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Omega 3 Fatty Acid as A Health Supplement: an Overview of its Manufacture and Regulatory Aspects

RAMESA SHAFI BHAT[1]*, ANWAR S. ALSUHAIBANI[2, 3], FAISAL S. ALBUGAMI[2, 3] and FAHAD S. ALDAWSARI[3]

[1]Biochemistry Department, College of Sciences, King Saud University, Riyadh, Saudi Arabia.
[2]Health & Herbal Supplements Section, Reference Laboratory for Medicines & Cosmetic Research and Laboratory Sector, Saudi Food and Drug Authority (SFDA), Riyadh, Saudi Arabia.
[3]Reference Laboratory for Medicines & Cosmetic Research and Laboratory Sector, Saudi Food and Drug Authority (SFDA), Riyadh, Saudi Arabia.

Abstract

Dietary supplements are used for potential health benefits and are mainly intended to provide those nutrients that may be insufficiently consumed through regular dietary intake. They are mostly made from natural sources and are readily available in the form of capsules, tablets, or liquid form. Nowadays, omega-3 fatty acids (n−3 FAs) supplements are in high demand and have gained noteworthy popularity as the human body cannot produce them, and need to be administrated externally. They are polyunsaturated fatty acids (PUFA) characterized by at least one cis double bond at the third and fourth omega-end carbons, with primary examples being α- linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) and are classified as functional foods. The natural sources of these supplements include aquatic organisms like freshwater and marine fish, microalgae, seaweeds; nuts and seeds plant oils, and fortified foods. n-3 FAs, being biologically active molecules, are highly susceptible to oxidation due to double bonds in their long chains, leading to degradation over time, during storage. Various encapsulation systems, such as gels, emulsions, and powders, have been developed to effectively encapsulate PUFAs to enhance their chemical stability, dispersibility, and bioavailability. These quality control measures are essential to guarantee the effectiveness and safety of products containing n-3 FAs. This paper explores various sources of omega-3 fatty acid supplements and summarizes the multiple manufacturing techniques used for the production of these products. Additionally, the article tried to correlate quality aspects with the regulations for such products in an

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CONTACT Ramesa Shafi Bhat rbhat@ksu.edu.sa Biochemistry Department, College of Sciences, King Saud University, Riyadh, Saudi Arabia.

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attempt to prevent exposure of consumers to harmful ingredients contained within these products. Lastly, potential recommendations for harmonizing the regulation and control of omega-3 supplements are proposed.

Introduction

Food consists of a diverse range of lipids and oils, with fatty acids (FA) being the main components of diet and a source of energy for humans.[1] Different types of FA are found in the blood, cells, and tissues of nearly all animals. They play a major role in many biological processes relevant to metabolism, hormone regulation, and many pathways in cells such as β-oxidation to produce energy and to depot fat storage.[2] They also regulate additional crucial activities such as the membrane permeability, transcription factor activity, expression levels of genes, and generation of bioactive lipid mediators.[2] FAs are carboxylic acids characterized by the presence of chemical double bonds, saturated or unsaturated. They consist of an even number of carbon chains ranging from two to thirty-six carbon atoms. Polyunsaturated fatty acids (PUFA) are a major group that is structurally constituted of double bonds arranged in a pentadiene configuration.[3] The occurrence of FA in the body is tissue and species-specific, for example, the most prevalent FA in animal and plant tissues with sixteen or eighteen carbon atoms, including palmitic, stearic, oleic, and linolenic acids.[4] Selectivity in mammals, FA are twelve to twenty-four carbon atoms long with up to six double bonds. Moreover, FAs longer than 22 carbon atoms or shorter than 14 atoms are present in very small amounts. Generally, saturated fatty acids are predominant in animal fats, whereas PUFA are more abundant in vegetable fats, with the exception of palm and coconut oils.[5]

In general, saturated fatty acids are predominant in animal fats, whereas polyunsaturated fatty acids are more abundant in vegetable fats, with the exception of palm and coconut oils. Normally, humans ingest 20 different kinds of FA daily with saturated, monounsaturated, or polyunsaturated carbon chains. Among them few are essential fatty acids (EFA) such as Omega-3 FAs (n−3 FAs) and Omega-6 FAs (n−3 FAs) are not synthesized within the human body and therefore need to be incorporated into the diet.[6]

n−3 FAs have unsaturated hydrocarbon chains with a double bond at the third and fourth positions, whereas in n−3 FAs, the double bond is situated at the carbon at position six.7 The carboxyl end is where most chemical changes take place, while the methyl group and its adjacent double bond, such as in n-3 and n-6, remain unchanged during physiological transitions in the body. n−3 FAs are generally PUFA, with cis double bonds such as ALA, EPA, and DHA, are classified as functional foods.[8] These FAs are found in a wide range of diet items. ALA is present in plant-based items like soybeans, flaxseed, chia seeds, and oils from canola. Alternatively, DHA and EPA, are mostly found in marine-based foods such as oily fish. Intake of long-chain n−3 FAs in humans is preferred because the biological conversion of ALA to EPA and DHA is either incomplete or extremely little *in vivo*.[9] Since the human body cannot produce ALA, it must be obtained from foods or supplements. n−3 FAs play a major role in cell membrane fluidity. Levels of DHA are particularly high in sperm, brain, and retinal (eye) cells. In addition, n−3 FAs play a regulatory role in numerous functions within the various organs like the heart and lungs; blood vessels; and immune and endocrine systems.[10,11]

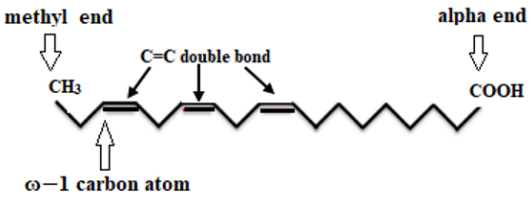
In recent years, there has been a notable gain in the market for fish oil-based food supplements, driven by the expectation of gaining similar health benefits as those derived from consuming fish. The global n-3 supplement market, valued at USD 5.18 billion in 2019, is projected to witness a compound annual growth rate of 8.4% from 2020 to 2027.[12] Despite the substantial turnover in this market, there is a noticeable scarcity of scientific studies dedicated to assessing the quality of these products.[13] Ensuring the quality of products containing n-3 FAs is a critical step in the manufacturing process. This is typically achieved through the implementation of quantitative chemical tests to verify compliance with international standards.[14] Ensuring the effectiveness and safety of n-3 supplements relies on crucial quality control measures, primarily focusing on the absence of

heavy metals (such as mercury) and trace metals (sodium, potassium, magnesium, iron, and calcium), as well as assessing the quality and quantity of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA).[15,16] Numerous projects are underway to scrutinize the quality of commercial omega-3 fish oil brands. These assessments encompass the examination of key quality attributes, including uniformity of weight, disintegration time, and DHA and EPA content. Additionally, they involve monitoring the presence of sodium, potassium, calcium, iron, magnesium, and mercury, even when not explicitly stated on product labels. Through risk assessment, particularly using the hazard quotient, it has been revealed that consumers using these products over an extended period may be at risk of potential adverse health effects.[17] The findings of these studies underscore significant concerns regarding the quality and safety of commercially available omega-3 supplements. This emphasizes the critical necessity for enhanced regulatory measures and increased consumer awareness to address and rectify these issues.

In this review, we have highlighted the general properties and manufacturing process for n-3 FAs namely chemistry, dietary sources, extraction, and encapsulating techniques. Further, we also compiled the major literature evidence relevant to the dosage, quality, and health advantages of n−3 FAs. Collectively, we attempted to leverage the potential risks associated with the use of these supplements while providing some recommendations for regulations sought for these dietary aids. We conducted a thorough search of research databases, including Pubmed, Medline, and Google Scholar, to gather peer-reviewed articles for writing this article.

Chemistry

Structurally, n−3 FAs contain double bond at the carbon three from the methyl (-CH3) end, called as omega where the alpha end consists of carboxylic acid functional group (-COOH) shown in Figure 1. Its chemical formula is $C_{60}H_{92}O_6$ with a molecular weight of 909.39 g/mol. Some of the most common n−3 FAs are α-linolenic acid (ALA; 18:3 n-3), stearidonic acid (SDA; 18:4 n-3), eicosapentaenoic acid (EPA;20:5 n-3), docosapentaenoic acid (DPA; 22:5 n-3), and docosahexaenoic acid (DHA; 22:6 n-3). Specifically, the three most important in terms of human physiology are ALA (all-cis-9,12,15-octadecatrienoic acid), EPA (all-cis-5,8,11,14,17-eicosapentaenoic acid) DHA (all-cis-4,7,10,13,16,19-docosahexaenoic acid) with eighteen, twenty , and twenty two carbon atoms with three, five , or six double bonds, respectively. Of note, all double bonds are in the cis-configuration, meaning that the two hydrogen atoms are on the same side of the double bond. Methylene bridges (-CH2) also act as double bond breaks, leaving two single bonds between each pair of adjacent double bonds.7 ALA is the parent n-3 FA which desaturases and lengthens to produce n−3 PUFAs. Due to the limitations of the enzyme delta-12 and delta-15 desaturase that inserts cis double bonds at carbon-carbon positions 12 and 15, n−3 PUFAs is not synthesised in body. ALA is a necessary 18-carbon unsaturated FA that undergoes both chain elongation and desaturation processes to yield EPA or DPA.[18]



α-linolenic acid (ALA; 18:3 ω-3)

Fig. 1: Chemical structure of n−3 FAs (here ALA)

Natural n−3 FAs Sources

Major dietary source of n−3 FAs comes from aquatic organisms especially the liver of cod and halibut, and the flesh from mackerel, menhaden, and salmon.[19] Moreover, essential FAs are present in seeds like flaxseed and chia seeds, as well as nuts, particularly walnuts. Plant oils, including flaxseed oil,[20] soybean oil,[21] and canola oil,[22] are also rich sources of these beneficial FAs. Additionally, fortified foods like specific brands of eggs, yogurt, juices, and milk provide sufficient amounts of these essential nutrients.[23] (Figure 2).

Marine Sources

Aquatic organisms are as a valuable reservoir of PUFA, notably n-3 FA, which the human body does not naturally produce.[24] EPA and DHA are the two key n−3 PUFAs derived from marine sources, whereas DPA is found at relatively low concentrations. Cold-water fish like salmon, mackerel, tuna, and

sardines are rich sources of natural n−3 FAs. In comparison to freshwater fish, marine fish contain high amounts of MUFA and PUFA.[25,26] Furthermore, cod flesh, halibut, and skipjack tuna are identified as having a substantial concentration of DHA, while flounder species and haddock exhibit a noteworthy level of EPA. Additionally, n−3 PUFA can be found in crustaceans, bivalves, and cephalopods. When compared to other cooked fish, salted mackerel had the highest concentration of EPA and DHA. Joint FAO/WHO Expert recommended having 2-3 servings of fatty fish each week to attain 250 mg of EPA and DHA per day.[27]

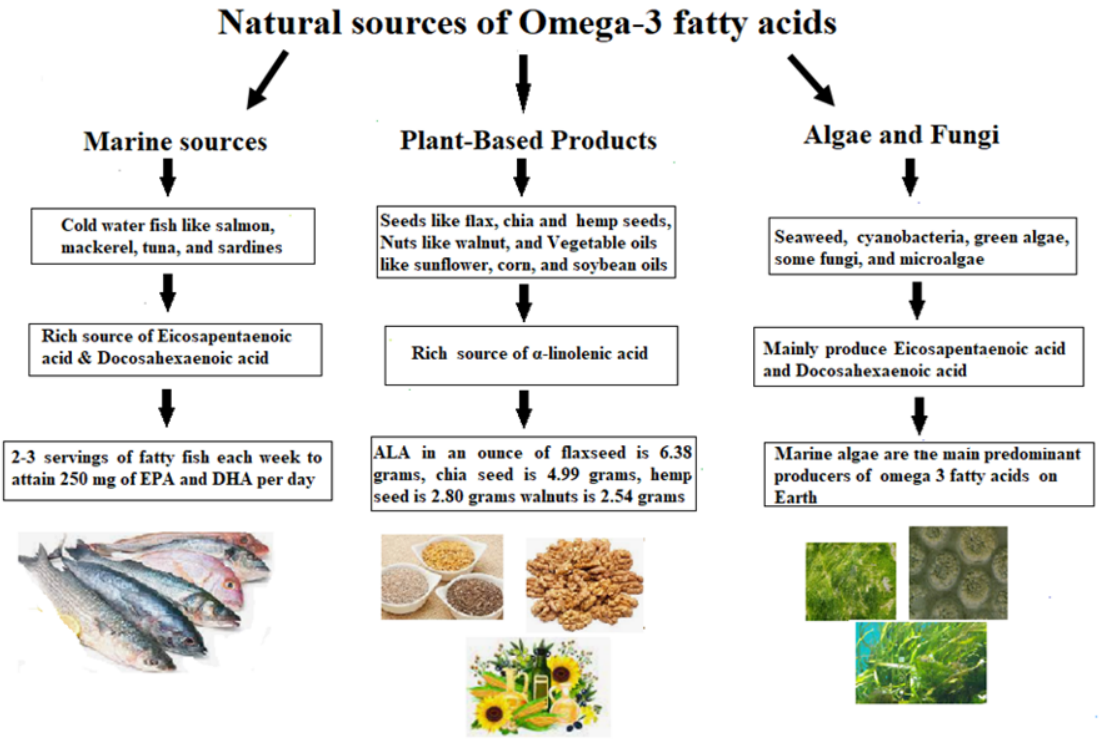


Fig. 2: Major dietary source of N−3 FAs in the human diet

Plant-Based Products

Plants are the main source of ALA, mostly present in seeds, nuts, and vegetable oils.[28] Moreover, flaxseed, chia, and echium seed oils in addition to walnuts as excellent sources of ALA. On the other hand, safflower, sunflower, corn, and soybean oils boast high linoleic acid content. A single ounce of flaxseed, chia seeds, hemp seeds, or walnuts supplies a sufficient quantity of ALA, meeting the recommended intake of 1.1 g/day for females and 1.6 g/day for males. In an ounce, flaxseed has 6.38 grams, chia seeds have 4.99 grams, hemp seed has 2.80 grams and walnuts have 2.54 grams of ALA, respectively.[28,29]

Microalgae, Seaweeds (Macroalgae), and Fungi

As a nutritional supplement, seaweed is becoming more and more popular all over the world in cookery.[30] Seaweed stands out as a functional food, offering a rich source of n3-PUFAs, protein, and minerals.[31] Notably, commercially available biomass extracts like Spirulina and Chlorella, derived from cyanobacteria and green algae, serve as excellent suppliers of n3-PUFAs. Furthermore, certain fungi and microalgae also contain n-3 PUFAs.[32] According to Senanayake and Fichtali [33] marine algae play a major role as the primary producers of long-chain -3 PUFA on Earth. DHA, a significant component, is found in various algae types such as *Crypthecodinium cohnii* and *Schizochytrium* spp., with the latter contributing to 55% and the former to 40% of the total FAs. Additionally, phytoplankton and algae generate -3 PUFAs, particularly EPA and DHA, which are then transferred through the food chain and accumulated in the lipids of fish and marine animals.[34]

Commercial Production of n−3 FAs

Just 5% of the global fish oil production is dedicated to extracting n−3 FAs for use in diet supplements.35 The remaining fractions that are produced are fed to animals. However, in the future, fishmeal and oils will be used in diets for aquaculture at lower levels, leading to rise in the availability of these crucial essential FAs for human consumption. The quality and composition of FAs isolated from any source will likely depend on the environmental factors and the implemented extraction techniques.[36]

Traditional solvent Extraction

Various techniques are being used to extract n−3 FAs. Whole fish or tissue that is rich in lipids is used to extract oils through traditional solvent-based techniques using an organic solvent with a mixture of methanol and chloroform with a ratio of 2:1. This technique can isolate both polar and non-polar oils from the tissue. At first, tissue is ground with the solvent followed by the addition of water to generate two distinct layers. Polar and nonpolar lipids are collected at the bottom chloroform layer and the residue is collected from the top methanolic and water layers. Finally, chloroform is evaporated leaving the lipids behind. This technique is mostly used to extract n−3 PUFAs from fish liver and muscle tissue.[37, 38]

Conventional Extraction

Usually, the wet pressing method is used to extract omega-3 fish oil. The process involves cooking the fish initially, followed by pressing, decanting, and centrifuging to separate the fatty and aqueous components. The collected oil fraction, rich in n−3 FAs, undergoes refinement stages, including neutralization, bleaching, degumming or water refining, winterization, and deodorization.[39] Quality of oil is maintained by reducing oil acidity, absorbing impurities or pollutants, separating phospholipids, and removing odorous. Finally, the yield of the n−3 FAs is increased by the molecular distillation process.[35]

Green Extraction Method

Although the yield of n−3 FAs with standard extraction techniques is acceptable, it has several disadvantages e.g. harmful organic solvent residues may persist in the last fraction, high temperatures that can destroy the heat-sensitive n−3 PUFAs and lastly environmental pollution caused by organic solvents and time-consuming procedures.[40-43] To ensure the purity and stability of these foodstuffs, more mild, secure, and ecologically friendly green chemical extraction techniques are being developed by industries. These methods are based on the idea of "green" extraction utilizing supercritical fluid, enzyme, microwave, pulsed electric fields, and ultrasound technology.[40,42] Supercritical fluid extraction is widely employed for extracting n−3 FAs due to its economic production of high-purity oils with substantial yields, all achieved without elevated temperatures or organic solvents.[44-46]

Biotechnological Approach for Synthesizing N-3 Fatty Acids

As a consequence of drawbacks associated with fish oil production, there is a continual quest for alternative [47] and sustainable sources of n-3 FAs like engineering plants to produce n-3 FAs in their seed.[48] However, this method requires months for harvesting and purifying the n-3 FA from the plant oil. Although there has been notable advancement in the biotechnological production of n-3 FA, significant challenges persist in overcoming the current supply shortage to meet the growing demand for this product. While genetic manipulation techniques in plants have led to the development of transgenic plants as potential sources of n-3 FAs, these processes are both expensive and labor-intensive when implemented on a large scale.[49]

Some cost-effective, time-efficient, environmentally friendly alternatives for n-3 FAs synthesis are being developed like industrial extraction of n-3 FAs from microalgae and other potential oleaginous microorganisms.[50] These microbial oils, also known as single-cell oils are produced by unicellular microorganisms, constituting 20–80% lipid per dry biomass. The production of single-cell oils is enhanced when organisms are grown at lower temperatures, promoting the process of fatty acid synthesis. Precision fermentation utilizes microorganisms, such as yeast or bacteria, as a production host to create specific molecules of interest. This approach holds promise for the animal-free production of PUFA. Oleaginous yeast, like Yarrowia lipolytica, is suitable candidate for large-scale fermentation process to synthesizing EPA due to its ability to accumulate significant amounts of intracellular lipids and utilize sugars from agricultural feedstock.[50]

The use of several microbes as cell factories for n-3 FAs production presents an avenue for improvement. Enhancing biosynthesis by optimizing carbon yield, balancing cofactors, and minimizing by-product formation holds the promise of elevating product quality and reducing bio-manufacturing costs. Exploring alternative carbon sources, such as economical sugars, waste oils/fats, offers an opportunity for increased product yield and cost efficiency. Advanced molecular biology tools can be leveraged to refine metabolic engineering, thereby boosting the yield of bio-manufactured n-3 FA. Additionally, cutting-edge technologies like continuous fermentation and AI-controlled fermentation can enhance downstream process engineering, increasing productivity while lowering operational and capital expenses. Collectively, these integrated strategies present a comprehensive roadmap for efficiently, cost-effectively, and sustainably elevating the production of omega-3 fatty acids.[47]

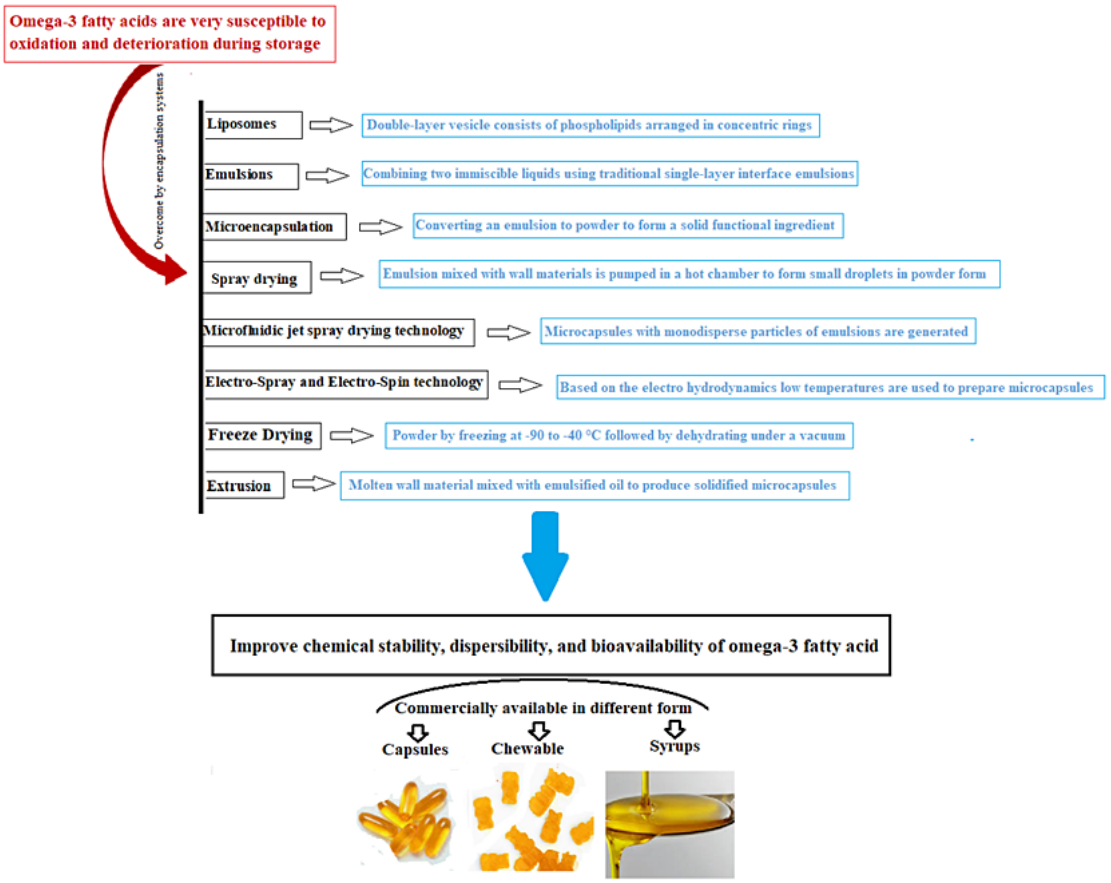


Fig. 3: Encapsulation systems designed to encapsulate n−3 PUFAs to overcome oxidation and deterioration during storage

Encapsulation System of Omega-3 FA

n−3 FAs are biologically active molecules and very prone to oxidation because of unsaturation in the long chain. The rate of lipid oxidation depends on the degree of unsaturation; thus DHA/EPA are more vulnerable to oxidation. Primary oxidation produces hydroperoxides and then subsequently lipid peroxides, which further generate a lot of free radicals (aldehydes/ketones).[51] These oxidation products are toxic it has negligible biological activity.[52] For instance, 4-hydroxyalkyl, one of the oxidized products of n−3 PUFAs, is toxic to the brain and retina.[53] Moreover, certain oxidized end products of n−3 PUFAs pose a risk of atherosclerosis, induce inflammation through the activation of pro-inflammatory molecules, and

adversely impact the body's cholesterol levels.[54] To address these concerns, various encapsulation systems, including gels, emulsions, and powders, have been developed and innovated. These systems aim to encapsulate these FAs so as to enhance their stability, dispensability, and bioavailability. (Figure 3).

Liposomes

Liposomes are extensively used in encapsulating n−3 PUFAs with a double-layer vesicle which usually consists of phospholipids arranged in concentric rings.[55,56] In terms of structure, liposomes encompass both polar and non-polar regions, allowing them to encapsulate substances that are both hydrophilic and hydrophobic. Liposomes are prepared and assembled through solvent evaporation, injection, and micro fluidization techniques each of which has a different commercial potential.[57] Liposomes enhance the oxidative stability of FAs while being stored however the high cost of the phospholipids and the low physical stability of liposomes due to fragile phospholipid membranes in complex matrices are some drawbacks of this technology.[58] To increase the stability of liposomes, phospholipid bilayers have been coated with wall material mainly for the pharmaceutical and food industries. Nanoliposomes is thought to be a useful technique for stabilizing and increasing the bioavailability of bioactive substances that are encapsulated.[59] Consequently, a variety of lipid-based nanocarriers, including nanoliposomes, were created by some researches to boost the bioavailability of n−3 FA.[60,61] Soy lecithin is used as a wall material for EPA and DHA nanoliposomes with increased encapsulation efficiency to 100%.[62] Researchers have reported β-sitosterol[63] and Poly-D, L-lactide-co-glycolide chitosan64 for DHA nanoliposomes where chitosan/gelatine (CH/GE)[65] was attempted for n−3 PUFAs nanoliposomes with increased encapsulation efficiency. Recently a thin-film hydration was used to encapsulate chia oil nanoparticles with 88.31% efficiency.[66] The encapsulated nanoliposome exhibited improved thermal stability and a prolonged release of n-3 FAs. Hence can be used as a supplement for both dietary and medicinal purpose.[66]

Emulsions

Emulsification is the process of combining two immiscible liquids with a smaller droplet of one liquid dispersed in the other liquid.[67, 68] Specifically, hydrophobic bioactive substances are encapsulated using traditional single-layer interface emulsions using water-in-oil and oil-in-water techniques. The encapsulation system used for n−3 PUFAs is usually either oil-in-water emulsion or nano-emulsion due to their hydrophobic nature. In emulsions, the average droplet diameter exceeds 100 nm, while it is less than 100 nm for nanoemulsions.[69] The use of nanoemulsions significantly enhances the capability and stability of the orally ingested n-3 oil. In this procedure, n-3 oil and water are homogenized with high-shear mixers, colloid mills, high-pressure valve homogenizers, microfluidizers, and sonicators in the presence of an emulsifier.[69] The emulsion acts as a physical barrier for reactive elements and protects n−3 PUFAs from oxidation.[70] The stabilization of the interface layer through emulsification can help alleviate the effects of adverse environmental factors on the oxidation of n−3 PUFAs. To address health risk concerns, chemical and synthetic emulsifiers are currently being avoided and substituted with natural interface stabilizers, including proteins, polysaccharides, and phospholipids. Recently, the stability of n−3 PUFAs-rich emulsions was examined using two milk proteins, namely sodium casein and whey protein isolate. Both proteins create an interface on the oil droplet film, resisting droplet breakage through robust repulsive interactions.[71] Furthermore, the stabilizing capabilities of hydrolysed whey protein (WPH), blue cod protein, and soy protein were also evaluated for n−3 PUFAs emulsions.[72] Recently Jagtap *et al*[73] has created an innovative formulation for the stabilized n-3 FA fortification and micronutrient enrichment with flaxseed oil, water, and sucrose ester (an emulsifier).[1] Important minerals, antioxidants, and plant-based n-3 FA were all included in the designed formulation. This product has the potential to improve the nutritional quality and bioavailability of n-3 FA in food and nutraceutical industries.[73] Zinc, vitamin E, magnesium, vitamin B3 and vitamin B6 were added by considering their metabolic effects.[1] The bioavailability of n−3 is significantly influenced by micronutrients and co-factors.[74-76] Fundamental aspects of emulsion formula and their subsequent use in different industries are being assessed in order to improve product quality and its potential for industrial applications [77]

Microencapsulation

Microencapsulation is a process of converting an emulsion to powder form through drying and subsequently encapsulation to form a solid functional

ingredient to improve its handling, storage, or stability.[78,79] Normally, oil containing n−3 PUFAs is emulsified with water and then dissolved in materials used for microencapsulation. The mixture is then dehydrated to powder form as a microcapsule of oil droplets surrounded by microencapsulated material.[80] The choice of wall material relies on factors such as packing, encapsulation efficiency, and chemical stability. It must be water-soluble and capable of forming a low-viscosity fluid, facilitating pumping during processes like spray drying or electro-spraying. Additionally, it should hinder gas diffusion and unwanted chemical reactions, particularly oxidation, and possess a suitable glass transition temperature. This temperature is crucial as the material is rigid and brittle below this point and soft and rubbery above it. Recently, Solomando *et al* [81] used chitosan and maltodextrine as a wall material to created fish oil microcapsules to be added in various commercial meat products. These microcapsules enhanced the amount of EPA and DHA in cooked and dry-cured meat products without changing the physico-chemical properties or oxidative stability of n-3 FA.

Spray Drying

The most common method employed for the industrial-scale microencapsulation of n−3 PUFAs is spray drying.[82] This process commences with the homogenization of oil-in-water to create an emulsion, which is then blended with wall materials such as casein, sugar beet pectin, lactose, dextrose, maltodextrin, starch, glucose syrup, gum Arabic and gelatine.[83,84] Subsequently, the mixture is pumped through a slender nozzle into a chamber that is heated, generating small droplets that swiftly undergo drying and are ultimately collected in powder form. The average diameters and morphology of the spray-dried powder had an impact on the stabilities n-3 FA. One of the crucial element in determining the surface-to-oil ratio in the spray-dried powder is the ratio of the oil droplet's diameter to the particle's diameter. Recently, Sultana *et al* [85] employs a kinetic model featuring fundamental mass transfer phenomena and chemical reactions to examine the stability of PUFAs in spray-dried powder. Degradation or oxidation kinetic constant and mechanism number can be used in the Avrami equation to evaluate the stability of n-3 FA in powder form.[85] Some of the major disadvantages of this method are the use of high temperatures and chemicals which can degrade n−3 PUFAs and reduce the nutrients. Also, the drying of the emulsion from the outside may leave the final product with some internal humidity.

Microfluidic Jet Spray Drying Technology

This technique produces microcapsules with uniform and larger particles of n−3 PUFAs emulsions. The method involves the preparation of whey protein isolate microcapsules of stabilized DHA oil, yielding uniform and consistent droplets. This is achieved using an airflow rate of 15 L/min at 160◦C through a microfluidic aerosol nozzle with a diameter of 75 µm. Whey protein isolate serves to shield DHA from elevated temperatures by creating a heat-induced cross-linked network over the oil droplet surface during the spray drying process 86 The electrospray technology based on the electrohydrodynamics of the solution and dispersed materials, is being used to produce n-3 PUFAs microcapsules in low temperature to decrease the impact of hot air flow on these particles. García-Moreno *et al*.,[87] used charged polymer carriers for the n−3 PUFAs and sprays them onto the opposing electrode in the electric field to disperse into smaller droplets polymer forms of n−3 PUFAs microcapsules after solvent evaporates.

Electro-Spray and Electro-Spin technology

Derived from the electrohydrodynamics of the solution, the electrospray technology uses low temperatures to prepare n−3 PUFAs microcapsules to reduce the effect of flow of heated air.on n−3 PUFAs. This method is considered a mild technique and is more suitable for n−3 PUFAs due to their unstable functional materials. n−3 PUFAs are carried by a charged polymer and sprayed in the electric field towards the other electrode to disperse into smaller droplets. During this process, the n−3 PUFAs disperse into smaller droplets while transitioning from one electrode to another, resulting in the formation of n−3 PUFA microcapsules. These microcapsules can take the form of particles (through electro-spraying) or fibers (via electro-spinning), depending on the specific operating conditions.[88-90] Rahmani-Manglano *et al* [91] used low molecular weight carbohydrates as encapsulating agents during the monoaxial or coaxial electrospraying and spray-drying processes to create the n-3 FA capsules. Electrospraying created encapsulates were reported with lower retention qualities as

compared to spray-drying method with higher encapsulation efficiency.[91]

Freeze Drying

This method converts omega-3 oil into a powder by freezing it at -90 to -40 °C followed by dehydrating it under a vacuum.[92] This method is impracticable than spray drying for the reasons that it is costly, time taking, and providing less efficient outputs.[93]

Extrusion

This technology uses extrusion to convert omega-3 oils into solids. The molten wall material is mixed with emulsified oil subjected to elevated pressure and then passed through a slender nozzle to produce hardened microcapsules.[94] Although this technique produce commercial microcapsules on a large scale, the use of high temperatures promotes the oxidation of the fish oil during the manufacturing process with significant capital and energy expenses.[95]

Quality System Uses to Maintain Omega 3 Supplements for Commercial Use

Quality control and the pharmaceutical ingredients of dietary omega-3 supplements have been well defined by pharmacopeia monographs and typically address the fatty-acid composition, oxidation states, and controlling the presence of any environmental pollutants.[96] n−3 FAs available these days are either non-prescription (e.g., fish oils) or prescription (Rx) medications that are not necessary the equivalent on the basis of their contents.[97] The United States Food and Drug Administration (USFDA) oversees the production of prescription pharmaceuticals and sets criteria for consistency and quality control.[98,99] It is crucial for both consumers and medical professionals to verify the qualitative and quantitative differences between FDA-approved prescription formulations and the dietary supplements that are commercially available.

Oxidative Status

n−3 FAs are among the best labile supplements due to high susceptibility to oxidation and deterioration over time during storage.[100] Exposure of this FA to oxygen in the air can lead to rancidity, deterioration issues, and change of taste and odour.[101] Upon prolonged storage, the nutritional value of the supplement decreases with the formation of unhealthy components such as 4-hydroxy-2-alkenal and 4-hydroxy-2-hexanal.[102] Some of the implemented precautionary procedures to prevent this kind of spoilage are to store the supplement at possible low temperatures, to encapsulate in safe polymer matrices, and to protect it from light.[103] This supplement is commonly available as a soft gel capsule that are mixed with antioxidants in order to reduce lipid oxidation. In addition, these supplements are manufactured using specialized techniques to assist in controlling the oxidation process, such as employing fresh raw ingredients of high quality and a low-temperature extraction method. Specifically, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butyl hydroquinone (tBHQ), propyl gallate, tocopherol, and ascorbyl palmitate are among the antioxidants commonly used in theses supplements to reduce the oxidation process. Such antioxidants can reduce the oxidation process but will likely not prevent it while the speed of oxidization is mostly depending on the composition of FAs, oxygen/ light contact, temperature, and the presence of water and heavy metals. The oxidation process in these FAs significantly affects the composition and produces lipid hydroperoxides as the primary oxidation by-product, which then degrades into secondary oxidation by-products such as aldehydes, ketones, and alcohols.[104,105] The level of secondary oxidation is determined by the para-anisidine value (AV), which provides a measurement of aldehydic compounds (predominantly 2-alkenes and 2,4-alkadienals) while hydroperoxide levels are determined by the peroxide value (PV) in the oxidized oil. The quality of n−3 FAs is determined by calculating the total oxidation (TOTOX) levels by measuring both PV and AV (primary and secondary oxidation levels.106 Although the maximum recommended levels for TOTOX for this supplement have been set by many organizations these standards are based on palatability rather than health.[107]

Actual Versus Claimed Content

EPA and DHA quantity and oxidative status in n−3 FAs supplements are the main indicator of their quality.[108] Many studies have reported the presence of a significant amount of saturated fat in commercially available N−3 FAs capsules which do not comply with the label.[104,109-115] The main reason may be the lack of a proper purification process for removing the impurities originally present in the fish oil. Many countries have extensively examined the composition of n−3 FAs supplements available in their

market. Some samples from the US did not meet the stated label claim of EPA or DHA.[114,115] Furthermore, samples from Poland showed lower levels of FA content compared to the claim stated in the label.[113] Moreover, Brazil reported large amounts of low-cost soybean oil added in EPA and DHA supplements.[109] Another study from New Zealand has found that only 3 out of 32 samples contained the exact or surplus amount of the label claim in terms of EPA and DHA.[116] Alghamdi and co-workers have recently analyzed the commercially available Omega-3 Supplements in three Arab Gulf Countries. Samples from the market of Saudi Arabia, Bahrain and Kuwait were measured for EPA and DHA and did not match the stated label amount.[117] The main reason was attributed to the negligence in monitoring for the quality of fish oil supplements by the manufacturers or companies. Preparation of omega-3 supplements involves impeccable purification levels to remove contaminations like free FAs, heavy metals, and coloured compounds present in the raw material. Accurate purification steps are potentially expensive, and are circumvented by the companies. To that end, the incompliance evidence observed in the literature emphasizes the importance of the post-market surveillance programs conducted by regulators in order to ensure the integrity for those supplements and to provide any corrections/notifications plans to manufacturers.

Harmful Contaminants

Omega 3 supplements are mainly extracted from marine organisms. These organisms are exposed to the dangerous contaminants present in water such as heavy metals and residual pesticides. Bio-accumulation of these harmful chemicals poses a higher risk of toxins in larger fish types. In fish oil harvested for such organisms, toxins in trace amounts remain in the final products.118 Some of the toxins found in these products include lead, mercury arsenic, cadmium, dieldrin, dichlorodiphenyltrichloroethane, selenium, and polychlorinated biphenyls.[118] Although studies have not reported any significant amount of toxins in these supplements, prolonged exposure to it can result in enduring health complications.[119]

Encapsulation

Encapsulating the oil supplement is one of the best ways to protect it from atmospheric oxygen to avoid oxidation.[117] Rancid odor and flavor are not detected in capsulated samples as compared to bottle-stored oil supplements due to the protective effect of packaging against oxidation. It is recommended to encapsulate omega-3 oils in polymer matrices such as Sunflower phospholipids, whey protein concentrate, maltodextrin, β-lactoglobulin, fibrils, and chitosan. Omega-3 products are commonly available in capsules, chewables, and syrups. Syrup forms are more susceptible to oxidation than capsules as capsules are better protected during the storage period. Common materials used to encapsulate n-3 oils are mentioned in section 5 of the present article.

Medical Use, Dosage, and Risk Factors

Currently, the US Food and Drug Administration (FDA) approved two types of prescription n-3 FA medications, one containing both EPA and DHA and with EPA only. Prescription n-3 FA medications are approved only after ample clinical safety and efficacy studies. Available n-3 FA products are icosapent ethyl (the ethyl ester of EPA) contains only EPA, n-3-acid ethyl esters containing both ethyl esters of EPA and DHA, n-3-carboxylic acids contain free FAs mainly EPA and DHA.120 FDA has approved n-3 FA medications only for the treatment of hypertriglyceridemia with a dose of 2–4 g/day depending on the severity of the disease.[112,113] Currently, a lot of research on the possible role of this medication in treating conditions and ailments such as cardiovascular disease, type 2 diabetes, cancer depression, brain development, rheumatoid arthritis, asthma, diabetic retinopathy, and non-alcoholic fatty liver disease is at the peak.[8,121-125] These medications are generally safe with mild side effects such as eructation, stomachache, diarrhea, nausea, and joint pain.[126,127] In some extreme cases adverse reactions such as arthralgia and oropharyngeal pain, increased ALT/AST, pruritus, and rash are also reported.[126-128] Prescription omega-3 FA medications should not be confused with dietary supplements of n−3 FAs which do not require FDA approval and don't need any safety trial or efficacy before marketing. n-3 FA dietary supplements are available without prescription with lower concentrations of EPA and DHA and are not designed to treat any disease.[120] Such supplements don't need FDA approval, hence EPA and DHA levels might differ widely between different brands and products and are derived from different sources mainly from fish and other marine organisms. Studies have reported the therapeutic and protective

role of these supplements against hypertension,[129] coronary heart diseases,[130] atherosclerosis,[131] memory loss, and neurodegenerative disorders such as autism spectrum disorder (ASD), Alzheimer's and Parkinson's diseases.[132-134] EPA and DHA supplements decrease oxidative stress due to their anti-inflammatory and antioxidant properties.[135,136] Mild side effects like unpleasant taste, bad breath, bad-smelling sweat, headache, and gastrointestinal symptoms are reported. However, patients on anticoagulants or nonsteroidal anti-inflammatory drugs should consult a physician before using them as omega-3 dietary supplements may prolong the time for bleeding to stop after a wound.[137,138]

Regulation of Omega-3 at Representative Jurisdictions

Globally, counties have different regulatory requirements for licensing and registration of omega-3 products. In addition, the specifications for the quality and safety of those products may vary according to the laboratory testing requested by a given regulatory authority. Consequently, these two factors may alter the regulatory process for importing countries as well as it could hinder consumer access to such products. We will highlight three representative examples of leading regulatory authorities in this area namely the Health Science Authority (HSA, Singapore), Health Canada (Canada), and Therapeutic Goods Administration (TGA, Australia). HSA, Health Canada, and TGA are classifying omega-3 supplements as health supplements, natural health products, and complementary medicines, respectively.[129,132] According to Health Canada, health supplements are subjected to product licensing to be legally sold in Canada.[140] Also, TGA requires health supplements to be registered in the Australian Register of Therapeutic Goods (ARTG).[142,143] However, HSA does not require licensing and it is the dealers' option to notify HSA of supplying their health supplements in Singapore.[139]

Regarding the quality, safety/efficacy, claims requirements and restrictions, authorities necessitate stringent requirements for manufacturers. Table 1 – summarize authorities' criteria for the aforementioned elements to guarantee omega-3 products compliance to regulations set by each country.

Table 1: Summary of Each Authoritie's Criteria for Quality, Safety/Efficacy and Claims.[139-143]

Requirements Aspect	Regulatory Authority Criteria		
	HSA	**Health Canada**	**TGA**
Quality	Stability and longevity of the product. Conditions for storage and distribution. Manufacturing, packaging, and assembly environments	Precise labeling and packaging specifications. Adherence to Good Manufacturing Practices (GMP).	All complementary medicines must adhere to the principles of Good Manufacturing Practice (GMP). Must undergo a full TGA pre-market evaluation of quality,
Safety and Efficacy	Prohibited substances in the health supplement (e.g., ingredients sourced from human parts, undis-closed active substances, active ingredients known to possess inherent pharma-cological properties). The product should adhere to specified heavy metal limits (As 5ppm, Cd: 0.3 ppm,Pb: 10ppm, Hg: 0.5ppm). The product should comply	Applicants are required to furnish comprehensive information to the authority about the product, encompassing details on medicinal ingredients, their source, dosage, potency, non-medicinal ingredients, and recommended use Substantiating the safety and efficacy of the product requires valid evidence,	safety, and efficacy by the TGA, if at least one of the following applies: • The products contain ingredients beyond those allowed for use in listed medicines or assessed listed medicines. • They carry higher-level indications not permitted for use in listed or assessed listed medicines.

	designated microbial limits: (total aerobic microbial count: NMT 105, yeast and mold: NMT 5×102, absence of Escherichia coli, salmonellae, and staphylococcus aureus).	such as clinical trial data, references to published studies, journals, pharmacopeias, and traditional sources.	• They are required to be sterile
Claims	Products should not be labeled, advertised, or promoted for any explicit medicinal intent, including claims that imply the treatment or prevention of any disease, disorder, or related conditions	While proper evidence is necessary, the specific type and quantity of supporting evidence depend on both the proposed health claim of the product and its overall associated risks.	Registered complementary medicines have the option to include a 'TGA assessed claim' on their label and other advertising indicating the TGA has assessed the evidence for the medicine's indications.

Quality Control of Omega-3 Supplements

Quality control for manufacturing finished products containing n−3 FAs is crucial process, which is typically achieved through performing quantitative chemical tests to ensure compliance to the pharmacopeias and/or international standards. Generally, there are a list of chemical tests required to assessment quality of products which fall under three main categories. Firstly, the assay of active ingredients (EPA & DHA) and the total N−3 FAs . Secondly, oxidation tests which can be measured and calculated by Peroxide value (PV) and Anisidine value (AV). Thirdly, the environmental contaminants particularly polychlorinated biphenyls (PCBs), dioxin (e.g. PCBs) and heavy metals tests.[144]

The determination and quantification of EPA, DHA and total n−3 FAs are heavily depending on source and type of dosage form of active substances (i.e. ethyl ester, triglyceride, free FA, or phospholipids). This fact is clearly shown through observing that fish and krill oil are characterized by a high EPA content, while DHA content is higher in algae oil.[117] Monitoring adherence to declared EPA and DHA levels on product labels is essential, ensuring compliance with regulatory thresholds. In New Zealand and Australia, for instance, regulations stipulate that a product must possess a minimum of 92% of the claimed content.. In contrary, in the USA where n -FAs are classified as natural products, their assay need to be at least 80% of the label claim. In addition, in the EU, a 20% tolerance for the nutrient is considered acceptable.[135] Discrepancies in the regulations between different jurisdictions might explain the regulatory violations in terms of incompliance to the label claim of FAs mentioned earlier in this review.[113-115,117] Consequently, establishing global harmonization and/or common agreement regards the acceptable label claim for those supplements is demanded.

According to different pharmacopeias, the oxidation tests should be performed to satisfy the good quality of products containing n−3 FAs. For example, in British Pharmacopeia (BP) and European pharmacopeia (Ph.Eur), the acid value (AV) acceptable limit is NMT 2.0 while in the United States Pharmacopeia (USP) the limit is NMT 3.0.[146,148] Moreover, the maximum limit of oxidations test of Anisidine value (AV), Peroxide value (PV) and Total oxidation value (TOTOX) is variable in different pharmacopoeias. Specifically, in the USP and BP, the peroxide value (PV) limit is NMT 10 mEq/Kg while in Health Canada and Australian TGA guidelines the maximum limit must be 5 mEq/Kg.149,150 Likewise, the Anisidine value (AV) in BP the maximum limit is 20.0 while in USP it is stated to be NMT 25.0. The slight variations within regulations might alter the quality attributes for those supplements and hence consumers could potentially affect particularly products authorized for prescription use.

Conclusions

n−3FAs supplements are manufactured using multiple industrial techniques with varied ingredients. In addition, such supplements are most sensitive to oxidation and can deteriorate if stored for a long period. Its quality mainly depends on its total

oxidative level and concentration of both EPA and DHA. Some preventative measures include storing the supplement at a low temperature, encapsulating it in secure polymer matrices, and protection against direct sunlight. There are some discrepancies among the quality control tests and the regulations pertaining to the marketing of these supplements, which perhaps raise the flag for the need for global regulatory harmonization. While compliance criteria may differ between different jurisdictions, consumers and healthcare professionals should verify the qualitative and quantitative attributes of n-3 FA dietary supplements before consumption.

Author Disclosure

Disclaimer

The views expressed in this paper are those of the author(s) and do not necessarily reflect those of the SFDA or its stakeholders. Guaranteeing the accuracy and validity of the data is the sole responsibility of the research team.

Author Contributions

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