Enzymatic Transesterification of Rapeseed Oil and Lauric Acid in a Continuous Reactor

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The optimum conditions for enzymatic transesterification of rapeseed oil and lauric acid in a fixed-bed reactor were studied. No solvent was used in the reaction mixture. A small amount of water was dissolved in the substrate mixture to maintain the activity of the enzyme at as high a level as possible. For maximum yield, the transesterification was performed with a residence time of about 20 min at a water content in the range of 0.1 to 0.2% and at the lowest possible temperature.

KEY WORDS: Continuous reactor, enzymatic, rapeseed oil, transesterification.

Enzymatic transesterification is a potential method for modification of the physical and chemical properties of edible fats and oils and is expected to become an alternative route to plant breeding of oil crops for the production of fats and oils with desired characteristics (1). The major advantage of lipase-catalyzed reactions over those carried out by chemical catalysis lies in the fact that a wide variety of specific products of different compositions and properties can be prepared, depending on the specificity of the lipase used (2). The use of enzymatic methods should also reduce environmental loading in comparison with conventional processes (3).

Lipase-catalyzed transesterification can be performed with free enzymes or immobilized enzyme preparations in stirred-tank or fixed-bed reactors with or without organic solvent in the reaction medium (4). When using organic solvents, the reactants and products are dissolved, and a rather wide temperature range can be used for catalysis. In solvent-free systems, the temperature must be high enough to maintain the reaction mixture in the liquid state. For practical applications, immobilized lipases and fixed-bed reactors, as well as solvent-free systems, should be used.

Hydrolysis of triglycerides and isomerization of diglycerides (acyl migration) are side reactions that should be minimized when performing transesterification (5). In some cases, hydrolysis cannot be totally avoided because lipases need a small amount of water to be active. Acyl migration causes changes in the fatty acid composition at the 2-position of triglycerides, even if 1,3-specific lipases are used. Practical applications should be conducted under conditions where side reactions are negligible.

Transesterification of lipids in continuous reactors has been studied mainly in systems in which substrates have been dissolved in organic solvents. The results have been reported mainly in the patent literature. The major effort has been to produce cocoa butter substitutes by transesterification of the mid-fraction of palm oil or sunflower oil and stearic acid in hexane at 40 or 50°C (6–8). Matsumoto *et al.* (9) claimed that the presence of ethanol reduced hydrolysis when transesterification was performed. Some studies concerning the activity and stability of an immobilized lipase preparation have been conducted in transesterifications of shea oleine or shea oil with fatty acid in hexane (10). Lipase-catalyzed transesterifications of vegetable oils with and without hexane have been investigated as a means of modifying their fatty acid composition (11).

Only a few studies concerning continuous transesterification in solvent-free systems have been published. Process configuration, catalyst lifetime and productivity were studied in the transesterification of olive or soybean oil and lauric acid (12). The results indicated that the most economical process for transesterification is the continuous reactor. The operational stability of the enzyme is highly dependent on the purity of the substrates, and optimization of the whole process is necessary for obtaining minimum costs. Studies concerning the effects of solvent concentration showed that a solvent-free continuous reactor filled with an enzyme immobilized on Hyflo Supercel (Johns Manville, Ltd., Richmond, United Kingdom) was impractical because of a high-pressure drop and difficulties in controlling the water content of the system (13). Trials in which solvent-free continuous transesterification was used to decrease the linolenic acid concentration of soybean oil and to produce cocoa butter equivalents have also been performed (14,15). Studies concerning the effects of mass transport and reaction parameters on transesterification of olive oil and trimyristin demonstrated that a solvent-free continuous process was acceptable above 60°C (16,17). A reactor model for continuous transesterification has also been presented (18). However, the published results do not indicate the optimum conditions for continuous transesterification in a solvent-free reactor.

We have previously studied the kinetics of enzymatic transesterification, as well as the transesterification of rapeseed oil and tallow for altering the melting properties of the mixture (19,20). The aim of the present work was to determine the optimum conditions for solvent-free transesterification catalyzed by 1,3-specific lipase in a continuous reactor.

MATERIALS AND METHODS

Materials. A 1,3-specific lipase preparation, immobilized on an anion exchange resin, (Lipozyme IM20, Novo Industry A/S, Bagsvaard, Denmark) was used as catalyst. Rapeseed oil, low-erucic acid rapeseed, was a refined, bleached and deodorized product, donated by the Raisio Group (Raisio, Finland), and lauric acid was from Fluka (61620; Buchs, Switzerland). Porcine pancreas lipase (Sigma L-3126; Sigma Chemical, St. Louis, MO) was used for the fatty acid analysis at the 2-position of triglycerides. All the solvents used for analysis of the products were reagent-grade.

Bioreactor. Transesterification of rapeseed oil and lauric acid was studied at a mass ratio of 3:1. No solvent was used in the reaction media. The fixed-bed reactor was a small glass column (2-cm diameter, 25-cm length) filled with the catalyst. The substrate mixture was fed upward into the column, and constant temperature was maintained by enclosing the whole system (column, feed- and product vessels and tubes) in an incubation cupboard.

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Residence time (τ) was calculated on the basis of porosity of the Lipozyme bed (Equation 1).

$$\tau = V_{tot} \cdot \varepsilon/v \quad [1]$$

where τ is the residence time, V_{tot} is the volume and ε = 0.43 the porosity of the enzyme bed (17), and v is the flow rate of the substrate. The batch experiments were performed in small glass vessels (4 g/batch) with magnetic stirring.

Analytical methods. The extent of transesterification was determined by measuring the lauric acid percentage of the modified rapeseed oil mixture by gas chromatography with flame-ionization detection (Micromat HRGC 314; Oriola, Espoo, Finland). Fatty acid methyl esters were prepared with sodium methoxide (21), and separation was performed with a polar 25 m × 0.20 mm × 0.3 μm column (HP-FFA; Hewlett Packard, Palo Alto, CA). Elution was carried out with temperature programming from 150 to 200°C at 5°C/min.

The degree of hydrolysis was measured by titrating free fatty acids (FFA) with 1 M NaOH by potentiometric titration (Titroprocessor 686 and Dosimat 665; Metrohm, Herisau, Switzerland). The samples were dissolved in acetone/ethanol/water, and the titration was performed to the end point of pH = 12. The percentage of FFA was calculated on the basis of the molecular weight of oleic acid.

The percentage of lauric acid at the 2-position of the modified rapeseed oil mixture was measured in the product samples by using a standard method (22), in which monoglycerides were fractionated from partially hydrolyzed samples by thin-layer chromatography and the fatty acids of the monoglycerides were analyzed after methylation.

The moisture content of the substrate mixture was measured by Karl Fischer titration (DL 18; Mettler, Zürich, Switzerland).

RESULTS AND DISCUSSION

Enzyme activity and stationary state of the process. Transesterification was first performed by feeding dry substrate mixture into the column. The extent of transesterification reached a constant level after about ten hours of operation, during which time the degree of hydrolysis decreased to zero [Fig. 1 (a and b)]. With a wet feedstream, the extent of both transesterification and hydrolysis remained at a higher level than without water addition [Fig. 1 (a and b)]. These demonstrated that the stationary state was reached in one day and that water, which was initially bound in the carrier resin, was consumed in hydrolysis during the first hours, thus causing inactivation of the enzyme. Similar results have been published earlier, and the time needed to reach the stationary state has been reported to vary from a few hours to two days (15,17,23). Posorske *et al.* (12) suggested that water saturation of oil is sufficient to maintain enzyme activity in a column filled with Lipozyme.

External mass transfer. To achieve maximum yield of transesterification, the process should be performed under conditions in which the reaction is kinetically controlled, *i.e.*, no mass transfer limitations are present. Our earlier studies showed that external mass transfer had no effect on the rate of transesterification at linear flow rates above

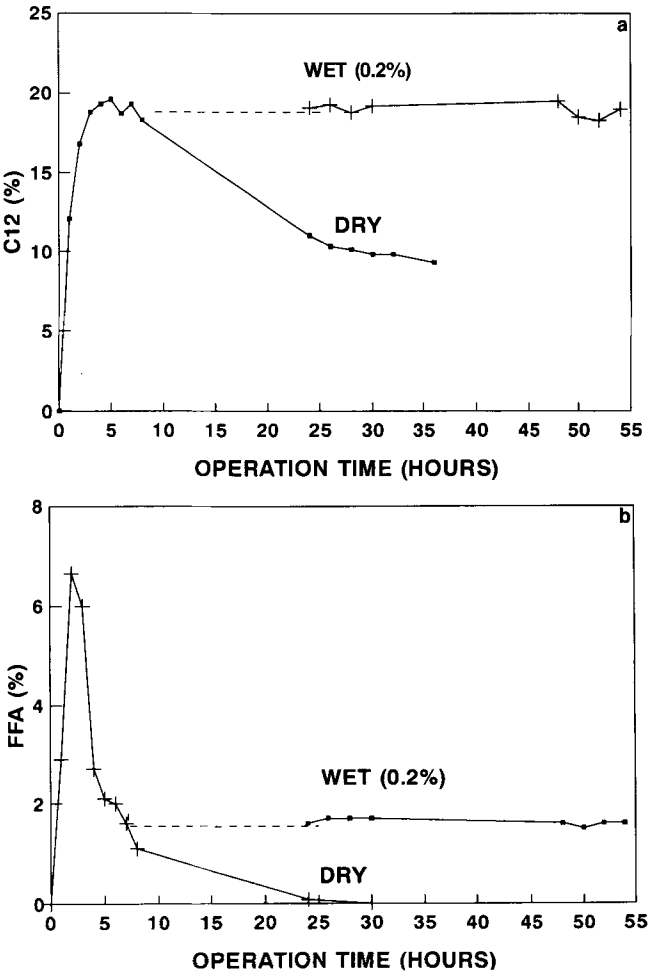

FIG. 1. The extent of transesterification (C12) (a) and hydrolysis [free fatty acids (FFA)] (b) as a function of operation time in the continuous reactor at 70°C. The residence time was about 30 min, and the substrate mixture was dry or a small amount of water (0.2%) was dissolved in the mixture.

$3 \cdot 10^{-5}$ m/s (24). This result was in accordance with the studies of Luck *et al.* (16,17), who performed solvent-free transesterification of lipids with Lipozyme. They used linear flow rates higher than $5 \cdot 10^{-5}$ m/s and observed no external mass transfer limitations.

Residence time and water content. The effect of residence time on the extent of transesterification and hydrolysis was studied at linear flow rates from 5 to $10 \cdot 10^{-5}$ m/s. The water content of the feedstream varied from 0.2 to 0.5%, and the process was operated at 70°C. The extent of transesterification reached a constant level at a residence time of about 30 min (Fig. 2). Because the water content of the feedstream was not constant in each experiment (the points in Fig. 2), the degree of hydrolysis was split around the hydrolysis curve (see also Fig. 3).

The effect of the feedstream water content on both the extent of transesterification and the degree of hydrolysis was studied separately at a constant residence time of 35 min. The extent of transesterification reached a constant level at a moisture content of about 0.2% and remained at this level, whereas the degree of hydrolysis was almost linearily dependent on the water content in the whole

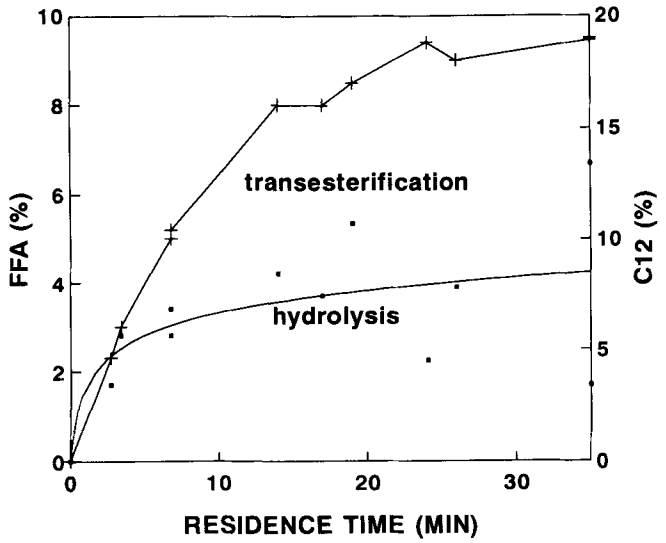

FIG. 2. The dependence of the extent of transesterification (C12) and hydrolysis [free fatty acids (FFA)] on the residence time at 70°C. The water content of the feedstream varied from 0.2 to 0.5%.

range studied (Fig. 3). The highest yield of transesterification was achieved by keeping the feedstream water content at 0.1–0.2%, which was sufficient to maintain the enzyme reactor active. Under these conditions, about 2% of FFA were formed by hydrolysis, which meant a loss of about 11% (w/w) of the triglycerides of rapeseed oil. This degree of hydrolysis is similar to that reported in the conventional transesterification with a chemical catalyst (12).

Residence times used in earlier studies have varied from 10 min to 2 h (6,8,11,15). In transesterification of palm oil mid-fraction and stearic acid in petroleum ether, the formation of 2% FFA and 5% diglycerides was measured (23). Hydrolysis has also been reported to result in a loss of about 14% (w/w) of the input triglycerides (2). Our results are in good agreement with these studies. Luck (16) performed transesterification of olive oil and trimyristin in a solvent-free reactor and observed maximum conversion with a residence time of 15 min at 60 and at 70°C. In this system the degree of hydrolysis did not depend on the residence time, and about 9 mol% of diglycerides were formed (17).

Acyl migration. Isomerization of diglycerides was studied at 70 and at 50°C at the optimum moisture content while varying the residence time. When the reactor was operated with a residence time of about 40 min, 5% (70°C) and 3% (50°C) of the fatty acids at the 2-position of the modified rapeseed oil were lauric acid (Fig. 4). At a residence time of about 20 min, only a small amount of acyl migration (less than 2% lauric acid at the 2-position) was detected at 50°C (Fig. 4). The degree of hydrolysis as well as that of transesterification was only slightly affected by the residence time over the range used (Table 1).

The extent of transesterification in the continuous reactor at a residence time of 25 min was about 20% (Table 1). In the batch reactor, the same extent of transesterification was achieved after two (70°C) and six (50°C) hours. The side reactions in the batch reactor proceeded in the same way as in the continuous reactor: at 20% conversion, 2 and 1% FFA were detected and 3 and 2% lauric acid at the 2-position were found at 70 and at 50°C, respectively (Fig. 5). These results may indicate that mass transfer effects were similar in both reactors, although the enzyme concentration was only 10% of substrate dry weight in the batch reactor.

Acyl migration is most often believed to be the reason for the fatty acid changes at the 2-position of triglycerides, and it has been demonstrated to depend on temperature, on the reaction time (enzyme concentration) and on the molar ratio of the substrates in transesterification of triglycerides and fatty acids (5,25). When ethyl stearate is used instead of stearic acid as acyl donor in transesterification, acyl migration decreases (26). The claim has been made that side activities are responsible for nonideal behavior of the reactions catalyzed by 1,3-specific lipases

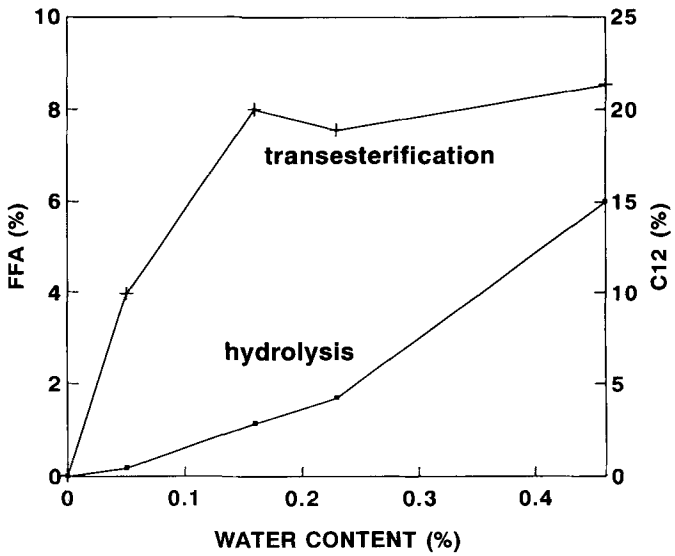

FIG. 3. The dependence of the extent of transesterification (C12) and hydrolysis [free fatty acids (FFA)] on the water content of the feedstream at a residence time of 35 min at 70°C.

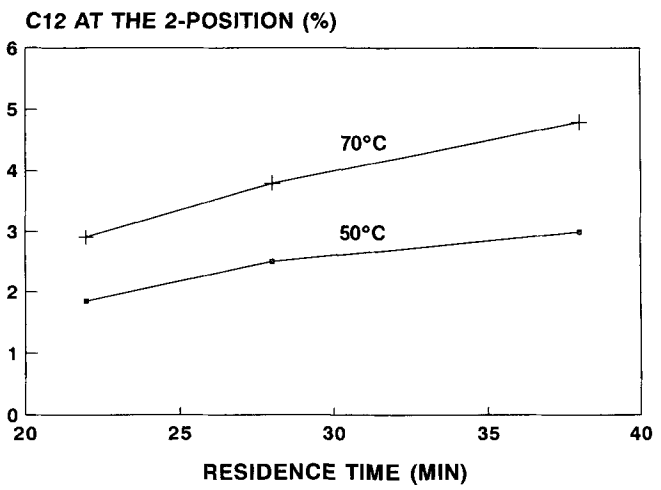

FIG. 4. The effect of temperature and residence time on the lauric acid percentage at the 2-position of the modified rapeseed oil.

TABLE 1

Dependence of the Extent of Transesterification (C12) and Hydrolysis [free fatty acids (FFA)] on the Residence Time (τ) and Temperature (T)

	22		28		38	
T (°C)	C12 (%)	FFA (%)	C12 (%)	FFA (%)	C12 (%)	FFA (%)
50	19	1.8	22	2.5	21	2.0
70	19	1.7	20	2.5	21	2.9

(16). According to the results of Macrae (23), stearoyl groups were incorporated exclusively into the 1- and 3-positions of triglycerides during transesterification of palm oil mid-fraction and stearic acid at a residence time of 10 min.

Whatever the reason for the changes at the 2-positions, our results clearly showed that the phenomenon is much slower than transesterification and hydrolysis, and that, after a long enough reaction time, random distribution seemed to be reached at higher temperatures (Fig. 5). Similar results were recently published concerning transesterification of cod liver oil and polyunsaturated fatty acids or their ethyl esters by Lipozyme (27). Furthermore, our studies demonstrated the direct dependence of the fatty acid composition at the 2-position on residence time (or the reaction time) and on temperature (Figs. 4 and 5). We also found that acyl migration was sensitive to the water content of the feedstream, with higher water content inducing more acyl migration (results not shown).

Optimum conditions and operational stability of Lipozyme. Transesterification should be performed under conditions in which maximum yield can be achieved. This means that hydrolysis and acyl migration should be minimized. The processing parameters for rapeseed oil and fatty acid should be chosen so that the linear flow rate is at least $5 \cdot 10^{-5}$ m/s, the residence time is 20 min or less, the water content of the feedstream is in the range of 0.1 to 0.2% and the temperature is as low as possible. The cost of the process is in any case largely dependent on the lifetime of the catalyst. The half life of Lipozyme

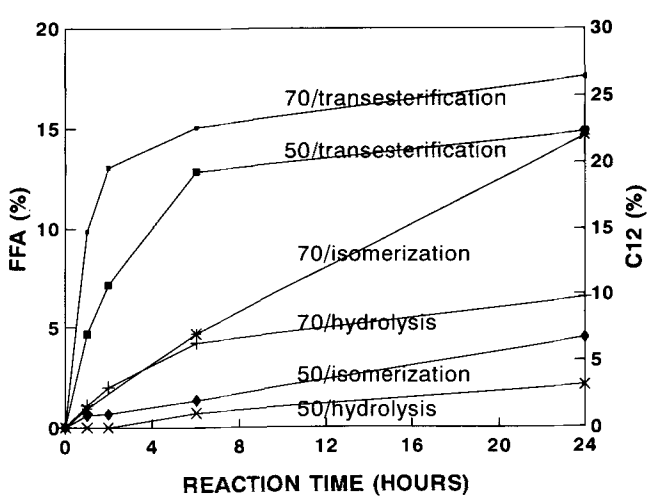

FIG. 5. Time course of transesterification, hydrolysis and acyl migration in a batch reactor at 50 and at 70°C. The enzyme concentration was 10% based on the substrate weight. FFA, free fatty acids.

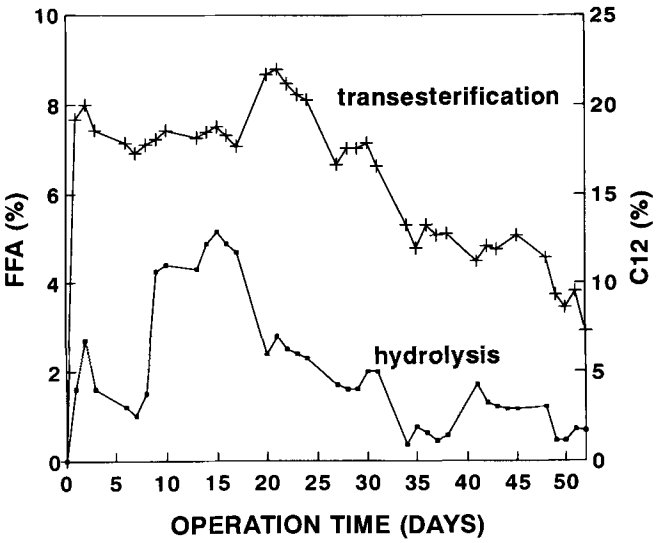

FIG. 6. The extent of transesterification (C12) and hydrolysis [free fatty acids (FFA)] as a function of operation time in the continuous reactor at 70°C at a residence time of about 35 min.

was measured at 70°C by using normal refined rapeseed oil and reagent-grade lauric acid. The half life was about 1.5 mon (Fig. 6). This confirms the results of Eigtved *et al.* (28), who reported the half life of Lipozyme in a solvent-free system to be 1.8 mon at 70°C.

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