

Synthesis of Monoglycerides by Glycerolysis of Rapeseed Oil Using Immobilized Lipase

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ABSTRACT: Reaction conditions for the synthesis of monoglycerides (MG) by enzyme-catalyzed glycerolysis of rapeseed oil using Lipozyme[®] IM have been studied. Silica gel was used to adsorb the glycerol to overcome the problems of low glycerol solubility in the organic phase. An experimental design was used where temperature, time, the ratio of silica gel to glycerol (w/w), the water activity (a_w), the isooctane concentration, and the ratio of glycerol to triglycerides (mol/mol) were varied. Response surface methodology was used to evaluate initial reaction rate and yield for the different products. The best yield of MG achieved under the studied conditions was 17.4% (mol fatty acid in substance/total mol fatty acid in mixture) (75°C, 20 h, silica gel/glycerol 2:1, $a_w = 0.17$, 48% isooctane, glycerol/triglycerides 6:1). The same conditions yielded 36.8% diglycerides, 13.6% free fatty acids (FFA), and 36.9% triglycerides. This is at the same level as the equilibrium yield. The yield of MG is low compared to the final yield achieved with solid-phase glycerolysis. However, in solid-phase glycerolysis the reaction mixture becomes solid, and therefore the solid-phase process is not suitable for industrial application. The formation of FFA was very fast compared to the synthesis of MG. Equilibrium for FFA was reached within 2 h, and the yield was strongly affected by the a_w . Increasing a_w greatly increased the formation of FFA. In the a_w ratio 0.06–0.3, the yield of FFA increased from 4 to 19% while the yield of MG was nearly unaffected. As FFA is an undesired product, it is important to keep the a_w as low as possible.

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KEY WORDS: Glycerolysis, immobilized enzyme, initial reaction rate, lipase, Lipozyme, monoglycerides, rapeseed oil, response surface methodology, water activity.

Monoglycerides (MG) are used as emulsifiers in the food, pharmaceutical, and cosmetics industries (1). MG account for 75% of the worldwide production of emulsifiers for the food industry (2). Today, MG are produced by chemical synthesis in which glycerol, fat, and an alkaline catalyst are mixed and heated to almost 250°C. $\text{Ca}(\text{OH})_2$ is used as catalyst in the production of MG for the food industry (3). This process only yields 40–60% MG; the rest are diglycerides (DG) and

triglycerides (TG) (2). As the diglycerides are not very good emulsifiers (4), high-vacuum distillation has to be used to obtain a final product with a high content of MG, normally 95% (2). Furthermore, the process is very energy consuming because of the high reaction temperature needed for the chemical synthesis of MG and the distillation required to obtain the product. The high temperature also changes the product's color and taste. Therefore, it is of interest to use lipase-catalyzed enzymatic synthesis, which is performed at a lower temperature and produces a better product. It is even possible to produce isomerically pure monoglycerides (5).

Different methods for the enzymatic synthesis of MG have been studied, such as esterification of glycerol with free fatty acids (FFA) (5–8) and selective hydrolysis of TG in a microemulsion system (9). Glycerolysis of natural fats and oils is of interest as the fat could be used without treatment, and 1 mol TG and 2 mol glycerol can give 3 mol MG. This reaction has been studied in different reaction systems, with organic solvents (5,10), without solvent (11–14) (solid-phase glycerolysis), with immobilized enzyme (10,13) and with free enzyme (5,11,12,14), or in microemulsions (15).

The enzymatic glycerolysis reaction has an equilibrium yield of only 25–35% (11–14). A higher final yield has only been achieved in processes where MG is removed from the reaction mixture. In solid-phase glycerolysis, the reaction temperature is reduced after a period of time and MG starts to crystallize directly in the reaction vessel. Final yields of 50–95% have been reported with this method (11–14). Berger and Schneider (5) used a system with a reactor and a cooled crystallization vessel, and they circulated the reaction mixture between the two vessels. In this way a final yield of 95–98% was achieved.

The solid-phase glycerolysis, with a reaction mixture that totally solidifies, is not a useful method from an industrial point of view. The removal of MG has to be accomplished outside the reaction vessel and unreacted material recirculated. To be able to develop an industrially applicable process, one must investigate factors that affect the equilibrium yield and the initial reaction rate of the glycerolysis reaction.

A problem with glycerolysis is that glycerol has a low solubility in fat. Berger and Schneider (5) present a solution to this problem using the adsorption of glycerol onto silica gel. This also solves another problem: If immobilized enzyme is

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used, free glycerol can make the enzyme aggregate, which leads to a loss in enzyme activity (13). Castillo *et al.* (16) have also shown that the adsorption of glycerol onto silica gel has a positive effect on the esterification of glycerol with fatty acid and suggest that the silica gel acts as a reservoir of glycerol.

Water activity (a_w) is a very important factor in the reaction system (10,17,18). Although the enzyme needs some water to retain enzymatic activity, too much water results in undesired hydrolysis (17). Unfortunately, researchers studying enzymatic synthesis use different ways of controlling the water content in their reaction mixtures, and comparing the water activity of the various reaction methods used is difficult. One good way of controlling the water activity is to adjust the water amount in the enzyme preparation and to keep the other components as dry as possible. A simple method of obtaining different water levels is equilibration of the immobilized enzyme over saturated salt solutions, which gives a defined water activity (19,20).

If silica gel is used as an adsorbent for glycerol, there is a risk that the enzyme may be dried by the silica gel (21). Therefore, it is important that the water activity of the silica gel/glycerol mixtures be adjusted in the same way as the enzyme.

We have studied the immobilized enzyme Lipozyme[®] IM in the glycerolysis of rapeseed oil, with the glycerol adsorbed onto silica gel and isooctane as solvent. The aim of the study was to investigate the equilibrium yield of MG, the initial reaction rate (calculated from the estimated yield at 2 h), and their dependence on the reaction temperature, reaction time, the ratio of silica gel to glycerol, the water activity, the amount of solvent, and the ratio of glycerol to triglycerides for this system.

EXPERIMENTAL PROCEDURES

Materials. Immobilized lipase from *Mucor miehei*, Lipozyme[®] IM (triglyceride hydrolase, EC 3.1.1.3), was purchased from Novo Nordisk A/S (Bagsvaerd, Denmark). Low-erucic rapeseed oil (99%) and lipid standards for calibration of the high-performance liquid chromatography (HPLC) system were bought from Larodan Fine Chemicals AB (Malmö, Sweden). The glycerol was of analytical grade and obtained from J.T. Baker (Deventer, Holland). Silica gel 60, 230–400 mesh, was from Sigma Chemical Company (St. Louis, MO). Lithium chloride, sodium chloride, and isooctane were all of analytical

grade, and *n*-heptane and 2-propanol were of HPLC grade, and they were all purchased from Merck (Darmstadt, Germany). Acetic acid (analytical grade) was from PROLABO (Paris, France). Potassium carbonate and magnesium chloride-6-hydrate were of analytical grade (Riedel-de Haën AG, Seelze, Germany). Silica gel with blue moisture indicator, internal surface 500–800 m²/g, particle size 3–6 mm, was from Fluka (Buchs, Switzerland).

Experimental design. The variables studied were temperature, time, the ratio of silica gel to glycerol (w/w), the water activity of the enzyme and of the silica gel/glycerol mixtures, solvent concentration, and the ratio of glycerol to TG (mol/mol). The amount of enzyme (10% w/w dry enzyme of the rapeseed oil mass) and the amount of rapeseed oil (200 mg) were kept constant throughout the experiments. The levels of the variables were selected according to a central composite design with fractional factorial design (2⁶⁻¹, 46 experiments) (22). The values of the variables are shown in Table 1. The solvent concentration is presented in weight percentage of rapeseed oil plus solvent.

Adsorption of glycerol onto silica gel. Glycerol and silica gel were mixed in different proportions (see Table 1) and mechanically stirred. A powder was obtained with equal amounts of glycerol and silica gel or an excess of silica gel. If there was an excess of glycerol, the final mixture was firm and wet.

Preparation of different water levels in the reaction system. Different levels of water in the system were obtained by controlling the water activity of the enzyme and the silica gel/glycerol mixtures and by keeping the reactants and the solvent as dry as possible. The rapeseed oil, the glycerol, and the silica gel were used as delivered whereas the isooctane was dried with anhydrous Na₂SO₄.

First, the enzyme and the silica gel/glycerol mixtures were dried with blue gel under vacuum. Then they were transferred to desiccators and equilibrated against saturated salt solutions for at least 24 h under vacuum at room temperature. The different salt solutions used were LiCl ($a_w = 0.11$), MgCl₂ ($a_w = 0.33$) and K₂CO₃ ($a_w = 0.44$) (23,24). Blue gel, dried at 105°C, was used to achieve the lowest water activity and was found to give a water activity of 0.06 by interpolation of the water content between $a_w = 0.11$ and P₂O₅, where P₂O₅ is set to $a_w = 0$.

TABLE 1
Levels and Values of the Variables for the Experimental Design

Variables	Levels				
	Very low	Low	Medium	High	Very high
Temperature (°C)	31	55	65	75	98
Time (h)	0.38	2	6.33	20	106
Silica gel/glycerol (w/w)	0.3:1	1:1	1.5:1	2:1	2:1
Water activity	0.06	0.06	0.11	0.33	0.44
Solvent concentration (% w/w)	0	43	56	64	72
Glycerol/triglycerides (mol/mol)	0.9:1	2:1	3.5:1	6:1	6:1

By weighing the samples until a constant weight was achieved it was shown that by using vacuum during the equilibration process, both the enzyme and the silica gel/glycerol mixtures reached equilibrium within 24 h, i.e., much faster than at atmospheric pressure.

Glycerolysis. The reaction was performed in test tubes (8 mL) with screw caps. Rapeseed oil, 200 mg (0.2 mmol), and glycerol, adsorbed to silica gel, 45–358 mg (0.18–1.2 mmol, according to the experimental design), were weighed in a tube. Enzyme, 20 mg (10% w/w dry enzyme of the rapeseed oil mass) was added and, in some of the experiments, isooctane was also added. The sealed tubes were put in a shaker and kept at the desired temperature. At the time indicated by the experimental design, the tube was taken from the shaker and 5 mL of a mixture of *n*-heptane and 2-propanol (20:1, vol/vol) was added. Some of the supernatant was withdrawn for HPLC analysis.

HPLC analysis. The composition of the reaction mixture (FFA, TG, 1,3-DG, 1,2-DG, MG) was analyzed in an HPLC system (model SCL 6B; Shimadzu, Tokyo, Japan) equipped with a diol column (LiChrospher® 100 diol, 5 µm, 4 × 125 mm; Merck) and an evaporative light-scattering detector (model 750/14; Applied Chromatography Systems Ltd., Macclesfield, Cheshire, England). The injected volume was 20 µL and the sample was eluted with *n*-heptane, acetic acid, and 2-propanol at 55°C, as described elsewhere (25). An integration system (Kontron Instruments, Milano, Italy) was used for data collection and integration. Calibration was made with known amounts of FFA, TG, 1,3-DG, 1,2-DG, and MG.

Calculations and evaluation. The yield was calculated from the analyzed molar amounts in each sample as the mole ratio of the fatty acid content in the substance to the total amount of fatty acids in the mixture.

For evaluation of the results, models were created that included most of the linear, cross-relating, and quadratic terms for the independent variables with the yield as the dependent variable, e.g.,

$$\text{yield} = b_0 + \sum_{i=1}^6 b_i x_i + \sum_{i=1}^5 \sum_{j=i+1}^6 b_{ij} x_i x_j + \sum_{i=1}^6 b_{ii} x_i^2 \quad [1]$$

Models were made for the yield of MG, DG, and FFA and for the remaining amount of TG. The least significant parameters were removed until a minimal standard error of estimate (SEE) was reached. Outliers were removed and a new estimation was made. The procedure was repeated until no outliers were identified (see Table 2). The computer program SYSTAT® 6.0 for Windows® (SPSS Inc., Chicago, IL) was used to estimate the parameters for the models using multiple linear regression and to present them graphically.

Some of the experiments have been excluded in the modeling due to difficulties of fitting good models. All these excluded experiments have been performed with one variable at the very low or very high level and the others at the medium level. Excluded experiments include: MG yield and MG ini-

tial reaction rate: very high temperature; DG yield and DG initial reaction rate: very low silica-to-glycerol ratio, very high temperature, and very high solvent concentration; FFA yield and FFA initial reaction rate: very high a_w and very high glycerol-to-TG ratio; TG yield and TG initial reaction rate: very low a_w , very high temperature, and very high solvent concentration.

In the modeling, a model dependent standard deviation is calculated and called the standard error of estimate (SEE). All of the experimental data can be found within the range $\pm k * \text{SEE}$, where k is a factor calculated from the residuals of the model. Differences in yield or in initial reaction rate less than $\pm k * \text{SEE}$ should be regarded as nonsignificant. The values of $k * \text{SEE}$ are given in the Figure legends. The distance between the lines in Figures 1–3 is set to $k * \text{SEE}$.

The variations in the yield over time of MG, DG, and FFA, and in the remaining amount of TG were studied to decide whether the reaction had reached equilibrium. The reaction rate had its highest value in the beginning of the reaction, therefore, the initial reaction rate was used as a measure of the maximal reaction rate. It was calculated from the estimated yield after 2 h and expressed as the amount of product/reactant per amount of enzyme and time ($\text{g/g}_{\text{enzyme}} \cdot \text{h}$).

RESULTS AND DISCUSSION

We have investigated reaction conditions for the synthesis of MG by glycerolysis of rapeseed oil with isooctane as solvent. The variables studied were reaction temperature, reaction time, the ratio of silica gel to glycerol, water activity, the amount of solvent and the ratio of glycerol to TG (levels and values shown in Table 1) and their effect on the initial reaction rate (calculated from the estimated yield at 2 h) and the yield of MG. In the reaction, MG was formed together with DG, and there was also a release of FFA. Some TG was left unreacted in the mixture. The results of the experiments and modeling are presented in Table 2 and illustrated in Figures 1–3. Good interpretations of the results can generally be made only between low and high levels (see Table 1). Very low and very high levels can only be considered when the remaining variables are at medium level. Therefore, the graph's scales only range from the low to the high level of each variable. Differences in initial reaction rate or in yield less than $\pm k * \text{SEE}$ should be regarded as nonsignificant.

Initial reaction rate. The highest initial reaction rate for MG achieved within our experimental range was $77.4 \pm 9.5 \text{ g/g}_{\text{enzyme}} \cdot \text{h}$ (temperature 75°C, silica gel-to-glycerol ratio 2:1, a_w 0.17, solvent concentration 43%, and glycerol-to-TG ratio 6:1; see Fig. 1). Temperature, silica-to-glycerol ratio, glycerol-to-TG ratio, a_w , and solvent concentration have a significant effect on the initial reaction rate. Increase in the first three variables gives a higher initial reaction rate. For a_w , the model shows a maximum for a_w 0.17 (see Fig. 1). However, this maximum is not significant. It is significant that a decrease in a_w from 0.33 to 0.17 gives a higher initial reaction rate for MG, but the increase in initial reaction rate achieved

TABLE 2
Estimated Parameters for the Models for Yield and Initial Reaction Rate Together with $k \cdot \text{SEE}$ for Each Model

Parameter ^a	MG yield	DG yield	FFA yield	TG yield	MG IRR	DG IRR	FFA IRR	TG IRR
b_0	47.1	—	-20.5	69.4	270	—	-93.7	146
b_1	0.445	0.595	0.581	-1.85	2.55	2.98	2.66	8.81
b_2	-84.5	16.5	8.14	102	-485	82.8	37.2	-485
b_3	8.74	-17.6	-3.36	-41.3	50.1	-88.2	-15.4	197
b_4	53.8	60.9	150	-189	309	305	684	900
b_5	-2.63	—	-0.0683	4.35	-15.1	—	-0.312	-20.7
b_6	—	19.3	-15.0	—	—	96.6	-68.6	—
b_{12}	—	—	-0.158	0.219	—	—	-0.724	-1.04
b_{13}	—	—	—	—	—	—	—	—
b_{14}	0.277	1.05	-0.996	—	1.59	5.25	-4.55	—
b_{15}	-0.00270	—	—	—	-0.0155	—	—	—
b_{16}	—	—	—	—	—	—	—	—
b_{23}	1.77	3.12	2.29	-7.06	10.2	15.6	10.5	33.7
b_{24}	—	-10.2	10.4	—	—	-51.2	47.5	—
b_{25}	3.35	—	0.0449	-5.21	19.2	—	0.205	24.9
b_{26}	-3.87	-12.2	—	16.0	-22.2	-61.0	—	-76.4
b_{34}	-13.4	-19.7	8.56	24.9	-76.8	-98.6	39.1	-119
b_{35}	-0.0721	-0.158	—	0.208	-0.414	-0.792	—	-0.994
b_{36}	5.79	4.50	4.63	-15.3	33.2	22.5	21.1	72.9
b_{45}	-0.142	-0.354	—	0.452	-0.815	-1.77	—	-2.16
b_{46}	-18.9	-17.4	35.4	—	-108	-87.2	162	—
b_{56}	0.0952	0.194	0.107	-0.572	0.546	0.969	0.490	2.73
b_{11}	-0.00203	-0.00453	-0.00285	0.0114	-0.0117	-0.227	-0.0130	-0.0542
b_{22}	0.996	-1.83	-2.24	5.21	5.71	-9.18	-10.3	-24.9
b_{33}	-1.46	7.49	—	12.2	-8.35	37.5	—	-58.1
b_{44}	-78.6	-156	-197	188	-451	-783	-899	-899
b_{55}	0.0259	—	—	-0.0379	0.149	—	—	0.181
b_{66}	-3.85	-17.2	—	26.6	-22.1	-86.1	—	-127
b_{255}	-0.0315	—	—	0.0478	-0.181	—	—	-0.228
$k \cdot \text{SEE}$	1.7	3.4	2.0	4.3	9.5	9.0	17.0	20.3

^aIndex: 1, Temperature (°C); 2, time (h, logarithmic scale); 3, silica gel/glycerol ratio (w/w); 4, water activity; 5, solvent concentration (% w/w); 6, glycerol/TG ratio (mol/mol, logarithmic scale). Abbreviations: IRR, initial reaction rate; MG, monoglyceride; DG, diglyceride; TG, triglyceride; FFA, free fatty acid.

when increasing a_w from 0.06 to 0.17 is not statistically significant. A decrease in solvent concentration from 58 to 43% gives a higher initial reaction rate.

Our results indicate that a temperature higher than 75°C should increase the initial reaction rate for MG (Fig. 1). However, in the experiment performed at the very high temperature (98°C), the yield of MG was very low, which means that at this temperature the enzyme had lost its activity. The experiment at 98°C had to be excluded during the modeling because it was not possible to fit a model that could describe this change in enzyme activity. Apparently, the maximal value for the initial reaction rate using Lipozyme[®] can be found when the temperature is in the range 75–98°C.

The formation of FFA is a very fast reaction. As can be seen in Figure 2, the reaction time has no effect on the yield of FFA for a_w below 0.25. This means that the equilibrium level is reached in a shorter time than 2 h and that the initial reaction rate for FFA is greater than $71.0 \pm 17.0 \text{ g/g}_{\text{enzyme}} \cdot \text{h}$ at a_w 0.25.

All the variables have a significant effect on the initial reaction rate for TG in the same way as for MG. The only difference is a_w . For MG, a decrease in a_w gives a higher initial reaction rate, but for TG an increase gives a higher initial reaction rate. This is explained by the formation of FFA. A higher a_w only results in a more rapid release of FFA.

Equilibrium yield. The yield of MG after 20 h was $17.4 \pm 1.7\%$ (mol FA in substance/total mole FA in mixture). It was obtained at a temperature of 75°C, a silica-to-glycerol ratio of 2:1, a glycerol-to-TG ratio of 6:1, an a_w of 0.17, and a solvent concentration of 48% (see Fig. 3). These conditions gave $36.8 \pm 3.4\%$ DG, $13.6 \pm 2.0\%$ FFA, and $36.9 \pm 4.3\%$ remaining TG.

Temperature, time, silica-to-glycerol ratio, and glycerol-to-TG ratio had a significant effect on the yield of MG. For the reaction temperature, the same trend is seen as for the initial reaction rate; a temperature higher than 75°C will give a higher yield. However, with the same argument as for initial reaction rate, the maximal value for the yield using Lipozyme[®] can be found when the temperature is in the range 75–98°C.

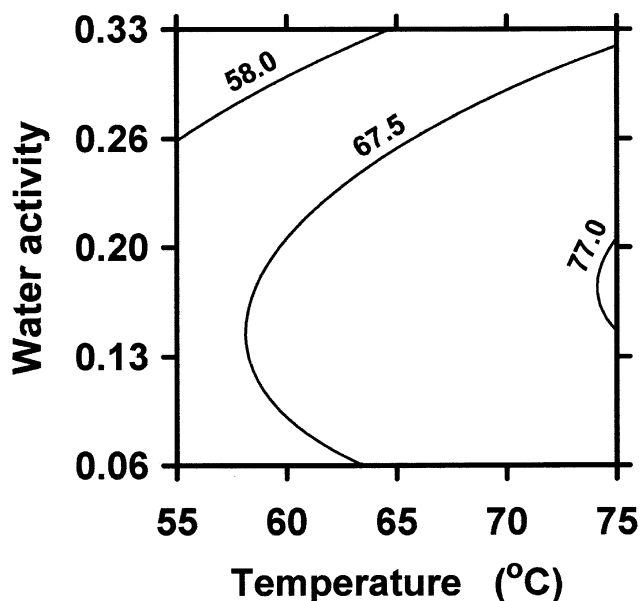


FIG. 1. Initial reaction rate for monoglycerides. Time 2 h, silica gel/glycerol ratio 2:1, solvent concentration 43%, glycerol/triglyceride ratio 6:1, $1.5 \cdot \text{SEE} = 9.5$, where SEE is the standard error of estimate.

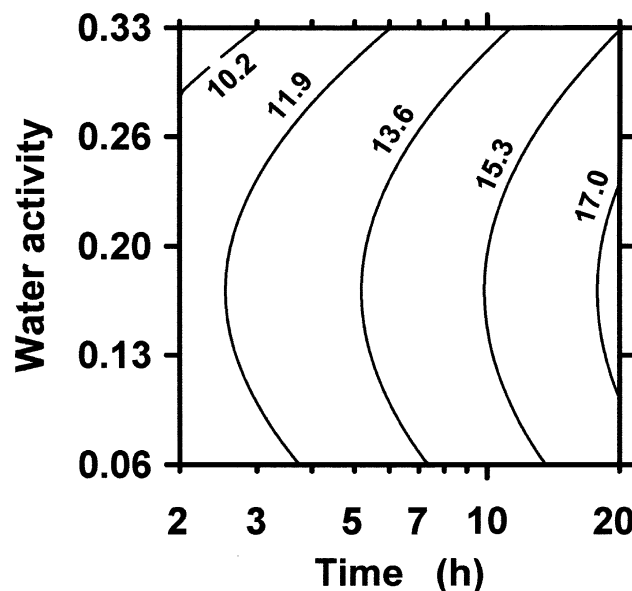


FIG. 3. Yield of monoglycerides. Temperature 75°C, silica gel/glycerol ratio 2:1, solvent concentration 48%, glycerol/triglyceride ratio 6:1, $1.5 \cdot \text{SEE} = 1.7$. For abbreviations see Figure 1.

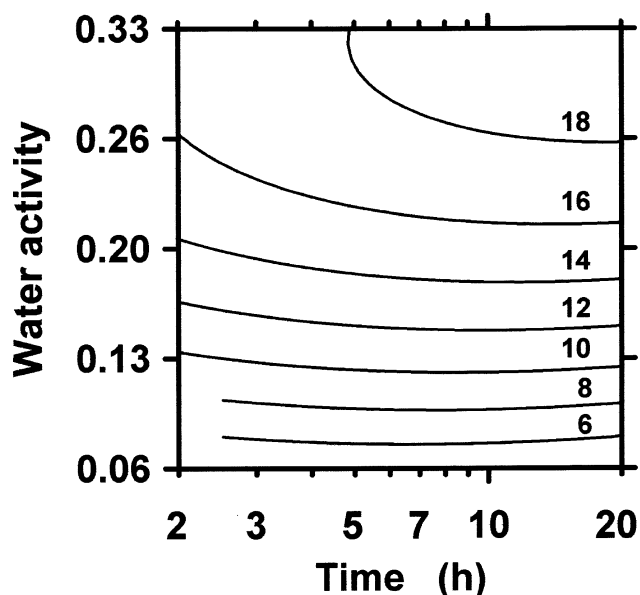


FIG. 2. Yield of free fatty acids. Temperature 75°C, silica gel/glycerol ratio 2:1, solvent concentration 48%, glycerol/triglyceride ratio 6:1, $1.8 \cdot \text{SEE} = 2.0$. For abbreviations see Figure 1.

For a_w , the model shows the same nonsignificant maximum at a_w 0.17 as it does for the initial reaction rate. The water activity has only a minor effect on the yield at a_w below 0.3 (see Fig. 3).

The yield of MG is very little affected by changes in solvent concentration between 43 and 50%. However, it is significant that a solvent concentration higher than 50% gives a lower yield of MG.

The yield of DG was increased in nearly the same way as the yield of MG. Therefore, it is difficult to find reaction conditions giving a high yield of MG and a low yield of DG.

The yield of FFA was significantly affected by the silica-to-glycerol ratio and by the a_w . An increase in silica-to-glycerol ratio gives a higher yield. As seen in Figure 2, a_w has a very strong influence on the formation of FFA. Increasing a_w gives a major increase of FFA. It is clearly seen from the Figures 2 and 3 that the water activity should be kept at a low level, as higher levels of a_w only result in the formation of FFA.

Figures 2 and 3 show the influence of time on the yield of MG and FFA. The time has almost no effect on FFA. This means that the equilibrium level of FFA has been reached during the first 2 h of the reaction. For MG, the time has a significant effect. From the graphs, it looks as if the yield of MG will increase if the reaction time is prolonged beyond 20 h, but equilibrium is probably reached very soon. The yield of MG is about 17% with the best conditions at 20 h. In the long-term experiment (106 h), the yield of MG is 19%. The other variables are not at the best levels in this experiment (temperature 65°C, silica-to-glycerol ratio 1.5:1, a_w 0.11, solvent concentration 56%, glycerol-to-TG ratio 3.5:1), but it is unlikely that there would be a drastic change in the yield if the variable levels were changed to the best conditions. This means that equilibrium is probably reached after a total reaction time of 25–30 h. This agrees well with the results of McNeill *et al.* (11), Stevenson *et al.* (13), and Ota *et al.* (14). They performed glycerolysis reactions with different types of fat and reported that equilibrium was reached in about 25 h. Their equilibrium yields are presented in Table 3.

Comparison with solid-phase glycerolysis. The yield of MG at equilibrium is 25–35% (mol/mol) (see Table 3). To obtain a higher yield, MG has to be removed from the reaction mixture. Crystallization is one possible technique (11–14). For the experiments presented in Table 3, McNeill *et al.* (11) reported a final yield of 70% for beef tallow if the temperature was 46°C or lower (11), and a final yield for rapeseed oil

TABLE 3
Comparison of Equilibrium Yield for Some Glycerolysis Reactions^a

TG	Glyc/TG (mol/mol)	Solvent	Time (h)	Temperature (°C)	Yield MG (%)	Reference
Beef tallow	2:1	No	25	48–50	30	11
Mutton tallow	2:1	No	24	50	35	13
Rapeseed oil	2:1	No	72	30	25	12
Sunflower oil	3:1	No	25	35	25	14
Rapeseed oil	2:1	Isooctane	20	55	23 ^b	Present paper
	3:1			55	24 ^b	
	6:1			55	25 ^b	
	6:1			75	28 ^b	

^aGlyc/TG, glycerol/TG. For other abbreviations see Table 2.

^bYield recalculated as normal mol percentage.

of 75% after 5 d, if the temperature was 5°C (12). Stevenson *et al.* (13) reported a final yield of 50% after 48 h if the temperature was reduced from 50 to 42°C after 24 h reaction time, and Ota *et al.* (14) reported a final yield of 95% after 80 h with a temperature program; 35°C (0–7 h), 15°C (7–18 h) and 10°C (18–80 h). In all four cases, MG started to crystallize owing to the decrease in temperature and therefore a high yield was achieved.

The solid-phase glycerolysis (11–14) gives high yields, but long reaction times are needed and the reaction mixture becomes solid. From an industrial point of view, this is not practical nor is it economically interesting. To be able to make an industrial glycerolysis process, it is more valuable to study the initial reaction rate and the factors influencing the equilibrium yield.

In our system, a change in the glycerol to TG ratio from 2:1 to 6:1 had a minor effect on the equilibrium. The yield increased from 23 to 25% (mol/mol). A change in temperature from 55 to 75°C changes the yield from 25 to 28% (see Table 3). How is it that the glycerolysis reactions with beef and mutton tallow performed at about 50°C and a glycerol-to-TG ratio of 2:1 give an equilibrium yield of 30–35% (Table 3, ref. 11,13) MG compared to 23% at 55°C and 2:1 in our system? The beef and mutton tallow contain mostly saturated fatty acids, yielding mostly saturated MG. Therefore, a temperature around 50°C is required to keep them in a liquid state. However, this temperature is very close to the melting point, so it may be that the MG have started to precipitate from the reaction mixture, though this precipitation has not been detectable because of the presence of solid enzyme particles. This could explain the higher equilibrium yield.

Comparison with chemical synthesis. In *Bailey's Industrial Oil & Fat Products*, Vol. 4 (26), is presented a table with theoretical equilibrium composition for the chemical reaction of fat and glycerol. For a MG content of 36.8% (mol/mol), the corresponding values for DG and TG are 44.9 and 18.3%, respectively. This reaction mixture contains no FFA. Recalculation of our best yield of MG and corresponding values of DG and TG to normal mole percentage (disregarding FFA) gives 36.2% (mol/mol) MG, 38.3 DG, and 25.6% TG. Comparing the figures, we have a lower level of DG and a higher level of TG, but we are close to equilibrium. The differences

in DG and TG could be explained by the fact that we also have FFA in the reaction mixture.

The table in *Bailey's* shows the equilibrium composition as a function of the glycerol-to-TG ratio provided that the glycerol forms a single phase together with the glycerides. This means that to obtain a higher yield of MG, more glycerol has to be dissolved in the reaction mixture. The solubility of glycerol in fat increases with increasing temperature, and this could explain why the yield of MG in our system increases with increasing temperature.

The ability of the reaction mixture to dissolve glycerol slowly increases as the reaction proceeds and MG is formed. This explains the slow progress of the reaction after about 15 h. However, this reaction is not fast enough to be used in an industrial process. The increase in yield for MG achieved at decreasing solvent concentrations could be explained by the poor solubility of glycerol in isooctane. As the glyceride mixture is a better solvent for glycerol, more glycerol is dissolved at a lower solvent content and therefore a higher yield is achieved.

From these findings we see that it is important to find a solvent that dissolves the glycerol. At the same time this solvent should not deactivate the enzyme and it should be harmless to the final product.

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