Enzymatic Transesterification of Palm Olein with Nonspecific and 1,3-Specific Lipases

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ABSTRACT: The enzymatic transesterification of palm olein was conducted in a low-moisture medium with nonspecific and 1,3-specific lipases from microbial sources. The enzymes were first immobilized on Celite, lyophilized for 4 h and then added to a reaction medium that consisted of 10% (wt/vol) palm olein in water-saturated hexane. The catalytic performance of the enzymes was evaluated by determining the changes in triglyceride (TG) composition and concentrations by reverse-phase high-performance liquid chromatography (HPLC) and the formation of free fatty acids by titration. Studies with lipase from *Candida rugosa* showed that the degree of hydrolysis was reduced by drying the immobilized preparation and that the best drying time was 4 h. In all cases, the transesterification process resulted in the formation of PPP, a TG initially undetected in the oil, and increases in the concentrations of OOO (1.3–2.1-fold), OOL (1.7–4.5-fold), and OLL (1.7–4.3-fold), where P, O, and L are palmitic, oleic, and linoleic acids, respectively. SOS (where S is stearic acid), another TG not detected in the oil, was synthesized by *Rhizomucor miehei* and *Pseudomonas* lipases, with the latter producing more of this TG. There was a corresponding decrease in the concentrations of POP, PLP, POO, and POL. PPP concentration ranged from 1.9% (w/w) for *Mucor javanicus* lipase to 6.2% (w/w) for *Pseudomonas* lipase after 24 h. The greatest degree and fastest rate of change were caused by *Pseudomonas* lipase, followed by the enzymes from *R. miehei* and *Aspergillus niger*. The effects of transesterification and hydrolysis of palm olein by the various lipases resulted in changes in the overall degree of saturation of the triglyceride components. There seems to be no clear correlation between the enzyme positional specificity and the products formed. Possible mechanisms for the formation of PPP, OOL, OLL, OOO, and SOS are discussed.

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KEY WORDS: Mechanisms of synthesis, nonspecific lipases, palm olein, PPP synthesis, 1,3-specific lipases, transesterification.

Microbial lipases are widely studied for a variety of applications, such as hydrolysis, interesterification, ester synthesis, production of biosurfactants, and resolution of racemic mixtures to produce optically active compounds. In nature, these enzymes catalyze the hydrolysis of glycerides to produce free fatty acids and glycerol. The other reactions are the reverse of hydrolysis and take place in environments of low water content. In terms of their substrate selectivity, lipases usually can be classified into three groups. They can be *sn*-1,3-regiospecific (e.g., lipase from *Aspergillus niger, Mucor javanicus, Rhizomucor miehei,* and various *Rhizopus* spp.) or nonspecific (e.g., lipase from *Candida cylindracea/C. rugosa, Corynebacterium acnes*, and *Staphylococcus aureus*) toward the position of the acyl group of triglycerides (TG) during hydrolysis, or they can possess selectivity toward particular types of fatty acids (e.g., *Geotrichum candidum*) (1–2). It is usually observed that the positional specificity is retained when lipases are used under conditions of low water content, such as in organic solvents.

In the literature, the term *interesterification* is often used interchangeably to describe reactions that involve the exchange of acyl radicals between an ester and an acid (acidolysis), an ester and an alcohol (alcoholysis), or an ester with another ester (transesterification) (3,4). Confusion, too, exists in the use of the term transesterification because it has been used on numerous occasions to describe reactions that are in fact acidolytic. Recent examples include the studies by Osterberg *et al.* (5), Forssell *et al.* (6), Goh *et al.* (7), and Murakami *et al.* (8).

Rearrangement of the fatty acid positions of TG molecules of fats and oils through transesterification processes can alter the initial physical properties of the oils and can lead to the formation of new products. Often, transesterification is carried out to counter problems associated with interchangeability or blending of fats and oils (9) and to reduce the melting points of oil mixtures. Some examples reported recently include the transesterification of tallow with sunflower oil, soybean oil (10), rapeseed oil (11), canola–palm oil mixtures (12), and triolein–tripalmitin mixtures (13). Lipases that are 1,3-specific, such as those from *R. miehei* and *Rhizopus delemar*, are usually used to catalyze these reactions.

In this paper, we report on the transesterification performance of lipases from several microbial sources, immobilized on Celite, on palm olein in water-saturated hexane. Both 1,3-

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and nonspecific lipases were used, and the products of reaction were analyzed by HPLC.

EXPERIMENTAL PROCEDURES

Materials. Refined, bleached, and deodorized palm olein was purchased from a local supermarket and was composed of 0.17, 1.07, 36.61, 3.98, 45.60, and 12.60% of lauric, myristic, palmitic, stearic, oleic, and linoleic acids, respectively. The composition of the oil was determined by gas–liquid chromatography (GLC) after conversion of oil to fatty acid methyl esters with sodium methoxide (14). Celite (analytical filter aid), which was used as the immobilization support, was obtained from BDH Limited (Poole, England). Amano Pharmaceuticals Co. (Nagoya, Japan) donated lipases (powder) from *Candida rugosa*, a *Pseudomonas* sp., *Rhizopus javanicus, Mucor javanicus, Aspergillus niger,* and *Rh. niveus. Rhizomucor miehei* lipase (Lipozyme IM 20), obtained already in the immobilized form and used as such, was donated by Novo Nordisk Industry (Copenhagen, Denmark). *Candida rugosa* lipase powder was also obtained from Sigma Chemical Co. (St. Louis, MO). Triglyceride standards were purchased from Sigma Chemical Co. All other chemicals were either of analytical or high-performance liquid chromatography (HPLC) grade.

Lipase immobilization. The immobilization of lipase on Celite was done by first dissolving 100 mg of lipase powder in 100 μL of cold deionized H_2O, followed by thorough mixing with 250 mg of Celite. The preparation was lyophilized for 4 h prior to the transesterification process. Each immobilization process was carried out separately for each replicate.

The effect of water content on transesterification and hydrolytic activities was studied by using immobilized Sigma *C. rugosa* preparations that were lyophilized for up to 8 h. Duplicate preparations were used for each time of lyophilization. Both activities were then assayed as described below.

Transesterification reaction. The reaction mixture (10 mL) was composed of 10% (wt/vol) palm olein in water-saturated hexane, contained in a modified screw-capped, long-neck, 50-mL conical flask. The reaction was started by adding and thoroughly mixing the Celite-bound lipase, which was prepared from 0.1 g of lipase powder and lyophilized for 4 h. Of the *R. miehei* lipase, 0.1 g was used per flask. The reaction mixture was then agitated in an orbital shaker at 200 rpm at 30°C. At various times during incubation, 1 mL of the reaction mixtures was withdrawn, and the TG composition and concentration were determined. Each transesterification reaction was done in duplicate.

Determination of transesterified products. The TG of palm olein (control and zero-time reaction mixtures) and enzyme-reacted samples were analyzed by nonaqueous reverse-phase HPLC in a Shimadzu liquid chromatograph LC-10AD and SLC-10A equipped with an auto-injector and a Shimadzu C-R4AX integrator (Shimadzu Corporation, Kyoto, Japan). A commercially packed RP-18 column (250 × 4 mm) with 5-μm particle size (E. Merck, Darmstadt, Germany) was used to separate the TG. TG were eluted from the column with an acetone/acetonitrile (63.5:36.5) mixture at a flow rate of 1 mL/min (15,16) and detected with a refractive-index detector (Gilson; Villiers-le-Bel, France). The injection volume for both standard and samples was 10 μL. The TG were identified based on the retention times of standards. For tripalmitin (PPP) and triolein (OOO), the concentrations were determined by reference to the peak areas of known standards.

Determination of hydrolytic activity. The ability of an immobilized lipase preparation after lyophilization to hydrolyze palm olein was determined by carrying out a set of experiments similar to those used for the transesterification process. At the end of 24 h incubation, 150 mL of diethylether/ethanol (1:1) mixture was added, and the amount of free fatty acids (FFA) present in the reaction mixture was determined by titration with 0.05 N NaOH to a phenolphthalein end point. The degree of hydrolysis is expressed as the percentage of FFA liberated and was corrected for the presence of the acid in controls. Duplicate runs were carried out for each lyophilization time.

Calculations. The total concentration of TG present in a reaction mixture was calculated by subtracting the concentrations of glycerides (eluted before 12 min) (17) from the total concentration of all glycerides recorded on the HPLC chromatogram. Percent remaining TG was then calculated based on the total concentration of TG after the reaction has occurred compared to the total concentration for an unreacted zero-time sample. Where applicable, the concentrations of PPP and OOO formed were calculated as actual concentration (% w/w oil) based on known concentrations of PPP and OOO standards; otherwise, their values are expressed as concentration in relation to the total concentration of TG.

The concentration of TG that increased in value, $[TGI]$, at reaction time, t, was calculated from the following formula:

$$[TGI_t] = \frac{\Sigma \text{peak area of TG that increased in concentration}}{\text{total peak area of TG in the reaction mixture}} \times 100 \quad [1]$$

The degree of transesterification is defined as the change in $[TGI_t]$ with respect to the value at the start of the reaction, $[TGI_0]$. The rate of transesterification (X) was calculated as shown below:

$$X\left(\text{h}^{-1}\right) = \frac{\text{initial velocity}(\%/\text{h})}{\text{enzyme activity }(\%)} \quad [2]$$

where initial velocity is $([TGI_t] - [TGI_0])/t$ at the linear range of reaction, and enzyme activity is the actual activity of the lyophilized immobilized enzyme used. The enzyme activity is expressed as the percentage of TG hydrolyzed (total TG minus remaining TG), determined here, rather than as the activity specified by the manufacturer. The activity was measured after 2 h incubation under the same reaction conditions described earlier for transesterification reaction because the rate of TG hydrolyzed was linear for all enzymes up to 6 h of reaction.

RESULTS AND DISCUSSION

The acyl groups of triglycerides can be repositioned (transesterified) by first removing them from the glycerol backbone *via* hydrolysis. Hydrolysis requires water, but it has been proven that too much water will discourage transesterification and instead promote hydrolysis. The effect of water on the processes was studied by lyophilizing the immobilized enzyme preparation for a known period of time and then determining both the percentage of TG remaining and the percentage of FFA formed during interesterification reactions. Studies with lipase from *C. rugosa* show that the best lyophilization time was 4 h (Fig. 1), where the percentage of triglyceride recovered was around 73.0%. This coincided with minimum hydrolytic activity where the FFA level in the reaction mixture was 8%. Little change in FFA concentration occurred upon further drying of the enzyme. If the immobilized preparation was used without drying, most of the TG was hydrolyzed, and the concentration of FFA liberated was 70%. Prolonging the time of drying caused the percentage of remaining triglycerides to decrease slightly, probably due to denaturation of the enzyme. Studies with other lipases, immobilized and dried similarly to the *C. rugosa* lipase, showed that the concentration of triglycerides that remained at the end of 24 h of reaction, compared to the concentration of triglycerides in the control, varied: *Pseudomonas* lipase, 80%; *Asp. niger* lipase, 75%; *C. rugosa* lipase (Amano), 71%; *Rh. niveus* lipase, 79%; *Rh. javanicus* lipase, 94%; and *M. javanicus* lipase, 81%. With *R. miehei* lipase, the value was 80%. The transesterification reaction was also always accompanied by diglyceride formation (peaks before 12 min on chromatograms; Fig. 2) because they are unavoidable intermediates in the reaction. The diglyceride concentrations differed slightly between lipases used, the highest being produced by *Pseudomonas* lipase.

Other workers have reported on the effect of drying on the degree of transesterification. Foglia *et al.* (10), who studied the transesterification of tallow and tallow–sunflower oil and of tallow–soybean oil mixtures with several types of lipases

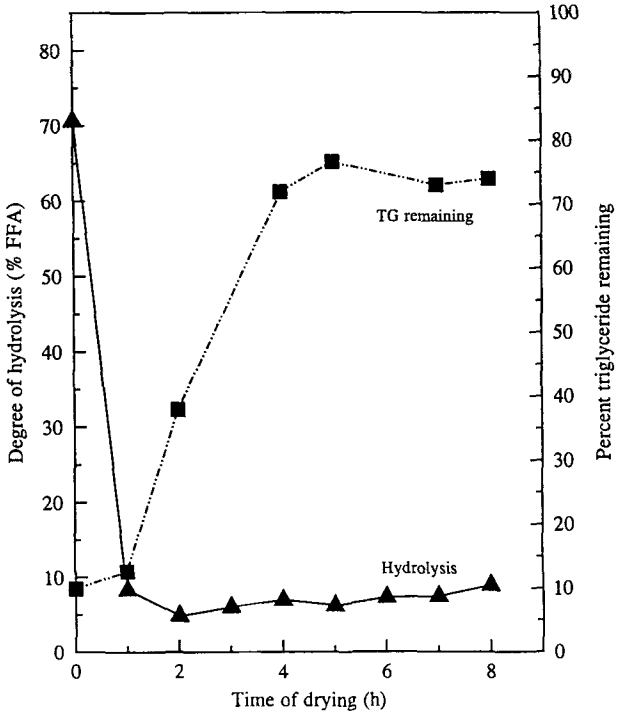

FIG. 1. Effect of lyophilization of immobilized *Candida rugosa* (Sigma, St. Louis, MO) lipase on the degree of hydrolysis and remaining triglyceride (TG) of palm olein. FFA, free fatty acid.

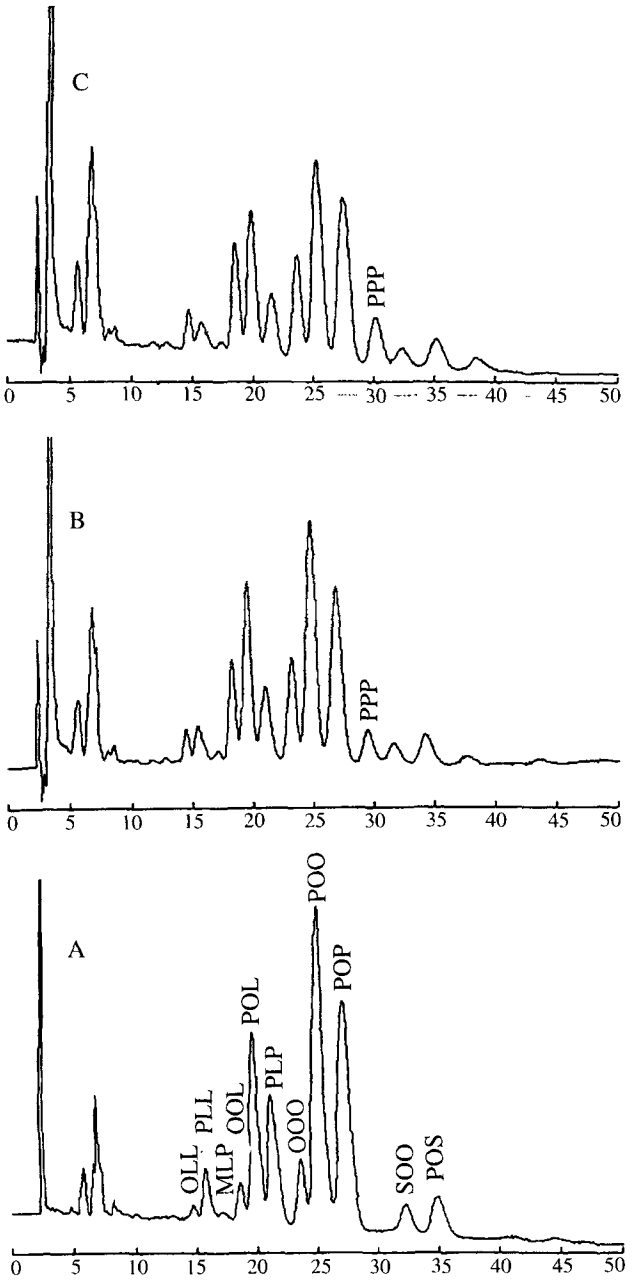

FIG. 2. Triglyceride profiles of palm olein at the beginning (A) and after 24 h reaction with *R. miehei* (B) and *Pseudomonas* (C) lipases. P, palmitic acid; O, oleic acid; L, linoleic acid; S, stearic acid.

containing 10% water, reported that between 4 and 13% hydrolysis took place in just 5 h of reaction at 60°C. The values initially obtained for *R. miehei* lipase (7–13%) decreased substantially when the water content of the enzyme was reduced by drying to less than 1%. Forssell *et al.* (11) also reported that there was a corresponding increase in the degree of hydrolysis when the moisture content in the reaction system they used to transesterify tallow with rapeseed oil was increased. Similarly, Yamane *et al.* (18) obtained maximum interesterification activity by lyophilizing the lipase powder used. Others either added molecular sieves to their reaction systems (12) or used them to dry the solvents they used (18,19) to reduce the incidence of hydrolysis. Therefore, in studies of this nature, it is important to reduce water content not only to make the transesterification process more efficient but also to ensure a high degree of remaining TG. The presence of high concentrations of FFA will lower the quality of oils; for example, smoke point of the oil is lowered and will incur costly product purification.

A simple way to judge whether transesterification has taken place would be to detect any real increase in the concentration of a TG present in the initial oil and/or the formation of new TG. In our studies, both of these occurred, and the results obtained are presented in Table 1 and Figures 2 and 3. Figure 2 shows the triglyceride profiles of palm olein before and after enzymatic transesterification with *R. miehei* and *Pseudomonas* lipase. These profiles are representative of those obtained with other lipases as well. Three TG experienced increases in concentrations, namely OLL (and its isomer), OOL (and its isomer), and OOO, where O and L are oleic and linoleic acids, respectively. Besides these increases, there was also the formation of PPP and SOS (where S is stearic acid), both of which were not initially detected in the oil sample used. PPP was confirmed from the retention time of PPP standard and spiking experiments (Fig. 4).

Figures 3A and B show the manner in which the sum concentrations of PPP, OOO, OLL, OOL, and SOS changed with respect to the total TG present in a reaction mixture when lipases with different specificities were used. The results provide further evidence that the specificity and the initial activity of the lipase preparation (Table 1) used may not play a big role in determining the overall changes that occur in a reaction such as the one studied here. The same trend with regard to PPP, OOO, OLL, and OOL was observed for all the enzymes examined. SOS was detected only when *Pseudomonas* and *R. miehei* lipases were used. Forssell *et al.* (11), who used *R. miehei* lipase in their studies, and Foglia *et al.* (10), who also used *R. miehei* lipase in addition to *Rh. delemar* lipase, have also reported the increase in concentrations of some TG and the formation of new TG in the oils they used.

Although the TG profiles of the reaction products were similar, the rate of transesterification catalyzed by the enzymes differed (Table 1). The bacterial lipase produced the fastest rate of reaction while intermediate rates were obtained for the lipases from *R. miehei* and *Asp. niger*. The values obtained showed that the rate catalyzed by *Pseudomonas* lipase was 2.7 and 3.7 times faster than the rate for *R. miehei* lipase and *Asp. niger* lipase, respectively. The reaction rates for the other lipases were between 5.6 and 13.0% that of *Pseudomonas* lipase, with the nonspecific *C. rugosa* lipase showing the poorest rate. Although from two different suppliers, the rates at which the latter catalyzed the transesterification of palm olein were similar. The differences in the observed rates can be ascribed to substrate preference, rather than to catalytic specificity. In a study on the transesterification of several vegetable oils by lipases from different sources, it was observed that bacterial lipases, including *Pseudomonas* lipase, showed a stronger preference, compared to fungal lipases, toward palm olein (and, to a lesser extent, palm kernel olein and coconut oil) than the other oils (unpublished results). The oils favored by the bacterial lipases generally contain a higher degree of saturated fatty acids.

Table 1 shows the actual concentrations of PPP and OOO formed at various times up to 48 h of reaction. PPP was not initially detected in the oil sample used, and the average initial concentration of OOO was 3.7% (w/w). As shown in the table, both the lipases from *R. miehei* and *Pseudomonas*, al-

TABLE 1
Rates of Transesterification and Concentrations of PPP and OOO with Time of Reaction by Lipases from Several Microbial Sources[a]

Source of lipase[b]	Specificity	Activity (% TG hydrolyzed)	PPP (% w/w)					OOO (% w/w)					Rate of trans. ($X h^{-1}$)
			2 h	4 h	6 h	24 h	48 h	2 h	4 h	6 h	24 h	48 h	
Candida rugosa (Sigma)	Random	8.4	0	0	0	1.6	3.0	4.0	3.3	3.1	3.5	4.2	3.3
C. rugosa (Amano)	Random	9.5	0	0	0	1.5	3.3	3.6	3.5	3.6	3.7	4.3	3.6
Pseudomonas sp. P	Random	8.0	4.8	5.4	6.0	6.3	6.2	6.3	7.0	6.3	5.8	6.0	59.4
Mucor javanicus M	1,3-Specificity	6.4	0	0	0	1.3	1.9	3.7	3.8	3.7	4.3	5.0	5.6
Rhizomucor miehei (Lipozyme IM 20)	1,3-Specificity	10.5	1.5	2.4	2.3	4.0	5.8	4.8	5.2	5.0	5.8	6.2	21.9
Aspergillus niger A	1,3-Specificity	11.8	0	1.4	1.8	1.9	3.3	4.6	4.8	4.8	5.2	5.3	16.3
Rhizopus javanicus F	1,3-Specificity	3.3	0	0	0	1.6	2.6	3.6	3.6	3.8	5.0	5.4	7.7
Rh. niveus N	1,3-Specificity	14.9	0	0	1.0	2.3	2.1	3.8	4.0	4.3	4.9	4.9	7.0

[a]P, palmitic acid; O, oleic acid; TG, triglyceride; trans., transesterification.
[b]Sigma (St. Louis, MO), Amano (Nagoya, Japan), Lipozyme IM 20 from Novo Nordisk (Bagsveard, Denmark).

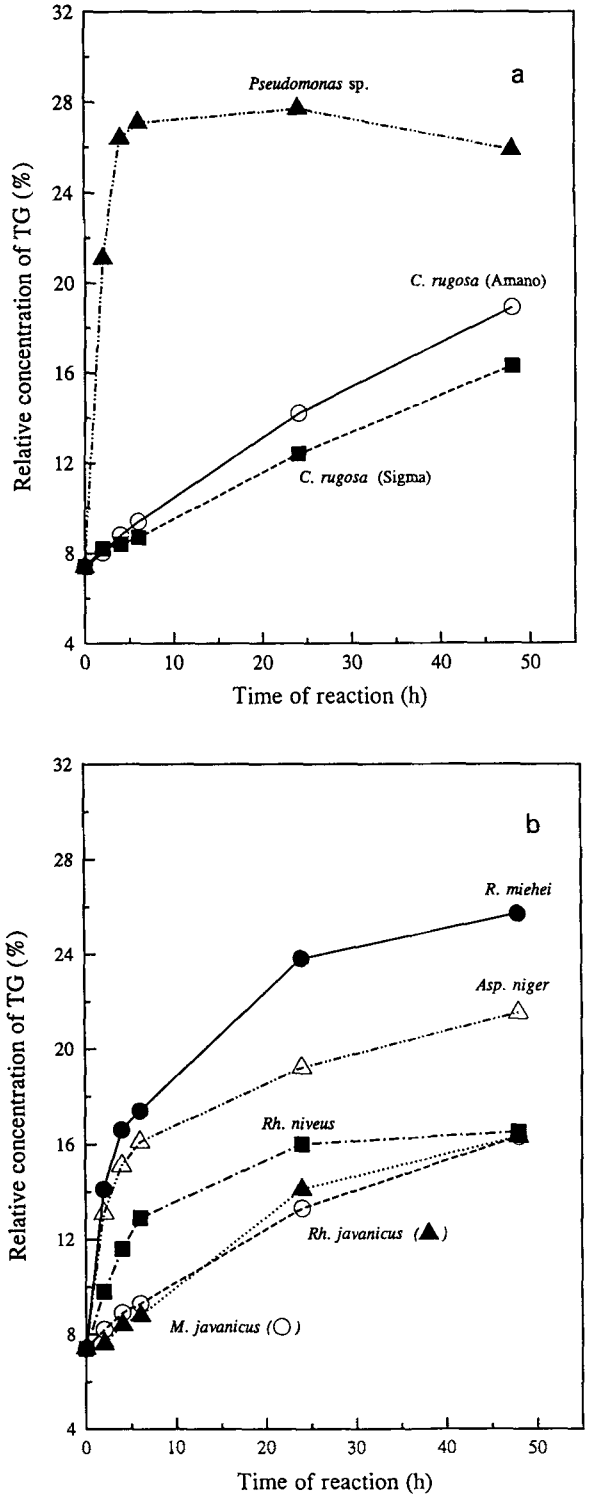

FIG. 3. Changes in the degree of transesterification based on the sum concentration of TG (PPP, OOO, OOL, OLL, and SOS) that experienced increases in content during transesterification with nonspecific lipases (A) and 1,3-specific lipases (B) with time. The initial combined concentration was 7.43%. Abbreviations as in Figures 1 and 2.

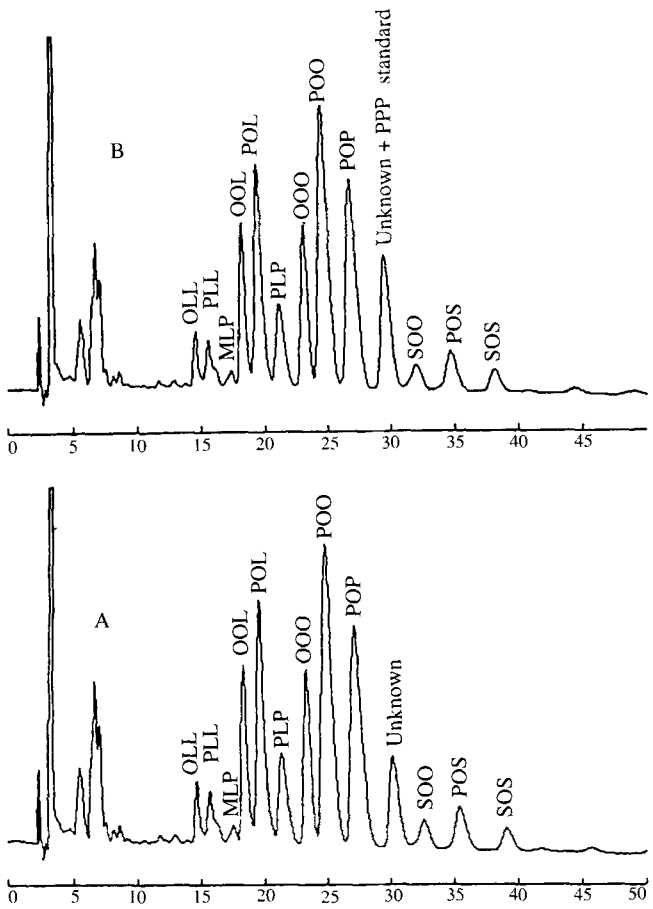

FIG. 4. Spiking experiment with PPP to determine the identity of the unknown peak on the high-performance liquid chromatography chromatograms of transesterified palm olein (60% in water-saturated hexane) with *Pseudomonas* lipase. Abbreviations as in Figures 1 and 2.

though different in specificity, caused the greatest and most rapid increase in PPP and OOO concentrations. The maximum concentration of PPP formed with the bacterial lipase was 6.3% (w/w). Some PPP was detected at 4 h of reaction with *Asp. niger* lipase. However, there was a much longer lag period before the increase in the concentration of OOO and the formation of PPP were detected when *C. rugosa, M. javanicus, Rh. javanicus,* and *Rh. niveus* lipases were used. In addition, some SOS were formed when *R. miehei* and *Pseudomonas* lipases were used, with detection being made earlier (2 h) for the latter compared to the former (24 h). The concentrations of SOS relative to the total concentration of TG after 24 h were 1.6 and 1.3% for *Pseudomonas* and *R. miehei* lipase, respectively.

There are several possibilities that contribute either singly or together to effect a rapid increase in PPP concentration. It can happen if *R. miehei* and *Pseudomonas* lipases are 2-specific and some dipalmitoyl-oleoyl-glycerol and dipalmitoyl-linoleoyl-glycerol present in palm olein are in the form of POP and PLP, respectively (Fig. 5, route 1). The enzymes would then hydrolyze the O or L in the 2-position and attach a P that forms and accumulates through hydrolysis, thus forming PPP. Because available data do not support the existence of 2-specific lipases, the next possibility would be route 2

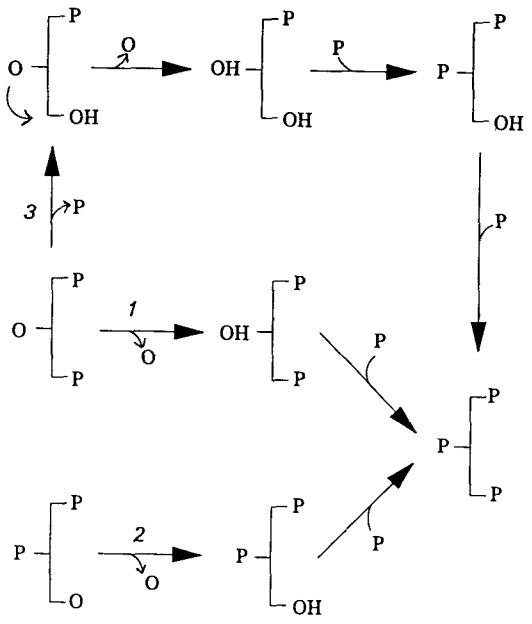

FIG. 5. Possible mechanisms for the formation of PPP during a lipase-catalyzed transesterification reaction. The same mechanisms can be used to explain the formation of OOO, OLL, OOL, and SOS from POO, SOO, and POL. Abbreviations as in Figure 2.

(Fig. 5). This pathway allows PPP to be formed rapidly if some of the dipalmitoyl-oleoyl-glycerol and dipalmitoyl-linoleoyl-glycerol present in palm olein are in the form of PPO and PPL, respectively. The enzyme removes the O or L from the outside position to form PP-OH, and then quickly adds a P to the diglyceride before 2-acyl migration of the middle P can take place. The third possibility (Fig. 5, route 3) is the stepwise removal of an acyl group from the outside position of POP or PLP followed by 2-acyl migration of P in the second position, its subsequent removal to form monoglycerol palmitate, and then the eventual addition of P to both the unoccupied hydroxyl groups of the monoglyceride. Random cleavage and reattachment of acyl groups of POP or PLP by the nonspecific lipases can also result in PPP formation but does not explain the difference in the rates of formation. Of course, it is also possible that the enzymes become specific toward the removal of certain fatty acids during the hydrolytic step and toward other fatty acids during the back reaction (acylation) in organic solvents. This is currently being examined by performing transesterification on oils other than palm olein and in different organic solvents. Regardless of the mechanism that operates, synthesis was always accompanied by a decrease in the concentrations of both dipalmitoyl-oleoyl-glycerol and dipalmitoyl-linoleoyl-glycerol (Table 1). A similar argument can be used for the observed increase in OOO, OOL, OLL, and SOS concentrations and the concomitant decrease in POO, SOO, POL, and PLL concentrations.

Similar inconsistency in results with respect to known and accepted specificities of lipases has been reported earlier by researchers such as Bloomer *et al.* (20) and Foglia *et al.* (10). The former partly attributed the dissimilarity in the effects of several 1,3-specific lipases on the formation of trisaturated TG during the production of cocoa butter equivalents from palm mid-fraction and ethyl stearate (or palmitic acid/methyl palmitate) to acyl migration of diglycerides. Foglia *et al.* (10), who studied the transesterification of tallow–sunflower (or soybean) oil mixtures, observed similar results but did not offer any explanation. Goh *et al.* (7) showed that the specificity of a 1,3-specific lipase can be altered by changing the polarity of the organic solvent used in the reaction medium. They found that *Humicola lanuginosa* and *R. miehei* lipases retained their 1,3-specificity in diethyl ether but not in hexane. This was ascribed to 2- to 1(3)-acyl migration in hexane, which did not occur in diethyl ether because greater quantities of 2-monoglycerides were detected in the latter.

The data in Table 2 describe the TG mixtures, resulting from the transesterification process, in terms of degree of unsaturation. The values shown are based on the total concentrations of TG only and obtained after correction to 100%. The values for PPP and OOO are relative concentrations instead of actual concentrations as used earlier. In general, the concentrations of fully saturated (PPP), triunsaturated (OOO and POL), and polyunsaturated (PLL, LOL, and LOO) TG increased, although to different degrees for the enzymes used. On the other hand, the concentrations of the monounsaturates (POP, POS, and SOS) and diunsaturates (POO, SOO, PLP, and MLP) decreased after the process. The combined concentration of the triunsaturated and polyunsaturated TG is also higher after transesterification compared to that before the reaction, indicating that perhaps the oil has become slightly softer with a lower melting point. A slight decrease in melting point was reported for tallow transesterified with either *R. miehei* lipase or *Rh. delemar* lipase (10). Similar results were also reported by Forssell *et al.* (11) for the interesterification of tallow and tallow–rapeseed oil mixtures with *R. miehei* lipase.

It is interesting that, of the enzymes used, *Pseudomonas* lipase actually caused an increase in the level of the higher-melting point triglycerides (sum of fully saturated and monounsaturated TG), albeit slightly, over the control value. It is perhaps this phenomenon that turned the oil in the reaction mixture to "solid" at the end of the reaction, although it could also be due to the presence of a slightly higher than usual concentration of diglycerides (Fig. 2). Studies are underway to obtain sufficient quantities of transesterified oils that are relatively free of other products of reaction, so that their physical characteristics can be determined. It would be of interest to obtain a harder product from palm olein because one might then obtain a sufficiently hard material that is free from *trans* fatty acids that are formed during hardening (hydrogenation).

Research abounds on the application of 1,3-specific lipases for the modification of fats and oils, especially on the production of cocoa butter equivalents. However, the results reported to date and from our studies indicated that positional specificity of the enzymes is probably a secondary factor in determining the properties of the end product of reaction in com-

TABLE 2
Triglyceride (TG) Composition and Concentration of Palm Olein Before (control) and After Transesterification for 24 h with Several Microbial Lipases (values shown have been corrected to 100% and are relative to the total concentration of TG in the reaction mixture)[a]

Source of lipase triglyceride (%)	Control	*Rhizomucor miehei*	*Pseudomonas* sp.	*Rhizopus niveus*	*Rh. javanicus*	*Aspergillus niger*	*Mucor javanicus*	*Candida rugosa*[b]	*C. rugosa*[c]
PPP	0	3.5	5.9	1.9	1.3	2.2	1.1	1.5	1.6
POP	25.1	20.2	20.3	23.0	23.3	21.4	23.1	23.7	22.7
POS	4.5	3.6	3.9	4.4	4.2	3.8	4.4	4.4	4.2
SOS	0.0	1.3	1.6	0.0	0.0	0.0	0.0	0.0	0.0
POO	30.8	25.2	22.8	26.4	27.6	26.6	28.1	29.9	29.2
SOO	3.1	2.2	2.1	2.3	2.7	2.2	3.1	2.9	2.2
PLP	11.2	7.5	6.3	9.0	9.6	7.8	9.2	9.0	8.7
MLP	0.8	0.7	0.6	0.9	0.5	0.5	0.7	0.7	0.7
POL	14.1	15.0	13.3	15.1	14.8	15.6	14.9	14.2	14.7
OOO	4.6	8.9	9.5	7.1	6.8	8.5	6.6	5.9	6.5
PLL	3.5	3.0	2.8	2.8	3.1	3.0	3.0	2.9	3.2
LOL	0.6	1.8	2.6	1.5	1.3	1.7	1.2	1.0	1.4
LOO	2.3	7.2	8.6	5.6	4.9	6.7	4.4	4.0	4.8
Total	100	100	100	100	100	100	100	100	100
Fully saturated (PPP)	0	3.5	5.9	1.9	1.3	2.2	1.1	1.5	1.6
Monounsaturated (POP, POS, SOS)	29.6	25.1	25.8	27.4	27.5	25.2	27.5	28.1	26.9
Diunsaturated (POO, SOO, PLP, MLP)	45.9	35.6	31.8	38.6	40.4	37.1	41.1	42.5	40.8
Triunsaturated (POL, OOO)	18.7	23.9	22.8	22.2	21.6	24.1	21.5	20.1	21.2
Polyunsaturated (PLL, LOL, LOO)	6.4	12.0	14.0	9.9	9.3	11.4	8.6	7.9	9.4

[a]Abbreviations as in Table 1. L, linoleic acid. [b]From Sigma Chemical Co. (St. Louis, MO). [c]From Amano Pharmaceuticals (Nagoya, Japan).

parison to acyl migration due to long reaction times (as is usually the case) and the hydrophobicity (21) of the organic solvent used as the reaction medium. The hydrophobicity of the reaction medium may determine the eventual products formed because the solubilities of the different fatty acids, partial glycerides, and TG would differ even in the same solvent and would thus affect the hydrolytic/esterification process. Also, it is felt that there is a need to take a closer look at the products of enzymatic transesterification because changes observed in the melting points and solid fat content of these products can be the result of either hydrolysis alone, synthesis, or both, as even a slight difference in the concentration of a TG in the oil can affect the overall physical properties of the oil significantly.

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REFERENCES

1. Macrae, A.R., *J. Am. Oil Chem. Soc. 60*:243A (1983).
2. Jensen, R.G., F.A. DeJong and R.M. Clark, *Lipids 18*:239 (1983).
3. Malcata, F.X., H.R. Reyes, H.S. Garcia, C.G. Hill and C.H. Amundson, *J. Am. Oil Chem. Soc. 67*:891 (1990).
4. Chaplin, M.F., and C. Bucke, *Enzyme Technology*, Cambridge University Press, 1990.
5. Osterberg, E., A.-C. Blomstrom and K. Holmberg, *J. Am. Oil Chem. Soc. 66*:1330 (1989).
6. Forssell, P., P. Parovuori, P. Linko and K. Poutanen, *Ibid. 70*:1105 (1993).
7. Goh, S.H., S.K. Yeong and C.W. Wang, *Ibid. 70*:567 (1993).
8. Murakami, M., Y. Kawasaki, M. Kawanari and H. Okai, *Ibid. 70*:571 (1993).
9. Young, F.V.K., *Ibid. 62*:372 (1985).
10. Foglia, T.A., K. Petruso and S.H. Feairheller, *Ibid. 70*:281 (1993).
11. Forssell, P., P. Kervinen, M. Lappi, P. Linko, T. Suortii and K. Poutanen, *Ibid. 69*:126 (1992).
12. Kurashige, J., N. Matsuzaki and H. Takahashi, *Ibid. 70*:849 (1993).
13. Marangoni, A.G., R.D. McCurdy and E.D. Brown, *Ibid. 70*:737 (1993).
14. Cocks, L.V., and C. van Rede, *Laboratory Handbook for Oil and Fat Analysts*, Academic Press, London, 1966.
15. Dong, M.W., and J.L. Dicesare, *J. Am. Oil Chem. Soc. 60*:788 (1983).
16. Swe, P.Z., Y.B. Che Man and H.M. Ghazali, *Ibid. 71*:1141 (1994).
17. Swe, P.Z., Y.B. Che Man and H.M. Ghazali, *Ibid. 72*:343 (1995).
18. Yamane, T., T. Ichiryu, M. Nagata, A. Ueno and S. Shimizu, *Biotechnol. Bioeng. 36*:1063 (1990).
19. Kirchener, G., M.P. Scollar and A.M. Klibanov, *J. Am. Chem. Soc. 107*:7072 (1985).
20. Bloomer, S., P. Aldercreutz and B. Mattiasson, *Ibid. 67*:519 (1990).
21. Laane, C., and J. Tramper, *CHEMTECH. 20*:502 (1990).

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