



Enzymatic production of structured lipids: reaction pathways, process optimization, functional significance, and industrial challenges

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Abstract

Structured lipids (SLs) are lipids that have been purposely manipulated in fatty-acid content, positional distribution, or molecular species to provide specific nutritional or physicochemical qualities. The lipase-catalyzed synthesis is especially useful when positional or substrate selectivity is required. The performance depends on the enzyme specificity, water activity, immobilization approach, reaction engineering, and downstream purification. The review critically discusses enzymatic pathways for SL generation, i.e., esterification, acidolysis, and interesterification, and assesses the process variables that govern the yield, selectivity, acyl migration, and catalyst lifetime. Lipase immobilization, ultrasound-assisted process intensification, molecular distillation, and emerging machine learning applications in lipase discovery, engineering, and bioprocess optimization are highlighted in particular. The nutritional evidence is presented by research type and physiological context, highlighting sn-2 palmitate in baby nutrition, diacylglycerol-rich oils, medium- and long-chain triacylglycerols, and structured phospholipids. Industrial and translational uses are human-milk-fat alternatives, cocoa butter equivalents, dietetic structured triacylglycerols, diacylglycerol-rich oils, and structured fat emulsions. To summarize, the analysis highlights the operational stability, continuous processing, recombinant

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lipase synthesis, and purification as critical factors for industrial practicality.

Keywords: structured lipids; enzymatic synthesis; lipase; interesterification; immobilization; medium- and long-chain triacylglycerols; human-milk-fat substitutes; process optimization

1. Introduction

Structured lipids (SLs) are lipids that have been specifically designed to have a certain fatty acid composition, positional distribution on the glycerol backbone, or molecular species profile so that they can obtain particular nutritional, metabolic, or physicochemical properties. The idea is more general than any particular lipid class. Triacylglycerols (TAGs), diacylglycerols (DAGs), monoacylglycerols (MAGs), and phospholipids can all be structurally modified for a particular purpose, but naturally occurring members of these groups are not automatically SLs. The interest in SLs has been increased due to the use of positional and compositional control to develop specialty fats, infant-nutrition lipids, dietetic oils, emulsifying lipids, and other high-value products (Lee and Akoh, 1998; Guo et al., 2020; Yan et al., 2026).

Chemical interesterification is a well-established technique for altering fat functionality, as it is relatively scalable and randomizes acyl groups among TAG species. The main drawback of this process for nutritional or stereospecific design is the lack of regioselective control, which may be an essential parameter when the placement of the fatty acid on glycerol is part of the desired product specification (Senanayake and Shahidi, 2005; Willis and Marangoni, 2002). Lipases offer an alternative strategy, as some enzymes have substrate, fatty-acid, or positional specificity, which allows more selective remodelling under very mild reaction conditions. However, these advantages do not make enzymatic processing always better, as reaction rate, enzyme stability, catalyst cost, water activity, substrate phase behavior, and downstream purification are still key process constraints (Bornscheuer, 2018; Kim and Akoh, 2015).

Representative items highlight the need to differentiate molecular and application categories. MLCTs are medium- and long-chain triacylglycerols (MLCTs) with medium- and long-chain fatty acids in a tailored TAG system. DAG-rich oils take advantage of variances in glyceride structure and metabolism. The objective of human-milk-fat substitutes is to achieve stereospecific fatty-acid distributions, such as enrichment of palmitate at the sn-2 position. Cocoa butter equivalents (CBEs), in contrast, are specialty confectionery fats meant to replicate a narrow TAG profile and thermal

behavior; they are not a lipid class comparable to emulsifiers or fat substitutes. The appropriateness of each product depends on a particular mix of molecular framework, processing objective, and evidence for function (Wei et al., 2019; Lee et al., 2022; Bahari and Akoh, 2018).

A translation for industry demands more than a good laboratory conversion. Process feasibility is affected by catalyst lifespan, immobilization method, substrate quality, mass transfer, acyl migration, reactor configuration, purification, and product value. Immobilized lipases can assist in catalyst recovery and can be reused or used continuously; however, support chemistry can also alter activity and selectivity. Process intensification tools and data-driven techniques can equally improve development processes but need to be assessed in terms of product quality and process metrics rather than being intrinsically sustainable technology. The following sections review SL classification, reaction routes, process optimization, nutritional evidence, application domains, and industrial constraints, utilizing primary evidence where claim-specific data exist.

2. Classification of Structured Lipids

For the purposes of this review, SLs are organized according to the molecular scaffold that is deliberately modified: MAGs, DAGs, TAGs, and phospholipid-based structured lipids. This classification should not be interpreted to mean that every naturally occurring MAG, DAG, TAG, or phospholipid is an SL. Rather, the term applies when composition, stereospecific distribution, or molecular species have been intentionally altered to achieve a target nutritional or technological function (Guo et al., 2020). Natural fats and oils are complex mixtures dominated by TAGs but also contain DAGs, MAGs, free fatty acids, phospholipids, sterols, and other minor components; they are therefore not accurately described as containing only TAGs.

2.1 Monoacylglycerols (MAGs)

Monoacylglycerols contain one fatty acid esterified to glycerol. They occur naturally as minor lipid components and digestion intermediates and are also produced synthetically for food, pharmaceutical, and surfactant applications. Their amphiphilic structure makes many MAGs effective emulsifiers. Enzymatic routes include glycerolysis, alcoholysis, and esterification, but the usefulness of a given route depends on equilibrium, acyl migration, and the efficiency of product isolation. In an SL context, MAGs are most relevant when the fatty-acid composition or isomer distribution is deliberately controlled rather than simply because the molecule belongs to the MAG class (Bornscheuer, 2018; Ghouila et al., 2019).

2.2 Diacylglycerols (DAGs)

Diacylglycerols contain two esterified fatty acids and occur mainly as sn-1,2- and sn-1,3-isomers. DAG-rich oils can be prepared by glycerolysis, esterification, or controlled hydrolysis followed by separation of residual MAGs, TAGs, and free fatty acids. Immobilized lipases and reactor design can improve process control; for example, Chen et al. (2020) demonstrated production of high-purity medium-chain DAGs using an immobilized-lipase bubble-column reactor. Human studies have also reported structure-dependent metabolic outcomes. In a double-blind controlled study, Nagao et al. (2000) observed greater reductions in several body-fat measures with DAG-rich oil than with a TAG comparator, while Maki et al. (2002) reported greater reductions in body weight and fat mass with DAG oil during a reduced-energy diet. These findings support study-specific physiological effects but do not justify the general statement that DAG oils prevent fat accumulation in all populations or dietary contexts.

2.3 Triacylglycerol-Based Structured Lipids

Triacylglycerols are the principal molecular scaffold for many SLs because three esterified positions can be manipulated in chain length, degree of unsaturation, and stereospecific distribution. Lipase-catalyzed esterification, acidolysis, and interesterification are commonly used to generate target TAG species. Examples include MLM-type lipids, MLCTs, human-milk-fat substitutes, and CBEs. The key design feature is not simply the presence of three fatty acids but the deliberate control of which fatty acids are incorporated and, where relevant, at which positions (Yang et al., 2014; Zhao et al., 2014).

2.3.1 Medium-Chain Triacylglycerols (MCTs)

Medium-chain triacylglycerols (MCTs) contain predominantly medium-chain fatty acids, commonly C8:0 and C10:0 and, depending on the definition used, other fatty acids up to C12:0. MCTs are often discussed together with SLs because medium-chain fatty acids are incorporated into many designed TAG systems; however, an MCT is not necessarily an SL unless its molecular composition or positional distribution has been deliberately engineered. Medium-chain fatty acids are handled differently from long-chain fatty acids during digestion and postabsorptive metabolism, but claims about rapid energy availability or body-fat reduction should be linked to specific formulations, doses, and populations rather than treated as universal properties (Lee et al., 2022).

2.3.2 Medium- and Long-Chain Triacylglycerols (MLCTs)

Medium- and long-chain triacylglycerols (MLCTs) contain both medium- and long-chain fatty acids within the same TAG system and can be produced by esterification, acidolysis, or interesterification. Their molecular design is intended to combine the distinct handling of medium-chain fatty acids with the nutritional or functional contributions of long-chain fatty acids. Enzymatic studies have optimized MLCT production using immobilized lipases in solvent-free systems (Yang et al., 2014; Zhao et al., 2014). Human intervention evidence should be interpreted conditionally: Kasai et al. (2003) reported greater decreases in body weight and several body-fat measures with an MLCT-containing diet than with a long-chain TAG control in healthy adults, while Xue et al. (2009) reported greater reductions in several body-composition measures and serum TAG concentrations in adults with hypertriglyceridemia. These outcomes support metabolic differentiation under defined conditions rather than an unconditional claim that MLCTs prevent fat accumulation.

2.4 Phospholipid-Based Structured Lipids

Structured phospholipids are phospholipid molecules in which the fatty-acid composition or positional distribution has been intentionally modified. Their amphiphilic architecture is important in membranes and interfacial systems, while enzymatic modification using phospholipases can introduce selected fatty acids into phospholipid species. Vikbjerg et al. (2007), for example, used immobilized phospholipase A2 to catalyze acidolysis of phosphatidylcholine and demonstrated incorporation of selected fatty acids under controlled reaction conditions. Evidence for neurological or cognitive effects must be stated with the appropriate evidence level. In rats, Chouinard-Watkins et al. (2019) found greater cortical phospholipid labeling after DHA was administered as phosphatidylcholine or phosphatidylserine than as triacylglycerol, indicating that carrier form can affect brain DHA targeting. Other animal studies have reported model-specific effects of DHA-enriched phospholipids on cognitive and neurobiological endpoints, but these findings should not be described as established improvement of human brain health.

3. Synthesis of Structured Lipids

Structured lipids can be produced by chemical or enzymatic modification of fats, oils, fatty acids, partial glycerides, and phospholipids. Chemical interesterification is effective for large-scale redistribution of acyl groups when positional control is not required, whereas lipase-catalyzed reactions are advantageous when substrate or positional selectivity is part of the product specification. The appropriate route therefore depends on the

target structure, required purity, reaction time, catalyst cost, and downstream processing rather than on a universal preference for one method (Senanayake and Shahidi, 2005; Bornscheuer, 2018).

3.1 Chemical Interesterification

Chemical interesterification redistributes fatty acyl groups among glycerides using chemical catalysts such as alkali metals or alkoxides under low-moisture conditions. The method is well established for modifying melting behavior and plasticity in edible fats and can be implemented at a large scale. Its main limitation for stereospecific nutritional design is that acyl redistribution is largely non-regioselective, so the resulting TAG population is determined by reaction equilibrium rather than by selective placement at sn-1, sn-2, or sn-3. Catalyst removal and post-reaction purification may also be required (Senanayake and Shahidi, 2005; Willis and Marangoni, 2002).

3.1.1 Randomization of Triacylglycerol Molecular Species

Because chemical interesterification redistributes acyl groups without the positional specificity provided by some lipases, it is particularly useful when the objective is to change overall TAG composition or melting profile rather than to preserve a defined stereospecific arrangement. Randomization can therefore improve technological functionality while remaining unsuitable for products whose specification depends on the location of a particular fatty acid on glycerol.

3.1.2 Limited Control over Positional Specificity

The main nutritional-design drawback of chemical interesterification is restricted control of the positioning of stereospecific fatty acids. This does not mean that chemically interesterified fats lack nutritional or functional utility; rather, they are less acceptable when a product demands enrichment of a defined fatty acid at a certain glycerol location, such as high sn-2 palmitate in human-milk-fat substitutes.

3.2 Enzymatic Synthesis

Lipase-catalyzed synthesis has become an essential approach for structured-lipid creation because enzyme specificity can be leveraged to change acyl composition or positional distribution under fairly mild circumstances. Its advantages are therefore best described in terms of catalytic selectivity and process conditions rather than by unqualified claims of being inherently eco-friendly or sustainable. In practice, the environmental and economic performance of an enzymatic route also depends on enzyme production, immobilization, solvent use, catalyst

lifetime, reaction productivity, and downstream purification (Bornscheuer, 2018; Causevic et al., 2022).

Enzymatic SL synthesis is not universally a two-step sequence of hydrolysis followed by re-esterification. Depending on substrates, water activity, and the target product, lipases can catalyze esterification, acidolysis, and interesterification/transesterification, while glycerolysis or controlled hydrolysis can be used when partial glycerides are desired intermediates or products. For example, Yang et al. (2014) optimized solvent-free esterification for MLCT synthesis, Zhao et al. (2014) produced MLCT-enriched structured lipids with an immobilized sn-1,3-specific lipase, and Ghidde et al. (2022) used a two-step acidolysis strategy to produce OPO-rich human-milk-fat substitutes. The major lipase-catalyzed pathways to modify acyl composition and positional distribution are summarized in Figure 1.

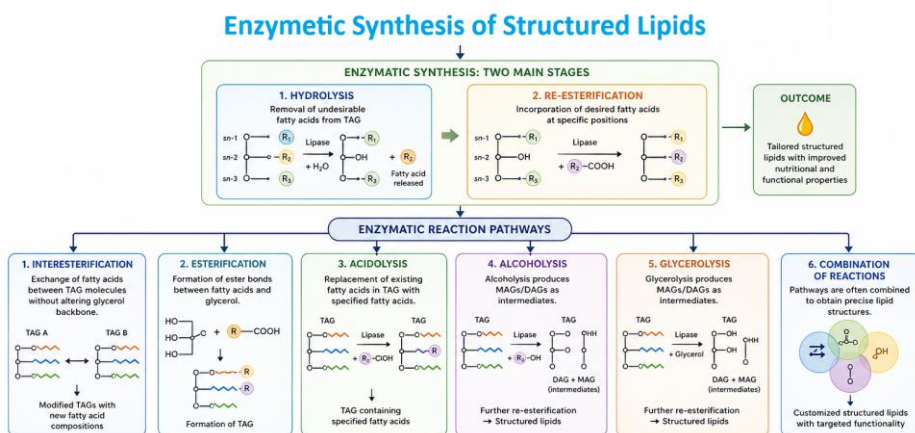


Figure 1: Principal lipase-catalyzed routes for structured-lipid synthesis. Route selection depends on substrate chemistry, enzyme specificity, water activity, and target structure (examples: Yang et al., 2014; Zhao et al., 2014; Ghidde et al., 2022; Mota et al., 2020).

4. Process Optimization in Enzymatic Synthesis

Process optimization must balance conversion, positional selectivity, enzyme stability, acyl migration, reaction time, and downstream purification. Temperature, water activity, substrate molar ratio, enzyme loading, mixing, and reactor configuration can affect both kinetics and equilibrium. Causevic et al. (2022) demonstrated that water activity and temperature influenced structured-lipid production in time-dependent ways, illustrating why an optimum identified at an early reaction time may not be equivalent to the optimum at equilibrium.

4.1 Immobilization of Lipases

Immobilization is widely used in lipid biocatalysis to facilitate enzyme recovery, enable repeated or continuous use, and improve operational stability; however, these benefits are not intrinsic to immobilization itself and depend strongly on immobilization chemistry, support properties, enzyme loading, reaction medium, and the target reaction. Common strategies include physical adsorption, covalent attachment, entrapment or encapsulation, and cross-linking. For lipases, adsorption onto hydrophobic porous supports is particularly common because interfacial interactions can stabilize an open-lid conformation. Covalent attachment can reduce enzyme leaching and improve mechanical robustness, but excessive or poorly oriented attachment may restrict conformational mobility and decrease activity. Immobilization should therefore be evaluated in terms of recovered activity, regioselectivity, stability, reusability, and product yield rather than being assumed to improve all performance attributes simultaneously (Causevic et al., 2023).

The importance of support selection has been demonstrated directly in structured-TAG synthesis. Causevic et al. (2023) compared *Rhizomucor miehei* and *Rhizopus oryzae* lipases immobilized on several hydrophobic adsorbents and an epoxy-functionalized support for transesterification of high-oleic sunflower oil with ethyl stearate. Adsorption generally produced higher catalytic activity than the tested covalent-binding preparation, while support identity also affected regioselectivity and by-product formation. Zhao et al. (2022) similarly showed that a magnetic biochar-based support could improve recovery and reuse in interesterification, illustrating how support chemistry can influence both catalytic performance and handling.

Operational stability is economically important because the contribution of an immobilized enzyme depends on how much product is generated over its useful lifetime. In dietetic SL production from spent coffee-ground oil, Mota et al. (2020) showed that deactivation depended on the immobilized preparation and reaction pathway. Continuous packed-bed production has likewise demonstrated that catalyst stability, substrate affinity, and specific productivity can differ markedly among commercial preparations (Souza-Goncalves et al., 2023). Reuse data should therefore be interpreted together with product yield, reaction time, catalyst loading, and productivity rather than reported only as the number of cycles.

Immobilization can also introduce transport limitations. TAGs and other hydrophobic substrates must diffuse through or around the support, so pore size, particle dimensions, viscosity, phase behavior, and mixing can constrain observed rates. Water activity must be high enough to maintain a

catalytically competent enzyme conformation while remaining low enough to limit competing hydrolysis in synthesis-oriented systems. As illustrated in Figure 2, immobilization can facilitate enzyme recovery and repeated use, but its effect on catalytic performance depends on the support and immobilization strategy.

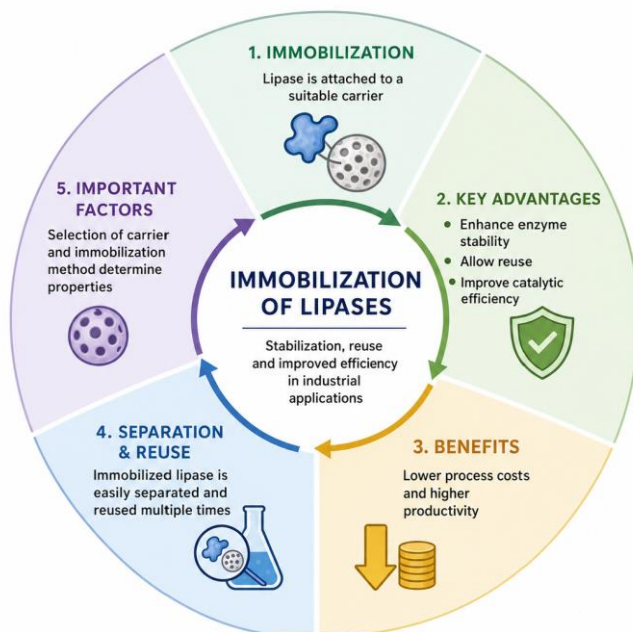


Figure 2: Lipase immobilization: potential gains and trade-offs relevant to structured-lipid synthesis. Support chemistry and immobilization methods can affect activity, selectivity, operational stability, mass transfer, reuse, and catalyst economics (Causevic et al., 2023; Zhao et al., 2022; Mota et al., 2020; Souza-Goncalves et al., 2023).

4.2 Key Reaction Parameters

Water activity is a central variable in non-aqueous lipase catalysis because a minimum hydration shell is required for enzyme flexibility, whereas excess water can shift the system toward hydrolysis. Causevic et al. (2022) showed that controlled water activity can affect initial reaction rate and equilibrium behavior differently, highlighting the need to specify the reaction time at which an optimum is defined. Temperature likewise influences substrate viscosity, mass transfer, reaction rate, enzyme deactivation, and acyl migration. A temperature that accelerates conversion

can become unfavorable if it increases enzyme inactivation or positional randomization.

Substrate molar ratio and enzyme loading should be optimized together with product concentration and productivity. Increasing the concentration of an acyl donor can shift reaction equilibrium, but excessive donor can alter solvent properties, inhibit the enzyme, or complicate purification. Higher enzyme loading often accelerates early conversion but does not necessarily increase final yield proportionally when mass transfer, equilibrium, or substrate inhibition becomes limiting. For industrial comparison, conversion should therefore be reported with reaction time, enzyme loading, product concentration, selectivity, and catalyst productivity rather than as an isolated percentage.

4.3 Process Intensification, Downstream Purification, and Data-Driven Approaches

4.3.1 Ultrasound-Assisted Process Intensification

Ultrasound can intensify lipase-catalyzed lipid reactions by improving dispersion and interfacial mass transfer in multiphase systems. Its effect is nevertheless dependent on power input, duty cycle, exposure time, substrate composition, and enzyme stability. In acidolysis of palm olein with caprylic acid, Jadhav et al. (2021) optimized both reaction and sonication variables and reported a maximum designer-lipid yield of 92% under their selected conditions. The immobilized biocatalyst was also evaluated over repeated cycles. This study provides direct evidence that sonication can intensify a structured-lipid reaction, but the optimum conditions should not be generalized to other lipases or substrates.

4.3.2 Molecular Distillation as a Downstream Purification Operation

Molecular distillation is better classified as a downstream separation technique than as an enzymatic synthesis technology. Under high vacuum, relatively volatile free fatty acids and other components can be removed at reduced thermal exposure. Cao et al. (2015) used a two-stage molecular-distillation process for low-calorie SL purification and reported a final acid value of 0.93 mg KOH/g and 60.25% purity under the optimized second-stage conditions. Li et al. (2018) combined molecular distillation with solvent fractionation and low-temperature crystallization to increase DAG content from 44.8% in the crude product to 86.6% in the final fraction. These examples show that molecular distillation is often one component of a broader purification train rather than a stand-alone solution.

4.3.3 Artificial Intelligence and Machine Learning: Demonstrated and Prospective Uses

Currently demonstrated applications of machine learning in lipase biotechnology are concentrated in enzyme discovery, enzyme engineering, and upstream bioprocess optimization rather than direct industrial structured-lipid manufacture. Ma et al. (2023) used machine-learning-assisted directed evolution to improve the methanol tolerance of *Proteus mirabilis* lipase; models trained on 266 combinatorial mutants were used to explore a larger sequence space, and the best reported variant showed up to a 13-fold increase in methanol tolerance relative to the wild type. Sarmah et al. (2022) used machine-learning models to predict *Candida antarctica* growth kinetics for lipase production and reported substantial reductions in experimental effort. Shahraki et al. (2022) combined sequence analysis with supervised machine learning to mine metagenomic data for lipolytic enzymes.

These demonstrated uses provide a realistic basis for prospective structured-lipid applications, including screening lipases for regioselectivity or solvent tolerance, prioritizing mutations that improve activity or stability, predicting the influence of water activity or substrate ratio, and coupling experimental design with data-driven process optimization. Such applications should be described as potential until they are validated directly in structured-lipid systems. Predictions of enzyme activity, substrate specificity, or process yield still require experimental verification because immobilization effects, phase behavior, mass transfer, and acyl migration may not be captured adequately by sequence- or structure-based models alone.

5. Nutritional and Functional Significance

The nutritional consequences of structured lipids depend not only on fatty-acid composition but also on stereospecific position, molecular species, dose, and physiological context. Pancreatic lipase preferentially hydrolyzes the sn-1 and sn-3 ester bonds of dietary TAGs, generating free fatty acids and 2-monoacylglycerols that are subsequently absorbed and re-esterified in enterocytes. Positional distribution can therefore influence the chemical form in which a fatty acid enters the intestinal lumen and its tendency to form insoluble complexes. The effect is fatty-acid specific and should not be summarized simply by stating that fatty acids in the sn-2 position are absorbed faster.

This principle is particularly relevant to palmitate-rich lipids used in infant nutrition. Human milk contains a high proportion of palmitic acid at sn-2, whereas many conventional vegetable-oil-based formulas contain

more palmitate at sn-1 and sn-3. Hydrolysis of outer-position palmitate produces free palmitic acid, which can form poorly soluble calcium soaps. In a randomized, double-blind, multicenter trial involving 488 healthy term infants, Beghin et al. (2019) compared formulas containing different proportions of sn-2 palmitate. Formulas with higher sn-2 palmitate produced softer stools during early infancy and were associated with higher bone mineral content at four months, while growth remained similar among formula groups. Shen et al. (2021) also reported lower fecal saponified fatty acids and stool calcium in infants receiving a high-OPO formula than in those receiving a standard formula. These findings illustrate a position-specific digestive effect but should not be generalized to all SLs or all age groups.

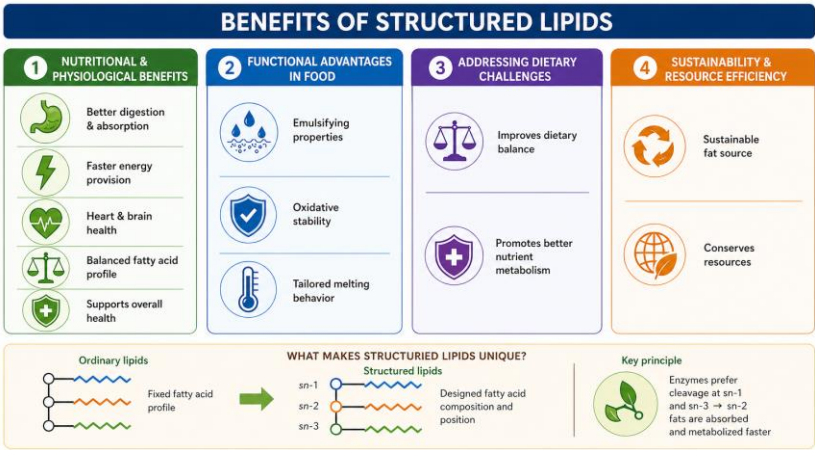


Figure 3: Nutritional, functional, and industrial significance of structured lipids

DAG-rich oils provide another example in which molecular structure can influence metabolic outcomes. Nagao et al. (2000) reported greater reductions in body-fat-related measures with DAG-rich oil than with a TAG comparator in a double-blind controlled trial. Maki et al. (2002) subsequently evaluated DAG oil in a 24-week randomized, double-blind study of overweight or obese adults consuming a reduced-energy diet; mean body-weight and fat-mass reductions were greater in the DAG group than in the TAG group. These trials support structure-dependent metabolic effects under defined intervention conditions but do not establish that DAG oils universally suppress fat accumulation (Figure 3).

MLCTs have also been investigated in human diets. Kasai et al. (2003) reported greater decreases in body weight and several body-fat measures with an MLCT-containing diet than with a long-chain TAG control after 12 weeks, while Xue et al. (2009) reported greater reductions in several body-composition measures and serum TAG concentrations in adults with

hypertriglyceridemia. These studies support discussion of MLCTs as metabolically distinct formulations, but the magnitude and reproducibility of their effects depend on fatty-acid composition, dose, baseline metabolic status, comparator oil, and dietary control.

Structured phospholipids may alter delivery and tissue availability of long-chain polyunsaturated fatty acids. In rats, Chouinard-Watkins et al. (2019) found substantially greater cortical phospholipid labeling after DHA was administered as phosphatidylcholine or phosphatidylserine than after DHA-triacylglycerol. Wang et al. (2019) likewise reported model-specific cognitive and neurobiological effects of DHA-enriched phosphatidylcholine in SAMP8 mice. These findings support a mechanistic rationale for structured phospholipids in neurological nutrition, but the evidence is predominantly preclinical and should not be described as established improvement of human cognition.

Functional significance also includes technological properties. Deliberate control of molecular species can alter melting behavior, crystallization, solid-fat content, interfacial activity, or product texture. These effects arise from the resulting composition and molecular structure rather than from 'structuring' alone. Figure 4 summarizes selected structure-function relationships for which mechanistic or intervention evidence is available; the effects are product- and context-specific rather than universal properties of SLs.

Molecular Design	Mechanistic / Physical Consequence	Example Evidence
High Sn-2 Palmitate	Less Free Palmitate Available For Calcium-soap Formation After Digestion	Infant-formula Trials
MCT / MLCT Incorporation	Altered Substrate Handling And Energy Use Under Defined Dietary Conditions	Human MLCT Studies
Dha-phospholipid Form	Altered Brain DHA Targeting / Tissue Delivery	Animal Studies
Targeted TAG Species	Tailored Melting And Crystallization Behavior	Cocoa Butter Equivalent Studies

Figure 4: Selected structure-function relationships supported by mechanistic, preclinical, or human intervention evidence. The evidence level differs among products and outcomes (Beghin et al., 2019; Maki et al., 2002; Kasai et al., 2003; Xue et al., 2009; Chouinard-Watkins et al., 2019; Bahari and Akoh, 2018).

6. Industrial and Translational Applications

Structured lipids are relevant to applications in which either the positional distribution of fatty acids or the resulting physicochemical properties must be deliberately controlled. Their industrial relevance differs among product classes. Some applications, such as specialty confectionery fats and structured nutritional lipids, are supported by direct process studies, whereas other proposed uses remain at laboratory or translational stages. Evidence of laboratory synthesis should therefore be distinguished from established commercial deployment.

6.1 Human-Milk-Fat Substitutes and Infant-Formula Lipids

Human milk-fat substitutes (HMFS) are a clear example of positional lipid design because human milk is enriched in TAGs containing palmitic acid at sn-2. Enzymatic synthesis can increase sn-2 palmitate while introducing oleic, linoleic, or medium-chain fatty acids at the outer positions. Ghide et al. (2022) used immobilized *Rhizomucor miehei* lipase in a two-step acidolysis process to produce OPO-rich TAGs, reporting 93.46% palmitic acid at sn-2 and 59.54% oleic acid incorporation at sn-1,3 under optimized conditions. Yuan et al. (2020) used enzymatic interesterification of catfish oil and coconut oil to produce SLs containing 62.14% MLCTs, including 39.85% MLL-type species, with 46.14% of palmitate located at sn-2. Manufacturing evidence should be considered separately from the clinical outcomes discussed in Section 5, and acyl migration remains an important process-control issue.

6.2 Cocoa Butter Equivalents and Confectionery Fats

Cocoa butter equivalents are specialty structured-fat products designed to reproduce a restricted TAG distribution and solid-fat/melting behavior. Bahari and Akoh (2018) synthesized a CBE from illipe butter and palm midfraction using Lipozyme RM IM; the optimized product exhibited a POP/POS/SOS profile and melting/cooling behavior comparable to cocoa butter. Huang et al. (2021) prepared a CBE from palm midfraction and stearic acid using *Rhizomucor miehei* lipase and reported a product yield above 92% under the tested conditions together with physicochemical characteristics similar to cocoa butter. These studies justify treating CBE as a specific confectionery-fat application rather than as a generic category alongside emulsifiers or fat substitutes.

6.3 Dietetic and Functional Structured Triacylglycerols

Dietetic SLs are often designed as MLM or related MLCT structures containing medium-chain fatty acids at selected positions while retaining

long-chain fatty acids in the molecule. Mota et al. (2020) produced dietetic SLs from spent coffee-ground oil using immobilized lipases and reported approximately 90% TAG conversion under selected acidolysis or interesterification conditions. Souza-Goncalves et al. (2023) demonstrated continuous packed-bed production of dietetic SLs from crude acidic olive-pomace oils over 70 h, with new-TAG yields of approximately 30-60% depending on the reaction system. These examples demonstrate feedstock valorization and continuous processing, but economic and environmental benefits remain process-specific rather than inherent properties of SL manufacture.

6.4 Clinical and Pharmaceutical Lipid Formulations

The term 'structured lipid' should not be conflated with nanostructured lipid carriers (NLCs), which are a distinct pharmaceutical nanocarrier technology. A defensible clinical application of structured TAGs is their use as components of structured fat emulsion injections. Zheng et al. (2020) identified and relatively quantified 47 TAG components in pharmaceutical structured TAGs and structured-fat emulsion injections by HPLC-high-resolution mass spectrometry. This supports discussion of structured TAGs in clinical lipid emulsions and pharmaceutical quality control, but it does not establish that enzymatically structured TAGs generally protect lipophilic drugs or increase drug bioavailability.

Representative application domains, their target structures, evidence, and important qualifications are summarized in Table 1.

Table 1: Demonstrated and translational applications of structured lipids.

Applicat ion domain	Target design	Primary evidence / relevance	Important qualificati on	Primar y source s
HMFS / infant- formula lipids	OPO-rich TAGs; high sn-2 palmitate; MLCT/ML L species	Enzymatic acidolysis/interester ification can enrich sn-2 palmitate and targeted outer- position fatty acids.	Manufactu ring evidence is distinct from clinical efficacy; acyl migration and positional	Ghide et al., 2022; Yuan et al., 2020

Applicat ion domain	Target design	Primary evidence / relevance	Important qualificati on	Primar y source s
			purity must be controlled.	
Cocoa butter equivalent s	Target POP/POS /SOS distributio n and cocoa- butter-like thermal behavior	Lipase-catalyzed interesterification can produce TAG and melting profiles similar to cocoa butter.	Physical similarity does not imply nutritional superiorit y; CBE and CBS terminolo gy should be distinguis hed.	Bahari and Akoh, 2018; Huang et al., 2021
Dietetic MLM/ML CT lipids	Medium- chain fatty acids incorporate d into selected TAG structures	Batch and continuous enzymatic systems can generate target new-TAG products from conventional or by-product oils.	Health effects need separate interventio n evidence; sustainabil ity is process- specific.	Mota et al., 2020; Souza- Goncal ves et al., 2023
DAG-rich functional oils	Increased DAG content / controlled DAG compositio n	Immobilized-lipase reactor systems can generate high-purity DAG fractions.	Do not infer health efficacy from process studies; human outcomes	Chen et al., 2020

Applicat ion domain	Target design	Primary evidence / relevance	Important qualificati on	Primar y source s
			belong in Section 5.	
Structured fat emulsions	Mixed medium- /long- chain structured TAGs meeting pharmaceu tical specificatio ns	Structured TAG mixtures have been directly characterized in structured-fat emulsion injections.	Distinct from NLCs; does not support generic lipophilic- drug delivery claims.	Zheng et al., 2020

7. Challenges and Industrial Considerations

The principal barriers to industrial enzymatic SL production are better considered as interacting processes and economic variables rather than as a fixed list of disadvantages. Enzyme purchase price can be important, particularly for immobilized commercial preparations, but its contribution to manufacturing cost depends on catalyst loading, operational lifetime, reaction productivity, product value, and the number of times the catalyst can be reused. This distinction is especially relevant for specialty lipids, for which the value of positional selectivity or high product purity may justify a more expensive catalyst if lifetime and productivity are sufficiently high.

Operational stability is therefore a central economic variable. Mota et al. (2020) demonstrated that immobilized-lipase stability during repeated SL synthesis depended on the reaction pathway and acyl donor. In continuous packed-bed production from crude acidic olive-pomace oil, Souza-Goncalves et al. (2023) observed stable operation over 70 h, new-TAG yields of approximately 30-60%, and specific productivity of 0.96-1.87 g new TAG h⁻¹ g⁻¹ biocatalyst depending on the system. The authors also reported that the purchase cost of Lipozyme RM IM was more than sevenfold that of Lipozyme TL IM in their comparison, while catalytic preferences and process performance differed. The appropriate economic question is therefore not whether enzymes are generically expensive, but

how catalyst cost per unit product changes with activity, lifetime, loading, and reactor configuration.

Scale-up introduces additional constraints. Viscous lipid phases, incomplete miscibility of substrates, water activity control, heat and mass transfer, pressure drop, and removal of free fatty acids or other by-products can alter performance when moving from laboratory reactors to larger batch or continuous systems. Continuous packed-bed reactors can reduce enzyme-separation requirements and improve catalyst utilization, but deactivation, channeling, fouling, and feedstock variability must be evaluated over sufficiently long operating periods before industrial conclusions are drawn.

Recombinant enzyme production provides another route for reducing dependence on enzyme extraction from native organisms. Zhao et al. (2008) scaled recombinant *Candida rugosa* lipase production in *Pichia pastoris* from 5 L to 800 L and reported approximately 14,000 IU/mL lipase activity at pilot scale. Eom et al. (2013) produced extracellular *Candida antarctica* lipase B (CALB) by fed-batch *P. pastoris* fermentation, obtaining 11,900 U/L activity and 1.18 g/L CALB in the culture supernatant; the recovered enzyme showed properties comparable to a commercial CALB preparation in the authors' tests. Yang et al. (2013) further showed that codon and secretion-signal optimization could markedly increase CALB expression. These studies demonstrate that recombinant expression and fermentation engineering are directly relevant to lipase sourcing, although purification, formulation, immobilization, and regulatory suitability remain process-specific.

Downstream processing can be equally important. Enzymatic reactions may simplify catalyst removal relative to some chemical routes, but SL products can still contain residual free fatty acids, partial glycerides, unreacted substrates, water, or solvent. Molecular distillation, fractionation, crystallization, or other separation steps add energy and capital requirements and can reduce overall product yield. Industrial evaluation should therefore integrate substrate cost and pretreatment, enzyme production or purchase, immobilization, reaction productivity, catalyst reuse, reactor operation, product recovery, purification, quality control, and the market value of the final specialty lipid.

8. Conclusion

Enzymatic synthesis provides a versatile platform for producing structured lipids when substrate selectivity, positional control, or mild processing conditions are important to product design. The strongest evidence supports its use in targeted TAG modification, human-milk-fat substitutes,

MLCT/MLM lipids, DAG-rich products, structured phospholipids, and specialty fats such as cocoa butter equivalents. However, catalytic selectivity alone does not determine industrial feasibility. Water activity, acyl migration, immobilization method, catalyst lifetime, mass transfer, reactor mode, and downstream purification must be considered together with product specifications and economic value. Nutritional claims likewise require evidence appropriate to the product and population: human trials support selected effects of sn-2 palmitate, DAG-rich oils, and MLCT formulations, whereas several neurological claims for structured phospholipids remain predominantly preclinical. Emerging machine-learning tools have demonstrated value in lipase discovery, engineering, and production, but direct application to structured-lipid process control remains a prospective area requiring experimental validation. Future progress will therefore depend on integrating molecular design, biocatalyst engineering, reaction engineering, evidence-based nutrition, and process economics rather than treating enzymatic synthesis as inherently sustainable or commercially superior.

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Conflict of Interest

The authors hereby declare no potential conflicts of interest with respect to the research, funding, authorship, and/or publication of this article.