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Recent advances in phospholipid-DHA: bioactivity for brain health and product development

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ABSTRACT

Docosahexaenoic acid (DHA) is an important ω-3 long chain polyunsaturated fatty acid, which plays important roles in brain development and preventing cardiovascular and cerebrovascular diseases. Phospholipid DHA (PL-DHA) is a form of DHA that has higher bioavailability, which is considered as a potential DHA molecules compared with triglyceride DHA and ethyl ester DHA. This review aims to summarize the recent progress of phospholipid DHA from its effects on human health and preparation methods. Firstly we will introduce the biological activities of DHA throughout human life, after which the absorption process of different structural DHA will be discussed systematically. Then, various preparation methods of PL-DHA, including formulation innovation, extraction, bio-enrichment and enzymatic catalysis, will be summarized in detail. Finally, the future challenges and prospects of PL-DHA will also be discussed.

1. Introduction

Docosahexaenoic acid (DHA) is an important omega-3 polyunsaturated fatty acid (ω-3 PUFAs) vital to brain health. It is crucial for cognitive function, memory, and overall brain health because it is a fundamental structural element of the brain and an important building block for brain cells[1]. Additionally, it contributes to the synthesis and transmission of neurotransmitters, which are chemical messengers that facilitate the neurons to communicate with each other[2-3]. Unfortunately, the human body cannot synthesize DHA independently; instead, it must be converted from precursors like α-linolenic acid through diet, but this process has a relatively poor conversion in human body[4]. Therefore, dietary supplements are still the primary way for the human body obtaining DHA.

In mammals and humans, the synthesis of PUFAs is primarily carried out in the liver through elongation and desaturation pathways. Multiple elongases and desaturases, including elongase 2 and 5, as well as Δ-6 desaturase (Δ-6D) and Δ-5 desaturase (Δ-5D), are required for this process. The ability to biosynthesize PUFAs is significantly influenced by the protein concentration and activity of these enzymes. Enzymatic activity is regulated by various factors such as nutritional status, hormone levels, and oxidative stress, all of which play a critical role in PUFA metabolism and ultimately affect the levels of PUFAs in tissues and blood[5-6]. Dietary supplementation with DHA can significantly alter the fatty acid composition of various phospholipids (PLs) molecules in the cerebral cortex and liver, effectively increasing DHA levels. In addition, the effects of dietary interventions involving different molecular forms of DHA on lipid profiles in the brain and liver vary, providing a theoretical foundation for DHA supplementation in dietary practices[7]. Triglyceride DHA (TAG-DHA) is primarily absorbed and utilized through the triglyceride metabolic pathway, while phospholipid DHA (PL-DHA), due to its association with PLs, is characterized by higher lipophilicity and can be more efficiently integrated into cell membrane structures.

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The differences in absorption and metabolic pathways form the theoretical basis for developing personalized DHA supplementation strategies. By tailoring different molecular forms of DHA to specific physiological needs, the biological effects on neural development, neuroprotection, and liver health can be more precisely optimized, and the clinical efficacy and metabolic efficiency of supplementation can be enhanced.

Fish oil DHA and algal oil DHA are the 2 most popular DHA supplements available on the market. The initial generation of DHA products is called fish oil DHA, and it is extracted from the tissues of oily fish like salmon, mackerel, sardines, and anchovies[8]. However, fish do not directly manufacture DHA, they accumulate DHA through the food chain by consuming marine algae or plankton. Subsequently, the second generation of DHA algal oil was proposed, primarily derived from microbial fermentation in conjugation with methods for lipid extraction and refining. In addition to these 2 products, another form of DHA that is bound to PLs is called PL-DHA[9], and it mimics the structure of human cell membranes. Research indicated that DHA levels in the bloodstream and other tissues were higher when PL-DHA supplements were used instead of other ω-3 formulations[10]. Because of its high absorption rate and enhanced bioavailability, PL-DHA is emerging as a new generation of DHA products. Therefore, PL-DHA has attracted much attention in recent years and become a highly anticipated form of DHA product.

This review extensively explores the bioactivity of DHA across different stages of human development. A comprehensive analysis of the diverse structural forms of DHA and the intricate absorption mechanisms was conducted. Significantly, considerable attention was dedicated to evaluating the present status of PL-DHA products. The findings of this review offer valuable insights to propel the advancement of PL-DHA products and provide guidance for the study of the physiological activities of PL-DHA.

2. Methodology

2.1 Literature search strategy

In order to gain a comprehensive understanding of the recent advancements and trends in PL-DHA research, a thorough search was conducted in the Web of Science database using "PL-DHA" as the primary keyword. This search was limited to publications from the period spanning 2013 to 2024. This time frame was chosen to capture the latest developments and innovations in the field, reflecting the ongoing research efforts and discoveries related to PL-DHA.

2.2 Literature selection criteria

To ensure the quality and relevance of the literature, strict inclusion and exclusion criteria were established. Original research and systematic reviews focusing on studies relevant to the research field and published in peer-reviewed journals were included. Unpublished research reports, conference abstracts, studies lacking complete data, non-English publications, and articles not aligned with the research topic were excluded. Through this systematic selection and evaluation process, the literature cited in the review was ensured to be both highly relevant and of high academic quality, providing readers with reliable conclusions.

2.3 Literature analysis

We utilized VOS viewer 1.6.17 software to perform a keyword co-occurrence analysis on the identified literature (Fig. 1). The analysis revealed that PL-DHA research can be broadly categorized into 4 main groups. Firstly, investigations focus on the structural aspects and compositions of PLs and fatty acids. Secondly, research delves into the physiological functions of PL-DHA, including its implications in brain development, Alzheimer's disease, and immune regulation, among others. The third category encompasses studies on the sources of PL-DHA, with a particular emphasis on Antarctic krill and various marine fish species. Finally, there is ongoing research on the production and preparation technologies related to PL-DHA, with a specific focus on lipases and analytical techniques.

3. Role of PL-DHA in brain development

3.1 Species of PL-DHA in the brain

PLs are amphipathic molecules, meaning they have both hydrophilic and hydrophobic regions (Fig. 2). They can be classified

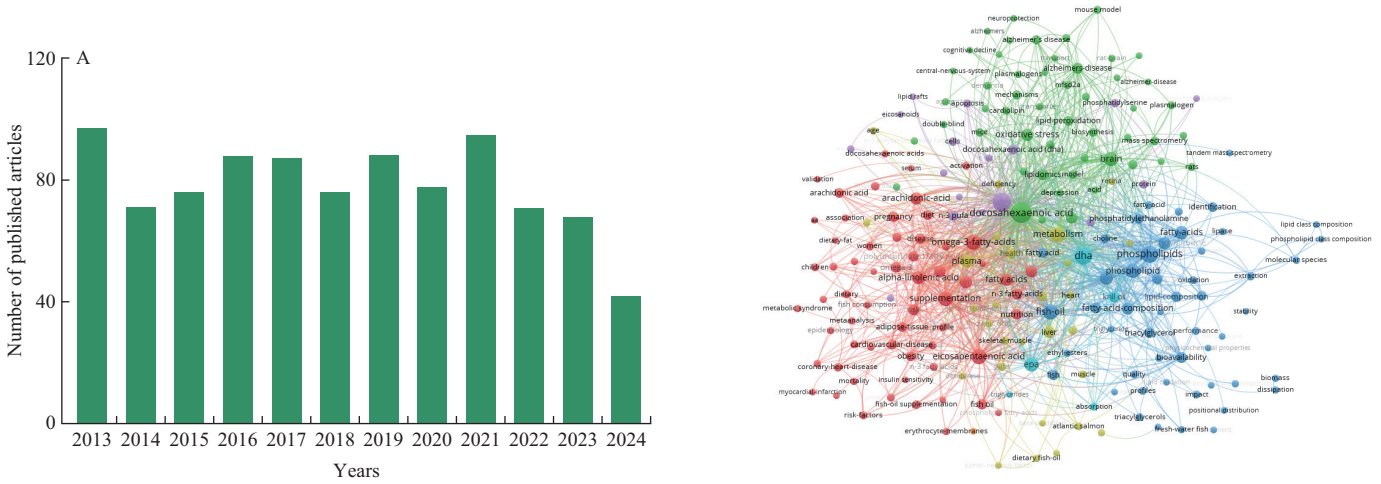

Fig. 1 Current research status of phospholipid DHA. (A) Number of phospholipid DHA scientific publications. (B) Cluster analysis of co-occurring keywords for phospholipid DHA from 2013 to 2024.

into several categories based on their head group and fatty acid composition[11]. Thus, PL-DHA can be divided into different types. According to the difference of head groups, we can see PL-DHA, phosphatidylethanolamine (PE)-DHA, phosphatidylserine (PS)-DHA, and phosphatidylinositol (PI)-DHA, each with unique properties and functions[12-13]. In addition, the fatty acids attached to the glycerol backbone of PLs can vary in length and saturation, further influencing the physical and chemical properties of the molecule. In addition, the positioning of DHA on PLs can vary, because DHA can be located in the first position the second position, or even present in both positions. This variability gives rise to different structural configurations of PL-DHA molecules. For example, lysophosphatidyl choline (LPC) is an important molecular structure of DHA if there is only one fatty acid attached to the glycerol backbone[14-15].

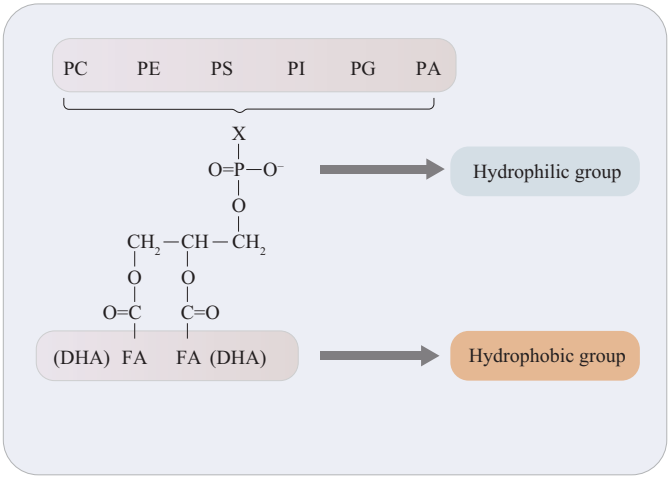

Fig. 2 Species of PL-DHA. If the hydroxyl group are not esterified with fatty acids, they will form *sn*-1 or *sn*-2 lysopholipids

3.2 Distribution of PL-DHA in the brain

The brain exhibits the highest concentration of PLs compared to other organs, with a diverse array of PL forms present. Notably, PE predominates in the brain of mice, making up 55% of the total PL content[16]. Phosphatidylcholine (PC) ranks as the second most abundant PL at about 31% of the total PL content. Additionally, the brain contains 8% PS, 5% PI, and 0.3% cardiolipin (CL). DHA and arachidonic acid (AA) are key PUFAs in various PLs forms within the brain. Taking the mice brain as an example, DHA is predominantly associated with PE and PS, while AA is typically abundant in PI. Specifically, PE species such as PE (18:0/22:6) and PE (18:0/20:4) constitute notable proportions, representing approximately 11% and 8% of brain PLs. On the other hand, PC is predominantly comprised of PC (16:0/18:1), and PS is primarily composed of PS (18:0/22:6) and PS (18:0/18:1), accounting for 51% and 26% of the composition, respectively[17]. These intricate interactions and distributions of PUFAs within various PLs species underscore the complexity of brain lipid metabolism and its crucial role in neurological function.

Furthermore, within the brain, DHA can undergo esterification with different PLs like PE and PC, leading to structural modifications in the PLs composition[18]. The enzymatic machinery required for fatty acid remodeling in PLs enables processes such as deacylation, reacylation, and transacylation of DHA in the brain[19]. Moreover, when DHA was supplemented to the adult mice, the proportion of ω-3 fatty acids in all the PLs species of the cerebral cortex significantly increaseds, meanwhile, the ratios for (n-6)/(ω-3) species in all 3 major PLs classes (diacyl-PE, PE-pl, and PC) showed significant decreases in the DHA-enriched group as compared with the control group[20]. These observations highlight the dynamic nature of DHA metabolism within brain PLs and emphasize the importance of PLs diversity in neuronal function and membrane composition.

3.3 PL-DHA is the most effective form for the brain

LPC results from the hydrolysis of PLs. PUFAs, especially those containing LPC with albumin, are highly required for the brain. According to research by Bazinet et al.[21], lyso-phosphatidylcholine-DHA (LPC-DHA) showed a higher brain-to-body distribution coefficient, which led to a higher build-up of DHA in the brain. Conversely, non-esterified DHA accumulated at a relatively higher rate in the heart and liver[21].

There is a direct correlation between the growth and development of the fetus during pregnancy and the infants throughout lactation[22]. Insufficient DHA throughout infancy might result in neurological problems. When mice were supplemented with TAG-DHA or PL-DHA, PL-DHA was considered as a better DHA supplement for inferior brain development caused by maternal ω-3 PUFA deficiency[23]. In addition, dietary supplementation with LPC-DHA during mouse development could not only increase the content of total fatty acids and major PLs (PC, PE, PI, and PS) in the heart and bone marrow, but also facilitate DHA recovery in the brain[24]. According to Patrick[25], apolipoprotein E4, which is associated with Alzheimer's disease, could impair the ability of the brain to transport free DHA but did not affect LPC-DHA transport. In conclusion, PL-DHA, especially LPC-DHA, is the type that targets the brain more effectively.

4. Bioactivity of DHA in brain development at different life stages

Lipids occupied approximately 50%–60% of the brain's weight, among of which are 35% of ω-3 PUFAs. In neuronal tissues, DHA constitutes over 40% of the total ω-3 PUFAs, especially in the grey matter of brain[26-27]. More and more evidence consistently demonstrated the crucial role of long-chain ω-3 fatty acids, particularly DHA, in preserving brain health and promoting brain development throughout human lifetime (Fig. 3).

4.1 Fetal and infant stages

Due to its presence in the central nervous system of the brain and its critical involvement in the formation and development of brain cells, DHA is important during the fetal and infant stages[28]. In 1980, Clandinin et al.[29] studied the quantity of fatty acids required in the last trimester of pregnancy for the brain development. According to studies on the fatty acid composition of fetal brain tissue, they found higher concentrations of long-chain ω-3 fatty acids during late pregnancy could effectively enhance brain cell proliferation during

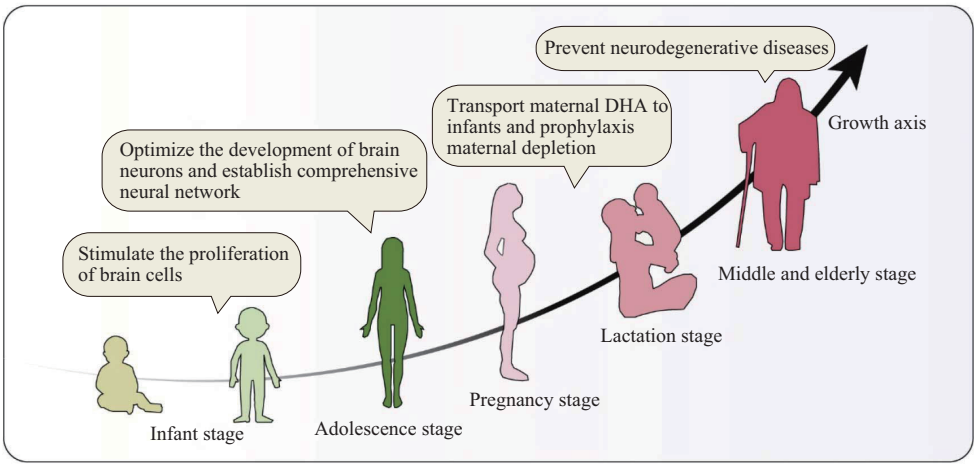

Fig. 3 Effects of DHA on the human brain at different stages.

this developmental phase[30]. It is reported that over 90% of the ω-3 PUFAs in the brain and 10%–20% of its total lipids are made up of DHA. In addition, the average concentration of DHA in breast milk (by weight percent is (0.32 ± 0.22)% (range: 0.06%–1.40%), which directly influences the accumulation of DHA in the infant's brain[31-32]. Thus, the intake of ω-3 PUFAs through the maternal diet is required to maintain adequate levels of DHA in breast milk. It has been estimated that approximately 3.9 kg of lipids are provided to the infant through breastfeeding during the first 6 months of life. These estimates are based on an assumed daily lipid intake of 21.42 g, which supplies about 190 mg of AA and 130 mg of DHA per day[33].

Additionally, higher levels of DHA have been observed in full-term infants compared to premature infants, with elevated DHA levels being correlated with enhanced development of brain microstructures. This development has been linked to larger cerebellum and brainstem volumes as well as superior language and motor skills[34]. Furthermore, it has been observed that myelination and synaptogenesis develop in the first 18 months following birth, with DHA supplementation significantly accelerating and extending neuronal development during this time[35]. After birth, the brain experiences a significant 12-fold increase in volume due to the rapid and continuous accumulation of DHA, which progresses at an accelerated rate until the age of 2[28]. Approximately 90% of brain development is completed by age 6. Therefore, adequate provision of DHA supplementation during this period has been suggested to maximize neuronal development and support the formation of a complete neural network[36].

4.2 Pregnancy and lactation stages

DHA has been recognized as crucial for women during pregnancy and lactation. The levels of DHA in the fetus during pregnancy are significantly influenced by the mother's dietary intake and DHA supplementation. When DHA intake is insufficient, negative effects on the brain development and function of fetuses and infants may be experienced. The levels of DHA in mother's milk can be as high as 1.4% of total fatty acids with a worldwide average of 0.32% of total fatty acids, depending on the mother's diet and number of pregnancies[1].

When DHA-enriched foods are consumed by the mother, the metabolism of fatty acids is improved, increasing DHA levels in critical components such as PLs and brain PLs. This increase has been linked to a protective effect on the offspring, reducing the potential risks of neuroinflammation and neurodegenerative disorders[37]. Furthermore, the proportion of long-chain PUFAs, in particular DHA, in breast milk becomes even more critical if the newborn is considered low birth weight because DHA is necessary for the normal ongoing development of the central nervous system[29].

Conversely, lactation has been shown to deplete maternal DHA stores and other vital nutrients, potentially leading to "maternal depletion syndrome". It has been observed that a lower risk of maternal depression is associated with higher levels of ω-3 PUFAs supplementation during early pregnancy[38-39]. Low maternal DHA status has also been linked to an increased risk of postpartum depression. According to the European Commission and the International Society for the study of fatty acids and lipids, pregnant and lactating women should consume at least 200 mg of DHA daily. DHA supplements can help fulfill this recommendation[40].

4.3 Middle and elderly stages

DHA levels in the brain gradually decline with aging after peaking in early adulthood[41], coinciding with the degeneration of the central and nervous systems and increasing the likelihood of developing neurodegenerative disorders such as Alzheimer's and Parkinson's disease.

Several studies have demonstrated that DHA has beneficial effects on certain neurodegenerative diseases. It has been found that approximately 4 mg of DHA is metabolized by the human brain per day, resulting in an estimated half-life of brain DHA of 2.5 years. Lower dietary intake of ω-3 PUFAs has been observed in early-stage AD patients compared to healthy individuals, indicating the beneficial effects of DHA on neurodegenerative diseases. In addition, 60% reduction in the risk of developing AD has been associated with a weekly intake of approximately 200 mg of DHA, and the total intake of DHA has also been identified as a key factor in reducing the risk of AD[3]. As an illustration of the neuroprotective function of DHA in an animal model of Parkinson's disease. Wu et al.[42]

discovered that supplementing with DHA could significantly increase DHA levels in the brains of defective mice and alleviate 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced neurotoxicity. In another study, Petursdottir et al.[43] used an accelerated aging technique to cause cognitive impairment in mice, which they supplemented with exogenous DHA. It was shown that incorporating DHA into brain PLs through supplementation could effectively delay the deterioration in cognitive performance. This study improved our comprehension of the role of DHA in cognitive health and emphasized its potential as a therapeutic intervention for slowing cognitive decline. Zhang et al.[44] reported that higher DHA intake was associated with a lower risk of Alzheimer's disease. In patients with mild cognitive impairment, daily supplementation of 2 g of DHA for 12 months was found to significantly improve cognitive function and reduce hippocampal atrophy. DHA supplementation has been shown to provide protective benefits to the brain[45]. High-dose DHA supplementation in APOE4 carriers before the onset of AD dementia can be a promising approach to decrease the incidence of AD. Given the safety, availability, and affordability of DHA supplements, refining an ω-3 intervention in APOE4 carriers is warranted[46]. Studied suggested that higher doses of ω-3 fatty acid (2 g/day or more of TG-DHA) were needed to ensure adequate brain delivery, particularly in APOE4 carriers[47-48].

5. Different structural forms of DHA and their absorption

DHA is found naturally in microalgae and marine species as triglycerides (TAGs)[49], or as a substrate in fish oil, where it is chemically or enzymatically changed to ethyl ester (EE) and free fatty acids, and it can also be found as PLs (Fig. 4). TAG-DHA, EE-DHA, PL-DHA, and free DHA, as 4 different forms of DHA products, can cause different digestion and absorption processes in the human body (Fig. 5).

5.1 TAG-DHA and EE-DHA

TAG-DHA must be emulsified with bile and PLs before being absorbed by the human body. They are subsequently degraded into *sn*-2 monoacylglycerols and free fatty acids by *sn*-1,3 pancreatic lipase. Through active or passive transport processes, these substances permeate into the intestinal epithelial cells, where they are absorbed. They then undergo re-esterification to become TAGs, cholesterol esters, or PLs. After TAGs bind to lipoproteins, they will be modified to create chylomicrons (CM)[50]. After exiting the intestinal epithelial cells, the CM enters the lacteals and moves *via* the lymphatic circulation. Eventually they enter the bloodstream *via* the thoracic veins and are carried to the liver and peripheral organ tissues[51].

Subsequently, lipoprotein lipase enzymes on the endothelium of these tissuessuch as fat and muscle will break down some TAG-CM molecules. Fatty acids are released during this process, and after being taken up by fatty acid translocase (CD36) membrane proteins on the endothelial cells, they move to the adjacent tissue cells at the base of the capillaries, where they will either completely degrade and release energy or be retained[51]. Approximately 5%–35% of the free fatty acids still remain in the bloodstream. Lyso-PLs and some free fatty acids are re-esterified to PLs, while some free fatty acids bind to TAGs and enter the bloodstream incorporated into the surface layer of chylomicrons, with TAGs being incorporated into the core of chylomicrons. A small proportion may also integrate into very low-density lipoproteins (VLDL). Once the TAG-rich particles of the chylomicron are broken down, PLs can be absorbed by the high-density lipoprotein (HDL) fraction[52-54]. A portion of DHA included in HDL can bind to LPC-DHA, the primary structure of DHA entering the brain. Major facilitator superfamily domain-containing protein 2a (Mfsd2a) is the major transport protein for transporting LPC-DHA across the blood-brain barrier (BBB) into the brain[55-57]. EE-DHA is hydrolyzed into free DHA, a process similar to the absorption of TAG-DHA but is less efficient than TAG-DHA[58].

5.2 Free DHA

Free DHA passively diffuses past the BBB endothelium and into the brain's parenchyma. Several proteins and enzymes, including fatty acid-binding proteins, fatty acid transport proteins, and acyl-CoA synthetases, facilitate this process. This transport mechanism stops the net flux of DHA across the membrane as soon as it achieves equilibrium at the membrane surface. For continual flux to be ensured, receptor proteins or DHA metabolism are needed. The fatty acid transport proteins family facilitates fatty acid uptake into tissues. Instead of transporting fatty acids, they accelerate the metabolism of fatty acids on the membrane, which lowers the amounts of fatty acids inside the cells and permits transmembrane transport to proceed[59-60].

5.3 PL-DHA

In contrast, DHA is more effectively absorbed in the form of PLs. As PL-DHA is amphiphilic, it can form micelles independently and move through an aqueous medium without bile. Phospholipase A2 in the small intestine may hydrolyze PL-DHA, releasing the fatty acids at the *sn*-2 position and generating LPC, in contrast to stomach lipase, which is unable to do so. Approximately 20% of intestinal PLs are directly incorporated into high-density lipoproteins by passive absorption without hydrolysis[61-63]. The released fatty acids can synthesize TAGs, and the lyso-phospholipids can be reacylated to produce PLs, which are then incorporated into CM, absorbed and degraded through circulation, and transferred to HDL. PLs are preferentially absorbed by HDL as compared to TAGs. Lastly, LPC-DHA is created by HDL and released into the bloodstream. BBB-crossing LPC-DHA derived from plasma can increase the amount of DHA in the brain and retina[64-65].

There is evidence for this complex process in many studies. The efficacy of enriching brain DHA was evaluated in a study by Sugasini et al.[66], who fed rats with TAG-DHA, PC-DHA, and LPC-DHA for 30 days. Results showed that DHA, or monoacylglycerol in the TAG form, was primarily absorbed as CM and mostly incorporated into adipose tissue and the heart. In contrast, PC-DHA produced free DHA and LPC-DHA during digestion, and LPC-DHA was transported to the brain without impacting the adipose tissue. Moreover, it has been indicated by studies that PLs rich in DHA are associated with superior efficacy in alleviating symptoms in MPTP-induced Parkinson's disease mice compared to TAG-DHA[67]. Thus, PC-DHA and LPC-DHA effectively increased brain-derived neurotrophic factors[66].

Among PLs containing DHA with different polar groups, greater therapeutic improvement was demonstrated by PS-DHA compared to PC-DHA[68]. It has been reported that both DHA-PC and DHA-PS

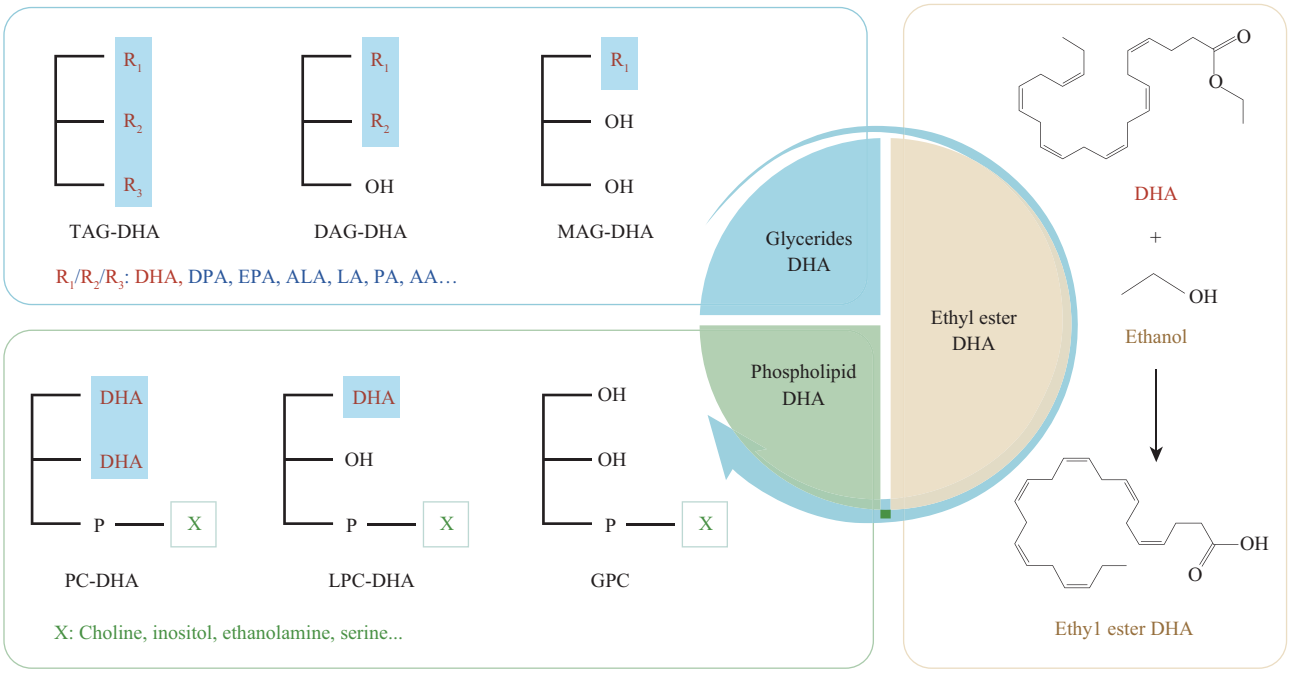

Fig. 4 Different types of DHA lipid carriers.

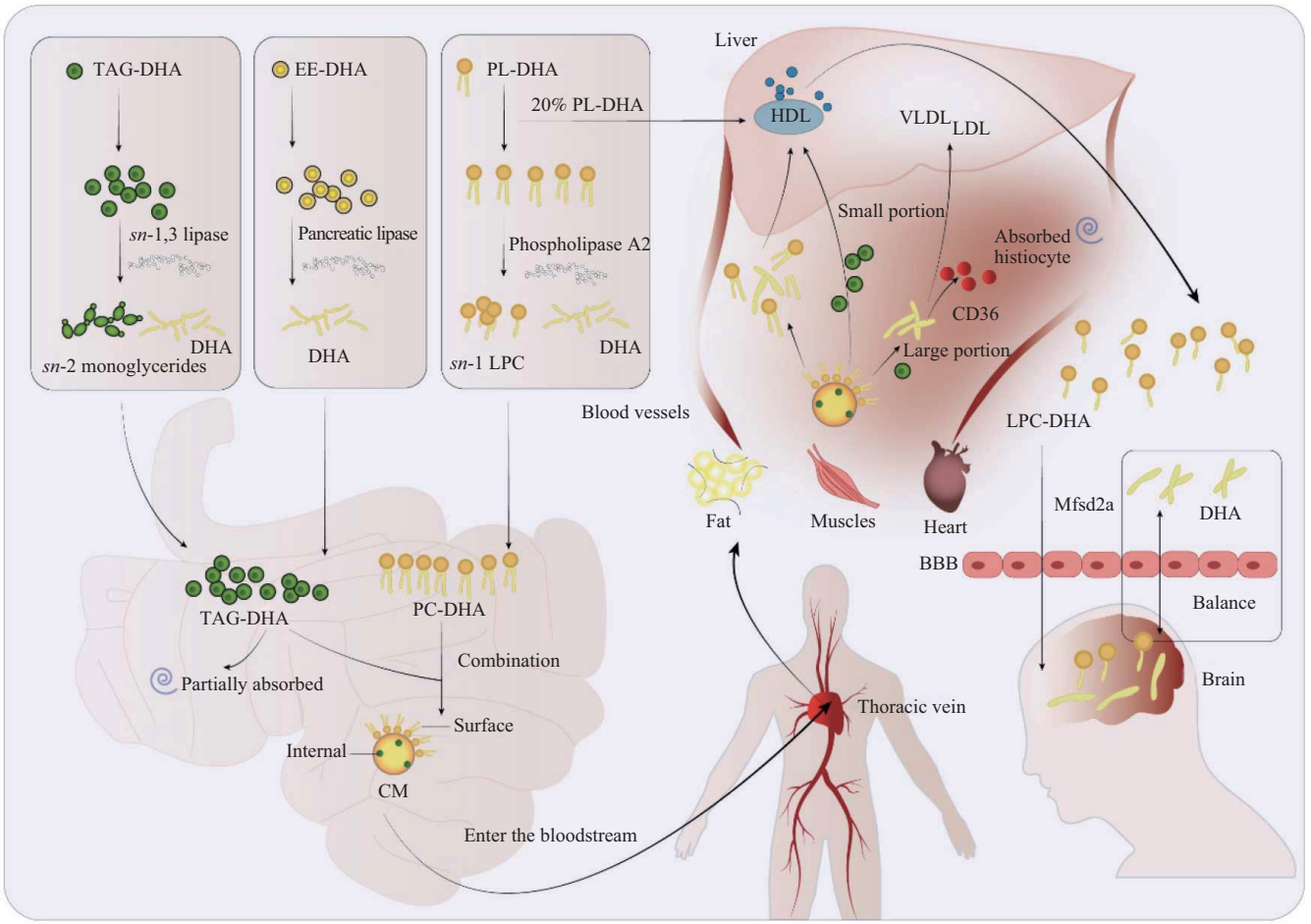

Fig. 5 Absorption processes of different structured DHA in the human body.

administration could remarkably mitigate cognitive deficiency in Aβ-induced rats, with better effect of DHA-PS than that of DHA-PC[67]. Furthermore, it was reported that both DHA-PC and DHA-PS significantly improved cognitive deficits in SAMP8 mice with a high-fat diet, as assessed by maze tests[69]. In addition, more notable benefits in inhibiting Aβ pathology, mitochondrial damage, and neuroinflammation were presented by DHA-PS in comparison to DHA-PC. DHA-PC mainly improves cognitive impairment through the cholinergic system, whereas signal transduction molecules might be the main reason for the effect of DHA-PS in improving memory function[70]. Additionally, it has been shown that different effects are exerted by DHA-PC and the recombination of DHA-EE with egg-PC on alleviating AD development, as assessed by the Morris water maze test and the Barnes maze test in SAMP8 mice, with effects equivalent to those of DHA-PC not being achieved by the recombination of DHA-EE and egg-PC[71]. Therefore, the significant bioactivity exhibited by PL-DHA may be attributed to its unique compositional structure and molecular form.

6. Strategies related to the development of PL-DHA products

The significant biological activities of PL-DHA has motivated researchers to develop a variety of PL-DHA products. For example, the PLs and DHA oil complexes, together with some products derived from PL shrimp and eggs, make up the majority of PL-DHA products available on the market at the moment[72-75]. Researchers have started to develop products from various marine sources in response to the growing demand for the PL-DHA. In addition, the enzymatic-catalyzed synthesis of PLs is also a hot topic in the development of PL-DHA products[76] (Fig. 6).

6.1 Combination of PLs and DHA

Strictly speaking, this is not a true PL-DHA. In situations where a large amount of PL-DHA cannot be obtained, PLs and TAG-DHA are combined and then processed into microemulsions or microcapsules to increase their bioavailability and nutritional value. For example, Wu et al.[77] compared several approaches to restoring DHA levels in mice lacking ω-3 fatty acids. They discovered that supplementing PC-DHA to mice lacking in DHA could quickly restore their DHA concentration. Moreover, TAG-DHA combined with egg PC or glycerylphosphorylcholine (GPC) could bring the DHA concentration of mice close to that of PC-DHA. Additionally, Ji et al.[78] prepared pea protein isolate-chitosan nanoparticles to create eicosapentaenoic acid (EPA)-delivering materials for digestion. The findings demonstrated that the EPA-encapsulated emulsions performed well in delivering EPA for digestive absorption through the digestive system and had a high retention rate during storage.

Nevertheless, while PLs and TAG-DHA in particular formulations may improve DHA absorption, they cannot completely replace PL-DHA. Research has demonstrated that in senescence-accelerated prone 8 mice, PL-DHA was more effective than EE-DHA and PC recombined PLs alleviating age-related memory loss and cognitive deficiencies[79].

6.2 PL-DHA from krill oil

Krill oil is a nutritional supplement made from the tiny marine crustaceans known as krill that resemble shrimp. These small organisms are an important component of the marine food chain and are primarily found in the frigid waters of the Antarctic. Krill oil has a high concentration of ω-3 fatty acids, particularly EPA and DHA, has become increasingly popular as a health supplement. Furthermore, the majority of ω-3 fatty acids are joined to PLs. Compared to conventional fish oil and algae oil, the unique PL structure of ω-3 fatty acids in krill oil is thought to improve their absorption and use in the body. According to reports, PLs accounted for around 40% of the total lipids in krill oil, with the PL component containing 80% of the PC[80].

The main extraction methods used to get krill oil are solvent extraction, supercritical or subcritical fluid extraction and enzymatic

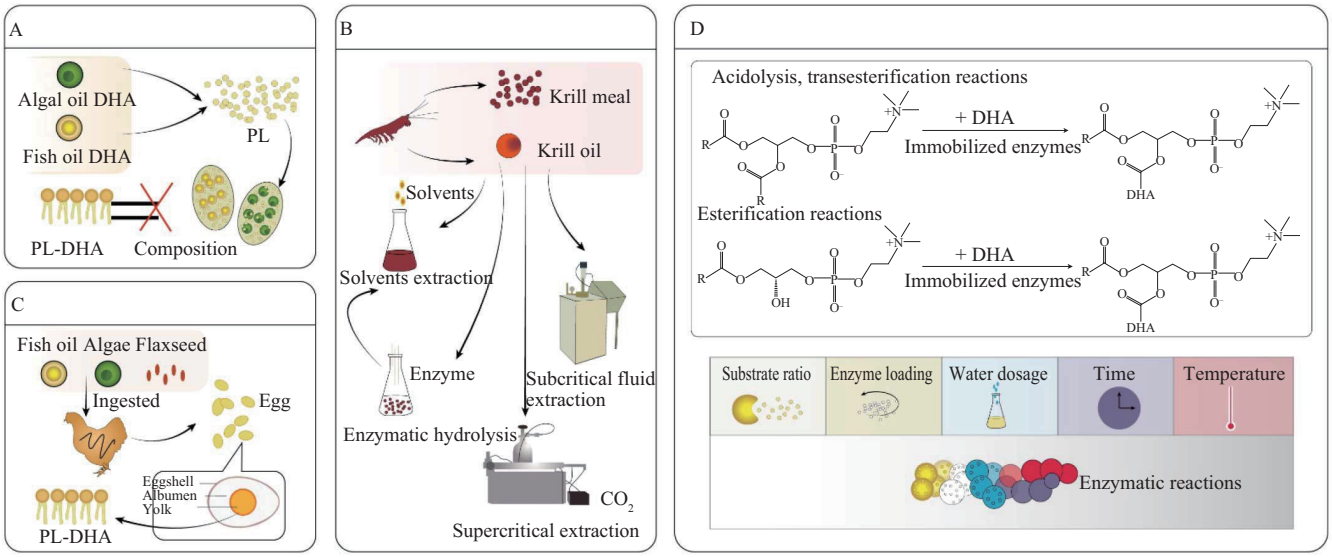

Fig. 6 Current PL-DHA product types and development methods. (A) Complexes of PL-DHA by combining TAG-DHA with PLs. (B) Extraction method of krill oil. (C) Enrichment method of DHA to increase the PL-DHA content in eggs. (D) Methods for catalyzing the synthesis of PL-DHA using different enzymes.

hydrolysis. Krill oil is easily extracted with organic solvents. Ethanol, hexane, and petroleum ether are frequently used as extraction solvents. Yin et al.[81] extracted krill oil from Antarctic krill using a variety of solvents. They shook the freeze-dried krill powder while immersed in different ratios of ethanol and hexane to facilitate extraction. The highest extraction rate of 18.75% was obtained when the ratio of ethanol and hexane was 1:2. Furthermore, the PL content of in the extracted krill oil ranged from 30.20%–64.82% of total lipids, and DHA made up 6.95%–9.22% of all fatty acids[81]. "Supercritical fluid extraction" describes a method for extracting and separating target components using supercritical fluids. Because of its extensive attention, stable qualities, and mild supercritical conditions, CO_2 is the most often used fluid. Ali-Nehari et al.[82] found that the PLs content of krill oil obtained by supercritical extraction was slightly lower than that of the hexane extraction method, but the peroxide value and acid value of the oil were significantly lower than those of solvent extraction methods.

Similarly, subcritical extraction technology could extract chemicals at normal temperature and specified pressure without harming thermosensitive components. Meanwhile, it requires comparatively less equipments than supercritical extraction. Liu et al.[83] proposed a subcritical dimethyl ether method to extract high-quality krill oil with low peroxide values from Antarctic krill. They reported that the oil contained 33.95% PC, 11.67% PE and 7.91% DHA, with an ideal extraction efficiency of 92.1030%.

Furthermore, enzymatic hydrolysis could dissolve proteins in krill thus hasten the release of krill oil. Wang et al.[84] investigated how various proteases affected the yield and quality of PLs extracted from krill. They hydrolyzed krill by proteases in distilled water and extracted them using anhydrous ethanol and petroleum ether. Results showed that the highest PL concentration of 50.19% was obtained when using alkaline protease, which was much higher than that in solvent extraction method. Teng et al.[85] proposed a two-step enzymatic-assisted extraction approach, in which the krill was pretreated with chitinase and protease before ethanol extraction. This extraction method obtained 112% improvement in lipid yield over the soxhlet extraction procedure. Meanwhile, the PL content in the lipid reached 246.05 mg/g and the free EPA and DHA concentration was as high as 80.96 mg/g.

To date, multiple safety evaluation authorities have affirmed the safety of PL-DHA derived from krill oil. In 2004, the U.S. Food and Drug Administration classified krill oil and its byproducts as compliant with the "Generally Recognized as Safe" standards. Additionally, in 2013, the Chinese National Health Administration Department officially approved krill oil as a novel food ingredient.

6.3 PL-DHA from egg yolk

Egg yolk is a valuable resource for developing PL-DHA products because it is an excellent source of PLs. PLs in egg yolk comprise around 78% (*m*/*m*) of PC and 18% (*m*/*m*) of PE. Palmitic acid, found at position *sn*-1, and oleic acid, found at position *sn*-2, are the primary fatty acids in the isolated egg yolk PLs. Nonetheless, it is important to remember that the hen's nutritional state and the kind of fatty acids it consumes can have an impact on the total amount of fatty acids and PLs in egg yolks[75]. Cruickshank demonstrated in 1934 that hens could directly deposit fatty acids that they had consumed into their egg fat[86]. Currently, modifying the diet of laying hens to increase the concentration of DHA in eggs is a hotspot of research.

Fish oil, algae, and flaxseed were usually used as feed supplements for laying hens to produce eggs with a higher DHA concentration[87]. Zhao et al.[88] increased the concentration of DHA and EPA in eggs by feeding laying hens with fish oil and krill oil supplements in their diets. The DHA and EPA content per egg increased to 348.5 mg with the addition of 1.5% fish oil. In addition, the addition of krill oil improved the PL build-up in the egg yolk. Moreover, they also found that DHA and EPA were esterified at the *sn*-2 position of the glycerol backbone due to the action of phospholipase A_2 hydrolysis. Microalgae is also a promise feed supplement for laying hens as they can provide eggs with a DHA level comparable to fish oil. Maina et al.[89] studied the effects of feeding DHA-rich microalgae oil on egg production and DHA enrichment. The findings revealed that eggshell thickness increased linearly with adding microalgae oil for 12 weeks. Moreover, the DHA concentration of the standard eggs increased from 84 to 297 mg/100 g in the DHA oil feeding group. An additional dietary supplement choice is flaxseed, a rich source of α-linolenic acid—a precursor for the synthesis of DHA. It can also increase the DHA content in laying hens[90]. According to Scheideler et al.[91], flaxseed was introduced to the hen feed. According to the results, the proportion of α-linolenic acid in the eggs increased from 2.31% to 6.83% after 8 weeks of feeding, when the flaxseed content in the diet increased from 5% to 15%. Concurrently, the DHA content in the yolk of the flaxseed feeding group increased to 1.83%. Additionally, PLs feeding may impact the enrichment of DHA in laying hen eggs. Magnuson et al.[72] supplemented various PLs into the diets of the hens, giving them high levels of DHA and choline, increasing the concentration of DHA in the egg yolk, and downregulating the expression of genes related to the synthesis of fat in the tissues. Supplementing the diet with serine and ethanolamine improved the concentration of DHA in the egg yolk and restored DHA-mediated modifications in gene expression.

6.4 PL-DHA from other marine sources

Researchers have discovered a variety of marine organisms that are rich in the PL-DHA, in addition to krill and egg yolks. Ahmmed et al.[92] discovered that salmon roe had high levels of PLs (26.53 μmol/g) and ω-3 fatty acids (43.32%), with the polar lipid fraction primarily containing EPA (7.99%) and DHA (34.47%), demonstrating salmon roe might be a valuable resource for developing products higher in the PL-DHA. PL-DHA can also be obtained from bluefin tuna roe, a great resource. Fish eggs contain a high concentration of ω-3 fatty acids (44.5% of the total lipid extracted), 11.3% EPA and 27.5% DHA, respectively[93]. In addition, PUFAs were observed in most of the PLs found in sea urchin gonads[94]. Besides, more marine organisms that show promise as PL-DHA sources include blue mussels[95], squid eggs[96], and clams[97].

6.5 Enzymatic synthesis of PL-DHA

Researchers have begun synthesizing PL-DHA using alternative approaches to address the limitations of few extraction resources. In order to chemically insert EPA and DHA into the *sn*-2 position of PC, Lyberg et al.[98] used 4-dime-thylaminopyridine as a coupling agent,

1-(3-dimethylami-nopropyl)-3-ethylcarbodiimide hydrochloride as a catalyst and a base. This intense chemical reaction process may disturb the natural structure of PLs. Furthermore, its safety profile is not optimal, which could lead to limitations in the food industry. Because of their favorable reaction conditions, high substrate selectivity, and low byproduct formation, enzymatic synthesis techniques have gained popularity. Esterification, transesterification, and acidolysis are examples of specific catalysis reactions (Table 1).

Fish oil fatty acids and soybean PC were used by Zhao et al.[99] as substrates for the acidolysis reaction. The immobilized phospholipase A1 was used as a catalyst to produce PC rich in *ω*-3 PUFAs and the greatest incorporation rate of *ω*-3 PUFA reached 57.4% with a PC yield of 16.7%. Similarly, Li et al.[100] synthesized the PC rich in DHA/EPA using immobilized phospholipase A1 in a solvent-free method to conduct the transesterification between PC and ethyl DHA/EPA. They improved the PC composition and the assimilation of DHA/EPA by raising the vacuum after 24 h. The maximum incorporation rate of DHA/EPA (31.72%) was attained at 30 h with a PC yield of 47.2%. Immobilized phospholipase A2 was utilized in a different investigation by Lilja-Hallberg et al.[101] to catalyze the esterification reaction involving LPC and *ω*-3 PUFAs. The primary solvent was fatty acids, with iso-octane or ethanol as backup solvents. In an environment with low water content, the PC yield in the iso-octane system peaked at 22%[101].

The choice of enzymes and several other parameters such as substrate ratio, enzyme loading, water content, temperature and reaction time were essential factors in the process of enzymatic catalysis and were the subject of numerous studies. Enzymes belonging to the lipase and phospholipase classes can catalyze modifications to the locations of fatty acids inside lipid structures. They can carry out transesterification processes and catalyze the hydrolysis of glycerides and PLs. Generally, high incorporation levels need screening of effective enzymes. Enzyme stability can also be improved by immobilized enzymes, enabling recovery and recurrent usage. As a result, these enzymes are frequently employed as biocatalysts in the immobilization process to prepare PL-DHA. This

Table 1
Summary of the literatures about PL-DHA prepared by enzymatic catalysis.

Substrates	Reaction type	Enzymes	Reaction conditions					Results	Reference
			Enzyme loading	Water dosage	Temperature (℃)	Time (h)	Substrate ratio		
Soy PC/*ω*-3 PUFA from fish oil	Acidolysis	Immobilized PLA1 on Lewatit VP OC 1600	20%	1.0%	55	24	1:8 (mol/mol)	57.4% *ω*-3 PUFA into PC 16.7% yield of PC	[99]
Soy PC/Ethyl esters	Transesterification	Immobilized PLA1 on D380	15%	1.25%	55	24	1:6 (g/g)	30.7% DHA/EPA into PC 74% yield of PC	[107]
Soy PC/Fatty acids from fish oil by saponification	Acidolysis	Immobilized PLA1 on Duolite	10%	0	50	24	1:8 (mol/mol)	35% *ω*-3 PUFA into PC	[108]
Soy PC/Ethyl esters	Transesterification	Immobilized PLA1 on macroporous resin	15%	1.1%	55.7	72	1.0:6.8 (g/g)	30.31% DHA/EPA into PC 47.2% yield of PC	[100]
PC/Fatty acids from *Schizochytrium* sp.	Acidolysis	PLA1	40%	0.4%	45	24	1.00:2.13 (g/g)	20.90% DHA into PC	[103]
PC/Fatty acids from fish oil by saponification	Acidolysis	PLA1	10%	-	55	24	1:10 (mol/mol)	28.0% *ω*-3 PUFA into PC	[109]
Egg-yolk PC/*ω*-3 PUFAs from cod liver oil	Acidolysis	Immobilized lipase B on macroporous acrylic resin (Novozym 435)	20%	-	55	48	1:10 (mol/mol)	45.6% DHA/EPA into PC	[110]
PC from Antarctic krill meal/Fatty acids from fish oil	Acidolysis	Immobilized PLA1 on macroporous resin DA-201	15%	1.0%	50	7	1:10 (mol/mol)	59.0% DHA into PC 27.5% yield of PC	[111]
PC/Algal oil from *Schizochytrium* sp.	Transesterification	PLA1	7 mg	0.7%	45	0.5	0.02:1.00 (g/g)	39.1% DHA into PC	[112]
LPC from soy lecithin/ Fatty acids/Glycerol	Esterification	Phospholipase A2/ Calcium chloride/ Formamide	60 mg/ 3 μmol/ 0.5 mL	-	40	48	0.11:0.18:5.50 (g/g)	89.5% yield of DHA-PC	[113]
Silica-adsorbed PC/ Supersaturated solution of DHA salts	Transesterification	Lipozyme CALBL	1 U	-	35	18	1:10 (g/g)	34.8% DHA into PC 80% yield of PC	[114]
Soy PC/EE	Transesterification	Immobilized lipase from *Rhizomucor miehei*	30%	4%	50	24	1:3 (g/g)	45.7% DHA/EPA into PC	[115]
Soy PC/Algal oil	Transesterification	Immobilized CALB on macroporous adsorbent resin HPD826	25%	0.02 g/mL molecular sieve	50	72	1:8 (g/g)	31.2% DHA into PC 43.6% yield of PC	[116]
Soybean PC/EE	Transesterification	Immobilized PLA1 on macroporous SiO_2 and SiO_2/Polymer composites	10%	-	60	20	1:6 (g/g)	74.8% DHA/EPA into PC 83.1% yield of PC	[117]

Note: - indicates that the component was not reported.

is one of the best ways to deal with the short lifespan of free enzymes and the high production costs in the business[102]. The amount of fatty acids present in the substrate directly affects the synthesis of PL-DHA, greater concentrations may enhance the possibility of interacting with PLs and foster the intended reaction up to the point at which the maximum content of PL-DHA reached. Nevertheless, adding DHA-enriched fatty acids would cause the reaction system to become more polarized at unreasonably high matrix-to-substrate ratios. Thus, the diffusion of substrates and the likelihood of molecular collisions involving fatty acids, PLs, and enzymes were hampered. Secondly, there is a chance that adding more enzymes will somewhat quicken the enzymatic reaction. However, because of problems with stirring and a decreased capacity for mass transfer, an excessive amount of enzyme loading can harm the PL-DHA content[103]. Thirdly, water must be present for the esterification reaction. On the other hand, too much water might have unintended side effects, including hydrolysis, which lowers the concentration of PC and increases the amount of LPC and GPC. Water can also affect enzyme activity, reaction rate, and substrate binding[104]. Lastly, the reaction temperature is critical because it influences both the activity of the enzyme and the stability of PL molecules. Furthermore, the extent of the reaction and the ensuing manufacturing costs are closely correlated with the reaction time. Accordingly, careful evaluation of these factors is crucial[105-106].

7. Conclusion and outlook

DHA is an important nutrient that is needed at every stage of life. It is crucial for brain health and development and has been shown to protect against several diseases. In recent years, with the increasing public awareness of health, wider applications of phospholipid-DHA have been observed in the food industry, demonstrating significant potential in health care and pharmaceuticals. It is indicated that when PL-DHA was used as a dietary supplement instead of other ω-3 formulations, higher levels of DHA were found in blood and other tissues. Due to its superior absorption rate and higher bioavailability, PL-DHA is regarded as a new generation of DHA, showing clear advantages over algal oil DHA and fish oil DHA. In 2004, it was declared by the U.S. Food and Drug Administration that high-concentration PL-DHA from krill oil and its by-products are "generally recognized as safe". In 2013, krill oil was approved by China's national health authorities as a new food ingredient. Additionally, it has been noted that PL-DHA can more directly and effectively influence brain function, supporting brain development and health while preventing neurodegenerative diseases. It is estimated that the market size for neurodegenerative diseases will grow from $51.45 billion in 2023 to $72.63 billion by 2028. Vast market potential is presented by PL-DHA as a functional ingredient in the pharmaceutical field, effectively preventing and alleviating human diseases. Furthermore, it can be used in combination with astaxanthin, carotenoids, and flavonoids to exhibit superior properties. Therefore, PL-DHA has broad application prospects.

Currently, extraction methods are used to generate most PL-DHA products. However, these procedures have limitations such as unstable materials, restricted sources, and potential allergen presence, which make large-scale application difficult. Furthermore, as the enzymatic synthesis technology of PL-DHA is still in its infancy, more efforts will be needed to optimize production efficiency and maximize yield. It is recommended that future studies focus on investigating novel enzymes, formulating procedures, and refining separation and extraction techniques to facilitate the industrial production of PL-DHA products. At present, significant attention has been drawn to the efficient production process of PL-DHA, and the need for large-scale production has become urgent. Therefore, the development of a novel and efficient production method is considered particularly crucial.

Conflict of interest

The authors report there are no competing interests to declare.

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