High-Yield Enzymatic Glycerolysis of Fats and Oils

Gerald P. McNeill, Shoichi Shimizu and **Tsuneo Yamane***
Laboratory of Bioreaction Engineering, Department of Food Science and Technology, Faculty of Agriculture, Nagoya University, Chikusa-Ku, Nagoya 464-01, Japan

Several triglyceride fats and oils were reacted with glycerol using lipase as catalyst. A batch system with magnetic stirring was used without the addition of any solvents or emulsifiers. In all cases a mixture of mono-, di- and triglycerides was obtained. However, the yield of monglyceride (MG) depended strongly on the reaction temperature: at higher temperatures approximately 30% MG was produced at equilibrium while at lower temperatures a yield of 65%–90% MG was obtained for most of the fats examined. The upper temperature limit below which a high MG yield could be attained was designated the critical temperature (T_c). The value of T_c depended on the fat type and was found to vary between 30°C and 46°C for naturally occurring hard fats. A high MG yield could not be obtained for fully hydrogenated tallow and lard under the conditions described here. Of the three liquid oils examined, rapeseed oil and olive oil had a T_c of 5°C and 10°C respectively whereas a high yield of MG could not be obtained with corn oil at 5°C or greater. The maximum yield of MG below T_c also depended on the fat type: the highest yields being obtained for olive oil (90%), palm stearin and milk fat (80%) and the lowest yield for palm oil (67%). In all cases a high yield of MG was accompanied by solidification of the reaction mixture. The effect of enzyme type on MG production was examined for palm oil and palm stearin and the effect of water concentration was examined for palm oil.

KEY WORDS: Fats, glycerolysis, lipase, monoglyceride, oils.

Both a large quantity of monoglycerides (MG) and their derivatives are synthesized every year for use as emulsifying agents in a wide range of foods (1). MG are usually manufactured by the glycerolysis reaction in which natural fats and oils undergo ester exchange with glycerol at temperatures greater than 220°C in the presence of an inorganic catalyst (2,3). A large molar excess of glycerol must be used and the yield of MG is 30–40%. Dark-colored by-products with an undesirable flavor are also formed due to the high reaction temperature and must be removed. To overcome these disadvantages, low temperature synthesis of MG using lipase enzyme (E C 3.1.3.3) has been attempted by several workers. In the case of reacting free fatty acid with glycerol in the presence (4) or absence (5) of an emulsifying agent, yields of MG were low. Selective hydrolysis of triglyceride or glycerolysis (6–9) also resulted in MG synthesis but produced low yields or required the use of organic solvents and surface active agents. Recently the authors reported that a high yield of MG (approximately 70%) could be obtained from tallow in a simple batch glycerolysis system by careful control of the reaction temperature (10). As organic solvents, emulsifiers and high temperatures are not required, this provides a practical alternative to chemical glycerolysis. Moreover, the substrates for enzymatic glycerolysis are triglyceride and glycerol in contrast to enzymatic ester synthesis which requires relatively expensive free fatty acid as substrate. The work reported here describes the application of our recently reported enzymatic glycerolysis process to the synthesis of MG from several kinds of fats and oils under a variety of conditions.

*To whom correspondence should be addressed.

EXPERIMENTAL

Glycerolysis. A mixture of glycerol, water, lipase powder and the fat or oil under investigation was prepared as previously described (10). Unless otherwise stated, water concentration in the glycerol phase was 3.6%, crude lipase from *Pseudomonas fluorescens* was used at 500 units/g fat or oil and a mole ratio of glycerol: fat of 2:1 was chosen. An enzyme reactor (model MS-50, Matsumoto Manufacturing Co. Ltd., Osaka, Japan) was used for temperature control and magnetic stirring at 800 rpm. Other lipase preparations were used under the same conditions and at the same activity.

Analysis. The course of glycerolysis was monitored by intermittent sampling (150 mg) followed by chloroform extraction. The extract was analyzed for triglyceride (TG), 1,3 diglyceride (1,3 DG), 1,2 diglyceride (1,2 DG), monoglyceride (MG) and free fatty acid (FFA) using a thin-layer chromatography (TLC)/flame ionization detector. Details of the extraction and analysis are described elsewhere (6). Results are expressed as percent peak areas and may differ slightly from the true weight percent as described by Tatara and co-workers (11).

Moisture contents were determined using a Karl-Fischer moisture meter (model MKS-1, Kyoto Electronics Ltd., Kyoto, Japan).

Initial Rates. Initial rate of appearance of 1,3 DG, 1,2 DG, MG and FFA is defined as follows: initial rate of appearance = fraction/hr. Initial rate of disappearance of TG is defined as: initial rate of disappearance = (1-fraction of TG)/hr. These were calculated from the tangential straight lines passing through the origin of the time course curves of the components.

Materials: Lipase activity was determined by the olive oil/surfactant nonaddition method as described previously (12). One activity unit is described as the amount of enzyme which liberates one micromole of free fatty acid per min at 37°C. Commercially available lipases (E C 3.1.1.3) were used and were obtained from the following companies: *Pseudomonas fluorescens* crude (lipase P), Amano Pharmaceutical Co. Ltd., Nagoya, Japan; *Chromobacterium viscosum*, Toyo Jozo Co. Ltd., Shizuoka, Japan; *Mucor miehei* and SP398, Novo-Nordisk Bioindustry Inc., Tokyo, Japan.

Refined fats and oils were provided by the following companies: Palm Oil Research Institute of Malaysia, Kuala Lumpur, Malaysia, palm olein and palm stearin; Fuji Oil Co. Ltd., Osaka, Japan, palm oil and coconut oil; Nippon Shokuhin Kako Co. Ltd., Shizuoka, Japan, corn oil; Ajinomoto Co. Inc., Tokyo, Japan, rapeseed oil (low erucic

acid); Taiyo Kagaku Co. Ltd., Yokkaichi, Mie, Japan, hydrogenated tallow and hydrogenated lard.

RESULTS

Determination of the critical temperature (T_c) for MG production. Figure 1 A, B and C shows the time course of monoglyceride production (MG) during the glycerolysis of palm oil, palm olein and palm stearin respectively in the temperature range 30°C to 50°C. For the three fats, the yield of MG increased as the temperature was reduced. The maximum temperature at or below which high yield was observed (T_c) was 46°C, 40°C, and 50°C with an MG yield of approximately 65%, 60%, and 80% for palm oil, palm olein and palm stearin respectively. In the case of palm oil and palm olein a sharp transition between the low and high yield equilibria was observed. In contrast,

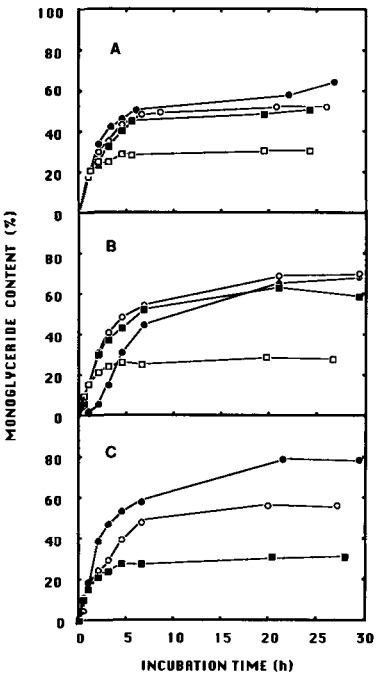

FIG. 1. The effect of temperature on monoglyceride production during enzymatic glycerolysis of palm oil and palm oil fractions. A, palm oil: 40°C (●), 44°C (○), 46°C (■), 48°C (□); B, palm olein: 20°C (●), 30°C (○), 40°C (■), 50°C (□); C, palm stearin: 40°C (●), 50°C (○), 60°C (■).

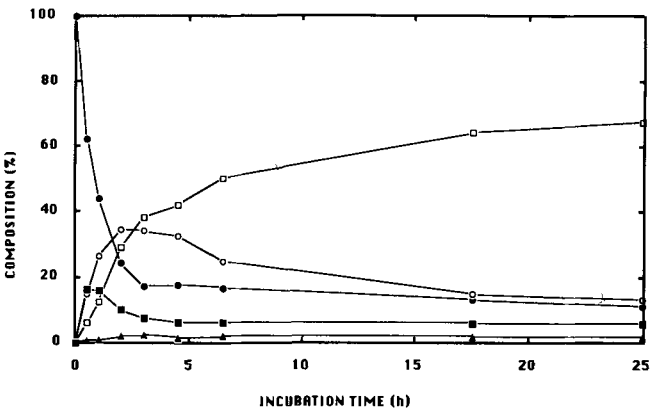

FIG. 2. The composition of the reaction mixture during enzymatic glycerolysis of palm oil at 40°C: TG (●), 1,3-DG (○), 1,2-DG (■), MG (□), FFA (▲).

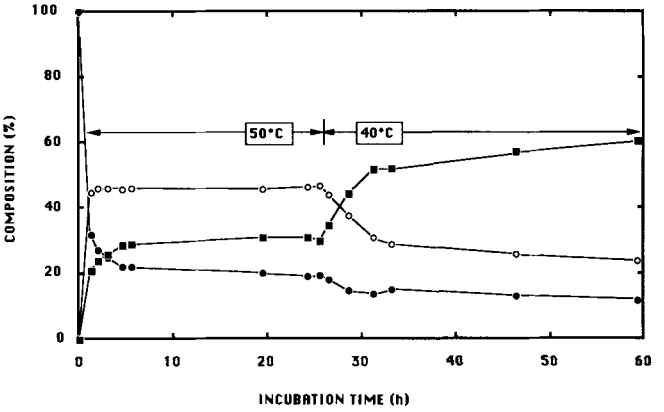

FIG. 3. Enzymatic glycerolysis of palm oil at 50°C followed by incubation at 40°C. Composition of the reaction mixture: TG (●), total DG (○), MG (■).

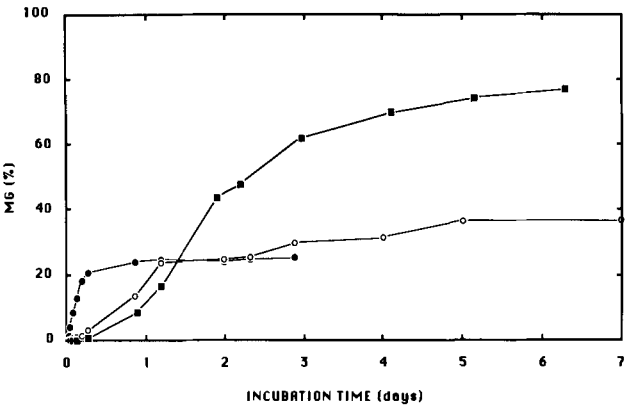

FIG. 4. The effect of temperature on monoglyceride production during enzymatic glycerolysis of rapeseed oil: 30°C (●), 10°C (○), 5°C (■).

palm stearin exhibited an intermediate yield equilibrium. At the T_c or lower, the reaction mixture became solid after about 3 hr for the three fats. The reaction mixture remained liquid (an emulsion) at reaction temperatures above the T_c.

The detailed change in composition of glycerides in the palm oil reaction mixture during the course of glycerolysis is given in Figure 2. Initially, the rate of MG synthesis was high but after 3 hr the rate decreased accompanied by an accumulation of 1,3 DG which was only slowly converted to MG. The concentration of 1,2 DG remained low throughout the reaction. Although the reaction mixture became solid after approximately 3 hr, synthesis of MG continued until a high yield was obtained.

Figure 3 further demonstrates the effect of altering the temperature during the course of the reaction on the equilibrium composition of palm oil. During incubation at 50°C, the equilibrium concentration of MG reached 30%. After adjusting the temperature to 40°C, a new equilibrium was reached in 35 hr with an MG concentration of 60%.

The effect of temperature on the synthesis of MG from rapeseed oil is shown in Figure 4. At 10°C or higher the MG concentration reached 25–35% but at 5°C the MG content

TABLE 1

Glycerolysis of Various Fats and Oils by *P. fluorescens* Lipase (500 units/g fat)

Fat or oil	Melting point (°C)	Optimum temperature[a] (°C)	Initial rate[b] (fraction/hr)	Maximum MG yield (%)
Beef tallow	46	42	0.14	76
Lard	33	30	0.13	69
Milk fat	30	32	0.09	80
Palm oil	38	40	0.15	67
Palm olein	20	30	0.16	71
Palm stearin	52	40	0.18	86
Coconut oil	25	30	0.05	77
Rapeseed oil	<5	5	0.01	77
Olive oil	<5	10	0.02	90
Corn oil	<5	5	0.01	42
Hydrogenated tallow	62	50	0.20	39
Hydrogenated lard	59	50	0.12	30

[a]Optimum temperature is the temperature at which the highest MG yield is obtained.
[b]Initial rate is the rate of MG production at the optimum temperature.

reached approximately 75%. Due to the low reaction temperature, final equilibrium was reached after 5 days. The effect of temperature on the equilibrium composition of other fats and oils is summarized in Table 1. It may be seen that corn oil, which is also a liquid oil, did not produce a high MG yield even at 5°C. With olive oil, an MG concentration of 90% was reached in 4 days at an incubation temperature of 10°C. This oil is unique among the fats examined because the only products of the glycerolysis at 5°C were MG and 1,3 DG, whereas at 10°C a substantial quantity of TG and 1,2 DG was also present. Other naturally occurring hard fats all possessed a T_c below which a high MG yield was obtained. In general, a high value of T_c was found for fats with a high melting point and a low T_c was found for low melting point fats. In the case of fully hydrogenated tallow and lard, a T_c could not be found and the maximum MG yield was only 30-40%.

The initial rate of MG synthesis at the optimum temperature (Table 1) was similar for most of the hard fats examined between 30 and 40°C. A notable exception was coconut oil with a low reaction rate (0.05 fraction/hr). This may be partly due to the presence of a high level of lauric acid (48%) in the triglycerides of this oil.

Effect of water content. Figure 5 shows the initial rates of formation of DG, MG and FFA and the initial rate of conversion of TG during glycerolysis of palm oil at 42°C with a water content in the glycerol phase ranging from 0.5% to 11%. The initial rate of conversion of TG was roughly proportional to the water content in the range 0.5% to 4.0%. From 4% to 11% the increase in rate was considerably lower. The initial rate of synthesis of both 1,3 and 1,2 DG was also proportional to water content between 0.5% and 3.5%, but above 3.5% the rate of DG synthesis was almost constant. There was essentially no difference in initial rate between 1,3 and 1,2 DG irrespective of water concentration. The initial rate of MG formation increased with increasing water content from 0.5% to 5.7%, but above 5.7% the water content had little effect on the rate of MG formation. In the case of FFA synthesis, the initial rate was very low and was hardly affected by moisture content between 0.5% and 5.0%. At high water content (5%-11%) there was a large increase in rate with increasing water content. However, even at the lowest water level (0.5%), a small amount of FFA (0.1%-0.2%) was synthesized.

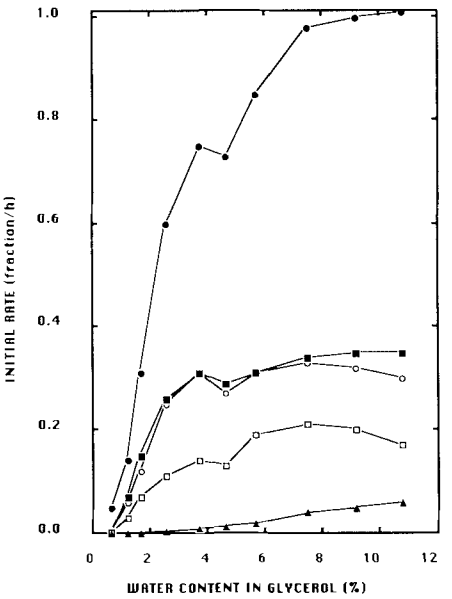

FIG. 5. The effect of initial water content on the initial rate of disappearance of TG and the initial rate of appearance of MG, DG and FFA during enzymatic glycerolysis of palm oil at 40°C: TG (●), 1,2 DG (○), 1,3 DG (■), MG (□), FFA (▲).

Glycerolysis activity of various enzymes. Table 2 shows the composition of the reaction mixture after glycerolysis of palm oil and palm stearin at 40°C using four different enzyme preparations. With both fats the highest yield of MG was obtained with *Pseudomonas fluorescens* and *Chromobacterium viscosum* lipases. The use of *Mucor miehei* and SP398 lipases resulted in high yields of MG (43-57% for palm oil and approximately 60% for palm stearin) but were a little lower than those obtained using *Pseudomonas fluorescens* or *Chromobacterium viscosum* lipases.

TABLE 2

Composition of the Reaction Mixture After Glycerolysis of Palm Oil and Palm Stearin at 40°C Using Four Different Lipase Preparations

Lipase preparation	Composition (%)				
	TG	1,3-DG	1,2-DG	MG	FFA
1. Palm oil					
Pseudomonas fluorescens	11	13	6	68	2
Chromobacterium viscosum	11	15	6	65	2
Mucor miehei	16	14	10	57	2
SP 398	25	17	8	43	6
2. Palm stearin					
Pseudomonas fluorescens	6	9	4	79	2
Chromobacterium viscosum	5	7	3	83	2
Mucor miehei	19	9	6	65	1
SP 398	33	6	13	47	2

DISCUSSION

In our previous publication it was shown that a high yield of MG (70%) could be obtained by glycerolysis of beef tallow using lipase as a catalyst (10). A simple batch system was used which avoided the use of organic solvents or emulsifiers. It was found that a high yield was only obtained when the reaction was carried out below a critical temperature (46°C). The object of the present work was to attempt to synthesize MG in high yield in a wide range of fats and oils using this process and to evaluate the effect of altering the reaction conditions.

In previous investigations of lipase-catalyzed glycerolysis (6,8), olive oil, corn oil and safflower oil were used, partly because of the ease of handling liquid oils. However, these oils are not used extensively for commercial MG production, and relatively low yields of MG were obtained. In the present study, in addition to liquid oils, several high melting point fats which are of greater relevance to commercial MG production (e.g. palm oil and lard) were investigated.

For all high melting point fats examined, except fully hydrogenated tallow and lard, a critical temperature (T_c) was found below which good yields of MG could be obtained. The fats became solid during the course of the reaction only if a high concentration of MG was synthesized. The value of T_c for palm oil (46°C) was similar to the T_c of 46°C found previously for tallow (10). The value of T_c for other fats depended on the fat type, and in general fats with a higher melting point also had a higher T_c. As demonstrated in the previous communication, the high MG yield is presumably due to lower solubility of MG in the reaction mixture resulting in preferential crystallization of MG shifting the equilibrium toward synthesis of more MG. In the case of high melting point fats, a greater proportion of high melting point MG will be synthesized. This MG should therefore exceed its solubility limit at a higher temperature resulting in a higher T_c. The low value of T_c for rapeseed oil (low erucic acid) and olive oil (5-10°C) is not surprising as the major MG to be synthesized will be the low melting point monoolein. For corn oil the major MG, monolinolein, did not precipitate from the reaction mixture at temperatures as low as 5°C; therefore the T_c could not be determined.

The yield of MG was dependent on the fat type. The highest yields of MG were obtained from olive oil (90%), palm stearin and milk-fat (approximately 80%). Palm stearin is a low cost fraction of palm oil and is therefore an obvious choice for the commercial application of the process described here. Efficient glycerolysis of rapeseed oil and olive oil at low temperature (5-10°C) eliminates the possibility of double bond oxidation which might easily occur at the reaction temperature of chemical glycerolysis (220°C). The reason for differences in MG yield among different fats is not easily explained but is probably due to differences in fatty acid and triglyceride composition. The absence of a high yield equilibrium state for fully hydrogenated lard and tallow is probably due to the inability of MG to preferentially crystallize from the mixture of high melting point TG and DG which are also present during the reaction.

In this study it was found that lipases from *P. fluorescens* and *C. viscosum* catalyzed the glycerolysis of palm oil and palm stearin most effectively. This observation was also made previously for glycerolysis of olive oil (6) and tallow (10). The intermediate yield of MG which was obtained using lipase from *M. miehei* and SP 398 lipase is similar to previous results obtained with tallow (10). As the latter enzymes possess 1,3 specificity and *P. fluorescens* and *c. viscosum* lipases are positionally nonspecific, this may explain the observed differences in MG yield. Previously, however (10), the nonspecific lipase from *C. cylindracea* was found to be inactive under glycerolysis conditions. Further research is required in order to understand why glycerolysis activity of lipases from different sources varies considerably.

In contrast to results obtained with tallow, the rate of MG synthesis from palm oil began to decrease during glycerolysis of palm oil at an MG concentration of about 40% (Fig. 2). The accumulation of 1,3 DG indicates that this glyceride is a poor substrate for the enzyme but further investigation is required to adequately explain this phenomenon. The effect of the concentration of water in the glycerol phase on the initial rate of glycerolysis of palm oil was found to be similar to that observed previously for tallow.

The work described here clearly shows that a high yield of up to 90% MG may be synthesized from a wide range of fat and oil types by lipase catalyzed-glycerolysis employing simultaneous fractional crystallization of the MG. The method is simple, is effective at relatively low tempera-

tures (5°C to 50°C) and provides a practical alternative to the conventional chemical method of synthesis.

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