REVIEW

# Enzyme-Catalyzed Modification of Oilseed Materials to Produce Eco-Friendly Products

**Douglas G. Hayes**

Department of Biosystems Engineering and Environmental Science, University of Tennessee, Knoxville, Tennessee 37996-4531

**ABSTRACT:** Novel products produced from seed oil materials (TAG, phospholipids, and minor components such as tocopherols, sterols, stanols, and fatty acyl esters of the latter two) by enzyme-mediated purification or chemical modification are reviewed. The primary focus is on "value-added products" of current and potential use (particularly in the food, cosmetics, and pharmaceutical industries) that require the selectivity of enzymes and mild operating conditions, the latter being beneficial for polyunsaturated and oxygenated acyl groups. The paper briefly reviews the biochemistry of enzymes in lipid modification (lipases, phospholipases, and lipoxygenases) and discusses and assesses the current and future applications, current state of the art, and areas for future research for the following enzyme-mediated processes: isolation of polyunsaturated and oxygenated FFA; formation of structured TAG as nutraceuticals; formation of MAG, saccharide-FA esters, and other polyhydric alcohol ester as emulsifiers and surfactants; isolation and/or modification of tocopherols and sterols as antioxidants; formation of hydroperoxides as chemical intermediates; and modification of phospholipids for use in liposomes.

Paper no. J10908 in *JAOCS 81,* 1077–1103 (December 2004).

**KEY WORDS:** Hydroxy fatty acids, lipase, lipoxygenase, nutraceuticals, phospholipases, phospholipids, polyunsaturated fatty acids, review, structured lipids, surfactants.

Agriculturally derived feedstocks will play an increasingly important role in North America and worldwide as the cost of petroleum continues to increase and its availability decreases. Lipids (TAG, phospholipids, sterols, etc.) are an example of such a feedstock. Although lipids are readily modified by chemical means (reviewed in Refs. 1–5) and can be synthesized from petroleum-based feedstocks (6), there is an increased interest in modifying lipids *via* biocatalysis, particularly to manufacture lipid-based products in the food, cosmetics, and pharmaceutical industries, and for applications focused on environmental friendliness, due to the mild reaction conditions and narrow product distributions. In addition, biocatalysis has also played a role in isolating natural products, for instance, by temporarily modifying the molecule of interest to facilitate downstream separations. Reviews on biocatalytic modification of lipids have appeared during the last 15 yr (7–11) and more recently in two monographs (12,13). In addition, a recent conference entitled Enzymes in Lipid Modification was held in 2003 in Greifswald, Germany (14). The organization and emphasis of the previous reviews focused mostly on the types of biocatalysts; the emphasis here is on the products formed. Preceding the discussion of products is a brief overview of the important biocatalytic tools to be used.

*To whom correspondence should be addressed at Department of Biosystems Engineering and Environmental Science, University of Tennessee, 2506 E.J. Chapman Dr., Knoxville, TN 37996-4531. E-mail: dhayes1@utk.edu

## BIOCATALYSTS USED FOR LIPID MODIFICATION

*Lipases (EC 3.1.1.3).* Lipases are specifically designed by nature for the hydrolysis and synthesis of ester bonds involving FA, and can form and/or hydrolyze amide, carbonate, and thioester bonds as well. Reactions catalyzed by lipases that involve the ester bond are diagrammed in Figure 1. Lipases are perhaps the most frequently used enzymes for biotransformations because of their ability to function in nonaqueous (reviewed in Refs. 15–19) and aqueous systems as well as at or near interfaces (their primary locale *in vivo*) without the need for cofactors or coenzymes in most cases. They narrowly control product distribution because of their regio-, stereo-, and substrate selectivities (reviewed in Refs. 20–22). As an example of regioselectivity, lipases are quite specific for specific hydroxyls on mono-, di-, and oligosaccharides (discussed below). Many lipases selectively discriminate against secondary OH acyl acceptors, such as the middle or 2-position of glycerol, whereas many other lipases do not have such selectivity (the latter referred to here as "random" lipases). Lipases have several potential and current applications in the pharmaceutical industries based on their ability to selectively esterify a specific enantiomer from racemic mixtures of alcohols or acids, such as the isolation of the biologically active S-ibuprofen (23)

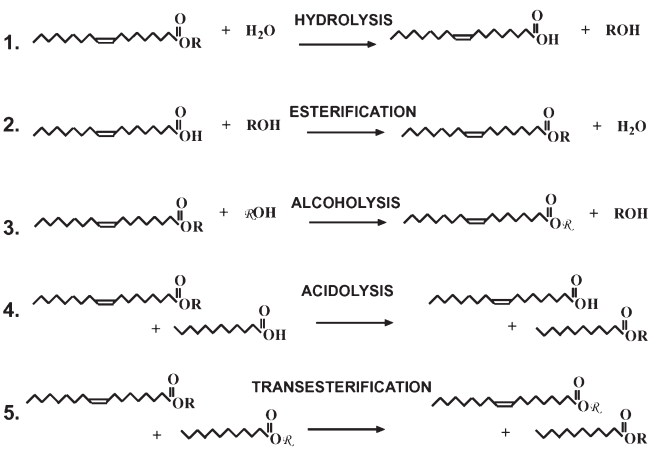


**FIG. 1.** Reactions catalyzed by lipases.

(reviewed in Refs. 24–28). (It should be noted that recent investigations have demonstrated that the position of substrates at an interface strongly influences the apparent enantioselectivity of lipases; see Ref. 21.) Lipases also have inherent selectivity toward the structure of acyl groups, i.e., their chain length and the position, type (*cis* or *trans*), and number of double bonds. An example is *Geotrichum candidum* lipase, which is highly selective toward substrates with *cis* unsaturation at the $C_9$ position, such as oleic, linoleic, and α-linolenic acids (ALA). Most lipases discriminate against acyl groups that possess double bonds near the carbonyl terminus, such as DHA (22:6-4*c*,7*c*,10*c*,13*c*,16*c*,19*c*), an important product from fish oil.

Several different lipases are commercially available (Table 1). Many of the lipases have been isolated from several different microorganisms, e.g., bacteria, yeast, and algae (35,36). Thermophilic and alkaline-stable lipases have been isolated from extremophiles (37,38). In several cases, the genes that express the lipases have been isolated and inserted into common recombinant hosts such as *Escherichia coli* for mass production. Improved immobilization technology has

**TABLE 1**
**Commercially Available Enzymes Useful for Biocatalytic Modification of Lipids**

| Product name | Enzyme type | Manufacturer | Application |
|---|---|---|---|
| Lecitase Ultra | Porcine pancreatic phospholipase $A_2$ | Novozyme, Inc. (Franklinton, NC)[a] | Degumming of oils |
| Lipase A "Amano" 12 | *Aspergillus niger* lipase | Amano Enzyme USA (Elgin, IL)[b] | 1,3-Selective; hydrolysis of short-chain acyl groups |
| Lipase AK 20 "Amano" | *Pseudomonas fluorescens* lipase | Amano Enzyme USA (Elgin, IL)[b] | High thermostability; "random" positional selective |
| Lipase AYS "Amano" | *Candida rugosa* lipase | Amano Enzyme USA (Elgin, IL)[b] | "Random" positional selective; selective against medium-chain acyl groups |
| Lipase CLEC | Cross-linked crystals of lipases[c] | Altus (Cambridge, MA) | Stereochemical modification of lipids |
| Lipase F-AP 15 "Amano" 15 | *Rhizopus oryzae* lipase | Amano Enzyme USA (Elgin, IL)[b] | Hydrolysis of short- and medium-chain acyl groups; 1,3-selective |
| Lipase G "Amano" | *Penicillium camembertii* lipase | Amano Enzyme USA (Elgin, IL)[b] | Hydrolysis of MAG and DAG |
| Lipase L9 | Immobilized *R. miehei* lipase | Biocatalytics (Pasadena, CA) | 1,3-Selective; interesterification (29) |
| Lipase M "Amano" 10 | *R. javanicus* lipase | Amano Enzyme USA (Elgin, IL)[b] | Designed to remove trace amounts of TAG in protein products; 1,3-selective |
| Lipase PS-C-II or –D-I "Amano" II | Immobilized *Burkholderia cepacia* lipase[d] | Amano Enzyme USA (Elgin, IL)[b] | Thermostable; enantioselective |
| Lipase R "Amano" G | *P. roquefortii* lipase | Amano Enzyme USA (Elgin, IL)[b] | 1,3-Selective; selective for short- and medium-chain acyl groups |
| Lipases, immobilized | Immobilized onto Sol-Gel-AK or sintered glass[e] | Fluka/Riedl de Haen Div. of Sigma-Aldrich (St. Louis, MO)[f] | Lipid modifications |
| Lipolase | *Thermomyces lanuginosa* lipase[g] | Novozyme, Inc. (Franklinton, NC)[a] | Stable at high temperature and pH; useful for synthesis and hydrolysis |
| Lipoxygenase | From soybean | Sigma-Aldrich (St. Louis, MO) | Formation of hydroperoxides from linoleic or arachidonic acid (AA) |
| Lipozyme RM IM | Immobilized *R. miehei* lipase | Novozyme, Inc. (Franklinton, NC)[a] | Highly thermostable 1,3-selective lipase (30) |
| Lipozyme TL IM | Immobilized (silica-granulated) *T. lanuginosa* lipase[e] | Novozyme, Inc. (Franklinton, NC)[a] | 1,3-Selective; Effective for interesterification (31–33) |
| Novozym 435 | Immobilized *C. antarctica* B lipase | Novozyme, Inc. (Franklinton, NC)[a] | Highly thermostable lipase with "random" positional selectivity (34) |

[a]www.novozymes.com.
[b]http://www.amano-enzyme.co.jp/english/productinfo/medical03.html.
[c]Lipases from *C. rugosa, B. cepacia, Geotrichum candidum*, porcine pancreatic.
[d]Formerly named *P. cepacia lipase*.
[e]From porcine pancreas, *A. niger, C. antarctica, C. rugosa, R. miehei, B. cepacia,* and *P. fluorescens*.
[f]http://www.sigmaaldrich.com/Brands/Fluka_Riedel_Home/Organic_Synthetic/Enzymes/Organic_Chemistry/Immobilization.html.
[g]Formerly named *Humicola lanuginosa* lipase.

led to lipases with enhanced operational stability and reusability, several of which are commercially available (Table 1). Lipases from plant species, such as rapeseed (*Brassica napus*), vernonia (*Vernonia galamensis*), and papaya latex (*Carica papaya*), may serve as low-cost alternatives to microbial lipases, particularly for their ability to purify acyl groups possessing Δ3–Δ6 unsaturation (39).

Mutagenesis techniques have been used to maximize the thermostability and activity of lipases for certain media such as organic solvents and detergent-rich systems and modify their enantio- and substrate selectivity (reviewed in Ref. 40). Such developments have been driven by the increased use of lipases as laundry detergent additives and as stereoselective biocatalysts in the pharmaceutical industry, which in turn have motivated fundamental investigations to understand how lipases function on a molecular level. As reviewed elsewhere (35,41,42), most lipases share the following common chemical motifs. Similar to other "serine proteases," their active sites consist of a catalytic triad: an activated serine (*Ser*) residue, which acts as a nucleophile, forming an acyl-enzyme intermediate, an acidic residue (either glutamic acid, *Glu*, or aspartic acid, *Asp*), and histidine (*His*). The latter two residues help stabilize the acyl-enzyme intermediate. Often, lipases possess a "lid" consisting of an α-helix region that covers the active site; the lid is retracted upon contact with an interface, exposing the active site for biocatalytic activity. Many lipases share a similar binding site structure shaped like the outline of a tuning fork with a bent handle. The "fork" part of the "tuning fork" consists of two hydrophobic pockets. The bent handle end also contains a hydrophobic domain. The position of the bend in the fork's handle corresponds to an oxyanion hole, a relatively hydrophilic region. The structure of the binding domains of lipases helps dictate their stereoselectivity.

*Lipoxygenases (EC 1.13.11.12).* As reviewed elsewhere (43–46), lipoxygenases (LOX) are nonheme iron dioxygenases that stereospecifically form an S-configuration hydroperoxide (HPOD) from $O_2$ and a PUFA that contain *cis* double bonds separated by a single methylene, or $CH_2$, group. Generally, for an FFA containing a *cis* double bond between carbon atoms at positions *n* and *n* + 1 and a second *cis* double bond between carbon atoms at position *n* + 3 and *n* + 4, LOX will add the $O_2$ group across one of the double bonds, resulting in a HPOD molecule containing either a peroxy (–OOH) group in position *n* with a *trans* double bond between positions *n* + 1 and *n* + 2 and a *cis* double bond remaining between position *n* + 3 and *n* + 4, or an –OOH group in position *n* + 4 with a *trans* double bond between position *n* + 2 and *n* + 3 and a *cis* double bond remaining between position *n* and *n* + 1 (Fig. 2). FFA are the natural substrates for LOX; however, certain LOX can operate on TAG and glycerolphospholipids (GPL).

Lipoxygenases are also very regioselective. For instance, soybean LOX isozyme-1 converts linoleic acid (18:2-9*c*,12*c*) into *S*-18:2-9*c*,11*t*-OOH-13 (thus being categorized as a "13-LOX" with respect to linoleic acid), whereas potato tuber

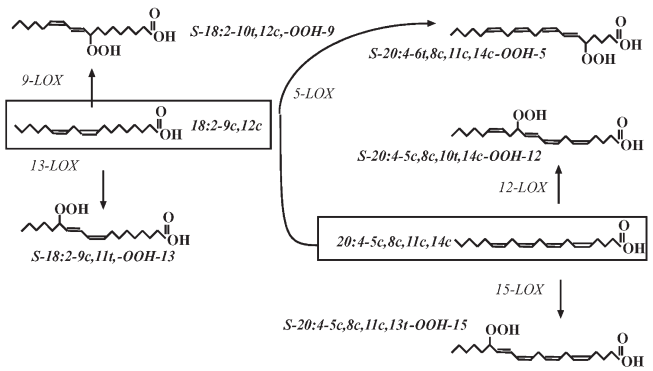


**FIG. 2.** Hydroperoxide products from linoleic and arachidonic acid catalyzed by lipoxygenases (LOX).

LOX produces almost solely *S*-18:2-10*t*,12*c*,-OOH-9 (categorized as a "9-LOX"; Fig. 2). Plant-derived LOX are classification with respect to the linoleic acid product since arachidonic acid (AA) rarely occurs in plants. With regard to AA (20:4-5*c*,8*c*,11*c*,14*c*), which is used to classify mammalian LOX, specific LOX are categorized as either 5-,12-, or 15-LOX (Fig. 2). (Soybean LOX-1 is an example of a 15-LOX.) The substrate specificity of LOX toward PUFA is reviewed elsewhere (47).

LOX fulfills myriad biological roles (reviewed in Refs. 44,45). Soybean LOX-1, the most thoroughly investigated LOX, consists of a single polypeptide strand (M.W. of 94 kDa) and contains a single iron atom. Six *His* residues are believed to bind with the Fe atom. The structural basis for stereo- and regioselectivities is fairly well known, allowing the use of site-directed mutagenesis to modify the selectivity of LOX (43).

Most reactions have used nonimmobilized LOX with a biphasic water–oil system in a closed, pressurized system under an $O_2$ atmosphere to maximize $O_2$ solubility. (Anaerobic conditions cause a loss of activity.) It is best if the aqueous phase is basic ($9 < pH < 10$) to allow formation of HPOD soaps, which act as surfactants to enhance PUFA substrate solubility in the aqueous phase (reviewed in Refs. 47). There has been significant interest in developing immobilized LOX preparations to allow for reuse and use in nonaqueous media (48–52) (discussed later).

*Phospholipases.* The biochemistry and enzymology fundamentals of phospholipases are reviewed in more detail elsewhere (53,54). The term "phospholipase" refers to five different enzyme types (phospholipases $A_1$, $A_2$, B, C, and D, abbreviated $PLA_1$, $PLA_2$, PLB, PLC, and PLD, respectively) that catalyze reactions involving different chemical moieties on the GPL molecule (Fig. 3). $PLA_1$ (EC 3.1.1.32) is rarely used for GPL modification because 1,3-selective lipases perform the same task more efficiently and are more readily available. $PLA_2$ (EC 3.1.1.4), which selectively hydrolyzes and esterifies acyl groups at the 2-position of GPL, is commonly encountered in secreted form in mammalian pancreatic fluids as well as in the cytoplasm of mammalian cells. Bovine pancreatic $PLA_2$, the phospholipase most frequently

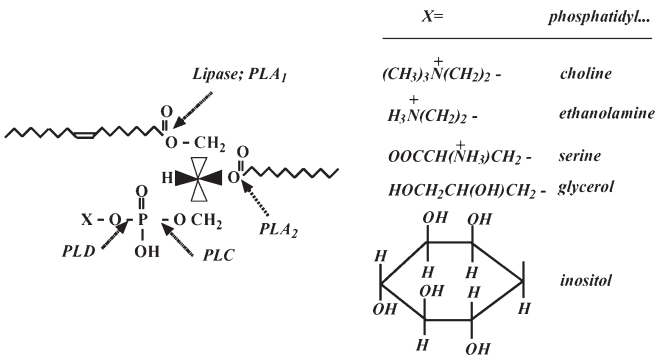


**FIG. 3.** Enzymes used to modify glycerophospholipids (GPL). $PLA_1$, $PLA_2$, PLC, and PLD refer to phospholipase $A_1$, $A_2$, C, and D, respectively.

used for GPL modifications, has a M.W. of 14 kDa and requires $Ca^{2+}$ for activity. It exists as a zymogen that is activated by proteolysis. This enzyme is rich (~50%) in an α-helix secondary structure and contains one active site that is highly hydrophobic and that uses structurally conserved aspartic *Asp* and *His* residues. A calcium-binding loop also occurs that perhaps stabilizes the transition state. Unlike with lipases, an acyl-enzyme intermediate does not form. As reviewed elsewhere (55), $PLA_2$ has been immobilized successfully for use in aqueous and nonaqueous media, as exemplified by Lecitase™, a pancreatic $PLA_2$ physically adsorbed onto a $SiO_2$-based matrix developed by Novozymes, Inc. (Franklinton, NC) for use as an aide in degumming (Table 1). PLB (EC 3.1.1.5) is not used in GPL modification mainly because of its broad substrate selectivity, hydrolyzing and forming ester bonds at the 1- and 2-glycerol positions.

PLC (EC 3.1.4.10 and 3.1.4.3) is a small metalloenzyme that cleaves on the glycerol side of the phosphodiester bond of GPL. *Bacillus cereus* PLC, readily expressed in *E. coli*, has received the most attention as a tool for lipid modification. It is a small (28.5-kDa) metalloenzyme, possessing three $Zn^{2+}$ groups that form a cluster in its active site, and is rich in α-helices. Mechanistically, the Zn moieties help bind the substrate in a configuration in which an activated water molecule in the active site can act as a nucleophile on the phosphate bond. Although PC is the preferred substrate, *B. cereus* PLC can also hydrolyze the phosphate bonds of PE and PS, thus demonstrating its broad substrate selectivity.

Phospholipase D hydrolyzes the diester bond of phospholipids at the ester bond dietal to the fatty acyl side of the diester linkage. The most abundantly used varieties of PLD (EC 3.1.4.4) are from cabbage (M.W. of 91.8 kDa) and, particularly in recent years, from *Streptomyces* sp. (54 kDa), both of which have been expressed recombinantly. The formation of GPL with unnatural head groups, and the introduction of head groups into alkyl phosphates without the glycerol backbone, have occurred *via* catalysis with PLD from cabbage, demonstrating the enzyme's broad substrate selectivity. PLD, and in particular its pH-activity profile, is highly dependent on $Ca^{2+}$, with 20–100 mM typically used. Although the biochemistry of PLD is less well known than that of the other phospholipases because of the difficulty in obtaining high-purity PLD isozymes and high-quality protein crystals, it is known that many PLD molecules share a highly conserved *His* group in the active site, which leads to the formation of a phosphor-enzyme intermediate, and a second *His* residue, which cleaves or forms the phosphodiester bond.

## FA-BASED PRODUCTS *VIA* ENZYME REACTIONS

*Uncommon fatty acyl species.* Most plant, animal, and microbial species possess common saturated and Δ9-unsaturated acyl groups such as palmitic (16:0), stearic (18:0), oleic (18:1-9*c*), linoleic, and ALA (18:3-9*c*,12*c*,15*c*). Such acyl groups, which are abundantly found in cooking oils such as soybean (33.4 megametric tons, or MMT, projected for 2004, at $0.50/kg; http://ffas.usda.gov/oilseeds_arc.html), sunflower (9.02 MMT, $0.70/kg), low erucic acid rapeseed (14.2 MMT) or canola, cotton (4.25 MMT, $0.70/kg), olive (2.6 MMT), corn ($0.70/kg), and beef tallow, are inexpensive and readily isolated and purified by conventional means. A similar comment can be made for $C_{12}$–$C_{16}$ saturates and $C_4$–$C_{10}$ saturates, which are abundantly found in palm oil and milk fat, respectively. However, other acyl groups found less abundantly have more highly specialized or "value-added" applications that would merit the higher operating costs of biocatalytic processes for their isolation and use as chemical feedstocks (Table 2). Generally, the list of "specialty" acyl groups falls into one of two categories: those possessing unusual and/or high degrees of polyunsaturation, and those possessing oxygenated moieties (hydroxyl or epoxide) in their alkyl backbones.

Polyunsaturated acyl groups with Δ3–Δ6 unsaturation, such as DHA, EPA (20:5-5*c*,8*c*,11*c*,14*c*,17*c*), AA, and GLA (18:3-6*c*,9*c*,12*c*), and their derivatives have received a great deal of attention recently as food additives ("nutraceuticals") and pharmaceuticals beccause of their medical and nutritional value. For instance, a literature search on patents involving DHA and AA from 1999 to the present resulted in 1014 and 878 hits, respectively. A few of the specific applications are listed in Table 2. Generally, a diet rich in PUFA is known to decrease blood TAG levels, blood pressure, and LDL (or "bad") cholesterol. The second category, hydroxy- and epoxy-containing acyl groups, has numerous applications as chemical feedstocks in lubricants, in paints and coatings, and as food and cosmetics emulsifiers. Knowledge of the utility of ricinoleic acid, the most commonly recognized member of this category, has existed for at least a century; for instance, a U.S. patent to use sulfonated ricinoleic acid in cleaning fluids was issued in 1908 (112). Many hydroxy FFA species and libraries of modified FFA are under investigation as anticancer agents (113). For both PUFA and oxygenated FFA, conventional means of isolation or utilization involve operating conditions that can lead to their chemical degradation. In contrast, biocatalytic processes involve mild operating conditions, such as low to moderate temperature and pressure and no extremes in pH.

**TABLE 2**
**FFA of Commercial Relevance That Are Isolated by or Employed as Acyl Donors for Lipase-Catalyzed Reactions**

| FFA product | Chemical structure | Sources | Application |
|---|---|---|---|
| AA | 20:4-5*c*,8*c*,11*c*,14*c* | Single-cell oils | Vision, brain, and nervous system development in infants (56,57); increase of skeletal muscle mass (58); reduction of intravenous hemorrhaging in premature infants (59); cognitive ability enhancer (60) |
| Crepenynic acid | 18:1-9c,12-yne | *Crepis alpine* oil | Chemical feedstock for free radical polymers in paints/coatings (reviewed in Refs. 61,62) |
| Dimorphecolic acid | *S*-18:2-10*t*,12*t*,OH-9 | *Dimorphotheca pluvialis* oil | Chemical feedstock for lubricants, coatings, and polymers (reviewed in Ref. 63) |
| DHA | 22:6-4*c*,7*c*,10*c*,13*c*,16*c*,19*c* | Fish oils; single-cell oils | Treatment of cardiovascular disease; vision, brain, and nervous system development in infants; anti-inflammatory, etc. (reviewed in Refs. 64–66) |
| EPA | 20:5-5*c*,8*c*,11*c*,14*c*,17*c* | | Same as DHA |
| Erucic acid | 22:1-13*c* | High erucic acid rapeseed or crambe oil | Slip agent for plastic film (erucamide) and other oleochemicals (reviewed in Ref. 67); photographic film (68); cosmetics and personal care products (67,69) |
| GLA | 18:3-6*c*,9*c*,12*c* | Blackcurrent (*Ribes nigrum*), borage (*Borago officinalis*), evening primrose (*Oenothera biennis*), or single-cell (fungal) oil | Anti-inflammatory, anticholesteremic; brain and nervous system development in infants (reviewed in Refs. 56,70) |
| Gorlic acid | 13:1-6*c*,13-(2-cyclopentenyl) | *Hydnocarpus wightiana* seeds | Possible organic chemical feedstock |
| Lesquerolic acid | *R*-22:1-11*c*,OH-14 | *Lesquerella fendleri* oil | Feedstock for nylon, lubricants, cosmetics, chemicals (71–74); potential replacement for ricinoleic acid |
| Petroselinic acid | 18:1-6*c* | Coriander (*Coriandrum sativum*) oil | Nutritional supplement (75); cosmetic additive (76); drug delivery (76); anti-wrinkle, anti-inflammatory agent for skin (77–79); rehydration of dry skin (80); hair growth (81) |
| Ricinoleic acid | *R*-18:1-9*c*,OH-14 | Castor (*Ricin comminus*) oil | Production of sebacic acid (nylon-11) and polyesters; polyurethanes (82,83); laxative, disinfectant, food emulsifier (polyglycerol polyricinoleate), lipstick, and other food- and health-related products (83,84); lubricants and other chemical feedstocks (83)[a] |
| Vernolic acid | 18:1-9*c*-epoxy-12,13*c* | *Vernonia galamensis* or *Euphorbia lagascae* oil | Paints and coatings (85,86); dibasic acid feedstock (87); oral drug delivery (88); antioxidant activity (89); nylon-11 and -12 precuror (90); other chemical feedstocks (91–93) |
| CLA | 18:2-9*c*,11*t*; 18:2-10*t*,12*c* | Milk fat; beef tallow; modification of ricinoleic acid (94); free radical isomerization of α-linolenic acid (95–98) | Diabetes treatment (99); reduces uptake of lipids; cancer treatment; improves metabolism of cholesterol; growth stimulant (reviewed in Ref. 100) |
| | 20:1-5*c*; 22:2-5*c*,13*c* | Meadowfoam (*Limnanthes alba*) oil | Oleochemicals (101–104); polymers (105); cosmetics for skin (106) and hair (107,108); surfactants (109,110) |
| | 20:3-5*c*; 11*c*,14*c*, 20:4-5*c*,11*c*,14*c*,17*c* | *Biota orientalis* oil | Possible lipid metabolism agent (111) |

[a]577 patents since 1990. For other abbreviation see Table 1.

*Isolation of FFA* via *hydrolysis ("lipolysis").* Acyl groups typically occur in seed oil and animal fat in the form of TAG. Prior to FFA isolation, seed oil is typically processed using the following steps: solvent extraction of the oil from the seeds, or use of pressing; degumming to remove GPL; alkali refining to remove residual FFA; bleaching to remove soaps and pigments; and steam deodorizing to remove off-flavors (114). (It is noted that great care must be used to prevent degradation of oxygenated acyl species during a solvent extraction or mechanical pressing operation, which is why su-

percritical carbon dioxide extraction is recommended; see Refs. 115–117.)

FFA are typically isolated from the processed oil by the Colgate–Emery process, in which steam hydrolyzes the TAG ester bonds at high pressure and temperature (typically 250°C and 5 MPa) (118,119). However, the harsh reaction conditions often lead to discoloration and to degradation of PUFA and oxygenated FFA (118,120). In addition, the Colgate–Emery process involves high energy costs, since about 800 kJ of energy is required per kg of oil processed (118,119). Although enzymatic lipolysis (reaction 1 of Fig. 1) has been investigated for treatment of several different oils to develop of a low-cost and environmentally safe alternative to the Colgate–Emery process, this approach would have a stronger impact in isolating the FFA listed in Table 1 because of their higher economic value (which would justify the materials cost of the biocatalysts) and the greater need to protect them from thermal degradation.

The use of lipases to recover oxygenated FFA *via* lipolysis is well-known, especially for ricinoleic acid (118,121). Although a nonpositionally selective, or "random," lipase would appear to be the rational choice, since ricinoleic acyl groups are present in all three glycerol positions in castor oil TAG, a 1,3-selective lipase is used to prevent formation of poly(ricinoleic acid) chains (122). Oils containing hydroxy acyl groups exclusively at the 1- and 3-TAG positions, such as lesquerella and dimorphotheca, are well-suited for 1,3-selective lipolysis. Immobilized *Rhizomucor miehei* lipase, or Lipozyme RM IM (Table 1), successfully hydrolyzed lesquerella oil partially diluted by isooctane in a stirred tank reactor at 60°C; maintenance of a high water activity for the reactor headspace through contact with a compartmentally separated saturated salt solution resulted in the liquid phase remaining saturated with the substrate water. Two-thirds of the maximum theoretical percent hydrolysis (66.7%) occurred, yielding 80% hydroxyl acids among the FFA (123). However, further hydrolysis reduced the purity of the hydroxy acids among the released FFA as a result of acyl migration (123). The occurrence of acyl migration would ultimately permit 90–100% hydrolysis (123). (As discussed in more detail in later sections, acyl migration refers to a nonenzymatic intramolecular acyl exchange within MAG and DAG, where acyl groups at the 2-glycerol position migrate to the 1- or 3-position or vice versa.)

Thermodynamic equilibrium drives the isomerization reactions in the directions of 2-MAG → 1(3)-MAG and 1,2 (2,3)-DAG → 1,3-DAG.] Lesquerolic acid-rich FFA were isolated from the residual MAG, DAG, and TAG by a saponification-extraction method at about 70–75% purity (124). Alternatively, lesquerella oil could be isolated from lipolysate by highly selective low-temperature crystallization (125). Derksen and coworkers (126–128) have successfully isolated dimorphecolic acid from dimorphotheca oil using a 1,3-selective lipase (from *R. javanicus*) in a hollow-fiber membrane reactor. The lipolysate was continuously recirculated in a stream that passed through a cold-trap unit, which selectively removed dimorphecolic acid by precipitation, thus enhancing product purity and shifting the thermodynamic equilibrium in favor of hydrolysis. The same approach was used to isolate erucic acid from the 1- and 3-positions of crambe oil TAG and vernolic acid from *Euphorbia lagascae* oil (127,129). FFA containing triple bonds, of which crepenynic acid (Table 1) serves as an example, is best released from TAG using Lipolase (*Thermomyces lanuginosa*) lipase (130).

Sehanputri and Hill (131) used lipolysis of corn oil by a "random" lipase to release 50% of acyl groups and achieve a high concentration of linoleic acid, taking advantage of the selectivity of lipases toward release of Δ9-unsaturated PUFA. Linoleic acid serves as a feedstock for CLA synthesis *via* chemical means.

In contrast to the PUFA, although erucic acid (22:1-13*c*) is not highly susceptible to thermal degradation *via* the Colgate–Emery process, there are inherent advantages to using hydrolysis to concentrate erucic acid *via* discriminatory lipolysis. For example, the strong discrimination of *G. candidum* lipase toward $C_{20}$ and $C_{22}$ FFA leads to the selective accumulation of erucic acid in the MAG/DAG/TAG during lipolysis of high erucic acid rapeseed (HEAR) oil (98% yield) (132,133). *Candida rugosa* lipase was not as discriminatory against erucic acid as was *G. candidum* lipase; however, precipitate formed that consisted of nearly 100% 1,2-erycyl DAG when lipolysis occurred at ~10°C (132,133). The recovery of erucic acid in the form of 1,2-erycyl DAG was lower than that resulting from *G. candidum*-directed lipolysis (132,133). The use of *G. candidum* lipase to selectively isolate erucic acid among the FFA fraction *via* the esterification of FFA derived from HEAR oil (produced *via* lipolysis using *P. cepacia* lipase), an approach discussed in the next section, resulted in a FFA product containing 85.4% erucic acid (134).

Lipases have been used to isolate PUFA with Δ3–Δ6 unsaturation because of their inability to rapidly hydrolyze PUFA acyl groups in TAG (reviewed in Refs. 135,136). Moreover, the PUFA are retained in the MAG, DAG, and TAG molecules, whereas non-PUFA acyl groups constitute the majority of the released FFA. The latter can be easily removed from the former by forming and removing the sodium soaps of the FFA. (An alternate method similar in concept is selective alcoholysis of the TAG; see reaction 3 of Figure 1. The resultant MAG/DAG/TAG are isolated from the FA esters by short-path molecular distillation; see Refs. 135,137–139. Whether any major differences exist in the selectivity between hydrolysis and alcoholysis is not clear.) The choice is dictated by the economics and effectiveness of the downstream purification. A summary of the most successful lipolytic isolations of PUFA is contained in Table 3. Generally, *C. rugosa* lipase has been the most successful biocatalyst for carrying out this task; however, degrees of purification have been rather low due to low to moderate degrees of hydrolysis. The latter may be caused by the occurrence of two or more Δ3–Δ6 acyl groups in many of the TAG molecules, which reduces their molecular flexibility to adopt a conformation that can fit within the active site of lipases (150–152).

**TABLE 3**
**Isolation of PUFA *via* Lipase-Catalyzed Hydrolysis of TAG**

| FFA product | TAG source[a] | Lipase | Result |
|---|---|---|---|
| AA | Single-cell (fungal) oil (25%) | *Candida rugosa* | 60% in recovered acylglycerols; 75% yield (two-step hydrolysis) (140) |
| DHA, EPA | Fish oils (20–30%) | *C. rugosa* | 56–75% in recovered acylglycerols; 63–90% yield (141–146) |
| GLA | Borage (23.6%) or evening primrose (9.4%) oil | *C. rugosa* | 46–51.7% in recovered acylglycerols; 59–90% yield (147–149) |
| 22:1-5*c*; 22:2-5*c*,11*c* | Meadowfoam oil (83.5%) | *C. rugosa* | 88.9% in recovered acylglycerols; 95% yield (150) |
| 20:3-5*c*,11*c*,14*c*; 20:4-5*c*,11*c*,14*c*,17*c* | *Biota orientalis* oil (15.6%) | *C. rugosa* | 40.8% in recovered acylglycerols; 64% yield (111) |

[a]Numbers in parentheses contain the amount of desired acyl group in the TAG source. For abbreviations see Tables 1 and 2.

The degree of discrimination has not been strongly affected by the type of reaction medium (150). The inclusion of a second step of discriminatory hydrolysis on the recovered MAG/DAG/TAG (subsequent to FFA removal) has led to only a modest improvement of purification (at the cost of reduced PUFA recovery) (140,144).

Lipase-catalyzed selective esterification has been more effective for PUFA isolation (discussed in the next section). *Pseudomonas* sp. lipases have generally been the most successful for achieving a high-percentage hydrolysis of PUFA-containing oils (79% or greater); thus, in many of the investigations cited, (135,136) *Pseudomonas* sp. lipolysate has been subjected to selective lipase-catalyzed esterification. The PUFA-rich MAG/DAG/TAG fraction resulting from selective *C. rugosa* lipolysis has been esterified with PUFA-rich FFA produced by a previous lipase-catalyzed selective esterification step to create PUFA-enriched TAG (144,147,149) (discussed below).

A comprehensive analysis of the methodology used to conduct lipolysis is beyond the scope of this paper. Reviews relating to reactor design and control of the operating parameters are available elsewhere (153,154). However, when one is designing a lipolysis bioreactor, the following criteria should be considered: (i) a means of isolating lipases from the reaction medium upon completion of the reaction, or retaining the lipases in a fixed position within the reactor (e.g., immobilized enzymes); (ii) a means of efficient mass transfer between aqueous and nonaqueous phases and a solid-phase lipase; (iii) a means of compartimentalizing or rapidly separating the aqueous and nonaqueous phases upon completion of the reaction and/or during the time course of the reaction; and (iv) related to item (iii), the continuous removal of the by-product glycerol to enhance the degree of lipolysis.

Membrane bioreactors have gained popularity because they compartmentalize the water and oil phases, hence facilitating continuous removal of the glycerol (143,154). However, overcoming the barrier to mass transport presented by the membrane and having an efficient means of immobilization of the lipase at the membrane surface are challenges that must be met. Reaction media based on emulsions provide high interfacial mass transport rates as well as interfacial area, but they possess the disadvantages of difficult isolation of the lipase, slow demulsification, and continuous energy input to maintain emulsion stability. Demulsification is made more difficult by the formation of FFA, a surface-active agent, during lipolysis. A process recommended by the author is to use a stirred-tank batch reactor containing immobilized lipase and water-saturated TAG/oil phase, with a means of continual water resaturation of the lipophilic liquid phase, e.g., by transporting the water molecules in a closed system from an external vessel containing a high-water-activity saturated salt solution to the lipophilic substrate solution through a shared vapor phase (123).

*Isolation of PUFA* via *selective esterification.* The use of lipase-catalyzed selective esterification (reaction 2 of Fig. 1) to isolate PUFA is summarized in Table 4 and reviewed in References 135, 137, 138, 173, and 174. Generally, this approach has been much more successful for PUFA isolation than has selective lipolysis. *Rhizopus* sp., *G. candidum*, *C. rugosa*, and *Chromobacterium viscosum* lipases are the most effective biocatalysts. Selective esterification typically occurs in a stirred-batch reactor containing the FFA source, an acyl acceptor, and the biocatalyst. One frequently desires to stop the reaction before 100% conversion occurs at a time that corresponds to an optimal extent of reaction (e.g., by removing the solid-phase biocatalyst *via* sedimentation). The subsequent step is to separate the (unreacted) PUFA-rich FFA from the FA ester by forming the insoluble soaps of the former using an alkaline aqueous solution. Alternatively, if long-chain acyl acceptors are used, short-path molecular distillation will separate fractions of unreacted alcohol, FFA, and FA esters. Frequently, an additional cycle of lipase-catalyzed selective esterification is applied to the PUFA-enriched FFA fraction recovered from the first cycle, which improves the purity of PUFA at the expense of reduced yield.

Uurea inclusion compounds (UIC) have also been used to purify the PUFA further (111,141,149,155,160,170,175). As reviewed elsewhere (176–178), this method involves the formation of solid-phase inclusion compounds that selectively remove FFA with long acyl chain lengths and a small degree of unsaturation *via* encapsulation. Both lipase-catalyzed selective esterification and UIC-based fractionation are complementary toward the isolation of PUFA, since the former removes oleic, linoleic, and linolenic acids *via* ester forma-

**TABLE 4**
**Isolation of FA *via* Lipase-Catalyzed Esterification**

| FFA product | FFA source[a] | Acyl acceptor; lipase type | Result |
|---|---|---|---|
| AA | Single-cell (fungal) oil (25%) | Lauryl alcohol; *Candida rugosa* lipase; two-step esterification | 75% AA in FFA; 71% yield (155) |
| CLA[b] | Derivatived from linoleic acid (95–98) | 1-Octanol or lauryl alcohol; *Geotrichum candidum* or *C. rugosa* lipase | 80–97% of 9*c*,11*t* isomer in esters; 82–89% 10*t*,12*c* isomer in FFA (156–161) |
| DHA, EPA | Fish oils (20–30%) | 1-Butanol; *Rhizopus* sp. lipase | 73–92% DHA and EPA in FFA; 60–84% yield (142) (reviewed in Refs. 135,137,162) |
| GLA | Borage (23.4%) or evening primrose (9.6%) oil | 1-Butanol, *R. miehei* or *G. candidum* lipase | 84–94% GLA in FFA, 64–75% yield (121,147,163–170) |
| Gorlic acid 18:2 (6*c*15*c*,cycl-14-18) | *Hydnocarpus wightiana* oil (12%) | 1-Butanol; *R. arrhizus* or *R. miehei* lipase | 70–80% enrichment (171) |
| Petroselinic acid (18:1-6*c*) | Coriander (*Coriandrum sativum*) oil (15.3%) | 1-Butanol; *G. candidum* lipase | 97% in FFA; 93% yield (121) |
| 20:1-5*c*; 22:2-5*c*,13*c* | Meadowfoam (*Limnanthes alba*) oil (83.5%) | 1-Butanol; *G. candidum* or *Chromobacterium viscosum* lipase | 95–97% 20:1 and 22:2 in FFA; 98–100% recovery (121,150) |
| 20:3-5*c*,11*c*,14*c*; 20:4-5*c*,11*c*,14*c*,17*c* | *Biota orientalis* oil (15.6%) | 1-Butanol; *C. rugosa* lipase | 61.5% 20:3 and 20:4 in FFA, 93% yield (111) |
| 16:0, 18:0*c* | Saturated FFA-rich product from palm oil FFA (87.8% 16:0, 8.7% 18:0) | Ethanol, *Burkholderia cepacia* lipase | 98.3% of 16:0 in ester fraction, nearly 100% yield (172) |

[a]Numbers in parentheses contain the amount of desired acyl group in the FFA source.
[b]Fractionation of 18:2-9*c* and 11*t*; 18:2-10*t*,12*c*, present at a 1:1 mole ratio.
[c]Fractionation of 16:0- and 18:0-rich acetone precipitate of palm oil FFA.

tion, whereas the latter selectively removes saturated and long-chain FFA.

The ability of lipases to discriminate against Δ3–Δ6 PUFA when forming alkyl esters has been used with reactions other than esterification. For instance, lipase-catalyzed alcoholysis (reaction 3 of Fig. 1) was applied to a FA ester mixture possessing an alcohol group that was significantly different in M.W. from the alcohol substrate used in the reaction (135,138,174). The FA esters were formed by alcoholysis of a PUFA-rich oil by *Pseudomonas* sp. lipase in a previous step (135,138,174). Short-path molecular distillation was used to fractionate unreacted alcohol type 1, FFA, and MAG/DAG/TAG after the first step, and unreacted alcohol type 2 (PUFA-rich) esters of alcohol type 1, and esters of alcohol type 2 after the second (selective esterification) step (135,138,174). A second alternative to esterification is the selective lipase-catalyzed hydrolysis of a FA ester mixtures, which isolates the PUFA among the unreacted esters (159,179).

In terms of choice of acyl acceptor, a comparative study found that among the 1-alkanols lauryl alcohol was optimal (180). When selecting the acyl acceptor, one must keep in mind that *n*-alkanols of chain length 1–4 can partition significantly into the microaqueous phase of the biocatalyst, resulting in a loss of activity, particularly when the acyl acceptor is present at high concentrations. However, short-chain alkanols have the advantage of being recovered from the reaction mixture *via* evaporation under reduced pressure, which is of relevance when a molar excess of acyl acceptor is used. A fed-batch approach, whereby a short-chain acyl acceptor is added in small-batch increments, is recommended. The mole ratio of acyl acceptor to FFA should be optimized for the given reaction; however, a stoichiometric excess of at least 100% for the acyl acceptor is common. Temperature and biocatalyst loading are other parameters that must be optimized (174). Selective esterification for the isolation of PUFA has been scaled up to kilograms of acyl donor (174).

Lipase-catalyzed selective esterification has been used recently to fractionate PUFA species present in the same mixture. Haraldsson and coworkers (181) utilized selective esterification to separate DHA from EPA. Fish oil-derived FFA was esterified with glycerol using immobilized *R. miehei* lipase in solvent-free media. The unesterified FFA contained 78% DHA and only 3% EPA, with a 79% recovery of DHA; the esters contained the majority of the EPA and the other acyl groups, with a 91% recovery of EPA (181). Lipases have also successfully fractionated the two most abundant species of CLA: 18:2-9*c*,11*t* and 18:2-10*t*,12*c* (Table 4).

*Structured TAG.* As reviewed in References 182–192, structured TAG can be defined as TAG containing mixtures of short- ($C_1$–$C_4$), medium- ($C_6$–$C_{12}$), and long- ($C_{14}$ or higher) chain acyl groups, with a given acyl group type frequently being confined to either the 1(3)- or 2-position on the glycerol backbone. TAG rich in medium-chain acyl groups have many applications, including in clinical treatments for patients with lipid absorption or digestion disorders and as high-energy nutraceuticals for athletes. The medium-chain acyl groups are readily metabolized *via* the portal vein and generally are not stored in adipose cells for long-term use.

One of the earliest commercial examples of structured TAG is Salatrim™ from Nabisco (now being manufactured by Danisco Cultor for use in dessert foods and salad dressings, among other uses). It consists of TAG of random positional distribution containing short- and long-chain acyl groups; each TAG molecule contains at least one long-chain saturate (e.g., 18:0) and one short-chain acyl group (193–195). Salatrim provides approximately one-half of the calories per serving compared with common fats. Other early examples are Caprenin™ and Caprucin™ from Procter & Gamble, which consist of TAG containing $C_8$, $C_{10}$, and behenic (22:0) acyl groups and $C_8$ and erucic acyl groups, respectively, with random positional distribution of the acyl groups. Caprenin, possessing physicochemical properties similar to cocoa butter, was used in M&M/Mars products (New Snickers™ bars) in the mid 1990s. Its use was discontinued shortly thereafter because clinical studies suggested it led to increased levels of LDL (or "bad") cholesterol (indicating enhanced risk factors for coronary disease) and decreased HDL (or "good") cholesterol levels. Captex 810-D™ (Abitec Corp, Columbus, OH) is similar to Caprenin; it contains random TAG of the 8:0, 10:0, and 18:2 acyl groups and is used for skin care, in lipsticks, and as a fat substitute. Fresenius-Kabi AB (Uppsala, Sweden) manufactures structured lipids (interesterified coconut oil and soybean oil) as components in their Structolipid™ product for parenternal delivery of nutrients.

There also has been expanded interest in structured TAG that segregate medium-chain (or short-chain) from long-chain acyl groups by their acylglycerol position [2- vs. 1(3)-], hence the interest in using 1,3-selective lipases to direct their syntheses. Examples are given in Table 5 and can be categorized as follows: (i) cocoa butter substitutes (1-palmityl,2-oleyl,3-steryl TAG, or P-O-S); (ii) infant formulas (palmitoleyl acyl group in the 2-acylglycerol position); and (iii) dietary supplements (1,3-medium-chain acyl group, 2-unsaturated acyl group TAG).

The use of 1,3-selective lipases to create a substitute for cocoa butter, an expensive and value-added product used in the manufacture of chocolate, from palm oil midfraction, an

**TABLE 5**
**Examples of Structured Triacylglycerols of Commercial Relevance[a]**

| TAG | Application | Approach |
|---|---|---|
| P-O-S | Cocoa butter substitute | Palm oil midfraction (P-O-P) + S-FFA; 1,3-selective lipase (196–198) |
| M-S-M, O-S-O | Margarine substitutes | S-S-S + $C_8$- or O-FFA; Lipozyme RM IM[b] (199) |
| MC-P-MC | Infant formula | P-P-P + MC-EE; papaya latex lipase (200) |
| Pu-P-Pu | Infant formula | P-P-P + PUFA-rich EE; Lipozyme TL IM[b] (201)<br>Butterfat + PUFA-rich FFA; Lipozyme RM IM[b] (202)<br>P-P-P + AA-FFA; immobilized *R. delemar* lipase (192) |
| O-P-O; Li-P-Li | Infant formula | Lard + soybean oil FFA; Lipozyme RM IM[b] (203)<br>P-P-P + O-rich FFA; Lipozyme RM IM[b] (172,204)<br>P-rich 2-MAG + O-FFA; *Rhizopus* sp. lipase (205,206) |
| MC-O-MC; MC-Li-MC, | Rapidly adsorbed fat with essential FFA in 2-position; treatment for pancreatic insufficiency | O-rich 2-MAG + $C_8$-FFA, immobilized *Rhizopus* sp. lipase (207)<br>Canola, olive, corn, or sunflower oil + $C_8$-FFA; Lipozyme RM IM[b] (201,208–211)<br>Chicken fat TAG + $C_8$-FFA; *Carica papaya* latex lipase (212)<br>LEAR oil + $C_6$ FFA-rich TAG; Lipozyme RM IM[b] (213)<br>LEAR oil + $C_{10}$-FFA; Lipozyme RM IM[b] (214)<br>O-O-O + $C_{12}$-FFA, Lipozyme RM IM[b] (215)<br>O-O-O + $C_8$-FFA; Lipozyme RM IM[b] (216) |
| MC-AL-MC | Rapidly adsorbed fat with essential FFA in 2- position | Perilla oil + $C_8$-FFA; Lipozyme RM IM[b] or Lipozyme TL IM[b] (217,401) |
| MC-Pu-MC | Rapidly adsorbed fat with essential FFA in 2- position | Fish oil + $C_{10}$-FFA; Lipozyme RM IM[b] (218)<br>Borage oil + $C_8$-FFA; Lipozyme RM IM[b] (219)<br>Tuna oil + $C_8$-FFA; immobilized *R. delemar* lipase (220)<br>Menhaden oil + $C_8$-FFA; Lipozyme RM IM[b] (221)<br>EPA-rich TAG + $C_8$-FFA; Lipozyme RM IM[b] (222)<br>Fish oil + $C_{10}$-FFA; Lipozyme RM IM[b] (223)<br>Pu- and $C_8$-rich TAG + $C_8$-EE; Novozyme[b] (224) |
| CL-Pu-CL | Nutraceutical | Menhaden oil + CL-EE; *B. cepacia* lipase (237) |
| CL-MC-CL | Nutraceutical | $C_8$-$C_8$-$C_8$ or coconut oil + CLA; Lipozyme RM IM[b] (402) |
| S-Ac-S | Low-calorie fat | Hydrogenated soybean oil + Ac-Ac-Ac; Lipozyme RM IM[b] (225) |
| Mixed ($C_8$, Er, $C_8$) | Caprucin™ mimic | Erucic acid-rich MAG + $C_8$-FFA; *B. cepacia* lipase (226) |

[a]Abbreviations for acyl groups: Ac, acetyl; AL, α-linolenic acid; $C_i$, saturated acyl group of chain length *i*; CL, conjugated linoleyl; Er, erucic; Li, linoleic (18:2); MC, medium chain (6:0, 8:0, 10:0, or 12:0); O, oleic; P, palmitoyl; Pu, polyunsaturated (DHA, EPA, GLA). Other abbreviations: EE, ethyl ester; LEAR, low erucic acid rapeseed.
[b]Identified in Table 1.

inexpensive material, was conceptualized and demonstrated by Macrae in the 1980s (120). The basic approach was the acidolysis of 1,3-palmityl,2-oleyl glycerol (POP)-rich TAG with stearic acid to create POS and SOS (1,3-dipalmityl,2-oleyl TAG), abundant TAG species in cocoa butter (120,190,227,228) (reaction 1 of Fig. 4). Regarding infant formula substitutes, OPO (1,3-dioleyl,2-palmityl TAG), a human milk fat substitute used in infant formulas for prematurely born and full-term infants, was the first commercial product in which lipases were used to create structured TAG (190,228,229) (Betapol™; Loders Croklaan BV, Wormerveer, The Netherlands). Betapol, which received generally regarded as safe (GRAS) classification from the U.S. Food and Drug Administration (FDA), was found to improve the absorption of dietary fat and calcium by infants and to reduce constipation. In addition, the product Bohenin™, which consists mostly of 1,3-behenyl,2-oleyl TAG, is manufactured by Fuji Oil Co. Ltd. (Osaka Japan) as a cocoa butter improver. It has also been suggested that infant formulas contain TAG with PUFA to enhance brain and nervous system development, and that the PUFA groups be located at the 1- and 3-positions to allow for proper digestion (230). The fact that groups at the 2-acylglycerol position are more strongly absorbed *in vivo* than those at the 1- or 3-position suggests that TAG with medium-chain acyl groups at the 1- and 3-positions and an essential acyl group, such as oleyl, linoleyl, DHA, or EPA, in the 2-position, may be a valuable nutraceutical as well as a nutrition source for patients with pancreatic deficiencies.

The main approaches for creating structured TAG are to interesterify a TAG using FFA or FA esters, or to transesterify long-chain-rich TAG with medium-chain TAG, both reactions catalyzed by 1,3-selective lipases (Fig. 4). An alternate approach developed by Schmid and coworkers (206,207) is to first perform 1,3-selective lipolysis of TAG, carefully isolate and crystallize 2-MAG to prevent acyl migration, and then 1,3-selectively esterify the 2-MAG. A 72–85% yield and

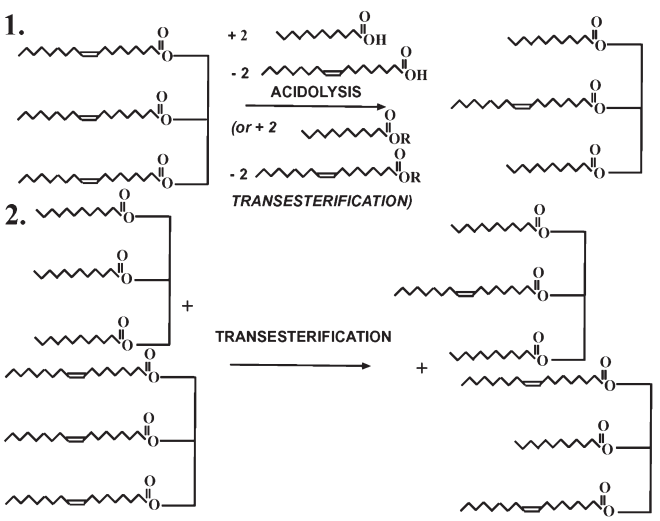


**FIG. 4.** Reactions catalyzed by 1,3-selective lipases to synthesize structured TAG.

>95% purity were obtained with such an approach. In addition, reactive separations were used to improve the yield of structured lipids. The acidolysis of TAG enriched with medium acyl chains by PUFA-rich FFA was enhanced by operating the reaction in a membrane bioreactor, which selectively permitted permeation of the released medium-chain FFA (231).

One of the most successful syntheses of structured lipids from raw lipid feedstocks was reported recently using glycerol and palmitic and oleic acid-rich FFA fractions of palm oil to produce OPO for infant formula (172). First, the palmitic acid-rich FFA fraction was further enriched by selective esterification by *B. cepacia* lipase, resulting in an ethyl ester product containing 98.3% palmitate, with the main impurity, stearic acid, remaining in the FFA fraction (172). Second, tripalmitin was synthesized by Novozym 435 (Novozyme, Inc.)-catalyzed glycerolysis of the ethyl palmitate-rich product (90% conversion), followed by removal of MAG and DAG by-products by column chromatography (172). Finally, tripalmitin was subjected to acidolysis by oleic acid-rich FFA, resulting in a product containing 97% TAG, of which 74% was OPO (172). This example demonstrates the important role of lipid separations in designing a scheme for synthesizing structured lipids from raw sources of FFA, as well as in using lipase-catalyzed selective esterification to purify PUFA.

When designing a reaction scheme for interesterification, the three main goals are high activity, low occurrence of the side-reaction hydrolysis (to prevent formation of DAG and MAG), and low occurrence of the acyl migration of DAG and MAG, which results in undesired products in the TAG fraction. A trade-off exists between a high percentage of interesterification and low degrees of acyl migration and hydrolysis; the former is promoted and the latter is hindered by an increase of the residence time, reaction temperature, and fatty acyl donor-to-TAG substrate ratio (232). An optimal water activity exists since an extremely low water content reduces biocatalytic activity, whereas a high water content promotes hydrolysis and enhances acyl migration (232). As reviewed elsewhere (233), acyl migration is also promoted by materials in the enzyme preparation, including the immobilization matrix. In a review, Xu (232) recommends the use of a continuous packed-bed bioreactor as opposed to a stirred-batch reactor since the former provides a lower extent of acyl migration due to the smaller residence time for substrates. A recent report illustrated that a lower degree of acyl migration occurred in supercritical $CO_2$ as compared with a solventless system operated under similar conditions (211). An additional problem with structured lipids prepared by lipases appears to be their relatively low oxidative stability (234–236).

*TAG enriched in PUFA.* As already discussed, TAG enriched in PUFA such as DHA, EPA, AA, and GLA would have applications as nutraceuticals in the specialty food and pharmaceutical markets (e.g., Clarinol™ and Marinol™, TAG rich in CLA and EPA/DHA, respectively, from Loders Croklaan B.V.). A common approach has been to use lipases, either 1,3-selective or "random," to enrich the content of

PUFA in TAG (or PUFA-enriched MAG/DAG/TAG *via* selective lipolysis; see Refs. 144,147,149,237) by interesterification using PUFA-enriched FFA (acidolysis, reaction 4 of Fig. 1) or FA methyl or ethyl ester (transesterification, reaction 5 of Fig. 1), or direct esterification between glycerol and PUFA-rich acyl groups (reviewed in Refs. 162,238,239). The problem of low yield is difficult to overcome because of the relatively poor selectivity of all lipases toward Δ3–Δ6 FFA, unless one uses a highly pure source of PUFA-rich acyl donor. *Pseudomonas* sp. (237,240) and *Rhizopus* sp. lipases (241,242) are among the least discriminatory toward Δ3–Δ6 acyl groups and are thus recommended for catalyzing the interesterification. [Novozym 435, a commercially available immobilized lipase from *C. antarctica* B listed in Table 1 (Novozyme, Inc.), has been successful for catalyzing interesterification at elevated temperatures; see Refs. 241–243.] An alternate approach recently applied by Hill and coworkers (29) was to use *C. antarctica* B lipase-catalyzed glycerolysis of PUFA-rich oil as a first step, resulting in the selective conversion of less PUFA-rich TAG into DAG and MAG. The resultant glyceride mixture was then interesterified using PUFA ethyl esters and the same lipase. To increase the yield of PUFA-rich TAG during acidolysis, the temperature can be reduced to ~10°C during the latter stages of the time course to remove the released FFA *via* crystallization, thereby thermodynamically driving acidolysis by PUFA in a forward direction (244).

*MAG and DAG.* MAG have numerous applications as biodegradable and biocompatible emulsifiers in the food, dairy, cosmetics (e.g., in toothpastes), and pharmaceutical industries (245,246). 1(3)-MAG are precursors for the synthesis of MAG sulfates, which are cosmetic surfactants with low irritability (247). MAG rich in lauric (dodecanoic) acid are potentially useful antibacterial compounds. As discussed above, 2-MAG are feedstocks for the lipase-catalyzed synthesis of structured lipids. Approval of MAG as GRAS was recently granted by the FDA (Code of Federal Regulations Title 21, Volume 3, Part 184.1505, http://www.accessdata.fda.gov/scripts/cdhrh/cfdocs.cfcfr/CFRSearch.cfm?fr=184.1505). DAG are used as additives to fats such as cocoa butter to reduce the extent of crystallization or "blooming." Isomerically pure DAG are possible feedstocks for the synthesis of GPL, glycolipids, and pharmaceuticals (247,248). Chemical means of MAG and DAG production (e.g., through glycerolysis directed by a heterogeneous catalyst), as reviewed elsewhere (4,249), involve high temperatures (~180–220°C, or 30–160°C if a high vacuum pressure of 200–400 Pa is applied; see Ref. 250) that may produce by-products that promote an off-flavor, -odor, or -color (248). Also, the product distribution from such a process is frequently broad, containing various MAG and DAG species, and perhaps TAG (substrate) since the yield is often low (245,248). Hence, molecular distillation must be applied, which increases process operating costs and can result in chemical degradation. Chemical means to produce regioisomerically pure MAG and DAG are tedious and not cost-effective for large-scale implementation (reviewed in Ref. 245). Therefore, enzymatic preparation of MAG and DAG may gain further interest if energy costs continue to increase, and will be of particular interest for products that contain degradation-susceptible PUFA and oxygenated acyl groups. A few examples of the lipase-catalyzed synthesis of PUFA (including CLA) (251–256) and hydroxyl (257,258) FFA-enriched MAG are contained in the literature.

As discussed more thoroughly in the cited reviews, the difficulty in synthesizing MAG *via* esterification and glycerolysis (Fig. 5) is the immiscibility of glycerol and the acyl donor, with the latter frequently acting as the bulk solvent. The use of polar solvents such as *tert*-butanol or acetone permits miscibility and increases the product distribution in favor of MAG rather than DAG (259), but lowers the catalytic rates of lipase relative to the nonpolar solvent phases. Common approaches to improve the miscibility of glycerol include polyhydric alcohol complexation agents such as phenylboronic acid (257), the use of protective groups such as isopropylidene [which "protects" two of the glycerol's three hydroxyls, resulting in regioisomerically pure 1(3)-MAG; reviewed in Ref. 247], water-in-oil microemulsions (260), and the suspension of silica gel saturated with glycerol (261), all of which are impractical for large-scale manufacturing. MAG can be removed from the reaction medium during the course of the reaction *via* selective adsorption or precipitation at moderately low temperatures, which enhances product yield by favorably shifting the thermodynamic equilibrium (reviewed in Ref. 245). *Penicillium camembertii* lipase applied to esterification will produce MAG and DAG but not TAG because of its unique substrate selectivity (255,262,263).

*Polyol esters.* Lipase has been used to catalyze FFA partial and FFA esters of glycols such as ethylene and propylene glycol, neopentanol, trimethylolpropane, and polyglycerol as low-cost and low-temperature alternatives to chemical processing for applications as biodegradable lubricants and emulsifiers in the food and cosmetics industry (Table 6). Perhaps the major value of lipases for synthesizing polyol esters

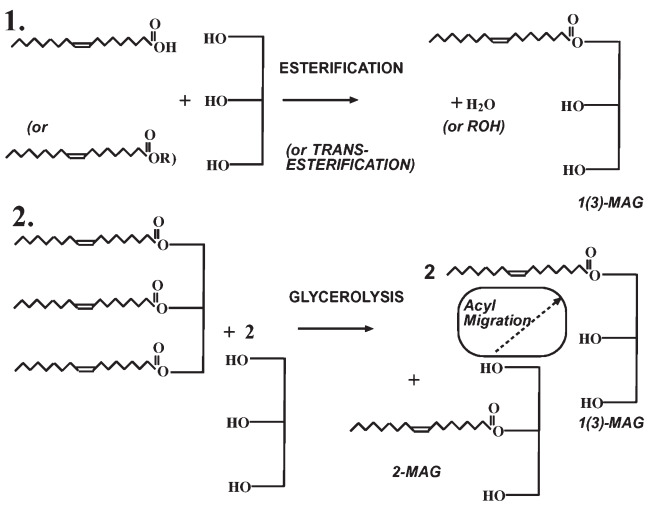


**FIG. 5.** Reactions catalyzed by 1,3-selective lipases to synthesize MAG.

compared with chemical procedures is the regioselective control of the product distribution. For instance, if one esterifies 1,2-propanediol using a 1,3-selective lipase, only the 1-monoester will be produced (268). In addition, parameters such as substrate ratio and water activity, among others, can be used to control the relative distribution of mono- and diesters (264,265).

Perhaps the regioselectivity of lipases has been most frequently applied in the synthesis of saccharide-FA esters as biodegradable and biocompatible surfactants (reviewed in Refs. 284,287). Sucrose esters, produced chemically at temperatures above 100°C and resulting in a broad product distribution (288–291), have numerous applications in the food and cosmetics industries as biodegradable and biocompatible surfactants (reviewed in Ref. 286). To use sucrose as an example, lipase from *C. antarctica* and *T. lanuginosa* can catalyze esterification at only the 6- and 6′-hydroxyl positions (with a preference toward 6-OH) (292–295). The use of isopropylidene protective groups to improve substrate miscibility (see foregoing discussion) leads to synthesis of only the 6′ esters since the protective group blocks access to the 6-hydroxyl (296). The 6′,6-*O*-diacyl ester occurred using Novozyme with a short-chain ($C_4$) acyl substrate but did not occur for a $C_{10}$ acyl substrate (295). Note that the 6- and 6′-positions constitute two of the three primary OH groups of sucrose. Generally, both nonselective and 1,3-selective lipases demonstrate a strong selectivity toward the primary OH groups. The third primary OH group, at position 1′, is esterified to a small extent (293). Of interest, a Spanish patent indicates the formation of 2-lauroyl sucrose monoester, an ester of a secondary –OH group, using vinyl esters as acyl donors and disodium hydrogen phosphate as catalyst at 40°C and 101 kPa (297).

Experimental approaches to lipase-catalyzed polyhydric alcohol ester formation follow along the lines already presented for MAG synthesis, mostly relating to overcoming the lack of miscibility between acyl donor and acceptor. Some additional approaches are worthy of mention. Hult and co-workers (298) report the use of room-temperature ionic liquids of high polarity for higher rates and conversions for glucose ester formation compared with results using polar solvents such as acetone. Second, the use of azeotropic mixtures of a polar and nonpolar solvent, such as ethyl methyl ketone and hexane, permits a

**TABLE 6**
**Polyol-FA Esters Synthesized by Lipases**

| Product | Approach | Applications |
|---|---|---|
| Ethylene glycol mono- and/or diesters of stearic acid (264) or lauric acid (265,266) | Esterification, hexane or isooctane as solvent, *Rhizopus* sp. lipases (264); use of ethylene glycol-based foam or reverse micelles (265); ethylene glycol/silica gel suspended in vinyl laurate, Lipozyme RM IM[a] (266) | Biocompatible surfactant |
| 1,2-Propanediol[b] monolaurate (267), monocaprylate (268), or mono-DHA or EPA (269) | Esterification, 65°C, no solvent, Novozyme[a] (267); polar solvents (268) or *tert*-butanol/hexane (269), Lipozyme RM IM[a] | Emulsifiers for cakes and whipped toppings |
| 1,3-Propanediol-oleic acid mono- and diester (270); lauric acid monoester (271) | Esterification, Lipozyme[a] (270); *R. delemar* lipase, reversed micelles (271) | Surfactant |
| Mono- and diesters of short-chain PEG and stearic or 12-hydroxystearic acid (272) | Esterification, no solvent (80°C), Novozyme[a]; PEG/silica gel suspended in vinyl laurate, Lipozyme RM IM[a] | Surfactants for cosmetics and pharmaceuticals |
| Diglycerol lauric acid monoester (273) | Esterification, no solvent, 80°C, Novozyme[a] | Biodegradable surfactants for foods, cosmetics, pharmaceuticals |
| Polyglycerol-oleic acid mono–tetra-esters (274) | Polyglycerol-saturated silica gel suspended in oleic acid methyl ester, Lipozyme RM IM[a] | Surfactants (275) |
| Trimethylolpropane-oleic acid (276) $C_8$ (277), or oligo(ricinoleic acid) triester (Kelly, A.R., and D.G. Hayes, unpublished data) | Alcoholysis, no solvent, immobilized lipase (276); esterification, dibutyl ether; Lipozyme RM IM[a] (277); esterification, no solvent, Novozyme[a] (Kelly, A.R., and D.G. Hayes, unpublished data) | Hydraulic fluid; lubricant |
| Erythritol lauric acid esters (278) | Esterification, acetonitrile, Novozyme[a] | Food product emulsifier |
| Sorbitol mono- and diesters (279–281) | Esterification, acetone (279,280), *tert*-butanol (282), or azeotropic mixture of hexane and *tert*-butanol (281), Novozyme[a] | Surfactants for foods, cosmetics, detergents, pharmaceuticals (“Span surfactants”) (283) |
| Xylitol monooleate (282,283) | Esterification, Novozyme[a], solventless | Surfactant |
| Fructose, glucose, sucrose, xylose, maltose, trehalose mono- and diesters (reviewed in Ref. 284) | Esterification or alcoholysis, polar solvent or solventless | Surfactants (285,286) |

[a]Identified in Table 1.
[b]Propylene glycol.

high reaction rate and allows removal of the esterification product, water, *via* azotropic distillation (299). Third, Schmid and coworkers (300–303) have successfully used "solid-phase synthesis" to produce saccharide monoesters at high yield. A polar solvent is used at a low temperature such that the monoester product precipitates from solution, driving ester synthesis in a forward direction and preventing diester formation. Fourth, Hayes and coworkers (304) have demonstrated that the presence of ~20% monoesters or greater significantly enhances the solubility of polysaccharides such that a polar solvent is not needed to induce miscibility. Eighty-five percent conversion of oleic acid and fructose at a 1:1 mole ratio of substrate using Lipozyme RM IM (Novozyme, Inc.) occurred when fructose was added in fed batch mode to stimulate increased maximum solubility of fructose during the time course of the reaction (Kelly, A.R., and D.G. Hayes, unpublished data). It should also be noted that glycosides, saccharide alkyl ethers produced *via* chemical and enzymatic means (glucosidases), are readily miscible with acyl donors and are thus effective acyl acceptor substrates (305–307).

*Monohydric alcohol esters.* Lipases have been used to synthesize wax esters such as oleoyl oleate as a substitute for jojoba oil, a well-known cosmetic agent (e.g., in antiwrinkle treatments for skin) (308–310), and for simple esters such as methyl- and ethyl-FA for use as diesel fuel (50,311–324), particularly from waste products such as lard, restaurant grease, and soapstock (reviewed in Ref. 320). It will be difficult for lipase-catalyzed reactions to be an economically viable choice in these areas in the short term because of the low selling cost of diesel fuel. FA alkanol esters are valuable materials for foods and fragrances; however, in most cases, there is no clear advantage to using lipases over chemical approaches. As an exception, Dordick and coworkers (325) presented a specific use of lipase-catalyzed reactions for this market that may be cost effective: the development of ester libraries from acyl donors and acceptors for their assessment as food flavors and fragrances. Also, lipases can esterify polymers of hydroxy FA without cleavage of the hydroxyacyl chains. Such esters possess improved properties as lubricant materials because of their increased viscosity indices (326,327; Kelly, A.R., and D.G. Hayes, unpublished data).

*Peroxidated FFA.* Peroxy FA, R-COOOH, formed by the esterification of FFA or by the alcoholysis of FA alkyl esters with $H_2O_2$, are oxidants used in the chemical industry to epoxidize double bonds, Baeyer–Villiger oxidation reactions, and the hydroxylation of aromatic rings and amines. Traditionally, peroxy acid synthesis occurs *via* catalysis using a strong acid; however, for long-chain and unsaturated FFA, harsh operating conditions (larger amounts of acid, higher temperatures) are required, and by-products frequently result. Thus, as reviewed elsewhere (328), lipase-catalyzed peroxy FFA formation may be a viable alternative. Enzymes are very susceptible to denaturation by $H_2O2$; however, Novozym 435 (Novozyme, Inc.) (Table 1) appears to possess the highest stability toward peroxide exposure, although it undergoes slow first-order inactivation (329,330). To reduce denaturation, a typical reaction involves the fed-batch addition of 30–60% aqueous $H_2O_2$ to the pure FFA or alkyl ester, and possibly the inclusion of a solvent such as toluene or dichloromethane. A novel approach is to continually feed a stirred-tank reactor with $H_2O_2$-saturated organic phase, conducted in an organic/aqueous mixer-separator vessel upstream of the reactor (329).

Of interest, during the time course of peroxidation of the unsaturated acyl groups, self-epoxidation, a nonenzymatic reaction, occurs concurrently (70–90% yield of epoxy-FFA from the original FFA or alkyl ester). For example, the intermediate product peroxyoleic acid self-epoxidizes into 9–10-epoxystearic acid. Lipase catalysis accompanied by self-epoxidation can result in epoxy-rich TAG, which are potentially important materials for paints, coatings, and disinfecting agents (sporicides) (331). Recently, the same approach was used to form a polymer containing epoxidized fatty acyl grafting (332).

LOX catalyze the formation of HPOD from PUFA such as ALA and AA. HPOD have a variety of applications (reviewed in Refs. 47,51,333–335) and can be easily reduced chemically into hydroxy acids, resulting in a ricinoleic acid substitute. HPOD are valuable precursors for prostaglandin and leukotriene synthesis; can act as fungicides in agricultural applications; are precursors of flavor ingredients such as ketones, alcohols, and aldehydes (*via* enzymatic cleavage); are feedstocks to create oxiranes; and are chiral synthons of possible drug discovery interest.

As discussed above, generally high conversions have been achieved using biphasic media in a pressurized container with an oxygen-rich atmosphere. Immobilization of LOX has led to increased thermostability and permitted reusability; however, a highly active and stable preparation has not been achieved. LOX has been suggested for inclusion in multienzyme systems: with lipase to convert TAG into HPOD (336), and with catalase to generate $O_2$ from the reduction of $H_2O_2$ (337).

## TOCOPHEROLS, STEROLS, AND STERYL ESTERS

Tocopherols, sterols, and stanols (depicted in Fig. 6) are minor components in seed oils and possess many applications in foods and pharmaceuticals as natural antioxidants (338). The most common plant sterol, or phytosterol, is ergosterol, whereas the most common animal sterol, and the one of greatest significance, is cholesterol. Phytosterols help lower the cholesterol level in blood, serve as feedstocks for hormone drugs, and help increase the response time and effectiveness of white blood cells against cancer (reviewed in Refs. 339–341). In addition, they are known to be effective in treating pulmonary tuberculosis, rheumatoid arthritis, HIV, and immune stress in marathon runners. Sterol esters possess the same capabilities but are oil-soluble, recently receiving GRAS status by the FDA for use in margarines and margarine substitutes [e.g., Benacol™, the first cholesterol-reducing margarine, from the Raisio Group (Raisio, Finland), which contains ~9% sitostanol esterified with acyl groups from canola oil, and Take Control™ from Unilever US

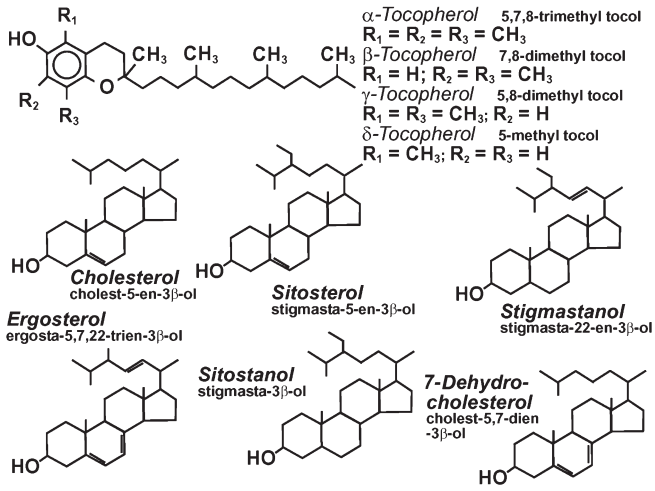


**FIG. 6.** Tocopherols, sterols, and stanols commonly found in the deodorizer distillates produced by purification of oils from the seeds of common crops.

(Englewood Cliffs, NJ)]. Tocopherols (e.g., α-tocopherol, a potent form of vitamin E) are well-known and commonly used antioxidants.

Tocopherols, sterols/stanols, and the esters of the latter, although present in nuts and in green, leafy plants, are mainly recovered from oilseeds during the deodorization process within the volatile by-product known as "deodorizer distillate" at amounts between 2 and 20%, with FFA, MAG, DAG, and TAG being the major components at ~30–50% (341,342). Isolation of the sterols and tocopherols from deodorizer distillate in high yield and purity remains a challenge. For instance, sterols and tocopherols possess similar M.W., making their separation by molecular distillation difficult. Extractive isolation of tocopherols by cold ethanol does not produce high yield and purity. Lipase-catalyzed esterification and hydrolysis have been proposed as alternatives. Shimada and coworkers (343) applied *C. rugosa* or *Pseudomonas* sp. lipase to soybean oil deodorizer distillate to efficiently esterify stigmastanol to its ester and concurrently hydrolyze MAG. The sterol esters can be isolated from the reaction mixture *via* molecular distillation (80%), but the latter method will not separate FFA and tocopherols. The subsequent addition of methanol to the lipase-treated deodorizer distillate in the presence of lipases results in the formation of FAME without modification of the steryl esters; FAME can be separated from tocopherols (and sterol esters) *via* molecular distillation (344). To isolate naturally occurring steryl esters from the deodorizer distillate, molecular distillation is applied to isolate sterol esters in a fraction rich with DAG and TAG; lipolysis is then conducted to selectively hydrolyze DAG and TAG, resulting in a mixture from which steryl esters are readily purified using molecular distillation (345). However, acyl exchange between glycerides and sterols occurs during the lipolysis process (345). Chu *et al.* (346) increased the concentration of a-tocopherol in palm distillate from 1–7% to 7–10% by Novozym 435 (Novozyme, Inc.)-directed lipolysis followed by removal of the FFA as salts.

Lipases have been used to transform cholesterol, dihydrocholesterol, 7-dehydroxycholesterol, sitosterol, stigmastanol, and ergosterol to their respective esters in yields from 70 to 100% (341,342,347–351). The typical reaction conditions consist of solventless systems using an acyl donor (e.g., methyl oleate or oleic acid) in stoichiometric excess and thermophilic immobilized lipases (or surfactant-coated lipases) at elevated temperatures (60–80°C) with means of maintaining low water activity. For polyhydroxylated steroids, regioselective esterification was observed (352).

The dietary requirement of ascorbic acid, or vitamin C, as a natural antioxidant is well known. Ascorbic acid esters, prepared regioselectively by lipase-catalyzed esterification or interesterification with FA alkyl esters, are potential lipophilic antioxidants with many current and potential uses, e.g., as antibrowning agents in apple juice, as sunscreen ingredients, and in the prevention of the linoleic acid oxidation (298,353–357).

## GPL-BASED PRODUCTS FROM ENZYME REACTIONS

*Lecithin and its applications.* GPL, depicted in Figure 3, are membrane lipids containing a glycerol backbone; unlike TAG, the third glycerol position contains a highly polar or charged group attached through a phosphodiester bond. The carbon at the 2-position of GPL is a chiral center; natural GPL exhibit L-optical rotation. The majority of GPL for commercial use are derived from soybeans during the degumming of the oil and are referred to as lecithin (present at only 3% in the oil). As reviewed elsewhere (358,359), crude lecithin, containing only 52% GPL (of which 23–35% is PC) and 35% TAG/DAG/MAG/FFA, is used for several industrial applications, e.g., as adhesives, adsorbants, animal feed, and mold-release agents for plastics, resins, concrete, and ceramics. The majority of the acyl groups contained within the GPL of lecithin are common $C_{16}$ and $C_{18}$ saturates or Δ9 unsaturates. Approximately 20% of the GPL produced from lecithin is used industrially; the majority of its use is in foods, cosmetics, and pharmaceuticals. Food-grade lecithin, produced by deoiling crude-grade lecithin *via* acetone extraction, contains 78% GPL. It has many applications, including as an emulsifier in cake mixes, cheese, candy, chocolate, dehydrated foods, ice cream, pasta, whipped toppings, and margarine, and as a nutritional supplement for fat adsorption, cholesterol metabolism, blood clotting, nervous system maintenance, pulmonary function, vision improvement, and prostaglandins biosynthesis. Ethanol-induced precipitation can be applied to food-grade lecithin to produce PC at 80–100% purity; the precipitant is rich in PE and PI. Lab-scale deoiling and ethanol extraction procedures have recently been published (360). Purified PC has specific applications as a precursor to acetylcholine, a neurotransmitter, and has been suggested as an additive to infant formula. PS, present at <1% in lecithin, is essential to nerve cell functioning in the brain (a "booster" for memory; see www.springboard4health.com), and effective in the treatment of dementia and Alzheimer's disease. Commercial PS prod-

ucts include Leci-PS™ from Lucas-Meyer, Inc., now the Texturant System Division of DeGussa (Atlanta, GA), and Brain Gum™ from Gum Tech International (Phoenix, AZ). PA is a common ingredient in cooking oils to prevent vegetable oils from burning during cooking; it leads to lower odor and off-color formation compared with PC (361). Phosphatidylglycerol (PG) is reported to be a more preferred surfactant than PC because of the ability of PG to form more hydrogen bonds (362). DHA-containing GPL may have potential medical applications for instance in promoting cell differentiation in leukemia, enhancing the survival of tumor-bearing mice, and preventing cerebral apoplexy. Additional sources of GPL include egg yolk and brain tissues.

*GPL products from enzymatic reactions.* Perhaps the application for which the selectivity of lipases and phospholipases would be of the most value is for the synthesis of highly pure GPL of a specific structure, i.e., containing specific acyl groups at the 1- and 2-position and a specific head group at position 3, for use in liposomes or gels as controlled-release agents in pharmaceutical, food, and cosmetics products. As reviewed elsewhere (363–365), an improved understanding of the relationship between GPL structure, the micro- and nanoscale structure of liposomes and gels, and release kinetic properties is taking shape. A related and more recent application is the use of GPL to solubilize membrane proteins for crystallographic mapping and biochemical experimentation.

The use of lipases and phospholipases to synthesize GPL of commercial interest is reviewed elsewhere (366–368). Perhaps the most common application is the hydrolysis (or alcoholysis) of GPL by $PLA_2$ to produce 1-acyl-lyso-GPL. Similar to its GPL parent, lyso-GPL is surface active but is more hydrophilic, hence more water-soluble. It also may be a useful component of liposomes. Along the same lines, $PLA_2$-catalyzed hydrolysis of GPL has been used to improve the degumming performance (369,370). Hydrolysis (or alcoholysis) of GPL by lipases results in the rapid formation of 2-acyl-lyso-GPL. Similar to 2-MAG, acyl migration of 2-acyl-lyso-GPL will occur, resulting in formation of the more stable 1-acyl-lyso-GPL; subsequently, lipase can cleave the ester bond at the 1-position, resulting in the formation of glycerol-GPL, a product of lower value. To avoid glycerol-GPL formation, Vulfson and coworkers (371) recommend rapid lipolysis of GPL, followed by removal of the lipase, then treatment with ammonia vapors to catalyze acyl migration into the more desired and more stable 1-acyl-lyso-GPL. Chemical methods of hydrolysis, such as acid-catalyzed hydrolysis by tetrabutylammonium hydroxide (372), will not selectively hydrolyze either the 1- or 2-acyl group. $PLA_2$ and lipases prefer PC over PE and PE, over PI and PA as the substrate (360).

Lipase-catalyzed interesterification or acidolysis in nonaqueous media (using a several-fold molar excess of acyl donor) has been used to produce GPL with specific acyl groups in the 1- and 2-acylglycerol positions for testing as amphiphiles in liposome formulations and for possible similar dietary applications as structured TAG (360,373). For instance, the body will absorb PUFA more readily when contained in the 2-position of GPL as compared with PUFA-enriched TAG (368); also, this "structured GPL" may have an enhanced effect on brain function as well as being anticancer and weight loss agents (368,374). Acyl migration and hydrolysis yield by-products, particularly for $PLA_2$-catalyzed reactions, because this enzyme requires a higher water activity for efficient operation (360,367). Yields are often slightly lower than desired because of the further hydrolysis of lyso-GPL into glycero-GPL, which precipitates from nonaqueous media (367). A two-step acyl exchange process, lipase-catalyzed hydrolysis, followed by lipase-catalyzed esterification, results in a more rapid acyl exchange than does lipase-catalyzed acidolysis but is more laborious (367). The extent of hydrolysis (60–75% yield of GPL) and acyl incorporation (38–53%) is similar for both approaches; almost all of the incorporation occurred at the 1-position, particularly when using immobilized 1,3-selective *Rhizopus* lipases (367). $PLA_2$ cannot perform acyl exchange *per se* since mechanistically it cannot form acyl-enzyme intermediates, but it can achieve this goal through a two-step hydrolysis/esterification process similar to that described for lipase.

The selling cost of highly pure "structured" GPL, mainly used as components of liposomes for drug delivery, is prohibitive. To tailor-make GPL with specific acyl groups in the 1- and 2-positions and a specific head group attached to the phosphate group for their assessment as liposome components, one author recently applied the term "toolbox" to the collection of enzymes (1,3-selective lipase, $PLA_2$, and PLD) needed to realize this goal (360). A recent publication demonstrated this approach in the creation of 1-decanoyl,2-hexanoyl PC from egg yolk PC (375). First, egg yolk PC was isolated from hen's eggs and was then hydrolyzed by Lipolase (Novozyme, Inc.), a commercially available porcine pancreatic $PLA_2$ product (Table 1), resulting in a crude 1-acyl lyso-PC product (80% conversion). Highly pure 1-acyl lyso-PC was subsequently obtained from the crude product by ethanol extraction (to isolate it from FFA), followed by silica gel column chromatography as a "polishing" step (to remove residual PC, PE, and lyso-PE). Hexanoic acid was subsequently incorporated into the 2-position *via* esterification catalyzed by immobilized Lipolase in toluene, resulting in a 49% yield and 7% acyl migration by-product. This reaction used a decreasing water activity program to optimize the yield. The acyl group at the 1-position of 2-hexanoyl PC was selectively removed by ethanolysis; this position was then esterified using decanoic acid. Both steps were catalyzed by a 1,3-selective immobilized *R. oryzae* lipase. The product, 1-decanoyl,2-hexanoyl PC, was 99% pure; the yield from crude egg yolk PC was 1%.

PLC has been used sparingly to produce products of interest. In all cases, it has been applied for hydrolysis. Anthonsen *et al.* (376) used covalently immobilized PLC to hydrolyze GPL, resulting in optically pure 1,2-DAG, which can serve as a diagnostic reagent for investigating signal cascades in medical biochemistry and as a feedstock for the synthesis of GPL. Other possible applications of PLC include resolution of racemic mixtures of GPL and the formation of organic

phosphate products from the released head group. For example, *O*-alkyl inositol 1-phosphates were produced from PI *via* two synthetic steps catalyzed by PLC. The first step, the hydrolysis of PI, resulted in the release of inositol 1-phosphate. PLC-catalyzed esterification of inositol 1-phosphate using an alkyl alcohol resulted in the final product.

PLD has been used with moderate success to catalyze the hydrolysis and transphosphatidylation of PC, the former resulting in the formation of PA and the latter in the introduction of new head groups (reviewed in Refs. 53,55). Transphosphatidylation is modeled by a ping-pong mechanism, similar to the acyl exchange catalyzed by lipases (377). The PLD-catalyzed conversion of PC into less-abundant and commercially relevant GPL, particularly PS and PG, has been used commercially (378–382). The formation of GPL with unnatural head groups, and the introduction of head groups into alkyl phosphates that lack the glycerol backbone, has occurred *via* catalysis with PLD.

Most recent reports of transphosphatidylation have used *Streptomyces* sp. PLD and biphasic media (e.g., water–ethyl acetate or water–ethyl ether). With such media being rich in water, the hydrolysis side-reaction, yielding PA as the by-product, occurs to a significant extent (383). Rich and Khmelnitsky (384) reported that salt-activation procedures used during lyophilization pretreatment of PLD enhanced the activity of PLD in nearly-anhydrous chloroform, resulting in several different successful transphosphatidylation reactions with minimal occurrence of hydrolysis. The same work suggests the importance of *in situ* removal of choline *via* adsorption to prevent inactivation of the biocatalyst (384). However, reaction rates remained slow, with at least 2 d required for 60–80% conversion (384), and neither reusability nor stability was examined (384). Recent reports suggest that aqueous media, in the absence of a nonpolar solvent, can be used in some situations to yield high conversions with minimal occurrence of hydrolysis. In purely aqueous media, covalently immobilized *Streptomyces* sp. PLD has been reported to transphosphatidylate 75% of PC into PG accompanied by ~10% conversion of PC into PA (385). Triton X-100, which is used to improve the solubility of PC, will impose downstream separation problems (385). (The use of a surfactant to enhance PC solubility in aqueous media was echoed in a recent patent; see Ref. 378.) Reaction times were on the order of 4 h (24 h in the absence of Triton) as compared with 2 h for biphasic aqueous–organic systems (385). A recent patent demonstrated 95% conversion of PC-rich GPL into PS with only a few percent conversion into PA for an aqueous-phase reaction, without information given on the rate of transphosphatidylation (383). The patent attributes its success to the PLD used, from *Streptoverticillium hachijoense* ATCC 19769, which apparently has a broader substrate selectivity than commercially available PC-selective PLD, and results in less hydrolysis of PE and other minor GPL components (383). Similarly, a recent investigation using a unique recombinant *S. antibioticus* PLD yielded 85% conversion of PC into PS and 12% conversion into PA in an aqueous medium containing calcium sulfate particles rich in adsorbed GPL. The particles acted as a reservoir of PC in the beginning of the reaction and as a storage device for PS during the latter stages of the reaction, for enhanced product recovery. Another major problem encountered with biphasic media is the formation of calcium salts at the interface (386) .

From the preceding discussion, one can infer a relationship between the physical state of the substrates and the yield and extent of hydrolysis. In support of this suggestion, the type of organic solvent is known to strongly influence the degree of transphosphatidylation (387). Furthermore, the amphiphilicity of phosphatidyl acceptors strongly controls substrate selectivity, such as the strong preference for 1-butanol rather than glycerol (387). The use of microstructured aqueous–organic media with large amounts of interfacial area, such as lamellar, lyotropic liquid crystals (the latter produced by homogenation of reaction mixtures in the presence of a TAG) has led to only modest degrees of conversion of PC into PS (386).

A major goal yet to be realized is the formation of an immobilized PLD preparation that is particularly active and stable in either aqueous, biphasic, or nearly anhydrous media (55). The best report of stability has been provided by Wang et al. (362), who demonstrated that PLD immobilized onto calcium alginate possessed a half-life of 25 batches.

## OTHER BIOCATALYTIC MEANS OF LIPID MODIFICATION

Schwaneberg and Bornscheuer (388) have reviewed the use of cytochrome P450 to hydroxylate acyl groups selectively, a process resulting in hydroxyl FFA for applications similar to those listed in Table 2 for ricinoleic acid. Intensive research has been done on the use of whole cells to produce oxygenated and polyunsaturated FFA and their ester products, fueled by an increased fundamental understanding of the synthetic metabolic pathways that produce double bonds and hydroxyl groups and a more efficient use of metabolic engineering and mutagenesis to "engineer" microorganisms to overproduce the lipid products of interest (reviewed in Ref. 389). The development of transgenic plants to produce PUFA- and hydroxy-rich FFA and structured lipids has been reviewed (390–399). As stated in a review by Jaworski and Cahoon (398) "the production of transgenic crop plants that efficiently synthesize and accumulate unusual FA is the next challenge" (p. 183). A comprehensive discussion of the topics contained in this section is beyond the scope of this review.

## FUTURE PROSPECTS FOR THE ENZYME-CATALYZED MODIFICATION OF LIPIDS

The use of biocatalysts to convert FFA into lipid products of interest will continue to grow in the upcoming years, especially in relation to purifying PUFA and synthesizing their esters and structured TAG. A more widespread use of enzymes as low-cost alternatives to chemical modification may also increase if petroleum prices continue to rise and vegetable oil prices remain steady. The use of lipases to modify lipids selec-

tively has reached maturity as a field. The further development of lipase bioreactor design and process scale-up technology will continue to occur. Development of multistep enzymatic and separation schemes to process lipids of interest, starting from raw feedstocks, has been an increasing trend in the literature during the past 5 yr and will continue to increase, as will further integration of reactions and separations. Development as such will also include the combined use of different enzymes in a process, or perhaps in the same process step, such as the combination of lipases and phospholipases to create structured phospholipids, or lipases and LOX to convert TAG directly into hydroperoxides.

Although by-products resulting from acyl migration and hydrolysis side reactions will be difficult to eliminate altogether, the programming of operational parameters such as water activity, solvent system polarity, and temperature will lead to enhanced yields and recovery. Greater sophistication in controlling such operational parameters will require developments in kinetic modeling and improved means of *in situ* control and rapid monitoring of water activity (an area of ongoing research; see Ref. 400) and substrate and product concentration. The development of a lipase that will not discriminate against Δ3–Δ6 and long-chain saturated FFA will improve the effectiveness of forming PUFA-rich esters at high yield. Advancements in mutagenesis, extremophile discovery and screening, and recombinant DNA technology will lead to more active and stable substrate-specific lipases. The use of enzymes to create libraries of lipid products for analysis as flavor and fragrance materials and surfactants, for instance, may become more widespread. The development of highly active and stable immobilized phospholipases and LOX that are on par with commercially available lipases is an important goal yet to be achieved.

In conclusion, further development of enzyme technology for the application of oilseed biomass as a feedstock for chemical and biological intermediates is required. Although alternate technologies (development of new and improved methods for lipid purification and chemical modification, use of transgenic plants, etc.) will improve, the selectivity of enzyme-based processing will continue to be an attractive choice as a unit operation in development of the "biorefinery" as a substitute for petrochemically based processes.

## REFERENCES


1. Lie Ken Jie, M.S.F., and M.K. Pasha, Fatty Acids, Fatty Acid Analogs and Their Derivatives, *Nat. Prod. Rep. 15*:607–629 (1998).
2. Biermann, U., W. Friedt, S. Lang, W. Luhs, G. Machmuller, J.O. Metzger, M. Rüsch gen. Klaas, H.J. Schafer, and M.P. Schneider, New Syntheses with Oils and Fats as Renewable Raw Materials for the Chemical Industry, *Angew. Chem., Int. Ed. Engl. 39*:2206–2224 (2000).
3. Knight, D.W., Carboxylic Acids and Derivatives, *Gen. Synth. Methods 14*:63–137 (1992).
4. Honydonckx, H.E., D.E. De Vos, S.A. Chavan, and P.A. Jacobs, Esterification and Transesterification of Renewable Chemicals, *Top. Catal. 27*:83–96 (2004).
5. Biondioli, P., The Preparation of Fatty Acid Esters by Means of Catalytic Reactions, *Ibid. 27*:77–82 (2004).
6. Khan, G.R., S. Waheed, S.A. Khan, C.M. Ashraf, and F. Scheinmann, Synthesis of Naturally Occurring Polyunsaturated Fatty Acids, *Sci. Intl. 13*:41–47 (2001).
7. Mukherjee, K.D., Lipase-Catalyzed Reactions for Modification of Fats and Other Lipids, *Biocatalysis 3*:277–293 (1990).
8. Eigtved, P., Enzymes and Lipid Modification, in *Advances in Applied Lipid Research*, Vol. 1, edited by F. B. Padley, JAI Press, London, 1992, pp. 1–64.
9. Adlercreutz, P., Enzyme-Catalyzed Lipid Modification, *Biotechnol. Genetic Eng. Rev. 12*:231–254 (1994).
10. McNeill, G.P., Enzymic Processes, in *Lipid Synthesis and Manufacture*, edited by F.D. Gunstone, CRC Press, Boca Raton, FL, 1999, pp. 288–320.
11. Gunstone, F.D., Enzymes as Biocatalysts in the Modification of Natural Lipids, *J. Sci. Food. Agric.* 79:1535–1549 (1999).
12. Bornscheuer, U.T. (ed.), *Enzymes in Lipid Modification*, Wiley-VCH, Weinheim, Germany, 2000.
13. Kuo, T.M., and H.W. Gardner, *Lipid Biotechnology*, Marcel-Dekker, New York, 2002.
14. Bornscheuer, U.T., Insights into Lipid Biotransformation, *Eur. J. Lipid Sci. Technol. 105*:561 (2003).
15. Halling, P., Enzymic Conversions in Organic and Other Low-Water Media, in *Enzyme Catalysis in Organic Synthesis,* 2nd edn., edited by K. Drauz and H. Waldmann, Wiley-VCH, Weinheim, Germany, 2002, pp. 259–285.
16. Hayes, D.G., How to Employ Proteins in Non-aqueous Environments, in *Modern Protein Chemistry: Practical Aspects*, edited by G.C. Howard, and W.E. Brown, Boca Raton, FL, 2000, pp. 179–225.
17. Hari Krishna, S., Developments and Trends in Enzyme Catalysis in Nonconventional Media, *Biotechnol. Adv. 20*:239–266 (2002).
18. Hari Krishna, S., and N.G. Karanth, Lipases and Lipase-Catalyzed Esterification Reactions in Nonaqueous Media, *Catal. Rev. Sci. Eng. 44*:499–591 (2002).
19. Carrea, G., and S. Riva, Properties and Synthetic Applications of Enzymes in Organic Solvents, *Angew. Chem., Int. Ed. Engl. 39*:2226–2254 (2000).
20. Villeneuve, P., and T.A. Foglia, Lipase Specificities: Potential Application in Lipid Bioconversions, *INFORM 8*:640–651 (1997).
21. Ransac, S., F. Carriere, E. Rogalska, R. Verger, F. Marguet, G. Buono, E.P. Melo, J.M.S. Cabral, M.-P.E. Egloff, H. van Tilbeurgh, and C. Cambillau, The Kinetics, Specificities and Structural Features of Lipases, in *Engineering of/with Lipases*, edited by F.X. Malcata, Kluwer Academic, Dordrecht, The Netherlands, 1996, pp. 143–182.
22. Jensen, R.G., and M. Hamosh, Selectivity of Lipases: Types and Determination, in *Engineering of/with Lipases*, edited by F.X. Malcata, Kluwer Academic, Dordrecht, The Netherlands, 1996, pp. 17–29.
23. Ducret, A., M. Trani, and R. Lortie, Lipase-Catalyzed Enantioselective Esterification of Ibuprofen in Organic Solvents Under Controlled Water Activity, *Enzyme Microb. Technol. 22*:212–216 (1998).
24. Williams, J.M.J., R.J. Parker, and C. Neri, Enzymatic Kinetic Resolution, in *Enzyme Catalysis in Organic Synthesis,* 2nd edn., edited by K. Drauz and H. Waldmann, Wiley-VCH, Weinheim, Germany, 2002, pp. 287–312.
25. Santaniello, E., P. Ferraboschi, and P. Grisenti, Lipase-Catalyzed Transesterification in Organic Solvents: Applications to the Preparation of Enantiomerically Pure Compounds, *Enzyme Microb. Technol. 15*:367–382 (1993).
26. Margolin, A.L., Enzymes in the Synthesis of Chiral Drugs, *Ibid. 15*:266–280 (1993).

27. Patel, R.N., Use of Lipases in Stereoselective Catalysis and Preparation of Some Chiral Drug Intermediates, in *Recent Research Developments in Oil Chemistry, Vol. 2*, anonymous editor, Transworld Research Network, Trivandrum, India, 1997, pp. 187–211.
28. Patel, R.N., Enzymatic Preparation of Chiral Pharmaceutical Intermediates by Lipases, in *Lipid Biotechnology*, edited by T.M. Kuo, and H.W. Gardner, Marcel Dekker, New York, 2002, pp. 527–561.
29. Torres, C., B. Lin, and C.G. Hill, Jr., Lipase-Catalyzed Glycerolysis of an Oil Rich in Eicosapentaenoic Acid Residues, *Biotechnol. Lett. 24*:667–673 (2002).
30. Hansen, T.T., and P. Eigtved, A New Immobilized Lipase for Interesterification and Ester Synthesis, in *World Conference on Emerging Technologies in the Fats and Oils Industry*, edited by A.R. Baldwin, American Oil Chemists' Society, Champaign, IL, 1986, pp. 365–369.
31. Pedersen, S., A.M. Larsen, and P. Aasmul, Large-Scale Preparation of a Water-Soluble Enzyme Immobilized in an Anhydrous Environment for Use in Non-aqueous Conditions, WO Patent 95-22,606 (1995).
32. Xu, X., T. Porsgaard, H. Zhang, J. Adler-Nissen, and C.-E. Høy, Production of Structured Lipids in a Packed-Bed Reactor with Thermomyces lanuginosa Lipase, *J. Am. Oil Chem. Soc*. 79:561–565 (2002).
33. Zhang, H., X. Xu, J. Nilsson, H. Mu, J. Adler-Nissen, and C.-E. Hoy, Production of Margarine Fats by Enzymatic Interesterification with Silica-Granulated *Thermomyces lanuginosa* Lipase in a Large-Scale Study, *Ibid. 78*:57–64 (2001).
34. Heldt-Hansen, H.P., M. Ishii, S.A. Patkar, T.T. Hansen, and P. Eigtved, A New Immobilized Positional Nonspecific Lipase for Fat Modification and Ester Synthesis, in *Biocatalysis in Agricultural Biotechnology (ACS.Symposium Series 389)*, edited by J.R. Whitaker, and P.E. Sonnet, American Chemical Society, Washington, DC, 1989, pp. 158–172.
35. Jaeger, K.E., B.W. Dijkstra, and M.T. Reetz, Bacterial Biocatalysts: Molecular Biology, Three-Dimensional Structures, and Biotechnological Applications of Lipases, *Ann. Rev. Microbiol. 53*:315–351 (1999).
36. Jaeger, K.-E., S. Ransac, B.W. Dijkstra, C. Colson, M. van Heuvel, and O. Misset, Bacterial Lipases, *FEMS Microbiol. Rev. 15*:29–63 (1994).
37. Demirjian, D.C., F. Moris-Varas, and C.S. Cassidy, Enzymes from Extremophiles, *Curr. Opin. Chem. Biol. 5*:144–151 (2001).
38. Sharma, R., Y. Chisti, and U.C. Banerjee, Production, Purification, Characterization, and Applications of Lipases, *Biotechnol. Adv. 19*:627–662 (2001).
39. Mukherjee, K.D., Plant Lipases as Biocatalysts, in *Lipid Biotechnology*, edited by T.M. Kuo, and H.W. Gardner, Marcel Dekker, New York, 2002, pp. 399–415.
40. May, O., C.A. Voigt, and F.H. Arnold, Enzyme Engineering by Directed Evolution, in *Enzyme Catalysis in Organic Synthesis, 2nd edn.*, edited by K. Drauz, and H. Waldmann, Wiley-VCH, Weinheim, Germany, 2002, pp. 95–138.
41. Derewenda, Z.S., Structure and Function of Lipases, in *Advances in Protein Chemistry*, Vol. 45, edited by V.N. Schumaker, 1994, pp. 1–52.
42. Marangoni, A.G., Lipases: Structure, Function, and Properties, in *Lipid Biotechnology*, edited by T.M. Kuo, and H.W. Gardner, Marcel Dekker, New York, 2002, pp. 357–386.
43. Feussner, I., and H. Kühn, Application of Lipoxygenases and Related Enzymes for the Preparation of Oxygenated Lipids, in *Enzymes in Lipid Modification*, edited by U.T. Bornscheuer, Wiley-VCH, Weinheim, Germany, 2000, pp. 309–336.
44. Pan, B.S., and J.-M. Kuo, Lipoxygenases, *Food Sci. Technol. 97*:317–336 (2000).
45. Brash, A.R., Lipoxygenases: Occurrence, Functions, Catalysis, and Acquisition of Substrate, *J. Biol. Chem. 274*:23679–23682 (1999).
46. Prigge, S.T., J.C. Boyington, M. Faig, K.S. Doctor, B.J. Gaffney, and L.M. Amzel, Structure and Mechanism of Lipoxygenases, *Biochimie 79*:629–636 (1997).
47. Iacazio, G., and D. Martini-Iacazio, Properties and Applications of Lipoxygenases, in *Enzymes in Lipid Modification*, edited by U.T. Bornscheuer, Wiley-VCH, Weinheim, Germany, 2000, pp. 337–359.
48. Kermasha, S., N. Dioum, B. Bisakowski, and M. Vega, Biocatalysis by Immobilized Lipoxygenase in a Ternary Micellar System, *J. Mol. Catal. B. Enzymat. 19–20*:305–317 (2002).
49. Hsu, A.-F., T.A. Foglia, and G.J. Piazza, Immobilization of Lipoxygenase in an Alginate-Silicate Solgel Matrix: Formation of Fatty Acid Hydroperoxides, *Biotechnol. Lett. 19*:71–74 (1997).
50. Hsu, A.-F., K. Jones, W.N. Marmer, and T.A. Foglia, Production of Alkyl Esters from Tallow and Grease Using Lipase Immobilized in a Phyllosilicate Sol-Gel, *J. Am. Oil Chem. Soc. 78*:585–588 (2001).
51. Hsu, A.-F., S. Shen, E. Wu, and T.A. Foglia, Characterization of Soybean Lipoxygenase Immobilized in Cross-Linked Phyllosilicates, *Biotechnol. Appl. Biochem. 28*:55–59 (1998).
52. Hsu, A.-F., E. Wu, T.A. Foglia, and G.J. Piazza, Kinetic Behavior of Soybean Lipoxygenase: A Comparative Study of the Free Enzyme and the Enzyme Immobilized in an Alginate Silica Sol-Gel Matrix, *J. Food. Biochem. 24*:21–31 (2000).
53. Ulbrich-Hofmann, R., Phospholipases Used in Lipid Transformations, in *Enzymes in Lipid Modification*, edited by U.T. Bornscheuer, WIley-VCH, Weinheim, Germany, 2000, pp. 219–262.
54. Servi, S., Phospholipases as Synthetic Catalysts, *Top. Curr. Chem. 200*:127–158 (1999).
55. Grunwald, P., Preparation and Application of Immobilized Phospholipases, in *Enzymes in Lipid Modification*, edited by U.T. Bornscheuer, Wiley-VCH, Weinheim, Germany, 2000, pp. 263–291.
56. Gill, I., and R. Valivety, Polyunsaturated Fatty Acids, Part 1: Occurrence, Biological Activities and Applications, *Trends Biotechnol. 15*:401–409 (1997).
57. Hoffman, D.R., E.E. Birch, J.A. Boettcher, and D.A. Schade, Use of Docosahexaenoic Acid and Arachidonic Acid to Enhance the Visual Development of Term Infants Breast-Fed up to the Age of Six Months, WO Patent 2004-048,926 (2004).
58. Llewellyn, W.C., Use of Arachidonic Acid as a Method of Increasing Skeletal Muscle Mass, WO Patent 2004-102,519 (2004).
59. Hansen, J.W., K.H. Knauff, and D.A. Schade, Method to Reduce the Incidence of Intraventricular Hemorrhage in Preterm Infants, U.S. Patent 6753350 (2004).
60. Akimoto, K., and Y. Koga, Compositions Containing Arachidonic Acid or Derivatives for Enhancement of Normal Responses of Cognitive Abilities of Healthy Humans, WO Patent 2004-028,529 (2004).
61. Samuelsson, J., and M. Johansson, Oxidation of FA with Alkene or Alkyne Functionalities Studied with Chemiluminescence and Real-Time IR Spectroscopy, *J. Am. Oil Chem. Soc. 80*:491–496 (2003).
62. Samuelsson, J., and M. Johansson, A study of Fatty Acid Methyl Esters with Epoxy or Alkyne Functionalities, *Ibid. 78*:1191–1196 (2001).
63. Muuse, B.G., F.P. Cuperus, and J.T.P. Derksen, Composition and Physical Properties of Oils from New Oilseed Crops, *Ind. Crops Prod. 1*:57–65 (1992).
64. Larsson, S.C., M. Kumlin, M. Ingelman-Sundberg, and A. Wolk, Dietary Long-Chain n-3 Fatty Acids for the Prevention

of Cancer: A Review of Potential Mechanisms, *Am. J. Clin. Nutr. 79*:935–945 (2004).
65. McColl, J., Health Benefits of Omega-3 Fatty Acids, *NutraCos 2*:35–40 (2003).
66. Li, D., O. Bode, H. Drummond, and A.J. Sinclair, Omega-3 (n-3) Fatty Acids, in *Lipids for Functional Foods and Nutraceuticals*, edited by F.D. Gunstone, Oily Press Lipid Library, Bridgwater, UK, 2003, pp. 225–262.
67. Leonard, E.C., High-Erucic Vegetable Oils, *Ind. Crops Prod. 1*:119–123 (1992).
68. Oyamada, T., Heat-Developable Photographic Film Containing Controlled Amount of Silver Behenate and Erucic Acid, WO Patent 2004-085,661 (2004).
69. Noguchi, Y., J. Oki, and M. Shakado, Esterification Product and Cosmetic Preparation Containing the Same, WO 2004-002,438 (2004).
70. Zilberberg, M.D., C. Levine, E. Komaroff, M. Guldin, M.J. Gordon, and S.D. Ross, Clinical Uses of γ-Linolenic Acid: A Systematic Review of the Literature, in *γ-Linolenic Acid: Recent Advances in Biotechnology and Clinical Applications*, edited by Y.S. Huang and V.A. Ziboh, AOCS Press, Champaign, IL, 2001, pp. 90–104.
71. Garmier, W.W., Biodegradable Lubricant Composition from Triglycerides and Oil Soluble Antimony, U.S. Patent 5,990,055 (1999).
72. Thames, S.F., and H. Yu, Synthesis, Characterization, and Application of Lesquerella Oil and Its Derivatives in Water-Reducible Coatings, *J. Coatings Technol. 68*:63–67 (1996).
73. Deguchi, F., Cosmetics Containing Lesquerella Oil or Its Fatty Acids, Japanese Patent 06,227,939 (1994).
74. Dierig, D.A., A.E. Thompson, and F.S. Nakayama, *Lesquerella* Commercialization Efforts in the United States, *Ind. Crops Prod. 1*:289–293 (1992).
75. Giordano, J.A., and C. Balzer, Compositions and Methods for Nutrition Supplementation, U.S. Patent 2004-0,109,901 (2004).
76. Hebert, R.F., Water-Soluble Stable Petroselinic Acid Dextran Salt, U.S. Patent 2003-228,378 (2003).
77. Barrett, K.E., M.R. Green, H.-L. Hu, P. Parmar, J.R. Powell, and A.V. Rawlings, Skin Care Composition Containing Petroselinic Acid and Phenols, WO Patent 2001-008,651 (2001).
78. Alaluf, S., K.E. Barrett, A.M. Brinker, F.W. Cain, M.R. Green, H.-L. Hu, K. Iwata, T.E. Januario, K.A. Ottey, L.R. Palanker, *et al.*, Petroselinic Acid-Containing Cosmetics for Treating Skin, WO Patent 99-47,110 (1999).
79. Malnoe, A., M. Baur, and L. Fay, Use of Petroselinic Acid for the Treatment of Inflammation of Superficial Tissues, European Patent 888,773 (1999).
80. Laugier, J.-P., and E. Picard, Cosmetics Containing an Oil Rich in Petroselinic Acid as Hydrating Agent, European Patent 709,084 (1996).
81. Takeoka, E., C. Hamada, J. Suzuki, Y. Nakazawa, T. Souma, M. Ogou, and M. Tajima, Hair Growth Phase Extender Composition, European Patent 867,169 (1998).
82. Heidbreder, A., R. Hofer, R. Grutzmacher, A. Westfechtel, and C.W. Blewett, Oleochemical Products as Building Blocks for Polymers, *Fett/Lipid 101*:418–424 (1999).
83. Schwitzer, M.K., Castor Oil, in *Proceedings World Conference on Oleochemicals: Into the 21st Century*, edited by T.H. Applewhite, AOCS Press, Champaign, IL, 1991, pp. 111–118.
84. Gaginella, T.S., F. Capasso, N. Mascolo, and S. Perilli, Castor Oil: New Lessons from an Ancient Oil, *Phytotherapy Res. 12*:S128–S130 (1998).
85. Perdue, R.E., Jr., K.D. Carlson, and M.G. Gilbert, *Vernonia galamensis*, Potential New Crop Source of Epoxy Acid, *Econ. Botany 40*:54–68 (1986).
86. Carlson, K.D., W.J. Schneider, S.P. Chang, and L.H. Princen, *Vernonia galamensis* Seed Oil: A New Source for Epoxy Coatings, in *New Sources of Fats and Oils*, edited by E.H. Pryde, L.H. Princen, and K.D. Mukherjee, AOCS Press, Champaign, IL, 1981, pp. 297–318.
87. Ayorinde, F.O., G. Osman, R.L. Shepard, and F.T. Powers, Synthesis of Azelaic Acid and Suberic Acid from *Vernonia galamensis* Oil, *J. Am. Oil Chem. Soc. 65*:1774–1777 (1988).
88. Russell-Jones, G.J., Systems for Oral Delivery, WO Patent 2000-022,909 (2000).
89. Zeinalov, E.B., G. Kossmehl, and R.R.K. Kimwomi, Synthesis and Reactivity of Antioxidants Based on Vernolic Acid and 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propionic Acid, *Angew. Makromol. Chem. 260*:77–81 (1998).
90. Ayorinde, F.O., E.Y. Nana, P.D. Nicely, A.S. Woods, E.O. Price, and C.P. Nwaonicha, Syntheses of 12-Aminododecanoic and 11-Aminoundecanoic Acids from Vernolic Acid, *J. Am. Oil Chem. Soc. 74*:531–538 (1997).
91. Grinberg, S., V. Kolot, and D. Mills, New Chemical Derivatives Based on *Vernonia galamensis* Oil, *Ind. Crops Prod. 3*:113–119 (1994).
92. Singh, C., N. Kapur, and B.L. Kaul, *Vernonia galamensis* (Cass) Oil—A Potential New Source of Epoxidized Triglyceride Oil, *Res. Ind. 38*:152–153 (1993).
93. Ayorinde, F.O., F.T. Powers, L.D. Streete, R.L. Shepard, and D.N. Tabi, Synthesis of Dodecanedioic Acid from *Vernonia galamensis* Oil, *J. Am. Oil Chem. Soc. 66*:690–692 (1989).
94. Berdeaux, O., W.W. Christie, F.D. Gunstone, and J.-L. Sébédio, Large-Scale Synthesis of Methyl *cis*-9,*trans*-11-Octadecadienoate from Methyl Ricinoleate, *Ibid. 74*:1011–1015 (1997).
95. Cawood, P., D.G. Wickens, S.A. Iversen, J.M. Braganza, and T.C. Dormandy, The Nature of Diene Conjugation in Human Serum, Bile, and Duodenal Juice, *FEBS Lett. 59*:280–283 (1983).
96. Saebo, A., C. Skarie, D. Jerome, and G. Haroldsson, Conjugated Linoleic Acid Compositions and Their Manufacture from Oilseed Oils, U.S. Patent 6,410,761 (2002).
97. Reaney, M.J.T., Method for Commercial Preparation of Conjugated Linoleic Acid from By-Products of Vegetable Oil Refining, U.S. Patent 6,414,171 (2002).
98. Reaney, M.J.T., Y.-D. Liu, and N.D. Westcott, Commercial Production of Conjugated Linoleic Acid, in *Advances in Conjugated Linoleic Acid Research*, edited by M.P. Yurawecz, M.M. Mossoba, J.K.G. Kramer, M.W. Pariza, and G.J. Nelson, AOCS Press, Champaign, IL, 1999, pp. 39–54.
99. Remmereit, J., J. Wadstein, and J. Klaveness, Conjugated Linoleic Acid in Treatment and Prophylaxis of Diabetes, U.S. Patent 6,440,931 (2002).
100. Pariza, M.W., Y. Park, and M.E. Cook, The Biologically Active Isomers of Conjugated Linoleic Acid, *Prog. Lipid Res. 40*:283–298 (2001).
101. Isbell, T.A., and S.C. Cermak, Synthesis of δ-Eicosanolactone and δ-Docosanolactone Directly from Meadowfoam Oil, *J. Am. Oil Chem. Soc. 78*:527–531 (2001).
102. Isbell, T.A., Development of Meadowfoam as an Industrial Crop Through Novel Fatty Acid Derivatives, *Lipid Technol. 9*:140–144 (1997).
103. Erhan, S.M., and R. Kleiman, Meadowfoam Oil Factice and Its Performance in Natural Rubber Mixes, *Rubber World 203*:33–36 (1990).
104. Mund, M.S., and T.A. Isbell, Synthesis of Chloroalkoxy Eicosanoic and Docosanoic Acids from Meadowfoam Fatty Acids by Oxidation with Aqueous Sodium Hypochlorite, *J. Am. Oil Chem. Soc. 76*:1189–1200 (1999).
105. O'Lenick, A.J., Jr., Free Radical Polymers Based on Meadowfoam Esters, European Patent 1,221,450 (2002).
106. Isshiki, T., M. Nakanishi, and T. Himeno, Skin-Lightening Cosmetics Containing Ellagic Acid and Taurines, Japanese Patent 2003-267,818 (2003).

107. Friedman, R.S., Method for Stimulating Human Hair Growth, WO Patent 2001-019,328 (2001).
108. O'Lenick, A.J., Jr., Preparation of Meadowfoam Amidoamine Oxide Nonionic Surfactants for Use in Hair Conditioners, U.S. Patent 5,907,049 (1999).
109. O'Lenick, A.J., Jr., Preparation of Meadowfoam-Based Quaternary Aminoamide Cationic Surfactants, U.S. Patent 5,932,754 (1999).
110. O'Lenick, A.J., Jr., Meadowfoam Based Sorbitan Esters, U.S. Patent 6,384,248 (2002).
111. Lie Ken Jie, M.S.F., and M.S.K.S. Rahmatullah, Enzymic Enrichment of $C_{20}$ *cis*-5 Polyunsaturated Fatty Acids from *Biota orientalis* Seed Oil, *J. Am. Oil Chem. Soc. 72*:245–249 (1995).
112. Ellis, C., Composition for Cleaning Finish, U.S. Patent 879,375 (1908).
113. Tronstad, K.J., K. Berge, R.K. Berge, and O. Bruserud, Modified Fatty Acids and Their Possible Therapeutic Targets in Malignant Diseases, *Expert Opin. Therapeut. Targ. 7*:663–677 (2003).
114. Jandacek, R.J., Commercial Applications of Fatty Acid Derivatives in Foods, *Food Sci. Technol. 96*:387–410 (2000).
115. Cuperus, F.P., G. Boswinkel, B.G. Muuse, and J.T.P. Derksen, Supercritical Carbon Dioxide Extraction of *Dimorphotheca pluvialis* Oil Seeds, *J. Am. Oil Chem. Soc. 73*:1675–1679 (1996).
116. Muuse, B.G., F.P. Cuperus, and J.T.P. Derksen, Extraction and Characterization of Dimorphotheca pluvialis Seed Oil, *Ibid. 71*:313–317 (1994).
117. Hayes, D.G., R. Kleiman, D. Weisleder, R.O. Adlof, F.P. Cuperus, and J.T.P. Derksen, Occurrence of Estolides in Processed *Dimorphotheca pluvialis* Oil, *Ind. Crops Prod. 4*:295–301 (1995).
118. Piazza, G.J., Jr., and M.J. Haas, Rapid Fat and Oil Splitting Using a Lipase Catalyst Found in Seeds, U.S. Patent Appl. 92-855,805 (1992).
119. Barnebey, H.L., and A.C. Brown, Continuous Fat-Splitting Plants Using the Colgate–Emery Process, *J. Am. Oil Chem. Soc. 25*:95–99 (1948).
120. Macrae, A.R., Microbial Lipases as Catalysts for the Interesterification of Oils and Fats, in *Biotechnology for the Oils and Fats Industry*, edited by C. Ratledge, P. Dawson, and J. Rattray, AOCS Press, Champaign, IL, 1985, pp. 189–198.
121. Foglia, T.A., K.C. Jones, and P.E. Sonnet, Selectivity of Lipases: Isolation of Fatty Acids from Castor, Coriander, and Meadowfoam Oils, *Eur. J. Lipid Sci. Technol. 102*:612–617 (2000).
122. Okumura, S., M. Iwai, and Y. Tsujisaka, Synthesis of Estolides During Hydrolysis of Castor Oil by *Geotrichum candidum* Lipase, *Yukagaku 32*:271–273 (1983).
123. Hayes, D.G., and R. Kleiman, 1,3-Specific Lipolysis of Lesquerella fendleri Oil by Immobilized and Reverse Micellar Encapsulated Lipases, *J. Am. Oil Chem. Soc. 70*:1121–1127 (1993).
124. Hayes, D.G., K.D. Carlson, and R. Kleiman, The Isolation of Hydroxy Acids from Lesquerella Oil Lipolysate Using a Saponification/Extraction Technique (SAPEX), *Ibid. 73*:1113–1119 (1996).
125. Frykman, H.B., and T.A. Isbell, Enrichment of Lesquerolic and Auricolic Acids from Hydrolyzed *Lesquerella fendleri* and *L. gordonii* Oil by Crystallization, *Ibid. 74*:699–702 (1997).
126. Cuperus, F.P., A.M. Krosse, G.B. Boswinkel, and J.T.P. Derksen, Processing of Oils from New Crops: A Hybrid Membrane Process for the Continuous Removal of Specific Fatty Acids, *Recents Progres Genie Proc. 6*:377–382 (1992).
127. Derksen, J.T.P., B.G. Muuse, F.P. Cuperus, and W.M.J. Van Gelder, New Seed Oils for Oleochemical Industry: Evaluation and Enzyme-Bioreactor Mediated Processing, *Ind. Crops Prod. 1*:133–139 (1992).
128. Derksen, J.T.P., A.M. Krosse, P. Tassignon, and F.P. Cuperus, Lipase-Catalyzed Production of Functionalized Fatty Acids from *Dimorphotheca pluvialis* Seed Oil, *Mededelingen Faculteit Landbouwwetenschappen, Universiteit Gent 57*:1741–1747 (1992).
129. Derksen, J.T.P., G. Boswinkel, and W.M.J. Van Gelder, Lipase-Catalyzed Hydrolysis of Triglycerides from New Oil Crops for Oleochemical Industries, in *Lipases: Structure, Mechanism, and Genetic Engineering*, edited by L. Alberghina, R.D. Schmid, and R. Verger, John Wiley & Sons, New York, 1991, pp. 377–380.
130. Lie Ken Jie, M.S.F., X. Fua, M.L. Lau Maureen, and M.L. Chye, Lipase-Catalyzed Hydrolysis of TG Containing Acetylenic FA, *Lipids 37*:997–1006 (2002).
131. Sehanputri, P.S., and C.G. Hill, Jr., Biotechnology for the Production of Nutraceuticals Enriched in Conjugated Linoleic Acid: I. Uniresponse Kinetics of the Hydrolysis of Corn Oil by a *Pseudomonas* sp. Lipase Immobilized in a Hollow Fiber Reactor, *Biotechnol. Bioeng. 64*:568–579 (1999).
132. McNeill, G.P., Enzymic Process for the Isolation of Erucic Acid from Vegetable Oils, U.S. Patent 5,633,151 (1997).
133. McNeill, G.P., and P.E. Sonnet, Isolation of Erucic Acid from Rapeseed Oil by Lipase-Catalyzed Hydrolysis, *J. Am. Oil Chem. Soc. 72*:213–218 (1995).
134. Sonnet, P.E., T.A. Foglia, and S.H. Feairheller, Fatty Acid Selectivity of Lipases: Erucic Acid from Rapeseed Oil, *Ibid. 70*:387–391 (1993).
135. Shimada, Y., A. Sugihara, and Y. Tominaga, Enzymatic Purification of Polyunsaturated Fatty Acids, *J. Biosci. Bioeng. 91*:529-538 (2001).
136. Shimada, Y., A. Sugihara, and Y. Tominaga, Enzymatic Enrichment of Polyunsaturated Fatty Acids, in *Lipid Biotechnology*, edited by T.M. Kuo, and H.W. Gardner, Marcel Dekker, New York, 2002, pp. 493–515.
137. Mukherjee, K.D., Fractionation of Fatty Acids and Other Lipids Using Lipases, in *Enzymes in Lipid Modification*, edited by U.T. Bornscheuer, Wiley-VCH, Weinheim, Germany, 2000, pp. 23–45.
138. Nagao, T., and Y. Shimada, Purification of Useful Oil- and Fat-Related Compounds Using Lipase Reactions, *Food Food Ingred. J. Japan 208*:259–269 (2003).
139. Haraldsson, G.G., and B. Kristinsson, Separation of Eicosapentaenoic and Docosahexaenoic Acid in Fish Oil by Kinetic Resolution Using Lipases, *J. Am. Oil Chem. Soc. 75*:1551–1556 (1998).
140. Shimada, Y., A. Sugihara, K. Maruyama, T. Nagao, S. Nakayama, H. Nakano, and Y. Tominaga, Enrichment of Arachidonic Acid: Selective Hydrolysis of a Single-Cell Oil from *Mortierella* with *Candida cylindracea* Lipase, *Ibid. 72*:1323–1327 (1995).
141. Gamez-Meza, N., J.A. Noriega-Rodriguez, L.A. Medina-Juarez, J. Ortega-Garcia, J. Monroy-Rivera, F.J. Toro-Vazquez, H.S. Garcia, and O. Angulo-Guerrero, Concentration of Eicosapentaenoic Acid and Docosahexaenoic Acid from Fish Oil by Hydrolysis and Urea Complexation, *Food Res. Intl. 36*:721–727 (2003).
142. Shimada, Y., K. Maruyama, A. Sugihara, S. Moriyama, and Y. Tominaga, Purification of Docosahexaenoic Acid from Tuna Oil by a Two-Step Enzymic Method: Hydrolysis and Selective Esterification, *J. Am. Oil Chem. Soc. 74*:1441–1446 (1997).
143. Rice, K.E., J. Watkins, and C.G. Hill, Jr., Hydrolysis of Menhaden Oil by a *Candida cylindracea* Lipase Immobilized in a Hollow-Fiber Reactor, *Biotechnol. Bioeng. 63*:33–45 (1999).
144. Huang, J., T. Aki, S. Kawamoto, S. Shigeta, K. Ono, and O. Suzuki, Enzymatic Preparation of Glycerides Rich in Docosahexaenoic Acid from *Thraustochytrid* Single Cell Oils by *Candida rugosa* Lipase, *J. Oleo Sci. 51*:447–455 (2002).

145. McNeill, G.P., R.G. Ackman, and S.R. Moore, Lipase-Catalyzed Enrichment of Long-Chain Polyunsaturated Fatty Acids, *J. Am. Oil Chem. Soc. 73*:1403–1407 (1996).
146. Moore, S.R., and G.P. McNeill, Production of Triglycerides Enriched in Long-Chain n-3 Polyunsaturated Fatty Acids, *Ibid. 73*:1409–1414 (1996).
147. Huang, F.-C., Y.-H. Ju, and C.-W. Huang, Enrichment of γ-Linolenic Acid from Borage Oil *via* Lipase-Catalyzed Reactions, *Ibid. 74*:977–981 (1997).
148. Syed Rahmatullah, M.S.K., V.K.S. Shukla, and K.D. Mukherjee, Enrichment of γ-Linolenic Acid from Evening Primrose Oil and Borage Oil *via* Lipase-Catalyzed Hydrolysis, *Ibid. 71*:569–573 (1994).
149. Huang, F.-C., Y.-H. Ju, and J.-C. Chiang, γ-Linolenic Acid-Rich Triacylglycerides Derived from Borage Oil *via* Lipase-Catalyzed Reactions, *Ibid. 76*:833-837 (1999).
150. Hayes, D.G., and R. Kleiman, The Isolation and Recovery of Fatty Acids with Δ5 Unsaturation from Meadowfoam Oil Using Lipase-Catalyzed Hydrolysis and Esterification, *Ibid. 70*:555–560 (1993).
151. Österberg, E., A.C. Blomstrom, and K. Holmberg, Lipase Catalyzed Transesterification of Unsaturated Lipids in a Microemulsion, *Ibid. 66*:1330–1333 (1989).
152. Tanaka, Y., T. Funada, J. Hirano, and R. Hashizume, Triglyceride Specificity of *Candida cylindracea* Lipase: Effect of Docosahexaenoic Acid on Resistance of Triglyceride to Lipase, *Ibid. 70*:1031–1034 (1993).
153. Murty, V.R., J. Bhat, and P.K.A. Muniswaran, Hydrolysis of Oils by Using Immobilized Lipase Enzyme: A Review, *Biotechnol. Bioproc. Eng. 7*:57–66 (2002).
154. Malcata, F.X., H.R. Reyes, H.S. Garcia, C.G. Hill, Jr., and C.H. Amundson, Immobilized Lipase Reactors for Modification of Fats and Oils—A Review, *J. Am. Oil Chem. Soc. 67*:890–910 (1990).
155. Shimada, Y., A. Sugihara, Y. Minamigawa, K. Higashiyama, K. Akimoto, S. Fujikawa, S. Komemushi, and Y. Tominaga, Enzymic Enrichment of Arachidonic Acid from *Mortierella* Single-Cell Oil, *Ibid. 75*:1213–1217 (1998).
156. Haas, M.J., J.K.G. Kramer, G. McNeill, K. Scott, T.A. Foglia, N. Sehat, J. Fritsche, M.M. Mossoba, and M.P. Yurawecz, Lipase-Catalyzed Fractionation of Conjugated Linoleic Acid Isomers, *Lipids 34*:979–987 (1999).
157. McNeill, G.P., C. Rawlins, and A.C. Peilow, Enzymatic Enrichment of Conjugated Linoleic Acid Isomers and Incorporation into Triglycerides, *J. Am. Oil Chem. Soc. 76*:1265–1268 (1999).
158. Nagao, T., Y. Shimada, Y. Yamauchi-Sato, T. Yamamoto, M. Kasai, K. Tsutsumi, A. Sugihara, and Y. Tominaga, Fractionation and Enrichment of CLA Isomers by Selective Esterification with *Candida rugosa* Lipase, *Ibid. 79*:303–308 (2002).
159. Yamauchi-Sato, Y., T. Nagao, T. Yamamoto, T. Terai, A. Sugihara, and Y. Shimada, Fractionation of Conjugated Linoleic Acid Isomers by Selective Hydrolysis with *Candida rugosa* Lipase, *J. Oleo Sci. 52*:367–374 (2003).
160. Nagao, T., Y. Yamauchi-Sato, A. Sugihara, T. Iwata, K. Nagao, T. Yanagita, S. Adachi, and Y. Shimada, Purification of Conjugated Linoleic Acid Isomers Through a Process Including Lipase-Catalyzed Selective Esterification, *Biosci. Biotechnol. Biochem. 67*:1429–1433 (2003).
161. Warwel, S., and R. Borgdorf, Substrate Selectivity of Lipase in the Esterification of *cis/trans* Isomers and Positional Isomers of Conjugated Linoleic Acid (CLA), *Biotechnol. Lett. 22*:1151–1155 (2000).
162. Haraldsson, G.G., Enrichment of Lipids with EPA and DHA, in *Enzymes in Lipid Modification*, edited by U.T. Bornscheuer, Wiley-VCH, Weinheim, Germany, 2000, pp. 170–189.
163. Syed Rahmatullah, M.S.K., V.K.S. Shukla, and K.D. Mukherjee, γ-Linolenic Acid Concentrates from Borage and Evening Primrose Oil Fatty Acids *via* Lipase-Catalyzed Esterification, *J. Am. Oil Chem. Soc. 71*:563–567 (1994).
164. Hills, M.J., I. Kiewitt, and K.D. Mukherjee, Enzymic Fractionation of Evening Primrose Oil by Rape Lipase: Enrichment of γ-Linolenic Acid, *Biotechnol. Lett. 11*:629–632 (1989).
165. Hills, M.J., I. Kiewitt, and K.D. Mukherjee, Enzymic Fractionation of Fatty Acids: Enrichment of γ-Linolenic Acid and Docosahexaenoic Acid by Selective Esterification Catalyzed by Lipases, *J. Am. Oil Chem. Soc. 67*:561–564 (1990).
166. Foglia, T.A., and P.E. Sonnet, Fatty Acid Selectivity of Lipases: γ-Linolenic Acid from Borage Oil, *Ibid. 72*:417–420 (1995).
167. Schmitt-Rozieres, M., G. Vanot, V. Deyris, and L.-C. Comeau, *Borago officinalis* Oil: Fatty Acid Fractionation by Immobilized *Candida rugosa* Lipase, *Ibid. 76*:557–562 (1999).
168. Van Heerden, E., and D. Litthauer, The Comparative Discriminating Abilities of Lipases in Different Media and Their Application in Fatty Acid Enrichment, *Biocatal. Biotransform. 16*:461–474 (1999).
169. Sovova, H., and M. Zarevucka, Lipase-Catalysed Hydrolysis of Blackcurrant Oil in Supercritical Carbon Dioxide, *Chem. Eng. Sci. 58*:2339–2350 (2003).
170. Shimada, Y., N. Sakai, A. Sugihara, H. Fujita, Y. Honda, and Y. Tominaga, Large-Scale Purification of γ-Linolenic Acid by Selective Esterification Using *Rhizopus delemar* Lipase, *J. Am. Oil Chem. Soc. 75*:1539–1543 (1998).
171. Jachmanian, I., E. Schulte, and K.D. Mukherjee, Substrate Selectivity in Esterification of Less Common Fatty Acids Catalyzed by Lipases from Different Sources, *Appl. Microbiol. Biotechnol. 44*:563–567 (1996).
172. Chen, M.L., S.R. Vali, J.Y. Lin, and Y.-H. Ju, Synthesis of the Structured Lipid 1,3-Dioleyl-2-palmitoylglycerol from Palm Oil, *J. Am. Oil Chem. Soc. 81*:525–532 (2004).
173. Wanasundara, U.N., and F. Shahidi, Biotechnological Methods for Concentrating Omega-3 Fatty Acids from Marine Oils, *Seafood Safety, Processing, and Biotechnology,* edited by F. Shahidi, Y. Jones, and D. Kitts, Technomic Press, Lancaster, PA, 1997, pp. 225–233.
174. Shimada, Y., Application of Lipase Reactions to Separation and Purification of Useful Materials, *INFORM 12*:1168–1174 (2001).
175. Breivik, H., G.G. Haraldsson, and B. Kristinsson, Preparation of Highly Purified Concentrates of Eicosapentaenoic Acid and Docosahexaenoic Acid, *J. Am. Oil Chem. Soc. 74*:1425–1429 (1997).
176. Hayes, D.G., Purification of Free Fatty Acids *via* Urea Inclusion Compounds, in *Handbook of Functional Lipids*, edited by C.C. Akoh, CRC Press, Boca Raton, FL, in press.
177. Hayes, D.G., Free Fatty acid Fractionation *via* Urea Inclusion Compounds, *INFORM 13*:832–834 (2002).
178. Hayes, D.G., Urea Inclusion Compound Formation, *Ibid. 13*:781–783 (2002).
179. Zander, L., S. Busch, C. Meyer, S. Both, and U. Schoerken, Lipase Catalyzed Hydrolysis of Conjugated Linoleic Acid Esters, WO Patent 2003-104,472 (2003).
180. Shimada, Y., A. Sugihara, H. Nakano, T. Kuramoto, T. Nagao, M. Gemba, and Y. Tominaga, Purification of Docosahexaenoic Acid by Selective Esterification of Fatty Acids from Tuna Oil with *Rhizopus delemar* Lipase, *J. Am. Oil Chem. Soc. 74*:97–101 (1997).
181. Halldorsson, A., B. Kristinsson, C. Glynn, and G.G. Haraldsson, Separation of EPA and DHA in Fish Oil by Lipase-Catalyzed Esterification with Glycerol, *Ibid. 80*:915–921 (2003).
182. Shimada, Y., T. Nagao, A. Sugihara, and Y. Tominaga, Production of Structured Lipids with Lipase, *Kagaku to Kogyo 76*:188–195 (2002).

183. Akoh, C.C., Structured Lipids, *Food Sci. Technol. 117*: 877–908 (2002).
184. Akoh, C.C., Structured Lipids for Parenteral Nutrition, US Patent 6,369,252 (2002).
185. Akoh, C.C., S. Sellappan, L.B. Fomuso, and V.V. Yankah, Enzymatic Synthesis of Structured Lipids, in *Lipid Biotechnology*, edited by T.M. Kuo, and H.W. Gardner, Marcel Dekker, New York, 2002, pp. 433–460.
186. Gupta, R., P. Rathi, and S. Bradoo, Lipase Mediated Upgradation of Dietary Fats and Oils, *Crit. Rev. Food Sci. Nutr. 43*:635–644 (2003).
187. Iwasaki, Y., and T. Yamane, Enzymatic Synthesis of Structured Lipids, *J. Mol. Catal. B. Enzymat. 10*:129–140 (2000).
188. Osborn, H.T., and C.C. Akoh, Structured Lipids—Novel Fats with Medical, Nutraceutical, and Food Applications, *Compr. Rev. Food Sci. Food Safety 1*:93–103 (2002).
189. Haumann, B.F., Structured Lipids Allow Fat Tailoring, *INFORM 8*:1004–1011 (1997).
190. Xu, X., Production of Specific-Structured Triacylglycerols by Lipase-Catalyzed Reactions: A Review, *Eur. J. Lipid Sci. Technol. 102*:287–303 (2000).
191. Akoh, C.C., Fat Replacers, *Food Technol. 52:*47–53 (1998).
192. Shimada, Y., A. Sugihara, and Y. Tominaga, Production of Functional Lipids Containing Polyunsaturated Fatty Acids with Lipase, in *Enzymes in Lipid Modification*, edited by U. Bornscheuer, Wiley-VCH, Weinheim, Germany, 2000, pp. 128–147.
193. Auerbach, M.H., P.W. Chang, S.L. Coleman, J.J. O'Neill, and J.C. Philips, Salatrim Reduced-Calorie Triacylglycerols, *Lipid Technol. 9*:137–140 (1997).
194. Kosmark, R., Salatrim: Properties and Applications, *Food Technol. 50*:98–101 (1996).
195. Smith, R.E., J.W. Finley, and G.A. Leveille, Overview of SALATRIM: A Family of Low-Calorie Fats, *J. Agric. Food Chem. 42*:432–434 (1994).
196. Undurraga, D., A. Markovits, and S. Erazo, Cocoa Butter Equivalent Through Enzymic Interesterification of Palm Oil Midfraction, *Proc. Biochem. 36*:933–939 (2001).
197. Coleman, M.H., and A.R. Macrae, Rearrangement of Fatty Acid Esters in Fat Reaction Reactants, U.S. Patent 2,705,608 (1977).
198. Matsuo, T., N. Sawamura, Y. Hashimoto, and W. Hashida, Cocoa Butter Substitute, U.S. Patent 2,946,565 (1980).
199. Yankah, V.V., and C.C. Akoh, Lipase-Catalyzed Acidolysis of Tristearin with Oleic or Caprylic Acids to Produce Structured Lipids, *J. Am. Oil Chem. Soc. 77*:495–500 (2000).
200. Gandhi, N.N., and K.D. Mukherjee, Synthesis of Designer Lipids Using Papaya (*Carica papaya*) Latex Lipase, *J. Mol. Catal. B. Enzymat. 11*:271–277 (2001).
201. Yang, T., M.-B. Fruekilde, and X. Xu, Applications of Immobilized *Thermomyces lanuginosa* Lipase in Interesterification, *J. Am. Oil Chem. Soc. 80*:881–887 (2003).
202. Christensen, T.C., and G. Hoelmer, Lipase Catalyzed Acyl-Exchange Reactions of Butter Oil. Synthesis of a Human Milk Fat Substitute for Infant Formulas, *Milchwissenschaft 48*:543–548 (1993).
203. Yang, T., X. Xu, C. He, and L. Li, Lipase-Catalyzed Modification of Lard to Produce Human Milk Fat Substitutes, *Food Chem. 80*:473–481 (2003).
204. Mukherjee, K.D., and I. Kiewitt, Structured Triacylglycerols Resembling Human Milk Fat by Transesterification Catalyzed by Papaya (*Carica papaya*) Latex, *Biotechnol. Lett. 20*:613–616 (1998).
205. Schmid, U., U.T. Bornscheuer, M.M. Soumanou, G.P. McNeill, and R.D. Schmid, Optimization of the Reaction Conditions in the Lipase-Catalyzed Synthesis of Structured Triglycerides, *J. Am. Oil Chem. Soc. 75*:1527–1531 (1998).
206. Schmid, U., U.T. Bornscheuer, M.M. Soumanou, G.P. McNeill, and R.D. Schmid, Highly Selective Synthesis of 1,3-Oleoyl-2-palmitoylglycerol by Lipase Catalysis, *Biotechnol. Bioeng. 64*:678–684 (1999).
207. Soumanou, M.M., U.T. Bornscheuer, and R.D. Schmid, Two-Step Enzymic Reaction for the Synthesis of Pure Structured Triacylglycerides, *J. Am. Oil Chem. Soc. 75*:703–710 (1998).
208. Xu, X., L.B. Fomuso, and C.C. Akoh, Synthesis of Structured Triacylglycerols by Lipase-Catalyzed Acidolysis in a Packed Bed Bioreactor, *J. Agric. Food Chem. 48*:3–10 (2000).
209. Mu, H., J.-P. Kurvinen, H. Kallio, X. Xu, and C.-E. Høy, Quantitation of Acyl Migration During Lipase-Catalyzed Acidolysis, and of the Regioisomers of Structured Triacylglycerols Formed, *J. Am. Oil Chem. Soc. 78*:959–964 (2001).
210. Fomuso, L.B., and C.C. Akoh, Lipase-Catalyzed Acidolysis of Olive Oil and Caprylic Acid in a Bench-Scale Packed Bed Bioreactor, *Food Res. Intl. 35*:15–21 (2002).
211. Kim, I.-H., S.-N. Ko, S.-M. Lee, S.H. Chung, H. Kim, K.-T. Lee, and T.-Y. Ha, Production of Strucutred Lipids by Lipase-Catalyzed Acidolysis in Supercritical Carbon Dioxide: Effect on Acyl Migration, *J. Am. Oil Chem. Soc. 81*:537–542 (2004).
212. Lee, K.-T., and T.A. Foglia, Synthesis, Purification, and Characterization of Structured Lipids Produced from Chicken Fat, *Ibid. 77*:1027–1034 (2000).
213. Zhou, D., X. Xu, H. Mu, C.-E. Hoy, and J. Adler-Nissen, Synthesis of Structured Triacylglycerols Containing Caproic Acid by Lipase-Catalyzed Acidolysis: Optimization by Response Surface Methodology, *J. Agric. Food Chem. 49*:5771–5777 (2001).
214. Xu, X., H. Mu, C.E. Høy, and J. Adler-Nissen, Production of Specifically Structured Lipids by Enzymic Interesterification in a Pilot Enzyme Bed Reactor. Process Optimization by Response Surface Methodology, *Fett/Lipid 101*:207–214 (1999).
215. Miura, S., A. Ogawa, and H. Konishi, A Rapid Method for Enzymatic Synthesis and Purification of the Structured Triacylglycerol, 1,3-Dilauroyl-2-oleoyl-glycerol, *J. Am. Oil Chem. Soc. 76*:927–931 (1999).
216. Paez, B.C., A.R. Medina, F.C. Rubio, L.E. Cerdan, and E.M. Grima, Kinetics of Lipase-Catalyzed Interesterification of Triolein and Caprylic Acid to Produce Structured Lipids, *J. Chem. Technol. Biotechnol. 78*:461–470 (2003).
217. Kim, I.-H., H. Kim, K.-T. Lee, S.-H. Chung, and S.-N. Ko, Lipase-Catalyzed Acidolysis of Perilla Oil with Caprylic Acid to Produce Structured Lipids, *Ibid. 79*:363–367 (2002).
218. Akoh, C.C., Structured Lipids—Enzymatic Approach, *INFORM 6*:1055–1061 (1995).
219. Shimada, Y., M. Suenaga, A. Sugihara, S. Nakai, and Y. Tominaga, Continuous Production of Structured Lipid Containing γ-Linolenic and Caprylic Acids by Immobilized *Rhizopus delemar* Lipase, *J. Am. Oil Chem. Soc. 76*:189–193 (1999).
220. Shimada, Y., A. Sugihara, K. Maruyama, T. Nagao, S. Nakayama, H. Nakano, and Y. Tominaga, Production of Structured Lipid Containing Docosahexaenoic and Caprylic Acids Using Immobilized *Rhizopus delemar* Lipase, *J. Ferment. Bioeng. 81*:299–303 (1996).
221. Xu, X., L.B. Fomuso, and C.C. Akoh, Modification of Menhaden Oil by Enzymatic Acidolysis to Produce Structured Lipids: Optimization by Response Surface Design in a Packed Bed Reactor, *J. Am. Oil Chem. Soc. 77*:171–176 (2000).
222. Gonzalez Moreno, P.A., A.R. Medina, F.C. Rubio, B.C. Paez, and E.M. Grima, Production of Structured Lipids by Acidolysis of an EPA-Enriched Fish Oil and Caprylic Acid in a Packed Bed Reactor: Analysis of Three Different Operation Modes, *Biotechnol. Prog. 20*:1044–1052 (2004).
223. Jennings, B.H., and C.C. Akoh, Enzymatic Modification of Triacylglycerols of High Eicosapentaenoic and Docosahexaenoic Acids Content to Produce Structured Lipids, *J. Am. Oil Chem. Soc. 76*:1133–1137 (1999).

224. Negishi, S., Y. Arai, S. Arimoto, K. Tsuchiya, and I. Takahashi, Synthesis of 1,3-Dicapryloyl-2-docosahexaenoylglycerol by a Combination of Nonselective and *sn*-1,3-Selective Lipase Reactions, *Ibid. 80*:971–974 (2003).
225. Mangos, T.J., K.C. Jones, and T.A. Foglia, Lipase-Catalyzed Synthesis of Structured Low-Calorie Triacylglycerols, *Ibid. 76*:1127–1132 (1999).
226. McNeill, G.P., and P.E. Sonnet, Low-Calorie Triglyceride Synthesis by Lipase-Catalyzed Esterification of Monoglycerides, *Ibid. 72*:1301–1307 (1995).
227. Lipp, M., and E. Anklam, Review of Cocoa Butter and Alternative Fats for Use in Chocolate—Part A. Compositional Data, *Food Chem. 62*:73–97 (1998).
228. Akoh, C.C., and X. Xu, Enzymatic Production of Betapol and Other Specialty Fats, in *Lipid Biotechnology*, edited by T.M. Kuo, and H.W. Gardner, Marcel Dekker, New York, 2002, pp. 461–478.
229. Quinlan, P., and R. Moore, Modification of Triglycerides by Lipases: Application to the Production of Nutritionally Improved Fats, *INFORM 4*:580–585 (1993).
230. Yamane, T., Lipase-Catalyzed Synthesis of Structured Triacylglycerols Containing Polyunsaturated Fatty Acids: Monitoring the Reaction and Increasing the Yield, in *Enzymes in Lipid Modification*, edited by U.T. Bornscheuer, Wiley-VCH, Weinheim, Germany, 2000, pp. 148–169.
231. Xu, X., A. Skands, G. Jonsson, and J. Adler-Nissen, Production of Structured Lipids by Lipase-Catalysed Interesterification in an Ultrafiltration Membrane Reactor, *Biotechnol. Lett. 22*:1667–1671 (2000).
232. Xu, X., Enzymatic Production of Structured Lipids: Process Reactions and Acyl Migration, *INFORM 11*:1121–1131 (2000).
233. Fureby, A.M., C. Virto, P. Adlercreutz, and B. Mattiasson, Acyl Group Migrations in 2-Monoolein, *Biocatal. Biotransform. 14*:89–111 (1996).
234. Timm-Heinrich, M., N. Skall Nielsen, X. Xu, and C. Jacobsen, Oxidative Stability of Structured Lipids Containing C18:0, C18:1, C18:2, C18:3 or CLA in *sn*-2-Position—As Bulk Lipids and in Milk Drinks, *Innovat. Food Sci Emerg. Technol. 5*:249–261 (2004).
235. Timm-Heinrich, M., X. Xu, N. Skall Nielsen, and C. Jacobsen, Oxidative Stability of Mayonnaise and Milk Drink Produced with Structured Lipids Based on Fish Oil and Caprylic Acid, *Eur. Food Res. Technol. 219*:32–41 (2004).
236. Nielsen, N.S., X. Xu, M. Timm-Heinrich, and C. Jacobsen, Oxidative Stability During Storage of Structured Lipids Produced from Fish Oil and Caprylic Acid, *J. Am. Oil Chem. Soc. 81*:375–384 (2004).
237. Torres, C.F., T.J. Nettekoven, and C.G. Hill, Preparation of Purified Acylglycerols of Eicosapentaenoic Acid and Docosahexaenoic Acid and Their Re-esterification with Conjugated Linoleic Acid, *Enzyme Microb. Technol. 32*:49–58 (2003).
238. Senanayake, S.P.J.N., and F. Shahidi, Structured Lipids Enriched with Omega-3 and Omega-6 Highly Unsaturated Fatty Acids, in *Food Factors in Health Promotion and Disease Prevention (ACS Symposium Series 851)*, edited by F. Shahidi, C.-T. Ho, S. Watanabe, and T. Osawa, American Chemical Society, Washington, DC, 2003, pp. 16–26.
239. Gill, I., and R. Valivety, Polyunsaturated Fatty Acids, Part 2: Biotransformations and Biotechnological Applications, *Trends Biotechnol. 15*:470–478 (1997).
240. Senanayake, S.P.J.N., and F. Shahidi, Structured Lipids: Acidolysis of γ-Linolenic Acid-Rich Oils with n-3 Polyunsaturated Fatty Acids, *J. Food Lipids 9*:309–323 (2002).
241. Martinez, C.E., J.C. Vinay, R. Brieva, C.G. Hill, Jr., and H.S. Garcia, Lipase-Catalyzed Acidolysis of Corn Oil with Conjugated Linoleic Acid in Hexane, *Ibid. 10*:11–24 (2003).
242. Ortega, J., A. Lopez-Hernandez, H.S. Garcia, and C.G. Hill, Jr., Lipase-Mediated Acidolysis of Fully Hydrogenated Soybean Oil with Conjugated Linoleic Acid, *J. Food Sci. 69*:FEP1–FEP6 (2003).
243. Torres, C.F., B. Lin, M. Moeljadi, and C.G. Hill, Jr., Lipase-Catalyzed Synthesis of Designer Acylglycerols Rich in Residues of Eicosapentaenoic, Docosahexaenoic, Conjugated Linoleic, and/or Stearic Acids, *Eur. J. Lipid Sci. Technol. 105*:614–623 (2003).
244. Yamane, T., T. Suzuki, and T. Hoshino, Bioreactors for Enzymic Reaction of Fats and Fatty Acid Derivatives, Part XV. Increasing n-3 Polyunsaturated Fatty Acid Content of Fish Oil by Temperature Control of Lipase-Catalyzed Acidolysis, *J. Am. Oil Chem. Soc. 70*:1285–1287 (1993).
245. Bornscheuer, U.T., Lipase-Catalyzed Synthesis of Monoacylglycerols, *Enzyme Microb. Technol. 17*:578–586 (1995).
246. Boyle, E., Monoglycerides in Food Systems: Current and Future Uses, *Food Technol. 51*:52–54, 56, 58–59 (1997).
247. Aha, B., M. Berger, B. Jakob, G. Machmuller, C. Waldinger, and M.P. Schneider, Lipase-Catalyzed Synthesis of Regioisomerically Pure Mono- and Diglycerides, in *Enzymes in Lipid Modification*, edited by U.T. Bornscheuer, Wiley-VCH, Weinheim, Germany, 2000, pp. 100–115.
248. Diks, R.M.M., and J.A. Bosley, The Exploitation of Lipase Selectivities for the Production of Acylglycerols, in *Enzymes in Lipid Modification*, edited by U.T. Bornscheuer, Wiley-VCH, Weinheim, Germany, 2000, pp. 3–22.
249. Krog, N., and J.B. Lauridsen, Food Emulsifiers and Their Associations with Water, *Food Sci. (NY) 5*:67–139 (1976).
250. Jeromin, L., G. Wozny, and P. Li, Process for Production of Monoglyceride by Glycerolysis of Methyl Esters, WO Patent 99-31,043 (1999).
251. Arcos, J.A., H.S. Garcia, and C.G. Hill, Jr., Continuous Enzymatic Esterification of Glycerol with (Poly)unsaturated Fatty Acids in a Packed-Bed Reactor, *Biotechnol. Bioeng. 68*:563–570 (2000).
252. Li, Z., and O.P. Ward, Lipase-Catalyzed Alcoholysis to Concentrate the n-3 Polyunsaturated Fatty Acid of Cod Liver Oil, *Enzyme Microb. Technol. 15*:601–606 (1993).
253. He, Y., and F. Shahidi, Enzymatic Esterification of ω-3 Fatty Acid Concentrates from Seal Blubber Oil with Glycerol, *J. Am. Oil Chem. Soc. 74*:1133–1136 (1997).
254. Arcos, J.A., C. Otero, and C.G. Hill, Jr., Rapid Enzymatic Production of Acylglycerols from Conjugated Linoleic Acid and Glycerol in a Solvent-Free System, *Biotechnol. Lett. 20*:617–621 (1998).
255. Watanabe, Y., Y. Yamauchi-Sato, T. Nagao, T. Yamamoto, K. Ogita, and Y. Shimada, Production of Monoacylglycerol of Conjugated Linoleic Acid by Esterification Followed by Dehydration at Low Temperature Using *Penicillium camembertii* Lipase, *J. Mol. Catal. B. Enzymat. 27*:249–254 (2004).
256. Watanabe, Y., Y. Yamauchi-Sato, T. Nagao, T. Yamamoto, K. Tsutsumi, A. Sugihara, and Y. Shimada, Production of MAG of CLA in a Solvent-Free System at Low Temperature with *Candida rugosa* Lipase, *J. Am. Oil Chem. Soc. 80*:909–914 (2003).
257. Steffen, B., A. Ziemann, S. Lang, and F. Wagner, Enzymatic Monoacylation of Trihydroxy Compounds, *Biotechnol. Lett. 14*:773–778 (1992).
258. Hayes, D.G., and R. Kleiman, Lipase-Catalyzed Synthesis of Lesquerolic Acid Wax and Diol Esters and Their Properties, *J. Am. Oil Chem. Soc. 73*:1385–1392 (1996).
259. Janssen, A.E.M., A. Van der Padt, and K. van't Riet, Solvent Effects on Lipase-Catalyzed Esterification of Glycerol and Fatty Acids, *Biotechnol. Bioeng. 42*:953–962 (1993).
260. Hayes, D.G., and E. Gulari, 1-Monoglyceride Production from Lipase-Catalyzed Esterification of Glycerol and Fatty Acid in Reverse Micelles, *Ibid. 38*:507–517 (1991).

261. Berger, M., and M.P. Schneider, Enzymic Esterification of Glycerol II. Lipase-Catalyzed Synthesis of Regioisomerically Pure 1(3)-*rac*-Monoacylglycerols, *J. Am. Oil Chem. Soc. 69*:961–965 (1992).
262. Yamaguchi, S., and T. Mase, High-Yield Synthesis of Monoglyceride by Mono- and Diacylglycerol Lipase from *Penicillium camembertii* U-150, *J. Ferment. Bioeng. 72*:162–167 (1991).
263. Watanabe, Y., Y. Shimada, Y. Yamauchi-Sato, M. Kasai, T. Yamamoto, K. Tsutsumi, Y. Tominaga, and A. Sugihara, Synthesis of MAG of CLA with *Penicillium camembertii* Lipase, *J. Am. Oil Chem. Soc. 79*:891–896 (2002).
264. Chand, S., P. Adlercreutz, and B. Mattiasson, Lipase-Catalyzed Esterification of Ethylene Glycol to Mono- and Diesters. The Effect of Process Parameters on Reaction Rate and Product Distribution, *Enzyme Microb. Technol. 20*:102–106 (1997).
265. Hayes, D.G., and E. Gulari, The Formation of Polyol-Fatty Acid Esters by Lipase in Reverse Micellar Media, *Biotechnol. Bioeng. 40*:110–118 (1992).
266. Berger, M., K. Laumen, and M.P. Schneider, Lipase-Catalyzed Esterification of Hydrophilic Diols in Organic Solvents, *Biotechnol. Lett. 14*:553–558 (1992).
267. Shaw, J.-F., H.-Z. Wu, and C.-J. Shieh, Optimized Enzymatic Synthesis of Propylene Glycol Monolaurate by Direct Esterification, *Food Chem. 81*:91–96 (2003).
268. Fevrier, P., P. Guegan, F. Yvergnaux, J. Pierre Callegari, L. Dufosse, and A. Binet, Evaluation of Regioselectivity of Lipases Based on Synthesis Reaction Conducted with Propyl Alcohol, Isopropyl Alcohol and Propylene Glycol, *J. Mol. Catal. B. Enzymat. 11*:445–453 (2001).
269. Liu, K.-J., and J.-F. Shaw, Synthesis of Propylene Glycol Monoesters of Docosahexaenoic Acid and Eicosapentaenoic Acid by Lipase-Catalyzed Esterification in Organic Solvents, *J. Am. Oil Chem. Soc. 72*:1271–1274 (1995).
270. Castillo, E., V. Dossat, A. Marty, J.S. Condoret, and D. Combes, The Role of Silica Gel in Lipase-Catalyzed Esterification Reactions of High-Polar Substrates, *Ibid. 74*:77–85 (1997).
271. Yang, C.-L., and E. Gulari, Enzymic Esterification of Diols in Reverse Micellar Media, *Biotechnol. Prog. 10*:269–276 (1994).
272. Viklund, F., and K. Hult, Enzymatic Synthesis of Surfactants Based on Polyethylene Glycol and Stearic or 12-Hydroxystearic Acid, *J. Mol. Catal. B. Enzymat. 27*:51–53 (2004).
273. Garcia, E., F. Ferrari, T. Garcia, M. Martinez, and J. Aracil, Optimization of the Enzymatic Esterification of Diglycerol and Lauric Acid, *J. Surfact. Deterg. 4*:257–262 (2001).
274. Charlemagne, D., and M.D. Legoy, Enzymic Synthesis of Polyglycerol-Fatty Acid Esters in a Solvent-Free System, *J. Am. Oil Chem. Soc. 72*:61–65 (1995).
275. Kato, T., T. Nakamura, M. Yamashita, M. Kawaguchi, T. Kato, and T. Itoh, Surfactant Properties of Purified Polyglycerol Monolaurates, *J. Surfact. Deterg. 6*:331–337 (2003).
276. Linko, Y.Y., T. Tervakangas, M. Lamsa, and P. Linko, Production of Trimethylolpropane Esters of Rapeseed Oil Fatty Acids by Immobilized Lipase, *Biotechnol. Technol. 11*:889–892 (1997).
277. Monot, F., Y. Benoit, D. Ballerini, and J.P. Vandecasteele, Enzymatic Synthesis of Neopentylpolyol Esters in Organic Media, *Appl. Biochem. Biotechnol. 24/25*:375–386 (1990).
278. Adachi, S., K. Nagae, and R. Matsuno, Lipase-Catalyzed Condensation of Erythritol and Medium-Chain Fatty Acids in Acetonitrile with Low Water Content, *J. Mol. Catal. B. Enzymat. 6*:21–27 (1999).
279. Torres, C.F., L.P. Lessard, and C.G. Hill, Jr., Lipase-Catalyzed Esterification of Conjugated Linoleic Acid with Sorbitol: A Kinetic Study, *Biotechnol. Prog. 19*:1255–1260 (2003).
280. Arcos, J.A., M. Bernabé, and C. Otero, Quantitative Enzymatic Production of 1,6-Diacyl Sorbitol Esters, *Biotechnol. Bioeng. 60*:53–60 (1998).
281. Sarney, D.B., M.J. Barnard, M. Virto, and E.N. Vulfson, Enzymatic Synthesis of Sorbitan Esters Using a Low-Boiling-Point Azeotrope as a Reaction Solvent, *Ibid. 54*:351–356 (1997).
282. Ducret, A., A. Giroux, M. Trani, and R. Lortie, Enzymatic Preparation of Biosurfactants from Sugars or Sugar Alcohols and Fatty Acids in Organic Media Under Reduced Pressure, *Ibid. 48*:214–221 (1995).
283. Ducret, A., A. Giroux, M. Trani, and R. Lortie, Characterization of Enzymatically Prepared Biosurfactants, *J. Am. Oil Chem. Soc. 73*:109–113 (1996).
284. Sarney, D.B., and E.N. Vulfson, Enzymatic Synthesis of Sugar Fatty Acid Esters in Solvent-Free Media, in *Enzymes in Nonaqueous Solvents: Methods and Protocols (Methods in Biotechnology Vol. 15)*, edited by E.N. Vulfson, P.J. Halling, and H.L. Holland, Humana Press, Totawa, NJ, 2001, pp. 531–543.
285. Vulfson, E., Enzymatic Synthesis of Surfactants, in *Novel Surfactants*, edited by K. Holmberg, Marcel-Dekker, New York, 2003, pp. 257–278.
286. Nakamura, S., Using Sucrose Esters as Food Additives, *INFORM 8*:866–874 (1997).
287. Lang, S., C. Syldatk, and U. Rau, Enzymatic Synthesis and Modification of Glycolipids, in *Enzymes in Lipid Modification*, edited by U.T. Bornscheuer, Wiley-VCH, Weinheim, Germany, 2000, pp. 363–393.
288. Feuge, R.O., H.J. Zeringue, Jr., T.J. Weiss, and M. Brown, Preparation of Sucrose Esters by Interesterification, *J. Am. Oil Chem. Soc. 47*:56–60 (1970).
289. Osipow, L.I., and W. Rosenblatt, Micro-emulsion Process for Preparation of Sucrose Esters, *Ibid. 44*:307–309 (1967).
290. Kaufman, V.R., and N. Garti, Organic Reactions in Emulsions—Preparation of Glycerol and Polyglycerol Esters of Fatty Acids by Transesterification Reaction, *Ibid. 59*:471–474 (1982).
291. Maag, H., Fatty Acid Derivatives: Important Surfactants for Household, Cosmetic and Industrial Purposes, *Ibid. 61*:259–267 (1984).
292. Woudenberg-van Oosterom, M., F. van Rantwijk, and R.A. Sheldon, Regioselective Acylation of Disaccharides in *tert*-Butyl Alcohol Catalyzed by *Candida antarctica* Lipase, *Biotechnol. Bioeng. 49*:328–333 (1996).
293. Ferrer, M., M.A. Cruces, M. Bernabé, A. Ballesteros, and F.J. Plou, Lipase-Catalyzed Regioselective Acylation of Sucrose in Two-Solvent Mixtures, *Ibid. 65*:10–16 (1999).
294. Potier, P., A. Bouchu, G. Descotes, and Y. Queneau, Lipase-Catalyzed Selective Synthesis of Sucrose Mixed Diesters, *Synthesis*:458–462 (2001).
295. Pedersen, N.R., R. Wimmer, J. Emmersen, P. Degn, and L.H. Pedersen, Effect of Fatty Acid Chain Length on Initial Reaction Rates and Regioselectivity of Lipase-Catalysed Esterification of Disaccharides, *Carbohydr. Res. 337*:1179–1184 (2002).
296. Sarney, D.B., M.J. Barnard, D.A. MacManus, and E.N. Vulfson, Application of Lipases to the Regioselective Synthesis of Sucrose Fatty Acid Monoesters, *J. Am. Oil Chem. Soc. 73*:1481–1487 (1996).
297. Plou Gasca, F.J., E. Pastor Martinez, M.A. Cruces Villalobos, M. Ferrer Martinez, and A. Ballesteros Olmo, Specific Enzymic Acylation of a Secondary Hydroxyl of Sucrose, Spanish Patent 2,141,670 (2000).
298. Park, S., F. Viklund, K. Hult, and R.J. Kazlauskas, Ionic Liquids Create New Opportunities for Nonaqueous Biocatalysis with Polar Substrates: Acylation of Glucose and Ascorbic Acid, in *Ionic Liquids as Green Solvents: Progress and Prospects (ACS Symposium Series No. 856)*, edited by R.D. Rogers, American Chemical Society, Washington, DC, 2003, pp. 225–238.
299. Yan, Y., U.T. Bornscheuer, and R.D. Schmid, Efficient Water

Removal in Lipase-Catalyzed Esterifications Using a Low-Boiling-Point Azeotrope, *Biotechnol. Bioeng. 78*:31–34 (2002).
300. Cao, L., U.T. Bornscheuer, and R.D. Schmid, Lipase-Catalyzed Solid Phase Synthesis of Sugar Esters, *Fett/Lipid 98*:332–335 (1996).
301. Cao, L., A. Fischer, U.T. Bornscheuer, and R.D. Schmid, Lipase-Catalyzed Solid Phase Synthesis of Sugar Fatty Acid Esters, *Biocatal. Biotransform. 14*:269–283 (1997).
302. Cao, L., U.T. Bornscheuer, and R.D. Schmid, Lipase-Catalyzed Solid-Phase Synthesis of Sugar Esters, IV: Selectivity of Lipase Towards Primary and Secondary Hydroxyl Groups in Carbohydrates, *Ibid. 16*:249–257 (1998).
303. Cao, L., U.T. Bornscheuer, and R.D. Schmid, Lipase-catalyzed Solid-phase Synthesis of Sugar Esters. Influence of Immobilization on Productivity and Stability of the Enzyme, *J. Mol. Catal. B. Enz. 6*:279–285 (1999).
304. Zhang, X., and D.G. Hayes, Increased Rate of Lipase-Catalyzed Saccharide-Fatty Acid Esterification by Control of Reaction Medium, *J. Am. Oil Chem. Soc. 76*:1495–1500 (1999).
305. Adelhorst, K., F. Björkling, S.E. Godtfredsen, and O. Kirk, Enzyme Catalysed Preparation of 6-*O*-Acylglucopyranosides, *Synthesis*:112–115 (1990).
306. Bousquet, M.P., R.M. Willemot, P. Monsan, and E. Boures, Enzymatic Synthesis of Unsaturated Fatty Acid Glucoside Esters for Dermo-Cosmetic Applications, *Biotechnol. Bioeng. 63*:730–736 (1999).
307. De Goede, A.T.J.W., M. van Oostero, M.P.J. van Deurzen, R.A. Sheldon, H. van Bekkum, and F. van Rantwijk, Selective Lipase-Catalyzed Esterification of Alkyl Glycosides, *Biocatalysis 9*:145–155 (1994).
308. Kapucu, N., A. Guevenc, U. Mehmetoglu, A. Calimli, and H. Kapucu, Lipase Catalyzed Synthesis of Oleyl Oleate: Optimization by Response Surface Methodology, *Chem. Eng. Commun. 190*:779–796 (2003).
309. Steinke, G., P. Weitkamp, E. Klein, and K.D. Mukherjee, High-Yield Preparation of Wax Esters *via* Lipase-Catalyzed Esterification Using Fatty Acids and Alcohols from Crambe and Camelina Oils, *J. Agric. Food Chem. 49*:647–651 (2001).
310. Garcia, T., M. Martinez, and J. Aracil, Enzymic Synthesis of an Analog of Jojoba Oil: Optimization by Statistical Analysis, *Enzyme Microb. Technol. 15*:607–611 (1993).
311. Xu, Y., W. Du, J. Zeng, and D. Liu, Conversion of Soybean Oil to Biodiesel Fuel Using Lipozyme TL IM in a Solvent-Free Medium, *Biocatal. Biotransform. 22*:45–48 (2004).
312. Shah, S., S. Sharma, and M.N. Gupta, Enzymatic Transesterification for Biodiesel Production, *Indian J. Biochem. Biophys. 40*:392–399 (2003).
313. Du, W., Y. Xu, and D. Liu, Lipase-Catalyzed Transesterification of Soya Bean Oil for Biodiesel Production During Continuous Batch Operation, *Biotechnol. Appl. Biochem. 38*:103–106 (2003).
314. Xu, Y., W. Du, D. Liu, and J. Zeng, A Novel Enzymatic Route for Biodiesel Production from Renewable Oils in a Solvent-Free Medium, *Biotechnol. Lett. 25*:1239–1241 (2003).
315. Lara Pizarro, A.V., and E.Y. Park, Lipase-Catalyzed Production of Biodiesel Fuel from Vegetable Oils Contained in Waste Activated Bleaching Earth, *Proc. Biochem. 38*:1077–1082 (2003).
316. Shieh, C.J., H.F. Liao, and C.C. Lee, Optimization of Lipase-Catalyzed Biodiesel by Response Surface Methodology, *Bioresour. Technol. 88*:103–106 (2003).
317. Belafi-Bako, K., F. Kovacs, L. Gubicza, and J. Hancsok, Enzymatic Biodiesel Production from Sunflower Oil by *Candida antarctica* Lipase in a Solvent-Free System, *Biocatal. Biotransform. 20*:437–439 (2002).
318. Watanabe, Y., Y. Shimada, A. Sugihara, and Y. Tominaga, Conversion of Degummed Soybean Oil to Biodiesel Fuel with Immobilized *Candida antarctica* Lipase, *J. Mol. Catal. B. Enzymat. 17*:151–155 (2002).
319. Shimada, Y., Y. Watanabe, A. Sugihara, and Y. Tominaga, Enzymatic Alcoholysis for Biodiesel Fuel Production and Application of the Reaction to Oil Processing, *Ibid. 17*:133–142 (2002).
320. Haas, M.J., G.J. Piazza, and T.A. Foglia, Enzymatic Approaches to the Production of Biodiesel Fuels, in *Lipid Biotechnology*, edited by T.M. Kuo, and H.W. Gardner, Marcel Dekker, New York, 2002, pp. 587–598.
321. Lee, K.-T., T.A. Foglia, and K.-S. Chang, Production of Alkyl Ester as Biodiesel from Fractionated Lard and Restaurant Grease, *J. Am. Oil Chem. Soc. 79*:191–195 (2002).
322. Fukuda, H., A. Kondo, and H. Noda, Biodiesel Fuel Production by Transesterification of Oils, *J. Biosci. Bioeng. 92*:405–416 (2001).
323. Watanabe, Y., Y. Shimada, A. Sugihara, and Y. Tominaga, Enzymatic Conversion of Waste Edible Oil to Biodiesel Fuel in a Fixed-Bed Bioreactor, *J. Am. Oil Chem. Soc. 78*:703–707 (2001).
324. Soumanou, M.M., and U.T. Bornscheuer, Lipase-Catalyzed Alcoholysis of Vegetable Oils, *Eur. J. Lipid Sci. Technol. 105*:656–660 (2003).
325. Kim, J., D.H. Altreuter, D.S. Clark, and J.S. Dordick, Rapid Synthesis of Fatty Acid Esters for Use as Potential Food Flavors, *J. Am. Oil Chem. Soc. 75*:1109–1113 (1998).
326. Hayes, D.G., The Catalytic Activity of Lipases Toward Hydroxy Acids (a review), *Ibid. 73*:543–549 (1996).
327. Hayes, D.G., and R. Kleiman, Lipase-Catalyzed Synthesis of Estolides and Their Esters, *Ibid. 72*:1309–1316 (1995).
328. Rüsch gen. Klaas, M., and S. Warwel, Lipase-Catalyzed Peroxy Fatty Acid Generation and Lipid Oxidation, in *Enzymes in Lipid Modification*, edited by U.T. Bornscheuer, Wiley-VCH, Weinheim, Germany, 2000, pp. 116–127.
329. Hilker, I., D. Bothe, J. Pruss, and H.J. Warnecke, Chemo-enzymatic Epoxidation of Unsaturated Plant Oils, *Chem. Eng. Sci. 56*:427–432 (2001).
330. Yadav, G.D., and K.M. Devi, Enzymatic Synthesis of Perlauric Acid Using Novozym 435, *Biochem. Eng. J. 10*:93–101 (2002).
331. Rüsch gen. Klaas, M., K. Steffens, and N. Patett, Biocatalytic Peroxy Acid Formation for Disinfection, *J. Mol. Catal. B. Enzymat. 19–20*:499–505 (2002).
332. Uyama, H., M. Kuwabara, T. Tsujimoto, and S. Kobayashi, Enzymatic Synthesis and Curing of Biodegradable Epoxide-Containing Polyesters from Renewable Resources, *Biomacromolecules 4*:211–215 (2003).
333. Gardner, H.W., Lipoxygenase Pathway in Cereals, in *Advances in Cereal Science and Technology*, edited by Y. Pomeranz, American Association of Cereal Chemists, St Paul, MN, 1998, pp. 161–215.
334. Sheldon, R.A., Synthesis of Oxiranes, in *Applied Homogeneous Catalysis with Organometallic Compounds*, edited by B. Cornils and W.A. Herrmann, Wiley-Canada, Mississauga, Ontario, Canada, 1996, pp. 411–423.
335. Hamann, H.-J., E. Hoft, and J. Liebscher, Preparation of Optically Active Hydroperoxides and Their Use for Stereoselective Oxygen Transfer, in *Peroxide Chemistry*, edited by W. Adam, Wiley-VCH, Weinheim, Germany, 2001, pp. 381–405.
336. Gargouri, M., and M. Dominique Legoy, A Two-Enzyme System for the Transformation of Unsaturated Oils to 9(S)-Hydroperoxy Fatty Acids, *Biotechnol. Lett. 24*:915–918 (2002).
337. Weiss, A., U. Schoerken, V. Candar, Y. Eryasa, M. Wunderlich, N. Buyukuslu, and C. Beverungen, Process for the Enzymic Generation and Recovery of Fatty Acid Hydroperoxides, European Patent 1,336,659 (2003).
338. Przybylski, R., Canola Oil: Physical and Chemical Properties, Canola Council of Canada, Winnipeg, 2001.

339. Ling, W.H., and P.J.H. Jones, Dietary Phytosterols: A Review of Metabolism, Benefits and Side Effects, *Life Sci. 57*:195–206 (1995).
340. Plat, J., D.A.J.M. Kerckhoffs, and R.P. Mensink, Therapeutic Potential of Plant Sterols and Stanols, *Curr. Opin. Lipidol. 11*:571–576 (2000).
341. Basheer, S., and D. Plat, Enzymatic Modification of Sterols Using Sterol-Specific Lipase, WO Patent 2001-075,083 (2001).
342. Weber, N., P. Weitkamp, and K.D. Mukherjee, Cholesterol-Lowering Food Additives: Lipase-Catalysed Preparation of Phytosterol and Phytostanol Esters, *Food Res. Intl. 35*:177–181 (2002).
343. Shimada, Y., S. Nakai, M. Suenaga, A. Sugihara, M. Kitano, and Y. Tominaga, Facile Purification of Tocopherols from Soybean Oil Deodorizer Distillate in High Yield Using Lipase, *J. Am. Oil Chem. Soc. 77*:1009–1013 (2000).
344. Watanabe, Y., T. Nagao, Y. Hirota, M. Kitano, and Y. Shimada, Purification of Tocopherols and Phytosterols by a Two-Step *in situ* Enzymatic Reaction, *Ibid. 81*:339–345 (2004).
345. Hirota, Y., T. Nagao, Y. Watanabe, M. Suenaga, S. Nakai, M. Kitano, A. Sugihara, and Y. Shimada, Purification of Steryl Esters from Soybean Oil Deodorizer Distillate, *Ibid. 80*:341–346 (2003).
346. Chu, B.S., B.S. Baharin, and S.Y. Quek, Factors Affecting Preconcentration of Tocopherols and Tocotrienols from Palm Fatty Acid Distillate by Lipase-Catalysed Hydrolysis, *Food Chem. 79*:55–59 (2002).
347. Weber, N., P. Weitkamp, and K.D. Mukherjee, Steryl and Stanyl Esters of Fatty Acids by Solvent-Free Esterification and Transesterification *in vacuo* Using Lipases from *Rhizomucor miehei*, *Candida antarctica*, and *Carica papaya*, *J. Agric. Food Chem. 49*:5210–5216 (2001).
348. Weber, N., P. Weitkamp, and K.D. Mukherjee, Fatty Acid Steryl, Stanyl, and Steroid Esters by Esterification and Transesterification *in vacuo* Using *Candida rugosa* Lipase as Catalyst, *Ibid. 49*:67–71 (2001).
349. Stamatis, H., V. Sereti, and F.N. Kolisis, Studies on the Enzymatic Synthesis of Lipophilic Derivatives of Natural Antioxidants, *J. Am. Oil Chem. Soc. 76*:1505–1510 (1999).
350. Osanai, S., Synthesis of Cholesterol Ester with Lipase in Organic Solvent and the Possibility of Repeated Use of the Recovered Enzyme, Yukagaku 35:955–957 (1986).
351. Norinobu, S., N. Senoo, S. Kaneko, F. Sato, and M. Mankura, Supercritical Preparation of Sterol Fatty Ester with Enzyme, Japanese Patent 2002-233396 (2002).
352. Bertinotti, A., G. Carrea, G. Ottolina, and S. Riva, Regioselective Esterification of Polyhydroxylated Steroids by *Candida antarctica* lipase B, *Tetrahedron 50*:13165–13172 (1994).
353. Yang, H., X. Xie, and P.A. Seib, Lipase-Catalyzed Synthesis of C-6 Saturated and Unsaturated Fatty Acid Esters of L-Ascorbic Acid, *J. Appl. Glycosci. 50*:373–378 (2003).
354. Maugard, T., J. Tudella, and M.D. Legoy, Study of Vitamin Ester Synthesis by Lipase-Catalyzed Transesterification in Organic Media, *Biotechnol. Prog. 16*:358–362 (2000).
355. Yan, Y., U.T. Bornscheuer, and R.D. Schmid, Lipase-Catalyzed Synthesis of Vitamin C Fatty Acid Esters, *Biotechnol. Lett. 21*:1051–1054 (1999).
356. Humeau, C., M. Girardin, B. Rovel, and A. Miclo, Enzymic Synthesis of Fatty Acid Ascorbyl Esters, *J. Mol. Catal. B. Enzymat. 5*:19–23 (1998).
357. Tang, L., Z. Hao, M.M. Shehate, and Y. Sun, A Kinetic Study of the Synthesis of Ascorbate Fatty Acid Esters Catalysed by Immobilized Lipase in Organic Media, *Biotechnol. Appl. Biochem. 32 (Pt. 1)*:35–39 (2000).
358. Parnham, M.J., The Importance of Phospholipid Terminology, *INFORM 7*:1168–1175 (1996).
359. Krawczyk, T., Lecithin: Consider the Possibilities, *Ibid. 7*:1158–1167 (1996).
360. Doig, S.D., and R.M.M. Diks, Toolbox for Exchanging Constituent Fatty Acids in Lecithins, *Eur. J. Lipid Sci. Technol. 105*:359–367 (2003).
361. Kawahara, Y., Progress in Fats, Oils, Food Technology, *INFORM 4*:663–667 (1993).
362. Wang, X., A. Qiu, W. Tao, and P. Shen, Synthesis of Phosphatidylglycerol from Soybean Lecithin with Immobilized Phospholipase D, *J. Am. Oil Chem. Soc. 74*:87–91 (1997).
363. Voinea, M., and M. Simionescu, Designing of "Intelligent" Liposomes for Efficient Delivery of Drugs, *J. Cell. Molec. Med. 6*:465–474 (2002).
364. Fattal, E., P. Couvreur, and C. Dubernet, "Smart" Delivery of Antisense Oligonucleotides by Anionic pH-Sensitive Liposomes, *Adv. Drug Deliv. Rev. 56*:931–946 (2004).
365. Banerjee, R., Liposomes: Applications in Medicine, *J. Biomat. Appl. 16*:3–21 (2001).
366. Iwasaki, Y., and T. Yamane, Phospholipases in Enzyme Engineering of Phospholipids for Food, Cosmetics, and Medical Applications, in *Lipid Biotechnology*, edited by T.M. Kuo and H.W. Gardner, Marcel Dekker, New York, 2002, pp. 417–431.
367. Adlercreutz, P., A.M. Lyberg, and D. Adlercreutz, Enzymatic Fatty Acid Exchange in Glycerolphospholipids, *Eur. J. Lipid Sci. Technol. 105*:638–645 (2003).
368. Takahashi, K., and M. Hosokawa, Production of Tailor-Made Polyunsaturated Phospholipids Through Biocatalysis, in *Lipid Biotechnology*, edited by T.M. Kuo and H.W. Gardner, Marcel Dekker, New York, 2002, pp. 517–526.
369. Gramatikova, S., G. Hazlewood, D. Lam, and N. Barton, Identification, Cloning and Sequences of Phospholipases from Environmental Sources and Their Use in Oil Degumming and Other Industrial Methods, WO Patent 2003-089,620 (2003).
370. Clausen, K., Enzymatic Oil-Degumming by a Novel Microbial Phospholipase, *Eur. J. Lipid Sci. Technol. 103*:333–340 (2001).
371. Sarney, D.B., G. Fregapane, and E.N. Vulfson, Lipase-Catalyzed Synthesis of Lysopholipids in a Continuous Bioreactor, *J. Am. Oil Chem. Soc. 71*:93–96 (1994).
372. Brockerhoff, H., and M. Yurkowski, Simplified Proparation of L-α-Glycerylphosphorlcholine, *Can. J. Biochem. 43*:1777 (1965).
373. Peng, L., X. Xu, H. Mu, C.-E. Høy, and J. Adler-Nissen, Production of Structured Phospholipids by Lipase-Catalyzed Acidolysis: Optimization Using Response Surface Methodology, *Enzyme Microb. Technol. 31*:523–532 (2002).
374. Hosokawa, M., T. Shimatani, T. Kanada, Y. Inoue, and K. Takahashi, Conversion to Docosahexaenoic Acid-Containing Phosphatidylserine from Squid Skin Lecithin by Phospholipase D-Mediated Transphosphatidylation, *J. Agric. Food Chem. 48*:4550–4554 (2000).
375. Adlercreutz, D., and E. Wehtje, An Enzymatic Method for the Synthesis of Mixed-Acid Phosphatidylcholine, *J. Am. Oil Chem. Soc. 81*:553–557 (2004).
376. Anthonsen, T., P. D'Arrigo, G. Pedrocchi-Fantoni, F. Secundo, S. Servi, and E. Sundby, Phospholipids Hydrolysis in Organic Solvents Catalyzed by Immobilized Phospholipase C, *J. Mol. Catal. B. Enzymat. 6*:125–132 (1999).
377. Ogino, C., Y. Yasuda, A. Kondo, N. Shimizu, and H. Fukuda, Improvement of Transphosphatidylation Reaction Model of Phospholipase D from *Streptoverticillium cinnamoneum*, *Biochem. Eng. J. 10*:115–121 (2002).
378. Nishina, A., H. Torada, and S. Hashizume, Phosphatidylserine Enzymic Manufacture, Japanese Patent 2002-218,991 (2002).
379. Kaneda, T., and Y. Inoue, Enzymic Manufacture of Phosphatidylserines Having Polyunsaturated Fatty Acid Residues, Japanese Patent 2001-186,898 (2001).
380. Kudo, S., H. Yamatoya, and M. Sakai, Phosphatidylserines Enzymic Manufacture, Japanese Patent 09,121,879 (1997).
381. Yamane, T., and S. Shimizu, Manufacture of Phosphatidylser-

ines with Phospholipase D from *Streptomyces*, Japanese Patent 02,079,990 (1990).
382. Fujita, K., S. Murakami, K. Iwanami, S. Tokuyama, and O. Nakachi, Enzymic Manufacture of Phospholipids Having Altered Basic Groups, Japanese Patent 63,036,791 (1988).
383. Kirschner, G., G. Menon, and S. Vaccaro, Procedure for the Preparation of Pure Phosphatides and Their Use in the Cosmetic, Pharmaceutical and Alimentary Fields, Japanese Patent 1231213 (2002).
384. Rich, J.O., and Y.L. Khmelnitsky, Phospholipase D-Catalyzed Transphosphatidylation in Anhydrous Organic Solvents, *Biotechnol. Bioeng. 72*:374–377 (2001).
385. Dittrich, N., and R. Ulbrich-Hofmann, Transphosphatidylation by Immobilized Phospholipase D in Aqueous Media, *Biotechnol. Appl. Biochem. 34*:189–194 (2001).
386. Sakai, M., R. Ebina, H. Yamatoya, and S. Kudo, Process for the Production of Phospholipids, WO Patent 2002-012532 (2002).
387. Hirche, F., and R. Ulbrich-Hofmann, The Interdependence of Solvent, Acceptor Alcohol and Enzyme Source in Transphosphatidylation by Phospholipase D, *Biocatal. Biotransform. 18*:343–353 (2000).
388. Schwaneberg, U., and U.T. Bornscheuer, Fatty Acid Hydroxylations Using P450 Monooxygenases, in *Enzymes in Lipid Modification*, edited by U.T. Bornscheuer, Wiley-VCH, Weinheim, Germany, 2000, pp. 394–414.
389. Hou, C.T., New Uses of Vegetable Oils: Novel Oxygenated Fattty Acids by Biotransformation, *SIM News 53*:56–61 (2003).
390. Huang, Y.-S., P. Mukerji, T. Das, and D.S. Knutzon, Transgenic Production of Long-Chain Polyunsaturated Fatty Acids, *World Rev. Nutr. Diet. 88*:243–248 (2001).
391. Budziszewski, G.J., K.P.C. Croft, and D.F. Hildebrand, Uses of Biotechnology in Modifying Plant Lipids, *Lipids 31*:557–569 (1996).
392. Murphy, D.J., Biotechnology and the Improvement of Oil Crops: Genes, Dreams and Realities, *Phytochem. Rev. 1*:67–77 (2002).
393. Roscoe, T., S. Maisonneuve, and M. Delseny, Production of Unusual Fatty Acids in Rapeseed, *Oleagineux 9*:24–30 (2002).
394. Napier, J.A., L.V. Michaelson, and A.K. Stobart, Plant Desaturases: Harvesting the Fat of the Land, *Curr. Opin. Plant Biol. 2*:123–127 (1999).
395. Murphy, D.J., Production of Novel Oils in Plants, *Curr. Opin. Biotechnol. 10*:175–180 (1999).
396. Napier, J.A., and L.V. Michaelson, Towards the Production of Pharmaceutical Fatty Acids in Transgenic Plants, *J. Sci. Food. Agric. 81*:883–888 (2001).
397. Sayanova, O.V., and J.A. Napier, Eicosapentaenoic Acid: Biosynthetic Routes and the Potential for Synthesis in Transgenic Plants, *Phytochemistry 65*:147–158 (2004).
398. Jaworski, J., and E.B. Cahoon, Industrial Oils from Transgenic Plants, *Curr. Opin. Plant Biol. 6*:178–184 (2003).
399. Kinney, A.J., Perspectives on the Production of Industrial Oils in Genetically Engineered Oilseeds, in *Lipid Biotechnology*, edited by T.M. Kuo and H.W. Gardner, Marcel Dekker, New York, 2002, pp. 85–93.
400. Kang, I.J., M.E. Rezac, and P.H. Pfromm, Membrane Permeation Based Sensing for Dissolved Water in Organic Microaqueous Media, *J. Membr. Sci. 239*:213–217 (2004).
401. Ko, S.-N., H. Kim, K.-T. Lee, T.-Y. Ha, S.-H. Chung, S.-M. Lee, and I.-H. Kim, Optimization of Enzymatic Synthesis of Structured Lipid with Perilla Oil and Medium Chain Fatty Acid, *Food. Sci. Biotechnol. 12*:253–256 (2003).
402. Rocha-Uribe, A., and E. Hernandez, Solvent-Free Enzymatic Synthesis of Structured Lipids Containing CLA from Coconut Oil and Tricaprylin, *J. Am. Oil Chem. Soc. 81*:685–689 (2004).

[Received July 29, 2004; accepted November 16, 2004]