REVIEW

Applications of Lipase

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ABSTRACT: Lipases are endowed with a substrate specificity that surpasses that of any other known enzyme. This confers on these enzymes an application potential that is literally boundless. Lipases can be employed in the production of pharmaceuticals, cosmetics, leather, detergents, foods, perfumery, medical diagnostics, and other organic synthetic materials. This review attempts to present a comprehensive discussion on the present status of this unique group of enzymes in industry, as well as the actual potential. It represents an endeavor to provide a sincere answer to the question, "What can be done with this enzyme?" as well as, "Can lipase be utilized for this purpose?" It is intended that the manuscript will cover or at least mention all known applications, based on the exploitation of a particular type of reaction catalyzed by lipases. An attempt will be made to cover as large a number of references as possible, so as to further underline the importance and significance of lipase action for industry.
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Lipases (E.C. 3.1.1.3) are enzymes that are primarily responsible for the hydrolysis of acylglycerides. However, a number of other low- and high-molecular weight esters, thiol esters, amides, polyol/polyacid esters, etc. are accepted as substrates by this unique group of enzymes. The wide berth for employment in a variety of reactions, endowed by this broad substrate specificity, is further enlarged by the fact that lipases are capable of catalyzing the reverse reaction of synthesis just as efficiently. In fact, some lipases are better suited for synthesis than for hydrolysis applications!

The two main categories in which lipase-catalyzed reactions may be classified are as follows:

(i) Hydrolysis:

$$RCOOR' + H_2O \Leftrightarrow RCOOH + R'OH \quad [1]$$

(ii) Synthesis:

Reactions under this category can be further separated:

(a) Esterification

$$RCOOH + R'OH \Leftrightarrow RCOOR' + H_2O \quad [2]$$

(b) Interesterification

$$RCOOR' + R''COOR^* \Leftrightarrow RCOOR^* + R''COOR' \quad [3]$$

(c) Alcoholysis

$$RCOOR' + R''OH \Leftrightarrow RCOOR'' + R'OH \quad [4]$$

(d) Acidolysis

$$RCOOR' + R''COOH \Rightarrow R''COOR' + RCOOH \quad [5]$$

The last three reactions are often grouped together into a single term, viz., transesterification.

Most applications where the potential for lipase is implicated presently entail the modification and/or derivatization of fats and oils and related substances by using classical chemical procedures.

For instance, medium-chain triglycerides are manufactured by esterification of acid with glycerol at high temperatures (200–250°C) and pressures and/or in the presence of an inorganic catalyst (1). This chemical reaction is tedious, nonselective and consumes a large amount of energy. The product obtained has to be purified further, by alkali washing, steam refining, molecular distillation, ultrafiltration, activated carbon treatment, etc.

Another process of industrial importance is the hydrolysis of vegetable oils, such as olive oil or coconut oil, to produce fatty acids and glycerol, both of which find widespread applications, especially in soaps and detergents, cosmetics, pharmaceuticals, and food. The current procedure is physicochemical and involves pressures of 3000–5000 kN/m^2 and temperatures of 250°C or more. About 96–98% of the hydrolysis of fats takes place in 2 h to yield a sweet water stream of 12% glycerol. The resultant fatty acids are unusable as obtained and need to be redistilled to remove color and by-products. The process is energy-consuming and gives rise to a variety of undesirable side reactions, such as polymerization of highly unsaturated fatty acids and production of ketones and hydrocarbons. The capital investment for this steam splitting process is high because a special splitter column, which must withstand high temperature and pressure as well as corrosive acids, is needed (2).

On the other hand, enzymatic fat splitting is carried out at ambient pressure and temperature (40–60°C), allowing for a lower energy cost. The overall cost is also brought down by the fact that reaction vessels need not be highly corrosion-resistant (mineral acids used as catalysts in conventional procedures are very corrosive), nor do they have to withstand severe environmental conditions. The products of such bioprocesses also have better odor and color, and are usually purer (owing to few or no side reactions) than those produced conventionally (3). This has the added advantage of lower cost resulting from the reduced amount of downstream processing required. The lower temperatures employed ensure minimal thermal degradation (4,5). Enzymes used typically for this purpose are lipases.

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A large amount of work has been carried out on the catalysis of reactions by lipases, and it would not be possible to do justice to the several hundred papers published annually in this frontline area during the last 20 yr or more. Nor would this fall within the scope of this review. The main objective of this manuscript is to give an overview of the numerous applications of lipases in almost every foreseeable area—ranging from utilization as a research tool in lipid structural analysis to cleaning and to mediating the synthesis of important organic products and/or their intermediates. This document therefore attempts to underline the importance of the enzyme lipase in almost every conceivable field.

Review of lipase applications is not a new idea. The literature records attempts by Nielsen (6), who wrote about hydrolysis of glycerides by lipases; Seitz (7), who discussed industrial applications of microbial lipases; Macrae and Hammond (8), who also reviewed certain aspects of lipase applications. In addition, Iwai and Tsujisaka (9) have listed a few applications of lipase-catalyzed hydrolysis, whereas Macrae (10) has reviewed the importance of biotechnology vis-à-vis the fats and oils industry, giving reference to some applications of lipase-catalyzed reactions. Yamane (11) has discussed various aspects of enzymes that are useful in the lipid industry, viz., lipases, phospholipases, and lipoxygenases—sources, properties, reactions catalyzed, some applications, and engineering aspects. Two other publications (12,13) merit mention here for their discussion of certain industrial applications for lipases. Godfrey and Reichelt (14) have also discussed some applications of lipases in industry. While these are some of the prominent reviews that have appeared in the literature since 1974, the list is by no means complete. However, despite the vast application potential expounded for lipases, the present scenario is rather dismal, probably due to ignorance about certain intricacies of enzyme reactions. Some of these were handled in an earlier review (Gandhi, N.N., S.B. Sawant, J.B. Joshi, and D. Mukesh, submitted for publication), which discussed the merits of using enzymatic (lipase-catalyzed) processes over conventional chemical reactions. This paper is intended to be the next step toward convincing skeptics of what is actually possible. This point will be emphasized and illustrated through numerous examples and references. It may also not be inappropriate to mention here that every year, more than 700–800 research papers and patents are published/issued, covering just a small area in this vast field offered by lipases. While justice cannot be done to all these workers for obvious reasons, this manuscript intends to impart a flavor of the enormous input into this field. Also, the large amount of work on lipases warrants a constant reviewing of the status to enable interested readers to be updated of the latest status.

HYDROLYSIS

This refers to the splitting of a fat/ester into its constituent acid and glycerol/alcohol in the presence of water. The product of interest may be either a specific acid or alcohol that is formed or, as shown below, the breakdown of the fat may be more important. A sample of various hydrolytic systems employed by workers in this area is presented in Table 1.

Lipolysis. This employs the "constructive" consequences of the ability of lipase to hydrolyze lipids so as to obtain fatty acids and glycerol, both of which have important industrial applications. For instance, fatty acids are used in soap production (15). Lipases used for this purpose include those from *Candida rugosa* (3), castor bean (process already operative on a commercial scale) (16), and *Pseudomonas fluorescens* (17).

The enzymatic method compares well with the chemical reaction (Colgate-Emery process of steam splitting) in terms of cost and it yields products with better odor and color (2). The latter is also considered superior owing to moderate reaction conditions, fewer working hazards, lower power consumption, absence of side reactions, etc.

Enzymatic hydrolysis occurs at ambient or moderate temperatures and pressures and can therefore be used to obtain fatty acids from unstable oils that contain conjugated or highly unsaturated fatty acids (17,18). This is generally difficult to achieve by conventional means owing to the high temperatures and pressures used, which could lead to undesired oxidation of the lipids. This is amply illustrated by the production of ricinoleic acid, a valuable starting material for a variety of technical products. It cannot be produced from castor oil by the conventional steamsplitting process owing to side reactions, such as dehydration, interesterification, etc. These can be avoided by using a lipase, such as that from castor seed, for hydrolysis of castor oil (19).

Lipases may also be used to hydrolyze wax esters, such as *p*-nitrophenyl acetate (20). This reaction is frequently used for lipase assays.

Leather manufacture. Unlike the above, the enzyme is used so as to make use of the ability of lipases to break down lipids. During processing of hides and skins, one important step is the removal of residual fats and protein debris that are associated with the hide and the hair. Such removal by chemical processes, such as liming, is not efficient (7). It has now become common practice to utilize a mixture of lipases and proteases for this purpose (known in technical jargon as the bating process) (13,21,22).

Muthukumaran and Dhar (23) have compared suede clothing leathers obtained from wooled sheep skins after *Rhizopus nodosus* lipase-catalyzed degreasing with those produced by treatment with solvents, such as kerosene, and found that the quality was comparable. Thus, degreasing can be carried out with enzymes rather than expensive and hazardous solvents.

Waste treatment. Lipases are utilized in activated sludge and other aerobic waste processes, where thin layers of fats must be continuously removed from the surface of aerated tanks to permit oxygen transport (to maintain living conditions for the biomass). This skimmed fat-rich liquid is digested with lipases (24), such as that from *C. rugosa*. The latter is manufactured by Meito Sangyo Co. (Nagoya, Japan) under the trade name Lipase-MY and is employed in sewage disposal plants in the United States (7). Lipases may also assist the regular performance of anaerobic digesters (14).

TABLE 1
Hydrolysis Catalyzed by Lipases

Lipase	Substrate	Remarks	Reference
Rhizopus delemar + Penicillium + Rhizopus niveus	Soybean oil	Combined lipase system	2
Candida rugosa, Aspergillus nige	Fish oil	Concentration of polyunsaturated fatty acids	95
C. rugosa	Soybean oil	Membrane reactor was used	153
C. rugosa	Cooking oil, Trex	Electrically enhanced dispersion	154
C. rugosa	*n*-Propylibuprofenate	Enantioselective hydrolysis in AOTmicroemulsions	155
C. rugosa	Olive oil	Lipase immobilized on Sephadex, Amberlite, etc. in isooctane	156
C. rugosa	Soybean oil	Hybrid membrane-emulsion reactor	157
C. rugosa	Butteroil	Spiral-wound membrane reactor	158
A. niger	Butteroil	Flat-sheet membrane reactor	159
C. rugosa	Tuna oil	Discrimination against docosahexaenoic acid	160
C. rugosa	Olive oil	Biphasic isooctane-aqueous system	161
Black cumin seed	Cumin seed oil	Kinetics determined	162
C. rugosa	Butter oil	Lecithin-isooctane reverse micelles	163
C. rugosa	Tributyrin	Kinetics determined	164
Mucor miehei	Soybean phosphatidylcholine	Solvents more polar than hexane better	165
M. miehei, Rhizopus arrhizus	*Lesquerella fendleri* oil	Different reaction systems compared	166
Pseudomonas putida	Olive oil	Cells as lipase source in organic-aqueous two-phase systems	167
C. rugosa	Fish oil	Lipase discrimination against docosahexaenoic acid	168
R. delemar	2-Naphthyl acetate, caprate, laurate, etc.	Interfacial kinetics determined	169
Pseudomonas	Hydrophobic diester	Stereospecific hydrolysis, a step in the synthesis of a selective leukotriene antagonist	170
C. rugosa, M. miehei, P. fluorescens, porcine pancreatic, lipoprotein lipases	Methyl-branched octanoic-acid thiol esters	Effect of branching and stereobias studied	171
Rat hepatic lipase	Neutral glycerides and phospholipids	Higher hydrolytic rate for neutral lipids than phospholipids	172
Chromobacterium viscosum	Olive oil, triolein	AOT-isooctane reversed micelles	173
Thermomyces lanuginosus	Beef tallow	Kinetics studied in a flat-plate immobilized lipase reactor	174
C. rugosa, M. miehei	Tallow, cod liver oil, etc.	Effect of amines on hydrolysis investigated	175
C. rugosa	Beef tallow, pork lard	Reactions in isooctane at temperatures lower than the melting points of substrates	176
R. niveus	Fish oil	Docosahexaenoic acid concentration due to discrimination	177
C. rugosa	Triacetin	Hollow fiber reactor	178
M. miehei	Oleyl oleate	Thermodynamics and kinetics studied	179
C. rugosa	Tributyrin	Various immobilization supports for lipase tested	180
P. fluorescens	Acyl nucleosides	Regioselective deacylation	181
C. rugosa	Olive oil	Hydrolysis enhanced by dimethyl β-cyclodextrin	182
R. delemar	Palm oil	Monoglyceride preparation in microemulsions	183
Porcine pancreatic	Glycidol esters	Enantiomers of glycidol-starting materials for manufacture of cardiovascular β-blockers, etc.	184
C. rugosa	Methyl-2-chloropropionate	Carbon tetrachloride allows for stereospecific hydrolysis	185

Effective breakdown of solids and the clearing and prevention of fat blockage or filming in waste systems are important in many industrial operations. Examples include: (i) degradation of organic debris—A commercial mixture of lipase, cellulase, protease, amylase, inorganic nutrients, wheat bran, etc. is employed for this purpose; (ii) sewage treatment, cleaning of holding tanks, septic tanks, grease traps, etc.

Effluent treatment is also necessary in industrial processing units, such as abattoirs, the food processing industry, the leather industry, and poultry waste processing (14). In fact, Tschoke (25) was granted a patent for enzymatic treatment of fats in wastewater treatment plants whereby scum that contains mainly triglycerides at the plant entrance is hydrolyzed by immobilized lipase-generating bacteria, with reasonable results—up to 90% removal.

Detergents. In line with the two examples above is another important industrial area where lipases are employed for their lipid degradation potential, viz., washing and cleaning. Ap-

plications of lipases and other enzymes in textile detergency were the subject for Nagayama's review (26).

Lipases function in the removal of (fat) stains from fabrics and are important components of detergent mixtures (27–35). Lipases used for this purpose include those from *Candida* (36) and *Chromobacterium viscosum* (37).

The superiority of detergents that contain lipase over those that do not was proven by Fujii *et al.* (38) for the removal of olive oil from cotton fabrics, which was 15–20% greater in the former product than in the latter. Therefore, surfactant systems used in laundering frequently contain lipases (39) in association with other enzymes, such as cutinases. Because laundering is generally carried out in alkaline media, lipases active under such conditions are preferred (28,30,40), for example, the *Aspergillus oryzae*-derived lipase.

Apart from detergents for cleaning fabrics, the other common commercial application for detergents is in dishwashing. Lipase-containing mixtures for this use have been patented by Fukano and Abe (41), Van Dijk (42), etc. The lipase component causes an increase in detergency and prevents scaling.

Other related applications for lipase that have obtained recognition through patents include: (i) a bleaching composition (43); (ii) decomposition of lipid contaminants in dry-cleaning solvents (44); (iii) liquid leather cleaner (45); (iv) contact lens cleaning composition to degrade deposits on the lens (46); (v) clearing of drains clogged by lipids in food processing or domestic/industrial effluent treatment plants (24); (vi) degradation of organic wastes on the surface of exhaust pipes/sewage pipes, toilet bowls, etc. (47); (vii) removal of dirt/cattle manure from domestic animals by lipases and cellulases (48); (viii) washing, degreasing, and water reconditioning by using lipases along with oxidoreductases, which allows for smaller amounts of surfactants and operation at low temperatures (49).

Flavor production in dairy and related industries. The use of lipases in flavor development for dairy products, such as cheese, butter and margarine, is well established (50–55). The aroma and texture of these milk products are a result of fat, protein, and lactose metabolism in milk (28). Therefore, enzymes, such as lipases and proteases, are extensively used for accelerating the maturation of cheese and for the production of typical flavors (13). During this process, there is formation of free fatty acids and soluble peptides and amino acids. Both act as flavors as well as flavor precursors (56), resulting ultimately in products of better flavor and acceptability than their untreated counterparts (57).

The flavor developed will depend on the enzyme used. For instance, pregastric esterase (PGE) is used in rennet paste preparation to curdle milk in Italy. Such cheeses, primarily provolone and Romano, exhibit a characteristic "piccante" flavor with a peppery characteristic. In contrast, cheese made with commercial animal rennets (pancreatic extracts) extracted from empty, washed vells or fermentation-derived rennets do not exhibit "piccante" flavor (55). On the other hand, blue cheese flavor development is due to enzymes from *Pencillium roqueforti* (58).

Enzyme-modified cheeses can be used in cheese spreads, cheese dips, cheese substitutes, etc. Butterfat modified by PGE is utilized to impart dairy flavor character to a wide range of processed foods (54). Margrove and McDonough (51) have used modified butterfat to promote flavor in cream that is used to improve skim-milk cheese. Lipase-treated milk fats are used in the production of butter/margarine flavors (52).

In coffee whiteners, lipases assist in imparting a rich creamy flavor (14). They enhance the buttery character of toffees and caramels and reduce excessive sweetness.

Apart from milk products, lipases also improve flavors of rice and alcoholic beverages, such as apple wine. Shay *et al.* (59) carried out continuous fermentation of *C. utilis* in the presence of beef extract/butteroil and lipase to a total mass of 800 kg. After heat treatment (at about 82°C) of the biomass, followed by spray-drying (in the absence of a centrifugation step), the powdered yeast had a beefy/blue cheese-like flavor. In the absence of these flavor-enhancing additives, the yeast had a bland flavor.

In the chocolate industry, the free fatty acid contribution to flavor of milk chocolate, caramels, toffees, and butter creams is appreciable. Such flavors can be obtained by the use of cultured broths or by lipases.

In summary, products flavored by addition of lipolyzed materials include: (i) bakery/cereal products, such as cake and cookie mixes, sweet doughs, cheese, cake mixes, pancake mixes; (ii) candy/confectioneries, such as milk chocolate, creams and cream centers, toffee, and caramel fudges; (iii) dairy, such as cheese dips, coffee whiteners; and (iv) miscellaneous, including margarines, popcorn oils, salad dressings, sauces, snack foods, and soups.

Foods. This is another related industry with enormous potential for lipase application. Biolipolysis is already being used for the production of fat-free meats (7,24,60). Fat removal during fish processing can be done with lipases (7).

Partial hydrolysis of triglycerides to increase the monoglyceride content by addition of lipase to bread dough reportedly leads to retardation of staling (61). Formation of mono- and diglycerides also allows for improvement of egg-white whipping properties (62).

According to Posorske (13), the palatability of dog food can be improved by partial hydrolysis of beef tallow with lipases. Haas and Lugay (63) reported that, when a component consisting of protein and emulsified fat is treated with a mixture of pancreatic lipase and a protease and incorporated into dog food, it greatly increases its palatability.

Lipases have also found application in soybean milk preparation, smoked carp processing, vegetable fermentation, and meat curing by virtue of their lipolytic ability (7).

Medical applications. Besides carbohydrates and proteins, the other major group of biomaterials consists of lipids. All three are ingested in substantial quantities by the body, and their digestion, absorption and assimilation constitute a major function. The primary enzyme for fat metabolism is lipase, and its deficiency would pose dire consequences to health.

The corrective measure would be external administration of lipase. Thus, lipases may be used as digestive aids (28,64).

The lipase level in blood serum is a diagnostic indicator for conditions such as acute pancreatitis and pancreatic injury (65,66) (the lipase assay is more sensitive than amylase estimation). Lipase activity/level determination is also important in the diagnosis of heart ailments (67).

Like many other enzymes, lipases may be immobilized onto pH/oxygen electrodes (in combination with glucose oxidase). These function as lipid biosensors (68,69) and may be used in triglyceride (70) and blood cholesterol determinations (71).

Lipase, being an activator of the Tumor Necrosis Factor, can be used in the treatment of malignant tumors (72). Other therapeutic applications of lipases, along with other components, have been found for treatment of gastrointestinal disturbances, dyspepsias, cutaneous manifestations of digestive allergies, etc. (73).

PGE preparations are reported to be useful in the treatment of calf scours and human malabsorption syndrome (54).

Pharmaceuticals and cosmetics. Lipase is a component of a hair-waving preparation (74) in which it promotes penetration of the preparation. Berrobi *et al.* (75) have filed a patent for cosmetic, pharmaceutical preparations that contain hyaluronidase and/or thiomucase enzymes in addition to lipases for use in skin inflammations.

"Slimming down," which is currently in vogue, can be carried out by fat removal by using lipase as a component of topical creams (76) or by its oral administration (77). Hirashima *et al.* (78) carried out the hydrolysis of acyl bonds at the 1-position of 1,2 diacylglycerophospholipids for the purification of plasmalogens with *R. delemar* lipase.

Structural analysis of triglycerides. Lipases can be classified on the basis of their substrate specificity. For instance, lipases from *Mucor miehei*, *R. delemar*, and porcine pancreas attack at the primary hydroxyl positions (1,3) of glycerol preferentially and are said to be 1,3-specific. On the other hand, lipases from *C. rugosa, Chromobacterium viscosum*, castor bean, etc. are nonspecific with respect to position. The lipase from *Geotrichum candidum* is selective toward *cis*-unsaturated (Δ9) fatty acids, such as oleic acid (79). Lipases from *Brassica napus* and *M. miehei* discriminate against polyunsaturated acids, such as γ-linolenic and docosahexaenoic acid (80), while adipose hormone-sensitive lipase preferentially releases polyunsaturated fatty acids from triglycerides (81).

Thus, the hydrolysis of lipids with different types of fatty acids at different positions will lead to hydrolyzates that are specific for a particular type of lipase. Hence, the regiospecificity and substrate selectivity of lipases can be advantageously exploited for their use in structural determination (82) of triglycerides, and for the synthesis of a specific and defined set of mono- and/or diglycerides (71,83–85).

Deinking of wastepaper. The addition of 200 units of lipase from *Pseudomonas* species (KWI-56) to a deinking composition for ethylene oxide—propylene oxide adduct stearate improved whiteness of paper from 56.5 to 58% and reduced residual ink spots from 18 to 7 cm (86). Patents for lipase as a deinking agent have also been granted to Sharyo and Sakaguchi (87) and Hagiwara *et al.* (88).

Resolution of racemates. Lipases have been used widely for the resolution of racemic acids and alcohols through asymmetric hydrolysis of corresponding esters as a result of the stereospecific characteristics of lipase activity (89–91). The preparation of a number of optically active secondary alcohols by enantioselective hydrolysis of racemic esters by lipases/esterases has been described by Roberts (92). Foelsche *et al.* (93) achieved the resolution of acyclic 2-azido alcohols by hydrolysis of racemic butyrates. Miyate and Sato (94) have utilized this property for the manufacture of optically active 2-hydroxy-4-phenylbutyrate esters which are useful in the synthesis of drugs, such as cilizapril.

Fatty acid fractionation. Hoshino *et al.* (95) developed a bioreactor for enriching triglycerides with n-3 polyunsaturated fatty acids (PUFA) in cod liver and sardine oils by more than 20% of the original by exploiting the inactivity of *C. rugosa* and *A. niger* lipases toward PUFA (other acids were preferentially formed during hydrolysis, leaving the triglyceride fraction rich in PUFA).

The selective preparation of petroselinic (*cis*-6-octadecenoic) acid from fennel oil was possible by hydrolysis with *R. arrhizus* lipase, which has a high selectivity for this acid (96).

Others reactions. (i) Mulberry silk refining by means of protease and lipase yields a product of better quality than alkali-refined silk (97); (ii) a solid mouth deodorant contains lipase, along with proteinases (98).

ESTER SYNTHESIS

The ability of lipases to catalyze the reverse reaction of synthesis is used in the manufacture of desired products. Esterification mixtures generally contain only the substrates and enzyme (solvent option may be foregone), and water is the only by-product of the reaction. Because the latter is generally easy to remove, this process is quite superior to other synthetic processes, which entail the use of hazardous solvents, corrosive acid catalysts, etc. (2,79), especially because esterification can proceed efficiently without the use of solvent (99) and because almost complete conversion is possible by adopting means such as continuous water removal. This enables an equilibrium shift in favor of the synthetic reaction. A large amount of work has been carried out to elucidate the mechanisms and kinetics of lipase-catalyzed esterification and the factors affecting them. They have been reviewed (Gandhi, N.N., S.B. Sawant, J.B. Joshi, and D. Mukesh, submitted for publication). Table 2 summarizes some esterification systems in the literature.

While esterification produces water and ester (usually the desired product), transesterification processes, such as alcoholysis, acidolysis and interesterification, give rise to alcohol, acid, or ester instead of water. Hence, transesterification becomes more lucrative when any of these are the desired products.

TABLE 2
Esterification Catalyzed by Lipases[a]

Lipase	Acid	Alcohol	Solvent	Reference
R. niveus, R. javanicus, C. viscosum, R. delemar, P. roqueforti, P. fluorescens, H. lanuginosa, etc.	C_4	C_2, C_4, isoamyl	Hexane	104
M. miehei	C_{12}, oleic	C_3–C_{12}	—	106
G. candidum, A. niger, R. delemar, P. cyclopium	Oleic	Terpene alcohols, primary alcohols (C_1–C_{12}), 2- and 3-substituted alcohols, benzyl alcohol, cyclohexanol, etc	Buffer + casein	108
A. niger, R. delemar, P. cyclopium, G. candidum	C_2–C_{18}, benzoic, oleic, ricinoleic, sebacic, succinic, etc.	Glycerol	Water	109
A. niger, R. delemar, P. cyclopium, etc.	C_3–C_6, isobutyric, etc.	Geraniol, farnesol, phytol, β-citronellol, etc.	—	110
M. miehei	C_4	C_4	Hexane	113
C. rugosa	Oleic	Sucrose, sorbitol, glucose, fructose, etc.	Buffer (pH 5.4)	115
C. rugosa	Oleic, isostearic, 12-hydroxystearic, stearic, etc.	Cholesterol	Cyclohexane	117
C. antarctica	Melted coconut acids	Ethyl *D*-glucopyranoside	—	119
P. cyclopium A, B, *C. rugosa, M. miehei, Aspergillus, C. rugosa,* PPL (Rohm, Kochlight), *Alcaligenes*	C_2–C_4	Isoamyl, geraniol	Heptane	132
M. miehei	Oleic, linoleic, α-linolenic, γ-linolenic, docosahexaenoic, etc.	C_2	Pentane	144
C. rugosa	C_4	C_2	Heptane	152
R. oligosporus	Oleic	C_1–C_8, 2-substituted alcohols (C_3–C_5)	Buffer (pH 5.5)	186
R. arrhizus, A. niger, C. rugosa, Mucor, H. lanuginosa, Pseudomonas, R. delemar, Myriococcum, Torulopsis ernobii, M. pusillus, etc.	Erucic, oleic, methylvaleric	C_1–C_{14}, isopropanol, erucyl, oleyl, prenyl, *sec*-, *tert*-butanol, isopropylidene glycerol, 2-ethylhexanol, diglycerol, 2-mercaptoethanol, sugars, 2-octyldodecanol, thiols, amines, etc.	Buffer (pH 5.6)	187
C. rugosa, M. miehei, Pseudomonas, Penicillium	α-Hydroxycaproic	C_4	Toluene	188
M. miehei, C. rugosa, M. miehei (esterase)	C_3–C_6	C_2, C_6	Hexane	189
Porcine pancreatic	γ-hydroxy C_4–C_{12} acids	—	Hexane	190
C. viscosum (in MBG)	Primary straight-chained, branched acids, etc.	Primary straight-chained, secondary and tertiary; branched and unsaturated, cyclic, etc.	Heptane	191
M. miehei	Oleic	Ethanol	Hexane	192
P. fluorescens	15-Hydroxy-C_{15}	—	Benzene	193
C. rugosa	Vinyl acrylate	β-Phenethyl, benzyl, C_4–C_8, etc.	Isooctane	194
M. miehei	Oleic	C_4	—	195
M. miehei	C_{14}	C_2	Supercritical CO_2	196
C. rugosa	C_3	Isoamyl	Hexane, solvent-free	197
M. miehei	C_{10}–C_{18}, oleic, etc.	C_{18}, oleyl, etc.	—	198
P. fluorescens	Oleic	Cholesterol	Isooctane	199
C. rugosa	C_{18}	Cholesterol	Cyclohexane	200
C. rugosa	Oleic	C_6	Hexane	201
M. miehei	Oleic	C_2	—	202

(continued)

TABLE 2 (*continued*)

Lipase	Acid	Alcohol	Solvent	Reference
M. miehei	C_4	Isoamyl	Heptane	203
C. rugosa, G. candidum, Aspergillus, M. miehei, R. arrhizus, etc.	C_{12}	Menthol, geraniol, etc.	Heptane	204
P. fragi (PEG-modified)	C_2–C_5	Citronellol, geraniol, farnesol, phytol, etc.	Benzene	205
M. miehei	C_4	Geraniol	—	206
P. fragi, Candida	C_4	Geraniol	Hexane	207
M. miehei	High free fatty acid rice bran oil	Glycerol	Chloroform, ethylene dichloride	208
C. viscosum, C. rugosa	Oleic, linoleic, linolenic	Glycerol	—	209
M. miehei	C_{14}–C_{18}	Acetone-glycerol	—	210
M. miehei	C_{10}	Glycerol	—	211
R. nodosus	C_8–C_{16}	Glycerol	Acetone/water (1:1)	212
Aspergillus	Oleic	Polyglycerol, diglycerol	Buffer (pH 5.4)	213
P. fluorescens	Caproic anhydride	2′-Deoxyuridine	DMSO, DMF	214
C. antarctica	C_8–C_{18}, oleic	Ethyl D-glucopyranoside	—	215
M. miehei	C_{14}	Sugar acetal	—	216
C. antarctica	Methyl caprate	Glucose	*tert*-Butanol	217
Candida	Oleic	Sorbitol	—	218
M. miehei	Oleic	Isosorbide	—	219
C. rugosa, G. candidum, Penicillium, Mucor sp., *Pseudomonas* (CES), *Pseudomonas* (PS-30), *M. miehei*	n-3 fatty acid concentrate	Glycerol	Hexane	220

[a]DMSO: dimethyl sulfoxide; DMF, dimethyl formamide; THF, tetrahydrofuran.

Whereas esterification refers mainly to the reaction of an acid with alcohol, lipases exhibit a much broader substrate spectrum (100,101), enabling them to catalyze the synthesis of sugar esters, thiol esters, peptides, fatty amides, etc. (102,103). The consequence of this phenomenon is reflected in an application potential for lipases that is much greater than for any other known enzyme!

Low- and medium-molecular weight esters. Low-molecular weight esters (104,105), such as geranyl acetate, isoamyl butyrate and benzyl propionate, are mainly used for their flavor and aroma qualities. For instance, butyl laurate is a component of flavor compositions, mainly for apricot and peach flavors (for fatty-oily notes). It may also be used in fruit flavors in conjunction with other low-boiling esters (106). Ethyl butyrate is known to impart a pineapple flavor (107). Terpenyl esters are used in fragrance preparations (108). Butyl oleate has applications in flavors (105). Certain volatile esters form bases for perfumes. Benzyl benzoate and benzyl salicylate are fixatives for artificial musks, pharmaceuticals, etc. Butyl oleate and butyl laurate are also used as plasticizers, lubricants, etc. (Gandhi, N.N., S.B. Sawant, J.B. Joshi, and D. Mukesh, submitted for publication). Thus, these esters find widespread use in perfumes, cosmetics, soaps, foods, etc. All can be prepared efficiently with lipases from *M. miehei*, *R. delemar*, *Penicillium cyclopium, G. candidum*, etc. (99,109,110). Miyamoto *et al.* (111) reported a process for the preparation of polyol fatty acid esters with mixed groups for cosmetics. Another use of such esters is for fuel; the lipase-catalyzed alcoholysis of oils with methanol and ethanol results in methyl and ethyl esters, which are excellent substitutes for diesel fuel (112).

Polyesters. Polymers of dimethyl terephthalate, vinyl acetate, butyl acrylate, etc. are used in plastics, coatings, adhesives, and laminates. Diisodecyl phthalate and ditridecyl phthalate are used in paints, lacquers, and coatings. Neopentyl polyol esters are important as high-temperature lubricants (113). Such esters/monomers of polyesters may be synthesized with lipases.

For instance, Morrow *et al.* (114) used porcine pancreatic lipase to catalyze polycondensations to prepare both a series of achiral polyesters from simple alkanedioates and diols and an optically active epoxy-substituted polyester with 95% stereochemical purity.

Foods. Lipases can be used in the synthesis of monoglycerides such as monolaurin, sugar esters (115–117) and fatty acyl amino-esters, such as *O*-acyl-L-homoserine, which find use as food emulsifiers (101). Such biosurfactants are used to stabilize emulsions, as in salad dressings (12,117,118). Sugar ester preparation with lipases has been studied by Adelhorst *et al.* (119). Sugar acyl esters, such as 3-stearoyl D-glucose, are useful as bread-softening agents. Apart from functioning as emulsifiers, monoglycerides (as also di- and triglycerides), such as those derived from octanoic and decanoic acids, are also used as base material for edible films or edible lubricants for food processing.

Such glycerides are frequently produced by transesterification by means of lipases, such as lipase from *P. fluorescens* (120). Applications of lipases and/or esterases in ester syn-

thesis and transformation of acyl groups have been reviewed by Antizak *et al.* (121), Luck and Bauer (122), and Bauky (123). Mukherjee (124) has discussed certain aspects of hydrolysis and esterification reactions for preparation of diverse products in food and nonfood industries. Acidolytic reactions have been used in the manufacture of shortenings (125). Margarine obtained by transesterification of hydrogenated fish oil with oils that contained unsaturated fatty acids, such as high-oleic sunflower oil, has a good texture (126).

Currently, an important lipid product with immense potential for lipase action is the synthetic fat substitute. It is in great demand because it can substitute for various high-caloric fats and oils. Other benefits offered by these products are high thermal stability, which allows for high-temperature applications, and the fact that the substitute is not absorbed by the body.

Some of these products that are on the production threshold include (127): (i) olestra—mixture of hexa-, hepta- and octaesters, formed by esterifying sucrose with long-chain fatty acids, and patented by Procter & Gamble (Cincinnati, OH); (ii) esterified propoxylated glycerols—developed by ARCO Chemicals (Greenville, DE) and CPC International (Englewood Cliffs, NJ). These are used in salad dressings, mayonnaise, ice cream, topping sauces; (iii) methyl glucose polyester—used in cheese, sausages, ice creams, and patented by Curtice Burns Foods (Rochester, NY); (iv) dialkyldihexadecylmalonate—ester of malonic and alkylmalonic acids, patented by Frito-Lay (Dallas, TX); (v) trialkoxytricarballylate—patented by CPC International, and produced by esterifying alcohols with tricarballyic acid; (vi) sorbitol fatty acid esters—developed by Pfizer (New York, NY), and can be employed in frozen desserts, salad oils, whipped toppings, baked goods, sauces, mayonnaise, pasta, etc.; (vii) caprenin—marketed by Procter & Gamble in 1992, and is a triglyceride esterified with caprylic, capric and behenic saturated fatty acids.

Pharmaceuticals and cosmetics. Mono-, di-, and triglycerides of octanoic and decanoic acids can act as dyes and perfume bases in cosmetics, toiletries, and pharmaceuticals. They are also known to dissolve gallstones in humans.

Menthyl salicylate is the sunscreen agent in many suntan preparations. Sorbitol and sugar monoesters of lauric and stearic acids possess antitumor and plant growth-inhibiting activity. Oleyl monooleate is used in bath oils, cosmetic creams and lotions, hair preparations, makeup, skin preparations, pharmaceuticals, etc.

Along with fatty esters, sugar esters, and glycerides, fatty hydroxamic acids, prepared by esterification of hydroxyl amine and fatty acids, are used as constituents of cosmetics, household cleaning products, pharmaceuticals, etc. (128).

Fatty acid fractionation/selective extraction/isolation. As mentioned earlier, the fatty acid discrimination property of *M. miehei* and *B. napus* lipases can be used to selectively enrich the medium with a particular acid, such as γ-linolenic and docosahexanoic acid, which are not acted upon by these lipases (80).

Resolution of racemates. Stereospecificity of lipases can be used to selectively transesterify particular enantiomers of chiral acids and/or alcohols (129–132) and in the asymmetric transformation of symmetrical compounds to obtain selectively the active isomers (12). Some examples from the literature are (i) kinetic resolution of 2,3-epoxy-8-methyl-1-nonanol, the key intermediate in the synthesis of gypsy moth hormone (133); and (ii) resolution of racemic tricarbonyl chromium complexes of 2-methyl, 2-methoxy, and 3-methyl benzyl alcohols by esterification with vinyl esters in toluene or by alcoholysis of the corresponding acetate in *n*-butanol with microbial lipases (134); Yamamoto and Oda (135) have reviewed certain applications of lipase-catalyzed asymmetric synthesis. Lipases may be used for kinetic resolution of racemic alcohols, binaphthol, cyanohydrins, and hydroperoxides by enantioselective acylation, for resolution of racemic carboxylates (with polyethylene glycol as acceptor), etc.

Synthesis of intermediates for diverse uses. Lipase-catalyzed alcoholysis can be employed in the production of fatty acid alkyl esters that are valuable intermediates in oleochemistry.

Optically active natural products, such as menalone-lactone, endo- and exo-brevicomin, may also be synthesized with lipase (136). Xier and Sakai (137) used the lipase from *P. fluorescens* for the preparation of a chiral building block, 1,3-*syn*-diol. Optically active fluoride-containing polyesters are prepared by stereospecific esterification with *Alcaligenes* and *Achromobacter* lipases. These esters are useful as intermediates for vitamins, cardiotonics, antibiotics, etc. A patent for this process was granted to Kitatsume and Kokusho (138). Azuma and Minamii (139) have obtained a patent for manufacture of optically active terphenyl derivatives that can be used as agrochemicals and intermediates for electric materials. Transesterification of racemic α-alkyl-substituted primary alcohols is carried out to obtain separate optical isomers (141) that may be used to manufacture antiinflammatory agents.

Dairy industry and confectioneries. The best-publicized use of lipase-catalyzed synthesis lies in the interesterification of fats to produce synthetic triglycerides with desirable characteristics (13,50,141–143). One such application is in the manufacture of a cocoa butter-equivalent from palm oil (144), which is an important ingredient for chocolates and confectioneries. Chang *et al.* (145) reported the synthesis of this cocoa butter-like fat by Lipozyme (commercially available immobilized preparation of the *M. miehei* lipase)-catalyzed reaction between cottonseed and olive oils. Such a defined triglyceride property modification is essentially possible because of the substrate specificity/regioselectivity of lipases, such as those from *C. viscosum, M. miehei,* and *P. fluorescens*, as explained earlier (146,147).

Peptide synthesis. The ability of lipases to act on the amide/peptide bond (C-N) has already been mentioned. However, unlike proteases, lipases can act on D-amino acids as well, allowing for the synthesis of peptide precursors of penicillin G and other penicillin analogs from starting materials

of both *D*- and *L*-configurations. This is not possible with proteases.

Another advantage of using lipases in protein synthesis is that they do not possess amidase activity, so that peptide bonds are not broken right after being made (12,100).

DISCUSSION

More than 95% of the total production of technical enzymes is accounted for by the hydrolases—proteases, carbohydrases, and lipases (103). While the first accounts for almost 60–70% of the technical market, 25% goes to the carbohydrates, and lipases account for a meager 5–10%. Considering the extremely high versatility of lipases (with respect to the diverse substrates and reactions they catalyze), compared to the other hydrolases (100), this is astonishing—a state of affairs that needs to be rectified!

Despite the enormous potential for lipase catalysis in industry and research, as evident from the detailed descriptions presented above, the actual tapping of this potential has been limited. It would not be an exaggeration to say that actual application of this technology is negligible compared to what is possible! This discrepancy is mainly due to problems and suspicions that may or may not be real.

For instance, there is the widespread belief that enzymes require aqueous media for activity. For lipases, this would shut out most if not all proposed reactions because their substrates are generally poorly soluble in water. However, the work of Zaks and Klibanov in the 1980s (148) has shown that this belief is highly misplaced. Lipases and a number of other enzymes are active in organic media. This finding has heralded the beginning of a new chapter in lipase technology. Many of the synthetic applications of this enzyme would be out of the question in aqueous media. In fact, synthesis, the reversal of hydrolysis, necessitates the reversal of the thermodynamic equilibria, which is possible by the maintenance of low water activity, as is possible in organic media.

The second major problem that is perceived is the instability of enzymes. This is easily manipulated by the use of organic media and/or enzyme immobilization (Gandhi, N.N., S.B. Sawant, J.B. Joshi, and D. Mukesh, submitted for publication; 99, 149, 150), both of which reduce drastically the vulnerability of lipases to deactivation (by heat, pH, etc.).

Immobilization of enzymes may in fact be considered a panacea for most ills that prevent widespread use of enzyme technology. The same is true for lipases. Apart from its effect on stabilization, it also enables facile recovery and reuse of the enzyme between batches and permits continuous operation. This results in increased cost effectiveness of the entire process because enzyme cost is a major contributor to process economics. It also solves the third major problem associated with enzymes, which prevents translation of potential into actual application. The same is also brought about by increasing production possibilities—thanks to an improved understanding of fermentation and downstream factors and to the strides made in genetic engineering, which will enable greater production owing to finding higher-yielding strains and the new-found ability to manipulate (microbial) genomes.

Therefore, prospects for the future are bright, and it would not be surprising if lipases take the top position in the enzyme application area. This, however, calls for a greater understanding of all aspects of lipase production and upstream and downstream processing, of factors affecting lipase activity and stability (Gandhi, N.N., S.B. Sawant, J.B. Joshi, and D. Mukesh, submitted for publication; 149), and last but not the least, the mechanism by which these factors influence the lipase three-dimensional structure and/or its ability to catalyze reactions. Factors such as immobilization and organic solvents also affect the catalytic efficiency of lipase with respect to its substrates, and this may be different for different substrates. Thorough understanding in these areas would enable the development of "tailor-made lipases" for specific applications in the long run and possibly open up newer vistas.

As of now, lipase technology is chiefly restricted to those operations where the cost of the product is high, making the enzyme cost low in relation, i.e., this technology is applicable to high-value fine chemicals. Apart from this, lipase employment may be attractive in processes that involve thermolabile substrates/products, such as phospholipids, or that may entail a number of side-reactions, such as oxidation, racemization, and dehydration, and in processes where high enantio- and/or regioselectivity is required. The latter is a problem frequently faced with the high temperatures and/or mineral acid catalysts, which are highly nonspecific with respect to the types of reactions they catalyze.

The other opportunity for lipase applications is in aroma and flavor industries, such as foods, dairy, perfumes and cosmetics, pharmaceuticals, and other medical fields. The production of various ingredients by natural processes assumes great significance in these fields. The reasoning behind the concept of natural identity is that the metabolism of the compounds that enter the body or come in close contact (such as creams) is in terms of individual chemical species, regardless of whether the source is natural or not. These species can have possible health implications. Thus, the International Organization of the Flavor Industry defines the natural flavoring ingredients as "those obtained by appropriate physical, enzymatic or microbiological processes from material of vegetable or animal origin, either in the raw state or after processing for human consumption" (151). Therefore, natural ingredients are in high demand. Compounds produced from natural substrates by biological synthesis are accepted as natural (152), while the same materials produced chemically are not. Hence, ingredients prepared by using whole cells and/or enzymes may command substantially higher prices (104)! This makes biocatalysis quite attractive. Thus, manufacture of various products *via* lipase catalysis will prove to be a boon to industry.

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REFERENCES

1. Kaufman, V.R., and N. Garti, Organic Reactions in Emulsions—Preparation of Glycerol and Polyglycerol Esters of Fatty Acids by Transesterification Reaction, *J. Am. Oil Chem. Soc. 59*:471–474 (1982).
2. Park, Y.K., G.M. Pastore, and M.M. DeAlmeida, Hydrolysis of Soyabean Oil by a Combined Lipase System, *Ibid. 65*:252–254 (1988).
3. Anonymous, Enzymes in the Fats and Oils Industry, *Jap-Chem Week*: 6–9 (January 9, 1981).
4. Linfield, W.M., R.A. Barauskas, R. Sivieri, S. Serota, and R.W. Stevenson Sr., Enzymatic Fat Hydrolysis and Synthesis, *J. Am. Oil Chem. Soc. 61*:191–195 (1984).
5. Kosugi, Y., H. Suzuki, and T. Funada, Hydrolysis of Beef Tallow by Lipase from *Pseudomonas* species, *Biotechnol. Bioeng., 31*:349–356 (1988).
6. Nielsen, T., Industrial Application Possibilities of Lipase, *Fat Sci. Technol. 87*:15–19 (1985).
7. Seitz, E.W., Industrial Applications of Microbial Lipases—A Review, *J. Am. Oil Chem. Soc. 51*:12–16 (1974).
8. Macrae, A.R., and R.C. Hammond, Present and Future Applications of Lipases, *Biotechnol. Genetic Eng. Rev. 3*:193–217 (1985).
9. Iwai, M., and Y. Tsujisaka, Fungal Lipase, in *Lipases,* edited by B. Borgstrom and H.L. Brockman, Elsevier, Amsterdam, 1984, pp. 443–469.
10. Macrae, A.R., Biotechnology in the Oils and Fats Industry, in *Proceedings of the World Conference on Emerging Technologies in the Fats and Oils Industry,* edited by A.R. Baldwin, American Oil Chemists' Society, Champaign, 1986, pp. 7–13.
11. Yamane, T., Enzyme Technology of Lipids Industry: An Engineering Overview, *J. Am. Oil Chem. Soc. 64*:1657–1662 (1987).
12. Gillis, A., Research Discovers New Roles for Lipases, *Ibid. 65*:846–850 (1988).
13. Posorske, L.H., Industrial-Scale Application of Enzymes to the Fats and Oils Industry, *Ibid. 61*:1758–1760 (1984).
14. Godfrey, T., and J. Reichelt, Industrial Applications, in *Industrial Enzymology—Applications of Enzymes in Industry,* The Nature Press, London, 1983, pp. 170–465.
15. Hoq, M.M., T. Yamane, S. Shimizu, T. Funada, and S. Ishida, Bioreactor for Enzymic Reaction of Fat and Fatty Acid Derivatives. III Continuous Hydrolysis of Olive Oil by Lipase in a Microporous Hydrophobic Membrane Reactor, *J. Am. Oil Chem. Soc. 62*:1016–1021 (1985).
16. Stirton, A.J., Fat Splitting, Esterification and Interesterification, in *Bailey's Industrial Oil and Fat Products,* edited by A.E. Bailey and D. Swern, John Wiley & Sons, New York, 1964, pp. 931–972.
17. Sonntag, N.O.V., Fat Splitting, *J. Am. Oil Chem. Soc. 56*:729A–732A (1979).
18. Stansby, M.E., Marine-Derived Fatty Acids of Fish Oils as Raw Material for Fatty Acids Manufacture, *Ibid. 56*:793A–796A (1979).
19. Mukherjee, K.-D., Plant Lipases and Their Application in Lipid Biotransformations, *Prog. Lipid Res. 33*:165–174 (1994).
20. Brahimi-Horn, M.C., M.L. Guglielmino, and L.G. Sparrow, Wax Esterase Activity in a Commercially-Available Source of Lipase for *C. cylindracea, J. Biotechnol. 12*:299–306 (1989).
21. Christner, J., E. Pfeiderer, and T. Taeger, Enzyme-Aided Soaking Process for Skins and Hides, United Kingdom Patent 2,233665 (1991).
22. Figurin, Y.V., I.S. Shestakova, T.F. Mironova, I.V. Shtein, and I.M. Arends, Method of Processing Hides and Skins, Union of the Soviet Socialists Republic Patent 1,567,634 (1990).
23. Muthukumaran, N., and S.C. Dhar, Comparative Studies on the Degreasing of Skins Using Acid Lipase and Solvent with Reference to the Quality of Finished Leathers, *Leather Sci. 29*:417–424 (1982).
24. Bailey, J.E., and D.F. Ollis, Applied Enzyme Catalysis, in *Biochemical Engineering Fundamentals*, 2nd edn., McGraw-Hill, New York, 1986, pp. 157–227.
25. Tschocke, C., Enzymic Treatment of Fats in Wastewater Treatment Plants, *Eau Ind., Nuisances 138*:63–64 (1990).
26. Nagayama, M., New Developments of Textile Detergency by the Use of Enzymes. The Present Situation of the Application of Enzymes for Detergent, *Senshobu Kogyo 37*:453–462 (1989).
27. Andree, H., W.R. Mueller, and R.D. Schmid, Lipases as Detergent Components, *J. Appl. Biochem. 2*:218–229 (1980).
28. Gerhartz, W., Industrial Uses of Enzymes, in *Enzymes in Industry—Production and Application,* VCH, Weinheim, 1990, pp. 77–148.
29. Starace, C.A., Detergent Enzymes—Past, Present and Future, *J. Am. Oil Chem. Soc. 60*:1025–1027 (1983).
30. Umehara, K., Y. Masago, T. Mukaiyama, and O. Okumura, Behaviour of Alkaline Lipase on Detergency, *Yukagaku 39*: 321–326 (1990).
31. Watanabe, T., K. Mukoyama, and K. Mizuno, High Bulk-Density Granular Detergent Containing Alkaline Lipase, Japanese Patent 2,178,397 (1990).
32. Aaslyng, D., E. Gormsen, and H. Malmos, Mechanistic Studies of Proteases and Lipases for the Detergent Industry, *J. Chem. Technol. Biotechnol. 50*:321–330 (1991).
33. Haverkamp, T., J.A. Van der Gugten, and A. Johanna, Particulate Detergent Composition with High Bulk Density and Enzyme Activity and Their Use, European Patent 381,397 (1990).
34. Armstrong, G.D., Lipase-Containing Detergent Composition for Washing Fabrics, European Patent 373,850 (1990).
35. Adams, C.J., G.D. Armstrong, and A.T. Hight, Lipolytic Enzyme-Containing Granular Detergent Composition, European Patent 366,589 (1990).
36. Nishioka, M., K. Joko, and M. Takama, Lipase Manufacture with *Candida* for Use in Detergents, Japanese Patent 2,92,281 (1990).
37. Minoguchi, M., and T. Muneyuki, Immobilization of Lipase on Polyacrylamide and Its Use in Detergents, Japanese Patent 1,285,188 (1989).
38. Fujii, T., T. Tatara, and M. Minagawa, Studies on Application of Lipolytic Enzymes in Detergency. 1. Effect of Lipase from *Candida cylindracea* on Removal of Olive Oil from Cotton Fabrics, *J. Am. Oil Chem. Soc. 63*:796–799 (1986).
39. El Sayed, M.Y., S.N. Lewis, S.A. Anderson, and R.J. Wiersema, Lipase and/or Cutinase Containing Surfactant Systems and Method Useful in Laundering, European Patent 399,681 (1990).
40. Satsuki, T., and T. Watanabe, Application of Lipase to Laundry Detergents, *Bio Ind. 7*:501–507 (1990).
41. Fukano, K., and S. Abe, Lipase-Containing Detergent Composition for Dishwashing Machines, Japanese Patent 2,77,498 (1990).
42. Van Dijk, W.R., Enzyme Dishwashing and Rinsing Composition Containing Lipase, European Patent 343,136 (1989).
43. Nakamura, K., and T. Nasu, Enzyme-Containing Bleaching Composition, Japanese Patent 2,208,400 (1990).
44. Abo, M., Method of Purifying Dry-Cleaning Solvent by Decomposing Liquid Contaminants with a Lipase, World Organization Patent 90 07,606 (1990).
45. Kobayashi, H., Liquid Leather Cleaners, Japanese Patent 1,225,700 (1989).
46. Bhatia, R.P., Contact Lens Cleaning Composition Containing an Enzyme and a Carboxylvinyl Polymer, United States Patent 4,921,630 (1990).

47. Moriguchi, H., J. Hirata, and T. Watanabe, Microorganism-Based Agent for Treatment of Organic Wastes, Japanese Patent 2,105,899 (1990).
48. Abo, M., H. Sakaguchi, H. Sato, M. Onishi, M. Hashida, S. Ikeda, and H. Orito, Dirt Removal from Domestic Animals with Enzyme, Japanese Patent 2,245,129 (1990).
49. Novak, J., B. Kralova, K. Demnerova, K. Prochazka, Z. Vodrazka, J. Tolman, D. Rysova, J. Smidrkal, and V. Lopata, Enzyme Agent Based on Lipases and Oxidoreductases for Washing, Degreasing and Water Reconditioning, European Patent 355,228 (1990).
50. Arnold, R.G., K.M. Shahani, and B.K. Dwivedi, Application of Lipolytic Enzymes to Flavor Development in Dairy Products, *J. Dairy Sci. 58*:1127–1143 (1975).
51. Margrove, R.F., and F.E. McDonough, To Promote Flavor in Cream Used to Improve Skim Milk Cheese, United States Patent 3,156,568 (1964).
52. Colburn, J.T., Lipase-Treated Milk Fats in Production of Butter/Margarine Flavors, United States Patent 3,477,857 (1969).
53. Nelson, J.H., Enzymatically Produced Flavors for Fatty Systems, *J. Am. Oil Chem. Soc. 49*:559–562 (1972).
54. Nelson, J.H., R.G. Jensen, and R.E. Pitas, Pregastric Esterase and Other Oral Lipases—A Review, *J. Dairy Sci. 60*:327–362 (1977).
55. Kosikowski, F.V., Flavor Development by Enzyme Preparation in Natural and Processed Cheddar Cheese, United States Patent 3,975,544 (1976).
56. Kinsella J.E., and D.H. Hwang, Enzymes of *Penicillium roqueforti* Involved in the Biosynthesis of Cheese Flavors, *Crit. Rev. Food Sci. Nutr. 8*:192–228 (1976).
57. Sood, V.K., and F.V. Kosikowski, Accelerated Cheddar Cheese Ripening by Added Microbial Enzymes, *J. Dairy Sci. 62*:1865–1872 (1979).
58. Farahat, S.M., A.M. Rabie, and A.A. Faras, Evaluation of the Proteolytic and Lipolytic Activity of Different *Penicillium roqueforti* Strains, *Food Chem. 36*:169–180 (1990).
59. Shay, L.K., T.J. Fisher, D.S. Banasiak, and E.H. Wegner, Enhancing the Flavor of Proteinaceous Products Derived from Microorganisms, European Patent 357,812 (1990).
60. Alford, J.A., D.A. Pierce, and W.L. Sulzbacher, Microbial Lipases and Their Potential Importance to the Meat Industry, *Proc. Res. Conf. Advisory Council Res. Am. Inst. Found. Univ. Chicago 15*:11–16 (1963).
61. Johnson, R.H., and E.A. Welch, Baked Goods Preservatives, United States Patent 3,368,903 (1964).
62. Wiseman, A., Industrial Practice with Enzymes: Applications and Sources of Industrial Enzymes, in *Handbook of Enzyme Biotechnology,* edited by A. Wiseman, Ellis Horwood Ltd., Chichester, 1975, pp. 252–259.
63. Haas, G.J., and J.C. Lugay, Animal Food of Increased Palatability, United States Patent 3,857,968 (1974).
64. Masuda, T., Digestive Agents Containing Amino Acid Hydrochlorides and Enzymes, Japanese Patent 1,238,538 (1989).
65. Gwozdz, G.P., K. Zuobi, and T. Bravdo, Lipase–Catalyzed Preparation of Optically Active γ-Butyrolactones in Organic Solvents, *J. Org. Chem. 55*:3546–3552.
66. Lott., J.A., and Lu, C.J., Lipase Isoforms and Amylase Isoenzymes—Assays and Application in the Diagnosis of Acute Pancreatitis, *Clin. Chem. 37*:361–368 (1991).
67. Schnatz, J.D., J.W. Ormsby, and R.H. Williams, Lipoprotein Lipase Activity in Human Heart, *Am. J. Physiol. 205*:401–404 (1963).
68. Karube, I., and K. Sode, Biosensors for Lipids, in *Proceedings of World Conference on Biotechnology in the Fats and Oils Industry,* edited by T.H. Applewhite, American Oil Chemists' Society, Champaign, 1988, pp. 215–218.
69. Spohn, U., P. Miethe, and H. Voss, Conductometric Biosensor for Use in Organic Solvents, East Germany Patent 278,869 (1990).
70. Iwai, M., Fundamentals and Use of Enzyme Lipase 7. Application of Lipase, *Yushi 43*:66–72 (1990).
71. Imamura, S., M. Takahashi, H. Misaki, and K. Matsuura, Method and Reagent Containing Lipases for Enzymatic Determination of Triglycerides, West Germany Patent 3,912,226 (1989).
72. Kato, K., S. Nakamura, T. Sakugi, K. Kitai, K. Yone, J. Suzuki, and Y. Ichikawa, Tumor Necrosis Factor and Its Activators for the Treatment of Malignant Tumors, Japanese Patent 1,186,820 (1989).
73. Mauvernay, R.Y., P. Laboreur, and M. Labrousse, Lipase Composition and Its Products, United States Patent 3,513,073 (1970).
74. Saphir, J., Permanent Hair Waving, West Germany Patent 1,242,794 (1967).
75. Berrobi, C., G. Manoussos, and S.A. Oreal, Cosmetic, Pharmaceutical Preparations Containing Lipase, Hyaluronidase and/or Thiomucase Enzymes, West Germany Patent 1,947,896 (1970).
76. August, P., Lipase-Containing Defatting Creams, West Germany 2,064,940 (1972).
77. Smythe, C.V., Microbiological Production of Enzymes and Their Industrial Application, *Econ. Botany 5*:126–144 (1951).
78. Hirashima, Y., A.A. Farooqui, E.J. Murphy, and L.A. Horrocks, Purification of Plasmalogens Using *R. delemar* Lipase and *Naja naja naja* Phospholipase A_2, *Lipids 25*:344–348 (1990).
79. Sonnet, P.E., Lipase Selectivities, *J. Am. Oil Chem. Soc. 65*:900–904 (1988).
80. Hills, M.J., I. Kiewitt, and K.D. Mukherjee, Enzymatic Fractionation of Fatty Acids: Enrichment of γ-linolenic Acid and Docosahexaenoic Acid. Selective Esterification Catalyzed by Lipase, *Ibid. 67*:561–564 (1990).
81. Gavino, V.C., and G.R. Gavino, Adipose Hormone Sensitive Lipase Preferentially Releases Polyunsaturated Fatty Acids from Triglycerides, *Lipids 27*:950–954 (1992).
82. Alford, J.A., and J.L. Smith, Production of Microbial Lipases for the Study of Triglyceride Structure, *J. Am. Oil Chem. Soc. 42*:1038–1040 (1965).
83. Brockerhoff, H., and R.G. Jensen, *Lipolytic Enzymes,* Academic Press, New York, 1974.
84. Brockerhoff, H., Unsolved Problem of Triglyceride Analysis, *Lipids 1*:162–163.
85. Jensen, R.G., J. Sampugna, and J.G. Quinn, Analysis of Some Triacid Triglyceride Mixtures, *Ibid. 1*:294–295.
86. Fukuda, S., S. Hayashi, H. Ochiai, T. Iiizumi, and K. Nakamura, Improvers for Deinking of Wastepaper, Japanese Patent 2,229,290 (1990).
87. Sharyo, M., and H. Sakaguchi, Deinking Used Paper with Incorporation of Lipase, Japanese Patent 2,160,984 (1990).
88. Hagiwara, M., Y. Okamoto, and J. Shinoda, Deinking Agents for Offset-Printed Wastepaper, Japanese Patent 2,80,684 (1990).
89. Ladner, W.E., and G.M. Whitesides, Lipase-Catalyzed Hydrolysis as a Route to Esters of Chiral Epoxyalcohols, *J. Am. Chem. Soc. 106*:7250–7251 (1984).
90. Kirchner, G., M.P. Scollar, and A.M. Klibanov, Resolution of Racemic Mixtures *via* Lipase Catalysis in Organic Solvents, *Ibid. 107*:7072–7076 (1985).
91. Matsumae, H., M. Furui, and T. Shibatani, Lipase-Catalyzed Asymmetric Hydrolysis of 3-Phenylglycidic Acid Ester, The Key Intermediate in the Synthesis of Diltiazem Hydrochloride, *J. Ferment. Bioeng. 75*:93–98 (1993).
92. Roberts, S.M., Production of Man-Made Molecules Using Nature's Catalysts, *Kem-Kemi 16*:250–251 (1989).
93. Foelsche, E., A. Hickel, H. Hoenig, and P. Seufer-Waserthal, Lipase-Catalyzed Resolution of Acyclic Aminoalcohol Precursors, *J. Org. Chem. 55*:1749–1753 (1990).

94. Miyata, A., and H. Sato, Manufacture of Optically Active 2-Hydroxy 4-Phenylbutyrate Esters by Enzymatic Asymmetric Hydrolysis, Japanese Patent 1,225,499 (1989).
95. Hoshino, T., T. Yamane, and S. Shimizu, Bioreactor for Enzymatic Reaction of Fat and Fatty Acid Derivatives. Part XII Selective Hydrolysis of Fish Oil by Lipase to Concentrate n-3 Polyunsaturated Fatty Acids (n-3 Pufa), *Agric. Biol. Chem. 54*:1459–1467 (1990).
96. Mbayhoudel, K., and L.C. Comeau, Selective Preparation of Petroselinic Acid from Fennel Oil by Enzymatic Hydrolysis, *Rev. Fr. Corps Gras 36*:427–431 (1989).
97. Liu, Q., X. Chang, K. Yue, and C. Wang, Refining Mulberry Silk with Protease and Lipase, *Fangzhi Xuebao 10*:116–119 (1989).
98. Sugawara, Y., E. Shionu, and S. Kaneuchi, Solid Mouth-Deodorant Containing Proteinase, Lipase and/or Bacteriolytic Enzyme, Japanese Patent 1,221,310 1989).
99. Gandhi, N.N., S.B. Sawant, and J.B. Joshi, Studies on the Lipozyme-Catalyzed Synthesis of Butyl Laurate, *Biotechnol. Bioeng. 46*:1–12 (1995).
100. Matos, J.R., J.B. West, and C.-H. Wong, Lipase-Catalyzed Synthesis of Peptides: Preparation of a Penicillin G Precursor and Other Peptides, *Biotechnol. Lett. 9*:233–236 (1987).
101. Nagao, A., and M. Kito, Synthesis of *O*-acyl-L-homoserine by Lipase, *J. Am. Oil Chem. Soc. 66*:710–713 (1989).
102. Bistline R.G., Jr., A. Bilyk, and S.H. Feairheller, Lipase-Catalyzed Formation of Fatty Amides, *Ibid. 68*:95–98 (1991).
103. Godtfredsen, S.E., K. Ingvorsen, B. Yde, and O. Andresen, The Scope of Biocatalysts in Organic Chemical Processing, in *Enzymes as Catalysts in Organic Synthesis,* edited by M.P. Schneider, Reidel Publishing Co., Philadelphia, 1986, pp. 77–95.
104. Welsh, F.W., R.E. Williams, and K.H. Dawson, Lipase-Mediated Synthesis of Low Molecular Weight Flavor Esters, *J. Food Sci. 55*:1679–1682 (1990).
105. Gandhi, N.N., S.B. Sawant, and J.B. Joshi, Specificity of a Lipase in Ester Synthesis: Effect of Alcohol, *Biotechnol. Progr. 11*:282–287 (1995).
106. Arctander, S., Application of Esters, in *Perfume Flavor Chemicals*—Vol. I, Weigner, Montclair, 1969, pp. 26–79.
107. Scott, D., Speciality Enzymes and Products for the Food Industry, in *Biocatalysis in Agricultural Biotechnology,* edited by J.R. Whitaker and P.E. Sonnet, ACS Symposium Series 389, Washington, DC, 1989, pp. 176–192.
108. Okumura, S., M. Iwai, and Y. Tsujisaka, Synthesis of Various Kinds of Esters by Four Microbial Lipases, *Biochim. Biophys. Acta 575*:156–165 (1979).
109. Tsujisaka, Y., S. Okumura, and M. Iwai, Glyceride Synthesis by Four Kinds of Microbial Lipases, *Biochim. Biophys. Acta 489*:415–422 (1977).
110. Iwai, M., S. Okumura, and Y. Tsujisaka, Studies on Lipase, Part XV Synthesis of Terpene Alcohol Esters by Lipase, *Agric. Biol. Chem. 44*:2731–2732 (1980).
111. Miyamoto, A., A. Shigeta, Y. Tanaka, H. Oomura, K. Masui, M. Katada, M. Asahi, T. Komori, and T. Sukuki, Process for Preparation of Polyol Fatty Acid Esters Having Mixed Acid Groups for Cosmetics, European Patent 319,126 (1989).
112. Mittelbach, M., Lipase-Catalyzed Alcoholysis of Sunflower Oil, *J. Am. Oil Chem. Soc. 67*:168–170 (1990).
113. Monot, G., F. Borzeix, M. Bardin, and J.-P. Vandecasteele, Enzymatic Esterification in Organic Media: The Role of Water and Organic Solvent in Kinetics and Yield of Butyl Butyrate Synthesis, *Appl. Microbiol. Biotechnol. 35*:759–765 (1991).
114. Morrow, C.J., J.S. Wallace, G.M. Bybee, K.B. Reda and M.E. Williams, Biocatalytic Synthesis of Polyesters by Enzyme Catalyzed Transesterification in Organic Media, *Mater. Res. Soc. Symp. Proc. 174*:197–208 (1990).
115. Seino, H., T. Uchibori, T. Nishitani, and S. Inamasu, Enzymatic Synthesis of Carbohydrate Esters of Fatty Acids (I) Esterification of Sucrose, Glucose, Fructose and Sorbitol, *J. Am. Oil Chem. Soc. 61*:1761–1765 (1984).
116. David, M.H.L., H.O.J. Lemmens, H. Gunther, and H.W. Roper, Surface-Active Acylated Alkylglycosides and Their Preparation by Esterification in Presence of an Enzyme, European Patent 334,498 (1989).
117. Ota, Y., and H. Machida, Manufacture of Sucrose Fatty Acid Esters with Lipase, Japanese Patent 2,60,591 (1990).
118. Nagao, A., and M. Kito, Manufacture of *O*-Acylated Amino Acids as Emulsifying Agents for Foods, Japanese Patent 2,17,156 (1990).
119. Adelhorst, K., F. Bjorkling, S.E. Godtfredsen, and O. Kirk, Enzyme-Catalyzed Preparation of 6-*O*-Acylglucopyranosides, *Synthesis 2*:112–115 (1990).
120. Zaks, A., and A.T. Gross, Production of Monoglycerides by Enzymatic Transesterification, World Organization Patent 90, 04,033 (1990).
121. Antizak, T., A. Krystynowicz, and E. Galas, Biosynthesis and Applications of Lipases and Esterases in Ester Synthesis and Transformation of Acylglycerols, *Kosmos 38*:95–99 (1989).
122. Luck, T., and W. Bauer, Application of Immobilized Lipases for the Interesterification of Lipids, *DECHEMA Biotech. Conf. 3*:631–636 (1989).
123. Bauky, T., Lipase Application in Lipid Chemistry I Transesterification of Triglycerides, *Elelmez Ip 44*:15–20 (1990).
124. Mukherjee, K.D., Lipase-Catalyzed Reactions for Modification of Fats and Other Lipids, *Biocatalysis 3*:277–293 (1990).
125. Meusel, D., A. Muschter, M. Tuelsner, and W. Paul, Lipase-Catalyzed Exchange of Fatty Acids in the Manufacture of Shortenings, *Lebensmittelindustrie 37*:63–66 (1990).
126. Nakai, E., T. Nezu, K. Suzuki, and W. Matsumoto, Modification of Hydrogenated Oils with Lipase, Japanese Patent 3,30,686 (1991).
127. Haumann, B.F., Food Technology, *INFORM 4*:1233–1235 (1993).
128. Servat, F., D. Montet, M. Pina, P. Galzy, A. Arnaud, H. Ledon, L. Marcou, and J. Graille, Synthesis of Fatty Hydroxamic Acids Catalyzed by the Lipase of *Mucor miehei, J. Am. Oil Chem. Soc. 67*:646–649 (1990).
129. Cambou, B., and A.M. Klibanov, Comparison of Different Strategies for the Lipase-Catalyzed Preparative Resolution of Racemic Acids and Alcohols: Asymmetric Hydrolysis, Esterification and Transesterification, *Biotechnol. Bioeng. 37*:1004–1009 (1984).
130. Whitesides, G.M., and C.-H. Wong, Enzymes as Catalysts in Synthetic Organic Chemistry, *Angew. Chem. Int. Ed. Engl. 24*:617–718 (1985).
131. Akiyama, A., M. Bednarski, M.-J. Kim, E.S. Simon, H. Waldmann, and G.M. Whitesides, Enzymes in Organic Synthesis, *CHEMTECH 18*:627–634 (1988).
132. Langrand, G., C. Triantaphylides, and J. Baratti, Lipase-Catalyzed Formation of Flavor Esters, *Biotechnol. Lett. 10*:549–554 (1988).
133. Fukusaki, E., S. Senda, Y. Nakazono, H. Yuasa, and T. Omata, Lipase-Catalyzed Kinetic Resolution of 2,3-Epoxy-8-methyl-1-nonanol, the Key Intermediate in the Synthesis of the Gypsy Moth Hormone, *J. Ferment. Bioeng. 73*:280–283 (1992).
134. Yamazaki, Y., and K. Hosono, Facile Resolution of Planar Chiral Organometallic Alcohols with Lipases in Organic Solvents, *Tetrahedron Lett. 31*:3895–3896 (1990).
135. Yamamota, Y., and J. Oda, Application of Enzymatic Reactions to Asymmetric Synthesis Using Lipase, *Kagaku, Zokan (Kyoto) 119*:125–132 (1991).
136. Sugai, T., H. Kakeya, H. Yamada, K. Matsumoto, S. Ohsawa, N. Suzuki, and H. Ohta, Synthesis of Naturally Optically Ac-

tive Products by the Aid of Lipases, *Tennen Yuki Kagobutsu Toroukai Koen Yoshishu 32*:563–570 (1990).
137. Xier, Z.F., and K. Sakai, Preparation of a Chiral Building Block Based on 1,3 *syn*-diol Using *P. fluorescens* Lipase and Its Application to the Synthesis of a Hunger Modulator, *Chem. Pharm. Bull. 37*:1650–1652 (1989).
138. Kitatsume, T., and S. Kokusho, Preparation of Optically Active Fluoride-Containing Polyesters by Enzymatic Esterification, Japanese Patent 2,40,343 (1990).
139. Azuma, T., and M. Minamii, Manufacture of Optically Active Terphenyl Derivatives with Lipase, Japanese Patent 1,16,731 (1989).
140. Bianchi, D., P. Costi, F. Francalonci, and W. Cabri, Enzymatic Separation of Optical Isomers of Racemic α-Alkyl Substituted Primary Alcohols, European Patent 328,125 (1989).
141. Macrae, A.R, Lipase-Catalyzed Interesterification of Oils and Fats, *J. Am. Oil Chem. Soc. 60*:291–294 (1983).
142. Tajima, I., and A. Kurashige, Fats and Oils Modified with Lipase for Margarines and Shortenings, Japanese Patent 2,219,581 (1990).
143. Richardson, G.H., Dairy Industry, in *Enzymes in Food Processing,* edited by G.H. Reed, Academic Press, New York, 1975, pp. 361–395.
144. Bloomer, S., P. Adlercreutz, and B. Mattiasson, Facile Synthesis of Fatty Acid Esters in High Yields, *Enzyme Microb. Technol. 14*:546–552 (1992).
145. Chang, M.K., G. Abraham, and V.T. John, Production of Cocoa Butter-Like Fat from Interesterification of Vegetable Oils, *J. Am. Oil Chem. Soc. 67*:832–834 (1990).
146. Haelck, L., and F. Spener, Enzyme Functionalization for Alternate Applications of Natural Fats, *DECHEMA Biotech. Conf. 3*:113–116 (1989).
147. Macrae, A.R., Microbial Lipases as Catalysts for Interesterification of Oils, *Biotechnol. Oils Fats Ind., AOCS Monogr. 11*:189–198 (1984).
148. Zaks, A., and A.M. Klibanov, Enzymatic Catalysis in Organic Media, *Science 224*:1249–1251 (1984).
149. Gandhi, N.N., S.B. Sawant, J.B. Joshi, and D. Mukesh, Lipozyme Deactivation by Butanol and Temperature, *Enzyme Microb. Technol. 17*:373–380 (1995).
150. Gandhi, N.N., Vijayalakshmi, V., S.B. Sawant, and J.B. Joshi, Studies in the Immobilization of the *M. miehei* Lipase on Ion Exchange Resins, *Chem. Eng. J.*, in press (1996).
151. Cowley, E., and J. Knights, Natural Flavors, in *Food Flavorings,* edited by P.R. Ashurst, Blackie, Glascow 1991, pp. 43–47.
152. Gillies, B., H. Yamazaki, and D.W. Armstrong, Natural Flavor Esters: Production by *Candida cylindracea* Lipase Adsorbed to Silica Gel, in *Biocatalysis in Organic Media,* edited by C. Laane, J. Tramper and M.D. Lilly, Elsevier, Amsterdam, 1987, pp. 227–232.
153. Tanigaki, M., M. Sakata, and H. Wada, Hydrolysis of Soybean Oil by Lipase with a Bioreactor Having Two Different Membranes, *J. Ferment. Bioeng. 75*:53–57 (1993).
154. Slaughter, J.C., L.R. Weatherley, and A. Wilkinson, Electrically Enhances Enzymic Hydrolysis of Vegetable Oils Using Lipase from *Candida rugosa, Enzyme Microb. Technol. 15*:293–296 (1993).
155. Hedstrom, G., M. Bucklund, and J.P. Slotte, Enantioselective Synthesis of Ibuprofen Esters in AOT/Isooctane Microemulsions by *Candida cylindracea* Lipase, *Biotechnol. Bioeng. 42*:618–624 (1993).
156. Yang, D., and J.S. Rhee, Continuous Hydrolysis of Olive Oil by Immobilized Lipase in Organic Solvent, *Ibid. 40*:748–752 (1992).
157. Pronk, W., M. Van Der Burgt, G. Boswinkel, and K. Van't Riet, A Hybrid Membrane-Emulsion Reactor for the Enzymatic Hydrolysis of Lipids, *J. Am. Oil Chem. Soc. 68*:852–856 (1991).
158. Garcia, H.S., F.X. Malcata, C.G. Hill Jr., and C.H. Amundson, Use of *Candida rugosa* Immobilized in a Spiral Wound Membrane Reactor for the Hydrolysis of Milk Fat, *Enzyme Microb. Technol. 14*:535–545 (1992).
159. Malcata, F.X., C.G. Hill Jr., and C.H. Amundson, Use of a Lipase Immobilized in a Membrane Reactor to Hydrolyze the Glycerides of Butteroil, *Biotechnol. Bioeng. 38*:853–868 (1991).
160. 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, *J. Am. Oil Chem. Soc. 70*:1031–1034 (1993).
161. Tsai, S.-W., G.-H. Wu, and C.-L. Chiang, Kinetics of Enzymatic Hydrolysis of Olive Oil in Biphasic Organic-Aqueous Systems, *Biotechnol. Bioeng. 38*:761–766 (1991).
162. Dandik, L., and H.A. Aksoy, The Kinetics of Hydrolysis of *Nigella sativa* (Black Cumin) Seed Oil Catalyzed by Native Lipase in Ground Seed, *J. Am. Oil Chem. Soc. 69*:1239–1241 (1992).
163. Chen, J.P., and K.-C. Chang, Lipase-Catalyzed Hydrolysis of Milk Fat in Lecithin Reverse Micelles, *J. Ferment. Bioeng. 76*:98–104 (1993).
164. Martinez, O., A.-M. Wilhelm, and J.-P. Riba, Kinetic Study of an Enzymatic Liquid-Liquid Reaction: The Hydrolysis of Tributyrin by *Candida cylindracea* Lipase, *J. Chem. Technol. Biotechnol. 53*:373–378 (1992).
165. Haas, M.J., D.J. Cichowicz, J. Phillips, and R. Moreau, The Hydrolysis of Phosphatidylcholine by an Immobilized Lipase: Optimization of Hydrolysis in Organic Solvents, *J. Am. Oil Chem. Soc. 70*:111–117 (1993).
166. Hayes, D.G., and R. Kleiman, 1,3-Specific Lipolysis of *Lesquerella fendleri* Oil by Immobilized and Reverse-Micellar Encapsulated Enzymes, *Ibid. 70*:1121–1127 (1993).
167. Kim, M.K., and J.S. Rhee, Lipid Hydrolysis by *Pseudomonas putida* 3SK Cultured in Organic-Aqueous Two-Phase System, *Enzyme Microb. Technol. 15*:612–616 (1993).
168. Tanaka, Y., J. Hirano, and T. Funada, Concentration of Docosahexaenoic Acid in Glycerides by Hydrolysis of Fish Oil with *Candida cylindracea* Lipase, *J. Am. Oil Chem. Soc. 69*:1210–1214 (1992).
169. Miyake, Y., M. Ohkube, and M. Teramoto, Lipase-Catalyzed Hydrolysis of 2-Naphthyl Esters in Biphasic System, *Biotechnol. Bioeng. 38*:30–36 (1991).
170. Junker, B.H., M. Bhupathy, and B.C. Buckland, Development of a Recovery and Recycle Process for a *Pseudomonas* Lipase Used for Large-Scale Enzymatic Synthesis, *Ibid. 42*:487–493 (1993).
171. Sonnet, P.E., and M.W. Baillargeon, Methyl-Branched Octanoic Acids as Substrates for Lipase-Catalyzed Reactions, *Lipids 26*:295–300 (1991).
172. Wilcox, R.W., T. Thuren, P. Sisson, G.L. Kucera, and M. Waite, Hydrolysis of Neutral Substrates by Rat Hepatic Lipase, *Ibid. 26*:283–288 (1991).
173. Prazeres, D.M.F., F. Lemos, F.A.P. Garcia, and J.M.S. Cabral, Modelling Lipolysis in a Reversed Micellar System: Part I. Conventional Batch Reactor, *Biotechnol. Bioeng. 42*:759–764 (1993).
174. Taylor, F., M.J. Kurantz, and J.C. Craig Jr., Kinetics of Continuous Hydrolysis of Tallow in a Multi-Layered Flat-Plate Immobilized-Lipase Reactor, *J. Am. Oil Chem. Soc. 69*:591–594 (1992).
175. Bilyk, A., R.G. Bistline Jr., M.J. Haas, and S.H. Feairheller, Lipase-Catalyzed Triglyceride Hydrolysis in Organic Solvent, *Ibid. 68*:320–323 (1991).
176. Virto, M.D., J.M. Lascaray, R. Solozabal, and M. De Renobales, Enzymic Hydrolysis of Animal Fats in Organic Solvents at Temperatures Below Their Melting Points, *Ibid. 68*:324–326 (1991).
177. Yadwad, V.B., O.P. Ward, and L.C. Noronha, Application of Lipase to Concentrate the Docosahexaenoic Acid (DHA) Fraction of Fish Oil, *Biotechnol. Bioeng. 38*:956–959 (1991).

178. Guit, R.P.M., M. Kloosterman, G.W. Meindersma, M. Mayer, and E.M. Meijer, Lipase Kinetics: Hydrolysis of Triacetin by Lipase from *Candida cylindracea* in a Hollow-Fibre Membrane Reactor, *Ibid. 38*:727–732 (1991).
179. Rostrup-Nielsen, T., L.S. Pedersen, and J. Villadsen, Thermodynamics and Kinetics of Lipase Catalyzed Hydrolysis of Oleyl Oleate, *J. Chem. Tech. Biotechnol. 48*:467–482 (1990).
180. Shaw, J.-F., R.-C. Chang, F.F. Wang, and Y.J. Wang, Lipolytic Activities of a Lipase Immobilized on Six Selected Supporting Materials, *Biotechnol. Bioeng. 35*:132–137 (1990).
181. Uemura, A., K. Nozaki, J-I. Yamashita, and M. Yasumoto, Regioselective Deprotection of 3′,5′-*O*-Acylated Nucleosides by Lipase and Esterase, *Tetrahedron Lett. 30*:3819–3820 (1989).
182. Chen, J.-P., Enhancement of Enzymatic Hydrolysis Rate of Olive Oil in Water by Dimethyl β-Cyclodextrin, *Biotechnol. Lett. 2*:633–636 (1989).
183. Holmberg, K., and E. Osterberg, Enzymatic Preparation of Monoglycerides in Microemulsions, *J. Am. Oil Chem. Soc. 65*:1544–1548 (1988).
184. Philippi, M.C., J.A. Jongejan, and J.A. Duine, Enantioselective Hydrolysis of Glycidol Esters: Analytical and Enzymatical Aspects, *Proc. Fourth Eur. Congr. Biotech. 2*:281–284 (1987).
185. Dahod, S.K., and P. Siuta-Mangano, Carbon Tetrachloride-Promoted Stereoselective Hydrolysis of Methyl-2-Chloropropionate by Lipase, *Biotechnol. Bioeng. 30*:995–999 (1987).
186. Ishii, T., T. Mori, J. Chen, Y. Itoh, S. Shimura, K. Kirimura, and S. Usami, Ester Synthesis by a Crude Lipase of *Rhizopus oligosporus* in an Aqueous System, *J. Ferment. Bioeng. 70*:188–189 (1990).
187. Lazar, G., A. Weiss, and R.D. Schmid, Synthesis of Esters by Lipases, in *Proceedings of the World Conference on Emerging Technologies in the Fats and Oils Industry,* edited by A.R. Baldwin, American Oil Chemists' Society, Champaign, 1986, pp. 346–354.
188. Dordick, J.S., D.R. Patil, S. Parida, K. Ryu, and D.G. Rethswisch, Enzymatic Catalysis in Organic Media. Prospects for the Chemical Industry, in *Catalysis of Organic Reactions,* edited by W.E. Pascoe, Marcel Dekker, New York, 1992, pp. 267–292.
189. Pecnik, S., M. Habulin, and Z. Knez, Fatty Acid Ester Synthesis Catalyzed by Immobilized Lipase from *Mucor miehei, Vestn. Slov. Kem. Drus. 38*:287–301 (1991).
190. Gutman, A.L., K. Zuobi, and T. Bravdo, Lipase-Catalyzed Preparation of Optically Active γ-Butyrolactones in Organic Solvents, *J. Org. Chem. 55*:3546–3552 (1990).
191. Rees, G.D., M.G. Nascimento, T.R.J. Jenta, and B.H. Robinson, Reverse-Enzyme Synthesis in Microemulsion-Based Organo-Gels, *Biochim. Biophys. Acta 1073*:493–501 (1991).
192. Chulalaksananukul, W., J.S. Condoret, P. Delorme, and R.M. Willemot, Kinetic Study of Esterification by Immobilized Lipase in *n*-Hexane, *FEBS Lett. 276*:181–184 (1990).
193. Yamane, T., Y. Kojima, T. Ichiryu, M. Nagata, and S. Shimizu, Intramolecular Esterification by Lipase Powder in Microaqueous Benzene: Effect of Moisture Content, *Biotechnol. Bioeng. 34*:838–843 (1989).
194. Ikeda, I., J. Tanaka, and K. Sukuki, Synthesis of Acrylic Esters by Lipase, *Tetrahedron Lett. 32*:6865–6866 (1991).
195. Knez, Z., M. Leitgeb, D. Zavrsnik, and B. Lavric, Synthesis of Oleic Acid Esters with Immobilized Lipase, *Fat Sci. Technol. 92*:169–172 (1990).
196. Dumont, T., D. Barth, C. Corbier, G. Branlant, and M. Perrut, Enzymatic Reaction Kinetic: Comparison in an Organic Solvent and in Supercritical Carbon Dioxide, *Biotechnol. Bioeng. 39*:329–333 (1992).
197. Carta, G., J.L. Gainer, and M.E. Gibson, Synthesis of Esters Using a Nylon-Immobilized Lipase in Batch and Continuous Reactors, *Enzyme Microb. Technol. 14*:904–910 (1992).
198. Miller, C., H. Austin, L. Posorske, and J. Gonzlez, Characteristics of an Immobilized Lipase for the Commercial Synthesis of Esters, *J. Am. Oil Chem. Soc. 65*:927–931 (1988).
199. Kosugi, Y., H. Tanaka, N. Tomizuka, K. Akeboshi, Y. Matsufune, and S. Yoshikawa, Enzymic Manufacture of Sterol Fatty Acid Esters, Japanese Patent 1,218,593 (1989).
200. Koshiro, A., Cholesterol Ester and Its Manufacture Using Microbial Lipase, Japanese Patent 62,296,894 (1987).
201. Kokusho, S., A. Tsunoda, N. Shimizu, H. Machida, and S. Iwasaki, Fatty Acid Ester Manufacture by Alkaline Lipase in an Anhydrous System, Japanese Patent 62,107,791 (1987).
202. Gatfield, I.L., The Enzymatic Synthesis of Esters in Nonaqueous Systems, *Ann. N.Y. Acad. Sci. 434*:569–572 (1984).
203. Mestri, S., and J.S. Pai, Synthesis of Isoamyl Butyrate by Lipase of *Mucor miehei, PAFAI J. 16*:24–30 (1994).
204. Baratti, J., G. Buono, H. Deleuze, G. Langrand, M. Secchi, and C. Triantaphylides, Enantioselective Synthesis of Fatty Acid Esters by Lipases, in *Proceedings of the World Conference of Emerging Technologies in the Fats and Oils Industry* edited by A.R. Baldwin, American Oil Chemists' Society, Champaign, 1986, pp. 355–358.
205. Takahashi, K., Y. Saito, and Y. Inada, Lipases Made Active in Hydrophobic Media, *J. Am. Oil Chem. Soc. 65*:911–916 (1988).
206. Gatfield, I., and T. Sand, Enzymic Production of Esters and Lactones, West Germany Patent 3,108,927 (1982).
207. Nishio, T., and M. Kamimura, Manufacture of Fatty Acid Esters with Lipase from *Pseudomonas* Species, Japanese Patent 1,174,391 (1989).
208. Bhirud, V.S., V.V.R. Subrahmanyam, and S.D. Vaidya, Influence of Reaction Media on Esterification Catalyzed by *Mucor miehei* Lipase, *J. Oil Tech. Assocn. India 23*:44–47 (1991).
209. Osada, K., K. Takahashi, and M. Hatano, Polyunsaturated Fatty Glyceride Syntheses by Microbial Lipases, *J. Am. Oil Chem. Soc. 67*:921–922 (1990).
210. Omar, I.C., H. Saeki, N. Nishio, and S. Nagai, Synthesis of Acetone Glycerol Acyl Esters by Immobilized Lipase of *Mucor miehei, Biotechnol. Lett. 11*:161–166 (1989).
211. Kim, S.M, and J.S. Rhee, Production of Medium-Chain Glycerides by Immobilized Lipase in a Solvent-Free System, *J. Am. Oil Chem. Soc. 68*:499–503 (1991).
212. Muthukumaran, N., and S.C. Dhar, Studies on Glyceride Synthesis by *Rhizopus nodosus* Acid Lipase, *Leather Sci. 30*:97–100 (1983).
213. Kiyono, H., T. Uchibori, M. Tsujita, Y. Nakao, I. Morita, and H. Nishitani, Polyglycerol Fatty Acid Esters by Enzymatic Esterification, Japanese Patent 61,187,795 (1986).
214. Uemura, A., K. Nozaki, J. Yamashita, and M. Yasumoto, Lipase-Catalyzed Regioselective Acylation of Sugar Moieties of Nucleosides, *Tetrahedron Lett. 30*:3817–3818 (1989).
215. Bjorkling, F., S.E. Godtfredsen, and O. Kirk, A Highly Selective Enzyme-Catalyzed Esterification of Simple Glucosides, *J. Chem. Soc., Chem. Commun.* 934–935 (1989).
216. Fregapane, G., D.B. Sarney, and E.N. Vulfson, Enzymic Solvent-Free Synthesis of Sugar Acetal Fatty Acid Esters, *Enzyme Microb. Technol. 13*:796–800 (1991).
217. Miyake, H., M. Hirano, H. Toda, and K. Kitano, Manufacture of Sugar Fatty Acid Ester Mixtures with Lipase, Japanese Patent 4,16,194 (1992).
218. Kobori, J., and Y. Hirota, Esterification of Fatty Acids and Alcohols in Presence of Lipase, Japanese Patent 63,133,991 (1988).
219. Mukesh, D., D. Sheth, A. Mokashi, J. Wagh, J.M. Tilak, A.A. Banerji, and K.R. Thakkar, Lipase-Catalyzed Esterification of Isosorbide and Sorbitol, *Biotech. Lett. 15*:1243–1246 (1993).
220. Li, Z.-Y., and O.P. Ward, Lipase-Catalyzed Esterification of Glycerol and n-3 Polyunsaturated Fatty Acid Concentrate in Organic Solvent, *J. Am. Oil Chem. Soc. 70*:745–748 (1993).

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