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Review Article

An Overview of Structured Lipid in Food Science: Synthesis Methods, Applications, and Future Prospects

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Structured lipids have attracted significant interest in industries, such as food, pharmaceuticals, and cosmetics, because they can be alternatives to traditional lipids. Enzymatic synthesis techniques utilizing lipases have gained attention for structured lipids synthesis because of their site and substrate specificity. However, most lipases discovered to date exhibit *sn*-1,3 regiospecificity or nonspecificity, limiting the selective range of natural oils and fats that can be used as raw materials. In this review, we provide an overview of the current synthesis methods and applications of structured lipids and the limitations of existing lipases in producing structured lipids. In addition, we discuss innovative approaches, including metagenomics, and machine learning, to discover, and classify new lipases and the use of gene editing technologies for lipase engineering. These efforts aim to overcome the limitations of existing lipases and expand the range of natural oils and fats that can be used for the production of structured lipids. Therefore, this study aims to promote a better understanding of structured lipids as a material with high-added value and provides insight into ongoing research efforts in the field.

1. Introduction

In the food, pharmaceutical, and cosmetic industries, materials are manufactured as goods based on natural products. However, the increasing demand for natural resources because of population upsurge, depletion of growing areas caused by environmental degradation, and extinction of plants caused by climate change necessitates a shift in strategic approaches to produce materials [1, 2]. As a response to these challenges, various new technologies have been developed to substitute natural products and ensure economic efficiency and widespread use of rare raw materials with high-added value [3–5]. Furthermore, progress is ongoing on the development of substitutable materials for the three major nutrients and functional substances.

Fat, one of the three major nutrients, provides necessary fatty acids and energy. It also plays a vital role in internal metabolic processes as a carrier that assists in the absorption of fat-soluble vitamins into the body [6], a precursor of hormones and neurotransmitters needed for the maintenance of body temperature, a buffering agent, and a component of intercellular communication [7–9]. Furthermore, microscopic levels of lipid derivatives are necessary components of brain function [10]. In the pharmaceutical and cosmetic sectors, lipids are also used as solvents for hydrophobic substances and emulsifiers for the homogenous mixing of water-soluble and fat-soluble substances [11, 12].

Structured lipids, as an alternative to conventional fats, have been receiving attention because of their customized functional properties and health benefits [13]. They can be

synthesized through different methods such as chemical or enzymatic catalyses [14]. In the synthesis of structured lipids, chemical catalysis is generated by reactions at high temperatures using catalysts such as sodium methoxide, sodium hydroxide, and lithium hydroxide [15–17]. This approach has a low-processing cost but requires a complex process, including the formation of byproducts and their elimination. By contrast, the production of structured lipids using biological enzymes does not lead to side reactions because of the substrate and site specificity necessary for the targeted production of structured lipids. In addition, commercially available immobilized enzymes are ecologically benign, react at comparatively low temperatures, do not encourage side reactions, are simple to reuse and recover, and offer economic benefits [18]. Recently, a manufacturing method using biological enzymes that immobilizes diverse enzymes has become the standard [19].

This review will discuss the trends of currently produced and investigated structured lipids, the advantages and disadvantages of different synthesis methods, and the present state of lipase excavation for the enzymatic generation of valuable structured lipids. In addition, this review will delve into the challenges and future prospects in the field, hoping that the discovery and creation of lipases for existing enzyme production will establish the direction of future structured lipid development through novel approaches for creating more advanced structured lipids.

2. Structured Lipids: Definition, Properties, and Synthesis Methods

2.1. Definition of Structured Lipids. Structured lipids are modified fats and oils that are chemically or enzymatically altered to enhance their physicochemical properties, nutritional values, and functional attributes. These lipids have a specific arrangement of fatty acids on the glycerol backbone, which allows for designing lipids with customized properties suitable for applications in the food, pharmaceutical, and cosmetic industries.

2.2. Advantages and Disadvantages of Structured Lipids

2.2.1. Advantages. Structured lipids offer numerous advantages, such as improved physicochemical properties, enhanced nutritional values, and customized functionality [14, 20]. These advantages enable the development of lipids with specific melting points, viscosity, and oxidative stability, making them more suitable for various applications [13]. By incorporating desirable fatty acids such as medium-chain fatty acids or polyunsaturated fatty acids (PUFAs), structured lipids can provide health benefits, such as reducing the risk of cardiovascular diseases and improving energy metabolism [21, 22]. Furthermore, structured lipids can be engineered to demonstrate specific functionalities, such as enhanced emulsification, solubility, or bioavailability, which are advantageous in food, pharmaceutical, and cosmetic formulations [23].

2.2.2. Disadvantages. Structured lipids also have some disadvantages. The synthesis of structured lipids, particularly through enzymatic methods, can be costly because of expenses related to enzyme production and purification [24]. Moreover, the majority of lipases employed in enzymatic synthesis are *sn*-1,3 regiospecific or nonspecific, limiting the ability to produce lipids with the desired fatty acid distribution [25]. Finally, the complexity of structured lipid synthesis necessitates the precise control of reaction conditions and the use of various catalysts or enzymes [26].

2.3. Synthesis of Structured Lipids. Manufacturing techniques of structured lipids can be chemical or enzymatic (Figure 1). This section explores the principles, advantages, and disadvantages of chemical and enzymatic synthesis methods.

2.3.1. Chemical Synthesis of Structured Lipids. Chemical catalysis of structured lipids primarily synthesizes lipids for use in the chemical industry, including specific-purpose synthetic oils and biodiesel [27]. Production processes of structured lipids employing chemical catalysts involving thermal decomposition, catalytic decomposition, microemulsification, and esterification reactions are characteristically irreversible [28]. Furthermore, they can proceed in a single direction, contrary to biological enzymatic reactions. However, chemical synthesis is conducted using a strong acid or a strong base that requires high temperatures and energy.

In a recent study, researchers utilized chemical interesterification (CIE) to fabricate trans-free cocoa butter alternatives (CBAs) from a blend of palm kernel stearin (PKS), coconut oil (CNO), and fully hydrogenated palm stearin (FHPS) [29]. In another study, the same chemical interesterification process was applied to a combination of palm stearin (PS) and olive oil (OO), resulting in fats with various degrees of plasticity, thus expanding the possibilities for the commercial utilization of PS and OO [30].

Despite these successful examples, it should be noted that chemical synthesis has improved in recent years, involving environmentally friendly and efficient processes that can be produced at low temperatures using physical methods, including sonication and cavitation [31]. Nevertheless, the use of organic solvents for quenching chemical catalyst reactions and considering the generation and elimination steps of byproducts is a very unfavorable process compared with synthetic biological engineering methods [32].

2.3.2. Enzymatic Synthesis of Structured Lipids. Lipases (EC. 3.1.1.3) generally catalyze reversible processes that can hydrolyze and dehydrate condensation reactions to decompose and synthesize bonds between carboxyl and hydroxyl groups [33]. In addition to decomposing and synthesizing lipids, lipases can also degrade and synthesize substrate-specific

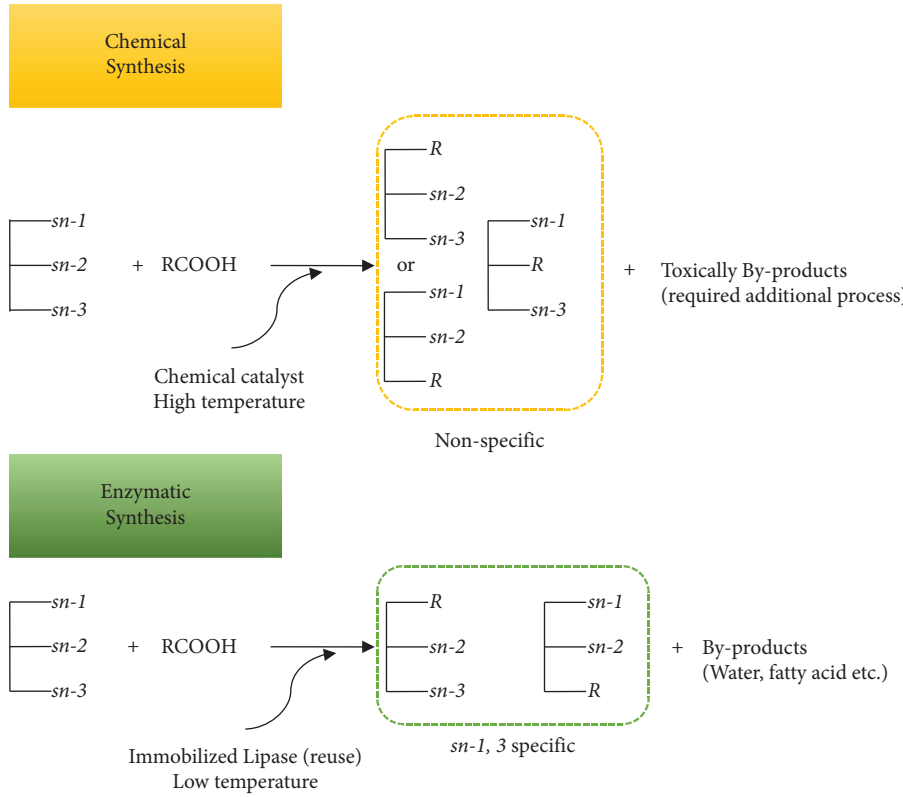

Figure 1: Scheme of chemical and enzymatic syntheses for producing structured lipids.

compounds and can be applied in several scientific and industrial fields.

In lipolysis and synthesis, lipases exhibit regiospecificity, though most commercially available lipases display *sn*-1,3 nonspecificity in lipids rather than *sn*-2 regiospecificity, which is the more desirable one. As a result, modifying the composition and distribution of fat are difficult.

Approximately four reactions exist when synthesizing structured lipids employing lipase as an enzyme, depending on the substrates used in the reaction mixture. Free fatty acids and glycerol are directly esterified, acylglycerol is transesterified intramolecularly or intermolecularly, triacylglyceride (TAG) is acidolyzed and transesterified with free fatty acids, and TAG is transesterified to produce monoacylglyceride (MAG) and diacylglyceride (DAG) [34–37] (Figure 2). In each of these methods, the water concentration, reaction temperature, and substrate are critical parameters that determine the direction of the lipase reaction.

Lipases, free and immobilized ones, are different based on the characteristics shown in the production process of structured lipids. First, free lipases are often inexpensive and can lower process costs; nevertheless, they are sensitive to extreme temperatures and pH, leading to decreased yields. In addition, the recovery of enzymes for reuse requires an additional complex procedure, and the activity of the recovered enzymes cannot be assured. By contrast, immobilized lipases alleviate the problem of enzyme yield loss and reaction stability induced by environmental changes such as pH, temperature, and organic solvents, allowing faster enzyme recovery and reuse [38].

Techniques for immobilizing enzymes include adsorption in a porous material, covalent binding between the enzyme and support, and enzyme conjugation through cross-linking and interenzyme coupling [39]. Furthermore, this method immobilizes enzymes through several physical binding interactions, including van der Waals forces, hydrophobic interactions, hydrogen bonds, and ionic connections [40].

Despite technological advancements, the practical use of immobilized lipases in structured lipids is largely limited by most lipases being *sn*-1,3 regiospecific or nonspecific [41] (Table 1). Two illustrative examples, however, show the potential applications of lipases. In one study, medium- and long-chain triglycerides (MLCTs) were synthesized by incorporating lauric acid into flaxseed oil via lipase-catalyzed acidolysis, demonstrating improved thermo-oxidative decomposition behavior [42]. Another study prepared structured TAGs rich in 1,3-dioleoyl-2-palmitoylglycerol (OPO) and 1-oleoyl-2-palmitoyl-3-linoleoylglycerol (OPL) through enzymatic acidolysis of fractionated palm stearin with free fatty acids, presenting high product yields and the potential for use in infant formulas [43].

Even though some lipases may extract fatty acids from the *sn*-2 position of TAG, these enzymes are quite uncommon for commercial immobilized lipases. Although specificity and selectivity for substrates, low energy consumption, and the creation of immobilized enzymes enable

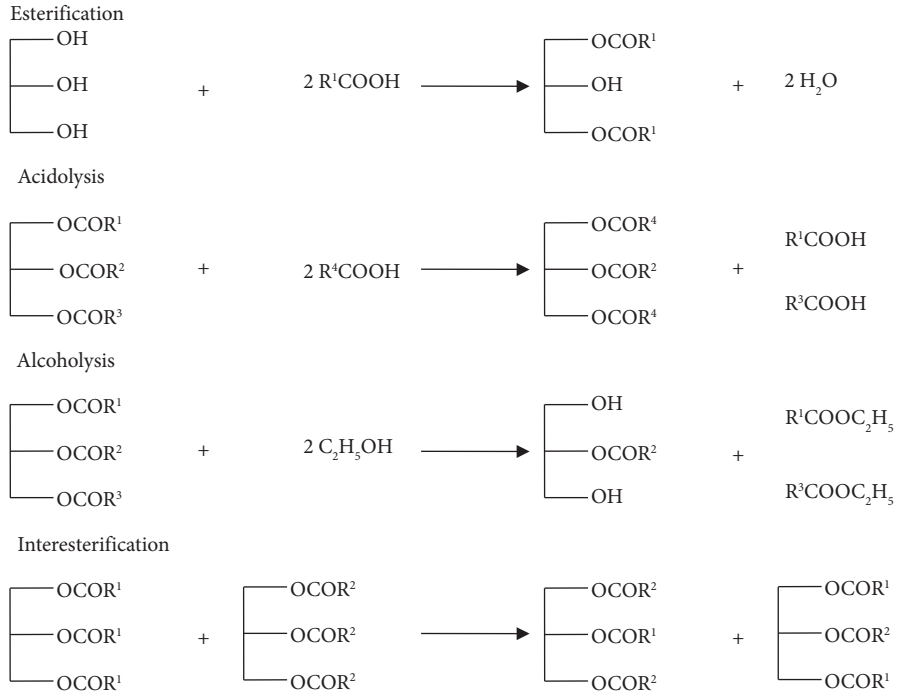

Figure 2: Types of lipase enzymatic synthesis processes for the synthesis of structured lipids.

Table 1: Current types and forms of immobilized enzymes manufactured commercially.

Product names	Source	Immobilization material	Development company
Novozyme 435	*Candida antarctica*	Resin	Novozymes
Lipozyme 435	*Candida antarctica*	Resin	Novozymes
Lipozyme TL IM	*Thermomyces lanuginosa*	Silica gel	Novozymes
Lipozyme RM IM	*Rhizomucor miehei*	Resin	Novozymes
Lipase Rd	*Rhizopus delemar*	MP 1000	Tanabe Seiyaku Co. Ltd.
Lipase QLM	*Alcaligenes* sp.	MP 1000	Meito Sangyo Co.
Lipase AK	*Pseudomonas fluorescens*	MP 1000	Amano Pharmaceutical Co.
Lipase D	*Rhizopus oryzae*	MP 1000	Amano Pharmaceutical Co.
Lipase DF	*Rhizopus oryzae*	MP 1000	Amano Pharmaceutical Co.

reusable successive processes, the enzymatic synthesis of structured lipids is limited(Table 2).

3. Functional Food Application of Structured Lipids

3.1. Cocoa Butter Equivalents (CBEs). Cocoa butter is a lipid obtained from cacao beans, and it has many commercial applications in chocolate, drinks, and baking. Regarding composition, cocoa butter differs from other lipids because it contains comparatively few TAGs. That is, >80% of the triglycerides that make up cocoa butter are composed of 1,3-dipalmitoyl-2-oleoyl-glycerol (POP, 13.8%–21.8%), 1-palmitoyl-2-oleoyl-3-stearoyl-rac-glycerol (POS, 26.3%–44.8%), and 1,3-distearoyl-2-oleoyl-glycerol (SOS, 20.0%–29.4%) with oleic acid bound to the *sn*-2 position [61]. The unique triglyceride fatty acid composition of cocoa butter reveals remarkable physical qualities in the final culinary product, including hard physical qualities at ≤25°C, soft physical properties, and a texture that melts at ≥30°C [62]. Given these properties, cocoa butter is more expensive than edible oils and fats. Owing to the increased international demand and lower yields caused by environmental problems, artificial lipid synthesis is being actively explored to obtain raw materials.

CBE is a structured lipid with a triglyceride and fatty acid profile comparable to cocoa butter, and it is manufactured from inexpensive vegetable oils [63]. Recent research on CBE synthesis has focused on POS-rich lipid synthesis, which accounts for approximately 40% of cocoa butter's triglycerides [64] (Table 3). Commercial CBE POS is synthesized using lipids rich in POP and SOS [71]. Palm oil midfraction, which can be derived through the fractional crystallization of palm olein, is the raw material of CBE for POP [72]. Fractional crystallization of SOS from tropical plants, including shea butter or a catalyst, is used to produce SOS.

CBE synthesis is limited by the requirement for oleic acid-rich lipids at the *sn*-2 position of the lipids. Most commercially available lipases are *sn*-1,3 regiospecific

Table 2: Overview of current structured lipids synthesized via enzymatic and chemical approaches.

No.	Enzymatic/ chemical	Catalyst/enzyme	Backbone	Fatty acids	Products	Ref.
1	Enzymatic	Lipozyme TL IM Lipozyme RM IM	Crude olive pomace oil	Caprylic acid (C8 : 0) Capric acid (C10 : 0)	Low-calorie structured triacylglycerols	[44]
2	Enzymatic	Lipozyme 435 Lipozyme RM Lipozyme TL IM	Medium-chain triglyceride	EPA-enriched fish oil (60% EPA, 15% DHA)	EPA-enriched medium- and long-chain triacylglycerol (MLCT)	[45]
3	Enzymatic	Lipozyme TL IM Lipase from Candida sp.	Palm stearin monopalmitoylglycerol	Oleic acid linoleic acid	Human milk fat substitutes (HMFS; OPL, OPO, and LPL)	[46]
4	Enzymatic	Lipozyme 435	Trioctanoate	*n*-Propyl gallate	1,2-Dioctanoylgalloylglycerol (DOGG)	[47]
5	Enzymatic	Novozyme 435	Linseed oil	Caprylic acid (C8 : 0) Capric acid (C10 : 0)	1,3-Dicapryl-2-linolenoylglycerol 1,3-Dicapril-2-linolenoylglycerol	[48]
6	Enzymatic	Lipase MAJ1	Tricaprylin	Methyl palmitate	LML type TAG	[49]
7	Enzymatic	Lipozyme RM IM	Grape seed oil	Capric acid (C10 : 0)	MLM type TAG	[50]
8	Enzymatic	Lipozyme RM IM	Palm kernel stearin	Coconut oil Fully-hydrogenated palm stearin	Cocoa butter alternatives (CBA)	[51]
9	Enzymatic	Lipozyme TL IM	Tripalmitin	Oleic acid Linoleic acid	Human milk fat substitutes (HMFS; OPL and LPL)	[52]
10	Enzymatic	Lipozyme RM IM	Coconut oil	Soybean oil	Improved bioavailability carrier oil	[53]
11	Enzymatic	Lipase TL100 Lipase RML Lipase CAL-A	Palm oil	Linolenic acid	Functional fatty acids in oil (ω-3)	[54]
12	Enzymatic	Lipozyme RM IM	Pomegranate seed oil	Conjugated linoleic acid Conjugated linolenic acid	Functional fatty acids in oil	[55]
13	Enzymatic	Lipozyme RM IM	Illipe butter	Palm midfraction	Cocoa butter equivalent (CBE)	[56]
14	Chemical	Sodium silicate (Na_2SiO_3)	Glycerol	Palm olein Palm stearin	MAG, DAG	[57]
15	Chemical	Sulfuric acid (H_2SO_4) HND-6 p-Toluenesulfonic acid (p-TSA)	Coconut oil	Caprylic acid (C8 : 0) Capric acid (C10 : 0)	Medium-chain triacylglycerols (MCTs)	[58]
16	Chemical	Sodium methoxide	Palm olein	Palm kernel oil Palm stearin	Trans-free plastic fats	[59]
17	Chemical	Sulfonated Zn-doped SBA-15 (Ph-SO_3H-Zn-SBA-15)	Soybean lecithin	Methyl butyrate	SCFA (short-chain fatty acid)-lecithin	[60]

Table 3: Types and triacylglyceride formulations of cocoa butter equivalent currently researched and developed.

	Product name	POP (%, w/w)	POS (%, w/w)	SOS (%, w/w)	Ref.
Natural product	Cocoa butter	13.8~21.8	26.3~44.8	20.0~29.4	[65]
CBE research	CBE from PMF*/PKO**/PS*** by *Thermomyces lanuginose* lipase	17.7	28.4	19.5	[66]
	CBE from PMF by *Rhizomucor miehei* lipase	30.7	40.1	14.5	[67]
	CBE from PO**** by *Rhizomucor miehei* lipase	26.6	42.1	18.0	[68]
	CBE from PMF by Lypozyme lipase	21	40	27	[69]
Commercialized CBE	ILLEXAO CB 40	34.2	13.8	31.4	[70]
	ILLEXAO SC 70	31.6	13.6	33.7	
	PALMY 50R	36.9	12.6	30.8	
	PALMY 20G	47.1	12.0	21.0	
	Coberine 507	43.8	12.9	21.7	
	Coberine 608	37.3	13.2	31.7	
	Chocovit 230	48.0	13.0	18.8	
	Chocovit 270	39.0	12.1	29.1	

*PMF: palm oil midfraction. **PKO: palm kernel oil. ***PS: palm stearin. ****PO: palm olein.

enzymes because they lack *sn*-2 site-specific reactions. Therefore, a considerable difference exists between the composition of natural cocoa butter and triglycerides, and substituting up to 5% of cocoa butter in food with triglycerides is acceptable.

3.2. Human Milk Fat Substitutes (HMFSs). Human milk fat is the primary energy source for babies, supplying 50% of their daily caloric intake [73], and is an essential source of vital fatty acids and fat-soluble vitamins. Oleic acid and palmitic acid are the main fatty acids in human milk fat, making up approximately 40% and 25%, respectively, and they exist as 1,3-dioleoyl-2-palmitoyl-glycerol [15]. Palmitic acid, a hydrolyzed free fatty acid, is used as an energy source. However, it forms an insoluble salt with the calcium and minerals needed for the growth of newborns and excretes them in the feces, preventing calcium absorption and causing constipation [74]. Therefore, in formulating HMFSs, the amount and composition of palmitic acid at the *sn*-2 position are important.

Commercial HMFSs are synthetic lipids with fatty acid contents and distribution comparable to human milk fat. Therefore, it is an important raw material in synthesizing infant food products (formula). Generally, HMFSs are manufactured by synthesizing free fatty acids obtained from hydrolyzing palmitic acid-rich fats and oils, tripalmitin, and oleic acid-rich vegetable oils, such as olive oil and sunflower oil [75]. In addition, they provide PUFAs that are important for the development of newborns and critical fatty acids are not found in human milk fat [76].

3.3. Trans-Free Plastic Fats. Many techniques such as margarine and shortening were created to ensure that vegetable fats, when used as animal fat substitutes, have the same physical qualities and texture as animal fat [77]. The liquid plant fat was converted into a solid with physical properties similar to animal fat. However, these vegetable fats that substitute animal fats have disadvantages [78]. The plastic fat used in margarine is produced through a hardening reaction involving the hydrogenation of vegetable oils, the primary raw materials [79]. The cis-type double-bond chain of unsaturated fatty acids is opened, and hydrogen is introduced during the curing process. Transfatty acids produced at this time increase the levels of total cholesterol and low-density lipoprotein cholesterol in the blood while lowering the levels of beneficial high-density lipoprotein cholesterol, causing cardiovascular and metabolic diseases [80]. Because of these problems, the level of transfatty acids in foods is closely limited, constraining the usage of hydrogenated oils [81].

Structured lipids can be practically produced through the enzymatic esterification of vegetable and solid fats to reduce transfatty acids while maintaining their physical qualities [82]. Saturated and unsaturated fats can be compared with partially hydrogenated oils in terms of their physical properties through esterification reactions that take place in the presence of lipases. Furthermore, margarine and shortening are produced by including helpful fatty acids, such as conjugated linoleic acid, without structured lipids, simply lowering transfat.

3.4. Low-Calorie Fats and Medium-Chain TAG. The calorific value of fat, a high-energy source, varies depending on the number of carbon atoms in fatty acids. Specifically, structured lipids with short- or long-chain fatty acids can be used as low-calorie fats. Short-chain fatty acids with 2–4 carbon atoms have a substantially lower caloric content than typical fat (9 kcal/g) [83]. Edible oils and fats, which are familiar sources of essential fatty acids, are mainly composed of long-chain fatty acids (L), which are stored in the body because of prolonged metabolic processes [84]. By contrast, medium-chain fatty acids (M), which include 8–12 carbon chains with hydrophilic properties, are easily oxidized and can be rapidly used by the body as an energy source. Unlike typical fat metabolism, medium-chain fatty acids are delivered directly to the liver through the hepatic portal vein and accumulate less frequently in tissues [85]. Therefore, they are efficient lipid substances for preventing metabolic diseases such as obesity, diabetes, and hyperlipidemia.

In addition, several edible oils (soybean oil, corn oil, olive oil, and canola oil) mainly comprise long-chain TAG- and medium-chain TAG-rich edible oils through enzymatic degradation and synthesis [86]. By reacting edible oils with heavy chain fatty acids in the presence of *sn*-1,3 stereospecificity lipase, other edible oils, including medium-long-medium (MLM) and medium-long-long (MLL), can be produced. Specifically, the MLM culinary sustenance provides better absorption of long-chain fatty acids, particularly essential ones, than other edible oils.

3.5. Functional Fatty Acids (PUFAs). Omega-3 fatty acids, including docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), efficiently improve brain and cardiovascular health; gamma-linolenic acid efficiently enhances skin health and blood circulation; and conjugated linolenic acid efficiently lowers body fat [87]. As the demand for functionally structured lipids containing high quantities of these helpful fatty acids increases, several studies are being conducted. By using the esterification process of perilla oil containing alpha-linolenic acid, linseed oil, and fish oils containing EPA and DHA in conjunction with lipase, studies have reported the synthesis of structured lipids containing large amounts of beneficial fatty acids [88–90]. These are beneficial because they are pure and contain many valuable fatty acids. In addition, fish oil can eliminate refusal to take it because of its objectionable odor .

3.6. Functional Emulsifiers. Emulsifiers are additives that bridge the combination of hydrophilic and hydrophobic chemicals and are employed to ensure the stability of several ingredients in the final food product. Characteristically, emulsifiers are composed of hydrophilic and hydrophobic molecules. In general, hydrophobic molecules have a long-chain structure similar to the molecular structure of fatty acids. Therefore, various emulsifiers use fatty acids as the hydrophobic molecular moiety. In particular, MAG and DAG are used in many industries as emulsifiers.

Lipases employed in reconstituted lipogenesis can synthesize emulsifiers by linking fatty acid chains to existing MAG, DAG, and other hydrophilic compounds with hydroxy groups. The emulsifier generated in this approach can be a multifunctional emulsifier with both hydrophilic molecules and fatty acid functionalities. Based on previous studies, multifunctional emulsifiers include sucrose palmitate, which is made of sucrose and palmitic acid, and emulsifiers that are made of fatty acids bound to ascorbic acid and erythorbic acid (Table 4) [96, 97]. These emulsifiers provide functions beyond those of existing emulsifiers. For example, they possess antibacterial and antioxidant characteristics. In addition, they are ecofriendly emulsifiers produced by enzymatic synthesis using natural materials and have low toxicity to the body [98].

Moreover, whether it is possible to maintain the functional features of hydrophilic and hydrophobic molecules while performing the role of emulsifiers to produce functional emulsifiers from structured lipids must be analyzed and assessed. Lipases react through dehydration condensation between hydrophilic head molecules and hydrophobic tail molecules; however, if they possess unique functionality at the binding site, they may lose their usefulness during synthesis. In addition, specifying the binding site when there are numerous binding sites for hydrophilic molecules other than lipids is impossible.

Table 4: Types of functional emulsifiers.

Product names	Enzyme	Properties	Ref.
Erythorbyl laurate	Novozyme 435	Antioxidant, antibacterial	[91]
Ascorbyl palmitate	Novozyme 435	Antioxidant	[92]
Sucrose palmitate	Lipolase 100 L	Nontoxicity	[93]
Sucrose laurate	Lipolase 100 L	Antibacterial	[93]
Fructose oleate	Lipozyme	Nontoxicity	[94]
Lauric arginate	Novozyme 435	Antibacterial	[95]

Briefly, structured lipids can be used in various food applications by customizing their composition and qualities. These lipids can create CBEs, HMFSs, trans-free plastic fats, low-calorie fats, medium-chain TAGs, and functional emulsifiers. Developing these functionally structured lipids could significantly affect the food industry and consumers by providing healthier and more diverse options. Research and innovation in this field will continue to expand the range of applications and benefits that structured lipids provide in functional foods (Figure 3).

4. Future Prospects

The market for extremophilic enzymes, such as lipases and esterases, isolated from microorganisms in extreme conditions, encompasses various applications in the food, pharmaceutical, and environmental industries, amounting to an enormous market size of approximately $600 million [99]. While the majority of these biocatalysts are discovered through traditional research methods such as metagenomics, innovative approaches are being developed to overcome cost and time constraints. Specifically, data science-based methods are being employed for classifying and systematizing the characteristics of known lipases and esterases and predicting the properties of new enzymes through phylogeny [100] and artificial intelligence and machine learning for predicting the properties of enzymes with specific amino acid sequences based on their three-dimensional (3D) structures [101]. From a genetic engineering perspective, third-generation gene editing technology [102], CRISPR/Cas9, offers a direction to address the challenges (e.g., reversibility/irreversibility, substrate specificity, temperature, and environmental adaptability) that existing enzymes possess through genetic manipulation [103].

4.1. Lipase Engineering Database (LED). Lipases (EC 3.1.1.3) and esterases (EC 3.1.1.1) can be categorized based on amino acid sequence similarity and physiological characteristics related to their ability to hydrolyze fats. However, classifying

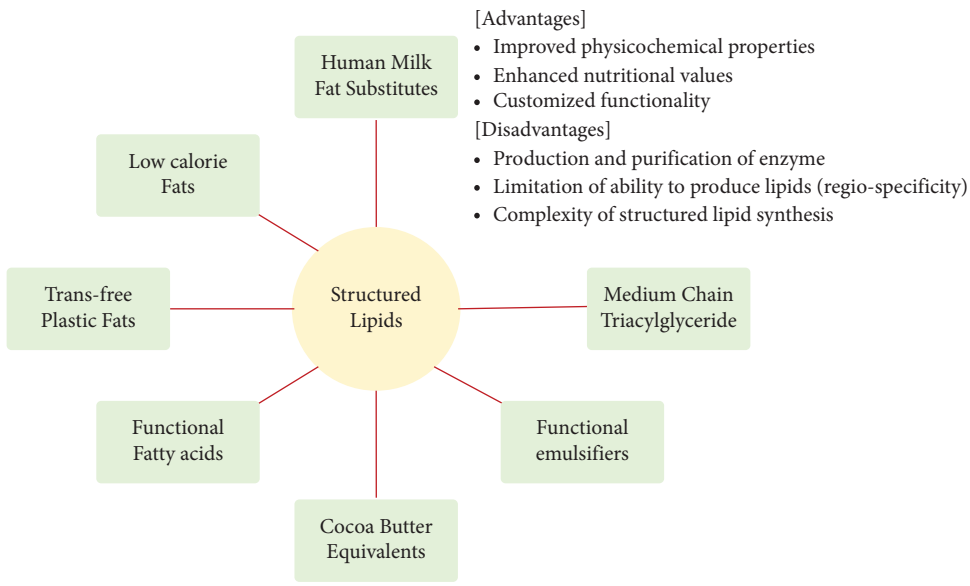

Figure 3: Overview of structured lipids in the food industry.

lipases accurately requires relying more than just on sequence similarity. The LED (https://led.biocatnet.de/) was developed to systematically organize information on lipases' sequences, structures, and functions and related proteins sharing the same α/β hydrolase fold. In particular, all lipases of the α/β hydrolase family consist of a catalytic triad and an oxyanion hole-containing core domain, a lid domain that protects the active site, a domain that supports the affinity of the active site, and an N- or C-terminal domain [104, 105]. These structural features can be utilized to classify lipases. In the latest version of the LED (ver. 4.1.0), 283,672 sequences and 1,590 structures have been categorized into 13 superfamilies. Although the LED remains a valuable tool for lipase classification, it requires regular database management and updates.

In addition to databases such as LED, various methods are employed to identify and classify the amino acid sequences of new lipases. These methods include metagenomic screening and reconstructing ancestral sequence approaches [106, 107]; however, these methods often involve costly and time-consuming validation processes. To overcome these drawbacks, automated tools for systematic classification are needed, typically relying on sequence similarity search tools such as BLAST. The functionality of these tools is limited by the quality of the search algorithm and the database scale used for searching. They may also involve some inherent errors within the program. Consequently, they need to provide a perfect solution for discovering and predicting new lipases.

4.2. Discovery of Lipases Using Machine Learning and Artificial Intelligence. Recent advances in machine learning and artificial intelligence have opened new opportunities for the classification of lipases and esterases. Techniques such as deep learning and artificial neural networks facilitate accurate classification and functional prediction of new enzymes based on sequence and structural information. This allows for overcoming the time and cost constraints associated with traditional approaches.

4.2.1. Prediction of Lipase 3D Structure. DeepMind's AlphaFold, the protein 3D structure prediction system developed by the creators of AlphaGo, has introduced new possibilities [101]. It employs artificial intelligence to predict amino acid sequences into 3D structures, improving accuracy through continuous iterative learning. After the first software release in 2018, AlphaFold2 was developed in 2020 [108]. AlphaFold contains approximately 3,000 lipase amino acid sequences; however, expanding to a more extensive database is necessary. While the 3D structure prediction of independent proteins is highly accurate, limitations in the prediction model arise from the need for more information on variables such as environmental conditions and proximity of other proteins, oligomers, metal ions, and cofactors.

RoseTTAFold, another protein 3D structure prediction system, provides more accurate predictions for specific regions, such as membrane proteins, by considering some of the variables mentioned above based on amino acid sequences [109]. However, similar to AlphaFold, the absence of machine learning for various variables results in reduced accuracy in predicting protein 3D structures under specific conditions [110].

Modeling methods for neural network-based protein 3D structure prediction have made significant progress in recent years, offering a new paradigm for protein structure prediction. Although systems such as AlphaFold and RoseTTAFold have many areas requiring improvement, their utility in various fields remains high. In discovering new lipases, clarifying structural features and phylogenetic classifications through prediction model systems can significantly shorten time constraints.

4.2.2. Prediction of Lipase Regiospecificity. Identifying novel lipases is essential for producing structured lipids with various functional properties. In addition to *sn*-1 regiospecific and nonspecific lipases, the discovery of *sn*-1(3) and *sn*-2 regiospecific lipases is significant. Despite several studies aiming to recognize lipases with *sn*-1(3) regiospecificity and *sn*-2 regiospecificity, obtaining experimental data, and research results takes time and effort. Various solutions can be proposed to overcome these limitations. For example, to determine enzyme inhibitors, Durai et al. [111] developed an *in silico* prediction model, predicting inhibitory potential based on structural compatibility and binding strength between natural substances and tyrosinase substrate interaction. These findings can be highly beneficial for identifying lipases that can synthesize structured lipids. Novel regiospecificity can be predicted based on structural compatibility and binding strength with each substrate after structural prediction for the active lipase site with known positional specificity to produce 1,3-DAG and 2,3-DAG with TAG and synthesize various structured lipids such as POS and POP. The outcomes of this prediction modeling may enable faster discovery of novel regiospecific lipases.

In addition to developing lipases with novel regiospecificity, ongoing research is needed to reduce the cost of commercially available lipases. Most commercially available lipases are expensive and derived from microorganisms [112]. Identifying new lipases, which are essential components of enzymatic synthesis methods for structured lipid production, is necessary. Despite active research on lipase extraction from diverse natural sources such as plants and fungi, only some industrial applications have been identified [113]. This is explained by the challenging mass production of lipases isolated from natural sources. Genetic engineering studies have offered potential solutions to these issues, such as finding novel lipases and producing lipases through genetic replication in various cultural environments, including bacteria and yeast, where mass production is more feasible. With active collaboration from discovery to mass production, further research on lipases that can synthesize structured lipids is expected.

In addition to discovering novel lipases, extensive research is ongoing on the multiple roles of lipids, anticipating the production of structured lipids with additional functionalities. As lipids with diverse properties such as antioxidant, antibacterial, and anti-inflammatory capabilities have been identified, producing multifunctional structured lipids by enzymatically synthesizing lipids with these properties and functions can add significant value. This can increase the availability of structured lipids for widespread use in medicine, cosmetics, and food as dietary ingredients.

In summary, machine learning and artificial intelligence hold great potential for discovering and classifying lipases and esterases. Techniques such as deep learning and artificial neural networks can facilitate accurate predictions of enzyme structures and functions, leading to more efficient discovery and development of novel lipases for the synthesis of structured lipids. Furthermore, ongoing research on the multiple roles of lipids and the development of *in silico* prediction models can contribute to the production of structured lipids with additional functionalities and expanded applications across industries.

4.3. Improvement of Lipases through Gene Editing Technologies. Gene editing technologies, particularly the CRISPR/Cas9 system derived from the bacterial immune system, are emerging as potential tools in various fields as third-generation gene scissors [102]. CRISPR/Cas9 comprises gRNA, which binds to a particular base sequence, and Cas9 nuclease, which acts as the scissors [103]. Compared with the first- and second-generation gene scissors, the CRISPR/Cas9 system allows for highly precise gene manipulation. It has been widely utilized because of its simplicity, ability to cut at any site, and cost-effectiveness. Moreover, many studies have tried to improve the function of lipases and esterases, and gene editing technologies can be employed to enhance the activity of specific enzymes or improve their stability in specific environments [114, 115]. This can lead to the development of improved enzymes through genetic manipulation, resulting in more cost-effective industrial products.

Despite the advantages of CRISPR/Cas9, concerns about the safety and ethical issues of gene manipulation are always raised [116]. The simplicity and precision of this technology have led to recent research findings presenting the risk of carcinogenesis because a portion of the genes is lost within cells [117]. In addition, a case in China where the technology was directly applied to humans faced ethical issues, and the research results were not recognized [118]. Consequently, CRISPR/Cas9, although a superior genetic engineering technology, has limited application because of ethical and legal concerns.

Various methods are available for discovering and developing biological enzymes to produce structured lipids, each with advantages and disadvantages. Traditional research methods, such as metagenomic screening and ancestral sequence reconstruction, may be more accurate but require significant time and costs. On the contrary, new paradigms such as machine learning, artificial intelligence, and CRISPR/Cas9 may present some potential for errors. Each technique can only be considered the best approach. Therefore, to derive and validate lipases that can synthesize structured lipids, combining various methods is necessary to complement the advantages and disadvantages of each approach in terms of research accuracy and efficiency.

5. Conclusions

Structured lipids produced using biological enzymes, particularly lipases and esterases, have become essential elements of food, pharmaceuticals, and cosmetics because of their numerous benefits over traditional chemical catalysts. The regiospecificity and substrate specificity of these enzymes, including the ability to selectively modify the composition of fatty acids and distribution of lipids, make them useful for the synthesis of structured lipids. In particular, the utilization of immobilized lipases as catalysts offers many advantages, such as reusability, high selectivity,

and low environmental effects, making them more attractive in commercial applications. However, the current range of *sn*-1,3 regiospecific lipases and available natural oils and fats for use in the synthesis of structured lipids is limited.

Therefore, further research and development of new lipases that can synthesize structured lipids with diverse functionalities and improvement of existing enzyme properties through genetic engineering and innovative screening methods are essential. Furthermore, the use of machine learning and artificial intelligence tools and the development of *in silico* prediction models can greatly aid in the efficient and accurate discovery of new lipases. With continued collaboration between academia and industry, the development and commercialization of structured lipids using biological enzymes will undoubtedly expand, offering wide-ranging benefits for various industries. The utilization of new technologies such as data science, machine learning, artificial intelligence, and CRISPR/Cas9 in discovering and enhancing lipases and esterases can promote the development of more efficient and cost-effective production methods for structured lipids. However, these novel technologies should be used responsibly, considering their potential ethical, legal, and safety issues.

In summary, structured lipids and their production using biological enzymes present numerous opportunities for improving human health and the environment. Future research should focus on discovering and enhancing novel enzymes, improving structured lipid production processes, and exploring new applications of structured lipids. The utilization of innovative technologies will accelerate the development of more effective and sustainable solutions for the synthesis of structured lipids.

Data Availability

All the data generated or analyzed during this study are included within this article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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References

[1] I. R. Lake, L. Hooper, A. Abdelhamid et al., "Climate change and food security: health impacts in developed countries," *Environmental Health Perspectives*, vol. 120, no. 11, pp. 1520–1526, 2012.

[2] P. Webb, T. G. Benton, J. Beddington, D. Flynn, N. M. Kelly, and S. M. Thomas, "The urgency of food system transformation is now irrefutable," *Nature Food*, vol. 1, no. 10, pp. 584-585, 2020.

[3] P. Kumar, M. Chatli, N. Mehta, P. Singh, O. Malav, and A. K. Verma, "Meat analogues: health promising sustainable meat substitutes," *Critical Reviews in Food Science and Nutrition*, vol. 57, no. 5, pp. 923–932, 2017.

[4] S. S. Shirai, O. Seneviratne, M. E. Gordon, C. H. Chen, and D. L. McGuinness, "Identifying ingredient substitutions using a knowledge graph of food," *Frontiers in Artificial Intelligence*, vol. 3, Article ID 621766, 2020.

[5] J. Zhou, Y.-Y. Lee, Y. Mao, Y. Wang, and Z. Zhang, "Future of structured lipids: enzymatic synthesis and their new applications in food systems," *Foods*, vol. 11, no. 16, p. 2400, 2022.

[6] P. Borel and C. Desmarchelier, "Bioavailability of fat-soluble vitamins and phytochemicals in humans: effects of genetic variation," *Annual Review of Nutrition*, vol. 38, no. 1, pp. 69–96, 2018.

[7] M. A. González Hernández, E. E. Canfora, J. W. Jocken, and E. E. Blaak, "The short-chain fatty acid acetate in body weight control and insulin sensitivity," *Nutrients*, vol. 11, no. 8, p. 1943, 2019.

[8] J. Donoso-Quezada, S. Ayala-Mar, and J. González-Valdez, "The role of lipids in exosome biology and intercellular communication: function, analytics and applications," *Traffic*, vol. 22, no. 7, pp. 204–220, 2021.

[9] Y. Guo, X. Zhu, M. Zeng et al., "A diet high in sugar and fat influences neurotransmitter metabolism and then affects brain function by altering the gut microbiota," *Translational Psychiatry*, vol. 11, no. 1, pp. 328–427, 2021.

[10] S. Laye, A. Nadjar, C. Joffre, and R. P. Bazinet, "Anti-inflammatory effects of omega-3 fatty acids in the brain: physiological mechanisms and relevance to pharmacology," *Pharmacological Reviews*, vol. 70, no. 1, pp. 12–38, 2018.

[11] G. Li, Z. Zhang, H. Liu, and L. Hu, "Nanoemulsion-based delivery approaches for nutraceuticals: fabrication, application, characterization, biological fate, potential toxicity and future trends," *Food & Function*, vol. 12, no. 5, pp. 1933–1953, 2021.

[12] C. M. O'Sullivan, S. Barbut, and A. G. Marangoni, "Edible oleogels for the oral delivery of lipid soluble molecules: composition and structural design considerations," *Trends in Food Science & Technology*, vol. 57, pp. 59–73, 2016.

[13] Y. Guo, Z. Cai, Y. Xie et al., "Synthesis, physicochemical properties, and health aspects of structured lipids: a review," *Comprehensive Reviews in Food Science and Food Safety*, vol. 19, no. 2, pp. 759–800, 2020.

[14] K. T. Lee and C. C. Akoh, "Structured lipids: synthesis and applications," *Food Reviews International*, vol. 14, no. 1, pp. 17–34, 1998.

[15] Z. Wang, L. Liu, L. Liu, T. Liu, C. Li, and L. Sun, "1, 3-Dioleoyl-2-palmitoylglycerol-rich triacylglycerol characterization by three processing methods," *International Journal of Food Properties*, vol. 22, no. 1, pp. 1156–1171, 2019.

[16] B. Dayal, G. Salen, B. Toome, G. Tint, S. Shefer, and J. Padia, "Lithium hydroxide/aqueous methanol: mild reagent for the hydrolysis of bile acid methyl esters," *Steroids*, vol. 55, no. 5, pp. 233–237, 1990.

[17] M. J. Sproston and C. C. Akoh, "Antioxidative effects of a glucose-cysteine Maillard reaction product on the oxidative stability of a structured lipid in a complex food emulsion," *Journal of Food Science*, vol. 81, no. 12, pp. C2923–C2931, 2016.

[18] V. L. Sirisha, A. Jain, and A. Jain, "Enzyme immobilization: an overview on methods, support material, and applications of immobilized enzymes," *Advances in Food & Nutrition Research*, vol. 79, pp. 179–211, 2016.

[19] P. Merlin Christy, L. Gopinath, and D. Divya, "A review on anaerobic decomposition and enhancement of biogas production through enzymes and microorganisms," *Renewable and Sustainable Energy Reviews*, vol. 34, pp. 167–173, 2014.

[20] C. C. Akoh, "Structured lipids," *Food lipids: Chemistry, Nutrition, and Biotechnology*, vol. 2, no. 2, pp. 877–908, 2002.

[21] P. E. Kankaanpää, S. J. Salminen, E. Isolauri, and Y. K. Lee, "The influence of polyunsaturated fatty acids on probiotic growth and adhesion," *FEMS Microbiology Letters*, vol. 194, no. 2, pp. 149–153, 2001.

[22] E. A. Trautwein, D. R. Rieckhoff, and H. F. Erbersdobler, "Dietary inulin lowers plasma cholesterol and triacylglycerol and alters biliary bile acid profile in hamsters," *Journal of Nutrition*, vol. 128, no. 11, pp. 1937–1943, 1998.

[23] H. Osborn and C. Akoh, "Structured lipids-novel fats with medical, nutraceutical, and food applications," *Comprehensive Reviews in Food Science and Food Safety*, vol. 1, no. 3, pp. 110–120, 2002.

[24] R. C. Rodrigues and R. Fernandez-Lafuente, "Lipase from Rhizomucor miehei as an industrial biocatalyst in chemical process," *Journal of Molecular Catalysis B: Enzymatic*, vol. 64, no. 1-2, pp. 1–22, 2010.

[25] C. C. Akoh, *Food Lipids: Chemistry, Nutrition, and Biotechnology*, CRC Press, Boca Raton, FL, USA, 2017.

[26] S. Sivakanthan and T. Madhujith, "Current trends in applications of enzymatic interesterification of fats and oils: a review," *Lwt*, vol. 132, Article ID 109880, 2020.

[27] M. Hájek, A. Vávra, H. de Paz Carmona, and J. Kocík, "The catalysed transformation of vegetable oils or animal fats to biofuels and bio-lubricants: a review," *Catalysts*, vol. 11, no. 9, p. 1118, 2021.

[28] A. G. Alsultan, N. Asikin-Mijan, Z. Ibrahim et al., "A short review on catalyst, feedstock, modernised process, current state and challenges on biodiesel production," *Catalysts*, vol. 11, no. 11, p. 1261, 2021.

[29] P. Ornla-ied, P. Podchong, and S. Sonwai, "Synthesis of cocoa butter alternatives from palm kernel stearin, coconut oil and fully hydrogenated palm stearin blends by chemical interesterification," *Journal of the Science of Food and Agriculture*, vol. 102, no. 4, pp. 1619–1627, 2022.

[30] R. C. da Silva, D. F. Soares, M. B. Lourenço et al., "Structured lipids obtained by chemical interesterification of olive oil and palm stearin," *LWT- Food Science and Technology*, vol. 43, no. 5, pp. 752–758, 2010.

[31] M. A. Bashir, S. Wu, J. Zhu, A. Krosuri, M. U. Khan, and R. J. Ndeddy Aka, "Recent development of advanced processing technologies for biodiesel production: a critical review," *Fuel Processing Technology*, vol. 227, Article ID 107120, 2022.

[32] P. C. Torres-Mayanga, D. Lachos-Perez, A. Mudhoo et al., "Production of biofuel precursors and value-added chemicals from hydrolysates resulting from hydrothermal processing of biomass: a review," *Biomass and Bioenergy*, vol. 130, Article ID 105397, 2019.

[33] S. C. Hong, K. M. Park, Y. H. Son et al., "AOT/isooctane reverse micelles with a microaqueous core act as protective shells for enhancing the thermal stability of *Chromobacterium viscosum* lipase," *Food Chemistry*, vol. 179, pp. 263–269, 2015.

[34] R. Rosu, M. Yasui, Y. Iwasaki, and T. Yamane, "Enzymatic synthesis of symmetrical 1, 3-diacylglycerols by direct esterification of glycerol in solvent-free system," *Journal of the American Oil Chemists' Society*, vol. 76, no. 7, pp. 839–843, 1999.

[35] P. S. Bisen, B. S. Sanodiya, G. S. Thakur, R. K. Baghel, and G. Prasad, "Biodiesel production with special emphasis on lipase-catalyzed transesterification," *Biotechnology Letters*, vol. 32, no. 8, pp. 1019–1030, 2010.

[36] A. Kawashima, Y. Shimada, M. Yamamoto et al., "Enzymatic synthesis of high-purity structured lipids with caprylic acid at 1, 3-positions and polyunsaturated fatty acid at 2-position," *Journal of the American Oil Chemists' Society*, vol. 78, no. 6, pp. 611–616, 2001.

[37] D. Goswami, J. K. Basu, and S. De, "Lipase applications in oil hydrolysis with a case study on castor oil: a review," *Critical Reviews in Biotechnology*, vol. 33, no. 1, pp. 81–96, 2013.

[38] K. R. Jegannathan, S. Abang, D. Poncelet, E. S. Chan, and P. Ravindra, "Production of biodiesel using immobilized lipase—a critical review," *Critical Reviews in Biotechnology*, vol. 28, no. 4, pp. 253–264, 2008.

[39] H. H. Nguyen and M. Kim, "An overview of techniques in enzyme immobilization," *Applied Science and Convergence Technology*, vol. 26, no. 6, pp. 157–163, 2017.

[40] N. R. Mohamad, N. H. C. Marzuki, N. A. Buang, F. Huyop, and R. A. Wahab, "An overview of technologies for immobilization of enzymes and surface analysis techniques for immobilized enzymes," *Biotechnology & Biotechnological Equipment*, vol. 29, no. 2, pp. 205–220, 2015.

[41] N. Sarmah, D. Revathi, G. Sheelu et al., "Recent advances on sources and industrial applications of lipases," *Biotechnology Progress*, vol. 34, no. 1, pp. 5–28, 2018.

[42] Y. Huang, W. Liu, X. Luo, M. Zhao, T. Liu, and F. Feng, "Synthesis and characterization of medium- and long-chain structural lipid rich in α-linolenic acid and lauric acid," *Food Bioscience*, vol. 52, Article ID 102363, 2023.

[43] X. Wang, C. Jiang, W. Xu, Z. Miu, Q. Jin, and X. Wang, "Enzymatic synthesis of structured triacylglycerols rich in 1,3-dioleoyl-2-palmitoylglycerol and 1-oleoyl-2-palmitoyl-3-linoleoylglycerol in a solvent-free system," *Lebensmittel-Wissenschaft und-Technologie*, vol. 118, Article ID 108798, 2020.

[44] J. Souza-Gonçalves, A. Fialho, C. M. F. Soares, N. M. Osório, and S. Ferreira-Dias, "Continuous production of dietetic structured lipids using crude acidic olive pomace oils," *Molecules*, vol. 28, no. 6, p. 2637, 2023.

[45] Y. Wang, W. Wei, R. Liu, M. Chang, Q. Jin, and X. Wang, "Synthesis of eicosapentaenoic acid-enriched medium- and long-chain triglyceride by lipase-catalyzed transesterification: a novel strategy for clinical nutrition intervention," *Journal of the Science of Food and Agriculture*, vol. 103, no. 10, pp. 4767–4777, 2023.

[46] Y. Li, Y. Zhang, Y. Zhou, Y. Zhang, and M. Zheng, "A novel and controllable method for simultaneous preparation of human milk fat substitutes (OPL, OPO and LPL): two-step enzymatic ethanolysis-esterification strategy," *Food Research International*, vol. 163, Article ID 112168, 2023.

[47] S. Zhang, J. R. Hyatt, and C. C. Akoh, "Lipase-catalyzed one-step regioselective synthesis of 1,2-dioctanoylgalloylglycerol in a solvent-free system: optimization of reaction conditions and structural elucidation," *Food Chemistry*, vol. 382, Article ID 132302, 2022.

[48] H. B. Jadhav, P. Gogate, and U. Annapure, "Process intensification of acidolysis reaction catalysed by enzymes for synthesis of designer lipids using sonication," *Chemical Engineering Journal*, vol. 428, Article ID 131374, 2022.

[49] R. Cui, X. Che, L. Li, D. Sun-Waterhouse, J. Wang, and Y. Wang, "Engineered lipase from Janibacter sp. with high thermal stability to efficiently produce long-medium-long

triacylglycerols," *Lebensmittel-Wissenschaft und-Technologie*, vol. 165, Article ID 113675, 2022.

[50] I. de Souza Correia Cozentino, A. Veloso de Paula, C. Augusto Ribeiro et al., "Development of a potentially functional chocolate spread containing probiotics and structured triglycerides," *Lebensmittel-Wissenschaft und-Technologie*, vol. 154, Article ID 112746, 2022.

[51] P. Ornla-ied, C. P. Tan, Y. Wang, and S. Sonwai, "Cocoa butter alternatives from enzymatic interesterification of palm kernel stearin, coconut oil, and fully hydrogenated palm stearin blends," *Journal of the American Oil Chemists' Society*, vol. 98, no. 1, pp. 53–64, 2021.

[52] N. Zhang, J. P. Zeng, Y. P. Wu et al., "Human milk sn-2 palmitate triglyceride rich in linoleic acid had lower digestibility but higher absorptivity compared with the sn-2 palmitate triglyceride rich in oleic acid in vitro," *Journal of Agricultural and Food Chemistry*, vol. 69, no. 32, pp. 9137–9146, 2021.

[53] S. Ji, J. Wu, F. Xu et al., "Synthesis, purification, and characterization of a structured lipid based on soybean oil and coconut oil and its applications in curcumin-loaded nanoemulsions," *European Journal of Lipid Science and Technology*, vol. 122, no. 10, Article ID 2000086, 2020.

[54] Q. Xia, T. O. Akanbi, R. Li, B. Wang, W. Yang, and C. J. Barrow, "Lipase-catalysed synthesis of palm oil-omega-3 structured lipids," *Food & Function*, vol. 10, no. 6, pp. 3142–3149, 2019.

[55] M. Shagholian, S. Goli, A. Shirvani, M. Agha-Ghazvini, and S. Asgary, "Liver and serum lipids in Wistar rats fed a novel structured lipid containing conjugated linoleic acid and conjugated linolenic acid," *Grasas Y Aceites*, vol. 70, no. 2, p. e307, 2019.

[56] A. Bahari and C. C. Akoh, "Synthesis of a cocoa butter equivalent by enzymatic interesterification of illipe butter and palm midfraction," *Journal of the American Oil Chemists' Society*, vol. 95, no. 5, pp. 547–555, 2018.

[57] I. P. Wangi, S. Supriyanto, H. Sulistyo, and C. Hidayat, "Sodium silicate catalyst for synthesis monoacylglycerol and diacylglycerol-rich structured lipids: product characteristic and glycerolysis–interesterification kinetics," *Bulletin of Chemical Reaction Engineering and Catalysis*, vol. 17, no. 2, pp. 250–262, 2022.

[58] S. Liang, X. Wei, M. Zhang, and C. Sun, "Preparation of structured lipid enriched with medium chain triacylglycerol by chemical catalyzed acidolysis of coconut oil: optimized by response surface methodology," *Journal of Oleo Science*, vol. 68, no. 12, pp. 1175–1185, 2019.

[59] Z. Zhang, J. Ye, W. J. Lee, C. C. Akoh, A. Li, and Y. Wang, "Modification of palm-based oil blend via interesterification: physicochemical properties, crystallization behaviors and oxidative stabilities," *Food Chemistry*, vol. 347, Article ID 129070, 2021.

[60] J. Zhang, K. Cheng, H. Li et al., "Efficient synthesis of structured phospholipids containing short-chain fatty acids over a sulfonated Zn-SBA-15 catalyst," *Journal of Agricultural and Food Chemistry*, vol. 68, no. 44, pp. 12444–12453, 2020.

[61] K. Yamada, M. Ibuki, and T. McBrayer, "Cocoa butter, cocoa butter equivalents, and cocoa butter replacers," in *Healthful Lipids*, AOCS Publishing, Urbana, Illinois, 2019.

[62] N. Biswas, Y. L. Cheow, C. P. Tan, and L. F. Siow, "Physical, rheological and sensorial properties, and bloom formation of dark chocolate made with cocoa butter substitute (CBS)," *LWT-Food Science & Technology*, vol. 82, pp. 420–428, 2017.

[63] M. Norazlina, M. Jahurul, M. Hasmadi et al., "Trends in blending vegetable fats and oils for cocoa butter alternative application: a review," *Trends in Food Science & Technology*, vol. 116, pp. 102–114, 2021.

[64] Z. Huang, Z. Guo, D. Xie et al., "Rhizomucor miehei lipase-catalysed synthesis of cocoa butter equivalent from palm mid-fraction and stearic acid: characteristics and feasibility as cocoa butter alternative," *Food Chemistry*, vol. 343, Article ID 128407, 2021.

[65] M. J. Haque Akanda, N. Mr, A. Fs et al., "Hard fats improve the physicochemical and thermal properties of seed fats for applications in confectionery products," *Food Reviews International*, vol. 36, no. 6, pp. 601–625, 2020.

[66] N. Biswas, Y. L. Cheow, C. P. Tan, and L. F. Siow, "Physicochemical properties of enzymatically produced palm-oil-based cocoa butter substitute (CBS) with cocoa butter mixture," *European Journal of Lipid Science and Technology*, vol. 120, no. 3, Article ID 1700205, 2018.

[67] I. O. Mohamed, "Lipase-catalyzed acidolysis of palm mid fraction oil with palmitic and stearic fatty acid mixture for production of cocoa butter equivalent," *Applied Biochemistry and Biotechnology*, vol. 171, no. 3, pp. 655–666, 2013.

[68] I. O. Mohamed, "Lipase-catalyzed synthesis of cocoa butter equivalent from palm olein and saturated fatty acid distillate from palm oil physical refinery," *Applied Biochemistry and Biotechnology*, vol. 168, no. 6, pp. 1405–1415, 2012.

[69] D. Undurraga, A. Markovits, and S. Erazo, "Cocoa butter equivalent through enzymic interesterification of palm oil midfraction," *Process Biochemistry*, vol. 36, no. 10, pp. 933–939, 2001.

[70] N. De Clercq, S. Kadivar, D. Van de Walle, S. De Pelsmaeker, X. Ghellynck, and K. Dewettinck, "Functionality of cocoa butter equivalents in chocolate products," *European Food Research and Technology*, vol. 243, no. 2, pp. 309–321, 2017.

[71] S. M. Ghazani and A. G. Marangoni, "The ternary solid state phase behavior of triclinic POP, POS, and SOS and its relationship to CB and CBE properties," *Crystal Growth & Design*, vol. 19, no. 2, pp. 704–713, 2018.

[72] B. Naik and V. Kumar, "Cocoa butter and its alternatives: a review," *Journal of Bioresource Engineering and Technology*, vol. 1, pp. 7–17, 2014.

[73] C. R. Martin, P.-R. Ling, and G. L. Blackburn, "Review of infant feeding: key features of breast milk and infant formula," *Nutrients*, vol. 8, no. 5, p. 279, 2016.

[74] I. Xinias and A. Mavroudi, "Constipation in Childhood. An update on evaluation and management," *Hippokratia*, vol. 19, no. 1, pp. 11–19, 2015.

[75] W. Wei, Q. Jin, and X. Wang, "Human milk fat substitutes: past achievements and current trends," *Progress in Lipid Research*, vol. 74, pp. 69–86, 2019.

[76] M. Makrides, M. Neumann, K. Simmer, R. Gibson, and J. Pater, "Are long-chain polyunsaturated fatty acids essential nutrients in infancy?" *The Lancet*, vol. 345, no. 8963, pp. 1463–1468, 1995.

[77] A. J. Martins, A. A. Vicente, R. L. Cunha, and M. A. Cerqueira, "Edible oleogels: an opportunity for fat replacement in foods," *Food & Function*, vol. 9, no. 2, pp. 758–773, 2018.

[78] M. N. Marikkar and Y. N. Abdul Manaf, "12- fats, oils, and emulsifiers," in *Preparation and Processing of Religious and Cultural Foods*, M. E. Ali and N. N. A. Nizar, Eds., Woodhead Publishing, Sawston, UK, 2018.

[79] W. Wongjaikham, G. Kongprawes, D. Wongsawaeng et al., "Production of low trans-fat margarine by partial

hydrogenation of palm oil using nature-friendly and catalyst-free microwave plasma technique," *Innovative Food Science & Emerging Technologies*, vol. 80, Article ID 103107, 2022.

[80] Y. Chen, Y. She, R. Zhang, J. Wang, X. Zhang, and X. Gou, "Use of starch-based fat replacers in foods as a strategy to reduce dietary intake of fat and risk of metabolic diseases," *Food Science and Nutrition*, vol. 8, no. 1, pp. 16–22, 2020.

[81] T. Nagpal, J. K. Sahu, S. K. Khare, K. Bashir, and K. Jan, "Trans fatty acids in food: a review on dietary intake, health impact, regulations and alternatives," *Journal of Food Science*, vol. 86, no. 12, pp. 5159–5174, 2021.

[82] D. K. T. Moreira, J. N. R. Ract, A. P. B. Ribeiro, and G. A. Macedo, "Production and characterization of structured lipids with antiobesity potential and as a source of essential fatty acids," *Food Research International*, vol. 99, pp. 713–719, 2017.

[83] P. Schönfeld and L. Wojtczak, "Short- and medium-chain fatty acids in energy metabolism: the cellular perspective," *Journal of Lipid Research*, vol. 57, no. 6, pp. 943–954, 2016.

[84] A. A. Papamandjaris, D. E. Macdougall, and P. J. H. Jones, "Medium chain fatty acid metabolism and energy expenditure: obesity treatment implications," *Life Sciences*, vol. 62, no. 14, pp. 1203–1215, 1998.

[85] J. O. Ebbert and M. D. Jensen, "Fat depots, free fatty acids, and dyslipidemia," *Nutrients*, vol. 5, no. 2, pp. 498–508, 2013.

[86] H. Takeuchi, S. Sekine, K. Kojima, and T. Aoyama, "The application of medium-chain fatty acids: edible oil with a suppressing effect on body fat accumulation," *Asia Pacific Journal of Clinical Nutrition*, vol. 17, no. 1, pp. 320–323, 2008.

[87] E. J. Baker, E. A. Miles, G. C. Burdge, P. Yaqoob, and P. C. Calder, "Metabolism and functional effects of plant-derived omega-3 fatty acids in humans," *Progress in Lipid Research*, vol. 64, pp. 30–56, 2016.

[88] E. M. Kurowska, G. K. Dresser, L. Deutsch, D. Vachon, and W. Khalil, "Bioavailability of omega-3 essential fatty acids from perilla seed oil," *Prostaglandins, Leukotrienes and Essential Fatty Acids*, vol. 68, no. 3, pp. 207–212, 2003.

[89] B. Diwan and P. Gupta, "Synthesis of MCFA and PUFA rich oils by enzymatic structuring of flax oil with single cell oils," *Lebensmittel-Wissenschaft und-Technologie*, vol. 133, Article ID 109928, 2020.

[90] S. Imanparast, J. Hamedi, and M. A. Faramarzi, "Enzymatic esterification of acylglycerols rich in omega-3 from flaxseed oil by an immobilized solvent-tolerant lipase from *Actinomadura sediminis* UTMC 2870 isolated from oil-contaminated soil," *Food Chemistry*, vol. 245, pp. 934–942, 2018.

[91] K. M. Park, M. J. Lee, S. K. Jo, S. J. Choi, J. Lee, and P. S. Chang, "Erythorbyl laurate as a potential food additive with multi-functionalities: interfacial characteristics and antioxidant activity," *Food Chemistry*, vol. 215, pp. 101–107, 2017.

[92] A. R. Hraš, M. Hadolin, Ž. Knez, and D. Bauman, "Comparison of antioxidative and synergistic effects of rosemary extract with α-tocopherol, ascorbyl palmitate and citric acid in sunflower oil," *Food Chemistry*, vol. 71, no. 2, pp. 229–233, 2000.

[93] M. Ferrer, M. A. Cruces, M. Bernabe, A. Ballesteros, and F. J. Plou, "Lipase-catalyzed regioselective acylation of sucrose in two-solvent mixtures," *Biotechnology and Bioengineering*, vol. 65, no. 1, pp. 10–16, 1999.

[94] N. Khaled, D. Montet, M. Pina, and J. Graille, "Fructose oleate synthesis in a fixed catalyst bed reactor," *Biotechnology Letters*, vol. 13, no. 3, pp. 167–172, 1991.

[95] Q. Ma, P. M. Davidson, and Q. Zhong, "Properties and potential food applications of lauric arginate as a cationic antimicrobial," *International Journal of Food Microbiology*, vol. 315, Article ID 108417, 2020.

[96] D. Reyes-Duarte, N. López-Cortés, M. Ferrer, F. J. Plou, and A. Ballesteros, "Parameters affecting productivity in the lipase-catalysed synthesis of sucrose palmitate," *Biocatalysis and Biotransformation*, vol. 23, no. 1, pp. 19–27, 2005.

[97] K.-M. Park, D. E. Lee, H. Sung, J. Lee, and P.-S. Chang, "Lipase-catalysed synthesis of erythorbyl laurate in acetonitrile," *Food Chemistry*, vol. 129, no. 1, pp. 59–63, 2011.

[98] G. L. Hasenhuettl, "Synthesis and commercial preparation of food emulsifiers," in *Food Emulsifiers and Their Applications*, G. L. Hasenhuettl and R. W. Hartel, Eds., Springer International Publishing, Berlin, Germany, 2019.

[99] O. Lopez-Lopez, M. E. Cerdan, and M. Siso, "New extremophilic lipases and esterases from metagenomics," *Current Protein & Peptide Science*, vol. 15, no. 5, pp. 445–455, 2014.

[100] M. Fischer and J. Pleiss, "The Lipase Engineering Database: a navigation and analysis tool for protein families," *Nucleic Acids Research*, vol. 31, no. 1, pp. 319–321, 2003.

[101] J. Jumper, R. Evans, A. Pritzel et al., "Highly accurate protein structure prediction with AlphaFold," *Nature*, vol. 596, no. 7873, pp. 583–589, 2021.

[102] M. R. O'Connell, B. L. Oakes, S. H. Sternberg, A. East-Seletsky, M. Kaplan, and J. A. Doudna, "Programmable RNA recognition and cleavage by CRISPR/Cas9," *Nature*, vol. 516, no. 7530, pp. 263–266, 2014.

[103] F. Jiang and J. A. Doudna, "CRISPR–Cas9 structures and mechanisms," *Annual Review of Biophysics*, vol. 46, no. 1, pp. 505–529, 2017.

[104] N. Miled, C. Bussetta, A. De caro, M. Rivière, L. Berti, and S. Canaan, "Importance of the lid and cap domains for the catalytic activity of gastric lipases," *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, vol. 136, no. 1, pp. 131–138, 2003.

[105] J. Pleiss, M. Fischer, M. Peiker, C. Thiele, and R. D. Schmid, "Lipase engineering database: understanding and exploiting sequence–structure–function relationships," *Journal of Molecular Catalysis B: Enzymatic*, vol. 10, no. 5, pp. 491–508, 2000.

[106] J. Chow, F. Kovacic, Y. Dall Antonia et al., "The metagenome-derived enzymes LipS and LipT increase the diversity of known lipases," *PLoS One*, vol. 7, no. 10, Article ID e47665, 2012.

[107] S. Verma, R. Kumar, and G. K. Meghwanshi, "Identification of new members of alkaliphilic lipases in archaea and metagenome database using reconstruction of ancestral sequences," *3 Biotech.*, vol. 9, no. 5, 2019.

[108] P. Cramer, "AlphaFold2 and the future of structural biology," *Nature Structural & Molecular Biology*, vol. 28, no. 9, pp. 704-705, 2021.

[109] F. Azzaz, N. Yahi, H. Chahinian, and J. Fantini, "The epigenetic dimension of protein structure is an intrinsic weakness of the AlphaFold program," *Biomolecules*, vol. 12, no. 10, p. 1527, 2022.

[110] C. Lee, B. H. Su, and Y. J. Tseng, "Comparative studies of AlphaFold, RoseTTAFold and Modeller: a case study involving the use of G-protein-coupled receptors," *Briefings in Bioinformatics*, vol. 23, no. 5, Article ID bbac308, 2022.

[111] P. Durai, Y. J. Ko, J. C. Kim, C. H. Pan, and K. Park, "Identification of tyrosinase inhibitors and their structure-activity relationships via evolutionary chemical binding

similarity and structure-based methods," *Molecules*, vol. 26, no. 3, p. 566, 2021.

[112] P. Chandra, R. Singh, and P. K. Arora, "Microbial lipases and their industrial applications: a comprehensive review," *Microbial Cell Factories*, vol. 19, no. 1, p. 169, 2020.

[113] M. F. Shahraki, F. F. Atanaki, S. Ariaeenejad et al., "A computational learning paradigm to targeted discovery of biocatalysts from metagenomic data: a case study of lipase identification," *Biotechnology and Bioengineering*, vol. 119, no. 4, pp. 1115–1128, 2022.

[114] Q. Li, J. Lu, G. Zhang et al., "CRISPR/Cas9-Mediated multiplexed genome editing in Aspergillus oryzae," *Journal of Fungi*, vol. 9, no. 1, p. 109, 2023.

[115] B. Song, J. Li, D. Meng, Y. Zhao, and J. Zhang, "Attenuating the triacylglycerol catabolism enhanced lipid production of Rhodotorula strain U13N3," *Applied Microbiology and Biotechnology*, vol. 107, no. 4, pp. 1491–1501, 2023.

[116] J. Rath, "Safety and security risks of CRISPR/Cas9," *Ethics Dumping: Case Studies from North-South Research Collaborations*, vol. 16, pp. 107–113, 2018.

[117] A. D. Nahmad, E. Reuveni, E. Goldschmidt et al., "Frequent aneuploidy in primary human T cells after CRISPR–Cas9 cleavage," *Nature Biotechnology*, vol. 40, no. 12, pp. 1807–1813, 2022.

[118] D. Cyranoski, "What CRISPR-baby prison sentences mean for research," *Nature*, vol. 577, no. 7789, pp. 154-155, 2020.