Acta Scientific NUTRITIONAL HEALTH (ISSN:2582-1423)

Volume 7 Issue 8 August 2023

Review Article

Nanoencapsulation of Omega-3 Fatty Acids and its Beneficial Health Effects

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DOI: 10.31080/ASNH.2023.07.1284

Received: July 03, 2023

Published: July 21, 2023

Abstract

This paper discusses the beneficial effects of omega-3 fatty acids on human health. Nutrient data of some types of fish that contain omega-3 fatty acids in the form of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), as well as the plant sources of omega-3 fatty acids which provide nutrients in the form of alpha- linolenic acid (ALA) are present. An overview of the techniques and formulations used for nanoencapsulation of omega-3 fatty acids or fish oil is given. Considering that omega-3 fatty acids are low water-soluble, and tend to rapid oxidation, nanoencapsulation is a very promising method that prevents their oxidation and instability. In addition, nanoencapsulation facilitates the release of food components and improves bioavailability and digestion in the human body. The creation of nanostructured bio-based delivery vehicles that enable better solubility, and bioavailability of bioactive peptides and poly-unsaturated fats are ensured with the nanoencapsulation. Different omega-3 fatty acids were added as nanoparticles or nanocapsules into the orange juice, probiotic fermented milk, whey protein, into pork meat for the fortification of food.

Keywords: Nanoecapsulation; Omega-3 Fatty Acids; DHA; EPA; ALA

Abbreviations

CAGR: Compound Annual Growth Rate; PUFAs: Polyunsaturated Fatty Acids (PUFAs)ALA: Alpha-Linolenic Acid; EPA: Eicosapentaenoic Acid; DHA: Docosahexaenoic Acid; FDA: Food and Drug Administration; USDA: United States Department of Agriculture; EFAD: Essential Fatty Acid Deficiency; EANS: European Academy of Nutritional Sciences; NO: Nitric Oxide; ADHD: Attention Deficit Hyperactivity Disorder; W/O: Water-in-oil; WPC: Whey Protein Concentrate; US: Ultrasound

Introduction

Omega-3 fatty acids play a significant role in achieving optimal health and in protection against diseases. The polyunsaturated fatty acids in vegetable oils and animal fats are responsible for the energy source, the structural unit of the cell membrane and important physical function. For this reason, these nutrients are extremely important for daily intake through human and animal nutrition [1]. According to the Market research report [2], the global omega-3 fatty acids market is projected to grow from $1.62 billion in 2021 to $2.24 billion in 2028 at a CAGR of 4.80% forecast period, 2021-2028. The recent recognition of the positive health effects of polyunsaturated fatty acids (PUFAs) has created a huge demand. The presence of the first double bond on the third or sixth carbon from the terminal methyl group determines n-3 (omega-3) or n-6 (omega-6) polyunsaturated fatty acids [3]. The three main omega-3 fatty acids are alpha-linolenic acid (ALA), $C_{18}H_{30}O_2$, eicosapentaenoic acid (EPA), $C_{20}H_{30}O_2$, and docosahexaenoic acid (DHA), $C_{22}H_{32}O_2$.

Docosahexaenoic acid (DHA) type dominated the market with the highest revenue share of 62.4% in 2022. This is attributed to the growing utilization of the product in infant formulas to improve the overall health of an infant. In the human diet, DHA is the most essential and abundant omega-3 fatty acid, which helps in maintaining the health of the human body. DHA is a crucial component for the development and maturity of an infant's brain and eyesight. Moreover, it is widely used in nutritional supplements and fortified foods. It is also used in treating dementia, attention deficit hyperactivity disorder, coronary artery disease (CAD), and Type 2 diabetes. The advantages of DHA for health and longevity have also resulted in its use in geriatric nutrition products.

The demand for Eicosapentaenoic acid (EPA) is anticipated to increase globally during the forecast period owing to several health benefits such as it helps in avoiding coronary heart diseases, high blood pressure, inflammation, and excessive triglycerides (blood fats). It is also used for treating diabetes, addressing chemotherapy-related side effects, and enabling recovery from surgeries among others.

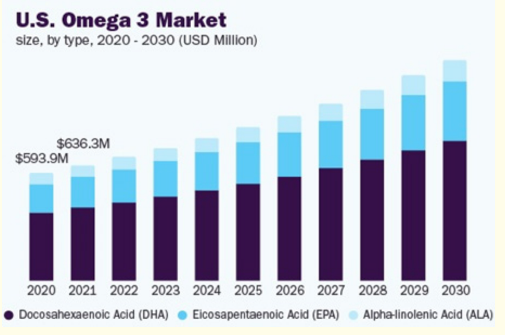

Figure 1: Prediction of the global omega 3 market by Grand view research.

The global omega 3 market was valued at USD 2.43 billion in 2022 and is anticipated to expand at a compound annual growth rate (CAGR) of 7.8% from 2023 to 2030 [4]. The demand for omega-3 fatty acids in the U.S. is projected to grow significantly over the next few years owing to rising consumer awareness in the country regarding the several health benefits offered, including lowering blood pressure, cholesterol, and risks related to heart diseases. According to the Grand view reseearch, the application of omega 3 is divided into a supplements and functional food, pharmaceuticals, infant formula, animal feed and pet food and other. The supplements and functional foods application segment dominated the market with the highest revenue share of 52.0% in 2022.

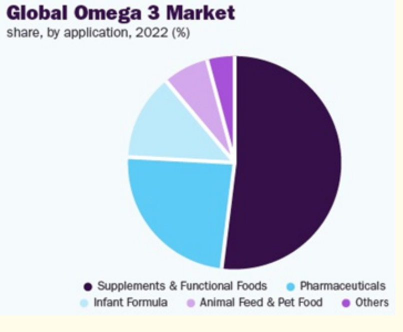

Figure 2: Application of omega 3.

ALA is found mainly in plant oils such as flaxseed, soybean, and canola oils. DHA and EPA are found in fish and other seafood. Omega-3s are found naturally in some foods and are added to some fortified foods (Table 1).

Inuit populations in Greenland that consumed large amounts of fish (and high levels of EPA and DHA) had a low incidence of cardiovascular disease and rheumatoid arthritis [6]. Eicosapentaenoic acid (EPA) is found in cold-water fatty fish, such as salmon. It is also found in fish oil supplements, along with docosahexaenoic acid (DHA).

Omega-3 fatty acids in some foods and fortified foods	Source
Fish and other seafood	Salmon, mackerel, tuna, herring, and sardines
Nuts and seeds	Flaxseed, chia seeds, and walnuts
Plant oils	Flaxseed oil, soybean oil, and canola oil
Fortified foods	Certain brands of eggs, yogurt, juices, milk, soy beverages, and infant formulas

Table 1: Omega-3 fatty acids in some foods and fortified foods [5].

The list of nutrient data of some types of fish that contain omega-3 fatty acids (DHA + EPA) in 3 ounces (oz.) or 85 gram provided by the U.S. Department of Agriculture (Table 2). These totals reflect the DHA and EPA content in raw (uncooked) fish.

Type of fish (3 oz. serving or 85 gram)	Omega -3 content (DHA + EPA), grams
Mackerel	2.0
Salmon (farmed, Atlantic)	1.7
Herring (Atlantic)	1.3
Anchovy	1.2
Salmon (wild, Atlantic)	1.2
Whitefish	1.1
Tuna (Bluefin)	1.0
Halibut (Greenland)	0.8
Sardines (Atlantic, canned in oil)	0.8
Tuna (Albacore, canned in water)	0.7
Bluefish	0.7
Stripped bass	0.6
Rainbow trout (wild)	0.5
Tuna (light, canned in water)	0.5

Table 2: Nutrient data of some types of fish that contain omega-3 fatty acids (DHA + EPA) in 3 ounces (oz.) or 85 gram.

If someone does not eat fish for certain reasons (allergy, vegetarian or vegan diet), then they can look for plant sources of omega-3, which provide nutrients in the form of ALA. One of the best sources of ALA is whole or ground flaxseed, which can be added in the amount of 2 tablespoons to food during the day (sprinkled in oatmeal, smoothies or yogurt). Other sources of ALA include: algae oil, canola oil, chia seeds, edamame, flaxseed oil, soybean oil and walnuts [7]. Food sources of omega-6s, also called LAs, include: canola oil, soybean oil, corn oil, sunflower oil, safflower oil, chia seeds, walnuts, hazelnuts, almonds and Brazil nuts.

The U.S. Food and Drug Administration (FDA) has established a Daily Value (DV) of 85 g for total fat but not for omega-3s. Thus, Table 3 presents the amounts of omega-3 fatty acids in grams per serving only and not the percent of the DV.

Food	Grams per serving		
	ALA	DHA	EPA
Flaxeed oil, 1 tbsp	7.26		
Chia seeds, 1 ounce	5.06		
English walnuts, 1 ounce	2.57		
Flaxseed, whole, 1 tbsp	2.35		
Canola oil, 1 tbsp	1.28		
Soybean oil, 1 tbsp	0.92		
Black walnuts, 1 ounce	0.76		
Mayonnaise, 1 tbsp	0.74		
Edamme, frozen, prepared, ½ cup	0.28		
Refried beans, canned, vegeterian, ½ cup	0.21		
Salmon, Atlantic, farmed, cooked, 3 ounces		1.24	05.9
Salmon, Atlantic, wiled, cooked, 3 ounces		1.22	0.35
Herring, Atlantic, cooked, drained, 3 ounces*		0.94	0.77
Sardines, canned in tomato sauce, drained, 3 ounces*		0.74	0.45
Mackerel, Atlantic, cooked, 3 ounces*		0.59	0.43
Salmon, pink, canned, drainned, 3 ounces*	0.04	0.63	0.28
Trout rainbow, wild, cooked, 3 ounces		0.44	0.40
Oysters, eastern, wild, cooked, 3 ounces	0.14	0.23	0.30
Shrimp, cooked, 3 ounces*		0.12	0.12
Lobster, cooked, 3 ounces*	0.04	0.07	0.10

Table 3: ALA, EPA and DHA content of selected food [8].

*Except as noted, the USDA database does not specify whether fish are farmed or wild caught.

**The USDA database does not specify whether beef is grass fed or grain fed.

Intake recommendations for fatty acids and other nutrients are provided in the Dietary Reference Intakes (DRIs) developed by the Food and Nutrition Board of the Institute of Medicine (IOM) (now called the National Academy of Medicine). Table 4 lists the current Adequate Intakes (Ais) for omega-3s in grams per day [9]. Human milk contains omega-3s as ALA, EPA and DHA, so the IOM established an AI for infants from birth to 12 months that is equivalent to the mean intake of omega-3s in healthy, breastfed infants. For infants, the AIs apply to total omega-3s. For ages 1 and older, the AIs apply only to ALA because ALA is the only omega-3 that is essential. The IOM did not establish specific intake recommendations for EPA, DHA or other LC omega-3s.

Age	Male	Female	Pregnancy	Lactation
Birth to 6 months*	0.5g	0.5g		
7-12 months*	0.5g	0.5g		
1-3 years**	0.7g	0.7g		
4-8 years**	0.9g	0.9g		
9-13 years**	1.2g	1.0g		
14-18 years**	1.6g	1.1g	1.4g	1.3g
19-50 years**	1.6g	1.1g	1.4g	1.3g
50 + years**	1.6g	1.1g		

Table 4: Adequate intakes (AIs) for Omega-3s [9].

*As total omega-3s

**As ALA

The product is used in various applications such as dietary supplements, functional foods, and pharmaceuticals.

Krill oil is one of the widely used sources of omega-3 owing to its superior properties as compared to traditional fish oil.

Regular consumption of adequate quantities of lipids rich in omega-3 fatty acids is claimed to provide a broad spectrum of health benefits, such as inhibiting inflammation, cardiovascular diseases, diabetes, arthritis, and ulcerative colitis.

Essential fatty acids are fatty acids the body cannot produce on its own. They play a key role in various bodily functions, including heart health, cancer prevention, cognitive function, skin health, and obesity prevention. Essential fatty acid deficiency (EFAD) is rare, especially in people who eat a varied diet. Certain conditions that affect absorption or metabolism may cause EFAD. Symptoms of EFAD may include:hair loss, a dry, scaly rash, slow wound healing and decreased growth [10].

Side effects from omega-3 fish oil may include: a fishy taste in mouth, fishy breath, stomach upset, loose stools and nausea. Taking more than 3 grams of fish oil daily may increase the risk of bleeding [11].

According to the Institute of Medicine at the National Institute of Health (NIH) in the USA the recommended intake of omega-3 oils for adults is 1.1 to 1.6 g per day of. However, the European Academy of Nutritional Sciences (EANS) recommends an average intake of 0.2 g of omega-3 oil per day [12].

Lipids isolated from many marine sources are a rich source of long-chain polyunsaturated fatty acids (PUFAs) in the omega-3 form and have high biological activities. Functional food products designed to enhance human health and wellbeing are increasingly being fortified with these omega-3 polyunsaturated fatty acids because of their potential nutritional and health benefits [13].

According to the number of published papers in the last ten years for the searched phrase "Nanoencapsulation of Omega-3 Fatty Acids" on 13.02.2023., it can be notice that this is the beginning of research, because the total number of research papers available on ScienceDirect at the starting of 2023 is only 244 since 2006. Diagram 1 shows the growth trend of published scientific and review papers in the last ten years. It can be concluded that more intensive research started two years ago [14].

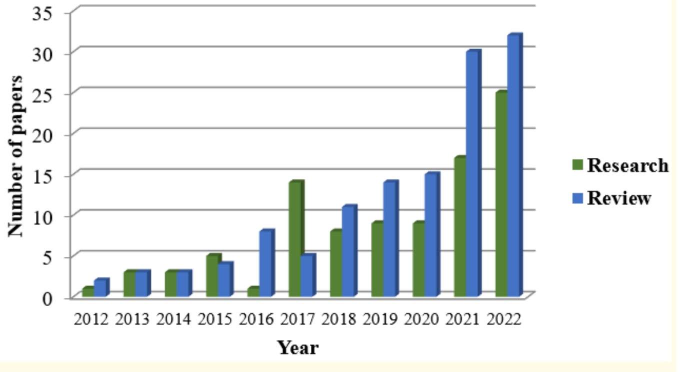

Figure 3: Number of published scientific and review papers in the last ten years.

Beneficial health effect of omega-3 fatty acids

The potential benefits of omega-3 have been studying for years. It has been shown that these nutrients are the key factor in reducing inflammation levels, a major risk factor for multiple chronic diseases[15,16]. In fact, omega-3 could be crucial to cardiovascular, nervous, and immune systems [17].

Researchers have hypothesized that higher intakes of omega-3s from either foods or supplements might reduce the risk of cancer due to their anti-inflammatory effects and potential to inhibit cell growth factors [18]. Some studies have shown associations between higher intakes and/or blood levels of omega-3s and a decreased risk of certain cancers, including breast and colorectal cancers [19-20]. Other studies have found no associations between omega-3s and cancer risk, and some have even found associations in the opposite direction, suggesting that omega-3s might *increase* the risk of certain cancers such as prostate cancer [21-23].

Omega-3 are fundamental in making hormones that regulate blood clotting, the contraction and relaxation of artery walls, and inflammation. Omega-3 fatty acids may also improve the functioning of the endothelium (a thin membrane that lines the inside of the heart and blood vessels). That is because they may help control how much fluid is carried with the blood, and how blood vessels dilate and constrict [24]. The research carried out by [25] has shown that nanoencapsulation of EPA:DHA 6:1 is an attractive strategy to enhance the beneficial effect at the vascular endothelium. They show that treatment of endothelial cells (Ecs) with coated EPA:DHA 6:1 nanoparticles cause greater and more sustained formation of nitric oxide (NO) and enhanced their anti-aggregatory effects.

Omega-3 fatty acids may affect the composition of our gut microbes, which in turn may have a positive impact on our gut health. Since that the digestive system is the first line of defense against harmful microbes, omega-3 may have an indirect, yet wide-ranging, effect on whole immune system. These fatty acids have also been shown to stimulate the production of antibodies and regulate the functioning of white blood cells [26].

Omega-3s may be essential for the proper functioning of our central nervous system, and the brain in particular. In fact, they have been shown to prevent or slow the progression of neurodegenerative diseases such as Parkinson's and Alzheimer's disease [27].

Omega-3 fatty acids are found in abundance in the brain cell membranes, and they may affect how neurons communicate with each other [28].

DHA is one of the key components of healthy brain and eye development. This particular fatty acid may also play a significant role in mental health throughout early childhood. Evidence suggests that low intake of omega-3 may increase the risk of developing Attention Deficit Hyperactivity Disorder (ADHD), autism, bipolar disorder, and depression [29].

Omega-3 is a significant structural component of the retina, and it plays an important role in eye health. Omega-3 optimizes the variability of photoreceptor membranes, retinal thickness, function and provides a protective role. Healthy levels of DHA also protect against damage from bright light exposure and oxidative stress.

Gong., *et al*. 2017 [30] suggested that omega-3 fatty acids may even protect against neovascular eye diseases, such as diabetic retinopathy and age-related macular degeneration. Both of these conditions may lead to blindness, and they both lack treatment options that would be free of adverse side effects.

One of the lesser known benefits of omega-3, and EPA in particular, is that it may contribute to healthier skin. Omega-3 may help improve the skin hydration and balance out its oil production, as well as reduce the risk of premature aging. In fact, these fatty acids may be the key to effective management of inflammatory skin diseases [31].

According to Balić et al. 2020, combined omega-3 and omega-6 supplementation appears to be effective at treating the symptoms of atopic dermatitis, psoriasis, and acne [32].

According to Orchard., *et al*., 2012 [33], omega-3 may help improve bone mineral density and several bone turnover markers. Omega-3 may particularly affect the levels of protein hormone osteocalcin involved in maintaining and regenerating bone tissue, and postmenopausal women are at particularly high risk of developing osteoporosis [34].

It has been recently found that different sources of omega-3 may have a different effect on bone remodeling processes. For example, a flaxseed oil may be better at improving bone structure, while fish oil may promote higher bone mineral density [35].

Encapsulation

Encapsulation is the process of entrapping, encloasing, coating, or surrounding a gas, liquid, or solid active compound within a material to achieve a more controlled release, protect the active ingredient compound from degradation before reaching the site of absorption or before reaching the site of action [36]. In general, embedding of bioactive ingredients into micro-/nanosized capsules to protect them against deteriorating environmental conditions such as high temperatures, oxygen, light, pH variations, undesired interactions with other compounds are known as micro-/nanoencapsulation processes [37,38].

Nanoencapsulation

There are five different nanoencapsulation technologies based on the main mechanism/ingredient used to make nanocapsules applicable to food bioactive ingredients and nutraceuticals. They include lipid-based techniques, nature-inspired techniques, specializedequipment techniques, biopolymer-based techniques [39], and other miscellaneous techniques [40]. Nanoencapsulation packs substances in miniature by using techniques such as nanocomposite, nanoemulsification and nanostructuration and provides final product [41-42]. Nanoencapsulation of food components is an emerging and innovative field for controlled and targeted delivery with various prophylactic activities. The technology encompassing nanoencapsulation facilitates the release of food components, improvement in bioaccessibility, and digestion in the human body [43].

Polyunsaturated fatty acids are characterized by low water-solubility, their tendency to rapidly oxidize, and their variable bioavailability. These challenges can be addressed using advanced encapsulation technologies, which typically involve incorporating the omega-3 oils into well-designed colloidal particles fabricated from food-grade ingredients, such as liposomes, emulsion droplets, nanostructured lipid carriers, or microgels. These omega-3-enriched colloidal dispersions can be used in a fluid form or they can be converted into a powdered form using spray-drying, which facilitates their handling and storage, as well as prolonging their shelf life [13].

Therefore, encapsulation of omega-3 fatty acids proved to be the most successful method for preventing their oxidation and instability, which greatly improved their use in various food products. In the following, a large number of researches for the formulation and creation of nanoencapsulation will be presented.

A stable proteinepolysaccharide complex between 0.1 g/100 ml of sodium caseinate and 0.2 g/100 ml of gum arabic at pH 4 and used for nanoencapsulation of fish oil has been prepared by Ilyasoglu *et al.*, 2014 [44]. Fish oil nanocomplex containing 40-50-60 mg EPA + DHA were used in the enrichment of 100 ml fruit juice. After in vitro digestion, the bioaccessibility of EPA, DHA and EPA + DHA were found as 56.16 ± 6.39, 36.25 ± 5.38 and 47.37 ± 10.65 percent, respectively ($p < 0.05$).

In other study, β-cyclodextrin (BCD) was used for encapsulation of fish oil and its addition into yogurt for fortification. The results showed that encapsulation of fish oil with BCD significantly increased DHA and EPA, while reduced the acidity, peroxide value, and syneresis of yogurt [45].

Different techniqs to improve encapsulation

da Silva Stefani., *et al*. 2019[46] prepared spray dried nanoparticles (SP LO-NP) by nanoencapsulation of linseed oil nanoparticles (LO-NP) and chia seed mucilage (CSM) used as structuring material for the enrichment of orange juice. Consumers have estimated that there is no significant differences between pure orange juice and orange juice with SP LO-NP. A good bioaccessibility to linseed oil after *in vitro* digestion was provided by the SP LO-NP, which represents an advantage to incorporate the nanoparticles in food.

Due to low solubility, bioactive peptides and polyunsaturated fats present in the marine environment are poorly bioavailable and have unfavorable interactions with the food matrix that can be overcome with nanostructured of bio-based delivery vehicles [47].

Fish oil was nanoencapsulated with gum arabic (GA) by freeze drying technique in order to protect their oxidation and degradation during its storage [48]. Fish oil 6% was dispersed in aqueous solutions of 20% and 25% of GA and sonicated at 24 kHz for 120 s to prepare a water-in-oil (W/O) emulsion. After freeze-drying, the nanocapsule were formed and incorporated into probiotic fermented milk. The highest encapsulation efficiency (EE) (87.17%)

was achieved with the nanoparticles encapsulated with 25% gum arabic and 4% emulsifier. Nanoencapsulated fish oil in fermented milk significantly increased the viability of *Lactobacillus plantarum* as well as (EPA) and (DHA) contents and probiotic bacterial count (8.41 Log CFU/mL).

The submicron droplets of EPA oil were encapsulated into whey protein concentrate (WPC) spherical microparticles by electrospraying assisted pressurized gas (EAPG). The encapsulated EPA-rich oil showed increased oxidative and thermal stability in comparison with the free oil. However, they showed a somewhat reduced organoleptic impact in contrast with the neat EPA oil using rehydrated powdered milk as a reference. An overall reduced organoleptic impact was observed for the mixtures of EPA and DHA-loaded microparticles compared to pure EPA [49].

Cationic and non-cationic nanovesicles of fish oil composed of 42% EPA and 16% DHA were prepared by using commercial Pronanosome preparations (Lipo-N and Lipo-CAT). The diffusion of microencapsulated fatty acids into pork meat was assested with ultrasound, while thin film hydration methodology was employed for encapsulation . Research results of ultrasound (US) treatment at 25 kHz of pork meat (*Musculus semitendinosus*) submerged in the nanovesicles suspension have shown that the content of omega-3 was increased following the US treatment which was higher for Lipo-CAT compared to Lipo-N nanovesicles. Higher levels of saturated fatty acids have shown samples subjected to Lipo-N compared to the Lipo-CAT. An improved fatty acid profile of pork was evident since the omega-6/omega-3 ratio was significantly reduced [50].

Conclusion

The small number of available published research papers on “Nanoencapsulation of omega-3 fatty acids” indicates that this is an early developing research area. In this work, a number of beneficial health effects of omega-3 fatty acids on human health have been demonstrated. Various nanoencapsulation methods and systems have been developed, although most of them have only been produced in the laboratory and only a few have reached the market. According to available economic research, the global increase in funds is estimated from $1.62 billion to $2.24 billion for the period from 2021-2028 for the global omega-3 fatty acids market. Physicochemical properties, required particle size, release properties, delivery method, process cost, and other factors influence the choice of nanoencapsulation technology. The lack of research studies on improving the quality and safety of food products significantly limits the application of nanoencapsulation technology for food products, as well as regulatory problems. Researchers should focus more on improving the shelf life and quality of food products with nanoencapsulated bioactive ingredients, such as delaying the oxidation of oils and fats.

Conflict of Interest

There are no any financial interest or conflicts of interes

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