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High *Sn*-2 Docosahexaenoic Acid Lipids for Brain Benefits, and Their Enzymatic Syntheses: A Review

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ABSTRACT

The normal development and maintenance of central neural functions are highly correlated with the amount of docosahexaenoic acid (DHA; ω-3 fatty acid) accumulated in the brain. DHA incorporated at the *sn*-2 position of lipids is well absorbed by intestinal mucosa and utilized efficiently *in vivo*. However, modern consumers have a reduced direct intake of DHA and increased intake of saturated fats or ω-6 fatty acid oils, resulting in behavioral and neurophysiological deficits. To provide an understanding of the integrated beneficial effects of DHA on the human brain, this review introduces the positional difference (*sn*-2 and *sn*-1,3 positions) of DHA on a glycerol skeleton in natural fats and oils, and further discusses the possible functional mechanism regarding DHA supplementation and the gut–brain axis. The multiple bidirectional routes in this axis offer a novel insight into the interaction between DHA supplementation, the gut microbiota, and brain health. To achieve high *sn*-2 DHA in diets, it is suggested that *sn*-2 DHA lipids be enzymatically produced in more efficient and economical ways by improving the specific activities of lipases and optimizing the purification procedures. These types of diets will benefit individuals with strong needs for *sn*-2 ω-3 lipids such as infants, children, and pregnant and lactating women.

1. Introduction

Docosahexaenoic acid (DHA), a 22:6 ω-3 fatty acid (FA), is abundant in the cell membranes of the human brain, and contributes to the normal development of neural and retinal tissues throughout the human life due to its unique structure and multiple double bonds [1,2]. DHA deficiency in the developing brains of fetuses, newborns, and children is generally linked to neuropathology (e.g., cognitive disorders and anxiety) and disorders related to visual function [3,4]. DHA also plays an important role in maintaining cognitive function and emotional performance during adulthood [5].

DHA is traditionally obtained by consuming α-linolenic acid (α-LNA; 18:3 ω-3)-rich diets and marine foods such as fish and algae. However, the conversion efficiency of α-LNA to DHA in individuals usually cannot meet daily requirements, especially for pregnant women and patients with liver or maple syrup urine diseases [3,6,7]. On the other hand, because the agricultural revolution and food industry have caused a shift in modern diets from marine or α-LNA-rich oils (flaxseed oil, etc.) to ω-6 FA-rich oils (soybean oil, palm olein, and corn oil, etc.) and saturated fats, there is a decreased intake of ω-3 FAs and further decreased concentrations of DHA in human milk [8,9]. Therefore, it has been suggested that preformed DHA from fish oils, algal oils, or high-DHA structured lipids (SLs) be added into foods [10]. Studies have shown that mothers who consumed preformed DHA diets accumulated many times more DHA in their milk, in comparison with the milk of vegans [11]. DHA in vegan milk is primarily synthesized from the ω-3 FAs present in vegetable oils.

In general, DHA is esterified to different positions (*sn*-1, 2, or 3) in a triacylglycerol (TAG) molecule depending on various food sources. After oral intake, TAGs are hydrolyzed by *sn*-1,3-specific pancreatic lipase, forming *sn*-2 monoacylglycerols (MAGs) and free fatty acids (FFAs) [12]. The *sn*-2 MAGs are then well absorbed through the intestinal mucosa and are preferentially used for the re-synthesis of TAGs or phospholipids (PLs; important components

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of the brain cell membrane) [13,14]. In contrast, no specific absorption is observed for FFAs hydrolyzed from the *sn*-1 and *sn*-3 positions [15]. Therefore, TAGs with DHA located at the *sn*-2 position are more favorable in terms of absorption and utilization compared with those that have a random DHA distribution [16]. Similarly, *sn*-2 DHA MAGs showed significantly higher absorption efficiency than other derivatives such as DHA-diacylglycerol (DAG) and DHA-ethyl ester [17,18]. However, most of the current DHA recommendations and supplementations do not pay attention to its positional distribution, and are only focused on the total amount of its daily intake.

Given that the positional arrangement of DHA in TAG and PL structures influences its pharmacological and nutritional benefits for human brain development and maintenance, it is worth providing a background on DHA distribution in common fats and oils, and on the brain benefits provided by high *sn*-2 DHA lipid diets. The technological procedures of enzymatic syntheses to produce *sn*-2 DHA-rich SLs and their typical analysis methods are also discussed in this review.

2. *Sn*-2 DHA in natural and synthesized lipids

DHA is generally provided by marine fish oils and single-cell oils [19]. There are four main types of DHA lipids from natural sources: *sn*-2 DHA TAGs, DAGs, MAGs in fish and algal oils, and *sn*-2 DHA PLs in krill oils and egg yolk (Fig. 1).

The position distribution of DHA on a glycerol skeleton in common fats and oil are summarized in Table 1 [20–31]. Single-cell algal oils (e.g., *Schizochytrium* sp. oil and *Crypthecodinium cohnii* oil) contain the highest total DHA levels, ranging from 44.89% to 48.20%, followed by various fish oils such as tuna oil, sardine oil, anchovy oil, and salmon oil (9.76%–26.85%). In contrast, the relative percentages of *sn*-2 DHA were higher in fish oils than in algal oils. Approximately 44.79%–72.99% of the total DHA in fish oil TAGs were esterified at the *sn*-2 position, while the numerical values were 31.66%–42.09% in algal oil TAGs. This difference might result from the absorption characteristics of *sn*-2 DHA lipids mentioned above. That is, the DHA synthesized in algal oil is eaten by fish through the food chain; *sn*-2 DHA MAGs or DAGs are then produced through digestion and absorption, and are further used to resynthesize TAGs, which increases the *sn*-2 DHA percentages in fish oils to some extent [15].

In particular, the lipids in egg yolk and krill oils are primarily present as PLs (Fig. 1), which are quite different from the lipids in fish and algal oils. Different lipid classes might influence DHA absorption and its concentration in the brain. Diets containing krill oil have been found to increase the DHA levels in rat brain as PLs, and PLs were found to be the major components of both the krill oil and brain cell membranes [32].

DHA also makes up a small proportion (0.36%–0.70%) of total FAs found in human milk fat (HMF) TAGs, and more than half (52.63%–65.15%) is incorporated at the *sn*-2 position (Table 1). However, the percentages decreased from colostrum to mature milks (0.56%–0.70%→0.36%–0.44%), while the relative percentages of *sn*-2 DHA increased from 52.63%–55.71% to 61.39%–65.15%. In addition, DHA levels were found to be progressively lower in nursing mothers who had given birth to twins or had given birth in rapid succession [33,34]. Clinical studies showed that feeding with α-LNA but without DHA over the first six months of life cannot sustain normal DHA concentrations in infant brains [35]. The low conversion rates of α-LNA to DHA in newborn and breast-fed infants were also confirmed in this case. It is further concluded from Table 1 that most of the current infant formula fats (IFFs) contain a lower total amount of DHA and *sn*-2 DHA (the relative percentages were 27.56%–48.17%) in comparison with HMFs. In 11 evaluated IFFs in Spain, only one IFF contained DHA at the *sn*-2 position [29]. However, 70–80 mg of DHA per day from breast milk is suggested to meet the increasing demand of the rapid growth of a baby's nervous system [34]. It is therefore suggested that DHA supplementation—especially of *sn*-2 DHA lipids—in

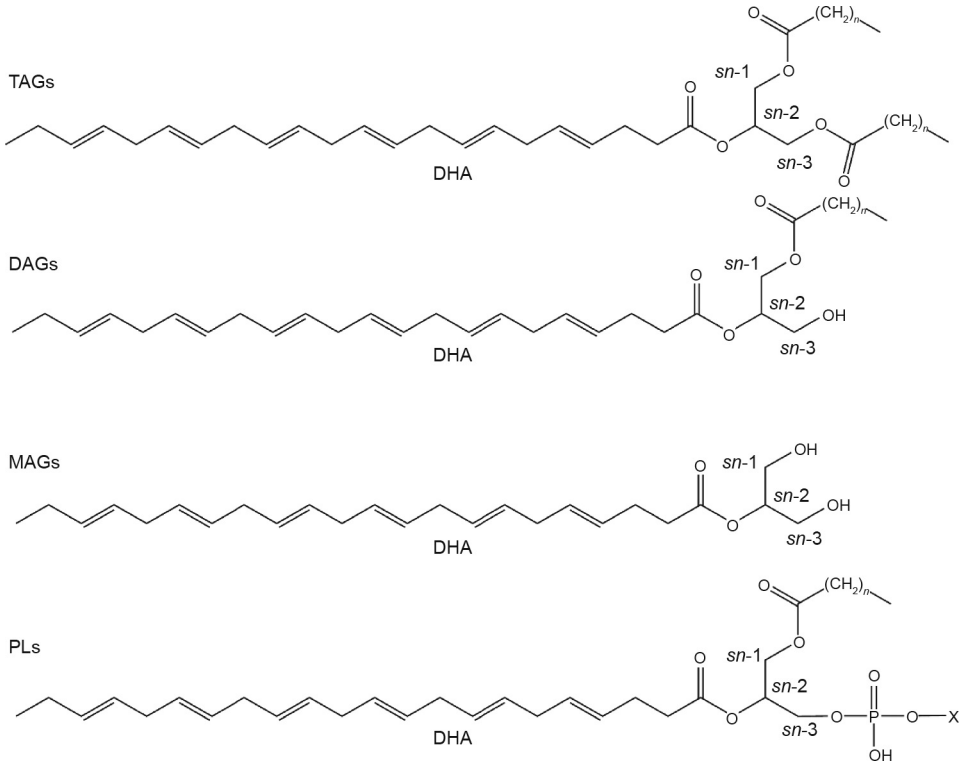

Fig. 1. Primary molecular structures of *sn*-2 DHA lipids. X: ethanolamine, choline, serine, inositol, etc.

Table 1
Position distribution of DHA on a glycerol skeleton in foods and infant formulas.

Sources	Total DHA (%)	*Sn*-2 DHA (%)	Relative percentage of *sn*-2 DHA[a] (%)
Salmon oil [20]	9.99	12.62	50.61
Anchovy oil [20,21]	9.76–10.04	11.59–20.88	49.28–71.31
Tuna oil [20,22]	21.94–26.85	25.88–36.08	44.79–49.00
Sardine oil [23]	10.30–13.90	21.10–29.40	60.67–72.99
Schizochytrium sp. oil [24]	48.20	60.86	42.09
Crypthecodinium cohnii oil [25]	44.89	42.64	31.66
Egg yolk PL [26][b]	2.74	2.89	—
Shrimp (*P. borealis*) oil [27][b]	8.3	7.1	—
HMF in Wuxi (China) [28][c]			
Colostrum	0.70	1.17	55.71
Transitional	0.61	1.07	58.47
Mature	0.44	0.86	65.15
HMF in Spain [29]			
Colostrum	0.56	0.93	52.63
Transitional	0.50	0.81	56.80
Mature	0.36	0.64	61.39
IFF in China [30]	—	0.09–0.21	27.56–33.13
IFF in Spain [29]	ND–0.20	ND–0.28	ND–48.17
IFF in America [31]	0.39	0.49	41.88

HMF: human milk fat; IFF: infant formula fat; ND: not detectable.
[a] Relative percentage of DHA at *sn*-2 position was calculated as [*sn*-2 DHA percentage/(DHA percentage in TAG × 3)] × 100% [30], or reported by the literature.
[b] The data was shown as mol%.
[c] HMF collected after birth at Days 1–5 was colostrum, at Days 6–15 was transitional, and at more than 15 days was mature.

maternal diets may protect infants from deficits in neurodevelopment [4].

3. Positive effects of *sn*-2 DHA on brains

3.1. DHA accumulation in brains by utilizing sn-2 DHA lipids

Lipids account for approximately 60% of the dry weight of brain tissue [34]. Although DHA is a critical component in maintaining proper brain and nervous functions, its location on a glycerol skeleton exhibits significantly different efficiencies in terms of absorption and utilization. It is much easier for DHA to be absorbed by the intestinal mucosa when it is incorporated at the *sn*-2 position than when it is randomly distributed at the *sn*-1,2,3 positions [16]. Further studies have revealed that DHA levels in brain PLs, such as phosphatidylserine and phosphatidylcholine (PC) of newborn rats fed *sn*-2 DHA diets, were significantly improved compared with those in rats that were fed milk diets (Table 2) [36]. Also, *sn*-2 lysophosphatidylcholine DHA was preferentially utilized in the rat brains in comparison with unesterified DHA (Table 2) [37]. In addition, large-scale trials have concluded that DHA supplementation through the consumption of large doses of marine oils is safe during pregnancy [38].

Table 2
Brain benefits of *sn*-2 DHA lipids.

Treatments	Findings	Reference
Newborn rats were fed diets containing 7.0% fat (3.70% DHA and 6.18% *sn*-2 DHA for structured oil group; 3.98% DHA and 3.57% *sn*-2 DHA for randomized oil group; and 0.66% DHA in rat milk for reference group)	DHA levels of brain phosphatidylserine and PC were significantly increased compared with the reference after three weeks, but no differences were observed in phosphatidylethanolamines and phosphatidylinositols	[36]
A solution containing *sn*-2 lysophosphatidylcholine DHA or unesterified DHA was injected into the tail veins of 20-day-old male rats for 30 s, respectively. Their tissue lipids were analyzed from 2 to 60 min after the injection	The developing (young) brain preferentially utilized *sn*-2 lysophosphatidylcholine DHA rather than unesterified DHA	[37]

3.2. DHA supplementation improves brain functions through the gut–brain axis

Emotional disorders, which are one of the results of brain function deficits, have been found to be specifically associated with gut microbiota alterations [39]. There has been recent interest in the possible correlation between brain problems (e.g., brain injury, declined cognition, schizophrenia, stroke, anxiety, stress, and depression) and intestinal microflora. The human intestines contain more than 1000 microbiota species with 100 trillion living microorganisms [40]. Bacterial colonization of different species could alter brain functions, and in turn, the central nervous system is speculated to indirectly influence the gut microbial composition. These integrative and bidirectional signaling pathways, which mainly involve the routes of the vagus nerve and spinal pathway, are defined as the gut–brain axis or the brain–gut–microbiota axis (Fig. 2) [41,42].

Previous evidence suggests that gut microbes play an important role in developing therapies for complex brain function disorders. In general, dietary interventions with DHA may have beneficial effects on behavioral and neurophysiological disorders due to alteration of the microbial composition in the intestines [43,44] as seen in Table 3 [45–49].

As shown in Table 3, DHA supplementation for early-life stressed, socially isolated, or aging mice restored and normalized their gut microbiota composition, by increasing the abundance of beneficial species such as *Lactobacillus*, *Bifidobacterium*, and *Bacteroides*, concomitantly decreasing the abundance of *Proteobacteria* (e.g., *Undibacterium*) and *Cyanobacteria*, among others, and subsequently alleviating the mice's brain-related disorders. In addition, García-Ródenas et al. [49] has suggested that psychological stress could be reduced by consuming DHA-containing diets through the normalization of gut permeability without the restoration of the intestinal microbiota. This difference indicates that the gut-brain axis includes various bidirectional routes, some of which have not yet been fully elucidated. More studies are required to explain the potential mechanism of the intestinal microbiome on DHA diet-induced effects on the brain. Also, further studies on

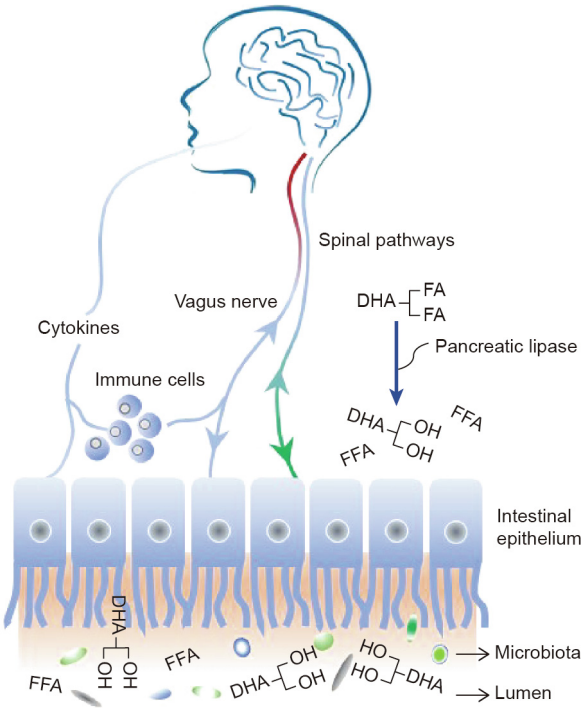

Fig. 2. The gut–brain axis: potential multiple bidirectional routes between the brain and the intestinal microflora [41,42].

the impacts of diets with a DHA positional difference (e.g., high *sn*-2 DHA lipid diets and randomly distributed DHA lipid diets) on the gut–brain axis are necessary.

4. Enzymatic synthesis of high *sn*-2 DHA fats and oils

Many infants and pregnant and nursing women consume foods containing only DHA precursors or limited DHA levels [11]. The decreased dietary DHA consumption that results from following a Western diet is responsible for this problem [50]. The production of modified fats and oils with abundant *sn*-2 DHA using low-pollution and highly efficient techniques such as enzymatic syntheses from saturated fats and the DHA-rich oils listed in Table 1 is encouraged. These processes mainly include the enzymatic reactions of acidolysis, interesterification, ethanolysis, and their combination.

4.1. Acidolysis reactions

Most of the developed methods to produce high *sn*-2 DHA SLs focus on the acidolysis of single-cell oils (e.g., DHA single-cell oil (DHASCO) from alga *Crypthecodinium cohnii*) and FAs (e.g., caprylic acid (C)) in a one-step reaction using *sn*-1,3 specific lipases or lipases with high activity on DHA.

As shown in the acidolysis reactions in Table 4 [25,30,31,51–61], optimal reactions are generally carried out with substrate mole ratios of 1:3–1:18 (oils to FFAs) at mild temperatures of 30–55 °C with 4%–15% enzymes for dozens of hours [51–54]. The *sn*-2 DHA levels vary significantly based on the enzyme species [62]. In some cases, the lipases, such as *Pseudomonas* sp. KWI-56 lipase, showed non-regiospecificity but were active toward DHA and docosapentaenoic acid (DPA), and may also cleave the DHA at the *sn*-2 position, resulting in acyl migration to some extent [52]. This side reaction might easily occur in the presence of caprylic acid and different lipases [63]. It is suggested that possible alternative or better lipases be developed in order to minimize acyl migration. In addition, recovery of the target SLs from these reaction products is usually complicated. Usually, for a small-scale reaction, FFAs are removed by neutralization with alkaline solution, followed by the extraction of TAGs with hexane; the solvent is then further evaporated to obtain the final SLs.

The other typical method to prepare *sn*-2 DHA SLs is to hydrolyze single-cell oils or marine fish oils to prepare DHA, followed by esterification with TAGs (Table 4). In this context, DHA is first released from the marine oils by saponification using potassium hydroxide and acidification using hydrochloric acid in the presence of antioxidants (e.g., butylated hydroxytoluene). Acidolysis of the prepared DHA and other oils is then conducted at substrate mole ratios of 1:5–1:18 (oils to DHA) and with an enzyme load of 10%, and the reaction is kept at 60–65 °C for around 24 h [25,31,55]. For large-scale and industrial reactions, the extra FFAs are commonly removed through short-path distillation.

4.2. Interesterification reactions

Interesterification between DHA-rich oils/ethyl ester and FA ethyl ester is another method to provide targeted SLs (Table 4).

Table 3
DHA absorbed through the intestinal mucosa improves brain functions through the gut–brain axis.

Treatments	Findings	Reference
Early-life stressed female rats were fed with DHA and EPA supplementation of 0.4 or 1.0 g·kg^{-1} daily for 17 weeks, and their fecal pellets were collected for microbiota analysis	High-dose DHA and EPA supplementation restored and normalized the gut microbiota composition of stressed rats. Levels of *Butyrivibrio* and several members of *Actinobacteria* were elevated, with a concomitant reduction of some *Proteobacteria*	[45]
Newborn male mice were fed with DHA and EPA diets for 13 weeks. Their social, depressive, and cognitive behaviors were tested, and fecal microbiota compositions were analyzed	The supplementation improved the neurodevelopment of the mice, with increases in beneficial *Bifidobacterium* and *Lactobacillus* in their gut. In contrast, DHA- and EPA-deficient mice showed social and emotional problems with an increased *Firmicutes*: *Bacteroidetes* ratio	[46]
Socially isolated male and female mice were supplemented with 0.1% or 1.0% by weight DHA. Their fecal pellets were collected for microbiota analysis at 0, 1, and 7 day(s) following the introduction of DHA supplementation	DHA intervention produced beneficial effects on anxiety in male mice, which were correlated with changes in gut microbiota relative abundances, e.g., an increase in *Allobaculum* abundance, which could decrease anxiety- and anhedonia-like behaviors	[47]
Aging mice received tuna oil and/or algal oil for 12 weeks. Their brain biochemical indices and fecal samples were evaluated	DHA-rich diets alleviated age-related decline in cognition by enriching the abundance of *Bacteroides*, *Tannerella*, *Coprobacter*, *Lactobacillus*, and *Prevotella*, and by decreasing the abundance of *Falsiporphyromonas* and *Cyanobacteria*	[48]
Early-life stressed male rat pups were fed with 100 g diets containing 2 g DHA or ARA, along with other components	The adapted diets reverted the negative imprinting of neonatal stress by normalizing intestinal permeability, and further restore the relevant growth rate	[49]

EPA: eicosapentaenoic acid; ARA: arachidonic acid.

Table 4
Enzymatic syntheses of high *sn*-2 DHA SLs.

Substrates	Enzymes	Technical procedure	Products	Reference
Acidolysis reactions				
DHASCO, and C	*Pseudomonas* sp.	SL was produced by esterification of the substrates and purified using hexane	*Sn*-2 DHA level was increased from 25.9% in unmodified oil to 39.9% in SL	[51]
Single-cell oil and C	*Pseudomonas* sp. KWI-56 lipase	Acidolysis was carried out using the substrates with more than 60 mol% lipase	SL contained 36% C-DHA/DPA-C and C–C-DHA/DPA, and the former accounted for 77%–78%	[52]
Tuna oil and C	*Rhizopus delemar*	SL was produced by acidolysis of tuna oil with C and FFA was neutralized with potassium hydroxide-hydroalcoholic solution	SL contained 16.2 mol% DHA, and its *sn*-2 position was occupied by 24.9 mol% DHA	[53]
Fish oil and capric acid	*Rhizomucor miehei*	Acidolysis reactions were carried out in hexane or solvent-free systems, respectively	DHA level obtained from the solvent-free system (28.3 mol%) was higher than that from the hexane system (23.5 mol%)	[54]
DHASCO, palm olein, etc.	Novozym 435	Preparation of DHA by hydrolyzing DHASCO, urea complexation, and solvent crystallization; then it was esterified with palm olein to produce SL	SL contained 17.2% DHA while 22.71% of it was incorporated at the *sn*-2 position[a]	[25]
DHASCO, tripalmitin, etc.	Lipozyme TL IM	DHA was prepared by saponification and acidification of DHASCO; then it was esterified with tripalmitin to produce SL	SL containing 4.80% *sn*-2 DHA was used in infant formula	[31]
DHASCO, olive oil, and tripalmitin	Lipozyme TL IM	FFAs were prepared by saponification of DHASCO and olive oil; SL was then produced by esterification of the mixed FFAs and tripalmitin	SL containing 1.79 mol%–2.57 mol% *sn*-2 DHA was used in infant formula	[55]
Interesterification reactions				
Ethyl DHA, ethyl caprylate, and tricapryloylglycerol	*Alcaligenes* sp. and Novozym 435	SL was prepared by interesterification of ethyl DHA and tricapryloylglycerol, followed by a regioselective ester reaction with ethyl caprylate	SL contained 76.4% C-DHA-C/C–C-DHA, and 82.7% of it was *sn*-C-DHA-C	[56]
Menhaden oil and ethyl caprate	Lipozyme 435	SL was produced by interesterification of the substrates using the Taguchi method	SL contained 9.83 mol%–10.57 mol% DHA, and its *sn*-2 position was occupied by 19.53 mol%–20.79 mol% DHA	[57]
DHASCO	Lipozyme TL IM and Novozym 435	Interesterification of DHASCO was done using mixed enzymes (weight ratio = 1:1) to increase the *sn*-2 DHA percentage	*Sn*-2 DHA in SL oil was improved from 34.3 mol% to 49.7 mol%	[58]
From *sn*-2 DHA MAG to *sn*-2 DHA lipids				
Bonito oil and ethyl caprylate	Novozym 435 and Lipozyme IM	*Sn*-2 MAG was prepared by the ethanolysis of bonito oil using Novozym 435; the MAG was then mixed with ethyl caprylate to produce SL using Lipozyme IM	Proportion of SL with DHA at the *sn*-2 position to that at the *sn*-1 (3) positions was more than 50:1	[59]
Cod liver oil, tuna oil, and C	Novozym 435 and *Rhizopus oryzae*	Novozym 435 was suitable for producing *sn*-2 MAG from fish oils, and *Rhizopus oryzae* was selected to catalyze the esterification reaction of the MAG and C to produce SL	Purified SL contained 37.9% DHA at the *sn*-2 position	[60]
Cod liver oil and C	Novozym 435 and *Rhizopus oryzae*	Alcoholysis of cod liver oil with Novozym 435 was used to prepare *sn*-2 MAGs, followed by incorporating the C at the *sn*-1,3 positions of the MAGs to produce SL	Purified SL contained 38.0% DHA at its *sn*-2 position	[61]

DPA: docosapentaenoic acid.
[a] Relative percentage of DHA at the *sn*-2 position was calculated as [*sn*-2 DHA percentage/(DHA percentage in TAG × 3)] × 100% [30].

The reactions require strict enzyme selection due to their positional specificities and the steric hindrance of DHA [52]. For example, in a two-step reaction, unspecific DHA-rich oil was first prepared from a nonselective reaction of DHA-ethyl ester and tricapryloylglycerol using *Alcaligenes* sp. lipase (50 °C, 90 h), followed by a *sn*-1,3 regioselective interesterification of the unspecific DHA-rich oil and ethyl caprylate using Novozym 435 to produce *sn*-1,3-dicapryloyl-2-docosahexaenoylglycerol (40 °C, 40 h) [56]. Both reactions were carried out in a nitrogen atmosphere to avoid oxidation, and extra esters and tricapryloylglycerol were removed by molecular distillation.

4.3. From sn-2 DHA MAG to sn-2 DHA lipids

Another typical strategy to obtain *sn*-2 DHA-rich lipids is to prepare *sn*-2 DHA MAG from marine oils, followed by the incorporation of needed FAs at the *sn*-1,3 positions of the MAG (Fig. 3 and Table 4).

To achieve this technical route, preparation of *sn*-2 DHA MAG from oils is a key step due to the oxidation problems of DHA, acyl migration during enzymatic catalysis, and the cost [64]. Conventional methods were carried out in an ethanol system with enzymes such as Novozym 435, which showed *sn*-1,3 regiospecificity in the presence of ethanol [59,60]. Recent research has reported a highly efficient approach to produce MAG enriched with ω-3 polyunsaturated fatty acids (PUFAs) at the *sn*-2 position using *Candida antarctica* lipase A in a more economical way [65]. In similar cases, *Candida antarctica* lipase A effectively concentrated the *sn*-2 DHA of anchovy oil from 20.88% in oil to 65.69% at *sn*-2 MAGs via catalytic reaction at low temperature (35 °C) for 12 h; the *sn*-2 DHA value in microalgae oil was increased from 3.24% to 22.20% in the same way [66]. This research demonstrated that *Candida antarctica* lipase A exhibits non-regiospecific and non-ω-3 PUFA preference in an ethanol system, and can thus selectively cleave non-target FAs and further keep the ω-3 PUFAs such as DHA on the glycerol backbone to form DHA-rich MAGs [21,65,66].

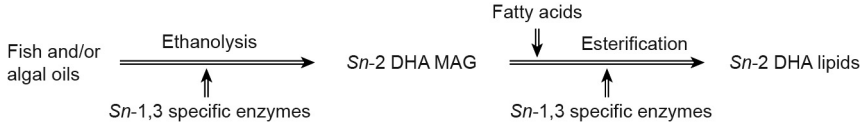

Fig. 3. Typical technique to produce *sn*-2 DHA SLs for various uses.

Table 5
Sn-2 PUFA compositions of fish oils determined by the pancreatic lipase method and the Novozym 435 method [71].

Methods	Cod liver oil		Tuna oil	
	EPA (%)	DHA (%)	EPA (%)	DHA (%)
Pancreatic lipase	10.8	23.4	7.5	27.1
Novozym 435	9.0	30.1	6.8	35.9

For purification, DHA-containing byproducts such as FFAs and their ethyl esters can be removed by short-path or molecular distillation for further re-utilization [67]. The advantage of this technique is its flexibility in manufacturing different fats and oils such as shortenings, margarines, spreads, IFFs, and bakery and confectionary fats using the *sn*-2 DHA MAG.

5. Analytical methods for *sn*-2 DHA

Regiospecific analysis of FAs in TAG molecules is generally conducted on a gas chromatograph equipped with a flame ionization detector. In brief, TAGs are first hydrolyzed by *sn*-1,3-specific lipases to form MAGs, followed by the isolation of *sn*-2 MAG using thin-layer chromatography and its conversion to FA methyl esters for further analysis [68]. Pancreatic lipase is a widely used lipase, which has been well confirmed through the determination of the *sn*-2 FA composition of many fats and oils. However, it should be noted that pancreatic lipase exhibits limited ability to hydrolyze all FAs, particularly PUFAs from marine oils [57]. Its ability for selective hydrolysis depends on the FA species and the location of the double bonds [69]. In contrast, *Candida antarctica* lipase B (Novozym 435 or Lipozyme 435) is suggested to be a better hydrolytic enzyme for this purpose [70,71]. Although Lipozyme 435 is a non-regioselective lipase in many cases, it behaves as *sn*-1,3-specific in the presence of excess ethanol [70]. Table 5 [71] shows the PUFA compositions of fish oils as detected by the Novozym 435 method and the pancreatic lipase method. Novozym 435 can release PUFAs from fish oils at different rates based on the degree of chain length and unsaturation. For example, eicosapentaenoic acid (EPA) levels detected using the pancreatic lipase method (7.5%–10.8%) were higher than those determined using the Novozym 435 method (6.8%–9.0%), while the contents of DHA exhibited the opposite trends [71]. That is, Novozym 435 shows exclusive selectivity for DHA compared with pancreatic lipase.

In general, the Novozym 435 method needs strict hydrolysis conditions, such as ethanol-to-oil ratio, reaction time, and temperature, to completely release the *sn*-1,3 FAs from TAGs; otherwise, the hydrolysis reaction might result in lower results compared with ^{13}C nuclear magnetic resonance (NMR) or predicted values. In a cod liver oil test, the result for *sn*-2 DHA by the Novozym 435 method was 69.4%, which was lower than that measured by ^{13}C NMR (72.5%); however, for analysis of tuna oil, the *sn*-2 DHA results were similar, at 53.1% for the Novozym 435 method and 52.0% for ^{13}C NMR [72].

6. Conclusion

Marine fish and algal oils are typical DHA sources with about half of their FA incorporated at the *sn*-2 position. Their unique structure makes it easier for DHA to be absorbed by the intestinal mucosa and to be used for the re-synthesis of TAGs or PLs *in vivo*, in comparison with molecules that have DHA located at the *sn*-1,3 positions. *sn*-2 DHA lipids, therefore, play important roles in the development of brain functions and in the mitigation of brain deficits such as anxiety, stress, declined cognition, schizophrenia, and stroke. A focus on the gut–brain axis is the most effective strategy to understand the beneficial effects of DHA supplementation on brain functions. It is suggested that brain problems could be alleviated by restoring and normalizing the gut microbial composition through DHA intervention. However, the multiple bidirectional routes of the gut–brain axis are not yet fully understood or explained. Further research is required on the impacts of dietary *sn*-2 DHA lipid supplementation on the gut microbiota and brain functions.

DHA accumulates in the human brain at a rapid rate from gestation to age two. However, although the amount of DHA in HMFs decreases to a low level 15 days after birth, the relative percentages of *sn*-2 DHA show increased trends, indicating the importance of *sn*-2 DHA in the brain development of infants and children. Therefore, it is suggested that preformed *sn*-2 DHA SLs containing *sn*-2 DHA be included in maternal diets; this could be done by preparing *sn*-2 DHA MAG from DHA-rich oils, and then incorporating selected FAs at the *sn*-1,3 positions of the MAG. For further study, it is suggested that novel lipases with high activity at the *sn*-1,3 positions or with a non-ω-3 PUFA preference be developed, together with mild reaction conditions and purification procedures to make the synthesis techniques and products more efficient and economical.

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Compliance with ethics guidelines

Jun Jin, Qingzhe Jin, Xingguo Wang, and Casimir C. Akoh declare that they have no conflict of interest or financial conflicts to disclose.

Nomenclature

α-LNA	α-linolenic acid
ARA	arachidonic acid
DAG	diacylglycerol
DHA	docosahexaenoic acid
DPA	docosapentaenoic acid
EPA	eicosapentaenoic acid
FA	fatty acid
FFA	free fatty acid
PL	phospholipid
HMF	human milk fat
IFF	infant formula fat
MAG	monoacylglycerol
NMR	nuclear magnetic resonance
PC	phosphatidylcholine
PUFA	polyunsaturated fatty acid
SL	structured lipid
TAG	triacylglycerol

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