附：酶法制备含ω-3 多不饱和脂肪酸油脂的国际研究进展（英语原文）

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The Global Research Progress of Enzymatic Processing of Oils with Omega 3 Polyunsaturated Fatty Acids

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Abstract: This paper has reviewed that Omega 3 polyunsaturated fatty acids (PUFAs), as an essential fatty acids (EFAs), mainly come from fish oil of marine products, has positive effects on treatment and/or prevention of several diseases. In this review, the recent developments by 2019 in the field of enzymatic modification of oils rich in omega 3 PUFAs have summarized. Several different products, such as structured lipids with a variety of FA compositions, nutritional aspects, omega 3 PUFA concentrates and phospholipids, have discussed from the point of process technology as well as possible applications. Enhancing omega 3 PUFA content in diet involves a number of strategies aiming to modify the content of such FAs in fats and oils. Due to the mild reaction conditions used, especially the lipase specificity, the position as well as content of omega 3 PUFAs in lipid molecules being of importance from the point of bioavailability, enzymatic processing of omega 3 PUFA oil is safe, efficient and preferred over chemical treatments.

Key words: Omega 3 polyunsaturated fatty acids; nutritional aspects; interesterification; enzymatic processing; structured lipids; PUFA concentrates; phospholipids; fish oil

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I Introduction

Polyunsaturated fatty acids (PUFA) serve as an important class of molecules, both structurally and functionally. The two predominant PUFA classes are omega 3 and omega 6 PUFA, which are defined by the first double bond proximate to the methyl end of the molecule. The primary omega 3 PUFA is α-linolenic acid (ALA, 18:3), whereas linoleic acid (LA, 18:2) is the dominant omega 6 PUFA. Both of these fatty acids (FAs) cannot be synthesized in the body, and therefore, are required nutrients, termed as essential fatty acids. Omega 3 and omega 6 PUFA share the same enzymes throughout their metabolism pathways. Therefore, the relative amounts of ALA and LA affect each other's metabolism efficiency[1].

Current evidence states that omega 3 PUFA, especially eicosapentaenoic acid (EPA, 20:5) and docosahexaenoic acid (DHA, 22:6), have beneficial effects on human health. It has been reported that only <8% of ALA was converted into EPA in adults, whereas conversion to DHA was negligible (<0.02%~4%). Thus, both of these FAs should be consumed via diet. The main source of omega 3 PUFA is marine products. They are synthesized by phytoplankton and algae, transferred through the food web and incorporated into lipids of fish and marine mammals[2].

In order to benefit from omega 3 PUFAs, incorporation of such FAs into foods and oils is suggested instead of fish meal consumption. Ward and Singh[3] stated that 60 to 135 g of salmon per day is needed to maintain a daily intake of 1 g of EPA and DHA, which raises the interest in omega 3 PUFA-rich oils.

Modification of oils can be performed via

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chemical or enzymatic processes. As an example, process flow charts of chemical and enzymatic interesterification reactions are compared in Figure 1. Since omega 3 PUFAs are highly prone to oxidation, due to their high level of unsaturation, chemical process which requires the use of high temperature is not favorable for handling such oils. Besides, the position of omega 3 PUFAs on the glycerol backbone has an important role in their bioavailability upon digestion. Providing omega 3 PUFAs esterified to the *sn*-2 position results in better protection of these FAs from oxidation[4] and provides better absorption of them in 2-monoacylglycerol (2-MAG) form[5]. Thus, selective enzymatic reactions are preferred to modify oils rich in omega 3 PUFAs.

This review will give a brief introduction to the nutritional importance of omega 3 PUFA oils, and will focus on the developments of their enzymatic modifications in the past five years.

II. Nutritional aspects of omega 3 PUFAs

Since 1970's, when the low incidence of coronary heart disease among Greenland Eskimos had been linked to their high level of oily fish intake[7], there have been a tremendous focus on health effects of omega 3 PUFAs. Omega 3 PUFAs function mainly by altering membrane lipid composition, cellular metabolism, signal transduction, and regulation of gene expression. They regulate the expression of genes in various tissues, including the liver, heart, adipose tissue, and brain[8]. According to the current knowledge, omega 3 PUFAs play an important role in the prevention and treatment of cardiovascular diseases, hypertension, diabetes, arthritis and other inflammatory and autoimmune disorders, as well as cancer, and are essential for normal growth and development, especially for the brain and retina.

Cardiovascular diseases. Cardiovascular disease is the common term for all diseases that affect the heart and the circulatory system. It is the leading cause of death in the Western societies and has been linked to the high fat intake, particularly saturated fat, common in Western diets. Regular consumption of fatty fish or fish oils containing omega 3 PUFAs has been shown to lower the rate of incidence and death from cardiovascular heart disease[9], confirmed by epidemiological studies as well[10]. It is noteworthy to mention that there has been some argument on the effects of omega 3 PUFA supplement form in order to prevent cardiovascular diseases[11]. It was suggested that the beneficial effects of fish consumption on the risk of cardiovascular diseases were the synergistic effects among nutrients in fish, not only the omega 3 PUFAs.

Cancer. There exist more than a hundred diseases that are grouped together under the term cancer. Omega 3 PUFAs have been shown to have anticarcinogenic effects, especially against breast [12], colon[13], and prostate cancers[14]. Furthermore, omega 3 PUFAs potentially increase the sensitivity of tumor cells to conventional therapies, possibly improving their efficacy especially against cancers resistant to treatment[15].

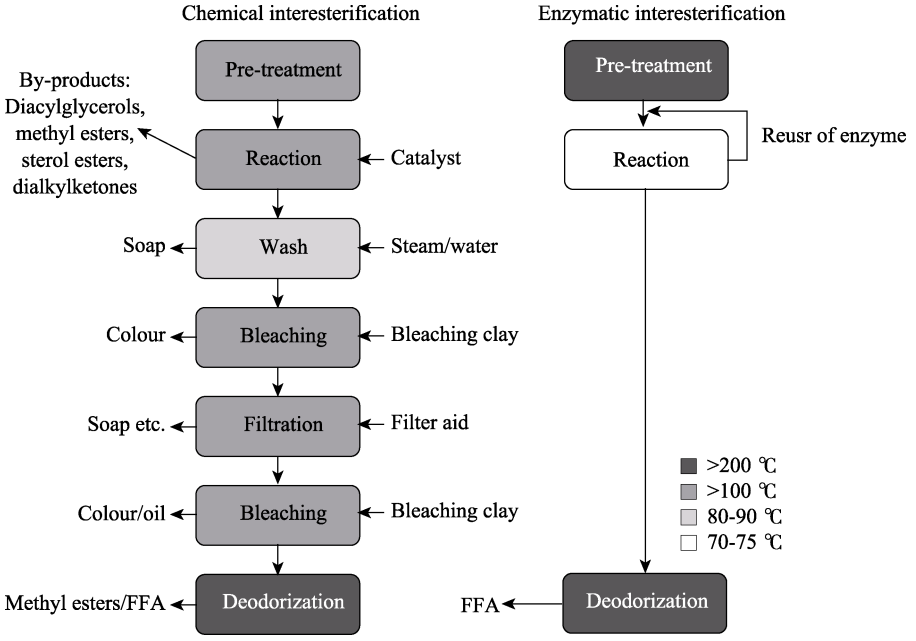

Fig.1 Comparison of process flow charts of chemical and enzymatic interesterification reactions between two TAGs (adapted from[6]). Enzymatic interesterification process is performed in a packed-bed lipase reactor, therefore, does not require the filtration of catalyst step

Diabetes. Insulin is involved in various aspects of FA metabolism at the cellular level, which are relevant to diabetes. Examination of large population studies revealed that the administration of omega 3 PUFAs for diabetic patients is advisable due to the favorable effects on hypertriglyceridemia, and to the modifications in cholesterol profiles[16]. It is an interesting finding that there is a rapidly increasing incidence of insulin resistance and glucose intolerance among Eskimos, which is associated with the shift from the traditional diet of fish and marine mammals, rich in omega 3 PUFAs and low in saturated FAs (SFA), to commercial foods, which are low in omega 3 PUFA and rich in SFA[17].

Inflammatory disorders. Inflammation is a component of a range of acute and chronic human diseases, and is characterized by the production of inflammatory cytokines. Arachidonic acid (AA)-derived eicosanoids have been shown to have pro-inflammatory effects. Omega 3 PUFAs, on the other hand, give rise to anti-inflammatory and inflammation resolving mediators called resolvins, protectins and maresins[18-19]. They act both directly (e.g., by replacing AA as an eicosanoid substrate and inhibiting AA metabolism) and indirectly (e.g., by altering the expression of inflammatory genes through effects on transcription factor activation) [18,20-21]. Consumption of oily fish was also effective in protecting against childhood asthma[22].

Nervous tissue development. Omega 3 PUFAs, especially DHA, are important components of human brain; about one-third of lipids in brain are composed of omega 3 PUFAs. Decreased DHA in the developing brain has been linked to deficits in neurogenesis, neurotransmitter metabolism, and altered learning and visual function and motor skills in animals[23]. Moreover, epidemiological studies have shown a relationship between low maternal DHA and increased risk of poor development of neural and visual systems in infants[24-26]. The presence of EPA and DHA in pregnant women's diet is important to ensure that their adipose tissue contain a reserve of these FAs for the developing fetus and the breast-fed newborn infant[27]. Another effect of omega 3 PUFAs on the nervous system is to protect against mental diseases. Although the etiology of such diseases are not completely understood, current evidence shows that consumption of omega 3 PUFAs reduces the risk of depression[25,28-29], as well as improving the symptoms of depression at chronic patients[30]. EPA concentration[31] as well as DHA concentration[31-32] are decreased in the membrane of erythrocytes and in the plasma of patients suffering from unipolar depression, seasonal winter affective disorder or social anxiety disorders[25].

Others. Several other reported effects of omega 3 PUFAs include enhancing the effects of weight loss on levels of serum lipids, glucose, and insulin in overweight patients[33-34], lowering blood pressure[35], reducing the inflammatory pain associated with rheumatoid arthritis, neuromuscular pain, inflammatory bowel disease, dysmenorrhea[36-37], reducing hot flashes in women during menopause[38], etc.

Mild dyspepsia and belching are the main adverse effects reported with the use of fish oil in the diet. Environmental toxins such as mercury and polychlorinated biphenyls may contaminate marine products such as fish and may result in potential harm to humans[39].

III. Structured lipids containing omega-3 PUFA

Structured lipids (SLs) are restructured triacylglycerols (TAGs) with special functional or nutritional properties. Modification of SL can be done via chemical or enzymatic processes to change the FA composition of TAG, or to redistribute FAs in the glycerol backbone.

SLs containing medium chain FAs (M) at the *sn*-1 and *sn*-3 positions and omega 3 PUFAs (L) at the *sn*-2 position are of interest due to their nutritional properties. Such lipids, called MLM-type SLs, are developed to combine the beneficial effects of FAs with different chain lengths, and their nutritional value lie in the metabolism route during digestion. Upon consumption, TAG is hydrolyzed into 2-monoacylglycerol (2-MAG) and two FAs by lingual, gastric, and mainly by pancreatic lipases. In the case of MLM-type SLs, digestion products will be 2-MAG rich in omega 3 PUFAs and medium chain free fatty acids (MCFFA), the latter of which have higher plasma clearance, higher oxidation rate, improved nitrogen-sparing action, and fewer tendencies to be deposited in the adipose tissue. Thus, they are suitable for providing energy quickly to patients suffering from malabsorption, short bowel syndrome, recovering from burn wounds, and for preterm infants as well. On the other hand, TAG oil rich in medium chain FAs would lack essential omega 3 PUFAs. Providing omega 3 PUFAs esterified to the *sn*-2 position results in better protection of such FAs from oxidation[4] as well as better absorption of them in the 2-MAG form[5]. MLM-type SLs are shown to have beneficial health effects on immune function, nitrogen balance, and lipid clearance from

the bloodstream, and they can be used not only as nutrition for patients with maldigestion and malabsorption of lipids, but also as high-value added nutraceuticals for the elderly[40].

Enzymatic synthesis of SLs is mainly performed by three reaction routes: (i) Acidolysis between a TAG rich in omega 3 PUFAs and medium chain FFAs. The aim is to conserve omega 3 PUFAs at the *sn*-2 position, since they are mainly found in this position naturally, and to replace the FAs at *sn*-1,3 positions with medium chain FAs. Non-reacted FAs can be removed after the reaction by either distillation or alkali extraction. (ii) Interesterification between two TAGs rich in omega 3 PUFAs and medium chain FAs, respectively. The product will be composed of TAGs exclusively, if hydrolysis can be suppressed. Thus, recovery of the product can easily be performed by removing the catalyst. However, the TAGs produced will include other combinations of restructured TAGs as well as MLM. Therefore, the strategy is not suitable for preparation of pure SLs. (iii) A two-step process involving alcoholysis of TAG to produce 2-MAG rich in omega 3 PUFAs, and further acidolysis of the product by medium chain FFA. This reaction route is the most suitable one for synthesis of high purity SLs, since by-products are removed after the first step. *Sn*-1,3-specific lipases, especially immobilized lipases from *Rhizomucor miehei* and *Thermomyces lanuginosus*, are used in most processes. Studies on the topic in the last five years of 2015-2019 are summarized in Table 1. Earlier works have been reviewed elsewhere[48-49].

Table 1 New literature review on enzymatic production of SLs with omega 3 PUFA in the last five years (2015-2019).

Reaction	Origin of lipase	Product properties	Reference
Acidolysis between EPA and palm oil	Immobilized *Rhizomucor miehei* lipase	35.1% oleic acid, 31.1% EPA	41
Acidolysis between fish oil and capric acid	Immobilized *Candida antarctica* lipase B	19.5% capric acid, 16.74% EPA, 11.69% DHA	42
Acidolysis between fish oil and capric acid	Immobilized *Rhizomucor miehei* lipase	9.81% capric acid, 20.70% EPA, 14.13% DHA	42
Acidolysis between soybean oil and FFA from sardine oil	Immobilized *Aspergillus niger* lipase	6.4% EPA, 5.3% DHA, and 67.9% PUFA	43
Acidolysis between soybean oil and FFA from sardine oil	Immobilized *Rhizopus javanicus* lipase	4.6% EPA, 2.6% DHA, and 59.7% PUFA	43
Acidolysis between caprylic acid and fish oil	Immobilized *Rhizomucor miehei* lipase	19.79-28.90% caprylic acid, 11.35-12.66% EPA, 10.57-10.75% DHA	44
Acidolysis between caprylic acid and fish oil	Immobilized *Candida antarctica* lipase B	20.27-30.29% caprylic acid, 11.25-11.56% EPA, 10.29-10.67% DHA	44
Acidolysis between stearic acid and fish oil	Immobilized *Rhizomucor miehei* lipase	19.32-29.25% stearic acid, 7.84-8.94% EPA, 7.62-8.69% DHA	44
Acidolysis between stearic acid and fish oil	Immobilized *Candida antarctica* lipase B	20.58-30.02% stearic acid, 11.15-12.88% EPA, 10.14-10.78% DHA	44
Esterification between omega 3 concentrated FFA from fish oil and dicaprylic glycerol	Immobilized *Candida antarctica* lipase B	24.13% capric acid and 41.5% PUFA	45
Interesterification between fish oil and ethyl caprate	Immobilized *Candida antarctica* lipase B	30.76% capric acid, 15.42% EPA, 9.83% DHA	42
Interesterification between fish oil and ethyl caprate	Immobilized *Rhizomucor miehei* lipase	28.63% capric acid, 16.25% EPA, 10.61% DHA	42
1. Ethanolysis of microalgae oil TAGs 2. Esterification of 2-MAG with caprylic acid	Immobilized *Thermomyces lanuginosus* lipase	8.52% capric acid, 34.90% PUFA	46
1. Ethanolysis of camelina oil TAGs 2. Transesterification of 2-MAGs and EPA or DHA FAEEs	1. Immobilized *Thermomyces lanuginosus* lipase 2. Immobilized *Candida antarctica* lipase B	81.0% DHA STAGs	47

The yield and purity of the products achieved depended on the selectivity of the lipase used. For instance, lipase from *Candida rugosa* was not suitable for production of SLs by acidolysis of omega 3 PUFA-rich oils[50-51], which is believed to be resulted from this lipase's unique FA selectivity. *Candida rugosa* lipase was reported to recognize not only the FAs at the positions of TAG separately, but also the whole TAG molecule[52-53]. It was suggested that hydrolysis reaction catalyzed by *Candida rugosa* lipase took place in two steps: TAG molecules without DHA were hydrolyzed at the earlier stages of the reaction. As the reaction progressed, DHA containing-TAG molecules were hydrolyzed as well. Since acidolysis of TAGs is consisted of a combination of hydrolysis and esterification reactions consecutively, *Candida rugosa* lipase-catalyzed acidolysis of omega 3 PUFA-rich oil would result in low yield. Additionally, positional specificity of lipases was effective on yield and purity of SLs produced. The

sn-1,3 specific *Rhizomucor miehei* lipase was the most effective in MLM-type SL production via acidolysis between lauric acid and seal blubber oil, when compared with *Candida antarctica* lipase B and *Psueodomonas* sp. lipase, both of which are non-specific[54].

Oxidative stability of SL including omega 3 PUFAs has been a major problem to solve, which has been reviewed[55]. Recently, the oxidative stability of emulsion-templated oleogels has also aroused researcher's attention especially when PUFA-rich oils are incorporated. For improving oxidative stability of SL and oils high in omega3 PUFAs, microencapsulated oleogels were examined. Oleogels were produced using a phytosterol blend of β-sitosterol/γ-oryzanol or a blend of sucrose stearate/ ascorbyl palmitate as oleogelators, and menhaden oil or SL prepared from menhaden oil and caprylic and/or stearic acid as the lipid phase. Results show that oleogelation and formation of microcapsules with oleogels improved the oxidative stability of the lipids[56]. A previous study also observed a similar trend in that both internal structuring (oleogel) and external coating (microencapsulation) led to improved oxidative stability, mostly due to protection against oxygen exposure which is a main factor in causing lipid oxidation[57]. It has been reported that oleogels of camellia oil (rich in oleic acids) structured with tea polyphenol-palmitate particles and citrus pectin showed higher oxidative stability compared to the liquid oil, probably as a result of both the formed gel structure and antioxidant properties of tea polyphenol - palmitate particles[58].

IV. Omega 3 pufa-enriched lipids by inter-esterification

Omega 3 PUFAs were incorporated into oils with longer chain FAs to improve the nutritional property. The main application of such lipids is the human milk fat substitutes (HMFS). Breast milk composition is unique since its *sn*-2 position is occupied dominantly by palmitic acid (approximately 60% of total *sn*-2). It has been shown that in breast milk fat, the large amount of palmitic acid esterified at the *sn*-2 position improves the infant's absorption of fat and calcium, while preventing the formation and disposal of calcium soaps. Optimal brain development during infancy and childhood requires an adequate and balanced dietary supply of omega 3 PUFAs. Among others, DHA is of special importance for early development of nervous system in infants. Several studies have demonstrated positive associations between blood DHA levels and improvements on tests of cognitive and visual function, with the effects lasting into childhood[59]. After birth, breast milk provides the sole source of omega 3 PUFAs, the level of which depends on the mother's diet. In the case of mother suffering from omega 3 PUFA deficiency and/or breastfeeding of infant is not possible, omega 3 PUFAs should be provided by formula. The most general method for the production of HMFS is the acidolysis reaction between tripalmitin or palmitic acid-rich SL at the sn-2 position and FFA from various sources. In addition to pure oleic acid, other FFA obtained from different vegetable oils such as olive oil, hazelnut oil, sunfower oil, soybean oil, saffower oil, and rapeseed oil, fish oils or microbial oils as a source of omega 3 PUFA (mainly DHA, and AA) have been used[60-61]. The majority of studies on the enrichment have focused particularly on DHA and ARA incorporation into the specific TAG structure of HMFS. The search for lipases capable of catalyzing reactions for the production of SL with specifc functional properties has greatly increased[62-63].

Besides HMFS, other edible oils have been treated enzymatically in order to enhance their nutritional value by incorporation of omega 3 PUFAs. Many examples from 2001-2009 include acidolysis of triolein[64-65], hazelnut oil[66], olive oil[67], soybean oil[68], and palm oil[69-70] with omega 3 PUFAs, acidolysis of menhaden oil with pinolenic acid[71], chicken fat FFA[72], and conjugated linoleic acid[73], esterification of 2-MAG rich in DHA with oleic acid[74], interesterification of palm stearin and palm kernel oil[75], and stearic acid ethyl esters (SAEE)[76] with omega 3 PUFA-enriched TAGs.

V. Omega 3 PUFA concentrates by enzymatic processing

Concentrated form provides higher omega 3 PUFA content while reducing the intake of saturated and monounsaturated fatty acids, as well as the total fat intake. Moreover, commercial fish oil capsules were shown to have mercury levels from non-detectable to negligible[77], which is a major concern related to adverse effects of fish consumption [78].

Main methods for concentration of omega 3 PUFAs are chromatographic separation, fractional or molecular distillation, low temperature crystallization, supercritical fluid extraction and urea complexation, which have been reviewed recently[79]. Enzymatic

methods have a number of advantages over the abovementioned methods. They do not involve extremes of pHs and high temperatures, which may partially destroy the natural all *cis* structure of omega 3 PUFAs by oxidation, *cis-trans* isomerization or double bond migration. Mild conditions used reduce the process cost as well. One specific feature of enzymatic process is that, it results in selective production due to substrate and positional specificities of lipases.

Lipase-catalyzed hydrolysis, alcoholysis and esterification reactions have been successively used to produce omega 3 PUFA concentrates. New literature review on production of omega 3 PUFA concentrates by lipase-catalyzed hydrolysis in the last five years of 2015−2019 have been seen in Table 2. Hydrolysis process is straightforward and is considered as environment friendly, since the substrates used are lipid and water. Product can be recovered simply by settling or centrifugation. The reaction is generally performed in a stirred tank reactor, which is a batch process, meaning that there is no removal of by-products (FFA). The accumulation of FFA decreases the reaction rate gradually. Removal of FFA from the product can be done by saponification, or more efficiently by distillation.

Table 2. New literature review on production of omega 3 PUFA concentrates by lipase-catalyzed hydrolysis in the last five years (2015−2019)

Origin of lipase	Substrate (Omega 3 PUFA content/%)	Omega 3 PUFA content of product[a]/%	Reference
Bacillus subtilis	Fish oil (29.89)	38.64	80
Candida antarctica	Cod liver oil (25.95)	36.65	81
Candida rugosa,	Cod liver oil (25.95)	48.88	81
	Kilka fish oil (23.19)	46.38	82
	Sardine oil (17.91)	50.79	83
	Fish oil (32.39)	48.01	84
Cryptococcus sp MTCC 5455 lipase	Sardine oil (33.3)	50.3	85
Thermomyces lanuginosa	Cod liver oil (25.95)	41.76	81
	Anchovy oil (29)	35.6	86
	Anchovy oil (29)	38.4	86

[a]Omega 3 PUFA content in glycerides fraction unless otherwise is stated.

Ethanol is the preferred alcohol in omega 3 PUFA-rich oils' treatment. In comparison with hydrolysis, ethanolysis carried out under water-deficient solvent-free conditions to concentrate omega 3 PUFAs substantially reduces the volume of the equipment used to carry out the process, and it is possible to employ ethanolysis in continuous systems, such as packed bed reactors. Moreover, the homogeneity of the substrate mixture is improved compared to hydrolysis, and separation of EE from the oil phase is easier by distillation. According to new researches in recent years, ethanolysis process has been used to produce omega 3 PUFA-enriched partial glycerides[87-88] as well as EE[89-90]. EE rich in omega 3 PUFAs have applications as pharmaceutical agents. From the nutritional point of view, partial glycerides are preferred over EE as the end product of ethanolysis reaction, due to better bioavailability of the former[91]. Besides, partial glycerides are promoted as "natural" products.

In alcoholysis reaction, ethanol can be replaced by glycerol to produce omega 3 PUFA-rich partial glycerides. Glycerolysis produces a different distribution of acylglycerols with no net loss of FA residues. The lipid class of interest can be extracted from the oil mixture afterwards. In the latest five years, diacylglycerols (DAG)[92-93] and MAG[93-95] with high omega 3 PUFA content as potential food ingredients have been produced by lipase-catalyzed glycerolysis; Mixture of acylglycerols is also possible to be produced by the same enzymatic process [96]; In a similar manner, direct esterification of omega 3 PUFAs in FFA form with glycerol can be used to produce enriched acylglycerols [97-98]. Halldorsson et al. [99] separated EPA and DHA from each other by selective esterification of the former with glycerol, while DHA was conserved in the FFA mixture.

VI. Phospholipids

Production of phospholipids (PL) enriched in omega 3 PUFAs has gained attention due to the studies showing that such PL provide novel functions other than the functionalities of PUFA itself, which have been reviewed elsewhere[100]. It has been reported that DHA esterified in the *sn*-2 position of phosphatidylcholine (PC) exhibited increased cell permeability, antitumor activity, and cytotoxicity. PL containing EPA, on the other hand, was shown to decrease adipose tissue weight of rats. Enzymatic production of such PL is mainly catalyzed by lipases

or phospholipases. Modification of the *sn*-1 position of PL is possible by acidolysis or transesterification of PL. Phospholipase A_1 (PLA_1)-catalyzed acidolysis of lecithin with a mixture of EPA, DPA and DHA resulted in a PUFA-rich lecithin where 35% of esterified FA was PUFAs[101]. Added water was shown to decrease the PUFA incorporation significantly. Results were unfortunately not reported, but water in the system is expected to decrease the product yield as well. Even without adding water, hydrolysis was unavoidable, resulting in 13.7% lysophosphatidylcholine (LPC) in the product. A similar work from the authors resulted in the same fashion [102]. Acidolysis resulted in higher incorporation of PUFAs into PC when the reaction was conducted longer than 6 h, increased from 21% to 28% in 24 h, but a parallel increase in the extent of hydrolysis resulted in a significant decrease in the PC yield. Considering both PC and LPC levels as well as PUFA incorporation, optimum a_w for acidolysis of PC catalyzed by immobilized PLA_1 was determined to be 0.65 [103]. In addition to PLA_1, lipases are used for production of PUFA-enriched PL. Regioselective incorporation of EPA and DHA into PC was carried out by lipase-catalyzed esterification of LPC [104]. PC yield was above 30%. Immobilized *Candida antarctica* lipase B was the only useful enzyme among the tested ones for incorporation of DHA, while incorporation of EPA was efficiently carried out using either this enzyme or immobilized *Rhizopus arrhizus* lipase. Peng et al.[105] compared the incorporation rate of different FAs into soybean PL by lipase-catalyzed acidolysis. Conjugated linoleic acid and caprylic acid had similar incorporation rates, but the rate of EPA and DHA incorporation remained relatively low.

In a work by Li et al in 2016[106], DHA/EPA-rich PC was successfully synthesized by immobilized PLA_1-catalyzed transesterification of PC and DHA/EPA rich EE in a solvent free system. The maximal incorporation was 19.09 % (24 h) under the following conditions: temperature 55.7 C, water addition 1.1 wt% and substrate mass ratio (ethyl esters/PC) 6.8:1. Immobilized PLA_1 was more active when water addition was above 0.5 wt%. The vacuum employed after 24 h significantly increased the incorporation of DHA/EPA and the composition of PC, and the highest incorporation (30.31%) of DHA/EPA was obtained at 72 h and the yield of PC was 47.2%[106]. Another study by Wang et al in 2019 shows that the modification of PC with omega 3 PUFA rich EE by immobilized MAS1 from marine *Streptomyces sp.* strain W007 lipase-catalyzed transesterification in the solvent-free system is possible. The maximum omega 3PUFA incorporation into PC was 33.5% at 24 h under the optimized conditions. These results indicate that immobilized MAS1 lipase is a promising biocatalyst with relatively high catalytic activity for the modification of phospholipids [107]. Phospholipase A_2 (PLA_2) catalyzes esterification of PUFAs into the *sn*-2 position of PL exclusively. PLA_2-catalyzed transesterification of PC with EPA-EE was carried out in various organic solvents [108]. The EPA incorporation yield was the highest in toluene. Water activity was shown to effect the reaction yield due to formation of LPC by the reverse reaction, hydrolysis. The highest yield of EPA incorporated to PC was 14.3%.

VII. Concluding remarks

The nutritional value of omega 3 PUFAs for human consumption, as well as their sensitivity to process conditions, has raised high interest in milder processing technology such as the use of enzymes. For the concentration of omega 3 PUFA from fish oils or any other product developments, all the enzymatic reactions could be conducted in ambient temperatures, normal pressure, and nitrogen-protected environment, regardless of hydrolysis, acidolysis, alcoholysis, ester-ester exchange, and esterification. Therefore, fish oil processing for the production of long chain omega 3 PUFAs by lipase-catalyzed reactions would be safer and more efficient than that of traditional methods.

References:

See in the Chinese version before.

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