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# Lipase-Catalyzed Synthesis of *Sn*-2 Palmitate: A Review

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ABSTRACT

Human milk fat (HMF) is an important source of nutrients and energy for infants. Triacylglycerols (TAGs) account for about 98% of HMF and have a unique molecular structure. HMF is highly enriched in palmitic acid (PA) at the *sn*-2 position of the glycerol backbone (more than 70%) and in unsaturated fatty acids at the *sn*-1,3 position. The specific TAG structure in HMF plays a valuable function in infant growth. *Sn*-2 palmitate (mainly 1,3-dioleoyl-2-palmitoyl-glycerol) is one of the structured TAGs that is commonly supplemented into infant formula in order to enable it to present a similar structure to HMF. In this review, the development of the lipase-catalyzed synthesis of *sn*-2 palmitate over the last 25 years are summarized, with a focus on reaction schemes in a laboratory setting. Particular attention is also paid to the commercialized *sn*-1,3 regioselective lipases that are used in structured TAGs synthesis, to general methods of TAG analysis, and to successfully developed *sn*-2 palmitate products on the market. Prospects for the lipase-catalyzed synthesis of *sn*-2 palmitate are discussed.



## 1. Introduction

*Sn*-2 palmitate refers to a group of structured triacylglycerols (TAGs) that are used as a human milk fat (HMF) substitute. A structured TAG is defined as an oil or fat based on a natural TAG that has been modified by artificial methods [1]. *Sn*-2 palmitate has been developed to mimic the fat and oil content of human milk.

Human milk is regarded as the best food for infants, and fats are an important component of it. Although HMF only accounts for 3%–5% of human milk, it supplies about 50% of the energy for breastfeeding infants. HMF is one of the most complex natural lipids. It contains 98%–99% TAGs, 0.26%–0.80% phospholipids, 0.25%–0.34% sterols (mainly cholesterols), and various minor components including monoacylglycerols (MAGs), diacylglycerols (DAGs), free fatty acids (FFAs), and other substances [2]. More than 400 different TAGs have been identified in HMF [3].

It is well known that the functional and nutritional properties of fats and oils are directly related to the type of TAGs they contain. A TAG molecule consists of a glycerol backbone esterified with three fatty acids (Fig. 1). Types of TAG are determined not only according to their fatty acid composition but also based on the positional distribution of fatty acids on an individual glycerol backbone.

Positions on the glycerol backbone are numbered using the stereospecific numbering (*sn*) system, where R′, R″, and R‴ are the fatty acids acylated to the *sn*-1, *sn*-2, and *sn*-3 positions, respectively. HMF is a natural fat with a unique TAG composition, which is characterized by the distribution of fatty acids on the glycerol backbone. The three primary fatty acids of HMF are oleic acid (OA; 18:1, $n-9$, about 33%), palmitic acid (PA; 16:0, about 24%), and linoleic acid (LA; 18:2, $n-6$, about 15%) [4]. In human milk, most PA (about 70%) is esterified at the *sn*-2 position [5]. The *sn*-1 and *sn*-3 positions are mainly occupied by unsaturated fatty acids (USFAs), such as those with 18:1 and 18:2 [6,7]. Long-chain polyunsaturated fatty acids (LCPUFAs), such as eicosapentaenoic acid (EPA; 20:5, $n-3$) and docosahexaenoic acid (DHA; 22:6, $n-3$), are mostly esterified to the *sn*-2 position [8]. The most abundant TAGs in HMF are 1,3-dioleoyl-2-palmitoyl-glycerol (OPO; 16%–29%) and 1-oleoyl-2-palmitoyl-3-linoleoylglycerol (OPL; 13%–20%) [3,6,9,10].

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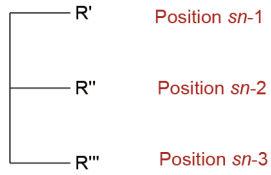


**Fig. 1.** A triacyl-*sn*-glycerol structure. R′, R″, and R‴ are the fatty acids acylated to the *sn*-1, *sn*-2, and *sn*-3 positions, respectively.

In contrast to HMF, vegetable oils, which are generally used in infant formulas, have different fatty acid distribution. In vegetable oils, PA is predominantly (> 80%) esterified to the *sn*-1 and *sn*-3 positions. Therefore, natural vegetable oils with classic processing of edible oil, physical modification (e.g., blending, fractionation), and chemical interesterification cannot achieve the same TAG structure as HMF.

The unique TAG structure in HMF plays a distinctive and important function in infant growth. The primary digestion of TAGs occurs in the stomach and small intestine, hydrolyzed by lipases. Since the stomach and pancreatic lipases are *sn*-1,3 regioselective, the hydrolysis of TAGs results in the formation of *sn*-2 MAG and FFAs [11]. The long saturated fatty acids (mainly PA) in HMF are generally absorbed as *sn*-2 MAG. Free PA forms insoluble soaps with calcium and magnesium, which will be lost through the feces [12–14]. Thus, modification of the TAG structure (by adding *sn*-2 palmitate) in infant formula fat could increase the efficiency of fatty acid absorption and reduce the symptoms caused by calcium palmitate [4,15,16].

The lipid absorption difference due to the positional variation of PA has led to interest being taken in the development and commercialization of the sources of *sn*-2 palmitate. Modification of oils and fats with the aim of improving their nutritional quality may take the form of changes in the fatty acyls esterified on the glycerol backbone [17]. The development of enzyme technology for lipid modification makes this possible. The *sn*-1,3-selective lipase-catalyzed interesterification of TAGs and acyl donors enables the modification of *sn*-1 and *sn*-3 fatty acids on the glycerol backbone to produce TAGs with specific structures. In addition to *sn*-2 palmitate, cocoa butter equivalents (CBEs), medium/long-chain triglycerides (MLCTs), and other structured TAGs are being studied.

During the last 20 years, numerous studies and patents on the lipase-catalyzed synthesis of *sn*-2 palmitate have been reported. Several commercial *sn*-2 palmitate products are now incorporated into infant formulas and follow-up formula as nutritional supplements. Researchers have published several reviews on regioselective lipases and their application to *sn*-2 palmitate [1,4,18–20]. However, few of these reviews have provided detailed information on TAGs analysis, which is critical to the evaluation of TAG structure. This review discusses the progress that has been made over the past 20 years on lipase-catalyzed *sn*-2 palmitate, with a particular focus on TAG analysis. We further provide an integrated view of the perspectives on *sn*-2 palmitate development in the laboratory and on an industrial scale.

## 2. Lipases used in the synthesis of *sn*-2 palmitate

Lipases (EC 3.1.1.3, TAG hydrolases) are one of the most-used biocatalysts in lipid modification [21]. Fats and oils are the natural substrates to lipases. Enzymatic reactions possess several recognized advantages, which include mild reaction conditions, reduced environmental pollution, and utility in the production of "natural" products. However, there is a particular need for the lipase-catalyzed synthesis of structured TAGs—namely, the opportunity for regioselective modification of TAGs [1]. Using specific lipases, lipids with a specific molecular structure can be produced, which are unobtainable by chemical interesterification methods, especially for food processing in large-scale production [1,22].

The three-dimensional structure and catalytic mechanism of lipases began to attract interest in the 1990s, and since then, lipases have been well understood as serine proteases. The serine hydroxyl group of the lipase makes a nucleophilic attack on the carbonyl carbon of the substrate, which leads to the formation of an acyl-enzyme as an intermediate [17]. As the nucleophile, the acyl-enzyme is hydrolyzed by water in an aqueous solution [1,23].

Although increasing numbers of microbial lipases have been successfully used as biocatalysts in the laboratory, only a limited number of lipases have been commercially developed. The commercialized lipases that are commonly used in the production of structured TAGs are listed in Table 1. To date, the most widely used lipases in the synthesis of *sn*-2 palmitate are Lipozyme TL IM and Lipozyme RM IM. More detailed information on the lipases used in *sn*-2 palmitate synthesis is also provided in Table 1.

The regioselectivity of lipases is of particular importance in the lipase-catalyzed steric modification of TAGs. However, the general mechanism of lipases' regioselectivity is still unclear. Most lipases exhibit regioselectivity to the *sn*-1 and *sn*-3 positions of the glycerol backbone and are thus known as *sn*-1,3-specific lipases. Lipases that have been confirmed to have *sn*-1,3 regioselectivity, including pancreatic lipase, pre-gastric lipase, and microbial lipases, are *Penicillium camembertii* lipase, *Rhizopus arrhizus* lipase, *Penicillium roquefortii* lipase, *Rhizomucor miehei* lipase (RML), and so forth. The regioselectivity toward the glycerol backbone depends on both the substrate and the type of lipase [24,25]. The *sn*-1,3 regioselectivity of RML is explained by the docking of the substrate to the binding site of the lipases [26]. Only a few lipases are reported to show *sn*-3 regioselectivity (e.g., rabbit gastric lipase) and *sn*-2 regioselectivity (e.g., *Candida antarctica* A lipase) [27]. However, it should be noted that the regioselectivity of lipase may be altered by the reaction conditions [28]. The regioselectivity of some known lipases has been well documented in publications by Xu [1], Adlercreutz [23], and Bornscheuer [29] among others; therefore, these lipases will not be covered in detail here.

The stability of lipases (i.e., long-term process stability, reusability, tolerance of organic solvents, etc.) is another critical property due to their high cost in industrial applications. Increased stability, especially thermostability, is also beneficial for storage and shipping. An effective and frequently used method to increase lipase stability is immobilization, which causes lipases to be more stable at high reaction temperatures than free enzymes while allowing them to be reused [30]. Most studies on the lipase-catalyzed synthesis of structured TAGs involve immobilized lipases—either commercialized products or lipases that have been immobilized in the laboratory. Protein engineering by means of directed evolution is a more effective method to increase enzyme stability [31,32]. Other processing methods, such as high hydrostatic pressure [33] and modification of the hydrophobicity of the

**Table 1**
Commonly used commercialized *sn*-1,3 regioselective lipases.

| Abbreviation | Source organism | Immobilization materials | Company |
|---|---|---|---|
| Lipozyme TL IM | *Thermomyces lanuginosa* | Silica gel | Novozymes (Denmark) |
| Lipozyme RM IM | *Rhizomucor miehei* | Ion-exchange resin | Novozymes (Denmark) |
| Lipozyme 435 | *Candida antarctica* | Macroporous anionic resin | Novozymes (Denmark) |
| NS40086 | *Aspergillus oryzae* | Macroporous acrylic resin | Novozymes (Denmark) |
| Lipase DF-15 | *Rhizopus oryzae* | — | Amano (Japan) |

oxyanion residue [34], can stabilize and increase the activity of some lipases.

## 3. Analysis of TAGs

The composition of TAGs in natural fats and oils is usually given as a list of the fatty acids present (i.e., the fatty acid amount and fatty acid composition) [2]. There has been increasing interest in TAG composition (i.e., fatty acids distribution on the glycerol backbone) due to the remarkable influence it has on lipid digestion [11], metabolism [35], and improving overall health. However, the analysis of TAGs is challenging work. TAGs are extremely complex mixtures containing glycerol molecules linked to three of the same or different fatty acids [36]. Nevertheless, studies on TAG analysis have significantly increased in recent decades [36], mainly due to improvements in state-of-the art analysis technologies—especially mass spectrometry (MS). When investigating *sn*-2 palmitate, TAG analysis is extremely important, because the different position distribution of the products will have a direct influence on their functional and nutritional properties.

The difference in the positional distribution of the fatty acids of the glycerol backbone was first demonstrated by means of enzymatic (mainly pancreatic lipase) hydrolysis, which can separate the fatty acids at the *sn*-2 position (regioselective analysis). The results reflect the fatty acid composition on the *sn*-1,3 and *sn*-2 positions, which cannot yield information on the TAG structure. However, this method is still used in some studies on *sn*-2 palmitate synthesis, due to its less expensive and simple calculation process. This method is often used at the cost of real information on TAG composition.

The most widely used and powerful methods are chromatographic methods, such as gas chromatography (GC) and liquid chromatography (LC), which are sometimes combined with MS [37]. The use of chromatographic methods in TAG profiling has been described in a number of excellent books and reviews, such as the review on chromatographic techniques by Buchgraber et al. [38], the review on thin-layer chromatography (TLC) by Fuchs et al. [39], the review on high-temperature GC by Ruiz-Samblás et al. [40], the review by Indelicato et al. [36], and the book by Christie and Han [41]. The present review summarizes the analysis methods that are generally used—especially in the study of structured TAG synthesis—with an emphasis on the quantitative determination of positional isomers.

The generally used TAG analysis methods for structured TAGs are high-temperature GC, non-aqueous reversed-phase (NARP) LC/evaporative light-scattering detector (LC-ELSD), and silver-ion-LC-ELSD. High-temperature GC is GC in which the capillary columns are maintained at high temperatures (> 350 °C). TAGs are separated based on their degree of unsaturation. However, a commonly used detector—the flame ionization detector—cannot provide information about TAG isomers. Thus, the identification of TAGs is based on the retention times compared with standards. For the analysis of structured TAGs, this method is generally combined with the analysis of *sn*-2-position fatty acids composition.

The LC that is widely used in TAG analysis can be divided into two kinds, depending on the relative polarity of the two chromatographic phases; namely, normal-phase (NP) LC and NARP LC. NARP LC uses a gradient elution and various mobile-phase systems [36]. The elution order depends on both the carbon numbers (CNs) and the double bonds (DB), which depend on the effective carbon number (ECN): $CN - 2 \times DB$. Under optimized chromatographic conditions, TAGs with the same ECN can be separated [42–44]. The sample preparation is simple, and the equipment is relatively cheap. Therefore, this method is the most widely used [42,43,45–95]. However, this method cannot separate TAG-region isomers.

The most-used NP LC in TAG analysis is silver-ion NP LC. Silver-ion LC can be applied for the separation of TAG isomers due to the weak complexes that form between silver ions of the stationary phase and the π electrons of DBs [96]. The separated TAGs are divided into groups that differ in DB number. Using this method, the exact content of each TAG isomer can be measured [44,45].

Another method that involves high-resolution $^{13}C$ nuclear magnetic resonance (NMR) can provide information on the fatty acid composition attached to the TAG and at the specific *sn*-2 position of the TAG, which can be used for the regioselective analysis of the fatty acids attached on the TAG [44]. Recently reported LC methods, including ultra-high-performance LC equipped with quadrupole time-of-flight MS [3], two-dimensional GC [97], and silver-ion atmospheric pressure chemical ionization (APCI) MS [98], can effectively separate the TAGs of the complex lipids matrix in natural fats and oils, as well as perform stereospecific analysis of TAGs, and should therefore be applied to structured TAG analysis in the future.

## 4. Reaction schemes

This review summarizes the studies that have been published on the lipase-synthesis of *sn*-2 palmitate between 1997 and 2018, as shown in Table 2. In general, there are three reaction schemes: acidolysis, transesterification, and alcoholysis and esterification. The acidolysis reaction is the most common method, followed by the transesterification reaction.

### 4.1. Acidolysis reaction

The typical scheme for an *sn*-1,3 regioselective lipase-catalyzed acidolysis reaction is shown in Fig. 2. The acidolysis reaction is generally carried out by TAGs rich in PA at the *sn*-2 position with an FFA or FFA mixture by the use of *sn*-1,3 regioselective lipases. Due to its high purity, tripalmitoylglycerol (PPP) is generally used as the substrate in the laboratory; however, since its price is high, cheap and natural oils (e.g., palm stearin, palm oil, butterfat, lard, etc.) are often considered as substitutes for PPP. FFA sources are usually OA, LA, gamma-linolenic acid (GLA), or an FFA mixture of vegetable oils (e.g., soybean oil, rapeseed oil, sunflower oil, palm kernel oil, coconut oil, hazelnut oil, etc.), fish oil, or single-cell oils rich in LCPUFAs.

As shown in Table 2, the acidolysis reaction was used in most of the studies over the past two decades, in which the contents of PA in the *sn*-2 position usually reached over 60%. Esteban et al. [72] used several *sn*-1,3 regioselective lipases (including lipase DF, Lipozyme RM IM, Palatse 20000L, Lipozyme TL IM, and lipase QLC) to catalyze the acidolysis reaction of PA-enriched TAGs with OA. The results showed that lipase DF could achieve relatively high incorporation of OA (50.4%) with a short reaction time (1 h) to maintain a high PA content in the *sn*-2 position (68.6%). After the optimization of various factors, a final structured TAG containing 67.2% OA and 67.8% PA at the *sn*-1,3 and *sn*-2 positions, respectively, was obtained.

Recently, our group also carried out the synthesis of *sn*-2 palmitate. Wei et al. [59] synthesized high-purity *sn*-OPO catalyzed by different *sn*-1,3 regioselective lipases (Lipozyme RM IM and Lipozyme TL IM) in both solvent (*n*-hexane) and solvent-free systems with a high purity of PPP and high OA, prepared from camellia seed oil. Lipozyme RM IM was found to be suitable for *n*-hexane, and Lipozyme TL IM was found to be suitable for solvent-free systems. The relative contents of PA in the *sn*-2 position reached 92.92% and 86.62%, while the contents of *sn*-OPO were 32.34% and 40.23%, respectively. Zou et al. [48] produced *sn*-2 palmitate from basa catfish oil and sesame oil fatty acids using a Lipozyme RM IM-catalyzed acidolysis reaction. The enzymatic product contained a

**Table 2**
A 25-year literature survey of the lipase-catalysis of *sn*-2 palmitate.

| Lipase | Substrate | Products | Reaction conditions | Reactor | TAGs analysis | Reference |
|---|---|---|---|---|---|---|
| Immobilized *Candida lipolytica* lipase | PPP and OA | 46.5% of OPO | 50 °C, *n*-hexane | Vial | Silver-ion LC | 2017 [45] |
| Novozym 435, Lipozyme 435, Lipozyme TL IM, Lipozyme RM IM | *Nannochloropsis oculata* and FFAs from *Isochrysis galbana* | 59.38%–68.13% PA in the *sn*-2 position, 13.92%–17.12%, *n* − 3, PUFA | 50/60 °C | — | — | 2017 [46] |
| Lipozyme RM IM | Lard and selected oils | 39.2% PA in the *sn*-2 position, 24.78% OPO | 50 °C | PBR | NARP LC | 2016 [47] |
| Lipozyme RM IM | Catfish oil and FFA from sesame oil | 67.7% PA in the *sn*-2 position, 23.2% LA | 40 °C | 25 mL RBF | NARP LC | 2016 [48] |
| Lipase *Candida* sp. 99–125 coupled with β-cyclodextrin | Lard and OA | 79.51% PA in the *sn*-2 position, 55.3% OPO | 40–50 °C | — | HPLC–ELSD | 2016 [42] |
| *Candida* sp. 99–125 lipase | Monopalmitin and OA | 75% PA in the *sn*-2 position, 40% OPO | 38 °C | Orbital shaker | GC | 2016 [49] |
| Immobilized *Rhizopus oryzae* lipase and Lipozyme RM IM | PPP and FFA from camelina oil | 42.6%–52% *sn*-2 palmitate, 67.7 mol% PA in the *sn*-2 position | 60 °C | Stirred batch reactor | GC | 2016 [50] |
| Lipozyme RM IM | Silkworm pupae oil and OA | 42.38% ALA in the *sn*-2 position, 20.11% PA, and SA | 60 °C | Conical flasks | — | 2015 [51] |
| Lipozyme TL IM | Palm stearin, high oleic sunflower oil, and tricaprin | 40% PA in the *sn*-2 position, 21.22% CA | 60 °C, *n*-hexane | Labeled Teflon-lined test tube | NARP LC | 2015 [52] |
| *Aspergillus oryzae* lipase | Palm stearin with OA | 55.08% PA in the *sn*-2 position, 45.65% C52 | 65 °C | 100 mL RBF | GC | 2015 [53] |
| Novozym 435 and Lipozyme RM IM | Lard and 2-MAG enriched in OA and LA | ~58.7% PA in the *sn*-2 position, 23.1% OPO | 37 °C, *n*-hexane | — | NARP LC | 2015 [54] |
| Lipozyme IM-20 | PPP and OA | 55.2% OPO | Isooctane | — | Silver-ion LC | 2015 [55] |
| Lipozyme RM IM | PPP and OA | 35.9% OPO | 50 °C, *n*-hexane | — | LC APCI MS/MS | 2015 [56] |
| Novozym 435 | 1,3-Diolein and PA | 94.8% OPO | Dichloromethane, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide and 4-dimethylaminopyridine | — | $^{1}$H NMR | 2015 [57] |
| Lipozyme RM IM | Catfish oil and high oleic sunflower oil | 50%–80% PA in the *sn*-2 position | 50 °C | 25 mL RBF | NARP LC | 2016 [58] |
| Lipozyme TL IM and Lipozyme RM IM | PPP and OA | ~85% PA in the *sn*-2 position, 32% OPO | 50/60 °C, *n*-hexane/no solvent | 25 mL RBF | NARP LC | 2015 [59] |
| Lipozyme RM IM | Lard and FAs from camellia oil | ~70% PA in the *sn*-2 position | 45 °C, no solvent | Orbital shaker | — | 2014 [60] |
| Novozym 435 | PA-enriched refined olive oil and DHA | 55.79 mol% of PA in the *sn*-2 position and 3.54 mol% total DHA | 60 °C, *n*-hexane | Screw-capped test tubes | — | 2014 [61] |
| Lipozyme RM IM | Lard and vegetable and single-cell oils | 60%–70% PA in the *sn*-2 position | 60 °C, no solvent | 50 mL RBF | NARP LC | 2014 [43] |
| Lipozyme RM IM | *Cinnamomum camphora* seed oil and OA | 78.69% PA in the *sn*-2 position | 60 °C, no solvent | 50 mL RBF | NARP LC | 2014 [62] |
| Lipozyme TL IM | PPP and FA from olive oil | ~60% PA in the *sn*-2 position | 65 °C, no solvent | Erlenmeyer flask | NARP LC | 2013 [63] |
| Novozym 435 | Hazelnut oil and PA; and ethyl palmitate | 63.5 mol% of PA and 71.1% of PA in the *sn*-2 position | 65 °C, *n*-hexane | Test tubes[a] | NARP LC | 2013 [64] |
| Novozym 435 and Lipozyme TL IM | PPP and FA from olive oil, AA), and DHA | > 50% PA in the *sn*-2 position | 60 °C, no solvent | Erlenmeyer flasks | NARP LC | 2013 [65] |
| Novozym 435 and Lipozyme RM IM | PA-enriched hazelnut oil and DHA, and ARA | 57.3% of DHA, 2.7% of ARA, 2.4% of PA, and 66.1% PA in the *sn*-2 position | 50/60 °C, no solvent | Screw-cap test tubes | — | 2012 [66] |
| *Carica papaya* lipase | PPP and OA | ≥ 70% PA in the *sn*-2 position, 22.1 mol% OA | 60 °C, no solvent | Closedcylindrical batch reactors | — | 2012 [67] |
| Lipozyme TL IM | PPP and SDA from hazelnut oil, commercial oil | 46.2% PA in the *sn*-2 position | 60 °C, *n*-hexane | Flasks with glass stoppers | — | 2012 [68] |
| Lipozyme RM IM | Palm stearin and mixed FA of SA and MA from rapeseed oil, sunflower oil, and palm kernel oil | 28.8% PA and 53.2% PA in the *sn*-2 position | 58 °C | PBR | NARP LC | 2012 [69] |
| Lipozyme TL IM | Lard and soybean oil | ~50% saturated fatty in the *sn*-2 position | 60 °C | PBR | GC, $^{13}$C NMR | 2011 [70] |
| Lipase DF, Palatase 20000L, Lipozyme RM IM, Lipozyme TL IM, lipase QLC | TAGs rich in PA and OA | 68.6% PA in the *sn*-2 position | 37 °C, no solvent | Flasks with silicone capped stoppers | — | 2011 [71] |

(*continued on next page*)

**Table 2** (*continued*)

| Lipase | Substrate | Products | Reaction conditions | Reactor | TAGs analysis | Reference |
|---|---|---|---|---|---|---|
| Lipozyme RM IM | PPP and FAs from hazelnut oil and Neobee | Rich in medium-chain FA | 57 °C, *n*-hexane | Orbital shaking water bath | — | 2011 [72] |
| Lipozyme RM IM | Lard and FAs from camellia oil | 43.72% OPO | 60/34 °C | A 25 mL conical flask | NARP LC | 2011 [73] |
| Novozym 435 and Lipozyme TL IM | PPP and SDA from soybean oil | ≥ 60% PA in the *sn*-2 position, 6.82% SDA | 50 °C, *n*-hexane | Teflon-lined test tubes | — | 2011 [74] |
| Lipozyme RM IM | Palm stearin and mixed FAs from rapeseed oil, sunflower oil, palm kernel oil, SA, and MA | 29.7% PA in the *sn*-2 position, 62.8% PA | 60 °C, no solvent | — | NARP LC | 2011 [75] |
| Novozym 435 | PA and palm oil stearin | 70.5% PA in the *sn*-2 position, 70.7% PA | 37/50 °C, *n*-hexane | Erlenmeyer flasks[a] | — | 2010 [76] |
| Lipozyme TL IM | PPP-rich fraction and ethyl oleate | 31.43% OPO, 64.9% PA in the *sn*-2 position, 80.6% PA | 50 °C | Erlenmeyer flask[a] | Silver-ion HPLC | 2010 [77] |
| Lipozyme TL IM, Lipozyme RM IM, *Candida parapsilosis* | PPP and OA/omega-3 PUFA | — | 60 °C, no solvent | PBR | — | 2010 [78] |
| Lipozyme RM IM | Lard and FAs from palm kernel oil, tea seed oil, and soybean oil | Similar to HMF | 60 °C, no solvent | Orbital shaker flask | — | 2010 [79] |
| Novozym 435 | Ethyl palmitate and amaranth oil | 76.6% PA in the *sn*-2 position, 45.5% PA | 60 °C, no solvent | Test tubes[a] | — | 2009 [80] |
| *Bacillus stearothermophilus* MC7 | PPP and OA | Conversion exceeded 50% | 60 °C, no solvent | — | Silver-ion TLC | 2008 [81] |
| Lipozyme RM IM | PPP, coconut oil, safflower oil, and soybean oil | 40.8% PA in the *sn*-2 position, 24.6% PA | 55 °C, *n*-hexane | Test tubes[a] | — | 2007 [82] |
| Lipozyme RM IM | FAs and lard | ~70% PA in the *sn*-2 position | 65 °C | PBR | — | 2006 [83] |
| Lipozyme RM IM | PPP and hazelnut oil FA, and omega-3 FA | 76.6% PA in the *sn*-2 position | 55 °C, *n*-hexane | Test tubes[a] | — | 2006 [84] |
| Lipozyme RM IM LIP1 (*Candida rugosa* lipase) | PPP and OA/methyl oleate | 49.4% OA (Lipozyme RM IM, methyl oleate) | 65 °C, 45 °C, *n*-hexane | Test tubes[a] | — | 2006 [85] |
| Lipozyme RM IM and Lipozyme TL IM | PPP and hazelnut oil FA, and GLA | 70% PA in *sn*-2 position, ~10% GLA, and ~ 44% OA | 55 °C, *n*-hexane | Test tubes[a] | — | 2005 [86] |
| Lipozyme RM IM | PPP and hazelnut oil FA, and SA | 42.5% OA, 7% SA, and 70% PA in the *sn*-2 position | 65 °C, *n*-hexane | Test tubes[a] | — | 2005 [87] |
| Novozym 435 and Lipase IM 60 | PPP and OA | 74% OPO, PA 90.7% in *sn*-2 position | 50 °C, no solvent | 10 mL glass vial | Silver-ion HPLC | 2004 [88] |
| Lipozyme RM IM | FAs and lard | 71% PA in the *sn*-2 position | 61 °C, no solvent | Conical flask | — | 2003 [89] |
| Immobilized *Fusarium* and *Rhizopus* lipase | PPP and OA | 36 mol% OPO | 50 °C, no solvent | Screw-capped vessel | 2 × NARP LC | 2001 [90] |
| Immobilized RDL | AA and PPP | 56.9/3.2 mol% of AA in *sn*-1,3/2 position, 75.9% APA | 40 °C, no solvent | Screw-capped vessel | NARP LC | 2000 [91] |
| Lipozyme IM, immobilized RDL, RML | PPP, 2-monopalmitin, and OA | OPO in up to 78% yield containing 96% PA in *sn*-2 position | Vacuum/*n*-hexane | Closed vessel | GC | 1999 [92] |
| Lipozyme IM and *papaya latex* | FAs and PPP | PA > 90% in *sn*-2 position | 60 °C, no solvent | Screw-capped tube | GC | 1998 [93] |
| Lipozyme IM, immobilized RDL, RJL, RNL | PPP, MAG, and OA | 72% OPO, PA 94% in *sn*-2 position, 84% yield at > 95% purity | Methyl-*t*-butyl ether | Closed vessel | GC | 1998 [94] |
| Immobilized M10™ from *Mucor jauanicus* | OA and butterfat | Modified butterfat | 40 °C, no solvent | Hollow-fiber reactor | NARP LC | 1997 [95] |

RBF: round-bottom flask; CA: capric acid; PPP: tripalmitoylglycerol; PUFA: polyunsaturated fatty acids; PBR: pebble-bed reactor; HPLC: high-performance liquid chromatography; ALA: α-linoleic acid; FA: fatty acid; AA: arachidonic acid; SA: stearic acid; MA: myristic acid; GLA: gamma-linolenic acid; SDA: stearidonic acid. RDL: *Rhizopus delemar* lipase; RJL: *Rhizopus javanicus* lipase; RNL: *Rhizopus niveus* lipase; APA: 1,3-arachiarachidonoyl-2-palmitoyl-glycerol C52: TAG with a carbon number of 52.

[a] Test tubes or flasks with screw-caps.

67.7% distribution proportion of *sn*-2 PA out of the total PA under optimal conditions. This approach is characterized by simple steps and fewer byproducts. The main byproduct of the acidolysis reaction is FFA, which can be effectively eliminated by molecular distillation. However, due to the presence of acyl migration, the fatty acid at the *sn*-2 position migrates to the *sn*-1,3 position, which affects the yield of the target structured TAGs. Therefore, the yield of pure structured TAGs obtained by the acidolysis reaction is relatively lower than that obtained by the alcoholysis reaction.

### 4.2. Transesterification reaction

The transesterification reaction is performed with TAGs rich in PA in the *sn*-2 position with FFA esters or oils, using *sn*-1,3 regioselective

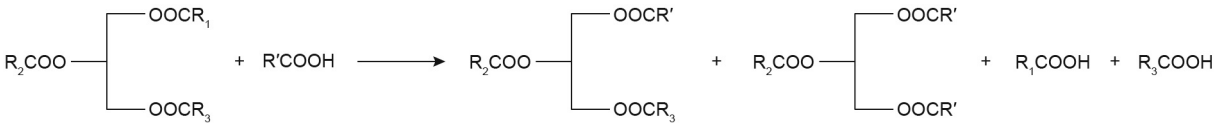


**Fig. 2.** Scheme for the *sn*-1,3 regioselective lipase-catalyzed acidolysis reaction.

lipases. Fig. 3 shows the scheme for the *sn*-1,3 regioselective lipase-catalyzed transesterification reaction. The FFA esters mainly include ethyl and methyl esters, while natural oils rich in OA/polyunsaturated fatty acid (PUFA) are usually used as the acyl donors. In this process, the selected materials are generally cheap and widely distributed, making it quite popular for the industrial production of structured TAGs. However, the final product is a mixture of different TAGs with similar physical properties, which makes it difficult to purify and obtain the structured TAGs. Therefore, it is essential to choose the appropriate kinds and proportions of oils.

With the aim of low cost, some studies selected lard as the substrate [47,70]. Zou et al. [99] produced *sn*-2 palmitate by means of the Lipozyme RM IM-catalyzed transesterification of lard with a vegetable oil mixture in a packed-bed reactor. The vegetable oil was a blend of high OA oils (e.g., sunflower oil and canola oil), microbial source oils (e.g., algal oil and microbial oil), palm kernel oil, and palm oil. The final product contained 39.2% PA in the *sn*-2 position and showed high degrees of similarity in its fatty acid profile with HMF. Srivastava et al. [85] synthesized structured TAGs by the transesterification of PPP with either OA or OA methyl ester using LIP1 or Lipozyme RM IM as the biocatalyst. The results showed that higher proportion incorporation of OA was observed with the OA methyl ester (transesterification) than with OA (acidolysis) in both lipases and that Lipozyme RM IM might be more suitable than LIP1 for preparing *sn*-2 palmitate.

### 4.3. Alcoholysis and esterification reaction

To overcome the drawbacks of the reaction schemes described above, and to obtain a higher yield of structured TAGs, the alcoholysis reaction has been proposed. The scheme for the *sn*-1,3 regioselective lipase-catalyzed alcoholysis reaction is shown in Fig. 4. This method is a two-step process, and *sn*-1,3 regioselective lipases are needed for both reactions. First, natural oil or fat is selected to react with alcohols, resulting in the formation of 2-MAG rich in PA. Second, the purified 2-MAG is esterified with FFA to obtain a higher yield of the target structured TAGs. Schmid et al. [94] produced *sn*-OPO by means of the alcoholysis reaction of PPP; in the first step, these researchers investigated the influence of different lipases (Lipozyme RM IM, *Rhizopus delemar*, and *Rhizopus javanicus*) on the yield and purity of 2-MAG enriched in PA. The results showed that *Rhizopus delemar* immobilized on celite gave the best yield, which was 95% 2-MAG after crystallization in methyl-*t*-butyl ether.

In the second step, the purified 2-MAG was esterified with OA in *n*-hexane using Lipozyme RM IM or *Rhizopus delemar* immobilized on celite. The final product contained 92%–94% PA in the *sn*-2 position and 83%–89% OA in the *sn*-1,3 positions, while the yield of *sn*-OPO reached 70%–72%. As shown in Table 2, the alcoholysis reaction has been used in only a few studies, in which an *sn*-OPO content greater than 70% was achieved, and in which the PA contents in the *sn*-2 position usually reached greater than 90%. This process avoids acyl migration and obtains pure structured TAGs. However, the complexity of the steps leads to an increase in cost. Therefore, it is not commonly used in industrial production.

## 5. Marketed products

Structured TAGs are designed to have desired nutritional, textural, or physicochemical properties for multipurpose applications in food products and in products for special medical purposes. Many studies have focused on the commercialization of structured TAGs. Today, commercialized products of *sn*-2 palmitate have been manufactured, and are commonly designed to mimic the composition and distribution of HMF. These products have been practically applied to infant formulas as a nutrient fortifier.

The production of *sn*-2 palmitate was first developed by Loders Croklaan [100]. In 1995, the product was approved as an infant

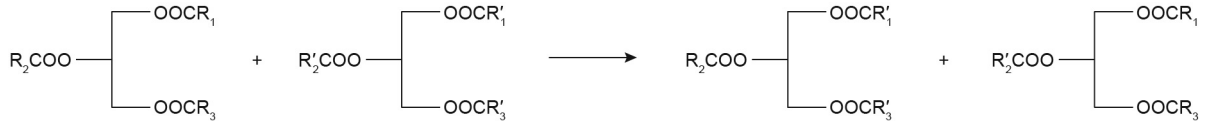


**Fig. 3.** Scheme for the *sn*-1,3 regioselective lipase-catalyzed transesterification reaction.

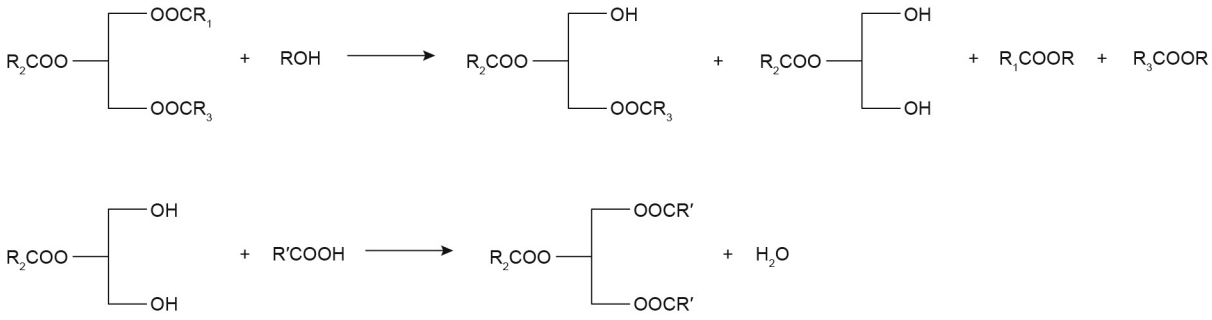


**Fig. 4.** Scheme for the *sn*-1,3 regioselective lipase-catalyzed alcoholysis reaction.

formula ingredient in Europe and began to be produced and commercialized as Betapol®. Betapol® is produced by the acidolysis of PPP-rich fats (palm stearin) with OA (FFAs obtained from high oleic sunflower oil) using immobilized *sn*-1,3 regioselectivity lipase (*Rhizomucor miehei*). The production is a two-stage process in two packed-bed reactors filled with the enzyme in order to increase the fatty acid conversion [101]. The TAG molecular weight distribution of Betapol® is mainly composed of an ECN:DB of 52:2 (33.4 mol%) and an acyl carbon number (ACN):DB of 52:3 (10.5 mol%), the main regio-isomers of which are *sn*-OPO (82.2 mol%) and *sn*-OPL/1-linoleoyl-2-palmitoyl-3-oleoylglycerol (LPO) (82.0 mol%), respectively [102].

At present, there are several commercial versions of *sn*-2 palmitates, such as INFAT®, which is manufactured by Advanced Lipids (Sweden). INFAT® contains 70%–75% PA esterified on the *sn*-2 position of the glycerol backbone. In the Chinese market, *sn*-2 palmitate products are being manufactured by Wilmar International (Singapore) and Zhejiang Beijia Bio-Technology Co., Ltd. (China), among others. Other commercial *sn*-2 palmitates include Bonamil (Wyeth Ayerst, United States), Alsoy (Nestlé, Switzerland), and Cow & Gate Premium (Nutricia, the Netherlands). For more information, readers are referred to the book chapters by Ferreira and Tonetto [101] and Happe and Gambelli [103].

## 6. Conclusions

In the past two decades, researchers have developed several structured TAGs using lipase catalyst technologies, such as CBEs, *sn*-2 palmitate, *sn*-BOB (where B refers to behenic and O refers to OAs), and more [1]. Among these, *sn*-2 palmitate is a successfully produced structured TAG that has been added to infant formula as a nutritional supplement by many manufacturers.

In addition to PA, it is necessary to study and modify the minor fatty acids to achieve a similar positional distribution to that of HMF. Some LCPUFAs (e.g., arachidonic acid (ARA) and EPA) are reported to be mainly attached to the *sn*-1,3 position, while a group of minor fatty acids, such as the branched fatty acids, have shown much higher content (up to 60%) at the *sn*-2 position [104]. A better understanding of the composition and structure of HMF will provide detailed information for the investigation of novel structured TAGs.

Lipase applications for the production of structured TAGs will have a broad market. Although lipases have obvious advantages compared with chemical catalysts for structured TAG synthesis, the industrial application has been relatively slow, mainly due to the high cost. Screening for cheaper *sn*-1,3 regioselectivity lipases and methods for improving their stability will be promising technologies for future research.

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## Compliance with ethics guidelines

Wei Wei, Cong Sun, Xiaosan Wang, Qingzhe Jin, Xuebing Xu, Casimir C. Akoh, and Xingguo Wang declare that they have no conflict of interest or financial conflicts to disclose.

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