0273-2289/02/98-100/0987/$12.50

# Enzymatic Synthesis of Medium Chain Monoglycerides in a Solvent-Free System

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## Abstract

The synthesis of monocaprin, monolaurin, and monomyristin in a solvent-free system was conducted by mixing a commercial immobilized lipase with the organic reactants (glycerol and fatty acids) in a 20-mL batch reactor with constant stirring. The effects of temperature, fatty acid/glycerol molar ratio, and enzyme concentration on the reaction conversion were determined. The addition of molecular sieves in the assays of monomyristin synthesis was also evaluated. The reactions were carried out for 5 to 6 h and the nonpolar phase was analyzed by gas chromatography. The best results in terms of selectivity and conversion (defined as the percentage of fatty acid consumed) were achieved when the stoichiometric amount of reagents (molar ratio = 1) and 9% (w/w) commercial enzyme were used and the reaction was performed at 60°C. The addition of molecular sieves did not improve the synthesis of monomyristin. Conversions as high as 80%, with monoglycerides being the major products, were attained. After 5 h of reaction, the concentration of monoglyceride was about twice that of diglyceride, and only trace amounts of triglyceride were found. The results illustrate the technical possibility of producing medium chain monoglycerides in a solvent-free medium using a simple batch reactor.

**Index Entries:** Medium chain monoglycerides; immobilized lipase; esterification.

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## Introduction

Monoacylglycerols (monoglycerides) are the most widely used emulsifiers in the food, cosmetic and pharmaceutical industries. Furthermore, they have a generally recognized as safe status, which contributes to their larger application. Mono- and diglycerides are consumed at an annual level of 85,000,000 kg in the United States, corresponding roughly to 70% of the total emulsifiers used in the food industry *(1)*. Besides bulk applications in the food and dairy industries, some other applications for special monoacylglycerols have been described *(2–4)*. Recently, the antimicrobial activities of particular types of monoglycerides such as monolaurin, monomyristin, monolinolein, and monolinolenin have been reported *(5,6)*. It has also been proposed that fatty acids and monoglycerides (lauric acid, monocaprin) may be used as intravaginal microbiocides for protection against sexually transmitted diseases *(7)*.

Monoacylgycerols are manufactured on an industrial scale by continuous chemical glycerolysis of fat and oils at high temperatures (220–250°C), employing inorganic alkaline catalysts under a nitrogen gas atmosphere. The product produced by this route has several drawbacks (e.g., low yield, dark color and burnt taste). The product is a mixture containing 35–60% monoacylglycerols, 35–50% diacylglycerols, 1–20% triacylglycerols, 1–10% free fatty acids, and their alkali metal salts *(8)*. High concentrations of monoacylglycerols are obtained from the mixtures by molecular distillation.

In the last decade, many approaches have been investigated for the enzymatic synthesis of monoacylglycerols, such as selective hydrolysis using 1,3-regiospecific lipases, esterification of fatty acids or transesterification of fatty esters with glycerol, and glycerolysis of fats or oils. The advantages of enzymatic synthesis are higher yields and mild reaction conditions, resulting in products of higher quality and lower energy consumption *(2,9)*.

When the production of a high-purity-degree monoglyceride is desired, an interesting route is the direct lipase-catalyzed esterification. Thus, the objective of the present work was to study the synthesis of monoacylglycerols in a medium solely composed of substrates by direct lipase-catalyzed esterification between glycerol and fatty acid, without any solvent or surfactant. Such a system avoids the problems of separation, toxicity, and flammability of organic solvents, thereby lowering the cost of the final product and permitting recovery of product without further complex purification or evaporation steps. Moreover, a solvent-free system was chosen because the products have a potential use in foods. This work reports the effect of some conditions that can affect the synthesis reaction, such as glycerol/fatty acid molar ratio, enzyme concentration, temperature, addition of molecular sieves, and chain length of the fatty acid.

## Materials and Methods

### *Materials*

Lipozyme IM-20 (*Mucor miehei* lipase immobilized on a weak anion-exchange resin) was a donation from Novo Nordisk A/S (Bagsvaerd, Denmark). All fatty materials (substrates and gas chromatography [GC] standards) were obtained from Sigma (St. Louis, MO). Analytical grade glycerol, *n*-hexane, ethyl acetate, ethanol, acetone, and molecular sieves (3 A) were purchased from Merck (Darmstadt, Germany).

### *Measurement of Lipase Activity*

The esterification activity of the enzyme was measured according to the method described by Langone and Sant'Anna *(10)*, which determines the rate of consumption of fatty acid at 60°C in a reaction system containing glycerol, lauric acid, and a given amount of the commercial enzyme preparation.

### *Nonpolar Phase Analysis*

Fatty acid and mono-, di-, and triglycerides were analyzed by capillary GC. Each sample of 20 µL was diluted (1000×) in a 1:1 hexane/ethyl acetate mixture, and 1 µL was injected into a Chrompack CP 9000 gas chromatograph with a flame ionization detector. The GC was fitted with a 10 m × 0.25 mm × 0.12 µm Chrompack Sil 5CB column. Helium was used as the carrier gas with a flow rate of 2 mL/min. The detector and injector temperatures were set at 350°C. The column temperature was set at 80°C for 1 min and was then programmed at 20°C/min up to 320°C, which was maintained constant for 2 min. Concentrations were expressed as molar fractions calculated from the peak area using calibration curves.

### *Esterification Experiments*

All experiments were carried out in a 20-µL batch reactor with constant stirring, using a magnetic stirrer. The reactor (jacketed beaker) was kept at the selected temperature by using a thermostatic water bath. Given quantities of glycerol and fatty acid were mixed together and preincubated at the selected temperature, followed by the addition of the immobilized lipase. The progress of the reaction was followed by withdrawing 20-µL aliquots at various time intervals and analyzing them by the GC, as previously described. The water produced by the reaction was spontaneously removed by free evaporation from the uncovered reactor. When water removal was performed by trapping the formed water onto molecular sieves, the reaction medium contained 0.6 g of 3 A molecular sieves (Merck), and the reactor was maintained covered during the experiment.

The following conditions were investigated: temperatures of 50–90°C, fatty acid/glycerol molar ratios of 0.5, 0.75, and 1 and enzyme concentration of 1, 3, 5, 7, and 9% (w/w).

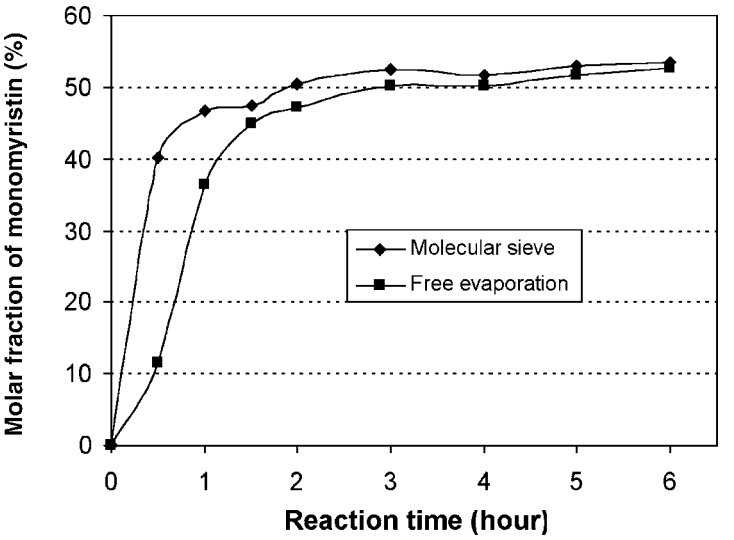


Fig. 1. Effect of addition of molecular sieve on monomyristin synthesis carried out at 60°C with myristic acid/glycerol molar ratio of 0.5 and 7% (w/w) lipozyme.

## Results and Discussion

### *Commercial Enzyme Preparation Activity*

A linear relationship between the initial rate of lauric acid consumption and the commercial enzyme concentration (% [w/w]) was observed for the range studied (1 to 9% [w/w]). Taking into account the definition of activity formulated by Langone Sant' Anna *(10)* and the stated relationship, it can be said that the commercial lipase preparation used in our experiments presented an activity of 0.03 U/g. For consistency, the enzyme amount per volume will be, hereinafter, expressed on a weight/weight percentage basis.

### *Effect of Addition of Molecular Sieve for Water Removal*

An initial set of experiments was performed at 60°C (the lower temperature investigated) for the synthesis of monomyristin (melting point of myristic acid = 51–54°C). Molecular sieves were used to lower the water activity of the reaction medium, thus shifting the equilibrium and pushing the reaction further. The results showed that the production of monoglyceride was not significantly affected by the addition of molecular sieves to the reaction medium, as illustrated in Fig. 1, which presents the reaction progress curves for two conditions (free evaporation and addition of molecular sieve). These results show that free evaporation is an effective mechanism to keep the water content low, a necessary condition for completion of reaction. Water evaporation was favored by operating an open batch reactor at 60°C.

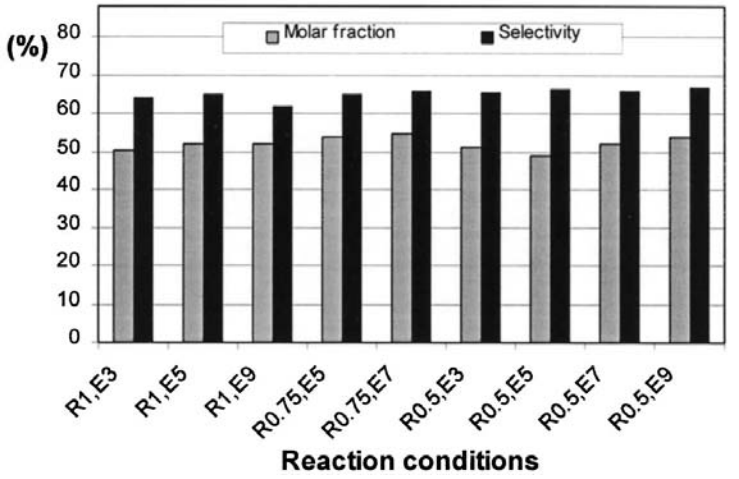


Fig. 2. Effect of fatty acid/glycerol molar ratio ($R$) and enzyme concentration ($E$) on selectivity and molar fraction of monomyristin. Synthesis was at 60°C, carried out for 6 h.

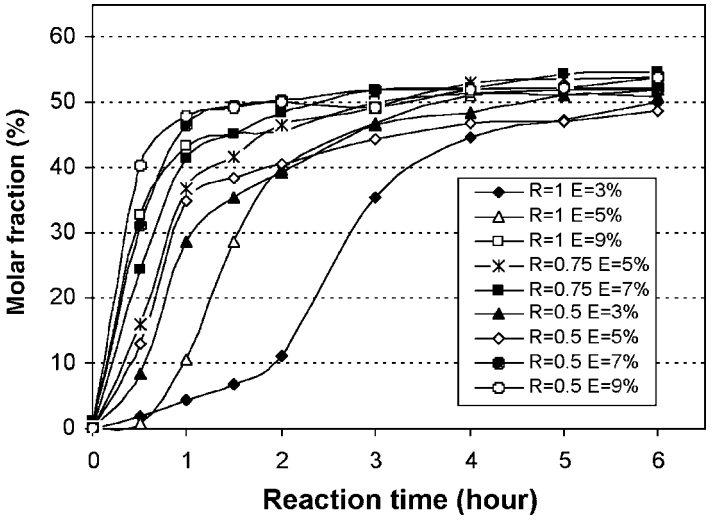


Fig. 3. Effect of fatty acid/glycerol molar ratio ($R$), and enzyme concentration ($E$) on monomyristin synthesis (molar fraction) at 60°C.

## *Monoglyceride Synthesis*

Experimental results of the synthesis of monoglyceride are summarized in Figs. 2–8. The results are expressed as molar fraction of the component in the nonpolar phase (fatty acid; mono-, di-, and triglyceride). The selectivity parameter, chosen to define the best reaction conditions, was defined as the ratio between monoglyceride and total glyceride (mono-, di-, and triglyceride) content on a molar basis.

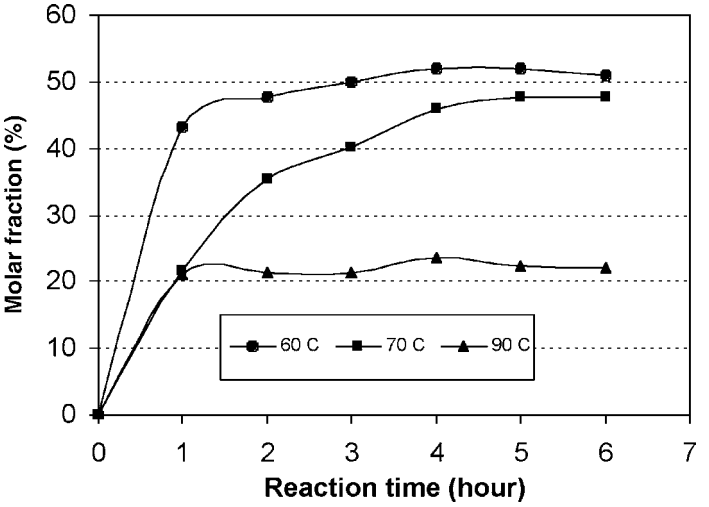


Fig. 4. Effect of temperature on monomyristin synthesis (molar fraction), carried out for 6 h, with fatty acid/glycerol molar ratio of 1 and 9% (w/w) lipozyme.

## Monomyristin

In a first set of experiments, the effect of enzyme concentration and myristic acid/glycerol molar ratio on monomyristin synthesis was evaluated at 60°C. These results are shown in Figs. 2 and 3. The maximum selectivity value (66.8%) was attained after 6 hours of reaction, using a molar ratio equal to 0.5 and an enzyme concentration of 9% (w/w) (Fig. 2). The utilization of an excess of glycerol, corresponding to molar ratios of 0.75 and 0.5 (the stoichiometric ratio is 1), did not result in a significant improvement in selectivity for monomyristin. Mono-, di-, and triglycerides were produced, approximately, in the same proportions, for the range of molar ratios investigated. Singh et al. *(11)* have observed a maximum conversion of monodiglycerides in water-in-oil microemul-sions, in experiments carried out with the stoichiometric molar ratio (oleic acid to glycerol). Thus, a surplus of glycerol in the reaction medium does not seem to favor the production of monoglyceride. On the other hand, when the production of triglyceride is targeted, the excess of fatty acid has a marked positive effect. Langone and Sant' Anna *(10)* observed a high triglyceride production rate and selectivity of 100% when a molar ratio of 5 was used for the production of trimyristin.

Enzyme concentration affected the initial reaction rate but did not significantly affect the final (after 6 h) selectivity (monomyristin), as shown in Figs. 2 and 3. Lower molar ratios also led to higher reaction rates; however, after 6 h of reaction, similar conversions were attained (Fig. 3). Equilibrium was reached after 2–5 hours, depending on the reaction conditions. Ultimate concentrations of monoglyceride were quickly attained when low molar ratios and high enzyme concentrations were employed.

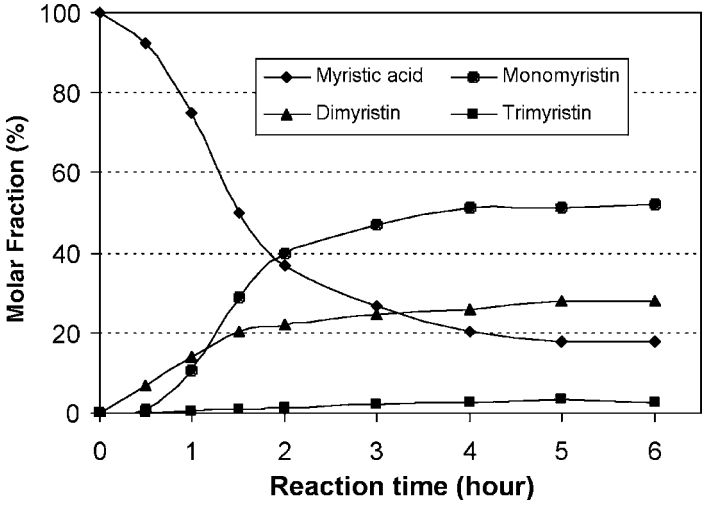


Fig. 5. Time course for monomyristin synthesis carried out at 60°C, with myristic acid/glycerol molar ratio of 1 and 5% (w/w)lipozyme.

The synthesis of monomyristin was investigated at different temperatures using the stoichiometric molar ratio, and an enzyme concentration of 9 % (w/w). Monomyristin synthesis was conducted at 60, 70, and 90°C, because these temperatures are above the melting point of myristic acid (51–54°C). As shown in Fig. 4, temperature had a great effect on the reaction performance, affecting both the initial reaction rate and the final monoglyceride concentration. Above 60°C enzyme denaturation was significant, becoming very severe at 90°C.

Since an appreciable monoglyceride yield was achieved when the reaction was performed at 60°C, using a moderate amount of enzyme (5% [w/w]) and a stoichiometric ratio (avoiding the utilization of a surplus of glycerol), time course experiments were carried out under these conditions. Figure 5 illustrates that after 5 h, the ultimate concentrations of mono-, di-, and trimyristin were 52, 28, and 2.6%, respectively. The residual concentration of myristic acid was 17.6%.

These results are similar to those obtained by chemical glycerolysis of fat and oils at higher temperatures *(8)*. The product produced by the chemical route contains 35–60% monoacylglycerols, 35–50% diacylglycerols, 1–20% triacylglycerols, 1–10% free fatty acids and their alkali metal salts.

According to the directives of the World Health Organization, the requirements for these mixtures for utilization as food emulsifiers are: 1) to have at least 70% of mono + diglycerides, 2) to have a minimum of 30% of monoglyceride, and 3) to present contents of both glycerol and triglyceride below 10% *(1)*. The enzymatic synthesis of monomyristin, as carried out in this work, resulted in a final mixture that fulfills these requirements.

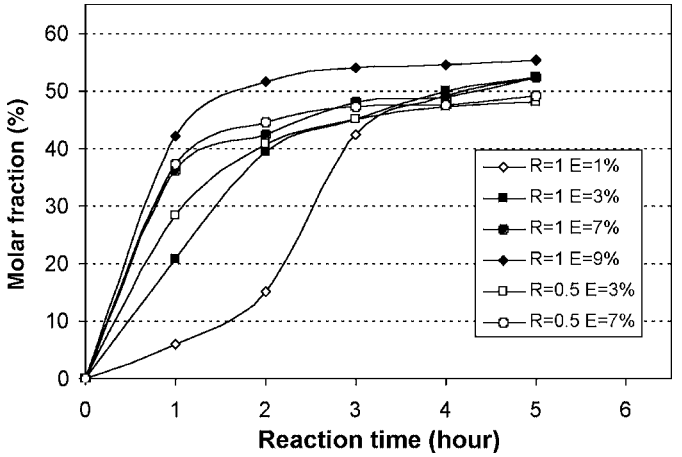


Fig. 6. Effect of fatty acid/glycerol molar ratio (*R*) and enzyme concentration (*E*) on monolaurin synthesis (molar fraction) at 60°C.

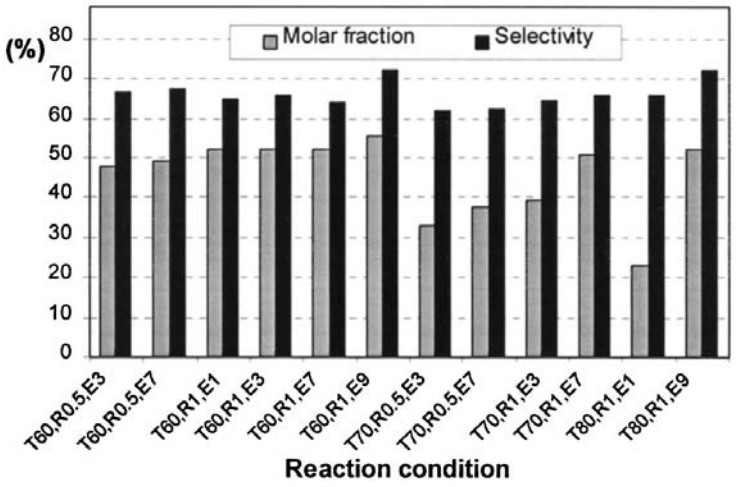


Fig. 7. Effect of temperature (*T*), fatty acid/glycerol molar ratio (*R*), and enzyme concentration (*E*) on selectivity and molar fraction of monolaurin. Synthesis was carried out for 5 h.

## Monolaurin

Results shown in Figs. 6 and 7 are very close to those obtained for monomyristin synthesis. An excess of glycerol did not promote a significant increase in final selectivity of monolaurin. Enzyme concentration affected the initial reaction rate, which increased in the range of 1–9%. Temperature also has a marked effect on monolaurin synthesis. Higher molar fraction values were attained at 60°C. The decrease in this parameter, which was pronounced at low enzyme concentrations, was presumably owing to the denaturation of the lipase, in such medium, at higher tem-

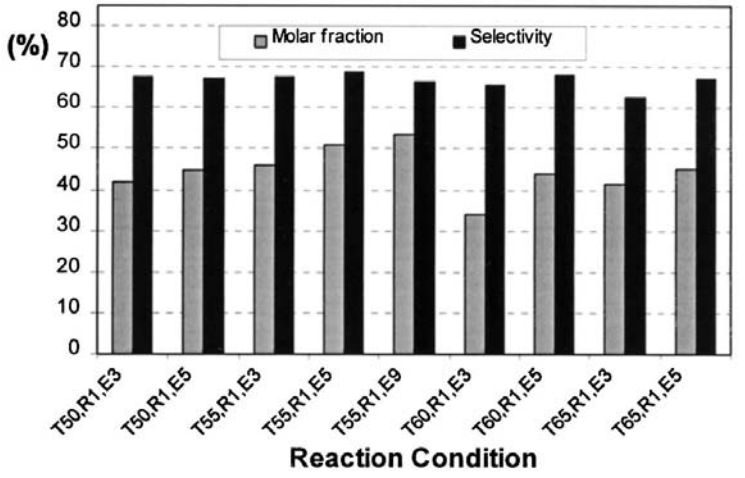


Fig. 8. Effect of temperature (*T*), fatty acid/glycerol molar ratio (*R*), and enzyme concentration (*E*) on selectivity and molar fraction of monocaprin. Synthesis was carried out for 5 h.

peratures. Temperature itself is not the unique factor affecting lipase denaturation. Langone and Sant' Anna *(10)*, using a similar reaction system, observed high yields of trimyristin and trilaurin at 80–100°C, in a reaction medium containing fatty acid and glycerol (molar ratio of 5) and lipozyme (5 or 9% [w/w]). Thus, the higher glycerol concentration required for the synthesis of monoglycerides seems to negatively affect the enzyme activity. In this case, the increase in glycerol, which is a polar substrate, may decrease the enzymes stability, because it is adsorbed on the lipozyme support, removing the water layer necessary for enzyme integrity. Some published works have shown that enzymes, such as lipases, retain high activity, mainly in nonpolar organic media *(12,13)*

### Monocaprin

The results obtained in the experiments on monocaprin synthesis are illustrated in Fig. 8. Although a high selectivity was observed, the molar fraction of monocaprin (at the end of the reaction) was lower, when compared with monolaurin and monomyristin synthesis reactions. The best conditions observed for the synthesis of monocaprin were a temperature of 55°C, a capric acid/glycerol molar ratio of 1, and an enzyme concentration of 9% (w/w).

## Conclusions

The results obtained in our experiments indicate the production feasibility of monocaprin, monolaurin, and monomyristin in a solvent-free medium. Removal of water was effectively conducted by free evaporation in an open reactor. The utilization of molecular sieve for water removal,

although effective, did not have a pronounced advantage over the simple evaporation technique.

Enzyme concentration was a relevant parameter that had a pronounced effect on the initial rate of monoglyceride synthesis. However, after 5 to 6 h of reaction, the ultimate molar fraction and the selectivity toward the product were relatively close for the different enzyme concentrations (1 to 9% [w/w]) tested. An excess of glycerol beyond the stoichiometric ratio did not promote a significant improvement in reaction yield parameters. Temperature had a marked effect on monoglyceride synthesis, and the selected values were 60°C for monomyristin and monolaurin, and 55°C for monocaprin.

Monoglycerides were produced at high yields and selectivities. Conversions (defined as the percentage of consumed fatty acid) reached 80%, with monoglyceride being the major product. Monoglyceride was produced at about twice the concentration of diglyceride, and only trace amounts of triglyceride were detected.

The final composition observed after 6 h of reaction in the experiments of monoglyceride synthesis fulfills the requirements for emulsifier utilization in food, cosmetic, and pharmaceutical industries. The results obtained by enzymatic synthesis under mild experimental conditions were similar to those obtained by chemical glycerolysis of fat and oils, without the drawback of producing some secondary products such as acrolein, polyethers, or polyesters of glycerol.

Finally, it should be stressed that the production of monoglycerides was successfully carried out in a very simple reaction system.

## Acknowledgments

This work was partially financed by the Brazilian agencies FAPERJ (no. E-26/150.418/97 and no. E-26/170.731/2000) and PADCT/CNPq (no.62.0160/91.8).

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