Technical News Feature

Process Considerations of Continuous Fat Modification with an Immobilized Lipase[1]

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Lipases will catalyze the incorporation of new fatty acids into the triglyceride component of a fat (acidolysis) or can rearrange/redistribute the existing fatty acid radicals in the fat's triglycerides (transesterification). Both processes can dramatically change the physical characteristics of the fat. Immobilization of the lipase allows for the use of the catalyst in a continuous column operation. Examples illustrating the commercial utility of such immobilized enzymes are presented. Process configurations, scale-up issues and catalyst lifetime/productivity are discussed.

The properties of a fat depend on its fatty acid structure, i.e. the types of fatty acid and their position on the glycerol backbone (1). The commercial value of one fat compared with another is based on this structure, and upgrading of fats consists of changing the relative amounts of the triglyceride molecules in a fat that contains particular fatty acid structures.

Traditionally, fats and oils processors have changed the fatty acid structure of their materials either by blending different triglyceride mixtures (combining of natural fats), by chemical modification of the fatty acids (such as by hydrogenation), or by rearrangement of the fatty acids on the glyceride backbone (interesterification, for example, of lard for shortening using alkali catalysis).

Recently, the use of more specific enzyme catalysts for interesterification has been proposed to give an additional level of flexibility in controlling the structure of modified fats. While alkali catalysts will randomize all of the fatty acids in a triglyceride mixture, certain enzymes can select some fatty acids and leave others alone (2,3). One type of enzyme that has this property is the 1,3-specific lipase (1-5).

Using a 1,3-specific lipase, a specific fatty acid can be incorporated into the outer positions without changing the fatty acid residues in the center position by a process known as acidolysis (4). In an alternate reaction scheme known as transesterification, the 1,3-specific lipase randomly rearranges the fatty acid residues on the outer positions without touching those on the center, or 2′-position (5). In contrast, alkali catalysts mix the fatty acid residues from all positions. These different processes can generate different physical properties in the resultant fat.

We have demonstrated this effect on a commercially available triglyceride mixture, cocoa butter stearine, using a 1,3-specific lipase from the fungus *Mucor miehei*. Cocoa butter stearine contains mainly oleic acid on the secondary hydroxyl, or 2′-position, and a mixture of stearic and palmitic acids on the outer hydroxyls, or 1′- and 3′-positions. Table 1 shows the results of a batch interesterification of this material, using either chemical or lipase catalysis. When we used a chemical catalyst, sodium methoxide, we saw dramatic changes in the distribution of fatty acids bound to the second hydroxyl of the glycerol backbone, because palmitic and stearic acids from the outer posi-

TABLE 1

Chemical vs Enzymatic Interesterification Cocoa Butter Stearine Treated in Batch Reactors

		Fatty acid composition in the 2-position[a]			
Catalyst	Treatment	C 16:0	C 18:0	C 18:1	C 18:2
None	None	1	2	94	3
Sodium methoxide	1.5 hr at 85 C[b]	25	37	38	0
Lipoxyme	1 hr at 70 C[c]	7	9	83	1

[a]Method 2.210, IUPAC Standard Methods for the Analysis of Oils, Fats, and Derivatives, 6th edn.

[b]3.0 g cocoa butter stearine was reacted with 1% sodium methoxide at 85 C under nitrogen in a Rotovapor. After 1.5 hr, the reaction was stopped by adding 0.6 ml of 1 M HCl, washed with 3 × 5 ml of hot deionized water and analyzed.

[c]1.7 g cocoa butter stearine was mixed with 250 mg of Lipozyme and 25 mg H_2O on a shaking water bath at 70 C. After one hr, the enzyme was removed by filtration, and the oil was analyzed.

[1]Presented at the symposium "The Biology, Biochemistry and Technology of Lipases" at the 78th annual meeting of the American Oil Chemists' Society held May 17-21, 1987, in New Orleans, Louisiana.

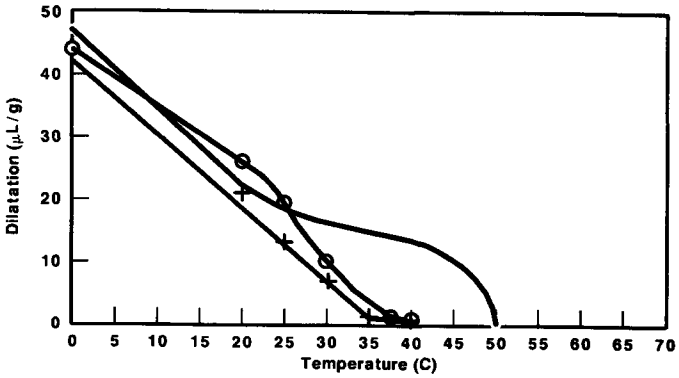

FIG. 1. Melting curves of a 60% palm stearine/40% coconut oil mix: chemical vs enzymatic interesterification. Reaction conditions as described in Table 2. Melting curves (Solid Fat Index) determined according to AOCS Official Method Cd 10-57. (—), no catalyst; (O), sodium methoxide catalyst; (+), Lipozyme catalyst.

tions were exchanged into the center. In contrast, interesterification catalyzed by the lipase resulted in little change in the distribution at the 2′-position, even though substantial rearangement occurred at the 1′- and 3′-positions.

The effect of interesterification on the properties of a fat is shown in Figure 1. This experiment was done with a mixture of 60% palm stearine:40% coconut oil. When the mixture was interesterified with an alkali catalyst, the melting curve was changed dramatically. When a 1,3-specific lipase was used, a somewhat different melting curve, with a lower melting point, was obtained. The existence of commercially available, 1,3-specific lipases offers the fat and oil processor the ability to choose between different melting curves when formulating a new product.

Two key questions must be asked about a new technology when deciding whether to use it in a manufacturing plant. The first is whether the process can produce the desired end product. This questions its technical feasibility. The second is whether the process can produce the product at a reasonable cost. This question deals with commerical feasibility. Technical feasibility can be demonstrated in small-scale batch reactions. However, commercial feasibility deals with things like the cost of a reactor needed to produce enough product (capital cost) and how much product can be produced by a given amount of catalyst (catalyst cost); answers to these commercial questions depend on data collected from models of the full commercial process.

To promote the use of a 1,3-specific lipase in commercial processing, we have begun to investigate on a laboratory scale the questions that have to be answered before large-scale use of lipases can occur. We have developed a continuous process model for use in studying these questions. Continuous processing allows the extensive reuse of an immobilized enzyme catalyst, reducing the catalyst cost. It also encourages careful control of the steady-state operating conditions, which promotes the quality of the product and increases the life of the catalyst, thereby also reducing costs (6–8).

The model system we used has been described by Hansen and Eigtved (5). It consists of two columns in series: one for the immobilized lipase, and a pre-column for conditioning the fat in the feed stream. There are reservoirs to contain the feed stream and the effluent, or product stream, and a pump to maintain a constant flow through the system. Because many triglycerides and fatty acids are solid at room temperature, the entire system is water-jacketed at constant, elevated temperature to maintain the process stream in liquid state throughout. The continuous process experiments that are discussed all have been done using this system.

The catalyst used in these experiments is a 1,3-specific lipase from the fungus *Mucor miehei*, which has been immobilized on a macroporous resin (9). It is available from Novo Laboratories Inc. (Wilton, CT) under the trade name Lipozyme. The immobilized particle has been designed for use in column reactors, and in the immobilized state, the enzyme can maintain activity during extended operation at 60–70 C. The particles have a size range of 200–600 μ and a wet bulk density of 0.28 g enzyme dry matter per ml of bed. As supplied, the enzyme contains 8–10% moisture.

On a molecular level, the mechanism of the interesterification reaction involves hydrolysis of the ester, followed by resynthesis. As a result, there must be at least catlytic amounts of water present in addition to the enzyme (2,10). Early experiments showed that when we operated an immobilized lipase bed continuously, oil in the reaction stream would absorb enough water to eventually dry out the bed, which stopped the reaction. To maintain continuous column operation over a long period, it is necessary to saturate the feed steam with water. This prevents the oil from drying out the enzyme bed and promotes increased productivity through longer operating life.

Control of water content in an oil stream can be accomplished on full-plant scale in a number of ways

TABLE 2

Effect of Water on Partial Glyceride Content Interesterification of Palm Stearine/Coconut Oil

Catalyst	Partial glyceride composition of batch reactions by Iatroscan analysis[a]			
	% FFA	% MG	%DG	%TG
No treatment	0	0	2	98
Sodium methoxide[b]	2	0	5	93
Lipozyme[c]	7	1	12	80
Lipozyne, 2nd use[c]	2	0	6	92

[a]Partial glycerides were separated by silica gel thin layer chromatography on Chromarod SII rods and quantitated by the flame-ionization detector of an Iatroscan TH-10 (Iatron Lab. Inc., Tokyo).

[b]20 g of 60% Palm stearine/40% coconut oil reacted with 1.5% sodium methoxide at 85 C under nitrogen in a Rotovapor. After two hr, the reaction was stopped by adding 7 ml of 1 molar HCl, washed with 3 × 20 ml of hot deionized water and analyzed.

[c]2.5 g (dry matter) of immobilized lipase was hydrated to contain 10% water and then mixed with 20 g of PS/CO in the ratio of 60/40. The mixtures were shaken in water bath at 70 C for two hr and filtered to separate the lipase from the reaction product. The lipases were reused without wash or hydration.

such as direct stream or water injection with in-line mixers. These are difficult to mimic on a laboratory scale. For the model system, we use a pre-column of water-saturated material that releases its water into the oil passing through it. When this pre-column has become exhausted, it can be replaced with fresh, water-saturated material, so the enzyme column always sees water-saturated oil. We have tried a number of materials in the pre-column, and we found hydratable silica of 40–60 mesh or zeolite molecular sieves with pore-size about 3-4 angstroms and particle size larger than 40 mesh both to be satisfactory.

There is another side to the effect of water content on a hydrolysis/resynthesis reaction mechanism. Because water is a reactant in each of the two partial reactions in the sequence, the water concentration will effect the equilibrium by mass-action (3,11). Thus, the relative amount of free fatty acids produced in the product stream will vary, depending on the water content of the immobilized enzyme.

This can be observed exerimentally in batch reactions. We measured the hydrolysis products present in batch interesterifiction reactions of palm stearine/coconut oil by quantitative thin layer chromatography. As Table 2 shows, chemical interesterification causes some increase in free fatty acids and diglycerides. Using fresh enzyme as the catalyst causes a substantial increase in all the hydrolysis products, driven by the initial water content of the enzyme preparation. However, the enzyme becomes equilibrated with reaction intermediates in the first reaction. So when the product from that reaction is drained off and fresh substrate is added, the level of hydrolysis products in a subsequent reaction is no greater than the level seen with chemical catalysis. By using a continuous flow system, one can quickly achieve the equilibrated state of the enzyme, and the reaction continues in the steady-state with an acceptable level of hydrolysis products (2,3).

As Figure 2 shows, the effect of water content, and therefore the amount of hydrolysis, on the solid fat index is less dramatic. The melting curve of product catalyzed by fresh enzyme, which contained a high level of partial glycerides, is not much different from the curve for the product of equilibrated, or reused enzyme.

The productivity, or how much product can be produced from a given amount of catalyst, is determined from decay curves of catalyst activity (5,12). The graphs in Figure 3 show the activity of the enzyme, or the amount of triglyceride treated in one hr by 1 g of enzyme, as it changes with the length of time the enzyme has been in continuous operation. Under use conditions, we have found that the activity of Lipozyme decays in approximately first order fashion. Plotting the log of the current activity against the time the column has been in continuous operation gives a straight line (Fig. 3b). The area under the curve represents the total amount of product that has been made. Factors that change the slope of the line, or that change the initial activity of the enzyme, which is the intercept, will change the area under the curve. These

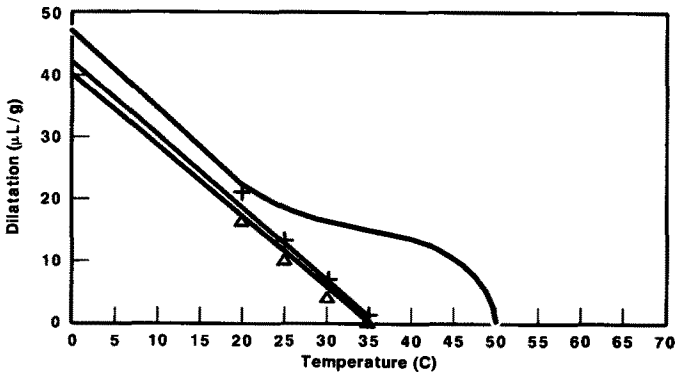

FIG. 2. Effect of water content on melting curves—interesterification of 60% palm stearine/40% coconut oil. Reaction conditions as described in Table 2. Melting curves (solid fat index) determined according to AOCS Official Method Cd 10-57. (—), no catalyst; (△), fresh Lipozyme catalyst; (+), Lipozyme, reused.

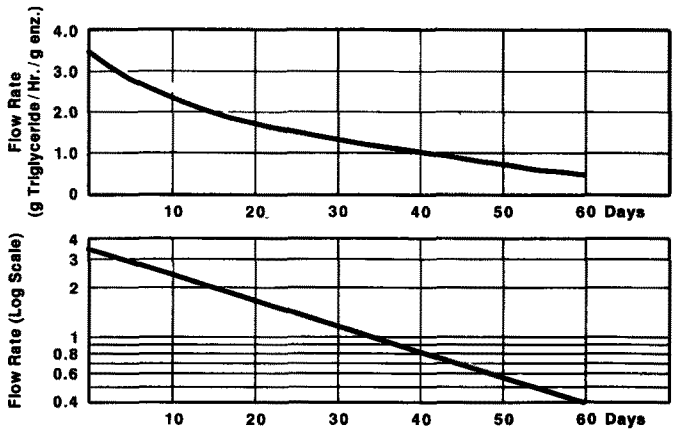

FIG. 3. Productivity of Lipozyme. Commercial soybean salad oil was mixed with reagent grade lauric acid in 5:2 weight ratio. The oil mixture was processed by Lipozyme in the reactor described (5) at 60 C. The effluent was sampled daily; the triglyceride fraction was separated by silica gel chromatography; and the fatty acid composition of the triglyceride fraction was determined by GLC of methyl ester derivatives. Flow rate was adjusted daily to maintain a level of 20% by weight lauric acid residues in the triglyceride fraction of the effluent.

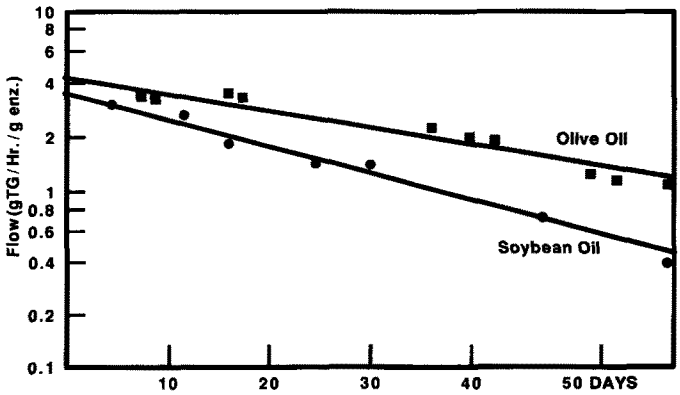

FIG. 4. Lipozyme productivity: comparison of two oils in a continuous, immobilized-enzyme reactor. Experimental conditions as described in Figure 3. The feedstocks were (●) soybean oil from a local market or (■) reagent grade olive oil from a lab supply house mixed 5:2 with lauric acid.

are factors that we want to identify and control because they can change the overall catalyst cost of the process.

One of the effectors of productivity is the water content of the enzyme, as was discussed earlier. For now, we feel that using water-saturated oil is sufficient to deal with this effect.

We also have looked at the effect that different feed oils, or substrates, have on productivity. Figure 4 shows decay curves for two different oils, run side-by-side in continuous column reactors. In both columns, oil was interesterified with reagent-grade lauric acid, and the activity of the enzyme is determined by measuring the rate of lauric acid incorporation into the triglyceride fraction. The substrate for the top curve was highly purified olive oil, purchased from a lab supply house, while the substrate for the lower curve was refined, bleached, deodorized soybean oil purchased at a local supermarket. The enzyme activity clearly decreases more quickly with the soybean oil than with the olive oil. This could be due to oxidation products in the soy, which is more susceptible to oxidation because it is more unsaturated, or to other enzyme catalyst poisons present in the oil. In any case, the result shows that substrates for this system should be highly refined, and that there may ultimately be trade-offs between catalyst cost and refining costs to determine the final minimum process cost overall. Wisdom et al. (13) reported similar differences in lipase performance when they interesterified different feed oils.

Another factor in determining productivity is the degree of conversion of the substrate molecules into product molecules that occurs in the column reactor. This can be controlled by changing the retention time of substrate in the column. A catalyst merely accelerates the approach to equilibrium, but it does not change the level of the equilibrium. It is possible to increase the amount of product molecules in the effluent stream by slowing down the flow rate through the column. However, because the relationship between flow and product concentration is not linear but rather an asymptotic approach to equilibrium, complete conversion of the substrate to product will not occur, at least on any practical time scale (6–8).

When we tested the effect of running our model system at different flow rates, we found a significant effect on productivity. As shown in Figure 5, the productivity of Lipozyme is substantially lower when the retention time is increased. The lower productivity observed when we increased the conversion of substrate to product by increasing the retention time indicates that to achieve a given concentration of particular triglyceride molecules in the final product, there will be a balance between two options: longer retention time in the enzyme reactor, which will increase catalyst costs due to lowered productivity, on one hand, and the cost of fractionating the effluent stream to increase the concentration of product molecules, on the other.

In the search for flexibility to control a product stream, the edible-oil technologist has a new tool, namely specific lipases, that can produce unique fats. Using these catalysts, it is possible to tailor the triglyceride to obtain properties otherwise not available.

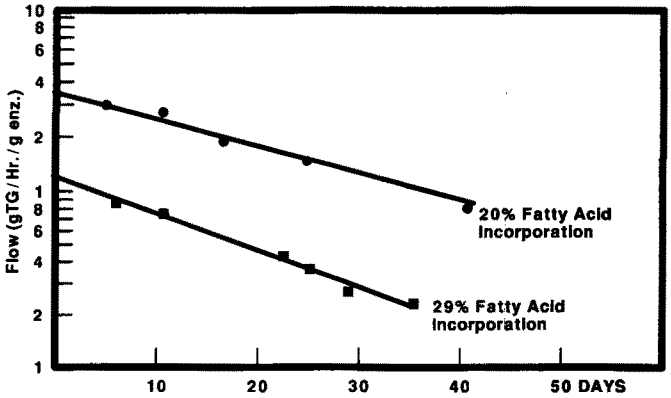

FIG. 5. Effect of retention times on Lipozyme productivity. Experimental conditions as described in Figure 3. The flow rate was adjusted to maintain either (●) 20% or (■) 29% incorporation of lauric acid into the triglyceride fraction of the effluent oil.

To use these catalysts in the most economically beneficial manner, the enzymes should be used in a continuous reactor. This is the way to get the largest amount of product per unit catalyst. By using a laboratory-scale continuous reactor, we have begun to identify the factors that will control the economics of commercial lipase reactors.

A number of the factors have been identified. These factors can be broken down into two lists: first, those characteristics that must be included in the design of the reactor to accommodate an enzyme catalyst, and secondly, those factors that must be considered in conjunction with the other units of the overall process to optimize the whole process for minimum cost.

When considering the equipment design, one must include facilities for temperature control, both to keep the process stream liquid and to prevent extreme temperatures that would inactivate the enzyme. One also must be able to decrease the flow rate through the reactor to maintain constant concentrations in the product stream, due to the decrease in activity of the catalyst over time. However, note that by operating a number of reactors in parallel, it is possible to achieve nearly constant flow through the plant, even though the flow through individual reactors is changing. We also have seen that a mechanism for hydrating the oil will be necessary.

Among the factors affecting optimum productivity, or operating life of the catalyst, one must include hydration. Highly purified feed oil also will prolong operating life, and the cost of refining will have to be balanced against the catalyst cost per unit production. In a similar manner, the extent of reaction (the retention time in the catalyst bed) must be balanced against the cost of fractionation of the product stream. While the enzyme makes it possible to obtain certain triglycerides, achieving the necessary concentration of them in the final product may be more economical by post-reactor fractionation.

The technical feasibility of producing specific triglycerides with a commercially available lipase catalyst can easily be tested in batch reactions. Use of small-scale continuous reactors, such as the one described, will permit the study of economic parameters in using the enzyme for commercial production.

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