

NEW PROCESSES FOR THE CONVERSION OF FATS AND OILS TO HIGHER VALUE-ADDED PRODUCTS

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Abstract

Fats and oils were once the primary sources of aliphatic carbon compounds used by industry. With the availability of inexpensive petroleum feedstocks, the consumption of these commodities has declined for most industrial applications. In addition, the economics have been exacerbated because of the large increase in world production of fats and oils without a similar increase in consumption. Moreover, health-related concerns continue to erode domestic and foreign market demand for edible fats and oils, especially animal fats. Despite their ready availability and competitive price the domestic non-food use of fats and oils continues to decline in almost all applications. To reverse these trends, our laboratory is evaluating the application of biocatalysis and biomimicry (chemical reactions that mimic enzyme reactions) to fats and oils with the goal of expanding current uses and identifying new uses of fats and oils in higher-value industrial applications. Particular areas of research in which we have developed expertise and continue to explore and develop include lipase reactions for fat and oil modification, biocatalytic oxygenation of fatty acids; biodiesel and biofuel additives, and biodegradable polymers.

Harvesting Erucic and γ -Linolenic Acids

The use of biocatalysts in transformations involving fats, oils, partial glycerides and fatty acids and their derivatives is well documented. One area where considerable effort is currently being expended is the study of the chemistry of lipases (triacylglycerol hydrolase, EC 3.1.1.3). More specifically, there has been a recent surge of interest in the application of lipases that exhibit either glycerol positional selectivity or fatty acid specificity. The expression of a lipase's selectivity for or against a given fatty acid structure can be exploited for the isolation of industrially or nutritionally important fatty acids from fats and oils (Figure 1). The expression of a lipase's selectivity is more pronounced in the esterification mode than in the hydrolysis mode. Using this selectivity concept, we recently developed a two-step process for obtaining highly enriched erucic acid fractions from high erucic acid rapeseed (HEAR) oil (1). Erucic acid was a targeted fatty acid because it already has several important industrial applications. The first step of the process was the total hydrolysis of HEAR oil using the lipase from *P. cepacia*. The latter lipase is a suitable catalyst for the total hydrolysis of triglycerides because the enzyme exhibits neither positional nor fatty acid selectivity. In the next step, the lipase of *G. candidum* was used to catalyze the esterification of the free fatty acids (FFA) of HEAR oil with 1-butanol. Because

the *G. candidum* lipase strongly discriminates against certain fatty acids, the erucic acid was concentrated in the FFA fraction. The results are summarized in Table 1. In a reaction conducted up to 52% conversion, the ester fraction contained 12.5% butyl erucate, and the residual FFA fraction contained 85.4% erucic acid. The HEAR oil used initially contained 47.5% erucic acid; therefore, the erucic acid content in the FFA fraction represented a total recovery of 86 % of the amount originally present in the oil. This two-step process also was used for the enrichment of γ -linolenic acid (GLA), an *n*-6-polyunsaturated fatty acid, in borage oil FFA (2). Borage oil is an excellent source of this nutritionally important fatty acid, as GLA comprises 25% of the fatty acids of this oil. The data in Table 1 show that in this manner one can obtain an acid fraction that contains >70% GLA with a total recovery of 95% of the GLA in the oil. Similar enrichments in GLA from primrose oil have been obtained with *M. miehei* lipase, though in lower absolute amounts because of the lower GLA (10%) content in the oil (3).

In the harvesting of a targeted fatty acid it is advantageous to use immobilized lipase preparations for practical considerations, such as enzyme reuse and ease of product isolation. To address this point, we used the supported lipases of *G. candidum* (4) and *M. miehei* (Lipozyme™) to obtain enriched GLA fractions. As shown in Table 1, the selectivities of both the supported lipases were equally effective in concentrating the GLA of borage oil FFA in esterification reactions. For both supported enzymes, GLA was recovered to the extent of about 80% in the FFA fraction. Additionally, the supported lipases could be recycled, and the recovery of GLA was about 70% after two additional reuses.

Biodegradable Polymers from Triglycerides

Poly(hydroxyalkanoates) (PHAs) are naturally occurring, optically active polyesters that accumulate in numerous bacteria as carbon and energy storage materials (5-7). In most cases the polymers contain β -linked repeat units and possess the general structure shown in Figure 2. The R group varies based on the bacterium and the carbon substrate from which the polymer was formed (8). Recently, there has been significant interest in the use of PHAs for biodegradable thermoplastics. Because they are viewed as "environmentally friendly," they are being studied as potential replacements for synthetic plastics in several applications. One major drawback to the use of these polymers is the cost involved in production. Generally, the cost to produce a given PHA polymer on an industrial scale is greater than for a comparable synthetic polymer. To make PHA production more economical, two avenues can be pursued: produce PHAs whose properties allow for their use in unique applications; lower the production costs either by increasing polymer yields or by using less expensive substrates. The latter possibility (and to some extent the former) can be achieved by using agricultural triglycerides as carbon substrates.

It is known that several bacteria (primarily pseudomonads) produce medium-chain-length PHAs from fatty acids (9-11). However, only recently have intact triglycerides been considered as feedstocks for PHA production. Three bacterial species have been shown to produce PHA from triglycerides. These include *Aeromonas caviae*, which produced a copolymer of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) from olive oil (12,13), *Pseudomonas aeruginosa*, which produced a complex PHA copolymer when grown on euphorbia or castor oil (14), and *Pseudomonas resinovorans*, which produced a PHA from tallow (11). In our laboratory we have

investigated the use of *P. resinovorans* to synthesize unique PHAs from other triglyceride substrates.

Six triglyceride substrates (lard, butter oil, olive oil, high oleic acid sunflower oil, coconut oil, and soybean oil) were screened as potential substrates for PHA production. A two stage fermentation was used to increase the number of viable cells prior to transfer into the polymer production medium in hopes that increased PHA yields could be achieved. Each triglyceride, whether animal fat or vegetable oil, supported cell growth (Table 2). This indicated that the organism showed no significant preference towards fats (solids), or oils (liquids) as substrates for growth and polymer production. After 48 h in the polymer production medium the cells were viewed under a phase-contrast microscope for the presence of phase-bright inclusions, evidence of polymer production. *P. resinovorans* produced an MCL-PHA from each triglyceride. This was evident by the presence of one or more PHA granules per bacterium that, when visually inspected, appeared to constitute approximately 50% of the cell mass. The cells were harvested by centrifugation and the cellular biomass, and PHA content and yield were determined (Table 2). The average PHA content for all tested triglycerides was 45%, and the average PHA yield was 1.5 ± 0.2 g/L. Thus our two stage fermentation system resulted in a 200% increase in PHA production compared to previously reported results (11). These results suggest that this system may be a viable means for commercial production of PHA polymers.

Catalytic Oxygenation of Fatty Acids to Reactive Intermediates

With one exception commercial fats and oils contain only double-bond and ester functionality, and for many non-food uses derivatization of a fat or oil to modify or increase its chemical functionality is required. The exception is castor oil, which contains the monohydroxyl fatty acid ricinoleic acid. The value that this hydroxyl imparts to castor oil is indicated by its market price, which is approximately three-fold higher than that of other vegetable oils. Another important industrial product is obtained by the epoxidation of vegetable oils. These derivatives are produced in excess of 200 million lbs per year in the US and are used mainly as stabilizer-plasticizers for PVC. Although the hydroxyl and epoxy functionalities are used in a number of applications, much of their value derives from their ability to be chemically transformed to other functional materials. Thus, for example, the hydroxyl group can be reacted to form a sulfate, endowing the fatty material with detergent properties, and the presence of the epoxide allows for easy crosslinking in plastics. Inexpensive ways of introducing oxygen into common fatty acids from US vegetable oils, e.g., soybean and cottonseed oils, in the form of hydroxy or epoxy functional groups has the potential to promote increasing utilization of these oils as industrial materials.

Our laboratory has an active research program designed to investigate novel methods for introducing oxygen into fats and oils. This research has revealed a number of promising avenues for the formation of oxygenated materials (Figure 3). We have previously reported our investigations on epoxidation of unsaturated fatty acids using the oxone method (15,16), and our use of the enzyme hydroperoxide lyase to produce intermediate length aldehydic materials (17). Here we will describe the results of our recent work to prepare fatty alcohol epoxides from polyunsaturated fatty acids.

The starting point for the synthesis is the preparation of a fatty acid hydroperoxide. This was accomplished using soybean lipoxygenase (LOX). This enzyme catalyzes the addition of oxygen to linoleic acid to form 13(*S*)-hydroperoxy-9(*Z*),11(*E*)-octadecadienoic acid (HPODE, Figure 3). LOX is a relatively unstable enzyme, and methods to immobilize this enzyme were sought in order to stabilize LOX and to allow for its recovery and reuse (18,19). Methods were devised to promote the formation of HPODE in organic solvents (20), and to promote the formation of hydroperoxide in esterified fatty acids such as those found in phospholipids and methyl esters (21).

After obtaining high yields of HPODE, methods of converting this material to useful chemical intermediates was sought. Any number of catalysts are capable of rearranging HPODE to alcohol epoxides, including strong acid and ferrous iron (22). However, what was desired was a catalyst that gives alcohol epoxy materials of specific structure, which limited the available catalysts to some enzymes and transition metal catalysts. A number of different metal catalysts were examined for their effect on the methyl ester of HPODE (Me-HPODE). The methyl ester was used because it was found that the free acid was non-reactive to these catalysts. It was also determined that very low water levels were required because water either inhibited the reaction or participated in the ring opening of the epoxide. In the rearrangement of Me-HPODE by titanium (IV) isopropoxide, the predominant methyl ester that was formed was the *threo* isomer, methyl 11(*R*),12(*R*)-epoxy-13(*S*)-hydroxy-9(*Z*)-octadecenoate (23), whereas Nb(OC₂H₅)₅ gave its *erythro* analogue, demonstrating that both catalysts selectively promoted the formation of an α -epoxide. Thus these results demonstrate that the combination of a regio- and stereospecific LOX and a specific metal ion catalyst will produce a fatty alcohol epoxide of specific structure. This specificity can be used to design homogeneous industrial products.

Vegetable Oils and Fats for Renewable Fuels

There is an increasing interest in the development of alternative fuels to reduce the dependency of the United States on imported petroleum and to reduce the environmental burden from petroleum-based fuels. Considerable effort has been spent on the use of renewable fats and oils (triacylglycerols) as alternative diesel engine fuels since heavy-duty diesel engines are high emitters of pollutants (24-26). However, the high viscosity of vegetable oils in general has been recognized as one major impediment to their use as neat diesel fuels. Previous studies have shown that the viscosity of fats and oils can be reduced when they are converted to their respective monoalkyl esters (27-31). More recently, additional evidence has suggested other beneficial effects of monoalkyl esters in the form of lower emissions (32-34), improved biodegradability, and a no net contribution to the greenhouse effect when used as fuels known as biodiesel (33-34). In addition to biodiesel fuels, lubricants and lubricant additives also can be derived from fats and oils.

Tallow and soybean oil were chosen as the major material in our studies since the United States produces more tallow and soybean oil than the rest of the industrialized world (35). The demand for tallow in the global market has gradually decreased due to health concerns and competition from other fats and oils. Value-added products, such as nutraceuticals, cleaning

solvents, and biofuels, need to be developed from tallow to improve its commercial value. Recycled restaurant grease also was studied since it can be less expensive than tallow.

Previously, Nelson and coworkers (36) demonstrated the application of lipase-catalyzed transesterification to the production of alkyl esters - including methyl esters from soybean oil, rapeseed oil, tallow, and recycled restaurant grease - that could be used as biodiesel (Figure 4). Nelson and coworkers also showed that the low-temperature properties of monoalkyl esters derived from tallow and grease were significantly improved when branched alcohols were used for transesterification (37). Among the large varieties of alkyl esters synthesized, three alkyl esters, namely ethyl tallowate, isopropyl tallowate, and ethyl greasate, were selected for scale-up production and diesel engine performance tests, since preliminary data suggested their potential as biodiesel fuels (37). Physical and low-temperature properties of the three monoalkyl esters and their 20% blends in No. 2 diesel fuel are shown in Table 3. Properties of methyl soyate, the main form of alkyl esters currently available in the U.S. biodiesel market, also are included for comparison. Kinematic viscosities of the esters were close to the proposed ASTM specification for biodiesel (1.9 - 6.0 mm²/s) (38). Viscosity values for the ester-diesel blends (20:80; v/v) were in the acceptable range of 3.1 to 3.3 mm²/s, compared to 3.0 mm²/s for 20% methyl soyate-No. 2 diesel blend and 2.8 mm²/s for No. 2 diesel fuel. Viscosity of isopropyl tallowate (6.4 mm²/s) was higher than ethyl tallowate (5.2 mm²/s) due to the increased molecular weight of the isopropyl esters, which paralleled previous findings (37). In general, monoalkyl esters derived from grease seemed to have better low-temperature properties than the tallow esters (Table 3). The crystallization onset temperature (T_{co}) was determined because it can be used to predict cloud point (CP), which strongly correlates with cold-temperature properties of fuels.

The heating values of the three neat esters also are shown in Table 3. All three esters had approximately the same level of gross heat as methyl soyate, around 40,000 kJ/kg. These values are close to the reported heating value of No. 2 diesel fuel (Table 3). The ignition delay time of a fuel when injected into the combustion chamber of a diesel engine is indicated by its cetane number (39). Cetane numbers of the three neat esters were estimated using a spreadsheet calculation based on the weighted average cetane number for each individual fatty ester. The calculated cetane numbers were 65.9, 62.8, and 54.3 for ethyl tallowate, isopropyl tallowate, and ethyl greasate, respectively. The relatively high cetane numbers of the two tallow esters probably were from their high palmitic acid (16:0) content. Similarly, cetane number for ethyl greasate was closer to 50 since this ester contained less palmitate than the two tallowates and higher amounts of unsaturated fatty acids.

Results from the diesel engine performance and emissions tests for the ester-diesel blends are summarized in Table 4. By comparing performance of the two cylinders of the test diesel engine, we found that the ester-diesel blends resulted in a 1 to 3% higher indicated mean effective pressure (imep) than those from the diesel fuel and therefore carried 1 to 3% more of the load and provided 1 to 3% more power than the diesel fuel. The isopropyl tallowate- and ethyl greasate-diesel blends showed shorter injection durations and thus lower fuel consumption than the diesel fuel. These two fuel blends also had higher combustion efficiency than diesel fuel since they provided equal or better power output. On the other hand, the ethyl tallowate-diesel blend had longer injection durations and higher fuel consumption for essentially equal power output as the diesel fuel. Examination of selected areas of injector nozzles, cylinder heads, and piston faces after running the engine for 5 h showed that all three ester-diesel fuel blends

generated less carbon buildup than did the No. 2 diesel. Emissions for all three ester-diesel blend fuels were similar, with slightly lower CO₂ emissions and slightly greater O₂ emissions than those from the reference fuel, the No. 2 diesel fuel, while no apparent change in CO, HC or NO_x emission was found between the ester-diesel blends and the No. 2 diesel fuel. It has to be kept in mind, however, that engine durability and exhaust emissions data collected in this study are preliminary owing to the short duration of testing.

The composition of biodiesel has also received attention. Biodiesels were analyzed by capillary gas chromatography (40), which accounted for esters, triglycerides, diglycerides, and monoglycerides in one run. Another report included analysis of glycerol by GC (41). In the work described in both papers (40,41), the hydroxy groups of the glycerides and glycerol were derivatized by silylation with *N*-methyl-*N*-trimethylsilyltrifluoroacetamide. Recently, a high performance liquid chromatographic (HPLC) method has been developed for quantifying reaction mixtures obtained from transesterified fats and oils (42). Advantages of the method are that derivatization of the sample is not required, analysis time is under 30 min and all neutral lipid classes, including alkyl esters, free fatty acids, triglycerides, 1,2- and 1,3-diglycerides and 1(2)-monoglycerides, are readily quantified.

This review therefore has shown the diverse applications of fats and oils when they are disassembled, reassembled, derivatized or even fed to bacteria. The United States produces enormous quantities of glycerides as coproducts of the oilseed processing, meat packing, and restaurant industries. Our research is giving industry the means to add value to these renewable commodities while giving the consumer new products that obviate the use of petroleum feedstocks.

1. Sonnet, P.E., T.A. Foglia, and S.H. Fearheller, Fatty Acid Selectivity of Lipases: Erucic Acid from Rapeseed Oil, *J. Am. Oil Chem. Soc.* 70:387-389 (1993).
2. Charton, E., and A. R. Macrae, Substrate Specificities of Lipase A and B from *Geotrichum candidum* CM/CC 335426, *Biochim. Biophys. Acta* 1123:59-64 (1992).
3. Syed Rahmatullah, M.K.S., 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.* 71:563-568 (1994).
4. Foglia, T.A., and P.E. Sonnet, Fatty Acid Selectivity of Lipases: γ -Linoleic Acid from Borage Oil, *J. Am. Oil Chem Soc.* 72:417-420 (1995).
5. Anderson, A.J., and E.A. Dawes, Occurrence, Metabolism, Metabolic Role, and Industrial Uses of Bacterial Polyhydroxyalkanoates, *Microbiol. Rev.* 54:450-472 (1990).
6. Brandl, H., R.A. Gross, R.W. Lenz, and R.C. Fuller, Plastics from Bacteria and for Bacteria: Poly(b-hydroxyalkanoates) as Natural, Biocompatible, and Biodegradable Polyesters, in *Advances in Biochemical Engineering/Biotechnology, vol 41*, edited by T.K. Ghose, A. Fiechter, Springer, Berlin, 1990, pp. 77-93.
7. Doi, Y., *Microbial. Polyesters*, VCH, New York, 1990.
8. Steinbuchel, A., H.E. Valentin, Diversity of Bacterial Polyhydroxyalkanoic Acids, *FEMS Microbiol. Lett.* 128:219-228 (1995).
9. Eggink, G., H. van der Wal, G.N.M. Huijberts, and P. de Waard, Oleic Acid as a Substrate for Poly-3-Hydroxyalkanoate Formation in *Alcaligenes Eutrophus* and *Pseudomonas Putida*, *Ind. Crops Products* 1:157-163 (1993).
10. Brandl, H., R.A. Gross, R.W. Lenz, and R.C. Fuller, *Pseudomonas Oleovorans* as a Source of Poly(b-hydroxyalkanoates) for Potential Applications as Biodegradable Polyesters, *Appl. Environ. Microbiol.* 54:1977-1982 (1988).
11. Cromwick, A.-M., T. Foglia, and R.W. Lenz, The Microbial Production of Poly(hydroxyalkanoates) from Tallow, *Appl Microbiol. Biotechnol.* 46:464-469 (1996).
12. Shimamura, E., K. Kasuya, G. Kobayashi, T. Shiotani, Y. Shima, and Y. Doi, Physical Properties and Biodegradability of Microbial Poly(3-hydroxybutyrate-co-3-hydroxyhexanoate), *Macromol.* 27:878-880 (1994).
13. Shiotani, T., and G. Kobayashi, Japanese patent application 93049, 1993.

14. Eggink, G., P. de Waard, and G.N.M. Huijberts, Formation of Novel Poly(hydroxyalkanoates) from Long-Chain Fatty Acids, *Can. J. Microbiol.* 41(suppl 1): 14-21 (1995).
15. Sonnet, P.E., and T.A. Foglia, Epoxidation of Natural Triglycerides with Ethylmethyldioxirane. *J. Am. Oil Chem. Soc.* 73:461-464 (1996).
16. Piazza, G. J., A. Nuñez, P. E. Sonnet, and T.A. Foglia, Two New Methods for Producing Epoxidized Oils for Industrial Uses, in *Proceedings of the 46th Oilseed Conference-- Processing Efficiency: Meeting the Challenge*, American Oil Chemists' Society, Agricultural Research Service, National Cottonseed Products Association, Champaign, IL., 1997.
17. Nuñez, A., T.A. Foglia, and G.P. Piazza, Improved Method for Extraction of Hydroperoxide Lyase from *Chlorella*, *Biotech. Techniques* 9:613-616 (1995).
18. Hsu, A.-F., T.A. Foglia, and G.J. Piazza, Immobilization of Lipoxygenase in an Alginate-Silicate Sol-Gel Matrix: Formation of Fatty Acid Hydroperoxide, *Biotechnology Letters*. 19:71-74 (1997).
19. Parra-Diaz, D., D.P. Brower, M.B. Medina, and G.J. Piazza, A Method for the Immobilization of Soybean Lipoxygenase, *Biotechnol. Appl. Biochem.* 18:359-367 (1993).
20. Piazza, G. J., D.P. Brower, and D. Parra-Diaz, Synthesis of Fatty Acid Hydroperoxide in the Presence of Organic Solvent Using Immobilized Lipoxygenase, *Biotechnol. Appl. Biochem.* 19:243-252 (1994).
21. Piazza, G.J., T.A. Foglia, and A. Nuñez, Soybean Lipoxygenase-Promoted Oxidation of Free and Esterified Linoleic acid in the Presence of Deoxycholate, *J. Am. Oil Chem. Soc.* 73:1045-1049 (1996).
22. Gardner, H.W., Oxygen Radical Chemistry of Polyunsaturated Fatty Acids, *Free Radical Biol. Med.* 7:65-86 (1989).
23. Piazza, G.J., T.A. Foglia, and A. Nuñez, Enantioselective Formation of an α,β -Epoxy Alcohol by Reaction of Methyl 13(S)-Hydroperoxy-9-(Z),11(E)-octadecadienoate with Titanium Isopropoxide, *J. Am. Oil Chem. Soc.* 74:1385-1390 (1997).
24. Mazed, M.A., J.D. Summers, and D.G. Batchelder, Peanut, Soybean and Cottonseed Oil as Diesel Fuels, *Transactions of ASAE* 28:1375-1377 (1985).
25. Samson, W.D., C.G. Vidrine, and W.D. Robbins, Chinese Tallow Seed as a Diesel Fuel Extender, *Ibid.* 28:1407 (1985).

26. Dunn, R.O. and M.O. Bagby, Low-Temperature Properties of Triglyceride-Based Diesel Fuels: Transesterified Methyl Ester and Petroleum Middle Distillate/Ester Blends, *J. Am. Oil Chem. Soc.* 72:895-904 (1995).
27. Bruwer, J.J., B.v.D. Boshoff, F.J.C. Hugo, J. Fuls, C. Hawkins, A.N. v.d. Walt, A. Engelbrecht, and L.M. du Plessis, In *Agricultural Energy*, Vol. 2, Biomass Energy/Crop Production, ASAE Publication 4-81, American Society of Agricultural Engineers, St. Joseph, MI, 1981, pp. 385-390.
28. Clark, S.J., L. Wagner, M.D. Schrock, and P.G. Piennaar, Methyl and Ethyl Soybean Esters as Renewable Fuels for Diesel, *J. Am. Oil Chem. Soc.* 61:1632-1637 (1984).
29. Natusch, D.F.S., D.W. Richardson, and R.J. Joyce, Methyl Esters of Tallow as Diesel Extender, *Proceedings, VI International Symposium on Alcohol Fuels Technology Conference*, 1-340-346, 21-25 May, 1984, Ottawa, Canada.
30. Richardson, D.W., R.J. Joyce, T.A. Lister, and D.F.S. Natusch, edited by W. Palz, J. Coombs, and D.O. Hall, Methyl Esters of Tallow as a Diesel Component, *Proceedings of the International Conference on Energy from Biomass*, 736-743 (1985).
31. Ali, Y., M.A. Hanna, and S.L. Cuppett, Fuel Properties of Tallow and Soybean Oil Esters, *J. Am. Oil Chem. Soc.* 72:1557-1564 (1995).
32. Ali, Y., M.A. Hanna, and L. I., Leviticus, Emissions and Power Characteristics of Diesel Engines on Methyl Soyate and Diesel Fuel Blends, *Bioresources Technology* 52:185-195 (1995).
33. Masjuki, H., A.M. Zaki, and S.M. Sapuan, A Rapid Test to Measure Performance, Emissions and Wear of a diesel engine fueled with Palm Oil Diesel, *J. Am. Oil Chem. Soc.* 70:1021-1025 (1993).
34. Sii, H.S., H. Masjuki, and A.M. Zaki, Dynamometer Evaluation and Engine Wear Characteristics of Palm Oil Diesel Emulsions, *Ibid.* 72:905-909 (1995).
35. Blanton, B., Market Report, *Render* 26:10-15 (1997).
36. Nelson, L.A., T.A. Foglia and W.N. Marmer, Lipase-Catalyzed Production of Biodiesel, *J. Am. Oil Chem. Soc.* 73:1191-1195 (1996).
37. Foglia, T.A., L.A. Nelson, R.O. Dunn, and W.N. Marmer, Low-Temperature Properties of Alkyl Esters of Tallow and Grease, *Ibid.* 74:951-955 (1997).

38. Howell, S., U.S. Biodiesel Standards - An Update of Current Activities, SAE Technical Paper Series 971687, In: *SP-1274, State of Alternative Fuel Technologies*, Society of Automotive Engineers, Inc., Warrendale, PA, 1997.
39. Owen, K. and T. Coley, *Automotive Fuels Reference Book*, 2nd ed., Society of Automotive Engineers, Inc., Warrendale, PA 1995.
40. Freedman, B., W.F. Kwolek, and E.H. Pryde. Quantitation in the Analysis of Transesterified Soybean Oil by Capillary Gas Chromatography. *J. Am. Oil Chem. Soc.* 63:1370-1375 (1986).
41. Plank, C., and E. Lorbeer. Simultaneous Determination of Glycerol and Mono-, Di- and Triglycerides in Vegetable Oil Methyl Esters by Capillary Gas Chromatography. *J. Chromatogr. A* 697:461-468 (1995).
42. Foglia, T.A. and K.C. Jones, Quantitation of Neutral Lipid mixtures Using High Performance Liquid Chromatography with Light Scattering Detection, *J. Liq. Chrom. Rel. Technol.* 20:1829-1838 (1997).

TABLE 1
Esterification of High Erucic Acid Rapeseed (HEAR) Oil and Borage Oil Fatty Acids by Lipases^a

Substrate	Lipase ^b	Conversion ^c	Acid ^d	Yield ^e
HEAR Oil	<i>G. candidum</i>	52E	12.5	86
		48A	85.4	
Borage Oil	<i>G. candidum</i>	67E	1.8	95
		33A	71.8	
	<i>G. candidum</i> /Si	64E	7.5	80
		36A	55.5	
<i>M. miehei</i>	59E	8.4	78	
	41A	47.2		

^aHEAR oil and borage oil free fatty acids esterified with 1-butanol in hexane (7, 10).

^b*G. candidum*/Si is *G. candidum* lipase supported on silica (6); *M. miehei* lipase is LipozymeTM.

^cConversion expressed as % acids esterified to butyl esters (E); % unreacted fatty acids (A).

^dwt% erucic acid or γ -linolenic acid in ester fraction and acid fraction, respectively.

^eTotal wt% recovery of erucic acid or γ -linolenic acid originally present in HEAR oil (47.5%) and borage oil (24.9%), respectively.

TABLE 2
Cell Dry Weights and Poly(hydroxyalkanoate) Polymer Content of *P. resinovorans* Grown on Triglyceride Substrates

Substrate	Cell Yield ^a (g/L)	PHA content ^b (% dry weight)	PHA yield ^c (g/L)
Control			
Oleic acid	3.8 (±0.3)	48.9 (±2.8)	1.9 (±0.2)
Animal fats			
Tallow	3.0 (±0.2)	39.8 (±2.0)	1.2 (±0.1)
Lard	3.6 (±0.3)	47.4 (±3.0)	1.7 (±0.2)
Butter oil	3.6 (±0.1)	47.0 (±2.3)	1.7 (±0.1)
Vegetable oils			
Olive	3.4 (±0.2)	43.1 (±2.2)	1.5 (±0.2)
Sunflower (high oleic)	3.1 (±0.2)	41.2 (±1.8)	1.3 (±0.2)
Coconut	3.8 (±0.3)	51.0 (±3.2)	1.9 (±0.2)
Soybean	2.9 (±0.2)	44.5 (±3.4)	1.3 (±0.2)
Averages (x) ^d	x = 3.3 (±0.2)	x = 44.9 (±2.6)	x = 1.5 (±0.2)

^aCell dry weight ± standard deviation (n = 3).

^bPHA per cell dry weight ± standard deviation (n = 3).

^cCalculated by multiplying the cell yield (g/L) by the PHA content (% dry weight) of the cells.

^dAverages do not include oleic acid values.

TABLE 3**Physical and Low-Temperature Properties of Alkyl Esters and Ester-Diesel Blends^{a,b}**

Fuel	Viscosity (mm ² /s, 40°C)	LTFT (°C)	CFPP (°C)	CP (°C)	PP (°C)	T _{co} (°C)	Gross heat (kJ/kg)
Ethyl tallowate (ET)	5.2	13	12	15	3	17.8	39,623
Isopropyl tallowate (IPT)	6.4	19	5	9	3	10.6	40,268
Ethyl greasate (EG)	6.2	9	0	5	-1	9.4	39,984
Methyl soyate (MS)	4.3	2	-3	0	-2	-	39,800
ET/D (20:80 v/v blend)	3.1	-1	-10	-6	-12	3.7	-
IPT/D (20:80 v/v blend)	3.2	12	-8	-10	-19	-5.3	-
EG/D (20:80 v/v blend)	3.3	-3	-12	-12	-21	-4.7	-
MS/D (20:80 v/v blend)	3.0	-12	-14	-14	-21	-	-
No. 2 Diesel fuel (D)	2.8	-14	-27	-16	-23	-8.5	45,200

^aLTFT - low-temperature flow test; CFPP - cold filter plugging point; CP - cloud point; PP - pour point; T_{co} - crystallization onset temperature.

^bData taken from reference 37.

TABLE 4**Diesel Engine Performance and Emissions Tests for Ester-Diesel Blends (20:80, v/v)^a**

Test	Summary of observations (blends vs. diesel fuel)
Performance	0 to 2% advantage in load-carrying capacity; 1 to 3% higher indicated mean effective pressure (imep); shorter injection durations (EG/D and IPT/D blends)
Carbon buildup	All three ester-diesel blends showed modest improvement over diesel in buildup characteristics
Emissions	0.25 to 0.5 % reduction in CO ₂ ; less than 1% increase in O ₂ ; No apparent change in CO, HC or NO _x

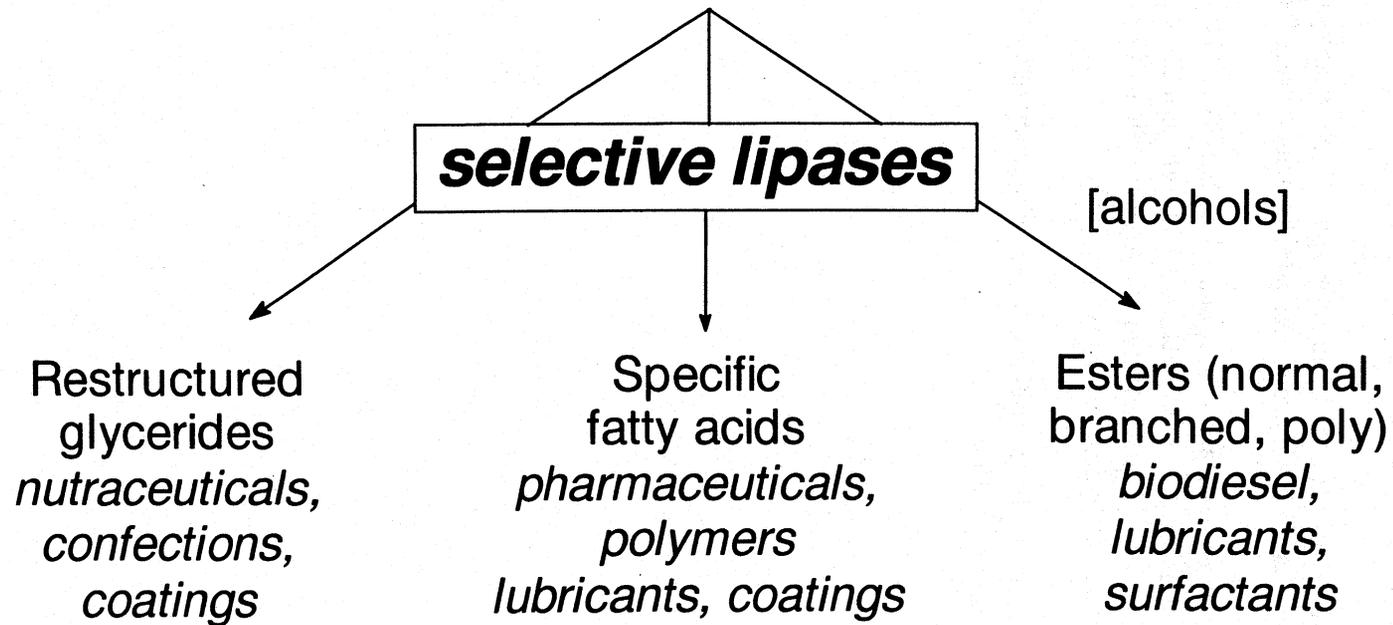
^aEster-diesel blends evaluated were ethyl, isopropyl tallowates and ethyl greasate.

Figure Legends

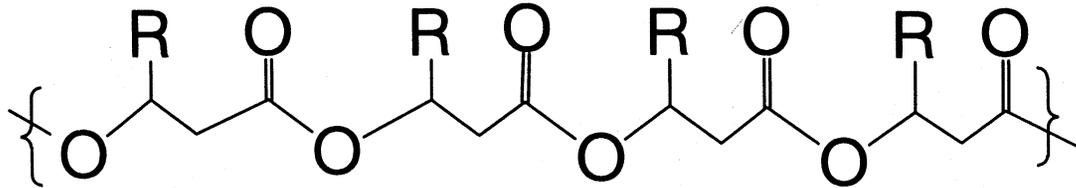
- Figure 1. Use of selective lipases to modify fats and oils to produce restructured glycerides, specific fatty acids and esters.
- Figure 2. The structure of short and long side-chain poly(hydroxyalkanoates) (PHA's). The long side-chain PHA's are produced from triglyceride feedstocks.
- Figure 3. Conversion of linoleate to oxygenated derivatives. The left side shows epoxide formation using the oxone method. The right side shows conversions using lipoxygenase to prepare the hydroperoxide. The hydroperoxide is then converted to alcohols, aldehydes, and epoxy alcohols.
- Figure 4. Characteristics of biodiesel produced using enzymes to prepare esters from tallow, greases, and soapstock.

FATS AND OILS

(tallow, lard, fish oils, vegoils, restaurant grease)



Poly(hydroxyalkanoates), PHA's



Short side-chain PHA's:

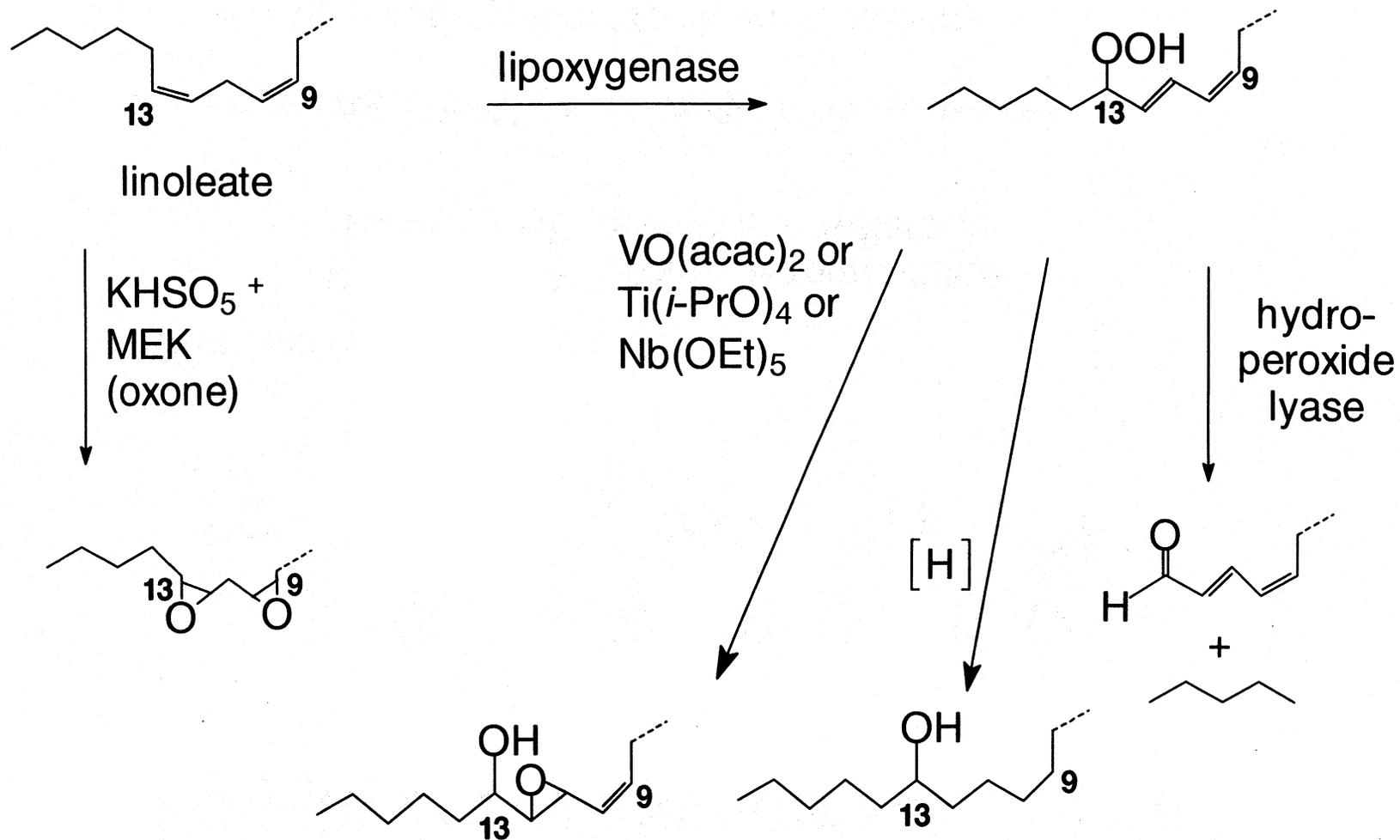
R = Me: PHB, poly(hydroxybutyrate)

R = Et: PHV, poly(hydroxyvalerate)

Long side-chain PHA's, from triglyceride feedstocks:

R = C₆₋₁₄ copolymers (some unsaturation)

Biocatalytic and biomimetic oxygenation



Biodiesel

- ❖ Enzymatic catalysis
- ❖ Inexpensive feedstocks
 - Tallow, Greases (High-FFA), Soapstock
- ❖ Branched alcohols (isopropanol, e.g.)
 - Normal alcohols (ethanol, e.g.)
- Adequate cold-temperature properties when blended 20:80 with petroleum diesel
- Tallow diesel: high cetane values, oxidative stability
- Grease: enzymes handle high FFA content