Kinetics of Continuous Hydrolysis of Tallow in a Multi-Layered Flat-Plate Immobilized-Lipase Reactor

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To investigate the feasibility of industrial hydrolysis of tallow in an immobilized-lipase bioreactor, a pilot plant was operated continuously at one gram/min for more than one year. A new thin-layer chromatography (TLC) method was employed for analysis of the composition of the product. Data were reduced by averaging, categorizing, and averaging again. The reduced data were used to calculate rates of the three individual reactions: hydrolysis of triacylglycerol (TG) to diacylglycerol (DG) and free fatty acid (FFA), hydrolysis of DG to monoacylglycerol (MG) and FFA, and hydrolysis of MG to glycerol and FFA. It was concluded that separation of mono- and diacylglycerol from FFA and triacylglycerol would be required to achieve a high concentration of FFA in the product.

KEY WORDS: Diacylglycerol, enzymatic fat splitting, monoacylglycerol, triacylglycerol.

Until now, utilization of fats and oils for fatty chemicals production has been carried out on a large scale, with industrial hydrolysis of tallow and other fats and oils *via* the high-temperature, high-pressure Colgate-Emery process as the mainstay of the industry. Recently, there has been greatly increased interest in carrying out rearrangements of lipids with an enzyme belonging to the general classification of lipase as the catalyst. Such catalysis permits more specific reactions as well as other advantages, not the least of which is safety because the reaction can take place at much lower temperatures and pressures. Limited industrial production of some specialty chemicals by means of enzymes has been reported (1). However, use of lipase for industrial hydrolysis on a scale approaching that of traditional fat splitting has not been shown to be economical.

In our laboratory, we have been engaged in an ongoing investigation of a process to produce high conversion of tallow by a single pass through a continuous-flow reactor or series of reactors (2). The three main problems inherent in such a system are the need to work at 50°C to keep the tallow melted, the need for a highly thermostable lipase so that the reactor can be operated for extended periods before deactivation of the enzyme, and the need to achieve close to 100% conversion to permit production of a triacylglycerol-free product.

Over the years we have solved the first and second of these problems through development of a flat-plate reactor containing immobilized lipase from *Thermomyces lanuginosus* (3). Our results compare favorably with those obtained by other workers (4–9). The main objective here was to achieve high conversion to free fatty acid. Toward this end, we carried out the operation with a system of three reactors in series and scaled up the flat-plate reactors further from ten to thirty layers per reactor. In this way, we were able to obtain as high as 83% conversion based on remaining triacylglycerol. More importantly, a new analytical procedure permitted us to routinely measure all lipid classes, including free fatty acid, monoacylglycerol, diacylglycerol and triacylglycerol. The kinetic analysis of these data as reported here may be useful for predicting the performance of larger reactors or more complex reactor systems.

MATERIALS AND METHODS

The flow pattern for continuous counter-current operation of three reactors in series is shown in Figure 1. The system was actually operated continuously for approximately 250 days with eight ten-layered reactors, and for approximately 170 days with four thirty-layered reactors. As shown in Figure 1, each of the three reactors in series had an associated oil-water separator, through which both liquid tallow and buffer (containing glycerol) were recirculated. Each stage consisted of one reactor, one separator and four pumps, oil and buffer feed and recycle pumps for each stage. Reactors were loaded with thermostable, nonspecific lipase as previously described (2) and always placed in the first stage after fresh loading, the first stage being the one into which the fresh tallow was pumped. Edible tallow was purchased from Ed Miniat, Chicago, IL, and fed to the system at 1 g/min. Other flow rates and details of the process are shown in Figure 1 and described elsewhere (3). At the time that a newly loaded reactor was placed in the first stage, the reactor that had been in the first stage became part of the second stage, the reactor that had been in the second stage became part of the third stage, and the reactor that had been in the third stage was removed for washing and reloading with enzyme. Some reactors were reloaded as many as three times. The buffer was the same as previously reported, 0.040 M sodium acetate with 0.02% sodium azide, pH 5.5. All pumps were of the positive displacement type, either peristaltic or piston-driven. The reactor stacks were assembled from alternating layers of 6 × 8-in polypropylene screens with vinyl borders and enzyme support material, and held tightly between 1-in stainless steel plates as previously described (3). The hold-up volume in the oil-water separator was approximately 150 mL of buffer and 50 mL of oil.

The measurement of lipid classes was done by a thin-layer chromatography (TLC) method recently developed in our laboratory (10). The measurement of free fatty acid was also carried out independently by placing about 1 g of water-free lipid in a clean, tared one-ounce vial, weighing, adding 7 mL of 2:1 hexane/ethanol, and titrating to pH 10 with 0.1 N NaOH in an automatic titrator. Agreement between the two methods was generally good (10). Data were analyzed by first separating them into discrete time periods corresponding to the times when new reactors were placed in the first stage, or when spent reactors were removed from the third stage. Then, for each

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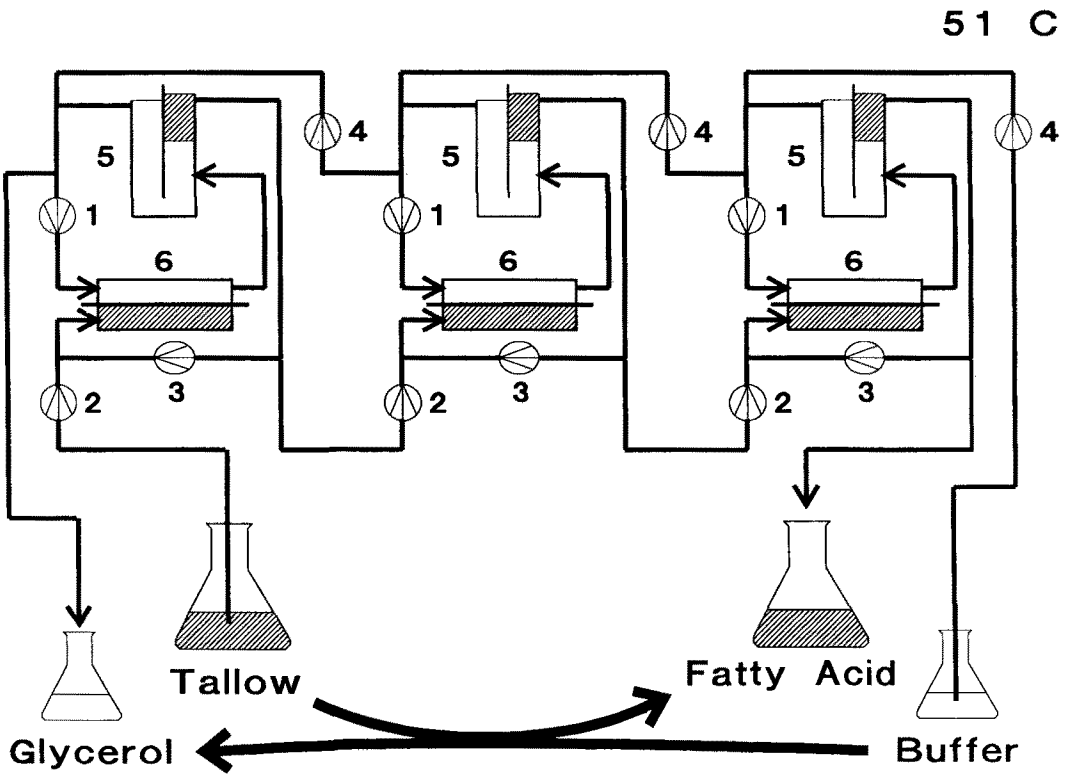

FIG. 1. Enzymatic fat splitting-continuous counter-current operation, three stages. 1—Buffer recycle pump, 5–10 g/min; 2—oil feed pump, 1 g/min; 3—oil recycle pump, 2 g/min; 4—buffer feed pump, 0.2 g/min; 5—continuous oil/water separator; 6—immobilized lipase reactor.

reactor operated during each time period, the product composition data were averaged. These averages were then grouped into categories depending on whether the reactor in question had been in the first, second or third stage, whether they pertained to ten or thirty layers, and whether they represented a new reactor or one that had been washed and reloaded. Finally, the individual averages for all reactors in a given category were averaged again.

The averaged data were used to calculate the rates of the individual reactions in the following scheme, where A = free fatty acid, T = triacylglycerol, D = diacylglycerol, M = monoacylglycerol and G = glycerol:

$$T \xrightarrow{r_1} A + D \quad [1]$$

$$D \xrightarrow{r_2} A + M \quad [2]$$

$$M \xrightarrow{r_3} A + G \quad [3]$$

where $dA/dt = r_1 + r_2 + r_3$; $dM/dt = r_2 - r_3$; $dD/dt = r_1 - r_2$. In this simplified kinetic scheme, the single arrows are not intended to indicate irreversible reactions. Data permit the calculation of an overall rate for each reaction that is actually the sum of individual forward (positive) and reverse (negative) reaction rates and may be either positive or negative.

The rates of formation of free fatty acid (FFA), monoacylglycerol (MG) and diacylglycerol (DG), dA/dt, dM/dt, and dD/dt, respectively, were calculated by first converting the compositions from weight percent to micromoles per gram by using an average molecular weight of free fatty acid of 282 g/mole. The corresponding molecular weights of monoacylglycerol, diacylglycerol and triacylglycerol were 356, 620 and 884 g/mole, respectively. The average composition entering each category of reactor was then subtracted from the average composition leaving that category of reactor. The fresh tallow entering the first stage was 100% triacylglycerol. All compositions were normalized for the loss or gain of weight due to water and glycerol entering and leaving the lipid phase. The rates of formation of the individual products, dA/dt, dM/dt and dD/dt (micromoles/min), were directly obtained from the compositions (micromoles/g) because the flow rate was 1 g/min. Then the rates of the individual reactions r_1, r_2, and r_3 were calculated from dA/dt, dM/dt and dD/dt by solving the above system of three equations and three unknowns as follows:

$$r_1 = 1/3\ (dA/dt + dM/dt) + 2/3\ (dD/dt) \quad [4]$$

$$r_2 = r_1 - dD/dt \quad [5]$$

$$r_3 = r_2 - dM/dt \quad [6]$$

RESULTS

Results are shown in Tables 1, 2 and 3. Table 1 shows the average product compositions for the ten-layered reactors. The free fatty acid content varied from 15% to 36% by weight. The monoacylglycerol content varied from 4% to

TABLE 1

Average Product Compositions for Ten-Layered Reactors

	FFA wt% (SD)[a]	MG wt% (SD)	DG wt% (SD)	TG wt% (SD)
New reactors in first stage	27.0 (0.9)			
	26.4 (3.7)			
	25.5 (0.9)			
	28.2 (3.2)			
	30.2 (2.6)	10.5 (2.1)	29.1 (4.3)	30.2 (5.4)
	30.1 (3.0)	10.7 (1.2)	25.2 (1.4)	34.0 (6.4)
	22.7 (4.3)	10.0 (0.6)	34.7 (0.5)	32.7 (6.1)
	21.4 (4.8)	6.5 (1.9)	29.0 (8.0)	43.1 (11.5)
Reactors in second stage; first and second stages both new	32.2 (3.6)			
	34.4 (3.4)			
	35.1 (2.4)	9.8 (1.6)	24.7 (1.5)	30.4 (0.6)
	30.0 (1.5)	8.8 (1.7)	34.1 (6.0)	27.0 (5.1)
	27.8 (4.6)	6.8 (2.1)	32.6 (6.1)	32.8 (10.5)
Reactors in third stage; all new	38.3 (4.5)	6.4 (1.9)	30.7 (3.0)	24.7 (5.7)
Washed and reloaded reactors in first stage	18.0 (1.0)	4.8 (1.9)	33.0 (2.9)	44.2 (4.7)
	14.7 (2.1)	4.5 (2.4)	26.8 (7.9)	54.0 (11.0)
	17.1 (1.0)			
	17.4 (3.3)	4.2 (2.1)	18.0 (2.1)	60.5 (4.0)
Reactors in second stage; one new, one washed and reloaded	29.4 (1.4)			
	29.0 (3.6)	6.5 (8.6)	27.1 (11.4)	37.4 (16.4)
	21.6 (2.3)	5.4 (1.6)	28.7 (3.7)	44.3 (4.8)
	30.2 (4.0)	7.6 (2.9)	30.3 (0)	32.0 (7.5)
	32.5 (0.6)			
	30.3 (2.4)			
Reactors in third stage; one or more washed and reloaded	34.8 (2.5)			
	27.4 (2.9)	6.9 (3.0)	27.4 (4.4)	38.3 (8.5)
	36.1 (1.2)			
	35.2 (1.2)			

[a]SD = standard deviation.

TABLE 2

Average Product Compositions for Thirty-Layered Reactors

	FFA wt% (SD)[a]	MG wt% (SD)	DG wt% (SD)	TG wt% (SD)
New reactor in first stage	33.1 (6.4)	11.8 (3.3)	20.0 (1.8)	35.2 (5.0)
	23.3 (4.5)	7.0 (3.0)	18.9 (3.8)	50.8 (10.7)
	28.6 (3.2)	5.8 (2.5)	33.3 (6.0)	32.3 (4.1)
	25.8 (5.5)	4.3 (3.8)	35.0 (4.3)	34.9 (8.0)
Reactors in second stage; first and second stages both new	35.6 (3.4)	6.2 (4.4)	20.7 (4.3)	37.4 (8.7)
	34.3 (3.4)	5.7 (2.5)	30.8 (5.0)	29.2 (3.3)
	34.6 (3.9)	4.5 (2.8)	33.6 (2.4)	27.4 (2.8)
Reactors in third stage; all new	42.7 (3.4)	7.4 (2.8)	23.8 (8.3)	26.2 (5.9)
	39.2 (5.2)	4.5 (2.8)	29.8 (3.2)	26.6 (3.5)
Washed and reloaded reactor in first stage	21.1 (3.2)	13.5 (2.6)	38.9 (3.2)	26.5 (4.9)
Reactor in second stage; one new, one washed and reloaded	28.8 (4.0)	12.8 (3.7)	36.5 (3.8)	21.9 (4.5)
Reactor in third stage; one washed and reloaded	39.4 (2.8)	12.5 (3.3)	31.0 (3.0)	17.1 (2.7)

[a]SD = standard deviation.

11%, diacylglycerol from 18% to 34% and remaining triacylglycerol from 25% to 61%. Conversion was generally less in washed and reloaded reactors than in new reactors, especially when they were in the first stage. This effect was due mainly to deterioration of the microporous structure of the support material. Thickness and internal surface area of the support layer were therefore reduced in washed and reloaded reactors. The effect was not as noticeable in the second and third stages where reaction rates were less because of the kinetics of the reaction. Table 2 shows the average product compositions of thirty-layered reactors. Free fatty acid varied from 21% to 43%

TABLE 3

Reaction Rates of Individual Hydrolysis Steps (micromoles/min)

	Stage	Reactor layers	r_1	r_2	r_3
Newly loaded	1	10	726	244	−23
	2	10	64	91	81
	3	10	25	−51	75
Washed and reloaded	1	10	526	103	−25
	2	10	183	149	95
	3	10	22	70	71
Newly loaded	1	30	690	250	43
	2	30	84	62	114
	3	30	57	86	72
Washed and reloaded	1	30	821	172	−220
	2	30	55	99	121
	3	30	58	153	165

by weight. Monoacylglycerol varied from 4% to 14%, diacylglycerol from 19% to 39% and remaining triacylglycerol from 17% to 51%. The fact that the conversion in thirty-layered reactors was only slightly more than in ten-layered reactors can be attributed to the fact that the oil feed rate was the same for both, 1 g/min. Therefore the oil flux was only one-third as much in the thirty-layered reactors. It has been previously shown that conversion is a nonlinear function of oil flux in these reactors (3).

Table 3 shows the average rates of the three individual reactions for the six different categories of ten-layered and thirty-layered reactors. These are net rates, which are actually the sum of the forward and reverse reactions. Hydrolysis of triacylglycerol in the first stage was by far the fastest reaction. Rates dropped off considerably for the second and third stages, as well as for the hydrolysis of diacylglycerol and monoacylglycerol in the first stage. Hydrolysis of diacylglycerol was faster in the second stage than in the third stage for washed and reloaded ten-layered reactors, and faster in the third stage than in the second stage for thirty-layered reactors either new or washed and reloaded. Hydrolysis of monoacylglycerol was slow or negative in all reactors. Negative rates indicate the net synthesis of monoacylglycerol and, in one case, diacylglycerol, in that category of reactors.

DISCUSSION

These data indicate that the reverse reactions, namely the synthesis of triacylglycerol, diacylglycerol and monoacylglycerol, take place at significant rates and can even exceed the rate of the corresponding hydrolysis reactions in reactors to which partially hydrolyzed tallow is fed. Therefore, it will be difficult to achieve close to 100% conversion, even with a larger number of counter-current reactors in series. A more successful approach to the problem of achieving high conversion would be to carry out an intermediate separation of mono- and diacylglycerols from free fatty acid and remaining triacylglycerol. Although free fatty acids are difficult to separate from their corresponding triacylglycerols, the mono- and diacylglycerols can be separated more easily due to their significantly higher melting points. The separated materials could then be further hydrolyzed more efficiently in separate reactors than could the combined mixture.

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