega-3 FAs given by various international health authorities.

Source	Year Published	Recommendation		
		Total Omega-3 [% of energy]	ALA [g]	EPA + DHA [g]
UK Committee on Medical Aspects of Food Policy	1994	0.2	-	0.1 - 0.2
US National Academies of Science, Institute of Medicine	2002	-	1.4	0.14
Eurodiet	2000	-	2	0.2
Health and Council of the Netherlands	2001	1	-	0.2
UK Dietary Guidelines		-	-	0.2
European Academy of Nutritional Sciences		-	-	0.2
New Zealand Heart Foundation	1999	-	-	0.2
Apports Nutritionnelles Conseilles [France]	2001	0.8 - 1	1.8	0.45
UK Scientific Advisory Committee on Nutrition	2004	-	-	0.45
International Society for the Study of Fatty Acids and Lipids [ISSFAL]	2004	-	1.6	0.5
North Atlantic Treaty Organisation workshop	1989	-	3	0.8
World Health Organization and Food and Agriculture Organization	2003	-	-	0.4 - 1
American Heart Association	2002	-	-	1 [CHD]
European Society of Cardiology	2003	-	-	1 [CHD]

3.5. Food Sources

The LC Omega-3 precursor ALA is found in high concentrations in terrestrial plants including green leafy vegetables, plant oilseeds especially flax and commonly consumed plant oils such as rapeseed and soya oils [Table 8]. Dietary surveys in the US indicate that average adult intakes for ALA range from 1.2-1.6 g/day for men and from 0.9-1.1 g/day for women. However due to the poor rate of bioconversion of ALA to EPA+DHA in humans [especially men] global health authorities also recommend daily intake of preformed EPA+DHA both for meeting essential Omega-3 requirements and addressing Omega-6:Omega:3 imbalances described earlier.

Table 8. Some Food Sources of Alpha-linolenic Acid [ALA; 18:3n-3]

Food	Serving	ALA [g]
Flaxseed oil	1 tablespoon	7.3
Walnuts, English	1 oz.	2.6
Flaxseeds, ground	1 tablespoon	1.6
Walnut oil	1 tablespoon	1.4
Canola oil	1 tablespoon	1.3
Soybean oil	1 tablespoon	0.9
Mustard oil	1 tablespoon	0.8
Tofu, firm	½ cup	0.7
Walnuts, black	1 oz.	0.6

Fatty marine fish especially carnivores such as salmon represent a unique source of preformed EPA+DHA and a list of the richest species are given in Table 9. Generally speaking, EPA+DHA content in wild versus famed fish are similar [Table 10] and directly related to diet. In the case of wild fish this will vary seasonally depending on the availability of different prey organisms[1] and in fed species varies with the rate of dietary inclusion of Omega-3 HUFA in aquafeeds. Due to static supplies and increasing prices however aquafeed manufacturers have considerably reduced fish oil inclusion rates for farmed marine fish in recent years falling from a global average of 15% total dry wt. of feed [salmon: 25%] in 1995 to 7% [salmon: 16%] in 2007.

The recently concluded EU project *Researching Alternatives to Fish Oils in Aquaculture* [RAFOA][2] for example highlighted substantial changes in the fatty acid compositions of farmed fish due to increased substitution of fish oils for vegetable oils in compound aquafeed. Here a 50% reduction in the amount of EPA+DHA in fillet flesh was observed in fish fed diets where between 75% to 100% of dietary lipids were substituted with vegetable rather than fish oil. Results show that in the case of salmonids fed 100% VO, flesh DHA & EPA concentrations were reduced by ~65%, in salmon and ~50% in trout such that the ISFFAL recommended weekly intake for EPA+DHA [3.5g] would no longer be met by consuming the recommended 2 portions per week [Fig. 7]. It is important to note that this effect was largely reversed with inclusion of a "finishing diet" high in fish oil.

Table 9. Some food sources of EPA [20:5n-3] and DHA [22:6n-3] [1 oz. = 28.5 g]

Food	Serving	EPA [g]	DHA [g]	Amount providing 1 g of EPA + DHA
Herring, Pacific	3 oz.	1.06	0.75	1.5 oz.
Salmon, Chinook	3 oz.	0.86	0.62	2 oz.
Sardines, Pacific	3 oz.	0.45	0.74	2.5 oz.
Salmon, Atlantic	3 oz.	0.28	0.95	2.5 oz.
Oysters, Pacific	3 oz.	0.75	0.43	2.5 oz.
Salmon, sockeye	3 oz.	0.45	0.60	3 oz.
Trout, rainbow	3 oz.	0.40	0.44	3.5 oz.
Tuna, canned, white	3 oz.	0.20	0.54	4 oz.
Crab, Dungeness	3 oz.	0.24	0.10	9 oz.
Tuna, canned, light	3 oz.	0.04	0.19	1 oz.

Table 10. Omega-3 FA content of various species of farmed and wild salmon

Salmon Species	Omega-3 FA [EPA+DHA/100g fish]
Wild Chinook	2
Farmed Atlantic	2
Farmed Chinook	1.8
Wild Atlantic	1.7
Farmed Coho	1.3
Wild Sockeye	1.3
Wild Coho	1.2
Wild Pink	1
Wild Chum	0.6

1. See for example: Gamez-Meza et al 1999. Lipids.34 : 639-642.
2. http://www.rafoa.stir.ac.uk/

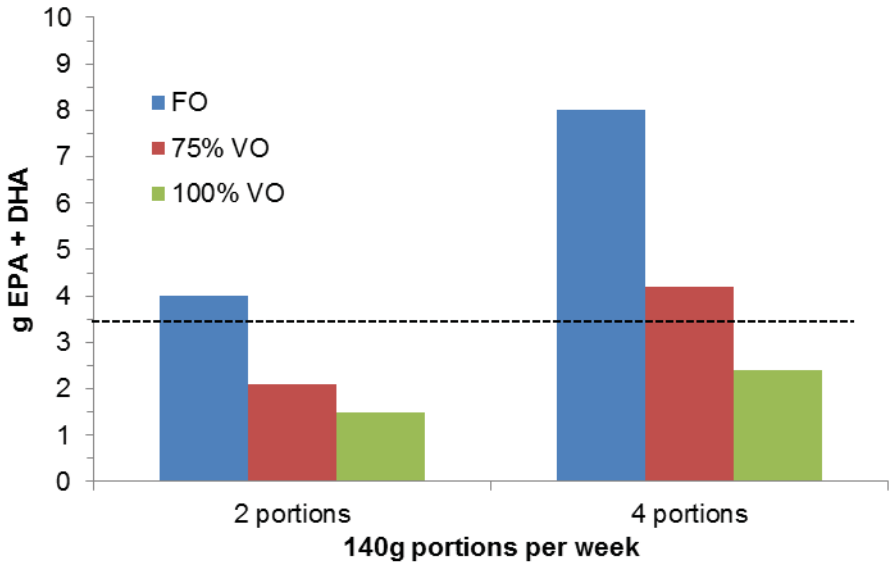


Fig. 7. Amounts of EPA + DHA provided by eating 2 or 4 UK Food Standards Agency portions of 140g of farmed salmon, produced using 100% fish oil [FO], 75% vegetable oil [VO] or 100% VO. Dashed line indicates ISSFAL recommended weekly intake [Source: EU Project RAFOA Final Report]

As well as being an excellent source of Omega-3 HUFA salmon [both farmed and wild] is a rich source of protein [15-20% total weight], amino acids, essential vitamins, anti-oxidants, minerals and trace metals that have numerous beneficial effects on human health. Table 12 provides a comprehensive list of typical levels found in a portion of farmed Atlantic salmon. Studies however show that most of the UK population consume very little fish with a recent survey indicating that more two thirds of adults ate no fish at all for the duration of the study[1]. Thus for those individuals who do not eat fish e.g. vegetarians, have limited access to variety of fish or cannot afford to purchase fish, a fish oil dietary supplement [FODS] may be a suitable alternative. FODS generally contain between 20% and 80% EPA+DHA by weight and depending on the purity of the finished oil contain little or no saturated or monounsaturated fats. Similarly levels of mercury, polychlorinated biphenyls [PCBs] and dioxins associated with wild fatty fish are low[2]. However while RDIs for Omega-3 HUFAs are readily met through FODs consumption[3] these do not provide any of the beneficial proteins, amino acids, vitamins and minerals embedded in a fillet of farmed or wild marine fish such as salmon [Tables 11 & 12]. Moreover, recent studies investigating bioavailability and health benefits of different chemical forms of refined Omega-3 oils suggest consumption of less oxidatively stable Ethyl Esters [EEs] of Omega-3 HUFA resulting from the oil concentration process may carry the risk of adverse side effects[4] though further study is required before this can be confirmed..

1. Henderson L, et al. The National Diet and Nutrition Survey: adults aged 19 to 64 years. Volume 1: Types and quantities of foods consumed. 2002, TSO, UK
2. Jimenez et al. 1996 http://dx.doi.org/10.1016/0045-6535(95)00233-2.
3. Pauga 2009 http://hdl.handle.net/10179/1230
4. Livar Froyland, et al. *Norwegian Scientific Committee for Food Safety (VKM)*, June 28, 2011.

Table 11. Total nutrient composition of salmon oil

Nutrient	Unit	Value per 100.0g	cup [218g]	tbsp. [13.6g]	tsp. [4.5g]
Proximate					
Water	g	0	0	0	0
Energy	kcal	902	1966	123	41
Protein	g	0	0	0	0
Total lipid [fat]	g	100	218	13.6	4.5
Carbohydrate, by difference	g	0	0	0	0
Fibre, total dietary	g	0	0	0	0
Minerals					
Calcium, Ca	mg	0	0	0	0
Iron, Fe	mg	0	0	0	0
Magnesium, Mg	mg	0	0	0	0
Phosphorus, P	mg	0	0	0	0
Potassium, K	mg	0	0	0	0
Sodium, Na	mg	0	0	0	0
Zinc, Zn	mg	0	0	0	0
Vitamins					
Vitamin C, total ascorbic acid	mg	0	0	0	0
Thiamine	mg	0	0	0	0
Riboflavin	mg	0	0	0	0
Niacin	mg	0	0	0	0
Vitamin B-6	mg	0	0	0	0
Folate, DFE	µg	0	0	0	0
Vitamin B-12	µg	0	0	0	0
Vitamin A, RAE	µg	0	0	0	0
Vitamin A, IU	IU	0	0	0	0
Lipids					
Fatty acids, total saturated	g	19.8	43.3	2.7	0.9
Fatty acids, total monounsaturated	g	29.0	63.3	4.0	1.3
Fatty acids, total polyunsaturated	g	40.3	87.9	5.5	1.8
Cholesterol	mg	485	1057	66	22

Table 12. Nutrient composition [per 100g] of farmed Atlantic salmon and recommended daily intakes for the UK.

Nutrient	Unit	Value	RDI [mg]	Nutrient	Unit	Value
Proximates				**Lipids**		
Protein	g	25.4	-	**Fatty acids, Total Saturated**	g	0.909
Total lipid [fat]	g	6.69	-	10:0	g	0.003
Ash	g	1.43	-	12:0	g	0.009
Minerals				14:0	g	0.155
Calcium, Ca	mg	12	700	15:0	g	0.016
Iron, Fe	mg	0.5	8.7	16:0	g	0.576
Magnesium, Mg	mg	36	~6.0	17:0	g	0.013
Phosphorus, P	mg	317	550	18:0	g	0.13
Potassium, K	mg	408	3,500	20:0	g	0.006
Sodium, Na a	mg	134	6,000	24:0	g	0.001
Zinc, Zn	mg	0.5	5.5 [min]	**Fatty acids, Total MUFA**	g	1.432
Copper, Cu	mg	0.072	1.2	16:1 undifferentiated	g	0.178
Manganese, Mn	mg	0.013	-	17:1	g	0.027
Selenium, Se	mg	0.0365	0.075	18:1 undifferentiated	g	0.858
Vitamins				20:1	g	0.362
Thiamin [B_1]	mg	0.215	1.0	24:1 c	g	0.008
Riboflavin [B_2]	mg	0.14	1.3	**Fatty acids, Total PUFA**	g	1.495
Niacin	mg	9.698	17	18:2 undifferentiated	g	0.376
Pantothenic acid	mg	1.371	-	18:3 undifferentiated	g	0.086
Vitamin B-6	mg	0.693	1.4	18:4	g	0.054
Folate, total	mg	0.009	0.2	20:3 undifferentiated	g	0.019
Vitamin B-12	mg	0.00567	0.0015	20:4 undifferentiated	g	0.021
Vitamin A	mg	0.69	0.7	20:5 n-3 [EPA]	g	0.268
Vitamin E [alpha-tocopherol]	mg	1.14	4.0	22:4	g	0.004
Vitamin D [D2 + D3]	µg	13.1	-	22:5 n-3 [DPA]	g	0.11
Vitamin D3 [cholecalciferol]	µg	13.1	-	22:6 n-3 [DHA]	g	0.524
Vitamin K	mg	0.0001	0.001 [per kg.body.wt]	**Fatty acids, Total Trans**	g	0.021
Total Amino Acids	g	25.5	-	Cholesterol	mg	63

The incorporation of stabilized fish oil into novel functional food products has also risen dramatically in recent years due largely to advances in preventing oxidation of highly labile EPA+DHA fractions. The three major forms of Omega-3 PUFA used for their fortification are ALA-rich vegetable oils, fish oils and algal oils rich in DHA and while their availability and usage is still limited in the United States, acceptance in Europe, South America and Australia is high [Fig. 9]. A snapshot of some Omega-3 enriched functional foods is given in Table 13 [Whelan and Rusty 2006].

Table 13. Selection of currently available functional foods as sources of Omega-3 HUFA [adapted from Whelan and Rusty 2006]

Food	Serving	ALA [mg]	EPA [mg]	DHA [mg]	Source of information
Bread/Pasta					
Wharburton Good Health Loaf for Women	100	-	-	30	http://www.nutraingredients.com Sold at Asda and Sainsburys in UK
Valfleuri Spaghetti	N/A	N/A	N/A	N/A	Sold in France Source of n-3: eggs derived from hens fed flaxseed oil Provides 20% of French recommendations
Milk					
St. Ivel Advance with Omega-3 semi-skimmed	250 mL	-	63	-	http://www.omega3.co.uk/omega3/pages/omega3_product>formula.php Sold in UK at Waitrose, selected Tesco's, Sainsburys and Morrison's supermarkets Source of n-3: Omega-3 in an emulsion format [containing sardine oils]
St. Ivel Advance with Omega-3 Whole	250 mL	-	113	-	
Marks and Spencers Super Whole Milk	250 mL	-	48	-	Sold in UK Source of n-3: fish oils incorporated into cow feed
Dawn Omega Milk Low-fat	250 mL	-	25	-	Sold in Ireland Source of n-3: DSM's *ROPUFA*
Whole Milk	250 mL	-	50	-	
Candia	250 mL	-	190	-	Sold in France Source of n-3: not revealed
Primevere Sterilized Drink	250 mL	210		47.5	Sold in France Source of n-3: colza [obtained from *Brassica rapa*] and fish oils
Eggs					
Stonegate	58	-	100	-	Sold in UK at Waitrose, Sainsbury, Ocado and Asda Source of n-3: Nu-Megas DHA-rich tuna oil
Juice					
Supajus	250 mL	-	-	100	Sold in UK at school vending machine Source of n-3: pure tuna oil
Nutrition bars					
Zoneperfect Lemon Yogurt	50	-	3	-	http://www.zoneperfect.com Sold in US at CVS Pharmacy, GNC, Ingles, Sam's Club & Walgreens Source of n-3: molecular distilled fish oil
Oh Mama! Nutritional Bar	50	-	-	115	http://www.ohmamabar.com/page/nutrition.html Sold in US at Motherhood Maternity, Buy Buy Baby or http:/www.ohmamabar.com Source of n-3: Martek's DHA algal oil

Table 13. *Cont'd*

Food	Serving	ALA [mg]	EPA [mg]	DHA [mg]	Source of information
IQ3 Brainstorm Bar	N/A	N/A	N/A	N/A	http://www.iq3-brainstorm.co.uk/brainstorm.php Sold in UK at most quality health food shops Source of n-3: vegetable margarine, microencapsulated tuna oil [31%]
Salad Dressing					
Omega-3 Mayonnaise	14	2000	-	-	Sold at local health food stores Source of n-3: flaxseed oil
Primevere Margarine	100	300	-	-	Sold in France Source of n-3: colza oil
Smart Balance Omega Plus Buttery Spread	100	-	150	-	http://www/smartbalance.com/product.html Sold in US by Reinbeck's, Bucklers, Roundy's and Wal-Mart supermarkets Source of n-3: menhaden oil
Infant Formula					
Similac by Ross	5 fl. oz.	-	-	8	Sold in US at local grocery and drug stores and Wal-Mart supermarkets Source of n-3: *Crypthecondinium cohnii* oil
Enfamil Lipil w/ iron by Mead Johnson	-	-	17	-	Sold in US at local grocery and drug stores and Wal-Mart supermarkets Source of n-3: *Crypthecondinium cohnii* oil
Nestle Gold Start w/ DHA and AA	-	-	N/A	-	Sold in US at local grocery and drug stores and Wal-Mart supermarkets Source of n-3: *Crypthecondinium cohnii* oil
Mother's Horlicks	75	-	-	60	Sold in India Source of n-3: powder–microalgae
Other					
Omega-3 Enriched Pizza	N/A	-	-	32	http://www.nardonebros.com Sold at www.nardonebros.com Source of n-3: Nu-Mega's microencapsulated tuna oil

3.6. Bioavailability: Food versus Supplements

Bioavailability is generally defined as the measurement of the extent to which a component reaches the systemic circulation or target site[1] and numerous clinical trials have reported variation in the bioavailability of LC PUFA depending on the chemical form in which it is delivered i.e. natural TG [nTG], chemically re-esterified TG [rTG][2], PL or EE[3]. The majority of these studies assessed HUFA absorption and bioavailability by measuring EPA+DHA in blood plasma after ingestion as either TGs or EEs with the overall evidence suggesting that EPA+DHA in TG fish oils are better absorbed than those in EE oils. This is thought to result from the limited digestibility of the ethanol-FA bond in EEs which is up to 50 times more resistant to the hydrolytic action of pancreatic lipase compared to the glycerol-FA bond in TGs. Consequently FFAs produced by EE hydrolysis must first be reconverted to TGs before being taken up by intestinal absorptive cells, enterocytes and transported into the blood. This reconversion requires a monoglyceride [MG] substrate present in TGs as a glycerol-FA bond but which is absent in ethanol-linked EEs. As a result MG substrates must be obtained from another source causing both a delay TG re-synthesis and increased likelihood of FFA oxidation in the small intestine with possible adverse side-effects[4].

From the numerous studies assessing EPA+DHA absorption from EE versus natural TG fish oils five main points emerge:

- nTG fish oil results in 50% more EPA and DHA in blood plasma after absorption in comparison to EE oils
- TG forms of EPA and DHA have been shown to be 48% and 36% better absorbed than EE forms respectively.
- EPA incorporation into plasma lipids was found to be considerably smaller and took longer when administered as an EE
- In rats, DHA TG supplementation led to higher plasma and erythrocyte DHA content than did DHA EE as well as a higher lymphatic recovery of EPA and DHA.
- Plasma lipid concentrations of EPA and DHA were significantly higher with daily portions of salmon in comparison to 3 capsules of EE fish oil

Interestingly, a single study by Wakil et al [2010] showed higher bioavailability of EPA+DHA components from rTGs compared to enzymatically produced high TG content fish oil [or eTG] where DGs and MGs of rTGs were thought to act as emulsifying agents in the stomach thereby increasing overall absorption and bioavailability. Similarly studies have found substantial differences in LC HUFA absorption between both TGs and EEs and PLs found in high concentrations in both krill and algal oils. For example, Schudhardt et al[5] found that while not statistically significant, a trend toward higher incorporation of EPA+DHA fractions into blood plasma when ingested as natural PLs [carried in krill oil] was followed by rTGs [as fish oil] and lastly EEs [also as fish oil]. This was thought to result from the higher proportion of EPA+DHA in krill oil PLs occurring as FFAs [22% and 21% respectively] which eliminates the need for hydrolysis by pancreatic lipases allowing more efficient absorption through the small intestine wall.

1. Mu, H. 2008 *Agro Food Industry Hi-Tech*, vol 19, no. 4, pp. 24-26.
2. Defined by European Pharmacopeia as mixture of MG, DG and TG with TG being the main component (>60%).
3. Sala-Vila et al. Prostaglandins, Leukotrienes and Essential Fatty Acids 74 (2006) 143h%28
4. Livar Froyland, et al. *Norwegian Scientific Committee for Food Safety (VKM)*, June 28, 2011
5. Schuchardt et al. Lipids in Health and Disease 2011, 10:145 http://www.lipidworld.com/content/10/1/145

Table 14 below provides results from a systematic clinical trial conducted by Sigma Tau Pharmaceuticals on the comparative bioavailability of Omega-3 PUFA in various chemical forms. In conclusion, encapsulated TG forms of DHA+EPA can be expected to give similar bioavailability to that of fish sources of DHA+EPA, which are mostly in the natural TG form. However, while recent long-term studies indicate a somewhat better bioavailability for TG versus EE form, the cost of high-omega-3 concentrates is generally much greater for the TG forms versus equal amounts of Omega-3 in the concentrated EE form. Future trials comparing the bioavailability of DHA+EPA from PL and FFA versus other forms in humans would be of interest particularly as the former are found in high concentrations in novel non-fish sources particularly krill and algae potentially making these a more nutritionally suitable source of Omega-3 HUFA for direct human consumption.

Table 14. Bioavailability of Omega-3 HUFA in various chemical forms.

Type of Oil	% Concentration of n-3 PUFA	% Bioavailability
nTGs	25-30 %	74-100 %
n- PUFA EEs	Up to 85 %	21-57 %
FFAs	Up to 65 %	51 %
rTGs	min. 70-75 %	98 %

4. The Global LC Omega-3 HUFA Ingredients Market

4.1. Introduction

While the uses of and demand for Omega-3 HUFAs have changed dramatically in the past half century, wild forage fish have remained the single largest commercial source of these nutrient-rich and highly sought after commodities. The wild forage fisheries on which the Omega-3 HUFA market depends so heavily however have reached their upper limit for sustainability with crude fish oil production remaining largely static over the past decade [1 MMT.yr-1]. Continued strong demand for fish oil from rapidly expanding aquaculture and health food sectors has resulted in a supply to use ratio of 1:1 for all source Omega-3 oils and with the price of crude fish oil soaring following the intense Pacific El Niño events of 1997/1998 [Fig. 8]. More recently fish oil prices increased by 130% between mid-2005 and mid-2008 reaching a decadal low of US$0.68.kg in 2009 before climbing to an all-time high of US$3.00.kg in the last quarter of 2010. This latest increase has followed severe catch quota reductions [~60% less than previous years'] amid forecasts of another strong El Niño event highlighting the sensitivity of these fragile stocks to fluctuations in ocean climate and consequent market price instability. Moreover, such climatic events have profound influence on the fatty acid profile of forage fish with increased water temperature during El Niño events substantially reducing the total Omega-3 content and ratio of EPA to DHA in fish body oils[1]. Additionally, UN initiatives aimed at mitigating the impacts of climate change on global poverty and food security could see a global cap placed on the amount of forage fish going to reduction which would severely curtail fish oil supplies and significantly impact aquaculture[2]. Forecasters are thus pointing to the emergence of substantial "fish oil trap" within the next five years if scalable, cost-effective and environmentally sustainable alternatives to wild forage fish oil are not found.

1. Galdos et al. 2002. http://dx.doi.org/10.1006/jfca.2002.1059.
2. http://www.un.org/millenniumgoals/

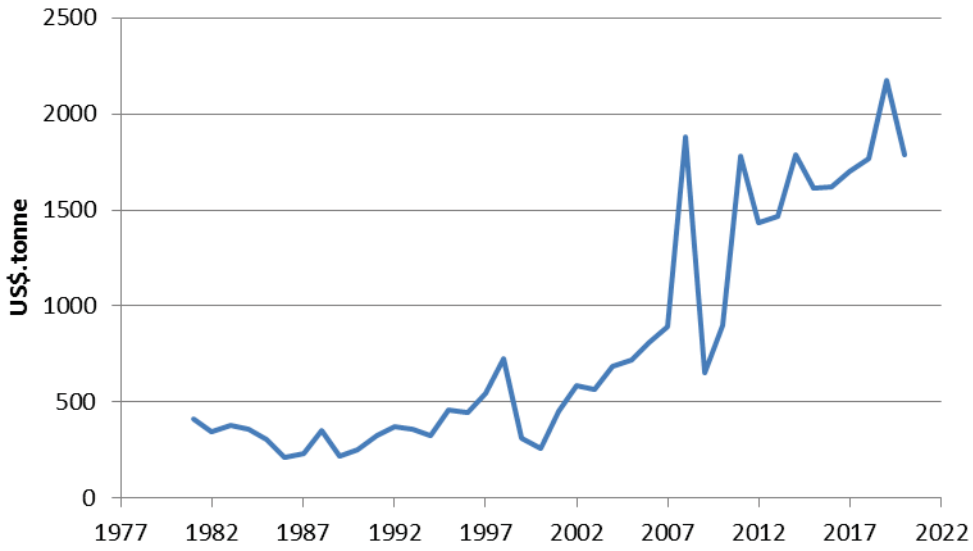


Fig. 8. Historic and forecasted trends in global market price of fish oil, 1981 to 2020.

4.2. Uses

In the mid-nineteen fifties the use of crude fish oil increased considerably [especially within countries such as Peru, Norway, and Japan] with the bulk of supply [>80%] going to the manufacture of hardened edible products particularly margarines, shortenings and cooking fats [Fig. 9]. Heightened public awareness of the deleterious effects of hydrogenated/trans fats on human health however has seen the virtual disappearance of hardened fish oils from the edible products market while traditional industrial uses such as leather tanning and as a drying agent in paint, varnish, lacquer and putty have similarly declined [3% in 2011]. Since 2000 the aquaculture sector has been the predominant user of marine-type lipids consuming on average 80% of total annual production for use in compound aquafeeds. The reason for this shift is mainly economic, with compound aquafeed manufacturers capable of paying a higher market price than their competitors over the past three decades. This is in turn is due to unprecedented growth in the aquaculture sector over the past three decades the primary drivers of which are increasing consumer demand for fish protein to feed a growing developing world population [FAO, 2000] and largely static production from global capture fisheries over the past decade.

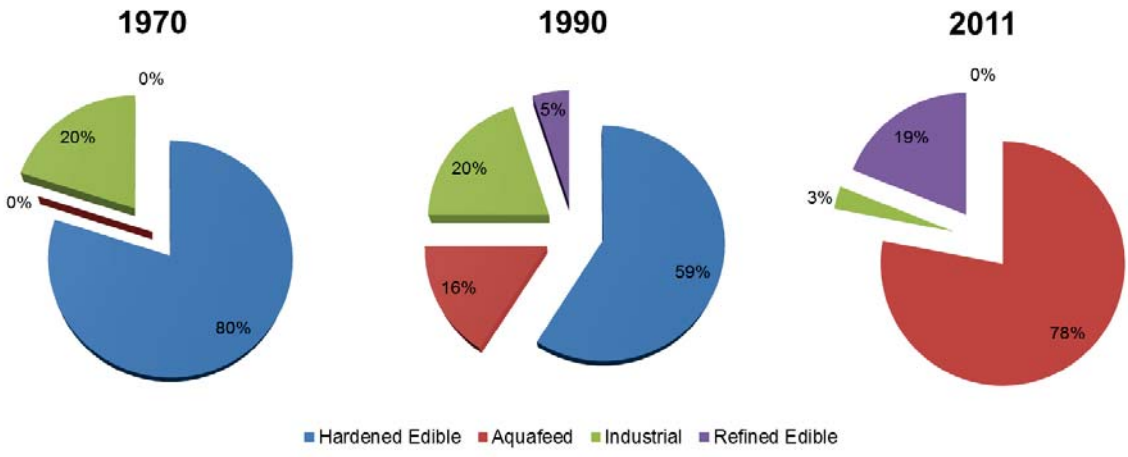


Fig. 9. Changing uses of fish and marine-type oils.

More recently however, strong growth in consumer demand for concentrated Omega-3 HUFA oils for the health food market has generated a four-fold increase in demand from the rapidly growing Refined Edible Products sector. Consequently a highly competitive marketplace has emerged with the REP sector consuming approximately between 1-150,000 MT of the available 6-700,000 MT of fish oil that satisfies the sustainability and quality requirements for aquafeed and the Omega-3 market. Moreover, due to the much price premium paid by REP consumers aquafeed manufacturers are losing an ever increasing share of available supplies to this capital-rich and rapidly expanding sector with some analysts forecasting a major supply gap or "fish oil trap" emerging in the next five years [see; http://www.nutraingredients.com/Consumer-Trends/GOED-chairman-Serious-omega-3-supply-issues-lie-ahead-as-demand-rockets].

4.3. Demand

4.3.1. Aquaculture

Aquaculture currently provides over 50% of all the fish and shellfish consumed globally with the great majority of this produced in Asia [89% in 2011]. Asian aquaculture is comprised primarily of omnivorous freshwater and diadromous species culture [95% total production] including carp, tilapia, catfish and milkfish [FAO 2011]. Overall demand for fish meal for aquafeed from this sector is substantial [45% total feeds used in 2010[1]] however demand for fish oil is relatively low. For example, dietary fish oil inclusion rates for major fed omnivorous species range between 0-13% total dry feed weight [Fig 10] and currently represents ~30% of total aquaculture consumption. By contrast high-value marine shrimp and carnivorous salmonids [including Atlantic salmon and rainbow trout] culture depends heavily on fish oil with inclusion rates ranging between 4 and 50% total dry feed weight. By contrast, European aquaculture in particular is heavily reliant on the supply of fish oil with expansion in total aquaculture production over the past three decades driven primarily by cage culture of salmon species [>60% total regional production in 2011; Fig. 11]. Furthermore, dietary inclusion levels for UK Atlantic salmon are among the highest in the world with Scottish aquaculture alone consuming ~10,000 $MT.yr^{-1}$ [SARF077]. Thus in light of recent increases in the price of fish oil and growing supply constraints sourcing a reliable and affordable alternative supply of this key nutrient is one of the most limiting factors for global aquaculture growth.

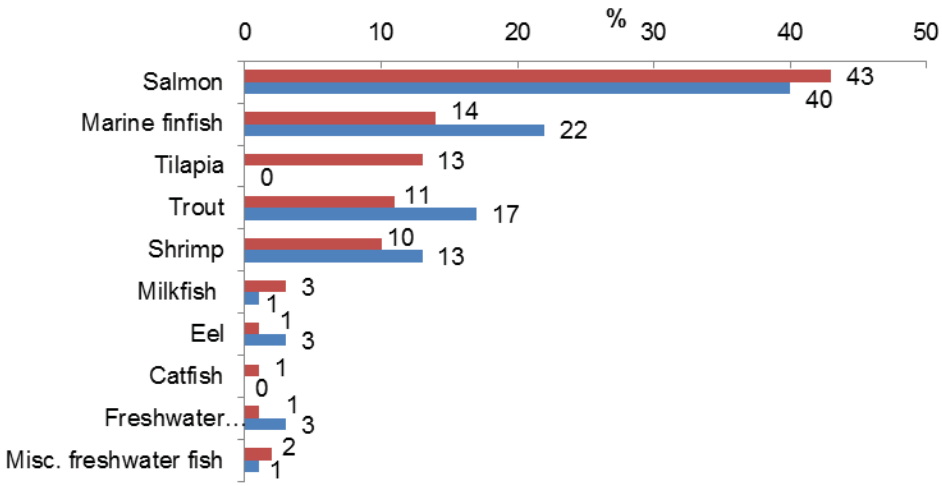


Fig. 10. Estimated alternative global use of fish oil within aquafeeds [percent dry feed basis] by species 2008. [Red, Tacon 2008; blue, IFFO 2007]. [Source: Tacon & Metian in prep; IFFO (2008)].

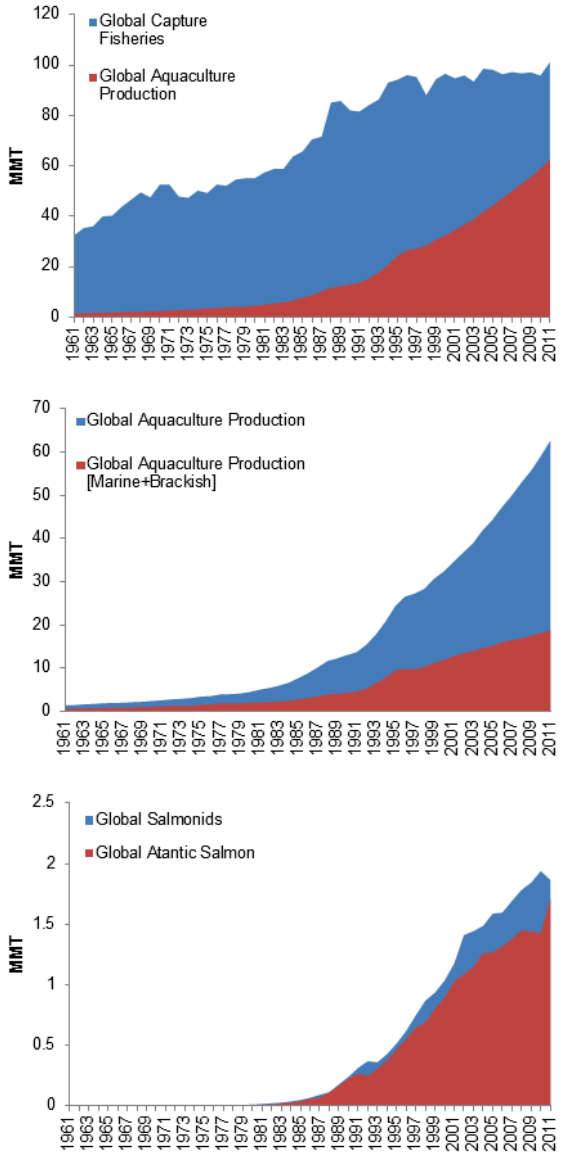


Fig. 11. Historic trends in a] global capture fisheries and aquaculture production b] marine and brackish water aquaculture production as a proportion of total annual production and c] the proportion of annual salmonid aquaculture production represented by Atlantic salmon. 1961 – 2011. [Source: FAO]

Fish oil inclusion rates for fed salmon have fallen steadily over the past decade decreasing from 25% to 16% between 1997 and 2007. Instead aquaculture producers are increasingly targeting fish oil as a specialty ingredient for use in higher value starter, finisher and broodstock feeds with dietary lipids for intermediate stage feeds substituted to varying levels with plant oils such as soya, rape, palm and flaxseed. However because farmed fish FA profiles reflect diet substitution of 75%+ dietary lipids with oils of terrestrial origin has been shown to decrease farmed salmon flesh HUFAs by 50% such that levels of EPA+DHA needed to maintain proper human health are not met by consumption of the recommended two portions of farmed fish per week. Disagreement exists however as to the impact of increased substitution on overall fish oil consumption by the aquaculture sector with the IFFO predicting a decrease of ~7.5% and the UK authority Seafish predicting an increase of ~18% on 2010 consumption by 2020. Nevertheless, continued

increases in crude fish oil prices are forecast due not only to static supplies from forage fisheries but also substantial growth in demand for Omega-3 HUFAs for direct human consumption. Consequently significant fish oil supply gaps [Fig 12] are anticipated within the next five years if affordable and sustainable alternative sources are not found.

Table 15. Estimated global use and demand ['000 T] for fish oil within compound aquafeeds between 1995–2020 [adapted from Tacon and Metian 2008]

Total production	**Total fed production**	**Total feeds used**	**Fish oil used [IFFO]**	**Fish oil used [SeaFish]**
1995	9092	3965	474	474
1996	10,293	4734	535	535
1997	11,477	5645	575	575
1998	12,502	6433	589	589
1999	13,850	7228	622	622
2000	14,900	8000	631	631
2001	16,267	8965	718	718
2002	17,310	9979	695	695
2003	19,744	11,586	768	768
2004	20,709	12,551	809	809
2005	22,341	13,729	843	843
2006	23,851	15,072	835	835
2007	25,708	16,575	778	778
2010	31,632	21,351	770	770
2015	44,143	31,578	756	845
2020	60,014	45,557	712	908

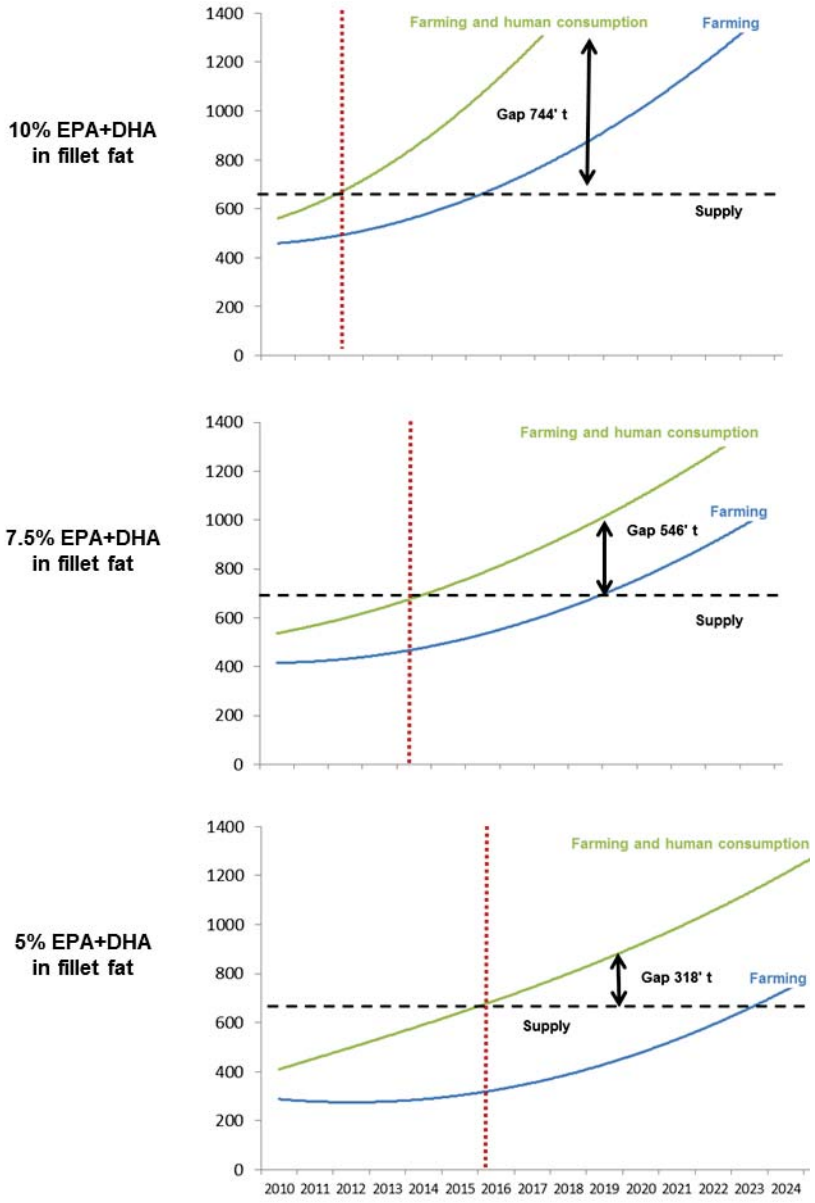


Fig. 12. The potential gap between the need for marine-type oil for the aquaculture sector and the combined needs of aquaculture and direct human consumption, based on a range of assumptions about desired Omega-3 HUFA content in salmon fillets. [Source SARF077]

4.3.2. Refined Edible Products

In the mid-1990s, the Omega-3 REP sector was dominated by functional foods such as dairy spreads and yogurts however recent technological advances in particular the development of emulsions to prevent PUFA oxidation have enabled the rapid expansion of this sector with a multitude of new Omega-3 REPs launched or in development [Fig 13]. Consequently, global demand for EPA+DHA ingredients has increased dramatically almost quadrupling from 5% to 19% of total supply in the past three decades [Fig 14]. The primary drivers here are increased public awareness of the numerous health benefits of LC Omega-3 HUFA consumption [particularly for early brain development and a cholesterol lowering agent] and increased affluence in developing world countries. Total Global consumer spending on Omega-3 enriched products is currently estimated at US$25.4 billion [2012;

GOED] while recent market analysis indicates growth to continue at a CAGR of 15% over the next five years such [Fig. 15]. Thus overall demand for marine-type lipids rich in EPA+DHA for the Omega-3 REP sector is estimated to reach nearly 0.5 MMT by 2018 signifying an urgent need for new and novel sources of this highly sought after commodity.

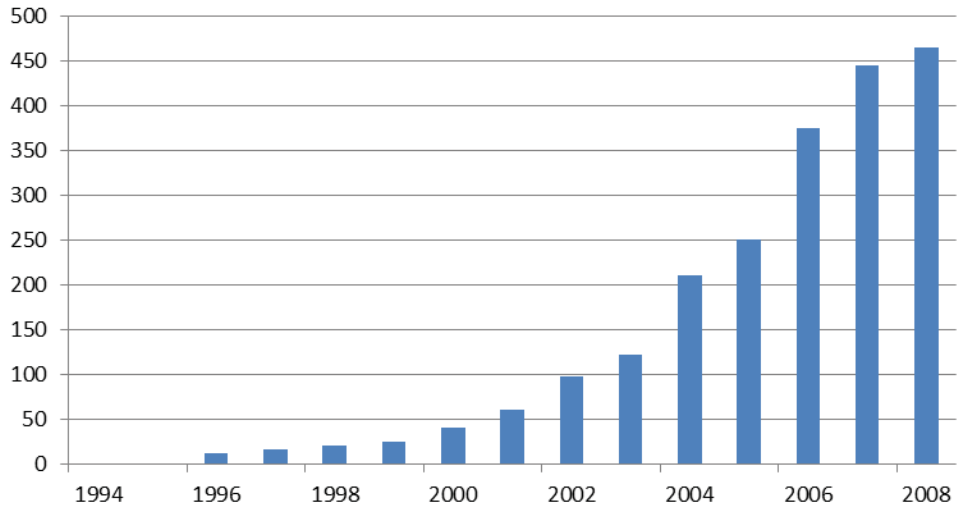


Fig. 13. Number of new Omega-3-enriched food product launches between 1994 and 2008.

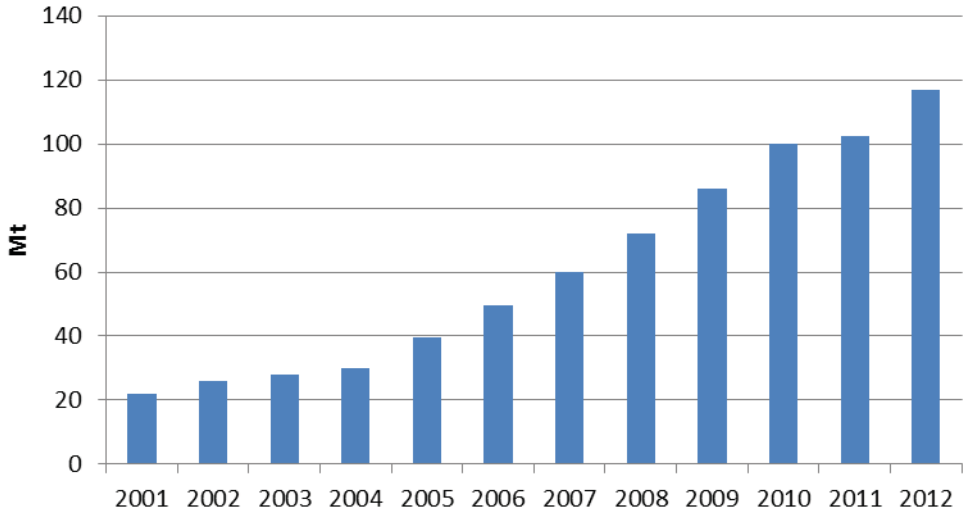


Fig. 14. Approximate annual growth in global consumption of EPA+DHA rich oils between 2001 and 2012 [Source: GOED; Frost & Sullivan 2009]

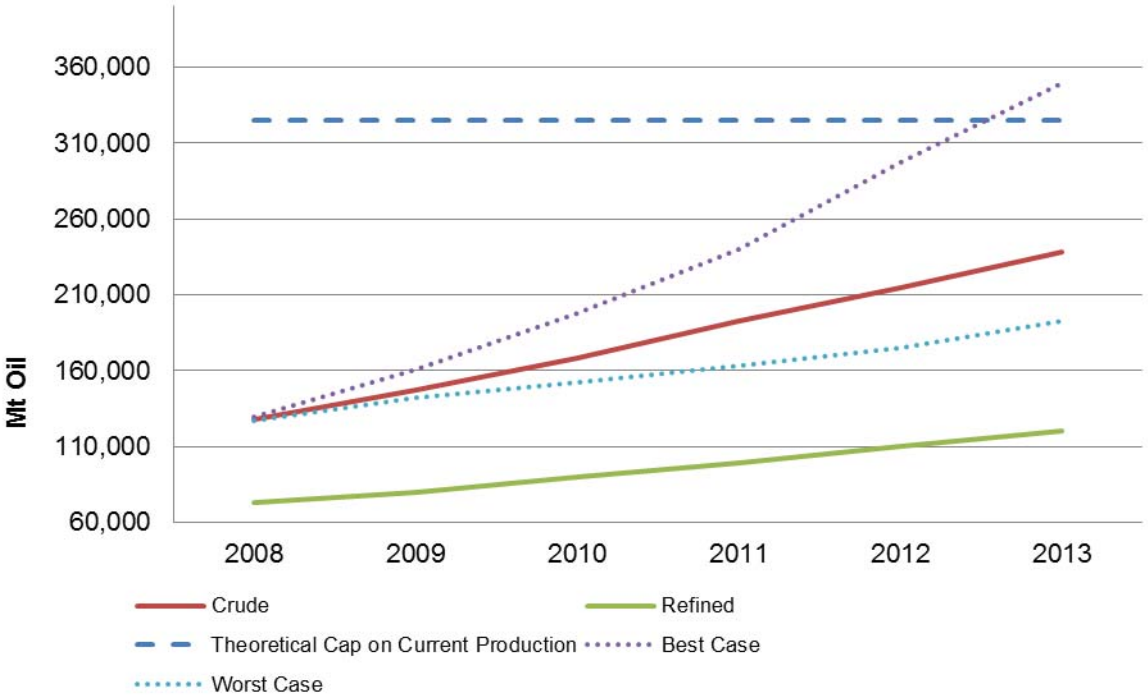


Fig. 15. Projected volume of the global Omega-3 REP market, 2008-2013. Dashed blue line shows theoretical cap for the sustainable reduction of wild forage fish to crude oil [GOED 2009]

In terms of applications approximately 61% of LC Omega-3 HUFAs consumed by the REP sector in 2011 went to the manufacture of dietary supplements [DSs] with the remainder going to animal feed [21%], functional foods and beverages [13%], infant and clinical nutrition [3%] and pharmaceuticals [2%] [Fig.]. In terms of total sales however infant nutrition generated the largest revenues [US$10.2 billion] followed by functional foods and beverages [US$7.9 billion], DSs [US$3.2 billion], pharmaceuticals [US$1.9 billion], clinical nutrition [US$1.3 billion] and pet foods [US$0.9 billion] [Fig. 16]. Of these the pharmaceuticals and infant formulas markets are expected to grow fastest and although sales slowed slightly in 2011, the value of the Omega-3 DSs market is expected to cross US$4.0 billion by 2018. In regional terms North America is the largest market for Omega-3 REPs [38% total global sales in 2011] followed by Europe [22%], China, [14%], Rest of Asia [12%] and Rest of World including Japan [14%]. However increasing affluence and public awareness are expected to drive significant growth in the Asia Pacific region over the next five years [Frost & Sullivan 2009].

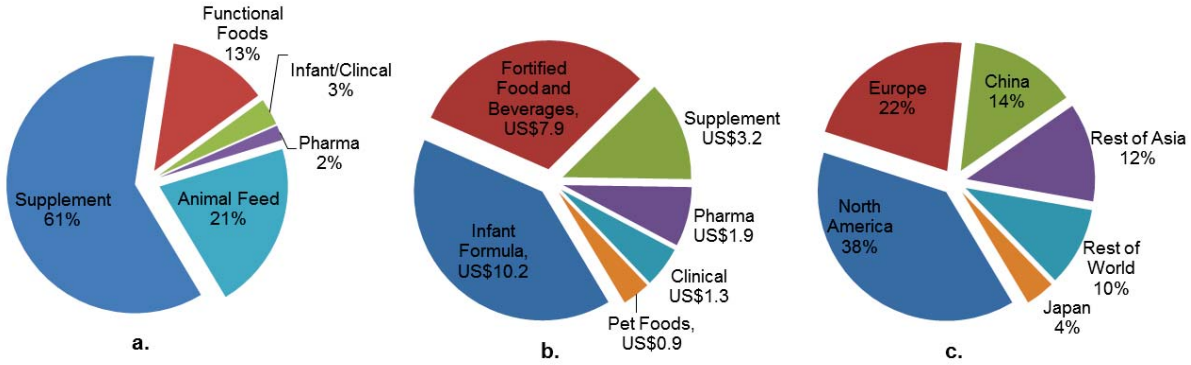


Fig. 16. Breakdown of global Omega-3 REP market by a] application b] revenues and c] geography, 2011.

In terms of EPA+DHA content, approximately 70% of the Omega-3 DSs manufactured today contain ~30% DHA+EPA in total lipids with concentrations of pharmaceutical grade supplements [i.e. ~10% of current DS sales] reaching between 80% and 90% EPA+DHA. The remaining 20% are classified “other” marine oils and generally consist of ethyl esters of Omega-3 HUFAs and are generated in significant quantities as a by-product of the concentration and refinement processes. In fact the wastage factor associated with the production of refined Omega-3 REPs and DSs in particular, constitutes one of the largest capital costs to the sector. Depending on the desired purity and concentration of EPA+DHA fractions in the finished product typical yields range between 50% and 10% of total crude oil input. For example, one report estimates an average loss across all REP sectors of about 33% of crude oil input or approximately 38,500 MT in 2012 [Frost and Sullivan]. Therefore the REP market continues to grow as fast as it has in the past decade, total available supply from global forage fisheries will not be sufficient to supply the demand for EPA+DHA ingredients by as early as 2014. The implication of this is that the more price-sensitive applications like pet foods and dietary supplements will be forced to find other sources of EPA and DHA while the least price-sensitive categories, like pharmaceuticals, will be forced to pay more for oil while retaining access to supply.

Global consumer awareness of the health benefits of regular LC Omega-3 HUFA consumption has increased steadily in the past decade and is generally high throughout the developed and developing world [≥60% adult population] [Fig. 17]. For example while only 55% of American consumers were aware of Omega-3 HUFAs in 2001 this figure currently exceeds 90%. This is thought to relate to increased research into and publication on Omega-3 health benefits [Fig. 18] thus generating greater media coverage which influence increased sales in various Omega-3 REPs. For example one study found a close correlation between US sales of dietary supplements and the number of on EPA+DHA related scientific papers published each year i.e. R-squared statistic explained 92% of the sales increase each year.

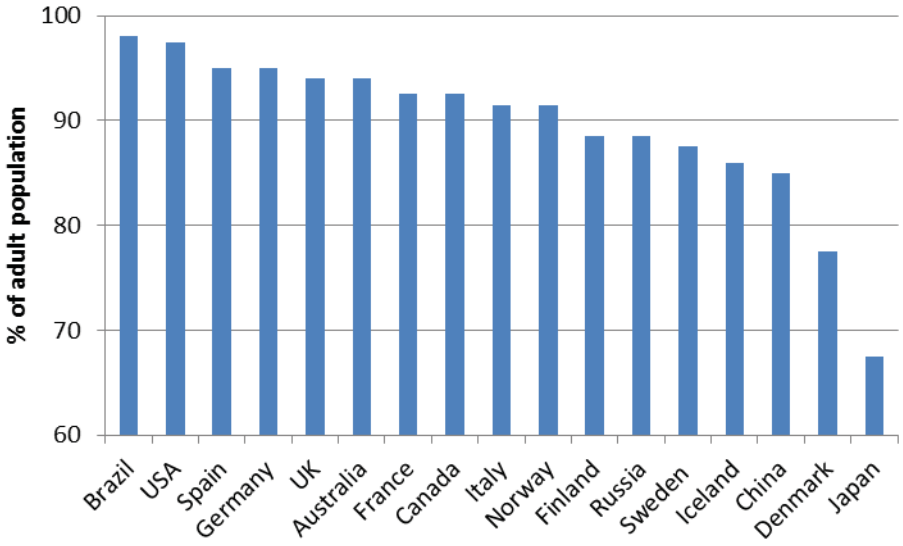


Fig. 17. Awareness [as % adult population] of the benefits of Omega-3 HUFA consumption in the developed and developing world countries [Source: GOED]

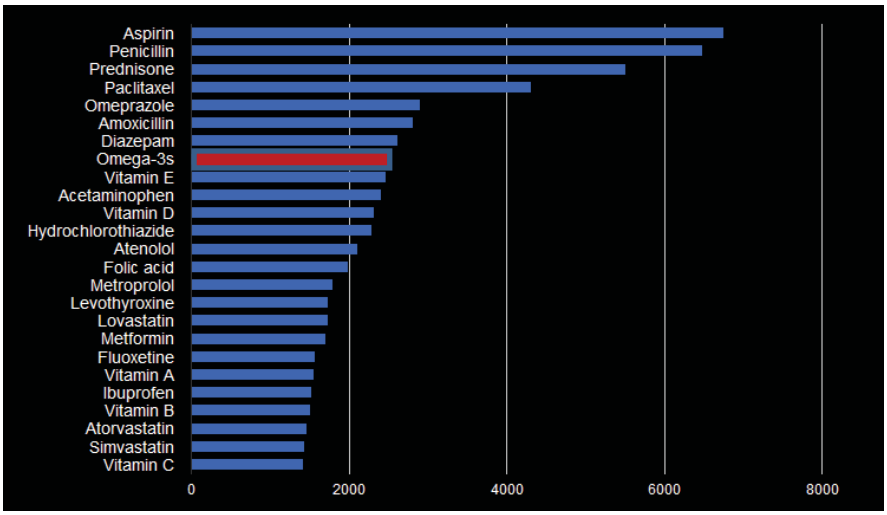


Fig. 18. Number of human randomized controlled trials investigating health effects of LC Omega-3 HUFAs relative to other compounds of pharmaceutical interest. 1967 to 2012 [Source: PubMed 2012; Ismail PPT].

Thus consumer awareness has been one of the primary drivers of recent growth in the REP market and a recent GOED commissioned survey[1] reveals some interesting international trends in overall usage and mode of consumption of Omega-3 REPs. For example, the highest percentage of adult population surveyed reporting regular Omega-3 HUFA intake is in Russia [77% adults surveyed] with the lowest in Canada [44% adults surveyed]. In terms of usage, over 65% of Russians surveyed reported seafood consumption as a means of meeting Omega-3 requirements while this was low [24%] among Canadians. Dietary supplements represent 35% of usage in both the US and

1. Leatherhead Food Research. Omega-3 - Fad or Future? Surrey: Leatherhead Food Research, 2008.

Australia while in France only 10% of those surveyed reported regular supplement use. Similarly France and America lag the rest of the world in terms of functional food usage with only 18% and 19% of respondents respectively reporting use. Interestingly however while Japan has the lowest percentage of adult population claiming awareness of Omega-3 health benefits overall intake here is the second highest globally [~1 g/d or 0.34 % total dietary energy].

4.4. Sources and Production of LC Omega-3 HUFAs

4.4.1. Introduction

In 2012 the REP sector utilized an estimated 242,330 MT of crude marine-type oil [GOED 2012] derived from a variety of sources including traditional forage fisheries, squid, algae, krill and yeasts [Fig 19]. The vast majority of this [>80%] comes from small pelagics including anchovy, herring, menhaden and hoki however the majority of these fisheries, specifically the Peruvian anchovy, have already reached their maximum sustainable limit. Thus should continuing growth in global aquaculture see demand for oil for aquafeed increase, major shortfalls in supply of EPA+DHA rich oils for both the REP and aquaculture sectors are a real possibility if reliable and affordable alternatives are not found soon.

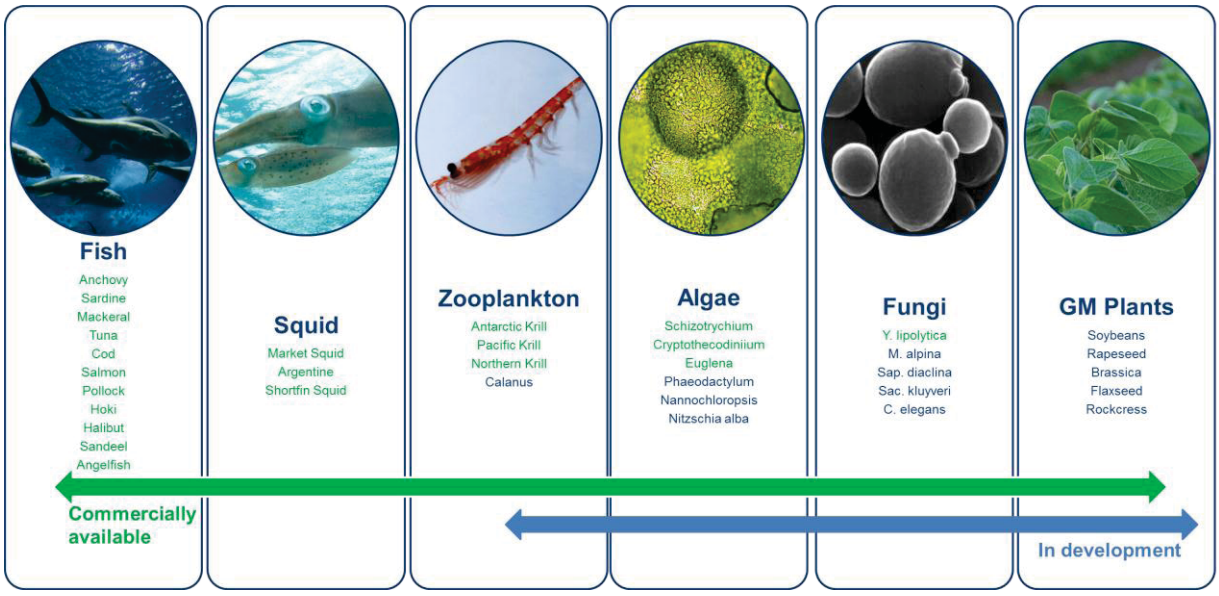


Fig. 19. Various existing and novel sources of Omega-3 HUFAs.

To this end, renewed and intensive research focused on optimisation of Omega-3 HUFA production from a variety of novel new sources has seen the tentative emergence of commercial production from various non-fish organisms such as microalgae, krill and yeasts [Fig. 20] primarily to supply the clinical and pharmaceutical markets with highly refined and concentrated EPA+DHA ingredients.

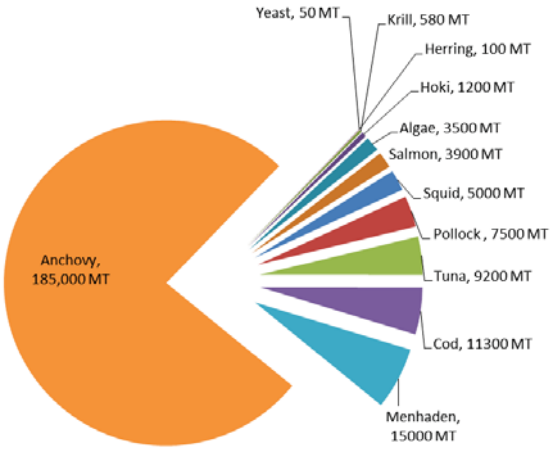


Fig. 20. Estimated Crude Oil Usage for Omega-3 Applications by organism, 2012 [GOED]

Given the consistently upward trend in fish oil prices over the past two decades, increasing consumer demand for high purity products and on-going improvements to Omega-3 production yields from non-fish sources the REP sector has significantly diversified its portfolio of Omega-3 sources in recent years and reduced dependency on crude fish oils [Fig. 21].

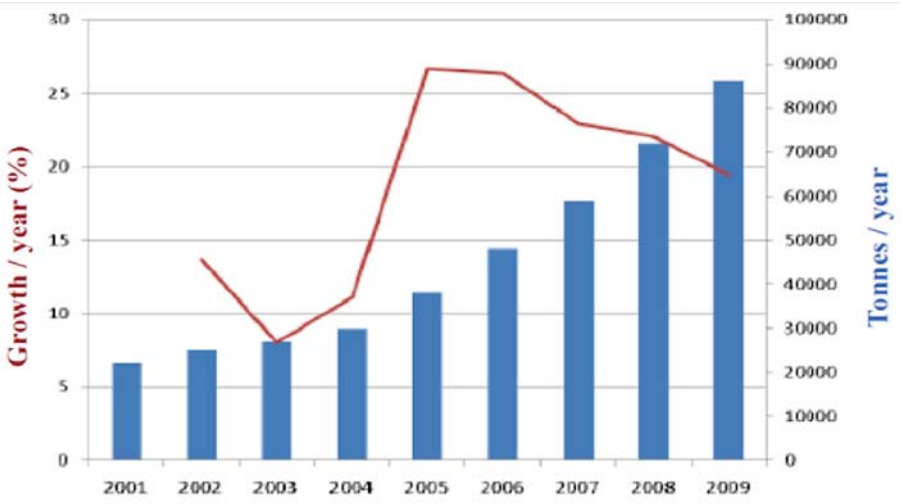


Fig. 21. Growth in the Omega-3 refining industry [red line] and the volume of fish oil consumed [blue columns], 2001-2009. [Source: GOED, 2010].

Furthermore, announcements from a number of multinational companies claiming breakthroughs in the genetic modification of terrestrial oilseeds for enhanced Omega-3 production has generated considerable interest as an inexpensive and scalable alternative to forage fish oils. Consumer fears over the safety of GM organism derived oils for direct human consumption however may stifle commercialisation for direct human consumption however they offer significant potential as a targeted replacement for fish oil in compound aquafeeds. Given the health costs of LC Omega-3 HUFA deficiency outlined in Section 3 however, the composition of GMO oils warrants consideration because as yet these organisms are only capable of synthesizing shorter chain AA and SDA in any significant quantity, and the human body has only a very limited capacity to convert these to more

beneficial LC EPA+DHA molecules. The following therefore is an overview of available and emerging sources of both short and long chain Omega-3 P/HUFAs and includes an assessment of current production technologies, costs and where applicable, associated bottlenecks limiting commercially viable industrial scale production.

4.4.2. Forage Fish

Reduction of marine forage fish represents the single most important source of LC Omega-3 HUFAs for human use today and in 2012 constituted >95% of total consumption by the REP sector [~234,300 MT]. And while numerous species are used including large demersal species such as cod, tuna and Pollock, small oleaginous pelagics from the orders Clupeiformes and Scombroidei such as menhaden and herring represent the single largest commercial source of crude fish oils since human use began. Anchovy in particular account for more than 85% of total global fish oil production [Fig 21] with ~76% of all LC Omega-3 HUFAs used by the REP sector last year coming from this single fishery [~185,000 MT in 2012]. Reasons for this include the high EPA+DHA content of anchovy body oils [Table 16] and the fact that the anchovy fishery represents the largest capture fishery in the world [~ 5 MMT]. Moreover, single species landings of *Engraulis ringens* or the Peruvian anchovetta accounts for an average of 30% of total global fish oil supply with almost 100% of both Peruvian and Chilean landings going for reduction to fish meal and oil for export to the international market. The remainder of crude fish oil supplies are generated from European [primarily herring and capelin] and Asian [primarily squid] fisheries [Fig. 22].

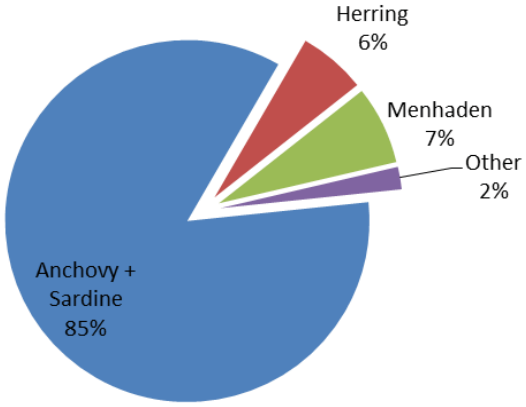


Fig. 22. Global crude fish oil production by species [five-year average 2005-2009] [Source: FAO].

Table 16. EPA and DHA content [% Total Lipids] of various marine fish [Modified from Catalina et al. 2012]

Species	EPA+DHA
Peruvian Anchovy	34.7
Hake	34.9
Pollock	41.4
Smelt	33.6
Rockfish	29.8
Pink Salmon	27.5
Capelin	17.8
Pilchard	44.1
Herring	17.3

Declining stocks due to both overfishing and climatic instability and consequent tightening of catch quotas however have resulted in substantially reduced forage fish landings since 2006[1] with over 90% of all forage fish species currently classified as fully exploited or overfished [Table 17]. According to the UN SOFIA report 2012[2] *"Increases in production from these overexploited stocks may be possible if effective rebuilding plans are put in place. The fraction of fully exploited stocks, [] has shown the smallest change over time, with its percentage stable at about 50% from 1974 to 1985, then falling to 43% in 1989 before gradually increasing to 57% in 2009".* More significantly in terms of fish oil production this report states that *"the two main stocks of anchovetta in the Southeast Pacific, Alaska pollock in the North Pacific and blue whiting in the Atlantic are fully exploited. Atlantic herring stocks are fully exploited in both the Northeast and Northwest Atlantic. Japanese anchovy in the Northwest Pacific and Chilean jack mackerel in the Southeast Pacific are considered to be overexploited. Chub mackerel stocks are fully exploited in the Eastern Pacific and the Northwest Pacific".*

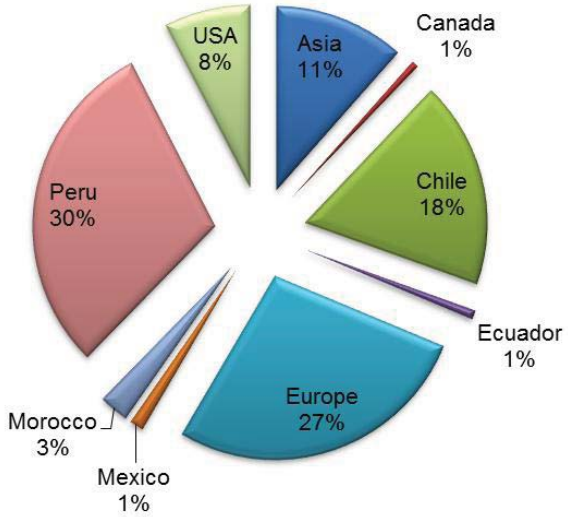


Fig. 23. Global fish oil production by country [five-year average 2005-2009] [Source: FAO].

Consequently fish oil production is forecast to remain largely static at ~1.0 MMT [~1.08 MMT in 2012] for the foreseeable future [Fig. 24] and as mentioned previously this coupled with increasing global demand has resulted in soaring increases in the price of crude fish oil over the past five years. For example, a recent paper by GOED Director A. Ismail[3] concluded that given the South American fisheries typical capacity to supply ~300,000 MT of crude oils in any given year [based on the catch allowed under Peruvian regulations] the available supply of oils from the South American anchovy fishery will not be sufficient to supply the demand for all Omega-3 applications by as early as 2013. However, more recently, this agency more recently highlighted that considerable quantities of EPA+DHA [~250,000 MT] are still available from wild stocks of marine fish which if carefully managed, can continue to satisfy almost 25% of market demand for the foreseeable future [Fig. 25].

1. http://www.huffingtonpost.com/2013/02/04/peruvian-anchovy-overfishing_n_2618275.html
2. http://www.fao.org/docrep/016/i2727e/i2727e.pdf
3. http://www.jle.com/e-docs/00/04/5E/27/article.phtml

Table 17. Stock status for fish destined for reduction in 2002 [based on FAO, 2005]. Multiple values of exploitation are due to multiple stocks within various FAO areas being in different states of exploitation [Watson et al 2006]

Target Stock	Order	FAO Area	State of exploitation
Chub mackerel	Scombroidei	EC Atlantic [34]	F
Atlantic mackerel	Scombroidei	NE Atlantic [27]	F
Norway pout	Gadiformes	NE Atlantic [27]	F
Sandeels/Sandlances	Trachinoidei	NE Atlantic [27]	F
		NE Atlantic [27]	F
European sprat	Clupeiformes	NE Atlantic [27]	F
Capelin	Salmoniformes	NE Atlantic [27]	F
Atlantic menhaden	Clupeiformes	NW Atlantic [21]	F
Pacific saury	Beloniformes	NW Pacific [61]	F
Japanese sardine [anchovy]	Clupeiformes	NW Pacific [61]	F
South African anchovy	Clupeiformes	SE Atlantic [47]	F
Pilchard	Clupeiformes	SE Atlantic [47]	F
		WC Atlantic [31]	F
Gulf menhaden	Clupeiformes	WC Atlantic [31]	F
Blue whiting	Gadiformes	NE Atlantic [27]	O
Pacific herring	Clupeiformes	NW Pacific [61]	?
South American pilchard	Clupeiformes	SE Pacific [87]	F-O
Chilean jack mackerel	Scombroidei	SE Pacific [87]	F-O
Hake	Gadiformes	SE Pacific [87]	F-O
Horse mackerel	Scombroidei	SE Atlantic [47]	M/F
Peruvian anchoveta	Clupeiformes	SE Pacific [87]	R-O
Atlantic herring	Clupeiformes	NW Atlantic [21]	U-F-R

*F=fully exploited; O=overexploited; U=underexploited; R=recovering; M= moderately exploited [FAO, 2005].

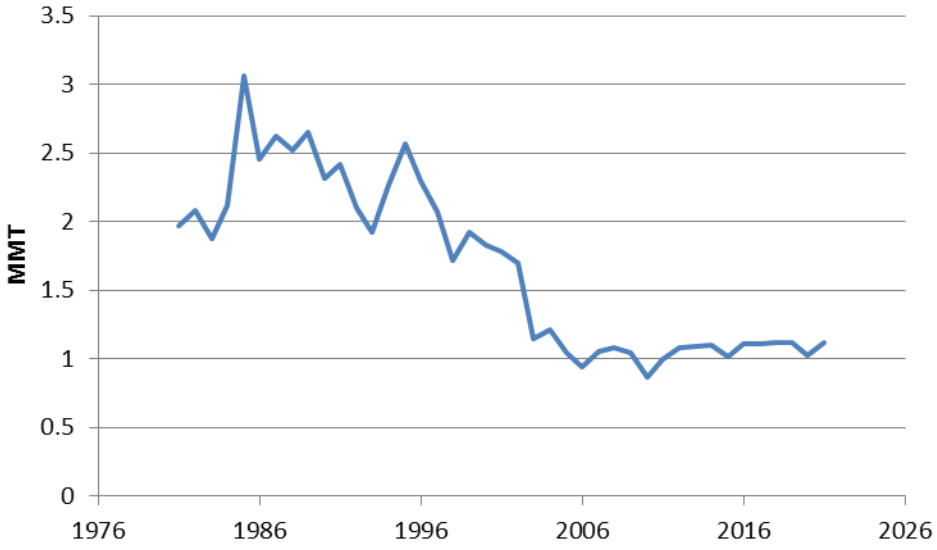


Fig. 24. Historic and forecast trends in global fish oil production [MMT] 1981-2021 [Source FAO]

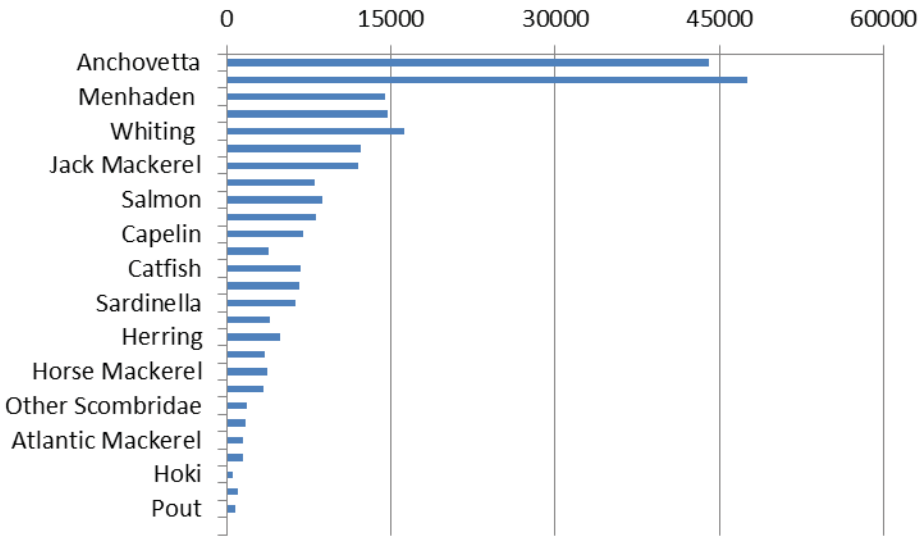


Fig. 25. Estimated availability of EPA+DHA ingredients from existing wild stocks of marine forage fish [Source: GOED].

Fisheries by-products, including discarded wild by-catch and trimmings from processing of food fish, however constitute a promising additional source of oil not yet fully exploited. For example, Alverson et al [1994] estimated an average of 27.0 MMT of fish are discarded globally each year by commercial fisheries while trimmings produced during processing can be as high as 50% total body weight. In the UK around 85% of fish processing by-products are rendered to produce fishmeal and oil [~730 MT.day], and from an industry point of view represents the most promising alternative to ensilement for discards not destined for human consumption. Due to the lack of monetised revenue and costs figures for processing of fish discards into fish oil and other products however it is difficult to assess the gross value added of utilising discards for oil production. However, disregarding variation in lipid content by species or body part, and using an estimated conversion efficiency of 7.14% given in a recent report by a leading global seafood company[1] rendering of an albeit unrealistic 100% of European wild marine only fisheries trimmings [i.e. total landings minus crustaceans and small pelagics ~ 9.98 MMT in 2011] could theoretically generate an additional 356,575 MT of crude fish oil per year. Furthermore, given the recent decision by the EU Commission to land all catch by 2015 European oil production from discards could potentially increase by 25% at current average discard rates[1]. Globally this equates to an additional 1.92 MMT of crude oil, more than double current volumes from all supply chains, which is currently unused.

4.4.2.1. Fatty Acid Composition

The composition of virtually all fish oils can be described by reference to eight fatty acids: myristic acid [14:0], palmitic acid [16:0], cis-9-hexadecenoic acid [16: 1 [n-7], palmitoleic acid], cis-9-octadecenoic acid [18:1n-9], oleic acid, cis-9-eicosenoic acid [20:In-ll], gadoleic acid, cis-11-docosenoic acid [22:In-ll], cetoleic acid, EPA and DHA [Table 18]. Climatic events in particular Pacific El Niño-La Niña cycles however crucially affect both the absolute amount and composition of Omega-3 HUFAs in fish body oils with producers reporting an increasing scarcity of oils containing the industry standard ratio of 18:12 EPA:DHA [18% EPA: 12% DHA] during strong El Niño events[2].

1. http://www.marineharvest.com/PageFiles/1296/2012%20Salmon%20Handbook%2018.juli_h%C3%B8y%20tl.pdf
2. Galdos et al. 2002. http://dx.doi.org/10.1006/jfca.2002.1059.

Table. 18. Principal Fatty Acids in Fish Oils (% Total Lipid) [Pike and Jackson 2010]

Fatty Acid	Capelin	Norway Pout	Mackerel	Sardine/pilchard	Horse mackerel	Anchovy
Myristic 14:0	7	6	8	8	8	9
Palmitic 16:0	10	13	14	18	18	19
Palmitoleic 16:1	10	5	7	10	8	9
Oleic 18:1	14	14	13	13	11	13
Eicosenoic 20:1	17	11	12	4	5	5
Cetoleic 22:1	14	12	15	3	8	2
LC Omega-3s						
EPA 20:5n-3	8	8	7	18	13	17
DHA 22:6n-3	6	13	8	9	10	9

4.4.3. Krill

Commercial operations only exist for Antarctic krill [*Euphausia superba*], with total landings estimated at 118,124 MT in 2007. Although REPs containing krill oil are commercially available [Fig. 26], information concerning total global production or market price is currently unavailable. The current Convention on the Conservation of Antarctic Marine Living Resources treaty allows 6.55 MMT to be caught in the major statistical areas but the average catch over the last 10 years has been about 120,000 MT [FAO, 2009]. Krill have very low oil content. If we assume that they contain 3% oil, and all the current krill catch is processed to produce oil, then about 3,600 MT of krill oil are potentially available annually. If the entire treaty volume were to be caught [6.55 MMT] and processed to make the oil, then there would be potential for about 197,000 MT of krill oil. This is unrealistic however because that level of catch would probably trigger a major environmentalist outcry. However interest in krill oil for inclusion in DS is growing as krill oil Omega-3 occurs largely in PL form which recent studies indicate may be more bioavailable than other forms[1].

Fig. 26. Selection of currently available krill oil supplements L-R: Cleanmarine Krill Oil, Superba Advanced Krill Oil, Nature's Way Krill Oil and MEGA RED® Omega-3 Krill Oil

1. Schuchardt et al. Lipids in Health and Disease 2011, 10:145

4.4.4. Single Celled Micro-Organisms [SCMOs]

4.4.4.1. Introduction

Oleaginous SCMO's including marine microalgae, protists, fungi, bacteria and yeasts exhibit a high degree of utility as alternative sources of LC Omega-3 HUFAs for the REP sector. For example, marine protists and dinoflagellates, such as species of Thraustochytrium, Schizochytrium and Crypthecodinium are rich sources of DHA, whereas microalgae like Phaeodactylum and Skeletonema are good sources of EPA. Species of lower fungi are also able to accumulate a high percentage of EPA in the lipid fraction[1] while several filamentous fungi belonging to the genus Mortierella were found capable of converting linseed oil to an oil containing EPA in their mycelia when grown at low temperature[2]. Similarly, yeasts such as *Yarrowia lipolytica* is capable of scavenging external HUFA and concentrating them internally and is currently being explored as a novel means of recovering lipids from fish processing waste and used frying oils[3]. As well as accumulating large quantities of EPA+DHA, SCMOs also have the advantages rapid growth [doubling biomass in less than 24 hrs.] and in the case of heterotrophic MOs are readily scalable using existing and well defined fermentation [closed] processes. However while large numbers of microalgae contain DHA, only a few species have demonstrated production potential on an industrial scale and there is still need for species specific optimisation of fermentation techniques. Several companies such as Martek Biosciences however currently use SCMO fermentation to produce commercial quantities of Omega-3 and 6 HUFAs with substantial improvements in cultivation techniques, biomass and lipid yields being made over the past decade.

4.4.4.2. Microalgae

A number of microalgal groups, including diatoms, crysophytes, cryptophytes and dinoflagellates produce large quantities of lipids [up to 80% of cell dry weight[1] with several species producing exceptionally high concentrations of LC Omega-3 HUFAs and low concentrations of Omega-6 HUFAs, MUFAs and SFAs [Table 19]. Microalgae also produce an array of other potentially valuable proteins, carbohydrates, pigments, anti-oxidants and other metabolites as well as large quantities of degradable organic carbon suitable for anaerobic biogas production[4]. Microalgal biomass productivity rates are also high with most species doubling their biomass within 24 h and as quickly as 3.5 h during the exponential growth phase[1]. For these reasons microalgae offer great potential as a source of both Omega-3 lipids and other functional ingredients[5] for the REP and nutraceutical sectors however realisation of this has been impeded by stubbornly high costs associated with processing harvested algae and the extraction and refinement of valuable components. Thus, the primary focus for commercialisation of microalgal lipid production over the past decade has been the optimisation of algal dewatering techniques or the development of more efficient technologies for extraction of lipids from wet biomass.

Microalgal fatty acid profiles are very much subject to modifications in the culture conditions with nitrogen limitation at the end of the growth cycle the most effective means of enhancing LC Omega-3 HUFA content. Other environmental stresses such as low

1. Ward & Singh, 2005; Biotechnological Applications of Microbes. I.K. International, New Delhi. pp 199-220.
2. Sakayu et al., 1988 Applied Microbiology and Biotechnology November 1989, Volume 32, Issue 1, pp 1-4
3. Bialy et al. 2011 http://dx.doi.org/10.1007/s11274-011-0755-x%l
4. Chisti, Y. http://dx.doi.org/10.1016/j.biotechadv.2007.02.001
5. Adarme-Vega et al. 2012 http://www.microbialcellfactories.com/content/11/1/96

temperature, change in salinity or UV radiation have also been shown to induce Omega-3 HUFA synthesis. For example significant increases in EPA content [+10%] have been reported for *P. tricornutum* cultured at low [10°C] compared to high temperatures [25°C] while a 20% increase was reported for the same species subjected to UV light stress. Similarly *Cryptothecodinium cohnii* increased its total DHA content up to 56.9% of total fatty acids when cultured at higher salinity [9 g/L NaCl]. Apart for environmental stresses, metabolic engineering is another promising approach to increase the production of fatty acids in microalgae [for a recent review see Schuhmann et al. [1991] and genes encoding key enzymes involved in fatty acid biosynthesis have been identified in *Ostreococcus tauri, Thalassiosira pseudonana, P. tricornutum* and the model organism *Chlamydomonas reinhardtii*. Commercial scale production of Omega-3 HUFA has also been demonstrated for genetically modified variants of the dinoflagellate species *C. cohnii* which currently provides some of the DHA found in US based Martek Biosciences Life'sDHA supplements [Fig. 27][1].

Several other pharmaceutical companies supply microlalgal oils primarily to the dietary supplements and infant formula markets [Fig. 25] however in terms of global production volumes FAO statistical coverage of microalgal biomass is limited to two freshwater species, *Spirulina platensis* [~73,000 MT 2011], *Haematococcus pluvialis* [205 MT 2011] neither of which are currently utilised for commercial lipid production. Nevertheless Benemann [2008] has estimated all species total global microalgal production at around 10,000 MT.yr which is broadly corroborated by more recent assessments by Brennan and Owende [2010][2] [Table 20]. Clear data on the proportion of microalgal derived LC Omega-3 HUFAs currently entering the REP sector however is difficult to obtain however estimates given by GOED for the 2012 fiscal year put this at ~3,500 MT or ~1.5% of total consumption by the REP sector[2]. Moreover, in terms of global sales of EPA+DHA ingredients, these ~3,500 MT of microalgal oils were estimated to have been worth US$330 million and despite their small volume produce significant economic value when compared to crude fish oil. Given this the high capital costs currently associated with microalgal biomass production are expected to decrease considerably within the next five to ten years with some analysts hypothesizing rapid growth in microalgal oils production as more efficient integrated production platforms and lipid extraction methods are developed, demand for high concentration pharmaceutical grade oils continues to increase and new uses and markets are found for the myriad components of dried algal biomass[2].

1. Mendes, A. et al. 2008 J Appl Phycol DOI 10.1007/s10811-008-9351-3
2. Brennan & Owende 2010; http://www.sciencedirect.com/science/article/pii/S1364032109002408

Table 19. Omega-6 and Omega-3 HUFA composition [% Total Lipids] in various microalga species [Modified from Catalina et al. 2012]

	n-6 HUFA					n-3 HUFA										
Species	**LA 18:2 n-6**	**GLA 18:3 n-6**	**DHGLA 20:3 n-6**	**AA 20:4 n-6**	**n-6**	**ALA 18:3 n-3**	**SDA 18:4n-3**	**EPA 20:5 n-3**	**DPA 22:5 n-3**	**DHA 22:6 n-3**	**n-3**	**n-6:n-3**	**HUFA**	**MUFA**	**SFA**	**Reference**
Amphidinium carterae	1.1			nd	1.1		34	23.3	1.4	18.2	76.9	0.01	78.8	1.2	18.8	Mansour et al 05
Rhodomonas salina	1.9			tr	1.9	25.5	20.3	17.6	0.7	8.9	73	0.03	77.6	5.1	14.3	Mansour et al 05
Dunaliella tertiolecta	3.7	2.1			5.8	34.6	1.3	36		0.7	72.6	0.08		15.5		Thompson et al 92
Rhodomonas lens	1.1			0.1	1.2	28.1	15.3	10.9		9	63.3	0.02	67.1	9	23.9	Milke et al 06
Chromophyte sp.	2.1			6.1	8.2			53.2		8.9	62.1	0.13				Kawachi et al 96
Cryptomonas sp.	1.6			0.2	1.8	24.4	24.1	7.4		3.8	59.7	0.03	63.3	4.4	27.5	Thinh et al 99
Rhodomonas sp.	1.3	0.2		0.3	1.8	19.8	28.1	7.7		3.8	59.4	0.03	64.7	8.7	25.2	Renaud et al 02
Isochrysis galbana	0.43	0.2		0.34	0.9	1.9	15.4	27.7		14.1	59.0	0.02	62.27			Fidalgo et al 98
Pavlova salina	1.8	0.7		0.4	2.9	1.3	15.2	28.2		10.9	55.6	0.05	63.7	5.8	30.5	Volkman et al 91
Chroomonas salina	10.5	2.6		0.9	14	14.2	21.3	11.9	0.3	5.2	52.9	0.26				Volkman et al 89
Skeletonema costatum	1.1				1.1		5.5	40.7		6.6	52.8	0.02	59.5			Servel et al 94
P. lutheri	0.6	0.5	0.7		1.8	2.1	10.9	27.6		7.9	48.5	0.04	54	17.5	26.7	Dunstan et al 93
Phaeodactylum tricornutum	2.6			0.9	3.5			25.7		1.8	27.5	0.13				Lopez Alonso et al 00

Fig. 27. Selection of some currently available algal oil dietary supplements L-R: Martek's Life'sDHA; Deva Omega-3 DHA-EPA; Nordic Naturals Algae Oil and Royal Green Omega-3 Algae Oil.

According to an FAO report, microalgal biomass production costs range between €4 and 300 per kg of dry biomass[1] with the majority of commercial operations located in China, Taiwan and India [Table 20]. Production costs, though high, are strongly dependent on the type of cultivation method used be it phototrophic or heterotrophic, open or closed systems [Fig. 28] and a detailed list of the various production metrics associated with photo- and hetero-trophic systems are given in Table 21. The current trend in the production of pharmaceutical grade algal oils however is for cultivation in closed fermentation type vessels such as those pictured in Fig 29. However production costs remain prohibitively high for most end users. To take a case in point Martek Biosciences produce algal DHA oil at a cost of €43 per gram of extracted oil [Table 20] for use in its Life'sDHA product while the maximum the aquaculture sector can currently afford to pay is €2.61 per gram[2].

Martek use the obligate heterotroph *C. cohnii* which uses reduced organic carbon sources such as glucose or acetate to provide energy for cell growth. In nature however, the vast majority of microalgae are obligate photosynthetic phototrophs that require light to convert CO_2 into glucose for growth. The difficulty with culturing phototrophs is that in order to minimize light limitation, the maximum cell density must be kept low while heating/lighting costs tend to be prohibitive on industrial scales. Thus all current commercial production of microalgal Omega-3 oils employ heterotrophic systems using existing infrastructure based largely on conventional "brewery/fermentation" models[3]. These use a hybrid of two techniques collectively termed semi-continuous fed batch culture where the fermentation process is split into a biomass density increasing stage and a lipid accumulation stage involving an initial exponential growth phase on a limited "batch" of nutrient media or substrate followed by regular additions of fresh substrate. Jasuja et al. [2010][4] estimated that high cell density batch fermentation processes results in biomass densities of at least 100.0 g dry cells/L in the fermentation broth and a minimum of 20% of dry cell weight as lipids. Heterotrophic production of microalgal LC Omega-3 HUFAs is also highly controllable allowing modification of the final lipid concentration and fatty acid profile via

1. http://www.fao.org/docrep/003/W3732E/w3732e06.htm
2. http://www.sarf.org.uk/cms-assets/documents/29524-222388.sarf077.pdf
3. http://www.emerging-markets.com/algae/Top_11%20Algae_Investment_Trends_%20from_%20Algae_%202020_%20Study.pdf
4. Jasuja et al. Asian Journal of Pharmaceutical and Clinical Research Vol. 3, Issue 4, 2010

subtle changes in culture environment, continuous “batch-wise” harvesting of biomass and none of the costly light inputs of phototrophic cultivation. Furthermore existing fermentation infrastructure can be readily adapted to microalgal production with several large companies such as Martek and Solazyme using heterotrophic microalgae to produce high purity LC Omega-3 HUFAs for direct human consumption.

Table 20. Present state of commercial microalgal production [Brennan & Oswende 2010[3]]

Microalgae	Annual production [MT.DW]	Producer country	Application and product	Price
Spirulina	3000	China, India, USA, Myanmar, Japan	Human nutrition Animal nutrition Cosmetics Phycobiliproteins	36 €.kg 11€.mg
Chlorella	2000	Taiwan, Germany, Japan	Human nutrition Cosmetics Aquaculture	36 €.kg 50 €.L
Dunaliella salina	1200	Australia, Israel, USA, Japan	Human nutrition Cosmetics B-carotene	215–2150 €.kg
Aphanizomenon flos-aquae	500	USA	Human nutrition	
Haematococcus pluvialis	300	USA, India, Israel	Aquaculture Astaxanthin	50 €.L 7150 €.kg
Crypthecodinium cohnii	240	USA	DHA oil	43 €.g
Schizochytrium	10	USA	DHA oil	43.€.g

Assman et al [2001] provide a comprehensive review of the relative merits of phototrophic versus heterotrophic production systems which are summarized in Table 21. Briefly, while much higher biomass productivities are achievable using a closed heterotrophic system, less energy intensive two-stage phototrophic processes have become the system of choice for present-day bio-refinery ventures such as those operated by Cellana and Solazyme. Two-stage phototrophic processes consist of a closed photobioreactor [PBR] stage [Fig 28. D - F] to maximize biomass density followed by second nitrogen starvation stage in an open outdoor raceway pond [ORP] [Fig 28. A - C] to enhance total lipid and EPA+DHA concentrations. Both the choice of PBR used and the siting of ORPs however are important considerations in terms of mass energy balance and overall production costs[1] with adequate supply of sunlight, flat land and freshwater necessary to offset the often high artificial light requirements associated with the PBR stage. Less energy intensive low tech PBR configurations such as simple polythene bags or various arrangements of horizontal or vertical tubes situated outdoors or in polythene tunnels [Fig. 28 D and F] however are capable of producing algal biomass for as little as 2.39 €.kg dry weight[2] using natural sunlight and are now widely used in the niche algal biomass industry.

1. http://www.lifesdha.com/
2. Norsker et al. Technikfolgenabschätzung – Theorie und Praxis 21. Jg., Heft 1, Juli 2012

The incapacity of heterotrophic microalgae to sequester CO_2 also adds to production costs in terms of the provision of an organic carbon source but also precludes growth on industrial CO_2 emissions thereby increasing overall process Greenhouse Gas [GHG] emissions[1]. Furthermore, while the overall land area required for heterotrophic production is much smaller than for open raceway pond cultivation, the latter utilizes arid land so does not compete with food production and where marine species are grown, does not compete with other utilities for available freshwater. Moreover, as microalgal production systems do not require arable land they do not compete with food production. For example, recent calculations estimate that the total US transport-fuel market could be satisfied by 25 M acres of algal systems or a mere 27% of the US area currently used in corn production.

De-oiled algal biomass from both heterotrophic and autotrophic systems is also suitable for anaerobic digestion to produce biogas however a major downfall of this is that lipid extraction prior to digestion requires algae to be dried to approximately 90% (w/w) equating to a greater investment than return of energy. In fact, algal biomass processing [harvesting, cell lysing and separations] accounts for up to seven times the energy required for biomass production[2] making this the most challenging obstacle toward sustainable and affordable microalgal lipid production today.

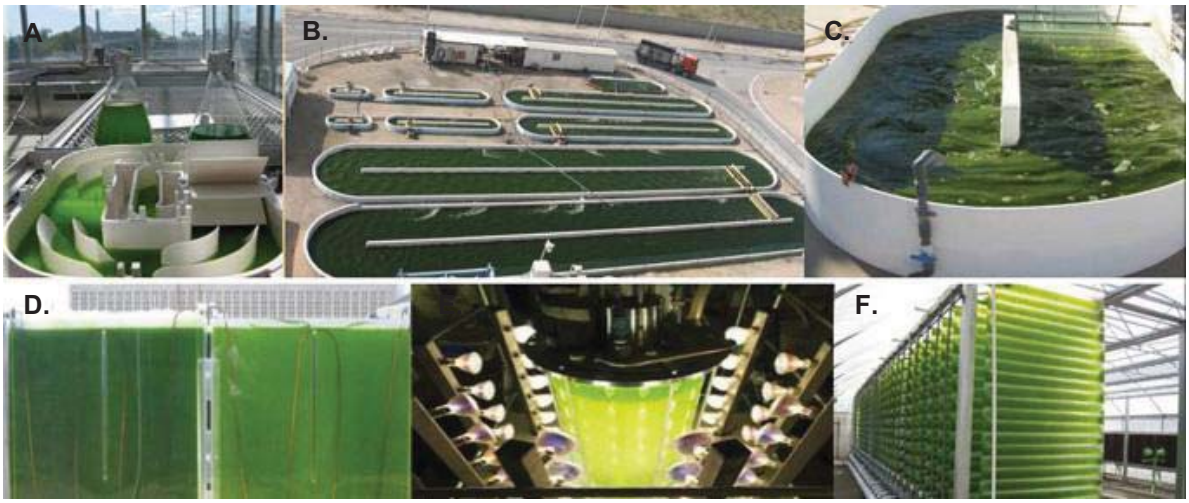


Fig. 28. Examples of open and closed systems for cultivation of phototrophic microalgae A) laboratory raceway pond culture B) commercial scale raceway pond. C) raceway paddle-wheel system. D) polythene bag culture E) photobioreactor F) tubular reactor system utilizing natural light.

1. For review see: http://www.biotechnologyforbiofuels.com/content/pdf/1754-6834-6-88.pdf
2. http://www.utexas.edu/research/cem/IEEE/Beal_EROIAlgae_BioEnergyResearch_OnlineFirst.pdf

Table 21. System specifications for A. autotrophic and B. heterotrophic production of *Chlorella* species [Assman et al 2011]

	System A: Autotrophic Cultivation		**System B: Heterotrophic Cultivation in closed bioreactors**
Organism	**Photobioreactor (Seed Production)** *Chlorella vulgaris*	**Open Raceway Pond (Lipid Production)** *Chlorella Vulgaris*	*Chlorella Protothecoides*
Biomass Density (kg/m3)	2.5	0.5	3 – 15
Lipid Content (% TL)	14%	35%	45% – 58%
Volume Productivity (kg biomass/m3.day)	0.60	0.07	14.71
TG Content	20%	85%	80%
Area (m2)	22	1344	6.7
Volume (m3)	1.6	324	20
Length (m)	193	100	3
Breadth/Diameter (m)	0.11	10	3
Depth (m)	N/A	0.3	N/A
Hydraulic residence time (days)	5	5	1
Water Source	Brackish (Aquifer)	Brackish (Aquifer)	Fresh
Water Recycling	No	Yes	No
Location	Southwest	Southwest	Urban/Suburban
Energy Source	Sunlight	Sunlight	Acetate
Carbon Source	Atmospheric CO2	Atmospheric CO2	Acetate
Nitrogen source	Urea		
Phosphorus source	Super Phosphate		
Potassium	Potash Fertilizer		
Harvest Efficiency N	N/A.	87	90
Lipid Extraction Efficiency	N/A.	70	70
TAG Refining Efficiency	N/A	80	80
Trans-esterification Efficiency	N/A	97	97
Evaporation (per month)	No	25% -100%	No
Pesticides	No	Yes	No
Waste Management	On-site Storage		Wastewater Treatment Plant

Fig. 29. Photograph of three fermenters (bioreactors) that are likely to be used in System B for the cultivation of heterotrophic algae *Chlorella protothecoides*. (topmachinebiz.com, 2011)

Omega-3 type oils from microalgae were recently granted GRAS [Generally Recognized as Safe] status by the US government and as well as being kosher and halal provide an added dimension of appeal to consumers as a vegetarian source of DHA[1]. Moreover, resurfacing of fears over the stability of fossil oil supply in the past decade has seen renewed efforts to overcome production bottlenecks and harness microalgae both phototrophic and heterotrophic as a feedstock for LC Omega-3 HUFAs. In order for this to happen however private and public research bodies need to develop more efficient harvesting, dewatering and component separation techniques that will allow maximum utilisation of raw biomass for biofuel as well as nutritional lipids, high value secondary metabolites, human and animal food proteins, a source of oxygen and as both a means of capturing industrial carbon emissions and removing nitrogen from various wastewater streams [Fig. 30]. Similarly concern over climate change and population growth has seen widespread and substantial investment aimed at the development of industrially scalable microalgal biomass production platforms both by national governments[2] and private enterprise[3] over the past decade. These endeavours bring together clusters of industry, government, academia, cleantech investors, and producers to collaborate on key challenges and opportunities with a number of government algae R&D ventures now phasing into pre-commercial ventures using open raceway pond, photo-bioreactor, and fermentation based production systems[4].

1. Conquer and Holub 1997 Lipids. 1997 Mar;32(3):341-5
2. http://apps1.eere.energy.gov/news/progress_alerts.cfm/pa_id=359]
3. http://www.bloomberg.com/news/2010-06-03/exxon-600-million-algae-investment-spurs-khosla-to-dismiss-as-pipe-dream.html
4. http://www.markets.com/algae/Top_11%20Algae_Investment_Trends_%20from_%20Algae_%202020_%20Study.pdf].

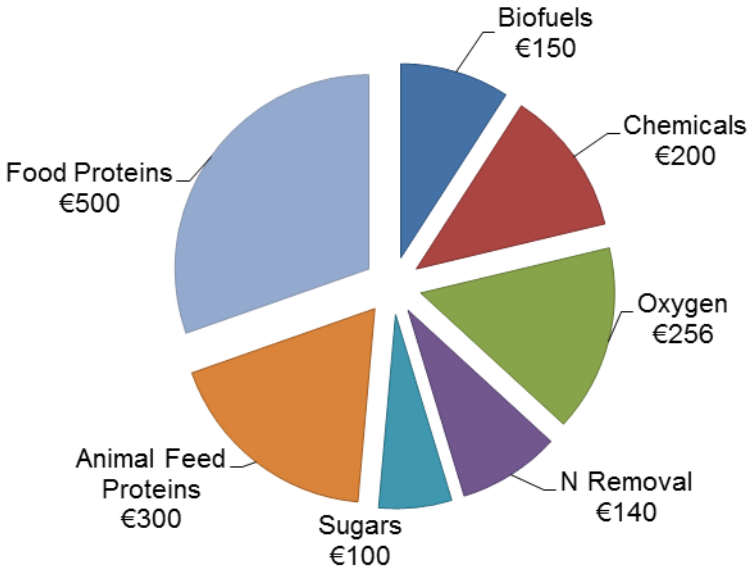


Fig. 30. Breakdown of value [EU€] of various components from 1 000 kg of refined algal biomass. Total value: €1,650 per MT biomass.

Calculated projections based on an integrated algal biorefinery approach such as that pictured in Fig. 31 suggest that with appropriate light and heat controls, a two-step [PBR/ORP] phototrophic algal "farm" grown on industrial CO_2-emissions could produce in excess of 6,000 gal biofuel/acre. Real time infrastructure and production costs based on vendor quotes and pilot scale studies by Molina-Grima however indicate that industrial scale phototrophic production of algal biomass is still many years away [Tables 22 and 23]. However, if the biofuels, food, pharma-, nutra-, and cosmeceutical potential of one MT of fully exploited algal biomass were to be realized this could potentially be worth €1,650 per MT making industrial production a viable and profitable commercial venture for the future.

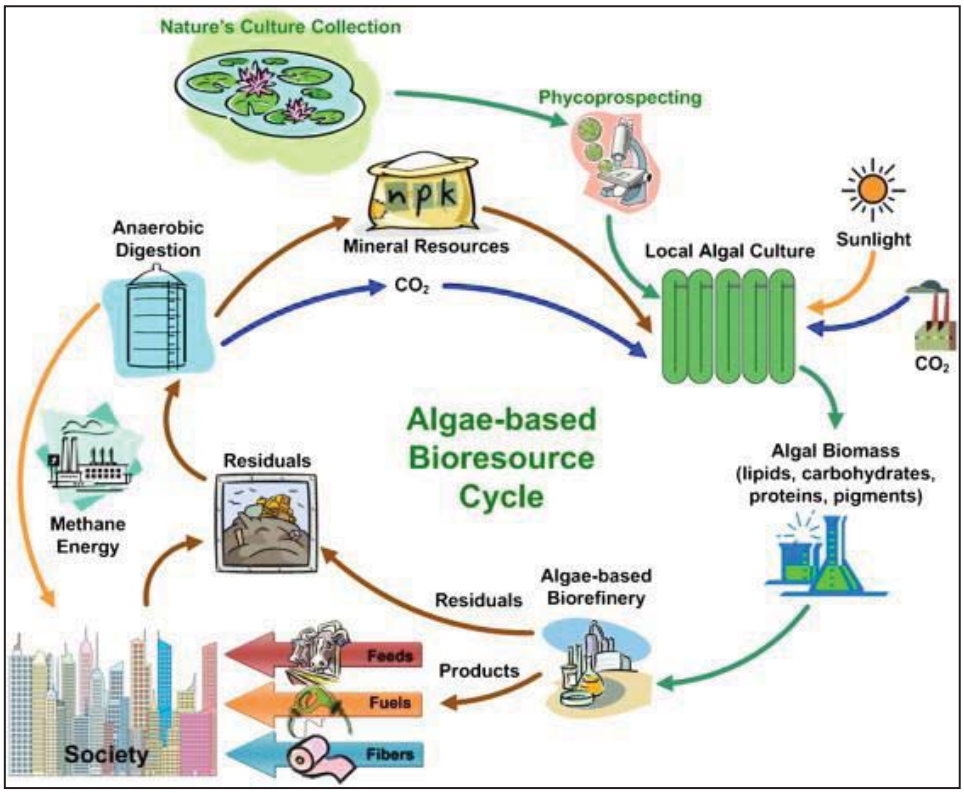


Fig. 31. The algae-based biorefinery concept. Algae with favourable characteristics are cultivated with resulting biomass processed in an algae-based biorefinery into consumable products. Sunlight drives phototrophic growth of algal cells using carbon dioxide and nutrients from local sources. Organic residuals from processing and consumption are then recycled through heterotrophically through anaerobic digestion producing energy and remineralizing elements required for further algal cultivation.

Table 22. Estimated capital Costs associated with microalgal biomass production using the Grima Closed System

Item	Cost (2003 dollars)
Equipment	
Photobioreactors x 75	264,300
Centrifuge x2	247,898
Medium filter unit	18,014
Medium feed pumps x75	26,175
Medium preparation tank x3	104,442
Harvest broth storage tank x3	104,442
Centrifuge feed pumps x2	1682
Air compressors x3	78,309
Harvest biomass conveyer belts x2	14,200
Seawater pump station	13,661
Carbon dioxide supply station	3006
Weighing station	2366
Biomass silos x2	2740
Construction	
Installation costs	264,371
Instrumentation and control	88,124
Piping	264,371
Electrical	88,124
Buildings	264,371
Yard improvements	88,124
Service facilities	176,247
Land	52,874
Engineering and supervision	220,309
Construction expenses	216,784
Contractor's fee	108,392
Contingency*	301,480
Total Capital Cost	**3,014,803**
Annual Cost	**429,240**

Table 23. Yearly operating and biomass production costs of the Grima Closed System

Input	Quantity	Cost (2003 dollars)
Culture medium (at $0.5883/kg)	65,500 kg	38,534
Carbon dioxide	96,940 kg	45,620
Media filters (at $70.59/unit)	210	14,824
Air filters (at $94.12/unit)	105	9883
Other consumables (at $117.65/kg)	13 kg	1529
Cooling water (included in pumping station)		0
Power (at $0.05883/kW h)	99,822k Wh	5873
Labour (at $16/h)	8760 hours	140,160
Supervision		28,032
Payroll charges		42,048
Maintenance		35,249
Operating supplies		442
General plant overheads		111,893
Tax		24,312
Contingency		5813
Wastewater treatment (at $0.59/m3)	10,480m	6183
Capital costs		332,093
Total Yearly Operating Costs		933,995
Biomass Costs ($/t)		**35,649**

However, relative to other crop based biofuels the algal fuels industry is very much in its infancy [Fig. 32] with no large scale production of algae-based biofuels occurring in Europe today. Three major industrial pilot projects supported by EU R&D funds are under construction however and are expected to deliver some hard figures in the next two years with respect to timescales for algae-to-biofuels production to become an economically viable prospect. However the costs to develop the necessary production infrastructure are considerable and the investment-driven development rate is most likely to be a function of the rate of price increase in crude oil. However opportunities for carbon mitigation could represent an additional driver for investment particularly within Europe where recent revisions to the EU biofuels policy have set the value of algal based biofuels at four times that for other crop based fuels.

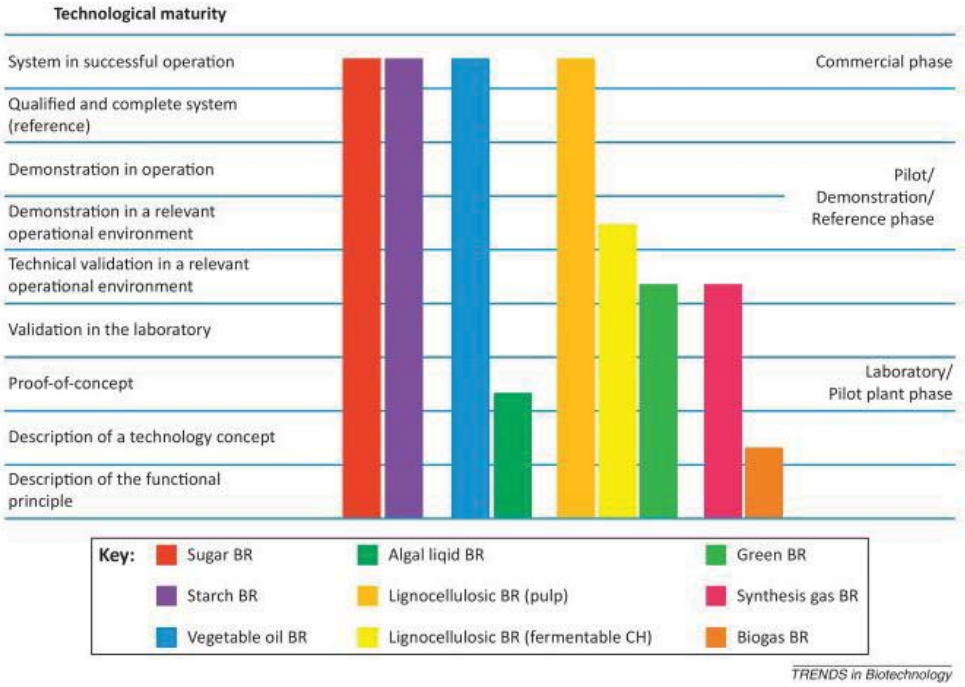


Fig. 32. The technological maturity of various biorefinery concepts, demonstrating that algal lipid biorefineries are among the least advanced and furthest from market[1]. Abbreviations: BR, biorefinery; CH, carbohydrate.

4.4.4.3. Thraustochytrids

Another group of micro-organisms offering considerable potential for supplying omega-3 lipids to the REP sector are the thraustochytrids. Thraustochytrids are heterotophic marine protists that contain both high lipid content and extremely high concentrations of DHA [Table 24]. Because their culture is relatively straightforward there exists considerable potential for industrial-scale HUFA production using either glucose or acetate in simple batch-fed or continuous anaerobic fermentation systems without light (Carter et al. 2003; Jasuja et al. 2010). Interestingly, recent research indicates that HUFA synthesis in thraustochytrids differs from other SCMOs and appears to occur via a polyketide synthase route [Fan et al. 2007] potentially more amenable to genetic manipulation for enhanced LC Omega-3 production.

1. Ritchie et al. 2013; http://www.sciencedirect.com/science/article/pii/S0167779913000218]

Table 24. Culture conditions and biomass, lipid, and DHA yields in different thraustochytrids [Reproduced from Jasuja et al. 2010]

Organism	Biomass(g .L^{-1})	% lipids in biomass	% DHA in lipids	DHA (g. L^{-1})
Thraustochytrium aureum ATCC 34304 (4 days growth)	4.9	20.3	51	0.5
T. aureum ATCC 34304;69 h growth	5.7	8	40	–
Thraustochytrium sp. 20892 (4 days)	6.1	15.2	53.1	0.7
T. roseum ATCC 28210 (5 days) fedbatch culture	17.1	25	50	2.1
Schizochytrium limacinum SR21;4 days in a fermenter	48	77.5	35.6	13.3
Schizochytrium limacinum SR21;5 days	38	5.0.0	43.1	4.2
Thraustochytrium sp. KK17- 3 (3 days)	7.1	19.9	52.1	0.3
Thraustochytrid strain 12B (3 days)	31	57.8	43.1	6.8
Schizochytrium limacinum SR21; 7 days	22.1	-	-	4.9
S. limacinum OUC88(5 day)	22.11	-	18.45	4.08
Schizochytrium limacinum SR 21	37.9	-	-	6.56

4.4.4.4. Bacteria, fungi [incl. moulds and yeasts]

Under appropriate culture conditions, numerous species of bacteria, fungi, moulds and yeasts are known to accumulate high concentrations of lipid as TGs [between 20% to 70% of biomass] [Table 25]. Omega-3 HUFA production in particular, was first reported for the marine bacterium *Flexibacter polymorphus*. This species was found to produce high quantities of EPA which secreted from cell wall membranes is thought to enhance flexibility and enable a characteristic gliding motility [Johns et al. 1977]. Similarly, Yazawa et al. [1988] reported EPA production of between 27.8% and 36.3% in the bacterial species *Pneumatophorus japonicas* isolated from the intestines of Pacific mackerel. Substantial DHA production has also been reported for several other bacterial species including *Shewanella* sp. strain SCRC-2738 [Wattanabe et al.1994] *Vibrio* sp. strain 5710 [Hammamoto 1994] and an unidentified strain SCRC 21406 isolated from smelt intestines [23% DHA in TFA] [Wattanabe 1997]. In fact several systematic screening efforts indicate widespread capacity for *de novo* synthesis of LC Omega-3 HUFA among marine bacteria and given their as yet unexploited species diversity, trophic versatility [aerobic, anaerobic, heterotrophic, phototrophic, mixotrophic], tolerance of environmental extremes and rapid exponential growth [hours] they offer huge potential as an alternative to fish oil for the REP sector[1].

Similarly, numerous species of oleaginous yeasts and fungi have been described in recent years and while these cannot produce LC Omega-3 HUFA *de novo* they are capable of efficiently scavenging EPA+DHA from their growth environment accumulating them as FFAs in cell lumen. Oleaginous yeasts also grow easily on a wide variety of agro-industrial wastes[2] including animal and vegetable fats and exhibit rapid growth rates similar to those for bacteria. Some examples include *Cryptococcus albidus, Lipomyxces lipofera, L. starkeyi, Rhodosporidium toruloides, Rhodotorula glutinis, Trichsporon pullulan* and of

1. Jensen and Fenical 1996 10.1007/BF01574765
2. Katre et al. 2012; http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3519684/

particular interest *Yarrowia lipolytica*[1]. GM *Y. lipolytica* strains capable of producing up to 25% EPA in total lipids have been engineered by pharmaceutical giant DuPont[2] while the granting of GRAS status[3] for these oils by the US Food and Drug Administration recently moves the likelihood of substantial additional volumes of GM SCMO generated Omega-3 HUFAs significantly closer to commercial reality with some analysts suggesting widespread availability within five to ten years [A. Jackson pers. comm].

Table. 25. EPA and/or DHA contents [as % total lipids] in various oleaginous bacteria and fungi [adapted from Catalina et al. 2012]

Organism	% EPA and/or DHA	Reference
Bacteria		
Shewanella putrefaciens	40.0 EPA	Yazawa K. Lipids 1996, 31[Suppl]:S297–S300.
Alteromonas putrefaciens	24.0 EPA	Yazawa K et al.. J Biochem [Tokyo] 1988, 103[1]:5–7.
Pnematophorus japonicus	36.3 EPA	Yazawa Ket al.. Nippon Suisan Gakkaishi 1988, 54[10]:1835–1838.
Photobacterium	4.6 EPA	Ryan J. et al. J Microbiol Methods 2010, 82[1]:49–53.
Fungi		
Mortierella	20.0 EPA	Jareonkitmongkol S, et al.. J Am Oil Chem Soc 1993, 70([2)]:119–123.
Mortierella	13.0 EPA	Jermsuntiea W, et al..J Oleo Sci 2011, 60([1)]:11.
Pythium	12.0 EPA	Athalye SK, et al. J Agric Food Chem 2009, 57([7)]:2739–2744.
Pythium irregulare	8.2 EPA	Liang Y, et al. Bioresour Technol 2012, 1:1.

4.4.5. Macroalgae

Relatively speaking total macroalgal lipid content is low [Table 26] with high concentrations of SFAs found in most species [Pereira et al 2012]. Within the Red and Green divisions however a handful of species produce considerable quantities of EPA+DHA. For example sizeable quantities of DHA are found in the Green alga *Ulva lobata* [0.15% TL] and three Red species *Chondrus crispus* [0.3% TL], *Palmaria Palmata* [0.5% TL] and *Gracilaria verrucosa* [0.2% TL] [Table 27]. Moreover, EPA constitutes almost 50% of the total lipid content of *Porphyra umbilicatus* [48% TL] and *P. palmata* [46.6% TL]. Examination of FA profiles across all three macroalgal divisions by Pereira et al. [2012] also indicate optimal dietary Omega-6:Omega-3 ratios ranging between 0.29 and 6.73 in 17 species examined. Thus while total macroalgal lipid content may be low certain species present attractive FA profiles for the production of rOmega-3 REPs. In addition they produce a range of other secondary metabolites [e.g., polysaccharides, vitamins, proteins etc.] of pharma- and nutra-ceutical interest however despite their relative ease of cultivation, remain largely underexploited.

1. http://www.google.com/patents/US7932077
2. MacKenzie et al. 2010 http://www.sciencedirect.com/science/article/pii/S0273230010001480

Table. 26. Average proximal composition of Brown, Green and Red macroalgae compared to that of other food sources [Source: SARF077].

	Composition [% Dry Weight]		
Division	**Protein**	**Lipid**	**Carbohydrate**
Brown [Average]	19	10	40
Green [Average]	18	2	40
Red [Average]	25	1	34
Meat	43	34	1
Fish	55	38	0
Soybean	37	20	30
Egg	49	45	3

Approximately 220 algal species are currently cultivated commercially with global production standing at ~18 MMT [wet weight] the vast majority [>95%] of which comes from Asia. The primary uses for macroalgae include food for direct human consumption [e.g. nori, wakame, kombu] and the production of hydrocolloids agar, alginate and carrageenan which have numerous industrial applications, such as gelling, stabilizing or binding agents. Almost all cultivation consists of species from the Brown and Red divisions most notably species from the genera Laminaria and Undaria [Browns] and Gracilaria and Porphyra [Reds] however despite their natural abundance, only a handful of Red species are cultured commercially. Recent global production volumes and prices for these are given in Table 28.

Table 27. LC Omega- 3 HUFA profiles of several macroalgae [% Total Lipids].

Division	Species	AA 20:4 n-6	ALA 18:3n-3	LC Omega-3 HUFA EPA 20:5n-3	LC Omega-3 HUFA DHA 22:6n-3
Brown	Egregia menziesii	-	7.625	4.6	-
Brown	Laminaria saccharina	13.7	3.6	6.2	-
Brown	Laminaria digitata	8	7.9	8.3	-
Brown	Fucus vesiculosis	8.7	5.2	4.7	-
Brown	Undaria pinnatifida	17.5	10.3	8.2	-
Brown	Halidrys siliquosa	11.5	5.8	3.6	-
Brown	Analipus japonicus	13.8	7.6	13.2	-
Brown	Laminaria dentigera	9.5	4.1	10.4	-
Brown	Hedophyllum sessile	9.6	2.1	3.1	-
Brown	Macrocystis integrifolia	14.3	6.5	8.7	-
Brown	Postelsia palmaeformis	7.9	5.9	7.2	-
Brown	Alaria marginata	14.2	8.7	15.5	-
Brown	Egregia menziesii	14.7	8.7	9.9	-
Brown	Fucus distichus	14.1	7.5	10.9	-
Brown	Cystoseira osmundacea	18.6	9.7	5.5	-
Green	Ulva lobata	0.625	21.7	0.8	0.15
Green	Ulva rotundata	0.3	9.6	1	-
Green	Enteromorpha intestinalis	0.7	15.5	1	-
Green	Ulva lactuca	0.3	11.1	1	-
Green	Enteromorpha compressa	0.5	21.9	1.4	-
Green	Chaetomorpha linum	1.2	0.5	1.3	-
Red	Chondracanthus canaliculatus	-	0.675	18.9	-
Red	Porphyra umbilicatus	10.9	0.2	48	-
Red	Chondrus crispus	22.5	1.1	18.7	0.3
Red	Palmaria Palmata	1.4	1	46.6	0.5
Red	Gracilaria verrucosa	5.3	0.5	25.8	0.2
Red	Prionitis linearis	23.4	0.2	27.8	-
Red	Prionitis lanceolata	19.8	0.2	32.2	-
Red	Iridaea cordata	5.3	0.2	45.4	-
Red	Gigartina harveyana	10.4	0.2	37.2	-
Red	Plocamium violaceum	8.2	0.5	37.2	-
Red	Odonthalia floccosa	14.8	0.2	31.6	-
Red	Cryptopleura violaceae	19.4	0.2	32.5	-

Interestingly three major petrochemicals firms Statoil, DuPont and ENAP have recently showed interest in macroalgae as a potential candidate for biofuel production. And while the species selected is/are not known primary drivers cited include high sugar content, greater capacity for atmospheric CO_2 absorption [compared to terrestrial plants], high productivity [compared to terrestrial plants], ease of harvest [compared to microalgae] and absence of indigestible lignin [negating the need for pre-treatment] as primary drivers. Seaweed biofuels include ethanol, methanol and biobutanol with collaboration of BP-DuPont's Butamax with BAL, a leader in the field to produce biobutanol for drop-in fuels and chemicals, planned in coming years[1].

1. http://www.biofuelsdigest.com/bdigest/2011/01/13/top-11-algae-market-trends-for-2011/

Table 28. Current annual production [wet weight] and market prices for a variety of commercially cultivated Red macroalgal species with potential for providing LC Omega-3 HUFA to the REP sector.

Region	Species	Scientific name	2010	2011	2010	2011
			MT		US$.MT	
Africa	Elkhorn sea moss	Kappaphycus alvarezii	128	621	24.88	22.30
	Eucheuma seaweeds nei	Eucheuma spp	10885	8299.8	67.60	37.47
	Gracilaria seaweeds	Gracilaria spp	580	130	470.53	830.47
	Spiny eucheuma	Eucheuma denticulatum	125029	129779	14.22	12.74
Americas	Elkhorn sea moss	Kappaphycus alvarezii	700	700	85.26	89.67
	Eucheuma seaweeds nei	Eucheuma spp	1.54	1.09	8148.05	7407.34
	Gracilaria seaweeds	Gracilaria spp	12180	14499	1297.01	1507.06
Asia	Elkhorn sea moss	Kappaphycus alvarezii	0	1.25	-	452.00
	Gracilaria seaweeds	Gracilaria spp	515581	630788	319.20	171.12
	Warty gracilaria	Gracilaria verrucosa	4888	4865.3	646.07	316.41
	Elkhorn sea moss	Kappaphycus alvarezii	1873749	2098824	141.44	167.85
	Eucheuma seaweeds nei	Eucheuma spp	3465196	4602713	327.10	231.22
	Gracilaria seaweeds	Gracilaria spp	37025	51823	478.67	486.03
	Japanese isinglass	Gelidium amansii	1200	-	450.00	-
	Laver (Nori)	Porphyra tenera	564234	608790.5	1940.71	1767.31
	Nori nei	Porphyra spp	1072350	1027450	59.09	61.92
	Spiny eucheuma	Eucheuma denticulatum	133583	136183	49.41	42.49
	Warty gracilaria	Gracilaria verrucosa	1147220	1513590	295.44	309.59
Europe	Dulse	Palmaria palmata	1	-	663.00	-
Oceania	Eucheuma seaweeds nei	Eucheuma spp	13305	12740	66.17	78.74

4.4.6. Terrestrial Oilseeds

The majority of UFA in higher plants occur as polyunsaturated C_{18} fatty acids, in particular oleic acid, LA and LNA and the enzymes necessary for conversion of PUFA to HUFA are generally absent [Table 29]. Therefore, even in the case of the most oil-rich tissues of terrestrial crops i.e. seeds, genetic manipulation is required to produce substantial quantities of Omega-3 HUFA. While several pharmaceutical companies have reported success in the genetic modification of various terrestrial oilseeds including rapeseed, canola and soybean, concerns regarding horizontal gene transfer from large plantations of GM crops to non-GM species represents a significant obstacle [not present in the case of enclosed GM SCMO culture] to large scale commercial production and could prove major obstacles to their becoming a viable alternative to fish oil. Current production of Omega 6 and 9 vegetable oils from oleaginous terrestrial plants however is substantial [Fig. 33] and given the potential Omega-3 yields from GM species on a purely commercial basis they represent an appealing alternative to fish oils for both the aquaculture and REP industries. However in addition to cultivation licensing delays as mentioned, further obstacles to commercial scale production include public acceptance and competition with food crops for arable land the latter having proven a considerable threat to food supply security for developing world peoples in recent years due to widespread government incentivisation of fuel crop cultivation[1].

Table 29. Fatty acid composition of oils from major oil crops [% Total Lipids] [Adapted from Dyer et al. 2008]

Seed Name	Fat Content in seed (%)	PUFAs ALA 18:3w3 (%)	PUFAs LA 18:2w6 (%)	PUFAs ALA+LA w3+w6 (%)	MUFAs Oleic 18:1w9 (%)	SFAs Stearic 18:0 (%)	SFAs Palmitic 16:0 (%)	Total (%)
flax	35	58	14	72	19	4	5	9
chia	30	30	40	70	-	-	-	-
kukui	30	29	40	69	-	-	-	-
hemp	35	20	60*	80	12	2	6	8
soybean	17.7	7	50	57	26	6	9	15
rape	30	7	30	37	54*	7	-	7
walnut	60	5	51	56	28	5	11	16
wheat germ	10.9	5	50	55	25	18	0	18
rice bran	10	1	35	36	48	17	-	17
neem	40	1	20	21	41	20	-	20

* Includes up to 5% erucic acid

1. http://www.ncsu.edu/cenrep/research/documents/food_v_fuel.pdf

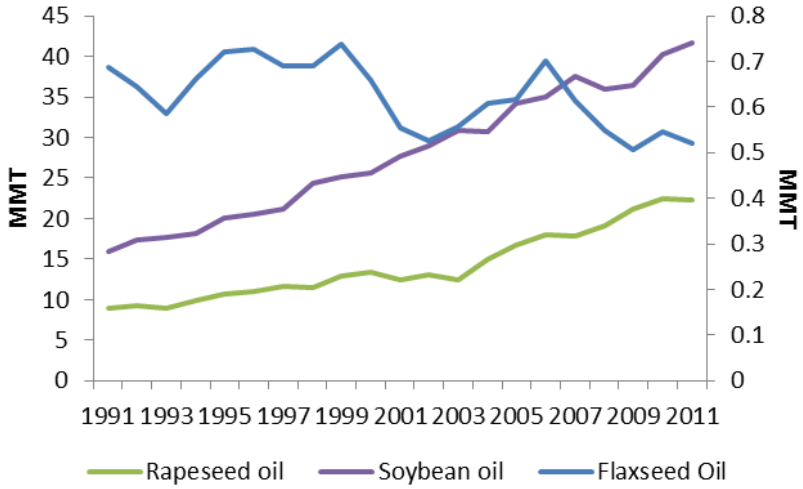


Fig. 33. Global production of terrestrial plant oils, 1991-2011 [note smaller flaxseed oil volumes on secondary vertical axis] [Source: FAO]

4.4.4.6.1. Rape seed oil

Rape seed oil is rich in C_{18} Omega-6 PUFAs LA and Omega-3 ALA and though concentrations vary seasonally and between strains typically occur at an average 20% LA to 12% ALA in total lipids providing close to the recommended ratio for human health of 2:1. Combined with a very low proportion of saturated fatty acids, rape seed oil is comparatively healthy and highly nutritious cooking oil that is commonly found in other foodstuffs such as margarine, salad dressings, mayonnaise, and baby food. Although rape oil however contains no longer chain Omega-3 HUFA, researchers have found it sufficient for salmonid well-being [growth and survival] provided the EPA and DHA are supplied separately, potentially as oil-residues associated with fishmeal[1]. Rape seed oil prices have very much the same price trend as fish oil. As there is an increasing demand for bio diesel, there will be continued pressure on price, including other types of vegetable oil[2].

4.4.4.6.2. Soybean oil

As with rapeseed oil, the major unsaturated fatty acids in soybean oil are the C_{18} Omega PUFAs, LA [~50%] and ALA [7-10%] as well as high concentrations of mono-unsaturated oleic acid [23%]. It also contains the saturated fatty acids, stearic acid [4%] and palmitic acid [10%]. Soy prices climbed to the highest level in 34 years in mid-2008 before falling slightly and remaining stable the last couple of years. The main reason for the 2008 price increase was because less soy was planted due to a shift from soy to corn in many regions, and a high demand for vegetable oil in general. High demand for corn in turn was due to increased demand for bioethanol produced from corn; coincident with significant food supply shortages in many developing regions due to competition between food and fuel crops for arable land[3].

1. http://www.thecrownestate.co.uk/media/211038/alternative_marine_sources_protein_oil.pdf
2. http://www.marineharvest.com/PageFiles/1296/2012%20Salmon%20Handbook%2018.juli_h%C3%B8y%20tl.pdf]
3. http://en.wikipedia.org/wiki/Food_vs._fuel

4.4.4.6.3. Flaxseed Oil

Flaxseed oil is currently used extensively as a fish-oil replacement in aquaculture. It again contains high proportions of C_{18} PUFAs however the exceptionally high concentrations of ALA [58% total lipids] combined with relatively low concentrations of both LA [14%] and SFA [9%] make flaxseed the most suitable replacement for fish oil for both humans and salmonids of all the terrestrial oilseeds currently available. In addition, salmon are able, at least to some extent, to convert these to the more health-beneficial longer chain HUFAs[1].

4.4.4.6.4. Genetically Modified Terrestrial Oilseeds

As higher plants do not possess the enzymes necessary to convert ALA to longer chain Omega-3 products LC PUFA biosynthetic genes need to be introduced into suitable oilseed crop hosts by genetic engineering [Table 30]. The resultant transgenic plants thus contain the 'trait' for LC PUFA synthesis providing a sustainable carbon-neutral and inexpensive alternative to fish oils in quantities needed to meet predicted future demand (Abbadi et al. 2001; Sayanova & Napier, 2004). The process of licensing for large scale cultivation of GMOs however is such that these oils are likely another 5 to 10 years from market [A. Jackson IFFO pers. comm.] while public acceptance of Omega-3s from GMOs introduces uncertainty to their commercial viability[2].

Table 30. EPA and/or DHA contents [as % total lipids] in various transgenic terrestrial plants [adapted from Catalina et al. 2012]

Plant [transgenic]	% EPA and/or DHA	Reference
Soybean	20.0 EPA	Kinney AJ et al. Patent WO 2004, 71467:A2.
Brassica carinata	25.0 EPA	Cheng B. et al. Transgenic Res 2010, 19[2]:221–229.
Nicotiana benthamiana	26.0 EPA	Petrie JR. et al. Metab Eng 2010, 12[3]:233–240.

Examples of current efforts to develop GM Omega-3 oilseeds are given in Table 31 and include efforts by Dow Agrosciences [in collaboration with Martek Biosciences] to insert genes from algae into other plants such as canola. These genes code for the three enzymes that synthesize DHA from ALA with DHA enriched canola oil being heralded as one of the "next generation of food industry oils"[3]. Monsanto, with The Solae Company, and its majority owner, DuPont have also reported breakthroughs in the modification of soybean to produce enhanced quantities of SDA[3,4] by inserting two genes, one from a plant related to primrose and one from a fungus. The modified soybean produces stearidonic acid, or SDA which although a shorter form Omega-3 fatty acid is more efficiently converted to LC forms than ALA [about 1/3 greater conversion] and has the added advantage of being more oxidatively stable than DHA or EPA. Furthermore the US Food and Drug Administration have recently verified the safety of oils derived from GM soya plants for human consumption[5]. Similarly Bayer CropScience is collaborating with the research group of Ernst Heinz at the University of Hamburg, Germany, to develop flax plants that produce omega-3 PUFA using algal genes.

1. Sargent and Tacon 1999 http://www.ncbi.nlm.nih.gov/pubmed/10466180
2. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1299184/pdf/5-7400289.pdf
3. http://www.bloomberg.com/apps/news?pid=newsarchive&sid=a4wl16vn_mOY]
4. http://www.foodnavigator-usa.com/Suppliers2/DSM-and-Monsanto-to-commercialize-soybean-oil-rich-in-omega-3-SDA.-But-will-anti-GMO-sentiment-hinder-its-progress
5. http://www.newscientist.com/article/dn18049-us-fda-says-omega3-oils-from-gm-soya-are-safe-to-eat.html#.UdX7L23-XYw

Table 31. Overview of projects currently underway to genetically modify terrestrial oilseeds for EPA+DHA production.

Project	Species	Investment
Rothamstead Research	Camelina	~$50 million
BASF & Cargill	Canola	$200 million
Dow & DSM	Canola	Unknown
CSIRO	Arabidopsis	$50 million
CSIRO & NuSeed	Canola	$50-100 million
DuPont	Soybean	Unknown

5. State of the Art in Omega-3 HUFA production

5.1. Crude fish oil production

With the exception of solvent extraction the reduction of forage fish and fish processing wastes to produce crude oil generally employs the same principles, techniques and equipment common to the production of the other edible fats and oils. In general, this is achieved by the wet reduction process in which the principal operations are cooking, pressing, separation of the oil and water with recovery of oil [Fig. 34]. As a well-established element of the capture fisheries supply chain, the crude fish oil production process is well organised with continuous processing from the time the fish are landed for optimal efficiency and maximum product quality [Bimbo et al. 1998]. Alkio et al [2000] estimated the cost for producing 1,000 kg DHA concentrate and 410 kg EPA from tuna at U.S. $550/kg DHA and EPA ethyl ester concentrate.

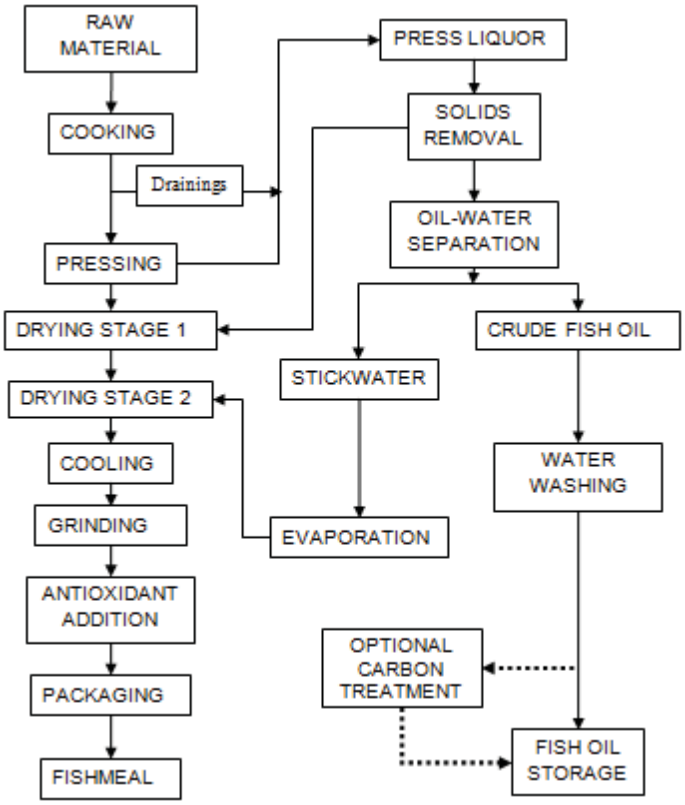


Fig. 34. Wet reduction process for production of crude oils from forage fish and fish processing wastes.

In addition to the wet reduction process there are a number other production methods that are being or have been used to produce crude oils. These include enzymatic [auto- or hydro-lysis], ensilation, dry rendering and solvent extraction and a brief overview given by Bimbo et al. [2005] is quoted below.

5.1.2. Enzymatic [Auto/Hydro-lysis]

Hydrolysed fish proteins are produced by employing proteolytic enzymes either from the fish themselves (autolysis) or from other sources [hydrolysis]. The enzymes can be of either animal, vegetable or microbial source and accelerate the breakdown of the proteins into smaller units (peptides). Hydrolysis can also be accomplished chemically under acidic or alkaline conditions. By using some of the newer enzymes available on the market, a process can be developed to recover fish peptides of various lengths with specific functionality. Although the process can be used with any fish, it is primarily used for white fish or offal low in oil. In cases where oily fish are hydrolysed, the processor must recover the oil phase without denaturing the proteins or face supplying a high fat hydrolysed protein product or a protein product with reduced functionality. It has been difficult to achieve a commercially viable product from fatty fish that is both functional and low in fat. The hydrolysis process is shown in Figure 35.

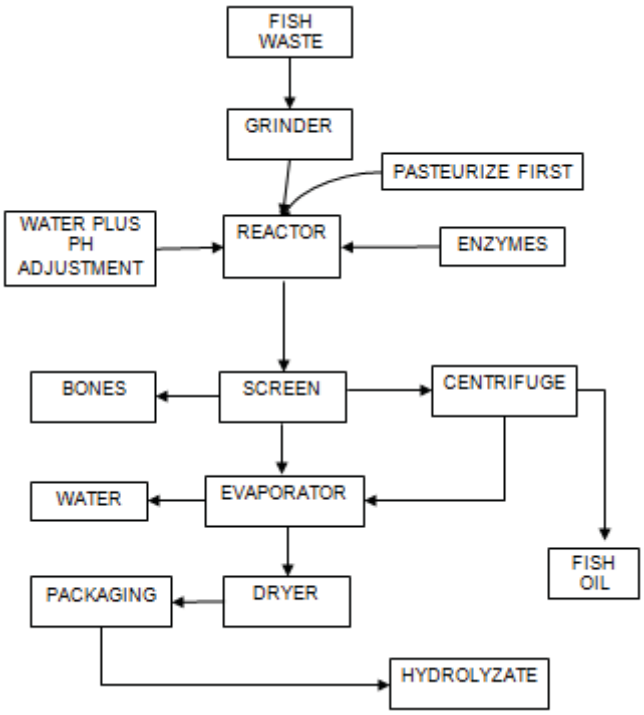


Fig. 35. Hydrolysis process to produce crude fish oil.

5.1.3. Ensilation

Silage production is a simplified, low cost, hydrolysis process. Fish silage is liquefied fish stabilized against bacterial decomposition by an acid. The process involves mincing of the fish followed by the addition of an acid for preservation. The enzymes in the fish gut break down the fish proteins into smaller soluble units and acid helps to increase their activity while preventing bacterial spoilage. Formic, propionic, sulphuric and phosphoric acids have been used. Normally, about 3-4% of acid is added so that the pH remains at or below 4.0.

Strong mineral acids require neutralization before feeding the final product. Silage might be defined as a crude form of hydrolysate.

Silage made from white fish offal does not contain much oil, but when made from fatty fish it is necessary to remove the oil. The composition of the silage will be very similar to the starting raw material. Fish silage of the correct acidity is stable at room temperature for at least 2 years without decomposition. The amount of free fatty acids increases in any fish oil present during storage. Silage production offers a solution to the handling of fish waste when the logistics of delivering the waste to a fish reduction plant are not economical. Silage can be produced in large or small containers both on the vessel and on shore. If the silage is processed quickly to recover the oil, it is possible to make an acceptable fish oil product. The ensilation process is shown in Figure 36; it can be carried out in 2 stages, producing a crude silage with very low capital expense and then producing a concentrated fish oil which requires a high capital expense. Various methods of concentration and refinement of the crude oils are discussed in Section

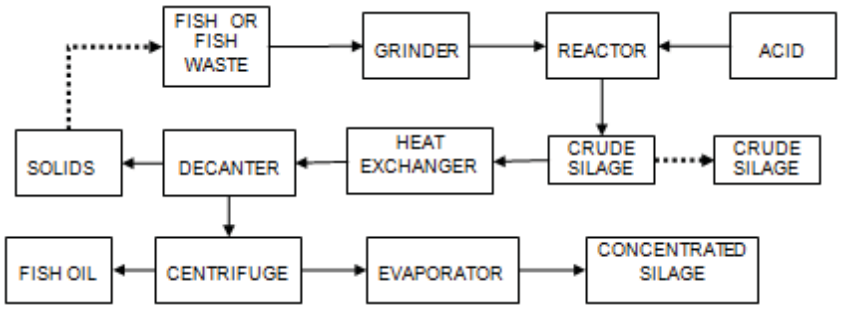


Fig. 36. Ensilation or autolysis process to produce crude fish oils.

5.1.4. Dry Rendering

The dry rendering process, which is commonly used to prepare animal proteins and fats, is not normally used in the manufacture of fishmeal and oil. However the process is used with catfish by-products. In this process the raw material is "cooked" to remove the water [essentially the drying process in the fishmeal wet rendering process]. The resultant dry cake is then pressed to remove any oil [Fig. 37]. Because the water has been removed, the lipid fraction can contain high levels of phospholipids. The phospholipids normally hydrate in the wet rendering process and are recovered with the water fraction. In the dry rendering process, they are not hydrated and therefore remain dissolved in the lipid or oil fraction. Since there is interest in the fish phospholipids, it is possible to produce a PL fraction by hydrating the oil [also called degumming].

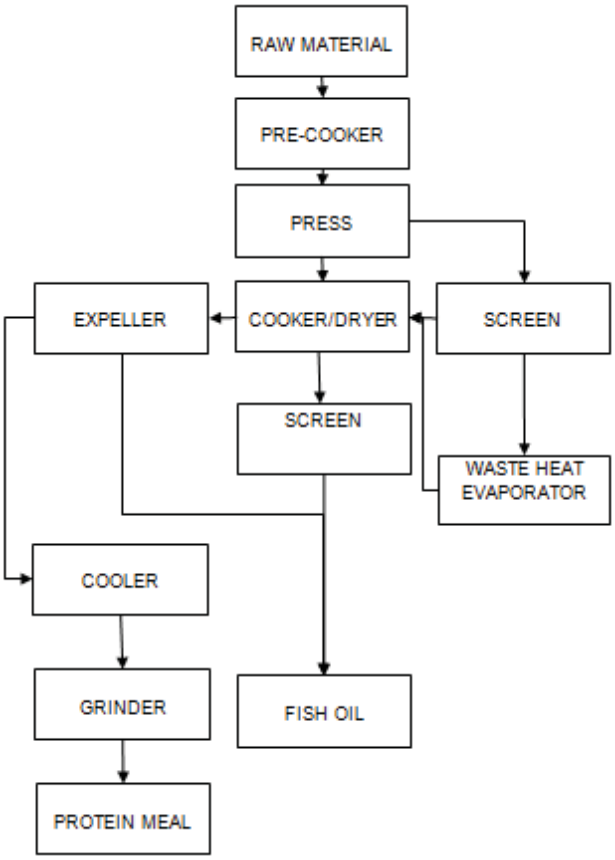


Fig. 37. The dry rendering process to produce crude fish oils.

5.1.5. Solvent Extraction

Solvent extraction to produce fish protein concentrate [FPC] is another process that could yield fish oil. FPC can be defined as any stable fish preparation intended for human consumption in which the protein is more concentrated than in the original fish. Methods developed so far are based mainly on the use of chemical solvents to remove water, fat and fishy tasting components either from the raw fish or from fishmeal. The solvents most successfully used to make FPC are ethanol, n-hexane, isopropanol, or ethylene dichloride. Normally the solvent is recovered and used over again. The recovered fat is usually mixed in an azeotropic mixture with water, solvent, and water soluble components. Separation of this azeotrope to recover the fat sometimes presents problems.

5.2. Concentration of Omega-3 HUFAs

Once the crude fish oil has been extracted certain additional processes are necessary to purify and enrich the EPA and the DHA to obtain a higher percentage of molecules of interest (up to 95% omega-3's when the natural concentration is ~20%). And while numerous methods are currently available all are costly and only a few suitable for large-scale production These Include adsorption chromatography, fractional or molecular distillation, enzymatic hydrolysis, low-temperature crystallization, supercritical fluid extraction and more traditionally, urea complexation. Moreover all of these processes are wasteful and while industry has flagged this as a research and development priority, current methods are only capable of recovering ~50% on average of the EPA+DHA fractions

available from crude oils. Moreover, production of highly concentrated oils for targeted clinical and pharmaceutical applications [>60% FAs as sum of EPA+DHA; see Table 32 for complete list of definitions] commonly result in losses in excess of 90% EPA+DHA available in the crude oil [Fig. 38]. However, consultation with representatives organisations IFFO [A. Jackson] and GOED [A. Ismail] during this study has highlighted development of new and improved extraction technologies which yield much higher concentrations of EPA+DHA from crude oil and which industry claims will dramatically reduce dependency on traditional forage fish supplies and thus lower demand from the REP sector in the near-term future [Fig. 39].

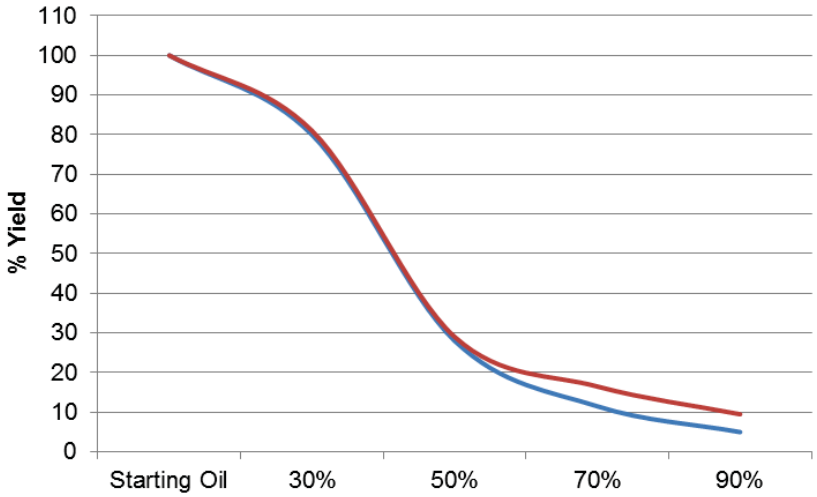


Fig. 38. Estimated percentage yield of refined EPA+DHA from crude starting fish oil to highly concentrated 90% Ethyl Ester Omega-3 using traditional urea complexation [blue line] and modern molecular distillation [red line] techniques.

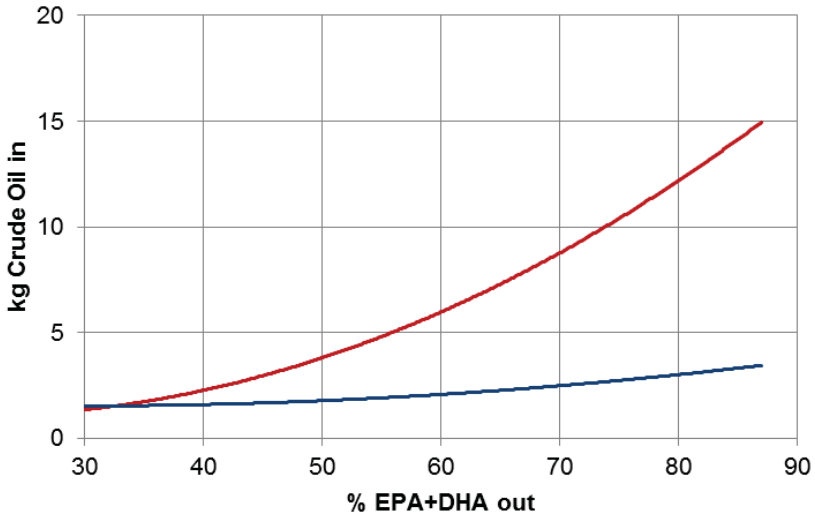


Fig. 39. The predicted effects of improved [blue line] versus traditional [red] extraction technology on crude fish oil demand [GOED]

Each of the traditional techniques listed in Section 5.1. has its own advantages and drawbacks however enzymatic hydrolysis and molecular distillation [often in sequence] are the methods of choice for the modern REP sector. Figure 40 provides a generalized overview of the molecular distillation method however one notable challenge in the concentration of EPA and particularly DHA using this method is the accumulation of mono and di-glycerides of various FAs in the final product. Currently no effective method for their removal exits and they are simply incorporated with EPA+DHA fractions contained in each of the four primary chemical forms of refined Omega-3 sold. Figure 41 provides a description of these forms and gives a generalized overview of the steps involved in their production i.e. natural TGs [nTG], ethyl esters of TGs [eTG], mixtures nTGs, eTGs, and mono- and di-glycerides or as re-esterified TGs [rTGs]

Table 32. Codex Committee on Fats and Oils standard definitions for the various grades and types of fish oils produced at various stages of the Omega-3HUFA supply chain.

Product Type	Description
Crude fish oils	Oils intended for human consumption after they have undergone further processing, refining and purification as applicable.
Concentrated fish oils [plural]	Derived from fish oils which have been subjected to processes such as hydrolysis, fractionation, winterization and/or re-esterification to increase the concentration of specific FAs.
Concentrated fish oil [singular]	Contains 40 to 60 w/w % FAs as sum of EPA+DHA and at least 50 w/w % of FAs are in the form of triacylglycerides.
Highly concentrated fish oil	Contains greater than [60 w/w %] FAs as sum of EPA+DHA, at least 50 w/w % of FAs are in the form of TGs.
Concentrated fish oil ethyl esters	Contain FAs as esters of ethanol of which [40 to 60 w/w %] are as sum of EPA+DHA
Highly concentrated fish oil ethyl esters	Contain FAs as esters of ethanol of which greater than [60 w/w %] are as sum of EPA+DHA
Virgin fish oils	Oils treated by heating not exceeding [70°C], washing with water, settling, filtering and centrifugation only. They may contain antioxidants and pigments naturally present in the raw material.
Extra low oxidised fish oils	Produced by mechanical maceration of the fresh raw materials at a temperature not exceeding 97°C, and a heating time not exceeding 20 minutes, and without using solvents. After centrifugation the oil may be processed by further purification steps.

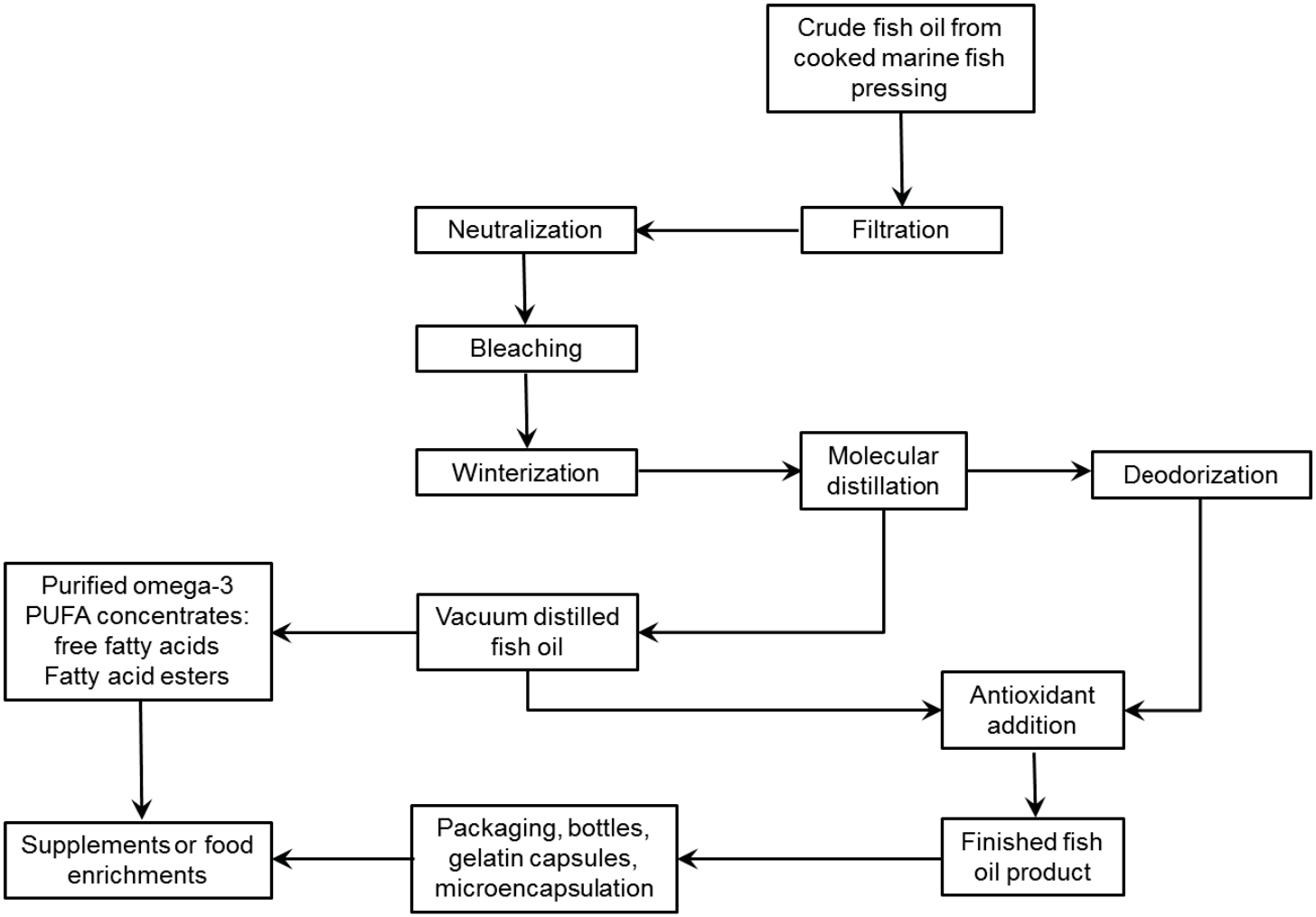


Fig. 40. Generalised flow chart showing production of food and pharmaceutical grade fish oil. [Redrawn from Bimbo 1998]

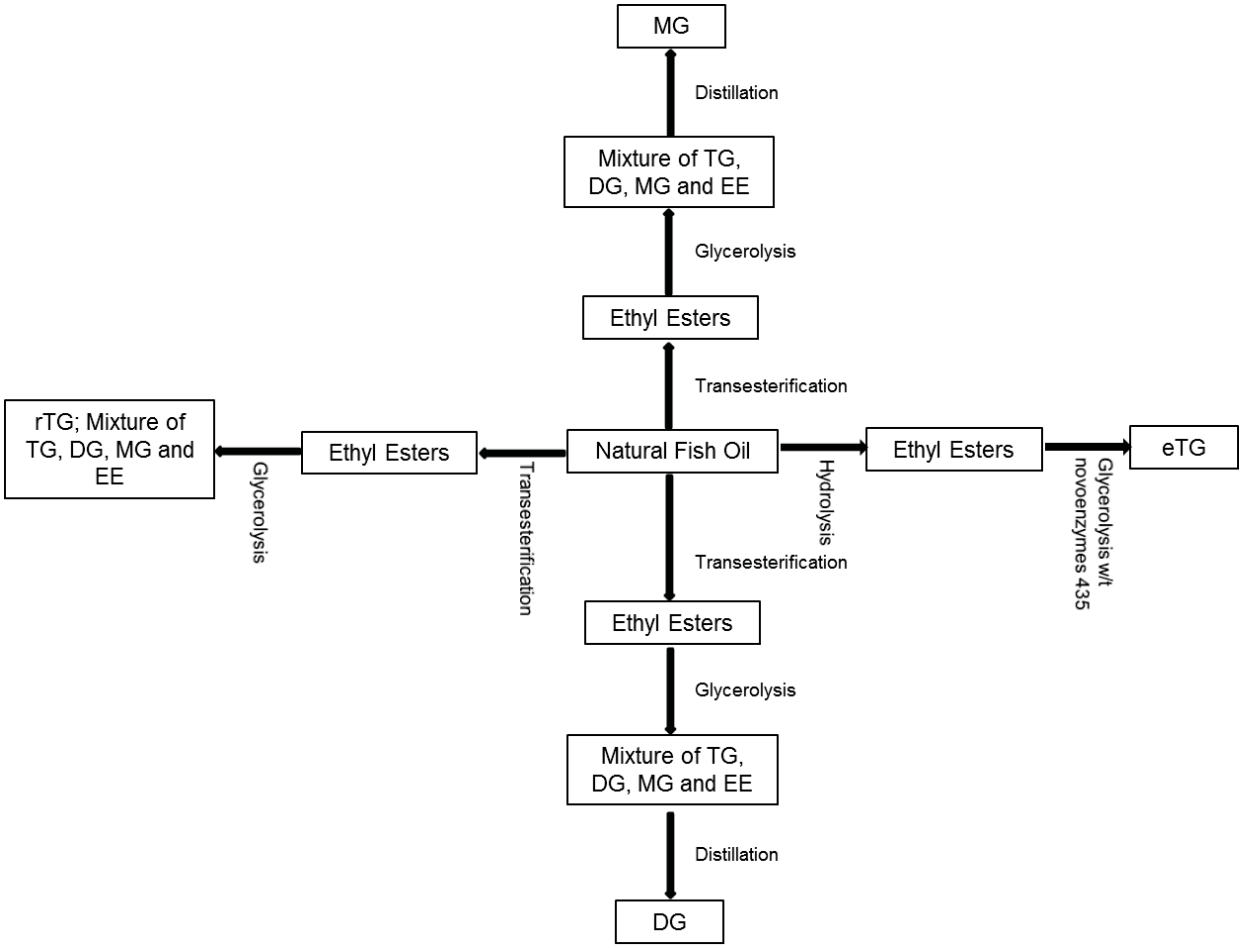


Fig. 41. The process of conversion of natural fish oil to the 4 available fish oil supplement types. nTG: natural triglyceride, rTG: re-esterified triglyceride, DG: diglyceride, eTG: triglyceride and MG: monoglyceride.

According to GOED at least three manufacturers claim to be on the verge of scaling up new methods which recover 100% of EPA+DHA in crude oil. Due to the highly sensitive commercial nature of such developments however real data on either the biotechnical modality or actual efficiencies of these methods are not available publicly while all industry stakeholders approached during this study [see Annex 1 Survey Questionnaire Results] declined to respond to questions relating to extraction yields. Nevertheless, a considerable amount of valuable oil is known to end up as waste from the concentration and refinement processes and crude approximations of these based on information provided by A. Ismail of GOED are given in Figure 42.

Finally, with the rapid escalation in demand for Omega-3 oils, ingredient buyers have had to increase their vigilance and knowledge accordingly to protect quality and efficacy. Thus the development of various government [EU, WHO etc.] and industry organisations [GOED, Eur Pharm etc.] standards on fish oil quality and their adoption by the Omega-3 REP sector has led to increased globalization of supply and the prevention of significant food safety incidents. These standards include measures of freshness/oxidation level (expressed as peroxide value, anisidine value and totox value); digestibility [i.e., acid value or percentage of free fatty acids]; purity from contaminants such as heavy metals,

pesticides, harmful microorganisms, etc.; and EPA and DHA level expressed as weight percentage [Table 33]. However the purification process is costly with separation of EPA+DHA components from organic solvents [e.g. hexane, methanol, ethanol etc.] commonly used in the initial fractionation process representing a major process cost to manufacturers.

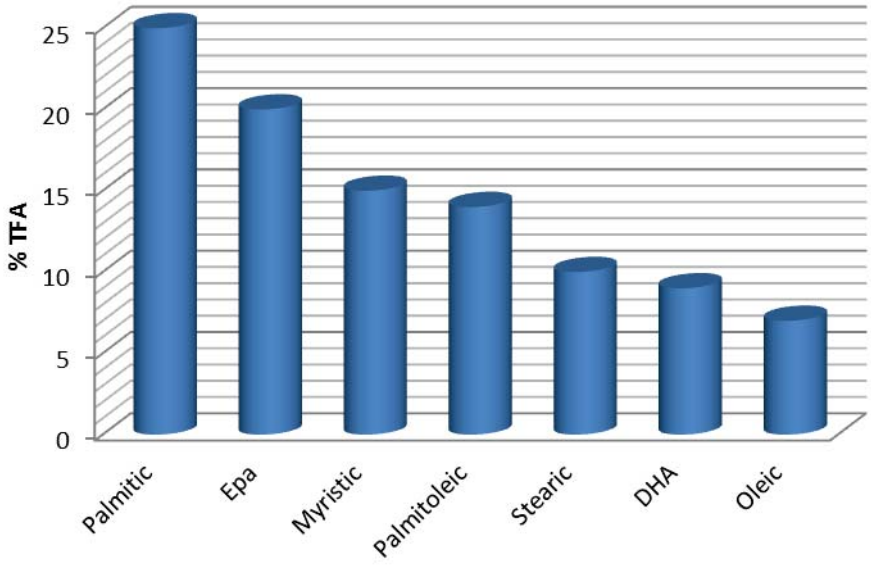


Fig. 42. Approximate FA composition [%TFA] of a generalized waste stream generated during production of concentrated food grade Omega-3 HUFAs.

Unsaturated oils are also susceptible to oxidation [Fig. 43]. For storage, all fish oils have to be out of contact with air, pro-oxidant metals, especially those high in iron and copper, and preferably treated. Ideally, free fatty-acid content should be below 2%, and there should be little oxidation. The degree of oxidation can be determined by methods which measure these – giving the so-called TOTOX value based on peroxide and anisidine values. Ideally, it should be below 20. For human use, further refining is required [Pike and Jackson 2005].

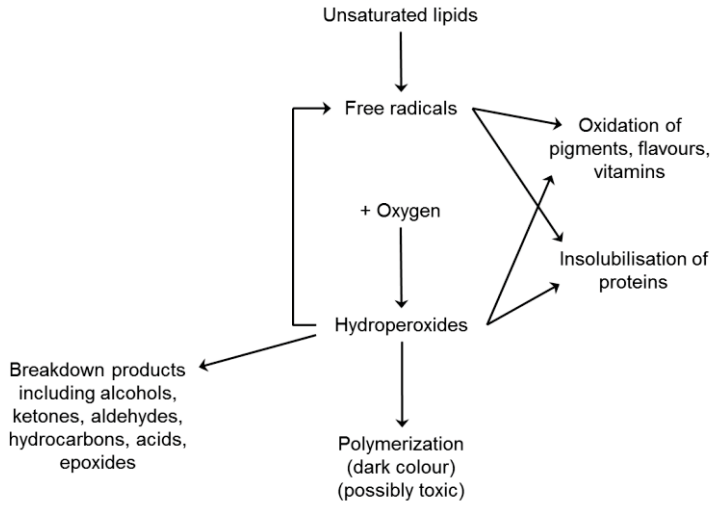


Fig.43. Oxidation of PUFA [Reproduced from Ohshima 2005]

Table 33. Quality and Safety limits of various national bodies governing production of Omega-3 FA for the REP sector

Quality Limits	EC Regulations	Eur Pharm Fish Oils	USP* Fish Oils	GOED** Voluntary Monograph	Australian Draft Guidance	Specific to Concentrates		
						Eur Pharm Omega-3 TGs	Eur Pharm Omega-3 EEs 60%	Eur Pharm Omega-3s EEs 90%
p-Anisidine Value, max	N/A	30.00	20.00	20.00	30.00	30.00	20.00	20.00
Peroxide Value, max	N/A	10.00	5.00	5.00	10.00	10.00	10.00	10.00
Unsaponifiable Matter, max	N/A	0.02	0.02	N/A	0.02	N/A	N/A	N/A
Stearin, Max	N/A	10mL	10mL	N/A	10mL	N/A	N/A	N/A
TOTOX, max	N/A	N/A	26.00	26.00	N/A	N/A	N/A	N/A
Safety Limits								
Dioxins and Furans, max pg WHO-PCDD/FTEQ/kg	2*	N/A	2.00	2.00	2.00	N/A	N/A	N/A
Dioxin-like PCBs, max pg WHO-TEQ/kg	8.0-10.0	N/A	8.0-10.0	3.00	8.0-10.0	N/A	N/A	N/A
PCBs, max mg/kg	N/A	N/A	N/A	0.09	N/A	N/A	N/A	N/A
Lead [Pb], max mg/kg	0.10	N/A	0.10	0.10	0.50	N/A	N/A	N/A
Arsenic 9As], max mg/kg	N/A	N/A	0.10	0.10	1.00	N/A	N/A	N/A
Mercury [Hg], max mg/kg	N/A	N/A	0.10	0.10	0.50	N/A	N/A	N/A
Cadmium 9Cd0, max mg/kg	N/A	N/A	0.10	0.10	0.50	N/A	N/A	N/A

* U.S. Pharmacopeial Convention

**Global Organization for EPA and DHA Omega-3

6. The LC Omega-3 HUFA Supply Chain

The global marine and algal oil supply chain is relatively fragmented with the top five firms including **BASF** [incl. Pronova and Equateq], **DSM** [incl. Martek and ONC] and **Nissui** [Fig. 43] controlling fifty percent of ingredient supply in 2010 compared to 70% in 2008. Within the European context, Pronova [recently consolidated with BASF subsidiary] is by far the largest consumer of refined DHA rich oils [30.5% of total] [Fig. 44]. However while the global fish oil supply chain represents an efficient, vertically integrated structure the nascent non-fish Omega-3 supply chain can be described as an insular closed loop system with multiple stages of production, extraction and refinement occurring internally within each of these major corporations. For instance, Christopher Shanahan of Frost & Sullivan consultants recently described the LC Omega-3 HUFA supply chain structure as "constantly evolving, with factors such as the nature of the products, economic and regulatory environment, the structure of downstream industries and upstream raw material supplies influencing changes in the value chain."[1]

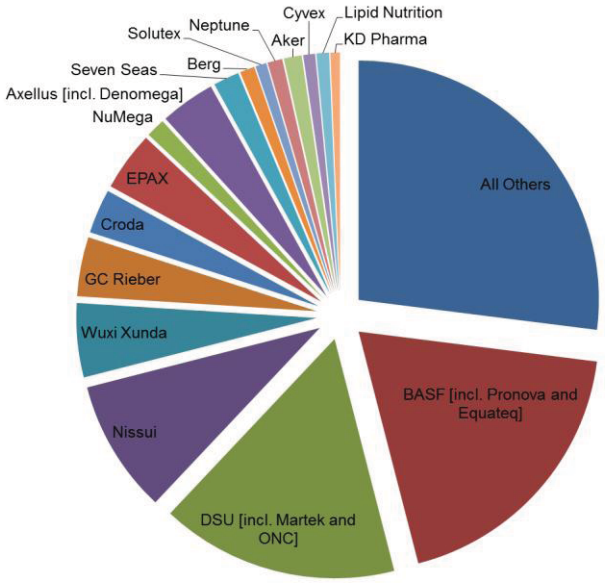


Fig. 44. Estimated global Omega-3 ingredients [EPA+DHA] use as % market share i.e. ingredients sales [US$], by the primary REP companies involved [Source: GOED]

Some out-sourcing to more specialised often smaller companies of ancillary services such as encapsulation, winterisation or functional food production does occur however. For instance, Cargill, UK, Lang Naturals, USA and SirioPharma, China represent three prominent Omega-3 encapsulators while NuMega Ingredients, Australia, Ocean Nutrition, Canada and AkerBiomarine, Norway are examples of companies involved in the development and production of Omega-3 enriched functional foods. However, in view of such strong market demand and diminishing forage fish resources competitive rivalry between companies tends to be high in both the marine and algal oils industry where the protection of proprietary advances particularly in extraction and refinement methods and thus improved yields, is a top commercial priority. Thus, while the author was at pains

1. Shanahan, C.: The Changing Dynamics of the Omega-3 Industry. Natural Products Insider, vol. 17 no. 9 September 2012.

[during direct consultation with commercial companies] to underline the anonymous and non-attributable nature in which survey results were to be collected and collated, only six of the nearly two hundred companies contacted [see Annex 2] completed the survey questionnaire supplied [see Annex 3]. A summary of survey responses is given in Annex 4.

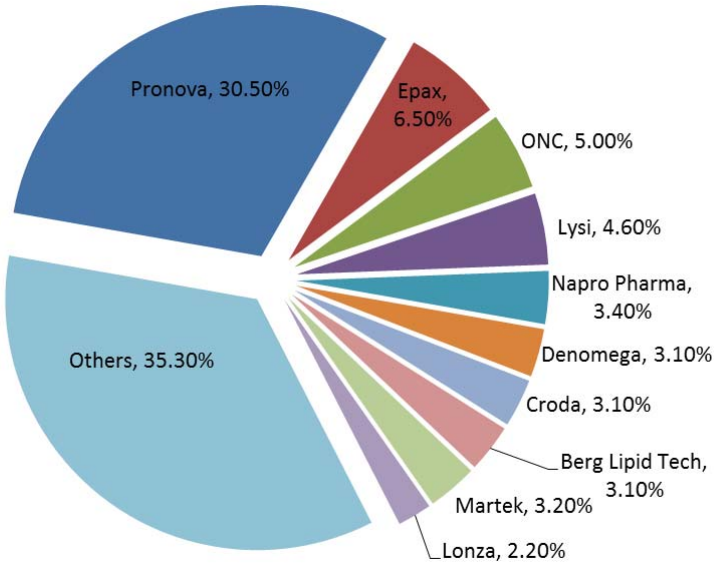


Fig. 45. European DHA Omega-3 ingredients market as % volume consumption by the primary REP companies involved.

Although survey responses were few [n = 6] these included three companies currently producing Omega-3 ingredients from non-fish sources, one microalgae and two krill [all producing <5,000 MT.yr]. Echoing the difficulties outlined in Section 5.1. however half of survey respondents cited refinement and/or concentration of EPA+DHA fractions as their most critical production challenge followed by extraction and oxidative stability. In terms of bulk extraction of Omega-3 type lipids [including AA, SDA, EPA+DHA] one respondent claimed an efficiency of approximately “90% depending on the conditions” while none of the respondents provided quantitative information on the efficiency of concentration of EPA+DHA fractions. Moreover while one respondent specified esterification as the preferred method of extraction all others declined to provide details of other, presumably improved techniques being used. Similarly, respondents were not willing to provide information on, in the case of refiners/concentrators, the price paid for crude oil or, in all cases; the fatty acid profiles of either the finished oils or wastes generated from refinement and/or concentration processes. In terms of total volumes of waste however, this is thought to be as high as 90% of crude oil processed [see Section 5] and while comprised primarily of unsaturated and monounsaturated fatty acids [e.g. Palmitic Acid, Myristic Acid and Oleic Acid] significant quantities of valuable EPA+DHA are also wasted. Only four respondent provided information on the fate of this waste however it is interesting to note that all companies involved in the refinement of crude fish oil utilize this waste for manufacture of value-added co-products such as low concentration refined Omega-3 oils or biodiesel. Finally, while all but one respondent voluntarily adhered to an industry oil quality standard, only one respondent thought the absence of approved guideline RDIs or consumer

awareness was a limiting factor to total production or sales. In terms of leveraging cost-effective industrial scale production from alternative non-fish sources, the microalgal oils manufacturer alluded to an effective implementation of research outcomes to ensure development of successful and profitable private companies while one krill oil manufacturer placed singular emphasis on the reduction of Cost Of Goods Sold [COGS] or put simply, the inhibitory high costs associated with the production of refined Omega-3 HUFAs from algal and other non-fish organisms.

2. Discussion, conclusions, and recommendations

7.1. Discussion: Meeting Future Demand

7.1.2. Supply Gap

The United Nations has predicted an approximate shortfall of 0.5 MMT in crude fish oil supply to all end-users by 2014. However consistently strong growth in demand from a rapidly evolving REP sector could yet see this grow even larger. For example, A. Ismail [GOED] recently pointed out that, assuming Omega-3 pharma-applications attain a market share equivalent to that achieved by very high concentration TG Omega-3 oils, this REP sub-sector [i.e. excluding functional foods, nutraceuticals, infant formula etc.] would require an additional ~0.46 MMT of crude fish oil containing at least 30% EPA+DHA to satisfy demand. Moreover, with current consumer dynamics ensuring market dominance by this rapidly growing REP sector, this industry will simply not accept the higher cost structures associated with production from SCMOs and algae. However such excess demand on limited fish oil supplies potentially provides a potent lever necessary to accelerate the diversification of raw materials supply to the REP sector.

For instance, one credible scenario exists wherein a constrained supply of crude oil from the dominant Peruvian anchovy fishery under pressure from increasing market demand, static stock size and attendant fish oil price increases establishes a threshold fish oil price beyond which industrial scale production of Omega-3 ingredients from non-fish sources becomes commercially attractive. And while determination of this price threshold was beyond the scope of this current study, summary analysis of figures given by GOED for 2012 wherein a single MT of algal derived Omega-3 oils retailed at approx. US$95,000, underlines the significant economic value of these resources despite their relatively small contribution to the global EPA+DHA ingredients market. Thus, while the Peruvian anchovy remains the dominant source of Omega-3 oils to the REP sector, the list of new and novel Omega-3 sources, both commercial and in development is getting ever longer with several new algae, new fungi and new zooplankton projects announced in the last year [A. Ismail, pers comm].

Similarly, continuous improvements on EPA+DHA extraction yields are expected to substantially reduce demand for crude fish oils from the REP sector within the next five years [a. Jackson IFFO pers comm]. As presented in Section 6 above, industry claims indicate an increase in EPA+DHA extraction yields of more than three times those currently achieved using molecular distillation/enzymatic hydrolysis techniques. Contingent with a reduction in the high capital costs associated with production from non-fish sources therefore, overall demand for crude fish oils from the REP sector may in fact stabilize despite forecasted sales growth and in the absence of alternatives. However, this seems optimistic and an alternative projection wherein supportive government policies and greater

consumer education leading to industrial production from non-fish sources represents a more pro-active strategy that could potentially decrease overall use and dependency on fish oils by the REP sector in coming decades.

7.1.3. Nutrition Gap

One of the most compelling drivers for a voluntary transition from traditional forage fish oil consumption to novel non-fish oil production by the REP industry is the widespread growing awareness of the costs to human health of dietary deficiency in EPA+DHA. As mentioned, GOED have estimated the capital costs to of this to global government at US$47 trillion over twenty years and although this figure includes costs associated with diseases that EPA+DHA are only putatively thought to play a role in preventing it nonetheless underlines the potential savings gained through the use of these oils in primary prevention care. For instance an active pursuit by national health authorities to promote greater Omega-3 intake either through increased fish consumption or the use of dietary supplements has the potential not only to improve life expectancy, reduce mortality rates in particular from heart attack [~16% of all deaths by heart attack in the US i.e. 13,400[1]] but also significantly improve the commercial feasibility of non-fish oil production by increasing demand for high concentration, highly refined EPA+DHA ingredients for clinical applications. Furthermore, as a vegetarian, halal, kosher and environmentally sustainable product, oils from SCMOs and microalgae in particular carry the inherent added value of being acceptable to several important consumer groups which as yet remain under-exploited by the REP industry.

7.2. Conclusions and recommendations

As detailed in this study, there exists a clear and present need for substantial additional supplies of marine-type lipids to meet ever growing demand for EPA+DHA ingredients from the rapidly expanding Omega-3 REPs sector. And while capital costs associated with industrial scale production from algal and other non-fish sources remain prohibitively high, necessary restriction of fish oil production from wild forage fish has created an environment wherein private companies, buoyed by strong consumer demand, are investing in research and development aimed at lowering costs through improved biomass drying and lipid extraction, refinement and concentration technologies. Efforts to enhance the Omega-3 potential of terrestrial oilseeds by introducing the novel trait for biosynthesis of Omega-3 long-chain EPA+DHA are also yielding promising results with successful modifications reported for soybean, rape and corn. Furthermore, given the large body of evidence supporting the myriad health benefits of Omega-3 intake, it is the opinion of the authors that this should be examined closer to lever a transition by the REP sector to alternative, non-fish supply chains. Given the static nature of traditional forage fish supplies such a move is expected to occur at a certain price threshold without government incentives however this could potentially be accelerated with the support of national health authorities. For example, this may take the form of active promotion by local health authorities of recommended daily intake minima such as that indicated by the UN World Health Authority, active promotion of the use of Omega-3 HUFAs as a standard component of management strategies for the treatment of the symptoms of CVD, CHD and high cholesterol and other chronic conditions such as auto-immune disorders and mental illness as well as increased clinical trialling to affirm the putative efficacy of Omega-3 HUFA in prevention and treatment of the various cancers. Under the current status quo however, commercial volumes of production from non-fish sources remain too low and production costs too high to significantly ease REP sector dependency on crude fish oil supplies such that the aquaculture sector can seize an

adequate share of existing supply needed to maintain product quality and support predicted future growth.

Thus, the following specific research is considered important to the protection of an adequate supply of affordable Omega-3 lipid resources for the UK salmonid aquaculture industry going forward:

- A critical pharma-economic analysis of the impacts of increased use of ultra-refined high concentration Omega-3 oils as a standard treatment for prevention of the symptoms of CVD, CHD etc. on the costs of production from algal and other non-fish sources and the potential such an approach has to increase supply share for less economically robust end users such as the aquaculture sector.

Appendix 1.

A. Fatty Acid Chemistry and Nomenclature

i. Introduction

Fatty acids [FAs] are aliphatic carboxylic acids that constitute a major component of most naturally occurring lipids. Their basic structure consists of a straight unbranched chain of hydrogen bonded carbon atoms with a distal methyl group and a terminal carboxyl group [Fig. 1]. Chains typically contain even numbers of between 2 and 24 carbon atoms with chains < 6 atoms long classified as short-chain [SC], 6 to 12 as medium-chain [MC], 14 to 20 as long-chain [LC] and >20 as very long-chain [VLC]. In nature, they are usually found esterified either as Triglycerides [TGs], Phospholipids [PLs] or due to a partial hydrolysis, as Free Fatty Acids [FFA]. TGs consist of three FAs attached to a glycerol backbone while PLs consist of two FAs attached to a glycerol group and a phosphorous group which is further attached to a head group. PL head groups can consist of choline, ethanolamine, glycerol, inositol or serine.

As aliphatic compounds FAs can occur in saturated or unsaturated form with saturated FAs [SFAs] consisting only single bonds between carbon atoms and unsaturated FAs [UFAs] consisting one or more carbon to carbon double bonds [Fig. 1]. FAs with one double bond are known as a monounsaturated fatty acids [MUFAs] whereas FAs with more than one double bond are known as polyunsaturated fatty acids [PUFAs]. The term “saturated” refers to the fact that SFAs carry the maximum number of hydrogen atoms along their chain length while “unsaturated” FAs do not. This is due to the molecular structure of double-bonds which restricts the number of hydrogen atoms that can attach to carbon atoms and depending on the specific geometry of the double bonds, UFAs can occur in either the *cis* or *trans* configuration.

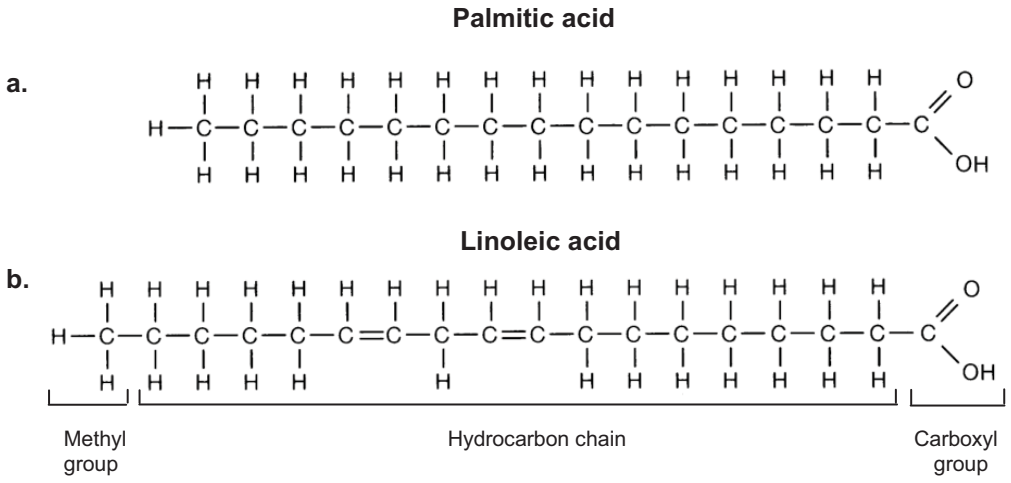


Fig. 1. Key molecular features of a] saturated and b] unsaturated fatty acids showing the distal methyl group, hydrocarbon chain and terminal carboxyl group.

Virtually all naturally occurring UFAs are in the *cis* configuration where adjacent hydrogen atoms are located on the same side of the carbon-to-carbon double bond resulting in bending of the FA molecule [Fig. 2]. The more double bonds the greater the degree of bending and thus “unsaturation”. In contrast, *trans*-configured UFAs possess a shape

similar to that of straight chain SFAs with hydrogen atoms occurring on opposite sides of the double bonds and in the case of UFAs, results almost solely from the chemical process of hydrogenation [i.e. addition of hydrogen atoms].

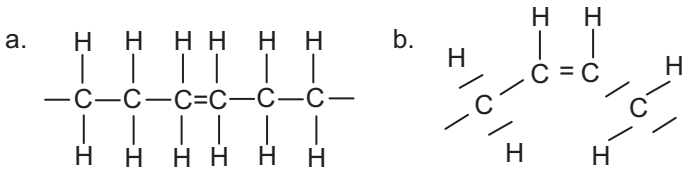


Fig. 2. Comparison of a] the *trans* isomer and b] the *cis*-isomer forms of unsaturated fatty acids.

The geometry of double-bonds has important implications for the physio-chemical properties of FAs and in particular influences the temperature at which they solidify. In the case of both SFAs and *trans*- configured UFAs however molecules can be closely packed within cells resulting in increased melting temperature and greater likelihood of crystallization [or solidification] at room temperature. In the *cis* configuration on the other hand bending of the carbon chain restricts the ability of UFAs to align closely within cell membranes decreasing melting point and increasing fluidity at low temperatures.

ii. Nomenclature

1. Geneva System

The Geneva system uses Greek terms whose prefixes describe the number of carbon atoms in the chain as counted form the carboxyl, *not* the methyl, end of the molecule with the carbon of the carboxyl acid considered carbon 1. For example, stearic acid which has 18 carbon atoms in its chain has the name octadecanoic acid; *octadec* meaning 18 and the suffix *anoic* [meaning oxygen deprived] signifying the absence of double bonds. Similarly palmitic acid which contains 16 carbon atoms is named hexadecanoic acid; *hexadec* meaning 16 and *anoic* indicating no double bonds. FAs containing double bonds are also named in this way with *octadec* prefixing Geneva names for oleic acid [octadecenoic acid, 18:1], linoleic acid [octadecadienoic acid, 18:2] and linolenic acid [octadecatrienoic acid, 18:3]. In each case the suffix is has been modified to reflect the number of double bonds with one double bond described by the suffix *enoic*, two double bonds by *dienoic* and three double bonds by *trienoic*. The exact position of double bond/s is then specified by placing the number of the first carbon of the first double bond [as counted from the carboxyl end] before the systematic name. For example, in the case of oleic acid the double bond located between carbon 9 and 10 is denoted *9*-octadecenoic acid while the PUFA linoleic acid becomes *9, 12*-octadecadienoic acid [i.e. double bonds located between carbon 9 and 10, carbon 12 and 13]. Geneva names also include a term indicating the *cis* or *trans* configuration of double bond/s with oleic acid for example becoming *cis,* 9-octadecenoic acid. More complete lists of FAs with their Geneva names, formulas and physical properties are given in Table 1.

2. Delta System

The Delta [Δ] system is the only nomenclature system that follows the International Union of Pure and Applied Chemistry [IUPAC] rules. It is an entirely numerical system that allows the number of hydrogen atoms, if any, on each carbon atom to be known unambiguously.

For example, arachidonic acid consists of 20 carbons and 4 double bonds and is denoted $20:4^{\Delta 5,8,11,14}$; where the numbers superscripted above the delta sign [Δ] specify the location of the lowest number carbon in each carbon double bond as counted from the carboxyl end.

3. Omega System

The Omega [n or ω] system is often used by biochemists to designate sites of enzyme reactivity or specificity on the FA molecule and appears in two very similar forms; the professional n-minus system and the lay omega [ω] system. Both systems identify carbon chain length and double bondedness numerically but in contrast to both the Geneva and Delta systems, the location of the last double bond is numbered according to its position relative to the methyl or N terminal, end and not the carboxyl end as with previous systems. Due to the reference made to molecular sites of bioactivity in nutritionally important UFAs these are commonly classified using the Omega system and five primary groups have been described. These include the saturated Omega-12 group the largely mono-unsaturated Omega-9 and Omega-7 groups and the more physiologically active polyunsaturated Omega-6 and Omega-3 groups. Unlike Omega-12s, -7 and -9 FAs, Omega-6 and Omega-3 FAs cannot be produced *de novo* in the human body and so are referred to as Essential FAs [EFAs] as they must be included in diet.

iii. Synthesis and Metabolism

The metabolism of Omega-3 PUFA begins in the small intestine with hydrolytic dissociation of FA molecules from the glycerol backbone via the activity of pancreatic lipases. In the case of fish oil, the resulting FFAs can include preformed EPA and DHA which can be incorporated directly into cell membrane phospholipid layers. Alternatively EPA+DHA can be synthesized *de novo* from the shorter chain EFA ALA however the capacity for *de novo* synthesis of Omega-3 HUFA is very limited in humans varying between 0.2 and 24% total ALA intake [Emmken et al. 2004]. This in turn is strongly influenced by factors such as gender, age and health status of the individual while recent studies indicate that not only does low dietary intake of ALA directly affect cellular concentrations of EPA+DHA but due to their competitive interactions low cellular concentrations of Omega-3 relative to Omega-6 PUFA can significantly inhibit EPA and DHA synthesis.

De novo synthesis of EPA+DHA occurs through a series of elongation and desaturation reactions occurring largely in the endoplasmic reticulum [Fig. 4]. The initial desaturation step involving the introduction of a double bond to the FFA molecule however is shared between Omega-3 and Omega-6 PUFA which compete for access to the catalytic enzyme Δ6-desaturase. Moreover while studies show that the affinity for Δ6-desaturase is higher in ALA the typically higher concentrations of the Omega-6 EFA LA relative to ALA in human cellular pools results in greater production of Gamma-Linoleic Acid [GLA; 18:3n-6] and thus AA compared to Stearidonic Acid [SDA; 18:4n-3] and thus DHA. Thus FFA desaturation by Δ6-desaturase is considered the rate-limiting step in EPA/DHA biosynthesis with high dietary intake of Omega-6 FAs rather than low Omega-3 intake currently considered a significant contributing factor to the widespread EPA+DHA deficiency reported in industrialised societies [Figs. 5 and 6; Hibbeln et al 2006; Cordain et al., 2005].

Subsequent steps in the production of Omega-3 and Omega-6 HUFA occur along separate pathways and in the case of EPA+DHA have been described in rat liver [Sprecher, 2002] and human neonates [Carnielli et al 1996]. Following the addition of the initial double bond to form Stearidonic Acid [SDA; 19:4n-3] a further two carbon atoms are added to form 20:4n-3. This is followed by further desaturation by ∆5-desaturase to form EPA [20:5n-3] and the addition of two additional carbon atoms to form DPA [22:5n-3]. Previously ∆4-desaturase was thought to be the mechanism for DHA synthesis from DPA but it is now widely accepted that this involves a series of separate reactions occurring outside the endoplasmic reticulum and include acyl chain shortening and the loss of two carbons from 24:6n-3 to form DHA [22:6n-3].

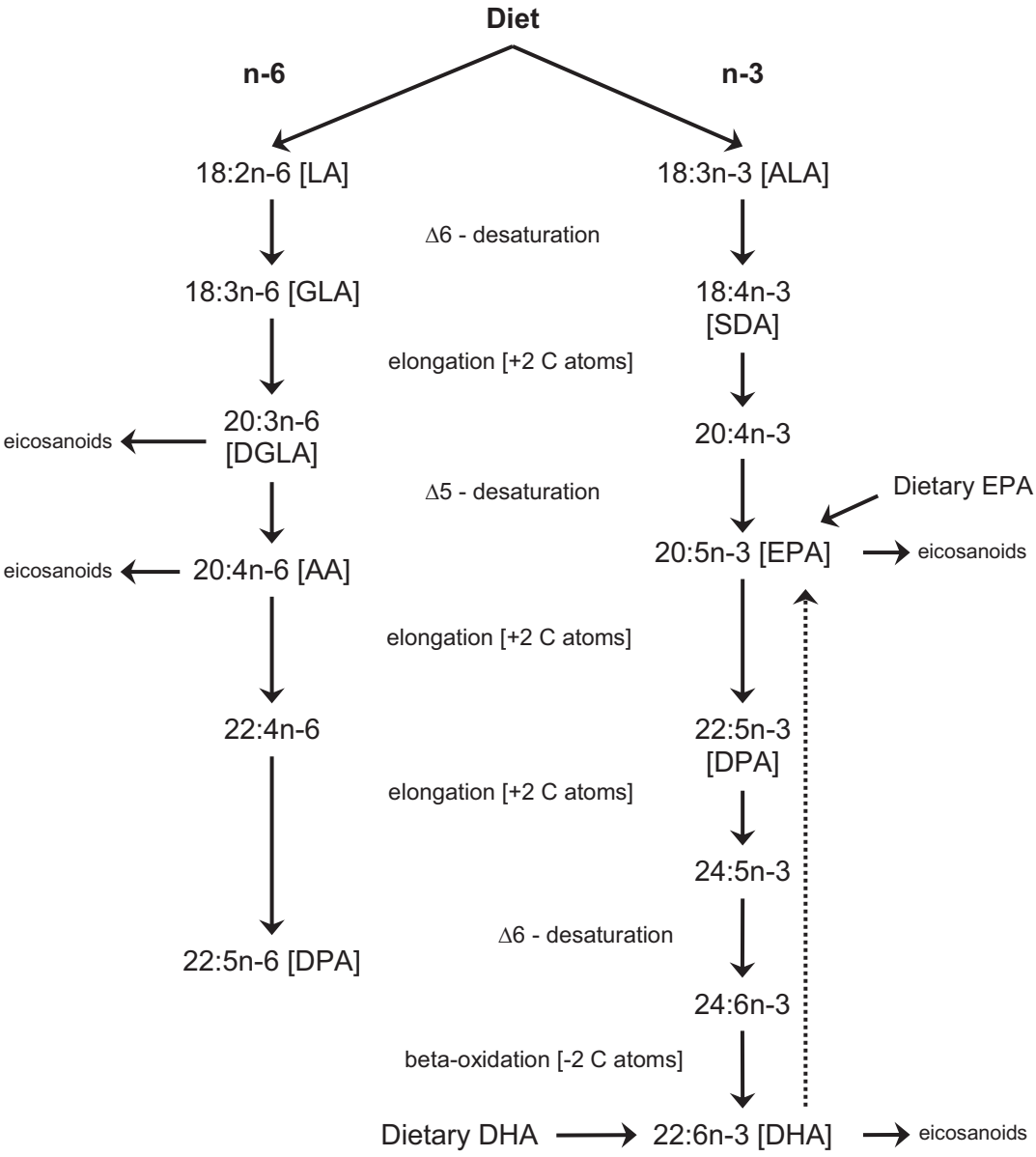


Fig. 4. Desaturation, elongation and retro-conversion of Omega-6 and Omega-3 PUFA [Holub and Holub 2004]

Finally, in addition to ALA competing with LA for access to ∆6-desaturase LC Omega-3 HUFA including ALA, also compete with Omega-6 HUFA both for incorporation into cell

membrane layers and access to enzymes that catalyse subsequent eicosanoid synthesis [Surette 2008]. This is important as inhibition of EPA+DHA synthesis due to excessively high intake of Omega-6 inhibits production of the anti-inflammatory and anti-thrombotic eico- and docosanoids so beneficial in the maintenance of proper cardiovascular and cognitive health. This is an increasing problem in developed countries where diet is increasingly dominated by vegetable oils and processed foods high in Omega-6 fatty acids [Fig. 5; Cordain et al. 2005]. For instance average daily intakes of Omega-6 LA range between 0.89% to nearly 9% of total energy compared to between 0.02% [Bulgaria] and 0.44% [Iceland] for LC Omega-3 HUFA intake [Fig. 6]. The optimum Omega-6:Omega-3 intake ratio is considered to be in the range 3:1 to 5:1 however in most westernized societies this currently frequently exceeds 15:1. [Pike and Jackson 2011].

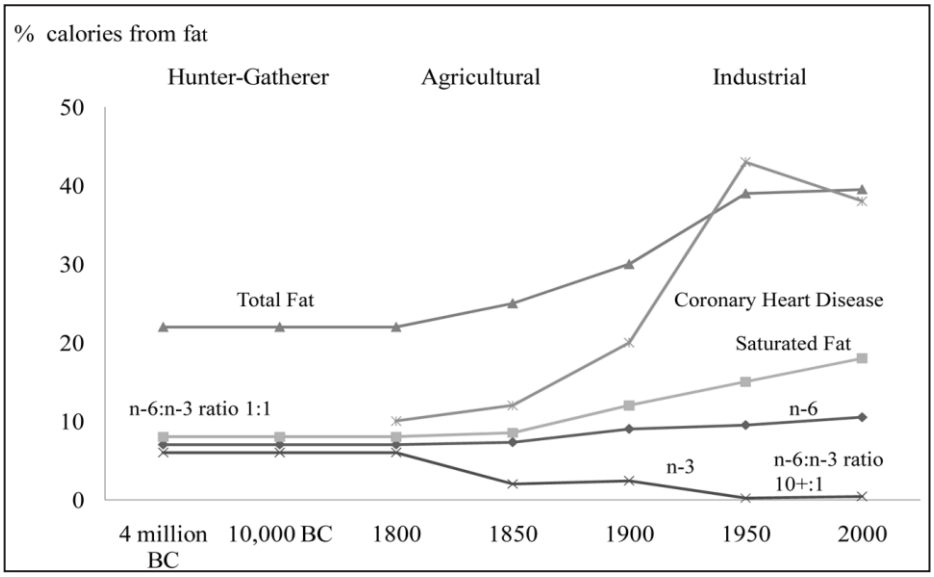


Fig. 5. Change in dietary intake of total, saturated, Omega-6 and 3 polyunsaturated fats and ratio of Omega-6 to Omega-3 PUFA from Palaeolithic period to present [Leaf and Weber 1987].

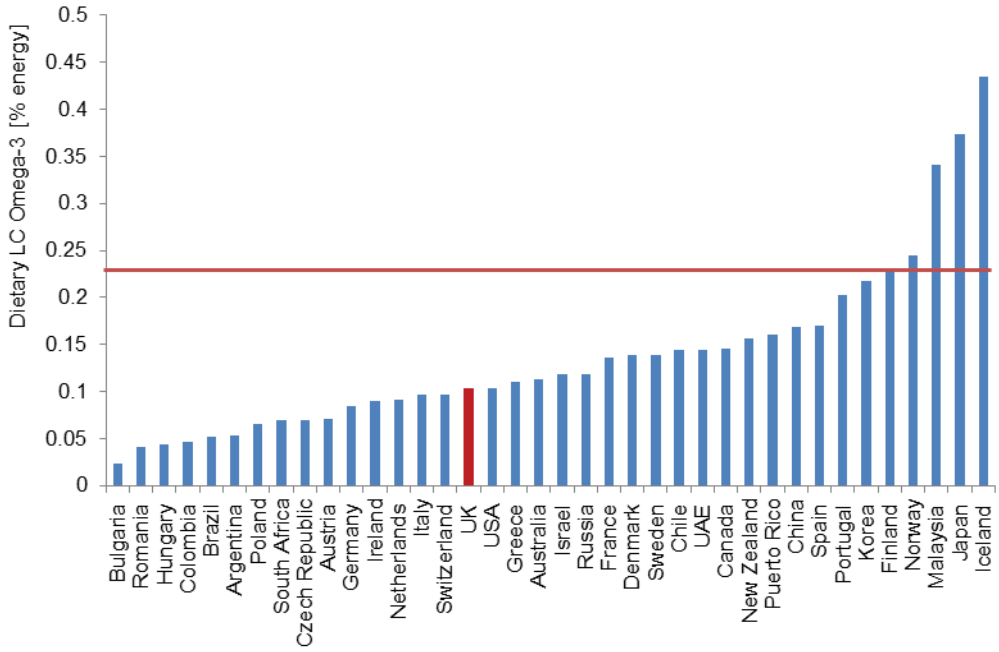


Fig. 6. Percentage of total dietary energy obtained from LC Omega-3 HUFAs by country [Recommended intake 0.22 % based on a 8,400 Kj diet] [Hibbeln et al. 2006]

Appendix 2.

Table 1. Details of raw materials producers contacted in the course of consultations.

Company	Country	Ingredient			Finished Product		Other Svcs	PUFA Source				Contact
		Supp	Food	Pharma	Supp	Food		Fish	Algae	Krill	Plant	
Nu-Mega Ingredients	Australia	✓	✓					✓				sales@nu-mega.com
NuSeed	Australia	✓						✓				none
Pathway International	Australia	✓						✓				sales@pathway.net.au
Acasti Pharma	Canada			✓						✓		j.ripplinger@acastipharma.com
Biodroga	Canada	✓						✓				info@biodroga.ca
Bioriginal	Canada	✓						✓				business@bioriginal.com
Ocean Nutrition	Canada	✓	✓					✓				via website
Golden Omega	Chile	✓						✓				Michael.Fisher@goldenomegausa.net
Natural Oils	Chile	✓			✓			✓				info@naturaloils.cl
Huatai Biopharm	China	✓			✓			✓				info@huatai-biopharm.com
Novosana	China	✓						✓				nothing available yet
Sinomega	China	✓						✓				purity@sinomega-biotech.com
Snahdong Keruier	China	✓			✓					✓		via website
Olvea	France			✓			Winterisation	✓				info@olvea.fr
Phosphotech	France	✓			✓			✓	✓			info@phosphotech.com
Polaris	France	✓						✓				contact@polaris.fr
Imperial-Oel-Import	Germany	✓	✓			✓		✓				info@imperial-oel-import.de
Lysi	Iceland	✓						✓				via website
Ocean Harvest	Ireland		✓				Aquafeed		✓			info@oceanharvest.ie
Bizen	Japan	✓	✓	✓				✓				via website
Maruha Nichiro Foods	Japan	✓	✓			✓		✓				biosection@maruha-nichiro.co.jp
Nippon Suisan	Japan	✓	✓			✓		✓				via website
Sojitz Corporation	Japan	✓						✓				nothing available yet
Felda Iffco	Malaysia	✓	✓					✓				via website
Winterisation Atlantic	Morocco	✓						✓				info[at]olvea.com
Kievit	Netherlands		✓				Encapsulators	✓				info@kievit.com
Lipid Nutrition B.V.	Netherlands	✓	✓					✓				via website
Aker BioMarine	Norway	✓	✓		✓					✓		Info-US@aker.hq
Ayanda	Norway	✓			✓			✓				info_ge@ayanda.com

Company	Country	Ingredient			Finished Product		Other Svcs	PUFA Source				
		Supp	Food	Pharma	Supp	Food		Fish	Algae	Krill	Plant	Contact
Nu-Mega Ingredients	Australia	✓	✓					✓				sales@nu-mega.com
NuSeed	Australia	✓						✓				none
Pathway International	Australia	✓						✓				sales@pathway.net.au
Denomega	Norway	✓	✓					✓				none
EPAX	Norway	✓						✓				epax@epax.com
GC Rieber Oils	Norway	✓	✓					✓				oyvind.sagli@gcrieber.no
Maritex AS	Norway	✓						✓				kari.raunedokken@tine.no
Pharmamarine	Norway	✓						✓				leif@pharmamarine.no
Pronova Biopharma	Norway			✓				✓				hamed.brodersen@pronova.com
Colpex	Peru	✓					Crude Fish Oil	✓				postmast@colpex.com.pe
Pesquera Diamante	Peru	✓	✓	✓				✓				via website
Equateq	Scotland	✓						✓				ursula.gieger @basf.com
EWOS	Scotland	✓						✓				customer.solutionsuk@ewos.com
AK Biotech	South Korea	✓						✓				tae026@gmail.com
Chemport	South Korea	✓	✓				Trading	✓				inaccesible webpage
BioSearch Life	Spain		✓			✓		✓				via website
Brudy Technology	Spain	✓	✓	✓				✓				info@brudylab.com
Solutex	Spain	✓						✓				solutex@solutex.info
Simris Alg AB	Sweden	✓	✓						✓			info@simrisalg.se
DSM Nutritionals	Switzerland	✓	✓					✓				via website
Lonza	Switzerland	✓	✓						✓			info@lonza.com
Cargill	UK		✓				Encapsulators				✓	via website
Croda	UK	✓	✓					✓				via website
Efamol	UK	✓		✓	✓			✓			✓	info@efamol.com
NuiQue	UK			✓	✓				✓			info@nuique.com
Vitabiotics	UK	✓			✓			✓				via website
Algenol	US	✓					Biofuels		✓			info@algenol.com
Arista Industries	US	✓						✓				via website
Aurora Algae	US	✓	✓						✓			via website
Azantis	US	✓	✓							✓		customerservice@azantis.com
Deva Nutrition	US	✓			✓				✓			info@devanutrition.com
DuPont (Solae)	US	✓							✓			via website

Company	Country	Ingredient			Finished Product		Other Svcs	PUFA Source				Contact
		Supp	Food	Pharma	Supp	Food		Fish	Algae	Krill	Plant	
Nu-Mega Ingredients	Australia	✓	✓					✓				sales@nu-mega.com
NuSeed	Australia	✓						✓				none
Pathway International	Australia	✓						✓				sales@pathway.net.au
Heliae	US	✓			✓				✓			info@heliae.com
Marine Ingredients	US	✓						✓				info@marine-ingredients.com
Martek Biosciences	US	✓	✓						✓		✓	marketing.dnpe@dsm.com
Novotech	US	✓			✓			✓				info@novotechnutrame.com
Nutrifinn Caps	Germany	✓					Encapsulators	✓				via website
Omega Protein	US	✓	✓					✓				inquire@omegagrow.com
Parabel	US	✓					Biofuels		✓			pasales@parabel.com
Pharmline	US	✓						✓				nothing available yet
PhycoBiosciences	US		✓			✓	Animal Feed		✓			info@phycobiosciences.com
Rainbow Light	US	✓			✓			✓				mayrao@rlns.com
Sapphire Energy	US	✓					Biofuels		✓			via website
Schiff Nutrition	US	✓								✓		Webpage unavailable
Solix Bio-fuels	US	✓					Biofuels		✓			via website
Tischon	US	✓					Encapsulators	✓				via website
Wright Group	US		✓					✓				customerservice@wenrich.com
Originates/Naturmega	US	✓						✓				info@originates.com

Table 2. Details of oil refiners and concentrators contacted in the course of consultations.

Company	Country	Ingredient			Finished Product		Other Services	PUFA Source				Contact
		Supp	Food	Pharma	Supp	Food		Fish	Algae	Krill	Plant	
Fit Bioceuticals	Australia				✓			✓				cs@fit.net.au
Bioglan	Australia				✓	✓		✓			✓	lsutherland@pegasuspr.co.uk
Lahana Naturals	Canada				✓			✓				info@lahananaturals.com
Ascenta Health	Canada				✓			✓	✓			via website
Neptune Technologies	Canada				✓	✓				✓		via website
Tharos	Chile						Consulting			✓		tharos@tharos.biz
Spes	Chile				✓			✓				
Yihai Kerry	China					✓		✓				info@wilmar-intl.com
KD Pharma	Germany	✓					Concentrators	✓				kdpharma@bioseutica.com
BASF Plant Sciences	Germany										✓	via website
Paradox Oil	Ireland				✓			✓				info@paradoxoil.com
Enzymotec	Israel				✓					✓		info@enzymotec.com
Qualitas	Israel	✓	✓						✓			info@qualitas-it.com
Taam Teva Altman	Israel				✓			✓				
UGA Nutraceuticals	Italy				✓			✓				gentian.selimi@uganutraceuticals.com
Smit & Zoon	Netherlands						Trading	✓				food@smitzoon.nl
Marvesa	Netherlands						Trading	✓				marvesa@marvesa.com
Unilever	Netherlands					✓		✓				via website
CHR Holtermann	Norway						Oil Brokers	✓				fishoil@holtermann.com
Skretting	Norway						Aquafeeds	✓				sales.invergordon@skretting.com
Axellus	Norway				✓			✓				jaroslava.juricova@awllus.no
Moller/Axellus	Norway				✓			✓				
Austral S.A.A.	Peru							✓				via website
TASA	Peru							✓				via website
Sifar Ilaclari	Turkey				✓			✓				sifar@sifar.com.tr
Equazen	UK				✓			✓				equazen@viforpharma.com
Holland & Barrett	UK				✓	✓		✓		✓	✓	healthinformation@hollandandbarrett.com

Company	Country	Ingredient			Finished Product		Other Services	PUFA Source				Contact
		Supp	Food	Pharma	Supp	Food		Fish	Algae	Krill	Plant	
Vertese	UK				✓						✓	info@bruhealth.co.uk
CleanMarine	UK				✓					✓		info@cleanmarinekrill.co.uk
Higher Nature	UK				✓			✓			✓	info@higher-nature.co.uk
Patrick Holford	UK				✓			✓				Jodie@imageboxpr.co.uk
New Horizons Global	UK				✓				✓			via website
Croda	UK			✓	✓	✓		✓				via website
Healthspan	UK				✓			✓				via website
Seven Seas	UK				✓			✓				via website
Healthspark	UK				✓					✓		via website
granoVita	UK				✓	✓					✓	via website
Cyanotech	US				✓				✓			brussell@cyanotech.com
JR Carlson Laboratories	US							✓				carlson@carlsonlabs.com
Lang Naturals	US						Encapsulators	✓	✓		✓	customer.service@ langpni.com
NBTY	US				✓	✓		✓	✓			emedia@nbty.com
Northwest Natural Products	US				✓			✓				info@nnpvitamins.com
Source Omega	US				✓				✓		✓	info@source-omega.com
Nordic Naturals	US				✓			✓			✓	jpsidaner@nordicnaturals.com
Activation [Oceans Alive Phytoplankton]	US					✓			✓			nothing available online
Pharmavite	US				✓			✓				nothing available online
Barleans	US				✓			✓	✓			questions@barleans.com
Organic Technologies	US							✓				Sales@organictech.com
Horizon Organic	US					✓			✓		✓	sara.loveday@whitewave.com
ForeverGreen	US					✓			✓			support@forevergreen.org
AlgaeBio	US					✓			✓			t.kimberley@algaebio.com
Cellana	US						Biofuels		✓			Unavailable via website
OmegaBrite	US				✓			✓				updates@omegabrite.com
Iceland Health	US				✓			✓				via website
Magna Pharma	US				✓			✓				via website
Nourish-Life	US				✓			✓				via website

Company	Country	Ingredient			Finished Product		Other Services	PUFA Source				
		Supp	Food	Pharma	Supp	Food		Fish	Algae	Krill	Plant	Contact
Nutri-Med Logic	US				✓			✓				via website
The Scoular Company	US						Trading	✓				via website
Monsanto	US										✓	via website
The Solae Company	US										✓	via website

Appendix 3

Example of structured email contact letter used:

To whom it concerns

Further to a telephone inquiry earlier today regarding your refined Omega-3 products I am writing to enquire if you would be willing to participate in a short industry survey we are conducting on behalf of the Scottish Aquaculture Research Forum [http://www.sarf.org.uk]?

Below is the link to this survey along with a brief description of the nature and purpose of our research: This survey forms part of a research project investigating production of n-3 marine-type lipids from algal and other non-fish sources [details at http://connelmarine.com]. The primary aim of this project is to identify critical supply chain wastage points within the rapidly expanding Refined Edible Products sector. However, as reliable data on existing systems are difficult to obtain we would be very grateful if you would complete our short survey by following the link below:

https://docs.google.com/forms/d/1Q82_b-1wjHEeKVMzcTmku153XUbWIhSjdOLE4JZhXN8/viewform

All responses will be treated in strictest confidence and collated on a non-attributable basis unless otherwise stated.

May I extend our sincerest thanks in advance for your cooperation with this important research project, the results of which will be made publicly available in due course at http://www.sarf.org.uk/reports.

Yours Faithfully,

Appendix 4

Survey Questionnaire

1. How long have you been active in the marine-type lipids industry?
    a. 0-5 years
    b. 5-10 years
    c. 10-15 years

2. Approximately what are your current/projected production volumes?
    a. Less than 5,000 Mt.yr
    b. Between 5,000 and 30,000 Mt.yr
    c. More than 30,000 Mt.yr

3. Where does the bulk of your raw material originate?
    a. Forage finfish
    b. Krill
    c. Microalgae
    d. Macroalgae
    e. Terrestrial plants
    f. Genetically modified organisms

4. What are your primary end products?
    a. Animal or Aqua feeds
    b. Nutraceuticals incl. functional foods
    c. Pharmaceuticals incl. nutritional supplements
    d. Biofuels

5. What are your most critical production challenges?
    a. Harvesting and component separation
    b. Extraction

c. Refinement/concentration

d. Oxidative stability

6. What is the conversion efficiency of your bulk extraction process i.e. raw material in:crude oil out?

7. Do you include a PUFA concentration step in your process and if so what is it?

    a. Chromatography

    b. Molecular distillation

    c. Hydrolysis

    d. Esterification

    e. Urea complexation

8. What is the conversion efficiency of your PUFA concentration process i.e. crude oil in:DHA/EPA out?

9. What is the fate of the associated waste?

10. Would you be willing to provide data on the fatty acid profiles of this waste?

11. Would you be willing to provide data on the fatty acid profiles of your primary end products?

12. Do you adhere to any voluntary quality standards and if so, which one?

    a. GOED

    b. European Pharmacopeia

    c. Council for Responsible Nutrition

13. Do you feel your current production volumes are constrained by the absence of approved Guideline Daily Amounts and/or lack of consumer awareness?

14. Do you agree that a supportive national policy would accelerate your industry's development e.g. funding for basic R&D, committed public-funding, tax-breaks, approved GDAs, other [please elaborate]?

15. In your opinion, what three key things need to happen to leverage scaled alternatives to n-3 oils from capture fisheries?

Appendix 5

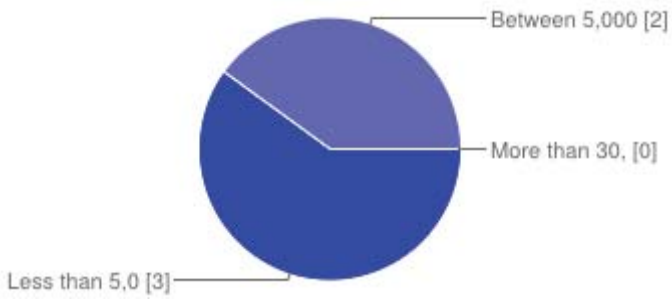
Survey Results Summary

Total number of respondents [*n*]: 6

1. How long have you been active in the marine-type lipids industry?

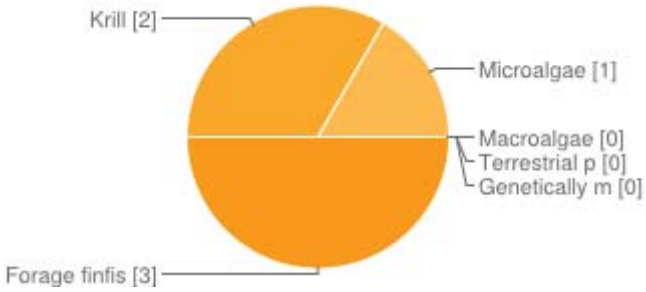
Optional response	*n*	Proportion of total
0-5 yrs.	0	0%
5-10 yrs.	2	33%
10-15 yrs.	3	50%
More than 75 years	1	17%

2. Approximately what are your current/projected production volumes?



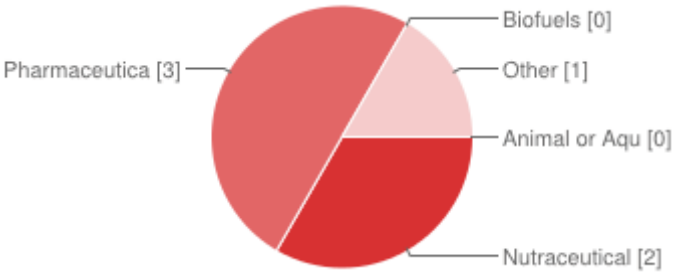
Optional response	*n*	Proportion of total
Less than 5,000 Mt.yr	3	60%
Between 5,000 and 30,000 Mt.yr	2	40%
More than 30,000 Mt.yr	0	0%

3. Where does the bulk of your raw material originate?



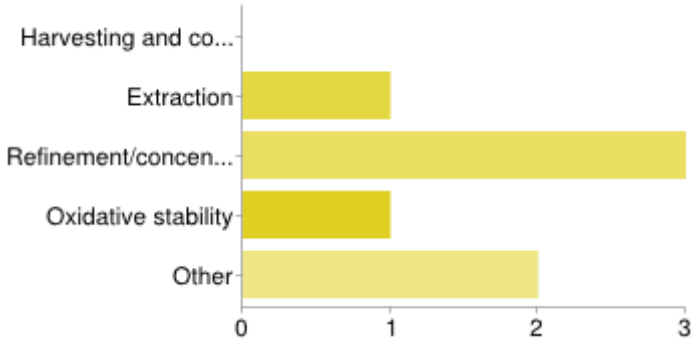
Optional response	*n*	Proportion of total
Forage finfish	3	50%
Krill	2	33%
Microalgae	1	17%
Macroalgae	0	0%
Terrestrial plants	0	0%
Genetically modified organisms	0	0%

4. What are your primary end products?



Optional response	*n*	Proportion of total
Animal or Aqua feeds	0	0%
Nutraceuticals incl. functional foods	2	33%
Pharmaceuticals incl. nutritional supplements	3	50%
Biofuels	0	0%
Other [details not provided]	1	17%

5. What are your most critical production challenges?

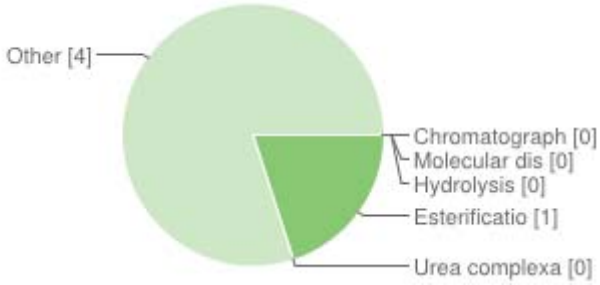


Optional response	*n*	Proportion of total
Harvesting and component separation	0	0%
Extraction	1	14%
Refinement/concentration	3	43%
Oxidative stability	1	14%
Other [incl. microalgal processing]	2	29%

6. What is the conversion efficiency of your bulk extraction process?

Response	*n*	Proportion of total
~90% depending on the conditions	1	17%
We use refined oil and concentrate it	1	17%
The efficiency is related to concentration. The final efficiency rate is confidential	1	17%
This varies greatly, approx. 5%	1	17%
N/A	2	33%

7. Do you include a PUFA concentration step in your process and if so what is it?



Optional response	*n*	Proportion of total
Chromatography	0	0%
Molecular distillation	0	0%
Hydrolysis	0	0%
Esterification	1	20%
Urea complexation	0	0%
Other [details not provided]	4	80%

8. What is the conversion efficiency of your PUFA concentration process?

Response	*n*	Proportion of total
Crude in: highly refined EPA & DHA out	1	17%
We do not use a PUFA concentration process	1	17%
N/A	2	33%
Non-respondents	2	33%

9. What is the fate of the associated waste?

Response	*n*	Proportion of total
Is sold as low concentration oil	1	16.67%
Used as biodiesel	1	16.67%
Various outlets	1	16.67%
We do not use a PUFA concentration process	1	16.67%
N/A	1	16.67%

Non-respondents	1	16.67%

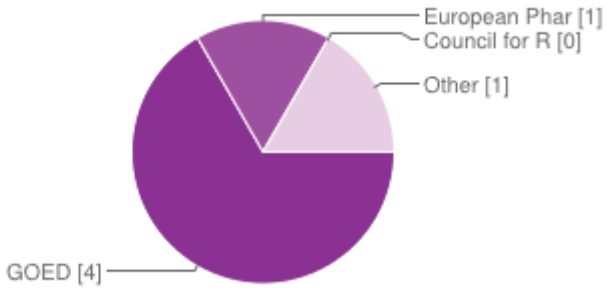
10. Would you be willing to provide data on the fatty acid profiles of this waste?

Response	*n*	Proportion of total
No	2	33%
N/A	1	17%
Non-respondents	3	50%

11. Would you be willing to provide data on the fatty acid profiles of your primary end products?

Response	*n*	Proportion of total
No	2	33%
N/A	1	17%
Non-respondents	3	50%

12. Do you adhere to any voluntary quality standards and if so, which one?



Optional response	*n*	Proportion of total
GOED	4	67%
European Pharmacopeia	1	17%
Council for Responsible Nutrition	0	0%
Other	1	17%

13. Do you feel your current production volumes are constrained by the absence of approved Guideline Daily Amounts and/or lack of consumer awareness?

Response	*n*	Proportion of total
Yes	1	17%
No	2	33%
N/A	1	17%
Non-respondents	2	33%

14. Do you agree that a supportive national policy would accelerate your industry's development?

Response	*n*	Proportion of total
Yes	2	33%
No	1	17%
Millions of dollars have been spent on this research to date, and the most important outcome at this point is for that research to lead to successful, profitable companies.	1	17%
Non-respondents	3	50%

15. In your opinion, what three key things need to happen to leverage scaled alternatives to n-3 oils from capture fisheries?

Response	*n*	Proportion of total
NO Opinion	1	17%
1. COGS 2. COGS 3. COGS	1	17%
1. Increased availability 2. Lower prices 3. Better lipid profiles of oils	1	17%
Non-respondents	3	50%

Scottish Aquaculture Research Forum

Charity Registration: SC035745
Company Registration: SC267177

SARF - Member Organisations

Industry

SEAFISH
Association of Scottish Shellfish Growers
Scottish Salmon SSPO
Shetland Aquaculture
British Trout Association

Government and Regulators

Scottish Natural Heritage
SEPA Scottish Environment Protection Agency
marinescotland
The Scottish Government
HIE Highlands and Islands Enterprise
Iomairt na Gaidhealtachd 's nan Eilean

Non-Governmental Organisations

The Crown Estate
Scottish Environment LINK
Rivers & Fisheries Trusts of Scotland
Safeguarding Scotland's Rivers & Lochs
ASFB The Association of Salmon Fishery Boards