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Bioprocess technologies for production of structured lipids as nutraceuticals

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9.1 Introduction

9.1.1 Lipids as nutraceuticals

Lipids are important components of a balanced diet, since they provide energy, essential fatty acids, liposoluble vitamins (A, D, E, and K), and other micronutrients. Nowadays, lipids are not only considered as components to satisfy hunger, prevent diet-deficiency diseases, or to provide the essential nutrition for body maintenance and/or tissues growth and repair. They can have an important proactive role in health, preventing several aging-related chronic diseases, and promoting both physical and mental health and wellness. Thus, several lipids can be considered as functional foods and/or nutraceuticals [1].

The category of "Foods for Specified Health Use" (FOSHU) was first legislated in 1991, in Japan, after a decade on systematic studies on food functionality. These foods must demonstrate a positive influence in physiological functions. The concerted action on "Functional Food Science in Europe" (FUFOSE), created by the European Commission in the 1990s, defined functional food as "a food that beneficially affects one or more target functions in the body beyond adequate nutritional effects in a way that is relevant to either an improved state of health and well-being and/or reduction of risk of disease" [2]. Thus, functional foods are consumed as conventional foods, incorporated in a normal diet. However, this concept of "functional food" is sometimes in contrast with the concept of "nutraceutical." The concept of nutraceutical is less clear because it can be described differently. Nutraceuticals have been defined as the products obtained from foods presenting demonstrated

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physiological benefits and/or protection against chronic diseases, which are sold in medical forms (e.g., capsule, solution, or potion) and used in higher dosages than those obtained from a normal diet [1,3]. In Canada, nutraceuticals are sold in medical forms not associated with foods and referred as "natural health products" [4,5]. In MedicineNet, nutraceutical is defined as "a food or part of a food that allegedly provides medical or health benefits, including the prevention and treatment of disease. A nutraceutical may be a naturally nutrient-rich or medicinally active food, such as garlic or soybeans, or it may be a specific component of a food, such as the omega-3 fish oil that can be derived from salmon and other cold-water fish" [Shield Jr., W.C.; https://www.medicinenet.com/script/main/art.asp?articlekey = 9474].

Considering these definitions, the European Union stated that both functional foods and food supplements can be considered as nutraceuticals because they are obtained from natural sources [5]. Functional foods represent a market opportunity. Formulating foods for health is one of the top trends in the food industry. In addition to these new foods, functional foods can include traditional foods which have benefits for health that have been recognized [1].

9.1.2 Definition and types of structured lipids

In a broad sense, structured lipid (SL) is defined as tailormade fats and oils with improved functional, technological, and/or pharmaceutical properties. These modified lipids, which do not exist in nature, are obtained either chemically or enzymatically by changing the composition and/or positional distribution of fatty acids in the glycerol backbone of acylglycerols. The SL comprise restructured triacylglycerols (TAG) or phospholipids (SPL) and, more recently, partial acylglycerols (diacylglycerols, DAG, and monoacylglycerols, MAG) and phenolic lipids [6–8]. Over the past few years, several SLs have gained interest in the food industry, namely: (1) human milk fat substitutes (HMFS), (2) low-calorie TAG, (3) cocoa butter equivalents (CBE), (4) *trans*-free plastic fats, and (5) enriched TAG [6–14]. Some of their characteristics and functional properties are summarized in Table 9–1.

The increasing concern about healthy lifestyles has driven the food industry towards innovation, in order to meet consumer demands. Recently, oleogels (also called "physically structured oils") have gained interest as SLs, due to their versatility and cost-effective production [14–16]. Oleogels are gels in which the continuous phase is an oil structured by oleogelators (e.g., phytosterols, phospholipids, vegetable oils, and partial acylglycerols) to mimic solid-like properties. Oleogels may be produced to fulfill specific requirements, namely to incorporate specific fatty acids, to reduce saturated fat content, control cholesterol levels, or carry bioactive compounds to be absorbed in the gastrointestinal tract [16].

9.2 General aspects of lipids: definition, structure, and properties

The rationale of SL production is based on the chemical and physical properties of natural lipids and on the knowledge of how they are related to their nutritional and biological properties. Fats and oils may be attractive due to their appearance, aroma, flavor, and

Table 9–1 Main characteristics and functional properties of different types of structured lipids (see list of symbols at the end of the chapter).

Type of structured lipid	Composition	Functional properties
Human milk fat substitutes	1,3-Dioleoyl-2-palmitoylglycerol (OPO)	Increase calcium absorption; Prevent constipation
	TAG enriched with long-chain PUFA, mainly EPA, DHA, and ARA	Improve cognitive functions; Neurological system development; Immunological function; Visual acuity; Brain development
	TAG with high levels of medium-chain fatty acids (mostly C8:0, C10:0, and C12:0)	Increase lipid absorption; Rapid energy source
Low-calorie TAG	SLS—short-chain fatty acids at the external positions of glycerol backbone and long-chain fatty acid at the *sn*-2 positions	Prevent fat malabsorption; Control obesity
	MLM—medium-chain fatty acids (M) at the external positions and long-chain fatty acids (L) at the *sn*-2 positions	
	MMM type	
Cocoa butter equivalents	TAG with saturated fatty acids (C16:0 and C18:0), in the external positions, and a MUFA (C18:1) at position *sn*-2	Rheological and sensory properties of cocoa butter
Trans-free plastic fats	Low/zero *trans* fatty acid contentFatty acid composition is maintained by interesterification	Prevent cardiovascular diseases; Improve cholesterol levels; Prevent type II diabetes
Structured phospholipids	Phospholipids with MCFA, n-3 PUFA (mainly DHA and EPA) and CLA at position *sn*-1 or *sn*2	Improve cognitive functions; Prevent cardiovascular diseases; Reduce inflammatory processes; Improve cholesterol levels
Partial acylglycerols	Monoacylglycerols and diacylglycerols obtained by lipase-catalyzed glycerolysis of TAG	Reduce obesity; Control total cholesterol, triacylglycerol, and glucose levels; Regulate appetite; Prevent fat malabsorption

texture, contributing to intensify and improve the organoleptic characteristics of the foods. The nutritional/physiological importance of lipids is based on their role as energetic molecules (37 kJ/g or 9 kcal/g triacylglycerol) and as a source of essential fatty acids, antioxidants, and vitamins. The physical, functional, nutritional, and organoleptic properties of a lipid are dependent, not only on its composition in fatty acids, but also on its positional distribution within the various TAG of oils and fats (*sn*-1,3 and *sn*-2) [17].

Lipids are a complex and chemically heterogeneous group of biomolecules, which have in common solubility in organic solvents, such as alcohols, ethers, and hydrocarbons, and high insolubility in water. Lipids can be divided into two main groups: the nonpolar lipids (acylglycerols, sterols, free fatty acids, hydrocarbons, alcohols, waxes, and steryl esters) and polar lipids (phosphoglycerides, glycosylglycerides, and sphingolipids) [18]. Lipids are also commonly

distinguished based on their physical state at room temperature. They are called oils when they are liquid at room temperature (20°C), and fats when they are solid or pasty at 20°C [19,20].

Based on their composition, they can be classified as simple or complex lipids. Simple lipids contain carbon, hydrogen, and oxygen, yielding fatty acids and an alcohol upon saponification. Simple lipids can be divided into TAG and waxes. Animal and vegetable oils and fats contain more than 95% TAG. TAG are esters formed by a glycerol molecule to which three fatty acids are esterified at different positions. These positions are represented by a stereospecific numbering system (*sn*): *sn*-1 (first external position), *sn*-2 (middle position), and *sn*-3 (third external position) [21]. In addition, oils and fats also contain monoacylglycerols (MAG), diacylglycerols (DAG), and free fatty acids (FFA), in much smaller amounts. MAG and DAG are important compounds for their emulsifying capacity [20].

Complex lipids contain one or more additional elements, such as phosphorus, nitrogen, and sulfur, yielding fatty acids, alcohol, and other compounds by saponification. Complex lipids are classified as phospholipids or glycolipids. Phospholipids are composed of fatty acids and a phosphate group. Glycerol-based lipids called phosphoglycerides contain glycerol, two fatty acids, and a phosphate group. The phosphoglyceride structure contains a hydrophilic (polar) head, the phosphate unit, and two hydrophobic (nonpolar) fatty acid tails. The polar head can interact strongly with water, while the nonpolar tails interact strongly with organic solvents. These structures generate three or more different compounds when hydrolyzed [19]. Glycolipids differ from phospholipids since they possess a sugar group in place of the phosphate group. Their structure is again the polar head and dual tail arrangement in which the sugar is the hydrophilic unit.

Moreover, small amounts of nonacylglycerol components are found in all oils and fats. Some of these components (e.g., phosphatides, sterols, waxes, insoluble hydrocarbons, pigments, tocopherols, lactones, and methyl ketones) are partially or completely removed during the refining process to make oils and fats edible. The fatty acids in TAG differ in the length of the chain, generally from 4 to 24 carbon atoms, and in the number of double bonds, from 0 (saturated fatty acid) to 6 (1: monounsaturated; >1: polyunsaturated fatty acid, PUFA). Since the rotation around the double bond is severely restricted, two types of isomers appear: *cis* and *trans*. Normally in nature, fatty acids are in *cis* form, although they can also exist in *trans* form. Industrial processing operations, such as refining and hydrogenation, can cause the change of *cis* isomers to the *trans* form. *Trans* isomers are undesirable as their presence has been related to cardiovascular diseases by a mechanism that lowers the HDL-cholesterol [22]. One of the objectives of the food industry is to reduce this form to the lowest level in processed fats and oils.

Regarding the chain length, fatty acids can be classified as short-chain fatty acids, when they have between two to six carbon atoms, medium-chain fatty acids (MCFAs), when they have between eight and 12 carbon atoms, and long-chain fatty acids (LCFAs) if they have between 14 and 24 carbons in their constitution, regardless of the degree of unsaturation [6]. The most common fatty acids in oils and fats are palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1), and linoleic (C18:2) acids. All vegetable oils contain at least four major fatty acids, which theoretically can give rise to 40 different combinations in the three positions of the glycerol molecule, that is, 40 TAG with different chemical and physical properties [20]. However, in nature, fatty acid esterification to glycerol backbone in TAG does not occur at random. Vegetable oils and fats

show a predominance of unsaturated fatty acids in the internal position of TAG, while saturated FA are mainly esterified at positions *sn*-1,3.

The physical properties, such as the melting point, the crystallization characteristics, the crystalline structure, and polymorphism of a fat, are very important parameters to predict and prevent undesirable changes during product processing and storage [23]. The melting point of a fat is the temperature at which, at atmospheric pressure, the melting of the lipid crystals begins [24]. It increases as the length of the fatty acid chain increases and is higher for saturated fatty acids than for unsaturated fatty acids with an equal number of carbon atoms [20].

TAG tend to crystallize in different ways, depending on the chemical composition of the fat phase and the cooling conditions that exist during crystallization. The distribution of FA in TAG will influence the physical properties of the fats [25]. In addition, it is also important to know its polymorphic behavior in relation to crystallization [23]. The polymorphic behavior is related to the way the crystals are organized, which may present greater or lesser stability. The three main forms that crystals can acquire are: α, β', and β, in increasing order of stability [26]. The polymorphic form α (crystals of the hexagonal system) has poorly ordered chains in space, is unstable, and has a low melting point. The α form appears in the initial phase of the crystallization process due to the sudden cooling of the fat, but quickly evolves into the β' and β forms. The β is the most stable form, with the highest melting point and latent heat. β crystals are of the triclinic system and due to their larger dimensions, they are responsible for a sandy and coarse texture of the crystallized fat. The β' form corresponds to orthorhombic twin-crystals responsible for a pleasant texture and mouthfeel of a fat [27].

9.3 The role of lipids in human nutrition

9.3.1 Functional/nutritional properties of lipids

Vegetable oils and fats, in adequate quantities and in a balanced diet, are essential for the human body. This is due to the need to consume some fatty acids that the organism does not produce in sufficient amounts for its needs. Omega-6 linoleic acid (C18:2 omega-6) and omega-3 linolenic acid (C18:3 omega-3) are considered essential fatty acids because they are not synthesized by the human body and they are absolutely necessary for human health. Thus, these FA must be included in the diet [9].

Animal organisms including humans can convert linoleic acid to arachidonic (C20:4 omega-6) and α-linolenic acid to longer chain omega-3 fatty acids such as eicosapentaenoic acid (EPA; C20:5 omega-3) and docosahexaenoic acid (DHA, C22:6 omega-3). EPA and DHA are omega-3 fatty acids found mainly in marine oils, with high benefits for human health. These fatty acids are important in the prevention and treatment of cardiovascular diseases, regulation of blood pressure, and the inflammation process [2]. DHA has proved to be very important in childhood development of the brain and nervous system and its normal functioning in adulthood [28,29].

Among the LCFAs, conjugated linoleic acid (CLA) and gamma-linolenic acid (GLA) also present important nutritional, physiological, and/or physical properties [9]. CLA, which is present in the milk and meat of ruminants, consists of a mixture of geometric and positional isomers of linoleic acid that contain conjugated double bonds in its carbon chain. Its intake has shown

anticarcinogenic, antioxidant, antiatherosclerotic, antidiabetic, antiobesity, and improved immunity effects [30]. GLA is present in the oil seeds from plants such as *Borago officinalis* L. and *Oenothera biennis* L. It has recognized antitumoral effects and is a precursor to prostaglandin 1 and 15-hydroxy di-homo gamma linolenic acid, which have antiinflammatory and antithrombotic properties [31].

The importance shown by PUFA in human health has led to the establishment of recommended daily intake doses for these compounds [9]. Also, several studies have shown the importance of the omega-6 FA/omega-3 FA ratio in the diet. Western food is deficient in omega-3 FA and has an excess intake of omega-6 FA, considering the genetic pattern and sedentary lifestyle. This situation promotes an increase in cardiovascular diseases, cancers, autoimmune and antiinflammatory diseases, and obesity [32,33]. A MUFA-rich diet shows recognized health benefits namely antiatherogenic and antithrombotic potential, increases the HDL/LDL cholesterol ratio, decreases oxidized LDL and total cholesterol, and reduces platelet aggregation [34]. The replacement of saturated fats by oils rich in oleic acid has benefits in the prevention of cardiovascular diseases and improves insulin sensitivity [35]. Olive oil, containing 55%−83% oleic acid, has been shown to have anticancer effects [36] and to modulate the inflammatory response [37]. Monounsaturated oils can also be beneficial in terms of weight control strategy since they induce a reduction in postprandial triglyceridemic compared to the consumption of saturated fats [38].

Lipids also contribute to better absorption of fat-soluble vitamins (A, D, E, and K), and are essential for human growth and development. However, the excessive intake of lipids also presents negative aspects, causing obesity problems due to their high caloric value. In addition, there is evidence that high-fat diets may increase the risk of colon cancer and cardiovascular diseases [20]. Excessive intake of animal fats, consisting essentially of saturated fatty acids, may cause problems with cholesterol accumulation in the arteries and consequently coronary problems. The ingestion of fats of vegetable origin, rich in unsaturated fatty acids, and oils from vegetable or marine animal origin, rich in omega-3 PUFA, promotes a decrease in the level of cholesterol.

9.3.2 Absorption and lipid metabolism

The composition of fatty acids, their position in the acylglycerol backbone and their physical and biochemical properties result in different physiological properties, demonstrated in terms of absorption in the gastrointestinal tract and in the metabolic process [6]. These characteristics are directly related to coronary diseases, to energy balance, insulin sensitivity, and postprandial metabolism with a subsequent impact on human health [21]. The first step in the digestion of fats occurs in the stomach and is catalyzed by lingual or gastric lipase. The main digestion products of this gastric phase are diacylglycerols and free fatty acids, which facilitate the intestinal digestion phase [39]. In the duodenum region, the hydrolysis is catalyzed by the pancreatic lipase (*sn*-1,3 regioselective) generating free fatty acids, and *sn*-2 monoacylglycerols (*sn*-2 MAG), which form micelles with the bile salts [40]. When these micelles, also containing phospholipids, approach the apical side of the intestinal epithelial cells, they release their content and enable the absorption of nonpolar lipids in the microvilli membrane. The absorbed lipids are reesterified to reform TAG in the smooth endoplasmic reticulum [41]. The rate of hydrolysis reaction carried out by the pancreatic lipase depends on the chain length and degree of unsaturation of the FA present in the *sn*-1 and *sn*-3 positions [6].

In nature, vegetable oils and some animal fats present mono- and polyunsaturated fatty acids (MUFAs or PUFAs) mainly esterified at position *sn*-2 of TAG. This profile promotes the absorption of these FAs in the *sn*-2 position, some of them essential for humans, such as *sn*-2 MAG, by the mucosal membrane, in the form of micelles with bile salts, avoiding FA deficiency [42]. Short-chain fatty acids are more quickly absorbed in the stomach than other fatty acids, due to their high volatility, water-solubility, and low molecular weight. As such, they are suitable for controlling obesity due to their low caloric value: acetic acid (C2:0), 3.5 kcal/g; butyric acid (C4:0), 6.0 kcal/g; and caproic acid (C6:0), 7.5 kcal/g [43].

MCFAs can be absorbed in the stomach, after hydrolysis by the gastric lipase [44], and can also be solubilized in the aqueous phase of the intestinal content, where they weakly bind to albumin and are transported to the liver through the portal vein [45]. About 80%−100% of the MCFAs present in the entire portal flow is captured by the liver and the remaining portion goes through the bloodstream, becoming available to peripheral tissues. Unlike LCFAs, they are not significantly incorporated into lipoproteins (chylomicrons and VLDL—very low-density lipoproteins), allowing them to be absorbed directly into the bloodstream. The rate of absorption in the intestines of MCFAs is similar to that of glucose and these are quickly oxidized in the liver and used by the body. As they do not undergo significant reesterification in TAG, they do not accumulate in adipose tissue, which allows obesity control [46]. For these reasons, MCFAs have been used as a quick source of energy in some metabolic syndromes, such as pancreatic enzyme deficiency (cystic fibrosis) [47]. However, since MCFAs are saturated fatty acids, their ingestion promotes the increase of blood cholesterol and should not be exclusive in the diet [46].

The digestive bioavailability of medium-chain TAG is greater than that of long-chain TAG. The hydrolysis of medium-chain TAG starts in the stomach more quickly and its absorption is faster and more efficient [6].

The high hydrophobicity resulting from the long hydrocarbon chains prevents the absorption and direct transport of LCFAs. These fatty acids leave the intestine in the form of TAG, via the lymphatic route, after incorporation into chylomicrons (formed by TAG, phospholipids, cholesterol, and apoproteins) and are finally secreted into the bloodstream [48]. A fraction of these chylomicrons undergoes intravascular hydrolysis, releasing most of LCFAs to extrahepatic tissues, while the remaining fraction is transported to the liver. LCFAs reach this organ as fatty acids linked to albumin or in the form of TAG. All FAs use the two transport systems in varying proportions. The longer the carbon chain of FA, the more it is found in the lymph and less in the portal blood. In the lymph, LCFAs circulate as TAG associated with chylomicrons. In portal blood, FAs are linked to albumin [49].

9.4 Production of structured lipids

SLs can be produced either by chemical- or enzyme-catalyzed reactions, namely by acidolysis, interesterification (ester-interchange), transesterification (alcoholysis), or direct esterification [8]. Chemical-catalyzed reactions of oils and fats are currently carried out at high temperatures, under reduced pressure. Due to the lack of selectivity of chemical catalysts, the final products

are contaminated by side-products with a subsequent decrease in product yield, the residual catalysts must be removed, and pollutant effluents are formed. Thus, products must be purified, increasing operation costs [50]. The replacement of chemical-catalyzed by enzyme-catalyzed processes, which are recognized as natural, is highly desirable. This is mainly due to the selectivity of biocatalysts and the mild reaction conditions used, resulting in highly pure products, higher yields, and environmentally friendly processes.

SLs production by enzyme-catalyzed processes has gained increased interest from the scientific community during the last 20 years. In fact, a search made in Scopus for "structured lipids and lipases" accounted for 517 documents up to the end of July 2021, showing the first publications in the late 1980s (accessed July 31, 2021).

9.4.1 Lipases and phospholipases

Lipases (acylglycerol acyl-hydrolases, EC 3.1.1.3.), phospholipases A1 (EC 3.1.1.32), and phospholipases A2 (EC 3.1.1.4), from microbial, animal, or plant origin, have been used to produce SLs [7,8,51]. Lipases and phospholipases are versatile biocatalysts that do not need cofactors and accept a wide variety of substrates. These enzymes are hydrolases that in aqueous media catalyze the hydrolysis of acylglycerols or phospholipids, respectively. Both lipases and phospholipases exhibit an interfacial activation kinetics [52–54]. It means that lipase and phospholipase activity is highly activated when the substrate concentration is higher than its critical micellar concentration and a lipid–water interface is formed (e.g., micelles, monomolecular or bimolecular layers of acylglycerols, or phospholipids).

These enzymes are also active in nonaqueous media at low water activity (a_w), where they can catalyze esterification, interesterification, alcoholysis, and acidolysis reactions, among others [55]. Particularly important is the selectivity exhibited by several lipases, either in aqueous or in organic media. They can be selective toward (1) the class of lipids (e.g., TAG, DAG, MAG, fatty acids methyl and ethyl esters); (2) the position of the fatty acids in acylglycerols (*sn*-1,3 regioselectivity; *sn*-2 regioselective lipases are not available from nature); (3) saturated, mono- or polyunsaturated fatty acids; (4) to an optical isomer; or (5) some combination of these situations [56]. The selectivity of each lipase can be affected by the water activity of the reaction medium and by the immobilization support used [57].

In the production of SLs, the use of an *sn*-1,3 regioselective lipase is of utmost importance to produce novel lipids with functional and/or nutraceutical properties that cannot be obtained by chemical catalysis. The *sn*-1,3 regioselective lipases will maintain the original fatty acids at the *sn*-2 position of TAG, since these lipases can only hydrolyze the *sn*-1,3 ester bonds. This is nutritionally desirable for faster absorption of the fatty acids at the internal position of the acylglycerols, in the form of *sn*-2 MAG. However, even using *sn*-1,3 regioselective lipases as biocatalyst, spontaneous acyl migration will occur, and undesirable TAG products will be obtained [7]. Thus, to minimize the extent of this undesirable side reaction, the following parameters must be controlled: reaction temperature, biocatalyst load, type of immobilization support, water content and water activity, solvent type, and reaction system [58]. When nonregioselective lipases are used, the obtained SLs are similar to those produced by chemical catalysis.

9.4.2 Biocatalyst immobilization

The commercial price of lipases and phospholipases is still higher than that of inorganic catalysts, which has been a constraint to the industrial scale-up of lipase-catalyzed processes in the food industry. The use of immobilized biocatalysts for SL production is especially important to decrease biocatalyst costs, by reusing it in successive batches or using it in continuous bioreactors. Cost reduction is particularly important to produce SLs such as interesterified fats with improved rheological properties or fats enriched in specific fatty acids, to be used as commodity fats. These SLs can only be industrially produced if the enzymatic processes are economically feasible.

The immobilized lipases must present both high specific activity and operational stability. In general, porous immobilization supports are preferred over nonporous supports since they allow the immobilization of higher amounts of enzyme molecules (at the surface and inside the porous matrix), intensifying the catalytic processes. Through the correct choice of the immobilization support, it is possible to create a microenvironment adequate to protect the enzyme and promote its activity and operational stability. Thus, the optimal support should foster the following conditions in the microenvironment: (1) high, but not inhibitory concentrations of substrates; (2) low product concentration to displace reaction equilibrium toward the synthesis; (3) low water activity, to favor the reactions of synthesis and avoid the reverse reaction of hydrolysis; and (4) protection of the biocatalyst against the toxicity effects of organic solvents and other inhibitory molecules (e.g., oxidation products, free fatty acids, pigments, phospholipids, and lipid polymers) [59,60].

However, during the immobilization process, stereochemical, and conformational modifications of the enzyme may occur, leading to a considerable decrease in the reaction rate. Also, internal diffusion and partition effects occur when porous supports are used. Mass transfer within solid particles, that is, the entrance of the substrates and the removal of the product away from the site of the reaction, only occur by molecular diffusion. Thus, the overall reaction rate can be significantly reduced if diffusion is slow, that is, under a diffusion-controlled reaction [61]. Partition effects between the bulk and the microenvironment of the enzyme can be estimated by applying the concept of partition coefficient between two immiscible liquid phases, K, adapted by Fukui et al. [62] to the system immobilization support/bulk medium, as follows:

$$K = \frac{C_{As}}{C_{Ab}} \tag{9-1}$$

where C_{AS} is the equilibrium concentration of component A in the support S (microenvironment) and C_{Ab} is the concentration of A in the bulk medium (macroenvironment), at equilibrium. The initial concentration of the component A in the reaction medium (C_{A0}) and its concentration in the reaction medium, C_{Ab}, after the addition of the support, the migration of A to the support until the concentration equilibrium is attained, can be easily quantified. Thus, considering the initial volume of the bulk medium, V_0, and the total volume of the system after addition of the support, V, the real volume of the immobilization support is $(V - V_0)$. Therefore, at equilibrium, K can be given by:

$$K = \left[\frac{C_{A0} - C_{Ab}}{C_{Ab}}\right] \times \left[\frac{V_0}{(V - V_0)}\right] \tag{9-2}$$

If $K > 1$, the component A shows higher affinity for the support than for the bulk medium and tends to enter in it. When $K \ll 1$, the compound A has low affinity for the support and hardly enters in it. Thus, in an ideal biocatalytic system, substrates must have $K \gg 1$, while products must have $K \ll 1$.

Several organic and inorganic supports have been tested for lipase immobilization with quite encouraging results in terms of operational stability and retention of activity. Hydrophobic supports have been preferred for lipase immobilization because they promote the entrance of the hydrophobic substrates into the pores of the matrix. However, the composition of the microenvironment must be optimized for each reaction system to avoid high inhibitory substrate concentrations near the enzyme [63]. The interactions of the immobilization support with the enzyme and medium components are important aspects on the optimization of the microenvironment of the catalyst and, therefore, on the reaction rate and yield.

9.4.3 Reaction systems

The majority of SLs are obtained by lipase-catalyzed (1) acidolysis of a pure TAG, oil, or fat with FFA (omega-3 PUFA, MCFA, LCFA), (2) interesterification of blends of TAG, fats, and oils, or (3) interesterification of a TAG, fat, or oil with fatty acid ethyl or methyl esters [8]. Structured phospholipids (SPL) can be obtained by (1) acidolysis of phospholipids with omega-3 PUFA, MCFA, LCFA, or (2) interesterification of phospholipids with FA ethyl or methyl esters, using immobilized lipases or phospholipases A1 or A2 (PLA 1 and PLA2, respectively). PLA1 and PLA2 can act at s*n*-1 and *sn*-2 positions of phospholipids, respectively [51].

The use of ethyl esters over methyl esters as acyl donors is preferred due to the toxicity of methanol released from the reactions [64]. Also, ethyl esters, being more volatile than FFA, are more easily recovered from the reaction media by distillation, decreasing downstream processing costs. The mechanisms of lipase-catalyzed interesterification, acidolysis, and alcoholysis reactions consist of hydrolysis of ester bonds in acylglycerols followed by reesterification [65]. Therefore, the optimization of these reactions results from a balance between the rates of hydrolytic and esterification reactions, which are reversible reactions. In the presence of excess water (high a_w), the equilibrium is displaced toward the hydrolysis step. Conversely, when low water amounts are present, the global reaction is shifted toward reesterification, which is desirable to achieve higher product yield. The product yield under reaction equilibrium is decided by the substrate ratio [58,66].

The choice of the adequate lipase (or phospholipase) for the synthesis of a specific SL will also depend on the substrates used and on the reaction conditions. With oils as substrates, which are liquid at 20°C, the preferred system consists of a blend of substrates in appropriate amounts (solvent-free media) and a mesophilic lipase (or phospholipase) as biocatalyst. If a fat is used as substrate, two options are possible: to solubilize the fat in an organic solvent and use a lipase (or phospholipase) from a mesophilic organism (reaction in organic solvent medium); or to use higher reaction temperature to melt the substrates and use a thermophilic lipase (or phospholipase) as biocatalyst in a solvent-free medium.

The choice of an organic solvent to be used as a continuous phase of the reaction medium, must consider physicochemical (e.g., solubility of substrates/products, density,

vapor pressure, melting and boiling points, viscosity, and superficial tension), biologic (toxicity for the biocatalyst), safety (toxicity for humans and flammability), logistic (availability, disposal of residues), and economic (cost) issues. Among them, the toxicity of the solvent for the biocatalyst is one of the most important parameters for the success of the biocatalysis.

The sensitivity to the nature of organic solvents varies with the lipase. Water is essential to the enzyme activity since it is responsible for the maintenance of native, catalytically active conformation of the enzyme, due to its role in the formation of hydrogen bonding and in van der Waals interactions. Zaks and Klibanov [67] demonstrated that the water monolayer bound to the enzyme corresponds to the essential water to maintain a lipase active in nearly anhydrous organic media. When a hydrophilic organic solvent is used, the water will be partitioned between the solvent and the enzyme, stripping the water monolayer around the enzyme, with subsequent loss of its catalytic activity. On the contrary, hydrophobic solvents are immiscible with water and will not strip the essential water from the enzymes. Thus, in enzyme-catalyzed processes in organic solvent, the interaction of the solvent with the enzyme-bound water is the main factor to consider for the success of the reaction [67].

Laane et al. [68] proposed the use of the Hansch parameter (log P) to assess solvent biocompatibility. Log P is related with solvent hydrophobicity. It is defined as the logarithm of the partition coefficient of the solvent in a standard biphasic system *n*-octanol/water. A sigmoid-shaped relationship between enzyme activity and log P value of the solvent has been observed. As a rule, biocompatible solvents have a log P higher than 4 (hydrophobic solvents); solvents with log P lower than 2 (hydrophilic solvents) will cause a strong inactivation of the biocatalyst, while the effect of solvents with a log P between 2 and 4 depends on the biocatalyst [68].

In the food industry, only food-grade organic solvents can be used. In this context, *n*-hexane is currently used in lipase-catalyzed reaction systems, despite presenting a log P value of 3.5. However, solvent-free systems are recommended for the food industry, due to solvent toxicity and negative effects on the environment and on enzyme activity and operational stability. In addition, the complexity of the system with organic solvents increases, as well as costs related with solvent acquisition and recovery and product purification.

9.4.4 Case studies on structured lipids production

In this section, several examples of SL production are presented: those where the original fatty acid composition is modified by incorporation of new fatty acids (e.g., dietetic low-calorie SL, HMFSs, and TAG enriched in specific fatty acids with health benefits) and fat blends where the original position of fatty acids is changed to reach specific rheological/functional properties.

9.4.4.1 Low-calorie triacylglycerols

Low-calorie TAG provide health and nutrition benefits by controlling fat malabsorption, obesity, and other metabolic disorders, due to their lower caloric density ($\sim$5 kcal/g) in comparison with conventional fats and oils (9 kcal/g). These SLs contain short- (S) or medium- (M) chain fatty acids, preferably esterified at external *sn*-1,3 positions, and long- (L) chain fatty acids esterified at the *sn*-2 position of the glycerol backbone [12,14]. Over the past few years,

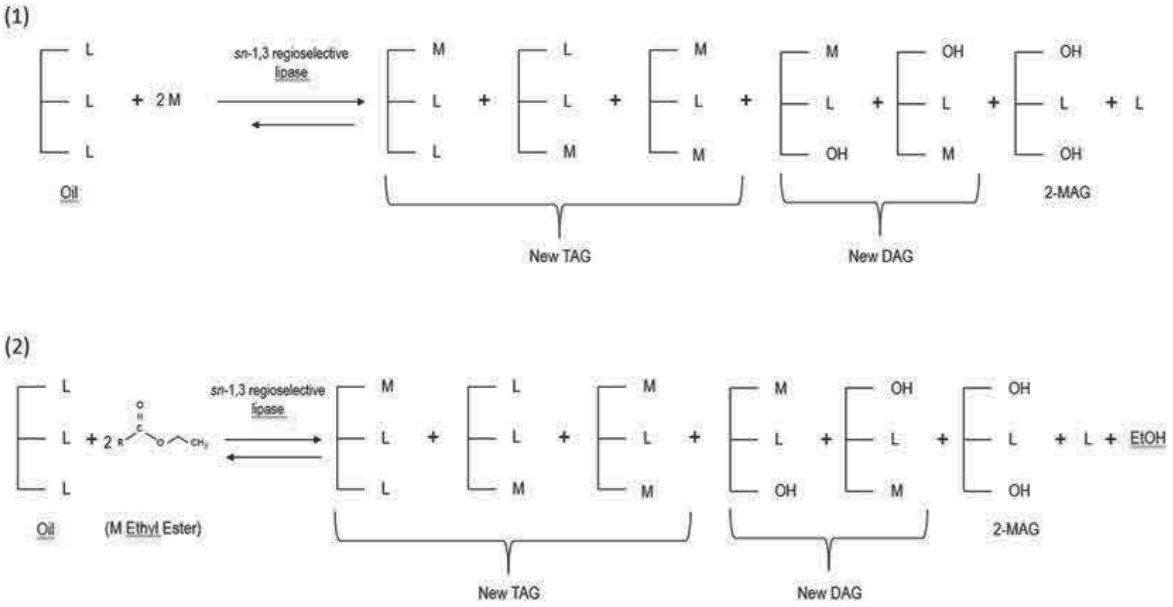

FIGURE 9–1 Schematic (1) acidolysis of a long-chain TAG (LLL) with medium-chain (M) fatty acids and (2) interesterification of a long-chain TAG (LLL) with medium-chain fatty acid ethyl ester, catalyzed by a *sn*-1,3 regioselective lipase (the L at position *sn*-2 is maintained) to produce low-calorie TAG (New TAG).

special interest has been devoted to MLM-type structured TAG. During digestion, medium-chain FA esterified at the *sn*-1,3 positions are released from TAG, by the action of pancreatic lipase, and transported to the liver where they are rapidly metabolized, providing a rapid source of energy [12,69]. The production of MLM SLs is usually carried out by (1) the acidolysis reaction between a TAG source rich in LCFAs (such as olive, olive pomace, grapeseed, avocado, soybean, and nut oils) and MCFAs, namely caprylic (C8:0) or capric (C10:0) acids or (2) interesterification of an oil with MCFA ethyl or methyl esters [12,69–74] (Fig. 9–1). Two-step reactions have also been implemented aiming at increasing structured TAG yield. A possible reaction scheme combines an alcoholysis, to get 2-MAG, followed by acidolysis between 2-MAG and MCFAs. Another approach uses esterification between glycerol and PUFA and the reaction product undergoes acidolysis with MCFAs to obtain MLM-type TAGs [12].

Since medium-chain FAs have melting points lower than 35°C, reactions can be carried out at near-room temperature, using mesophilic *sn*-1,3 regioselective lipases, in the absence of a solvent. Recent examples of low-calorie TAG produced either by acidolysis and interesterification reactions are presented in Table 9–2. Several SLs with low-calorie values have been commercialized, namely SALATRIM (Nabisco, United States), Olestra and Caprenin (both produced by Procter and Gambler, United States) [69].

9.4.4.2 Human milk fat substitutes

Human milk fat (HMF) is one of the main sources of nutrients and energy for the newborn, comprising TAG (98%–99%), phospholipids (0.26%–0.80%), sterols (0.25%–0.34%, mainly cholesterol), and trace amounts of partial acylglycerols (DAG and MAG) and free fatty acids

Table 9–2 Examples of MLM-type structured lipid production by batch acidolysis or interesterification (see list of symbols at the end of the chapter).

Reaction	Biocatalyst	Substrates	References
Acidolysis	ROL immobilized in MNP	Crude oils from spent coffee grounds and olive pomace + C10:0	[74]
	Lipozyme TL IM		[74]
	Novozym 435,	ARA-rich single cell oil from *Mortierella alpine* + capric acid	[73]
	Lipozyme 435		[73]
	Lipozyme TL IM		[73]
	Lipozyme RM IM		[73]
	NS 40086		[73]
	Lipozyme TL IM	Grapeseed oil + C8:0	[72]
	Lipozyme RM IM	Grapeseed oil + C10:0	[72]
	Novozym 435		[72]
	rROL in Amberlite IRA 96	Grapeseed oil + C8:0	[71]
	CPL self-immobilized in papaya latex	Grapeseed oil + C10:0	[71]
Interesterification	ROL immobilized in MNP	Crude spent coffee grounds oil + C10:0 ethyl ester	[74]
	Lipozyme TL IM	Crude olive pomace oil + C10:0 ethyl ester	[74]
	Lipozyme RM IM	Fish oil + C10:0 methyl ester	[75]
		Fish oil + medium-chain TAG	[75]

[76]. The FA composition of HMF is highly dependent on the mother's diet, on the time and frequency of breastfeeding, as well as on psychological and environmental factors that may affect breastfeeding practices. A total of 54 different fatty acids have been reported for HMF composition, with the following being the most abundant: palmitic (C16:0, 18%−25%), oleic (C18:1; 24%−39%), linoleic (C18:2 *n*-6; 8%−18%), linolenic (C18:3 *n*-3; 0.4%−2%), lauric (C12:0; 4%−14%), myristic (C14:0; 3%−12%), stearic (C18:0; 5%−8%), and capric (C10:0; 1, 5%−2.5%). Long-chain PUFA, such as arachidonic acid (C20:4 omega-6) and DHA (C22:6 omega-3), account for 0.1%−0.5% of total FA in mature milk [77].

The fatty acid distribution in HMF has a unique structure, characterized by the predominance of TAG containing unsaturated FAs in the *sn*-1 and *sn*-3 positions (c. 80%−90% of oleic acid) and with saturated FAs (about 60%−70% of palmitic acid) in the internal position, conversely to that observed in other natural fats. This structure is crucial for the efficient absorption of palmitic acid as monoacylpalmitate [78].

In infant formula, produced with vegetable oils or ruminant milk, palmitic acid and the remaining saturated FAs are predominantly esterified at the *sn*-1 and *sn*-3 positions of the TAG molecules. Saturated fatty acids, released by the action of the *sn*-1,3 regioselective lipase, may form insoluble calcium complexes, known as calcium soaps, which contribute to the poor absorption of calcium and FAs by children fed with infant formulas. This is also one of the causes of constipation in the first months of life. It is worth noting that, unlike free palmitic acid, monoacylglycerol *sn*-2-monopalmitate is efficiently absorbed [79−82].

The production of HMFS, to incorporate in infant formula, mimics the rather unique structure of HMF and has been extensively investigated over recent years [11,13]. The synthesis of HMFS may be attained by lipase-catalyzed acidolysis or interesterification reactions, performed batchwise or in continuous mode, either in solvent or solvent-free media (Fig. 9−2).

Both reaction systems require a TAG source rich in palmitic acid at *sn*-2 position, and *sn*-1,3 regioselective lipases as biocatalysts. Tripalmitin, palm stearin, palm oil, fractionated palm stearin, butterfat, and lard have been used as suitable substrates. Lard is a fat of low commercial value with a TAG structure very similar to that of human milk. However, it has a lower content of long-chain PUFA, namely linoleic and linolenic acids [83]. Nevertheless, ethical, religious, and cultural issues can be an obstacle to its use in the production of HMFS. In laboratory studies, tripalmitin is often used, but its implementation on a large scale is compromised owing to its high cost. Due to the high melting point of tripalmitin (66°C−68°C), and of the fats rich in palmitic acid at position *sn*-2, the production of HMFS in solvent-free media has to be performed at temperatures higher than the melting point of the reaction medium, using thermophilic *sn*-1,3 regioselective lipases. To produce HMFS at near room temperature, catalyzed by mesophilic lipases, the substrates must be solubilized in a biocompatible hydrophobic solvent (e.g., *n*-hexane).

In the acidolysis reaction, several substrates have been used as sources of FAs, namely vegetable oils (such as olive, sunflower, and hazelnut oils), MUFA, or omega-3 PUFA [83−86]. Blends of vegetable oils rich in long-chain FAs or methyl or ethyl esters (namely ethyl oleate) have been used as acyl donors in the interesterification reaction for HMFS production [66,87,88]. In order to obtain higher yields of structured TAG, two-step reactions have been carried out, using *sn*-1,3 regioselective lipases as catalyst. This multistep process comprises an alcoholysis to obtain

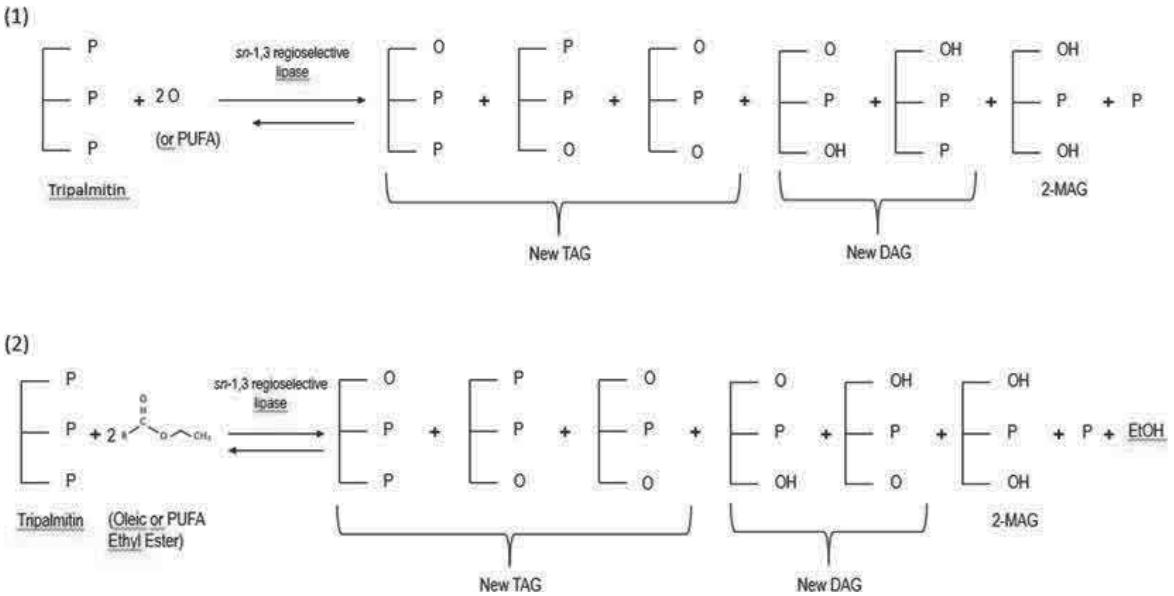

FIGURE 9–2 Schematic (1) acidolysis of tripalmitin (PPP) with oleic acid (O) or polyunsaturated fatty acids (PUFA) and (2) interesterification reaction of tripalmitin (PPP) with ethyl oleate or PUFA ethyl esters, catalyzed by a *sn*-1,3 regioselective lipase (the L at position *sn*-2 is maintained) to produce HMFS (new TAG).

Table 9–3 Examples of HMFS production by batch acidolysis or interesterification (see list of symbols at the end of the chapter).

Reaction	Biocatalyst	Substrates	References
Acidolysis	Lipozyme RM IM	Fungal oil (from *Mortierella alpina* ALK) + fractionated palm stearin + C18:1	[90]
	Novozym 435; Lipozyme 435; Lipozyme TL IM; Lipozyme RM IM	Microalgae oil from *Nannochloropsis oculata* rich in C16:0 at *sn*-2 position + FFA enriched in C18:2, SDA and DHA from the microalga *Isochrysis galbana*	[91]
	rROL; Lipozyme RM IM	PPP + FFA from camelina oil	[86]
	rROL in Accurel MP 1000; Novozym 435; Lipozyme TL IM; Lipozyme RM IM	Lard + omega-3 PUFA	[83]
Interesterification	Lipozyme RM IM, Lipozyme TL IM; NS 40086, DF Amano 15	Basa catfish oil (*Pangasisus haniltoa*) + coconut fat	[92]
	CpLip2 immobilized on Accurel MP 1000	PPP + ethyl oleate	[66]
	Lipozyme TL IM	Fractionated palm stearin + fish oil	[93]

sn-2-MAG rich in palmitic acid, followed by an esterification reaction between the purified *sn*-2-MAG and FFA [89]. Recent research on HMFS production is summarized in Table 9—3.

Nowadays, there are several HMFS produced by lipase-catalyzed reactions in the market. Betapol was the first product to reach the market, to mimic human milk fat for infant formula, developed by Bunge Loders Croklaan, using lipase-catalyzed processes (http://europe.bungeloders.com/applications/infant-nutrition/). More recently, other products such as Infat (Advanced Lipids, Sweden, Karlshamn), Alsoy (Nestlé), Cow & Gate Premium (Nutricia), and Bonamil (Wyeth Ayerst) are also commercialized [13].

9.4.4.3 *Interesterified* trans*-free fat blends and triacylglycerols rich in specific long-chain fatty acids*

The interesterification of fats and oils consists in the exchange of fatty acid moieties between the different TAG of oil or fat blends. This reaction is currently performed to obtain SLs with improved physical and functional properties (e.g., melting point, solid fat content, and crystallization pattern of fat blends) which is important for margarines and edible shortenings production [23]. In fact, the interesterified fat blends, obtained by chemical catalysis and currently used in the margarine industry, are SLs. In terms of physical properties, these interesterified fats may have a consistency ranging from creamy to hard and the texture must be smooth and not grainy.

By interesterification, ester-interchange occurs among TAG, without changing their original fatty acid composition (e.g., essential FAs and PUFA contents). Also, no *trans* fatty acids are produced, in contrast to what happens when the physical properties of fat blends are modified by hydrogenation [50]. The hydrogenation of vegetable oils destroys the unsaturated FAs and causes isomerization of nonhydrogenated *cis*-double bonds of unsaturated

vegetable oil into their *trans* form with recognized negative impact on human health (c.f. 9.3.). However, when chemical catalysts are used (e.g., sodium, sodium methoxide), ester interchange occurs at random and does not respect the original structure of natural TAG.

Enzyme-catalyzed interesterification has emerged in recent years as a possible alternative to chemical interesterification, due to the benefits related to the use of biocatalysts (c.f. 9.4.1). When a nonregioselective lipase is used, the ester interchange occurs at random and the obtained product is similar to that obtained by chemical interesterification. When an *sn*-1,3 regioselective lipase is used, the *sn*-2 FAs are preserved, which has absorption benefits, in addition to the advantages related to the use of enzymatic processes. Fig. 9−3 shows the schematic interesterification catalyzed by these two types of lipases.

A wide variety of raw materials has been used for the production of these interesterified fat blends with the desirable plastic properties: low-cost vegetable fats rich in saturated fatty acids (e.g., palm fat and palm stearin), fats rich in lauric acid (C12:0) (e.g., palm kernel and coconut fats), and polyunsaturated vegetable oils (e.g., sunflower and soybean oils). In solvent-free systems, when the reaction media contain considerable amounts of fats with high melting point, the reaction temperature must be high enough to melt the fat blends and thermostable lipases must be used. Otherwise, an organic solvent must be used in the reaction medium.

The incorporation of omega-3 PUFA, especially EPA and DHA, essential FA, or other PUFA with recognized benefits in human health, in food products more readily available for consumption than marine fish oil, such as vegetable oils, dressings, and margarines, may be an interesting option for the human diet [6,32]. Thus, the use of vegetable and/or fish oils rich in PUFA as raw materials, will increase the functionality of the obtained interesterified fat blends. Therefore, SLs can be designed with different goals and approaches, namely nutrition, medical, nutraceutical use, or for functionality in foods.

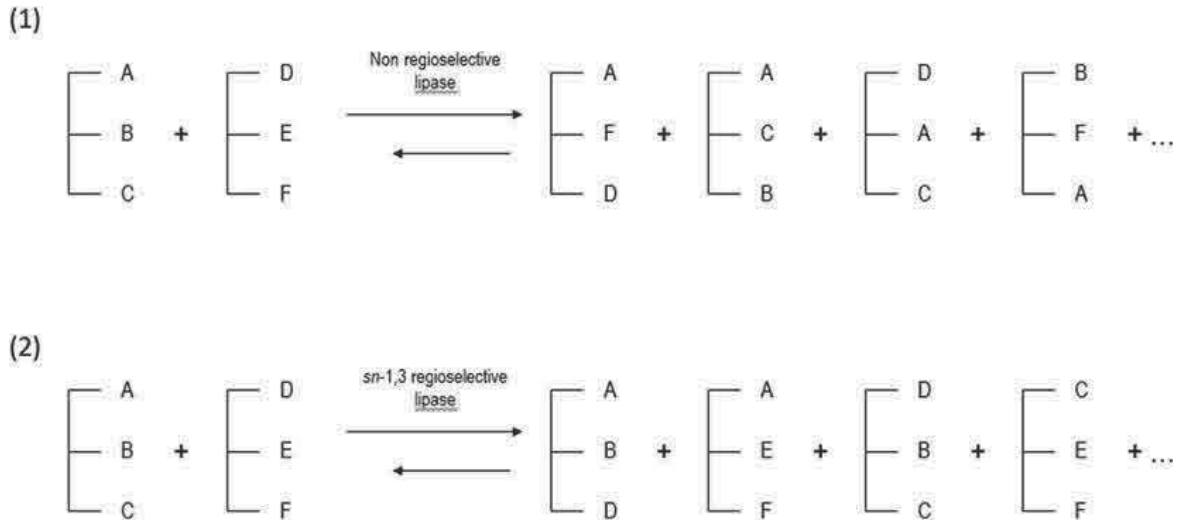

FIGURE 9–3 Schematic interesterification (ester interchange) between TAG with different fatty acid residues (A, . . .F) catalyzed by (1) a nonregioselective lipase (the reesterification occurs at random) or (2) by a *sn*-1,3 regioselective lipase (the FAs at position *sn*-2 are maintained).

The supply of essential FAs and/or omega-3 PUFA can also be performed by replacing several FAs of natural oils and fats by these specific FAs. These novel TAG can be produced either by acidolysis with FFA or interesterification with those FA ethyl (methyl) esters, as reported for the production of HMFS or MLM dietetic TAG. Several studies have been carried in batch or continuous operation mode for the production of (1) low-*trans* or *trans*-free plastic fats by interesterification or (2) TAG rich in specific LCFAs, using commercial immobilized lipases such as Novozym 435 (*Candida antarctica* lipase) [94,95], Lipozyme RM IM (*Rhizomucor miehei* lipase) [31,96−98], and Lipozyme TL IM (*Thermomyces lanuginosus* lipase) [98−102]. In most of these examples, reaction equilibrium was attained in less than 3 h, which is comparable to the time needed for chemical interesterification.

However, these immobilized lipases are rather expensive when used to produce these commodity fats, which has been a constraint for industrial process implementation. Other lipases, such as the noncommercial *Candida parapsilosis* lipase/acyltransferase [103,104] and *Rhizopus oryzae* lipase immobilized in a hybrid silica-organic support [95] have been tested in solvent-free media, as alternatives to the high-cost commercial biocatalysts (Table 9−4).

There are already some examples of commercialized products obtained through enzymatic interesterification that meet new specific needs of the industry with different applications (e.g., production of margarines, shortenings, bakery, and pastry products). Some examples of these

Table 9–4 Examples of enzymatic synthesis of TAG rich in specific long-chain fatty acids and interesterified TAG blends, in solvent-free media, in batch or continuous bioreactors (see list of symbols at the end of the chapter).

Operation mode	Biocatalyst	Substrates	Product type	References
Batch	Lipozyme RM IM	Borage oil + Blend (50:50, wt.%) of PK and palm olein (1:9, substrate molar ratio)	TAG rich in GLA and C18:2	[31]
	Lipozyme TL IM	PS + PK + TAG rich in omega-3 PUFA (55:35:10, wt.%) and (45:45:10, wt.%).	SL rich in omega 3 PUFA for *trans*-free table margarines	[99]
	CPLip2	PS + PK + TAG rich in omega-3 PUFA (45:45:10, wt.%).	SL rich in omega 3 PUFA for *trans*-free table margarines	[103]
	Lipozyme TL IM	High-stearate soybean oil + stearidonic acid soybean oil (2:1, molar ratio)	SL rich in stearidonic acid for *trans*-free table margarines	[100]
	Lipozyme RM IM	Glycerol + sardine oil FAs	Omega-3 FA-rich oil	[96]
Continuous	Novozym 435	PS + soybean oil (55:45, wt.%)	SL for *trans*-free margarines	[94]
	Lipozyme TL IM	PS + PK + SO(55:25:20, wt.%);PS + PK + TAG rich in omega-3 PUFA(55:35:10, wt.%)	SL for *trans*-free margarines	[101]
	CpLip2	PS + PK + TAG rich in omega-3 PUFA (45:45:10, wt.%)	SL rich in omega 3 PUFA for *trans*-free table margarines	[104]
	Lipozyme RM IM	Rice bran oil + C8:0 (1:6, molar ratio)	Rice bran oil SL for *trans-free* plastic shortenings	[97]
	Lipozyme TL IM	Mustard oil + fish oil (2:1, molar ratio)	PUFA-rich mustard oil	[102]
	Lipozyme TL IM; Lipozyme RM IM	PS + PK oil + OO(45:30:25, wt.%)	*Trans*-free table margarines	[98]
	Novozym 435; ROL in organic-inorganic hybrid support	Milk fat + SBO (65:35, wt.%)	SL for *trans*-free margarines	[95]

commercial products include products from the Crokvitol line (Crokvitol Stand, Crokvitol Allround, Crokvitol Vitality) from Bunge Loders Croklaan (http://europe.bungeloders.com/applications/spreads/) and ADM's products (https://www.adm.com/products-services/food/oils).

In addition, it is possible to find on the market oils enriched in PUFA produced by biocatalysis. An example is Marinol D-40 from Stepan Lipid Nutrition (https://www.stepan.com/products-markets/product/MARINOLD40.html), which is a concentrate of natural fish oil with a high content of docosahexaenoic acid in glyceride form. This product can be used via tablet or capsule supplements, or incorporated in several types of foods (e.g., fruit sticks for children or convenient breakfast drinks for the elderly; fruit beverages and dairy drinks; margarines and dairy spreads; frozen desserts; fish cakes, sticks and fillets; soups, pastas, and pasta sauces).

9.4.5 Operational stability in batch and continuous bioreactors

Lipase-catalyzed processes are in general simple and require a lower investment than chemically catalyzed processes, since a lower number and less complex unit operations are needed. The implementation of an enzymatic process aimed at producing SL needs to be economically feasible. SL can be produced batchwise and the biocatalysts reused in successive batches or used in continuous bioreactors (e.g., packed-bed or fluidized-bed bioreactors), to reduce biocatalyst-related costs. Thus, low-cost biocatalysts presenting both high activity and operational stability must be used.

When compared to other enzymes, lipases are highly stable even under adverse conditions such as organic solvents and high temperatures. Nevertheless, like any enzymatic process, even in nonconventional media, the use of lipases is limited to a relatively narrow range of temperature, and inhibition by high concentrations of substrates and/or products, inactivation by heavy metals, lipid oxidation products, free fatty acids, and solvents, may occur. In fact, these major constraints will result in lower conversions and, therefore, in longer reaction times with low volumetric productivities, when compared with the chemical route. This may be a dramatic situation when inhibition effects and thermal deactivation occur simultaneously.

Several deactivation models have been fitted to biocatalysts during SLs production, either in consecutive batches or in continuous bioreactors. The most used are first-order deactivation model, and the series-type deactivation kinetics model proposed by Sadana [105] described by a parabolic profile. In some cases, the initial activity is maintained for a certain period, represented by a plateau, decreasing thereafter following a first-order deactivation model. This is known as a time-delay inactivation model [106] (Fig. 9−4).

The respective model equations and equations to estimate half-life times are given by Eqs. (9−3)−(9−6), respectively. The half-life time ($t_{1/2}$) of the biocatalyst corresponds to the operation time required for half the biocatalyst activity to be lost by deactivation.

First-order deactivation kinetics model:

$$A_t = Ae^{-k_d t} \tag{9-3}$$

where A_t is the biocatalyst residual activity (%) at time t, A is a constant representing the initial activity of the enzyme before deactivation, and k_d is the deactivation rate constant, expressed in t^{-1}.

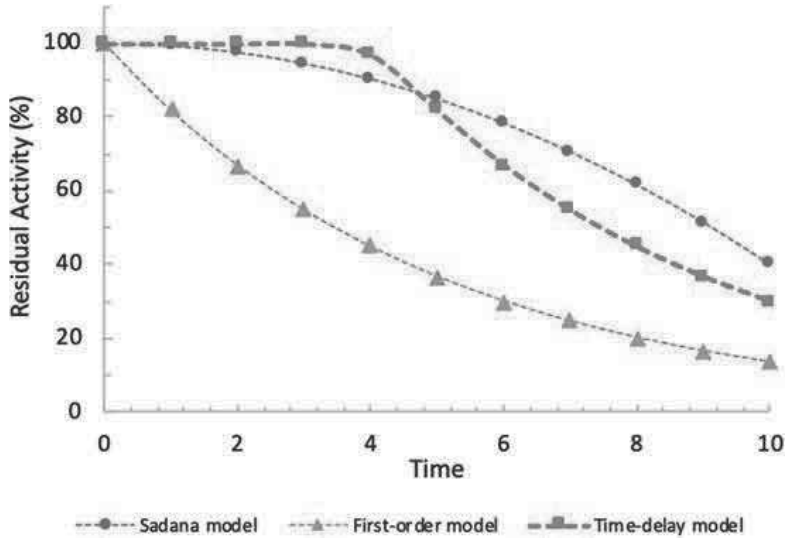

FIGURE 9–4 First-order, Sadana series-type, and time-delay first-order deactivation models.

When the biocatalyst follows a first-order deactivation profile, the half-life time is given by Eq. (9−4):

$$t_{1/2} = \frac{ln2}{k_d} \tag{9-4}$$

Series-type deactivation kinetics model of Sadana:

$$A_t = 100 - 50 \times k_d t^2 \tag{9-5}$$

The half-life time of a biocatalyst following a Sadana deactivation kinetics is given by the following equation:

$$t_{1/2} = k_d^{-1/2} \tag{9-6}$$

If the biocatalyst is reused in successive batches, in the equation models, the time t is replaced by the number of batch, n.

Tables 9−5 to 9−7 show some examples of the operational stability tests of commercial and noncommercial immobilized biocatalyst used in batch or continuous production of SLs in solvent-free media. In the production of low-calorie TAG from olive oil, for the same biocatalyst, the use of caprylic (C8:0) or capric acid (C10:0) leads to a different stability and/or deactivation profile (Table 9−5). When commercial immobilized lipases were tested (Lipozyme RM IM, Lipozyme TL IM, and Novozym 435, from Novozymes), half-lives varied from 47 to 299 hours. With Lipozyme RM IM, in the presence of olive oil and capric acid, no deactivation was observed during 10 reuses of 23 hours each [107]. As alternatives to the expensive commercial immobilized lipases, several noncommercial lipases were immobilized and used in consecutive batches. When the noncommercial recombinant lipase from *Rhizopus oryzae* (rROL) was immobilized in different synthetic

Table 9–5 Batch operational stability of some biocatalysts used in the production of low-calorie SL (MLM) in solvent-free media (n.d., not determined; see list of symbols at the end of the chapter).

Reaction	Biocatalyst	Substrates	Deactivation model	Half-life time (h)	References
Acidolysis	Lipozyme RM IM	Olive oil + C8:0	First-order	299	[107]
	Lipozyme RM IM	Olive oil + C10:0	No deactivation	n.d.	[107]
	Lipozyme TL IM	Olive oil + C8:0	First-order	50.4	[107]
	Lipozyme TL IM	Olive oil + C10:0	First-order	47	[107]
	Novozym 435	Olive oil + C8:0	First-order	217	[107]
	Novozym 435	Olive oil + C10:0	First-order	225	[107]
	rROL in Eupergit C	Olive oil + C8:0	Time-delay	159	[108]
	rROL in Eupergit C	Olive oil + C10:0	Time-delay	136	[108]
	rROL in Eupergit C + rehydration	Olive oil + C8:0	First-order	39	[109]
	rROL in Eupergit C + rehydration	Olive oil + C10:0	First-order	54	[109]
	rROL in Lewatit VP OC 1600	Olive oil + C10:0	First-order	49	[109]
	rROL in Lewatit VP OC 1600 + rehydration	Olive oil + C10:0	Sadana series-type	234	[109]
	rROL in Amberlite IRA 96	Grapeseed oil + C8:0	First-order	166	[71]
	rROL in Amberlite IRA 96	Grapeseed oil + C10:0	First-order	118	[71]
	CPL	Grapeseed oil + C8:0	First-order	96	[71]
	CPL	Grapeseed oil + C10:0	First-order	81	[71]
	ROL in MNP	Crude olive pomace oil + C10:0	Sadana series-type	163	[74]
Interesterification	ROL in MNP	Crude olive pomace oil + C10:0 ethyl ester	Sadana series-type	220	[74]

Table 9–6 Batch operational stability of some biocatalysts used in the production of HMFS by acidolysis in solvent-free media (see list of symbols at the end of the chapter).

Biocatalyst	Substrates	Deactivation model	Half-life time (h)	References
Lipozyme RM IM	PPP + C18:1	No deactivation	n.d.	[110]
Lipozyme RM IM	PPP + omega-3 PUFA	Linear	276	[110]
Lipozyme TL IM	PPP + C18:1	Linear	154	[110]
Novozym 435	PPP + C18:1	Sadana series-type	253	[110]
Novozym 435	PPP + omega-3 PUFA	Sadana series-type	322	[110]
CPLip2	PPP + C18:1	First-order	35	[110]
CPLip2	PPP + omega-3 PUFA	Sadana series-type	127	[110]
Carica papaya Lipase	PPP + C18:1	Sadana series-type	80	[84]
rROL in Lewatit Accurel MP 1000	PPP + C18:1	Linear	35	[85]
rROL in Lewatit VP OC 1600	PPP + C18:1	Linear	64	[85]
rROL in Lewatit VP OC 1600 + rehydration	PPP + C18:1	Sadana series-type	202	[85]
rROL in Accurel MP 1000	Lard + omega-3 PUFA	First-order	112	[83]

supports and reused in batch, for the synthesis of MLM from olive oil or grapeseed oil, different deactivation profiles were observed and the half-life times varied from 39 to 234 hours [71,108,109]. Also, the low-cost self-immobilized *Carica papaya* lipase in the latex and the commercial lipase from *Rhizopus oryzae* immobilized in ferromagnetic nanoparticles showed high

Table 9–7 Operational stability of some biocatalysts used in the production of triacylglycerols modified in the original position of the fatty acids by interesterification in solvent-free media (see list of symbols at the end of the chapter).

Operation mode/ bioreactor type	Biocatalyst	Substrates	Deactivation model	Half-life time	References
Continuous fluidized-bed reactor	Novozym 435 (fresh and reused)	PS + SBO (55:45, wt.%).	Sadana series-type	17 days	[94]
Continuous packed-bed reactor	Lipozyme TL IM	PS + PK + SO (55: 25: 20, wt.%)	First-order	135 h	[101]
Continuous packed-bed reactor	Lipozyme TL IM	PS + PK + TAG rich in omega-3 PUFA (55:35:10, wt.%)	First-order	77 h	[101]
Continuous fluidized-bed reactor	CPLip2	PS + PK + "EPAX 4510 TG" (45:45:10, wt.%)	First-order	9 h	[104]
Batch	CPLip2	PS (45 wt.%) + PK (45 wt.%) + "EPAX 4510TG" (10 wt.%)	First-order	10 h18 h (water addition between batches)	[104]
Continuous packed-bed reactor	Lipozyme RM IM	PS + PK + OO (45:30:25, wt.%)	First-order	60 h	[98]
Continuous packed-bed reactor	Lipozyme TL IM	PS + PK + OO (45:30:25, wt.%)	First-order	88 h	[98]
Continuous fluidized-bed reactor	Novozym 435	Milk fat + SBO	No deactivation during 168 h operation	n.d.	[95]
Continuous fluidized-bed reactor	ROL in organic–inorganic hybrid support	Milk fat + SBO	Sadana series-type	190 h	[95]

batch operational stability in acidolysis of grapeseed oil [71] and acidolysis or interesterification of crude olive pomace oil [74], respectively.

In the production of HMFS in solvent-free media (Table 9–6), the same behavior was observed: in the system tripalmitin/oleic acid, no deactivation was observed for Lipozyme RM IM, in 10 consecutive reuses of 23 hours each. However, when oleic acid was replaced by a concentrate of omega-3 PUFA, a considerable change in the deactivation profile of Lipozyme RM IM was observed [110]. Also, rROL immobilized in different supports showed different inactivation profiles and stability in the acidolysis of tripalmitin with oleic acid or of lard with omega-3 PUFA, with half-lives from 35 to 202 hours [83,85].

Other examples of SL are the interesterified fat blends enriched in specific fatty acids or with improved functional properties for the food or pharmaceutical industries. Commercial immobilized lipases and noncommercial immobilized lipases were used as catalysts for the interesterification of different fat blends in the absence of solvent, either in consecutive batches or in continuous bioreactors (Table 9–7). Again, the operational stability and inactivation profile varied with the biocatalyst and, for the same biocatalyst, varied with reaction medium composition and operation conditions. The highest stability was observed for Novozym 435 with a half-life of 17 days in the interesterification of palm stearin with soybean oil in a continuous fluidized-bed reactor [94]. The same biocatalyst maintained its activity along 168 hours continuous operation in a fluidized-bed reactor for the interesterification of milk fat with soybean oil [95]. Using other commercial

immobilized biocatalysts, half-life times varied from 60 to 135 hours. As alternative to these biocatalysts, *Rhizopus oryzae* lipase immobilized in an organic−inorganic hybrid support, showed excellent results in terms of operational stability with a half-life of 190 hours.

The results obtained with several noncommercial immobilized lipases, concerning their operational stability, are rather promising toward cost reduction and industrial implementation of enzymatic processes for SL production.

9.5 Conclusions and perspectives

Over the past few years, SLs have been recognized for their technological, functional, and health benefits. The use of lipases and phospholipases as catalysts for SL production offers several advantages in comparison with the chemical route, namely enzyme selectivity and mild operational conditions used. The *sn*-1,3 regioselectivity exhibited by some lipases and phospholipases allows for the production of SLs, which are impossible to obtain by chemical catalysis (e.g., low-calorie TAG, HMFS, and oils enriched in specific fatty acids in the positions *sn*-1,3). Nevertheless, the high cost of commercial enzymes remains a main constraint for industrial production of commodity fats (e.g., margarines and shortenings). The implementation of sustainable processes for SL production has been a challenge for the food industry. Promising results have been achieved in terms of yield and productivity of SLs, in solvent-free media, either in batch or continuous reactions. Also, high operational stability was observed with some biocatalysts in different reaction systems.

Several SLs obtained by lipase-catalyzed reactions are already in the market to be used incorporated in foods or sold as supplements. It is of utmost importance to search for novel low-cost enzymes with both high catalytic and operational stability, as well as to promote the use of cheap raw materials in the synthesis of SLs.

List of symbols

a_w	water activity
ARA	arachidonic acid (C20:4 omega-6)
CLA	conjugated linoleic acid
CPL	*Carica papaya* lipase
CpLip2	*Candida parapsilosis* lipase/acyltransferase
DAG	diacylglycerol(s)
DHA	docosahexaenoic acid (C22:6 omega-3)
EPA	eicosapentaenoic acid (C20:5 omega-3)
EPAX 4510 TG	TAG concentrate rich in EPA and DHA
FA	fatty acid(s)
FFA	free fatty acid(s)
GLA	gamma-linolenic acid
HMF	human milk fat
HMFS	human milk fat substitute
L	long-chain fatty acid(s)
LCFA	long-chain fatty acid(s)
Lipozyme RM IM	commercial immobilized lipase from *Rhizomucor miehei*

Lipozyme TL IM	commercial immobilized lipase from *Thermomyces lanuginosus*
M	medium-chain fatty acid(s)
MAG	monoacylglycerol(s)
MCFA	medium-chain fatty acid(s)
MLM	TAG containing medium-chain FAs at positions *sn*-1,3 and a long-chain FA at position *sn*-2
MMM	medium-chain TAG(s)
MNP	magnetic nanoparticles
MUFA	monounsaturated fatty acid(s)
Novozym 435	commercial immobilized lipases from *Candida antarctica*
O	oleic acid
OO	olive oil
PK	palm kernel fat
PL	phospholipid(s)
PO	palm oil
PPP	tripalmitin
PS	palm stearin
PUFA	polyunsaturated fatty acid(s)
ROL	commercial *Rhizopus oryzae* lipase
rROL	noncommercial recombinant lipase from *Rhizopus oryzae*
SBO	soybean oil
SDA	stearidonic acid (C18:4 omega-3)
SL	structured lipid(s)
SO	sunflower oil
SPL	structured phospholipid(s)
TAG	triacylglycerol(s)

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