Optimization of the Chemoenzymatic Epoxidation of Soybean Oil

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ABSTRACT: The lipase *Candida antarctica* (Novozyme 435) immobilized on acrylic resin was used as an unconventional catalyst for *in situ* epoxidation of soybean oil. The reactions were carried out in toluene. The peracid used for converting TG double bonds to oxirane groups was formed by reaction of FFA and hydrogen peroxide. The reaction conditions were optimized by varying the lipase concentration, solvent concentration, molar ratio of hydrogen peroxide to double bond, oleic acid concentration, and reaction temperature. The kinetic study showed that 100% conversion of double bonds to epoxides can be obtained after 4 h. The addition of free acids was not required for the reaction to proceed to conversions exceeding 80%, presumably owing to generation of FFA by hydrolysis of soybean oil. The enzyme catalyst was found to deteriorate after repeated runs.

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KEY WORDS: Enzyme, epoxidation, lipase, oleic acid, soybean oil.

Epoxidized oils are considered as promising intermediates for a broad range of applications such as polyols for polyurethane foams, coatings (1,2), casting resins (3), adhesives, inks, lubricants, and the like (4).

Standard industrial production is based on *in-situ* epoxidation, in which peracid is generated by reacting acetic or formic acid with hydrogen peroxide in the presence of strong mineral acids such as H_2SO_4 and H_3PO_4 (5,6). However, using strong mineral acids as catalysts has several disadvantages. Strong acids are nonselective, they cause equipment corrosion, and they must be neutralized and removed from the end product. Another drawback is that strong acids can initiate undesirable oxirane ring-opening reactions with water, leading to formation of secondary hydroxyl groups on the FA backbone or oligomerization through the ether linkage (7). Typical conversions of double bonds to epoxy groups are about 90% owing to the partial consumption of epoxy groups in side reactions.

Many other types of catalysts such as methyltrioxorhenium (8,9), ammonium molybdate, ion exchange resins (10), Venturello's catalyst (11), phase transfer catalysts such as quaternary ammonium tetrakis(diperoxotungsto) phosphates, and crown ethers have been studied for the purposes of improving the selectivity and increasing the conversion and yield of epoxidation.

Lipase-catalyzed chemoenzymatic oxidations have been developed relatively recently. They appear to provide a new and very promising technique for epoxidation of double bonds (12,13). These techniques have several important advantages over ones using inorganic catalysts, including the following: (i) mild reaction conditions, i.e., neutral pH of the reaction mixture, (ii) formation of stable hydroperoxides directly from FA, i.e., no need for acetic or formic acid addition, (iii) high regio- and stereoselectivity, (iv) significant suppression of side reactions, and (v) high conversion.

The research in this field has been focused mainly on the study of enzymatically catalyzed epoxidations of unsaturated FFA rather than TG. Since enzymes require a neutral environment, it has been discovered that FFA, as weak acids, do not affect the pH of the reaction mixture and can be efficiently converted to peroxoacids in the presence of lipases. The unsaturated peroxo FA themselves are being epoxidized, becoming part of the product. The highest lipase catalytic activities are observed when the epoxidations are carried out in nonpolar organic solvents such as toluene and hexane (14). The enzymatic epoxidations of PUFA are not accompanied by any side reactions and therefore are suitable for model studies. However, in the case of epoxidation of vegetable oils, lipases also may catalyze partial hydrolysis of the TG molecules simultaneously with peracid formation.

The objective of this work was to optimize reaction parameters for the enzymatic synthesis of epoxidized soybean oil catalyzed with *Candida antarctica* lipase immobilized on acrylic resin. Soybean oil containing from 75 to 93% unsaturated FA is one of the most common vegetable oils used for chemical modifications. The worldwide market for soybean oil is stable and estimated to be approximately 224 million metric tons in 2004 (15).

A major part of our work was focused on the effect of reaction conditions on kinetics of soybean oil epoxidation. Our goal was to shorten the reaction time, generally recognized as one of the limiting factors for the application of enzyme-catalyzed synthesis. Other targets were to maximize the conversion, increase the end-product yield, and decrease the production costs by decreasing the amount of reaction components added to the oil. Additionally we investigated the influence of reaction conditions on the extent of by-product formation and the purity of the final product.

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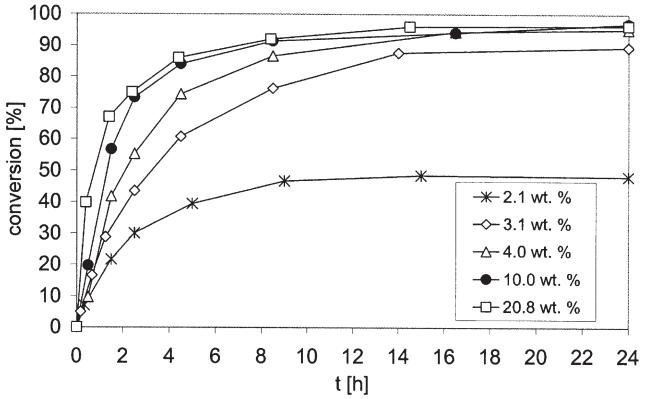

FIG. 1. Effect of catalyst concentration (relative to oil) and time on conversion of double bonds to epoxy groups. Conditions: H_2O_2/double bonds molar ratio = 2:1, oleic acid concentration = 8.0 wt% (related to oil), toluene concentration = 440 wt% (related to oil), reaction temperature = 50°C.

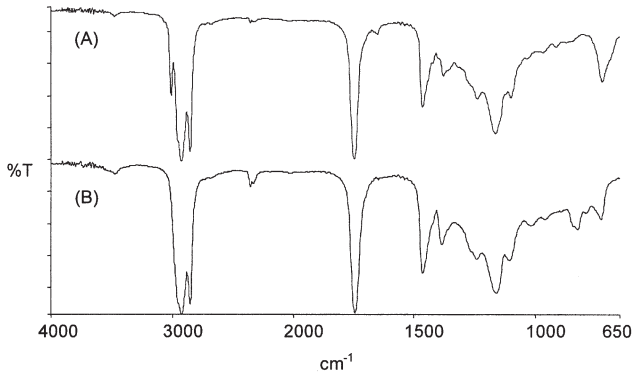

FIG. 2. FTIR spectra of soybean oil before epoxidation (A) and after 24 h of epoxidation (B).

EXPERIMENTAL PROCEDURES

Materials. Novozyme 435 Lipase B from *C. antarctica* physically adsorbed on macroporous polymethacrylate resin beads (Lewatit) was purchased from Sigma-Aldrich (Milwaukee, WI). Hydrogen peroxide was purchased from Acros Organics (Fairlawn, NJ) as 35 and 60% w/w solution. Soybean oil having an iodine value (IV) of 131 g I_2/100 g was supplied by ADM (Decatur, IL). Oleic acid (IV 85.3 g I_2/100 g), acetic acid (glacial), potassium phosphate monobasic–sodium hydroxide buffer (for pH 7.00 at 25°C), toluene (HPLC grade), and sodium sulfate anhydrous were purchased from Fisher Scientific (Pittsburgh, PA).

Synthesis procedure. The epoxidations were carried out in a 250-mL three-necked round-bottomed reaction flask equipped with an electric heating mantle and a digital temperature controller. In the first step, 25.0 g of soybean oil, 2.0 g (8.0 wt%) of oleic acid, 5.2 g of catalyst, and 110 g of toluene as nonpolar solvent were added to the reactor in the order as listed. Then the reaction mixture was heated to 50°C and homogenized at 350 rpm with a polytetrafluoroethylene blade stirrer on a glass shaft connected to an IKA mixer (IKA, Wilmington, NC). In the second step, 25.1 g of 35% hydrogen peroxide was added dropwise in 5 min to the reaction mixture through a funnel. The reaction of hydrogen peroxide with oleic acid as catalyzed by lipase gives oleic peracid, which oxidizes double bonds in soybean oil and oleic acid itself to epoxy groups. All syntheses were carried out for 24 h. Conversion of double bonds to epoxy groups was followed by FTIR and titration. After finishing epoxidation, catalyst beads were removed from the reaction mixture by filtration using a Büchner funnel, filter paper (No. 1), and a heavy-wall filtering flask connected to a water vacuum pump. Enzyme catalyst beads were rinsed several times with toluene, then air-dried and stored in a glass bottle for reuse. The filtered reaction mixture was washed four times with a large excess of distilled water. The separated organic layer was mixed for about 1 h with anhydrous sodium sulfate (10 wt%) to adsorb remaining moisture and decompose traces of unreacted peroxide. Solids were removed by the filtration procedure described above. An oil rotary evaporator at vacuum below 1 mmHg was used to distill off solvent and remaining traces of water as the final step of the product purification. The high-vacuum distillation was carried out at 90°C for 2 h.

Instrumental methods. Determination of M.W. distribution was carried out by size exclusion chromatography (SEC) consisting of a 510 Waters pump (Waters, Milford, MA), a set of four Phenogel columns, 300 × 7.8 mm (Phenomenex, Torrance, CA) covering a M.W. range from 10^2 to 5×10^5, and a 410 differential refractometer (Waters). ASTRA software (Wyatt Technology Corp., Santa Barbara, CA) was used for data collection and processing. THF at a flow rate of 1 mL/min was used as a mobile phase. The samples were injected as 0.3% (wt/vol) solutions in the mobile phase using a manual 20 mL injector (Rheodyne, Rohnert Park, CA). The measurements were carried out at room temperature.

TABLE 1
Properties[a] of Epoxidized Soybean Oils Prepared with Different Catalyst Concentrations

Sample	Catalyst (wt% of oil)	Conversion (%)	EOC (wt%)	IV (g I_2/100 g)	AV (mg KOH/g)	OH # (mg KOH/g)
Exp. 1	2.1	48.14	3.43	65.34	15.54	1.46
Exp. 2	3.1	89.40	6.37	11.59	15.23	1.41
Exp. 3	4.0	95.16	6.78	1.65	16.15	0.64
Exp. 4	10.0	98.98	6.91	0.66	15.77	2.08
Exp. 5	20.8	96.28	6.86	0.00	26.70	6.76

[a]EOC, epoxy oxygen content; IV, iodine value; AV, acid value; OH #, hydroxyl number.

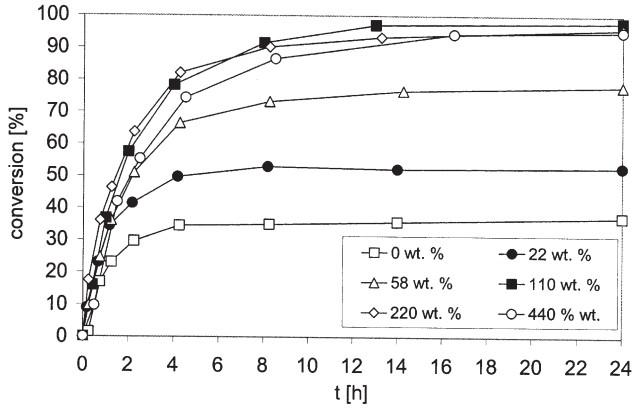

FIG. 3. Effect of time and solvent concentration on conversion of double bonds to epoxy groups. Conditions: H_2O_2/double bonds molar ratio = 2:1, oleic acid concentration = 8.0 wt% (related to oil), catalyst concentration = 4.0 wt% (related to oil), reaction temperature = 50°C.

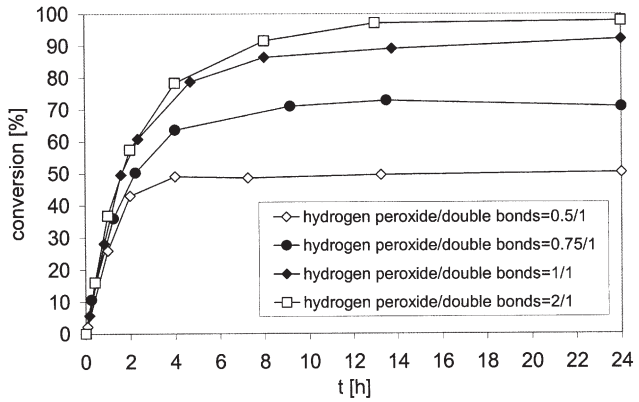

FIG. 4. Effect of time and hydrogen peroxide/double bond molar ratio on conversion of double bonds to epoxy groups (0.5, 0.75, 1, and 2:1 of 35% H_2O_2 relative to oil). Conditions: oleic acid concentration = 8.0 wt% (related to oil), catalyst concentration = 4.0 wt% (related to oil), toluene concentration = 110 wt%, reaction temperature = 50°C.

The disappearance of double bonds (3009 cm^{-1}) and the formation of epoxy groups (doublet at 822 and 833 cm^{-1}) were monitored during the synthesis by an FTIR spectrometer (model Spectrum 1000; PerkinElmer). Samples were spread as a thin film on KBr plates.

Epoxy oxygen group content (EOC) determination was carried out according to the standard procedure for oils and fats (16). IV was determined by the Hanus method (17). The hydroxyl group content (OH #) was determined according to ASTM titration method E 1899-97 using the reaction with *p*-toluenesulfonyl isocyanate and potentiometric titration with tetrabutylammonium hydroxide. Acid value (AV) was determined according to the AOCS Official Method Cd 3d-63 (18).

RESULTS AND DISCUSSION

The process of epoxidation of soybean oil, catalyzed by Novozyme 435, was studied in terms of varying the synthesis parameters (catalyst concentration, solvent, peroxide and acid content, and reaction temperature).

The first step was focused on the determination of the minimum amount of lipase catalyst necessary for reaching the highest possible conversion of double bonds to epoxy groups. The starting recipe used in this set of experiments was based on the preliminary tests, when determination of a zero IV confirmed total consumption of double bonds. The reaction mixture consisted of 25.0 g of soybean and 2.0 g of oleic acid dissolved in 110 g of toluene. The catalyst concentration used in the first experiment was 20.8 wt% of oil. To the reaction mixture was slowly added 25.1 g of a 35% water solution of hydrogen peroxide (molar ratio H_2O_2/double bonds = 2:1). The number of double bonds per molecule of soybean oil was 4.51. Conversions were calculated both from the IV and the epoxy oxygen content. For kinetic studies, i.e., the conversion–time plots, we used values of conversions obtained from the epoxy oxygen content titration method. These values are more realistic than conversions obtained from the amount of residual unreacted double bonds since no side reactions of epoxy groups were observed.

Figure 1 shows that the rate of formation of epoxide groups is strongly affected by the catalyst concentration if the amount of lipase used is lower than 4.0 wt% of oil. This concentration was taken as critical for reaching conversions exceeding 95%. The efficiency of epoxidation rapidly decreases below this critical point. In the region of higher catalyst concentrations, maximal conversions from 95 to 99% can be reached. Comparison of kinetic curves in Figure 1 clearly shows that the reaction rates are high in experiments with catalyst concentrations over 4.0 wt%, when conversion of 50% is obtained in less than 2 h.

TABLE 2
Properties of Epoxidized Soybean Oils Prepared at Different Solvent (toluene) Concentrations[a]

Sample	Solvent (wt% of oil)	Conversion (%)	EOC (wt%)	IV (g I_2/100 g)	AV (mg KOH/g)	OH # (mg KOH/g)
Exp. 6	0	37.20	2.65	NA	NA	NA
Exp. 7	22	52.77	3.76	60.41	16.85	4.70
Exp. 8	58	78.18	5.57	29.82	15.87	6.81
Exp. 9	110	97.96	6.98	0.52	16.09	4.14
Exp. 10	220	96.00	6.84	1.61	16.61	2.85
Exp. 3	440	95.16	6.78	1.65	16.15	0.64

[a]NA, not analyzed; for other abbreviations see Table 1.

TABLE 3
Properties[a] of Epoxidized Soybean Oils Prepared at Different Concentrations of Hydrogen Peroxide

Sample	H_2O_2 (mol%)	H_2O_2/double bonds	Conversion (%)	EOC (wt%)	IV (g I_2/100 g)	AV (mg KOH/g)	OH # (mg KOH/g)
Exp. 9	200	2:1	97.96	6.98	0.52	16.1	4.14
Exp. 11	100	1:1	92.21	6.57	8.13	23.8	5.81
Exp. 12	75	0.75:1	71.02	5.06	36.58	38.3	6.81
Exp. 13	50	0.5:1	50.39	3.59	64.33	40.0	11.40

[a]For abbreviations see Table 1.

A Novozyme 435 content of 4.0 wt% was used for further experiments. The transformation of double bonds to epoxy groups was monitored also by FTIR analysis. Comparison of FTIR spectra of soybean oil at the beginning and after 24 h of epoxidation (see Fig. 2) clearly shows the formation of epoxy groups in the latter, as reflected by the doublet at 822 and 833 cm^{-1}. The consumption of double bonds is indicated by the decrease of the peak at 3009 cm^{-1}.

Increasing the amount of lipase catalyst caused partial hydrolysis, which is reflected in the increase of OH # and AV (see Table 1). This side reaction has a reasonable explanation. Novozyme 435 is a highly enantioselective and versatile biocatalyst, designed to catalyze esterification among other reactions. In the presence of Novozyme 435, water from the hydrogen peroxide solution can easily attack relatively weak ester bonds of vegetable oils, resulting in a mixture of FFA, DG, and TG. The hydrolysis effect will also be discussed later in this work. We also studied the possibility of a ring-opening reaction of epoxidized oil in the presence of different hydroxy functional components (water, methanol, etc.) and Novozyme 435 catalyst. Ring-opening did not take place in any case since no decrease of epoxy groups or appearance of hydroxyl groups could be registered.

In the study of the effect of solvent on epoxidation, we found that synthesis in bulk is possible, but that the catalytic activity in these conditions is very low. The maximal conversion was below 40%. Figure 3 shows that in this case of bulk reaction, the conversion increases only during the first 4 h. After that it stays constant. This can be explained by deactivation of Novozyme 435. The kinetic studies (see Fig. 3) revealed that addition of toluene to the reaction mixture significantly improves and extends the lipase catalytic activity. The conversion increases linearly with increasing dilution of the reaction mixture up to its maximum of 98%, when the solution is saturated. Further additions of toluene do not affect the conversion and contribute mainly to undesirable lowering of the yield. However, high dilutions of epoxidation mixture can partially suppress hydrolysis, observed as decreasing hydroxyl values from 4.14 to 0.64 mg KOH/g (compare Experiments 3 and 9 in Table 2). Use of nonpolar solvents in enzymatic epoxidations is important because the solubility of hydrogen peroxide in such solvents is higher than that of water, which results in reduced formation of carboxylic acids (19). Addition of 110 wt% of toluene to the reaction mixture was found to be optimal and was used as a constant parameter in further experiments.

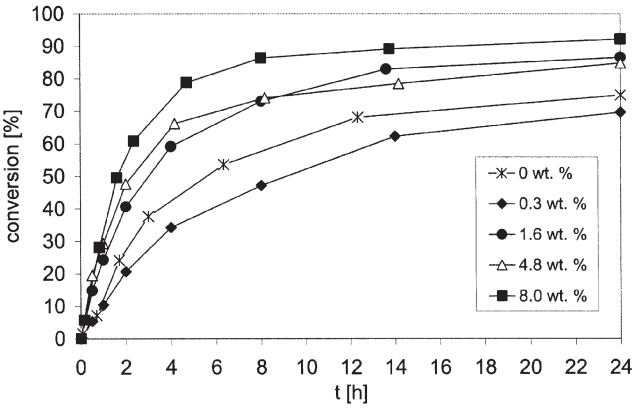

FIG. 5. Influence of reaction time and oleic acid concentration (0.0, 0.3, 1.6, 4.8, and 8.0 wt% relative to oil) on conversion of double bonds to epoxy groups. Conditions: H_2O_2/double bonds molar ratio = 1:1, catalyst concentration = 4.0 wt% (related to oil), toluene concentration = 110 wt%, reaction temperature = 50°C.

The optimization of the hydrogen peroxide content is important mainly for lowering the cost by reducing the amount of unreacted peroxide at the end of the reaction. The best results were obtained at a 2:1 molar ratio of hydrogen peroxide/double bonds, when the conversion reached 98% (see Fig. 4). Lower ratios lead to a relatively slow decline in conversion to reach 92% at an equimolar ratio. A further reduction in the amount of peroxide added to the reaction mixture causes again a linear, but more rapid, decline of conversion. Low concentrations of peroxide can lead to significant hydrolysis, parallel with peracid formation. Table 3 shows that side reactions are taking place as observed from increasing OH # and AV. We also tried to use a 65% water solution of hydrogen peroxide, but the experiments were not successful. Peroxide in high concentrations seems to deactivate Novozyme 435. The equimolar ratio of hydrogen peroxide and double bonds was chosen as the most nearly optimal, giving acceptable conversions over 90% with a minimum of unreacted H_2O_2.

In the next step, oleic acid was used in concentrations ranging from 0 to 25.0 mol% of TG (or 0–4.8 wt% oil) to study of the influence of FFA concentration on conversion. The best results, 95% conversion after 24 h of synthesis, were obtained with the highest content of oleic acid (see Fig. 5). Higher amounts of oleic acid were not used, because no significant contributions to the further increase of conversion were expected. Also, a high content of FA remaining in the final product is undesirable. FA can be removed from the reaction mixture by alkali treatment, for example, but this procedure re-

TABLE 4
Properties[a] of Epoxidized Soybean Oils Prepared at Different Concentrations of Oleic Acid

Sample	Oleic acid [wt(mol)%]	Conversion (%)	EOC (wt%)	IV (g I_2/100 g)	AV (mg KOH/g)	OH # (mg KOH/g)
Exp. 11	8.0 (25)	94.74	6.57	8.13	23.8	5.81
Exp. 14	4.8 (15)	84.78	6.19	12.47	21.2	7.94
Exp. 15	1.6 (5)	86.56	6.50	13.66	15.4	4.20
Exp. 16	0.3 (1)	69.69	5.30	NA	4.7	0.86
Exp. 17	0 (0)	80.99	6.18	20.00	7.1	3.23

[a]For abbreviations see Tables 1 and 2.

quires an extra production step, which makes the epoxidation less attractive. The most important finding was that the epoxidation takes place up to about 80% conversion even when the synthesis is running without any acid added to the reaction mixture. Figure 5 shows that experiments carried out with smaller amounts of FA had lower reaction rates. The concentration of epoxy groups generated after 24 h of synthesis was lower. The epoxidation of soybean oil having a zero content of oleic acid was explained by the presence of some FFA formed by hydrolysis. Rüsch gen. Klaas and Warwel (19) showed that hydrolysis can be reduced by the addition to the reaction mixture before the synthesis of approximately five molar percent of FFA. They indicated that a small amount is sufficient to suppress undesirable formation of difficult-to-remove DG and MG. This is, however, contrary to our findings. SEC analysis detected 12.1 wt% of residual FA in the sample prepared with 25 mol% of oleic acid. After deduction of the 8.0 wt% of oleic acid added at the beginning of the synthesis, we obtained 4.1 wt% of FFA (137 mmol) formed by hydrolysis during the epoxidation. The epoxidized oil prepared without added oleic acid contained approximately 4.6 wt% of FFA (153 mmol). This result shows that the hydrolysis is taking place to the same extent, independently of the amount of oleic acid added to the reaction mixture at the beginning of the synthesis. The SEC analysis did not confirm the presence of MG as claimed by Rüsch gen. Klaas and Warwel. The extent of hydrolysis measured by OH # values, summarized in Table 4, is not clear. The experiment carried out with 25 mol% oleic acid gave the highest conversion of about 95% at a constant extent of hydrolysis.

We tried to run the epoxidation using glacial acetic acid without and with monobasic potassium phosphate–sodium hydroxide buffer to adjust the reaction mixture to a neutral pH. Figure 6 clearly shows the strong influence of high acidity of the epoxidation mixture on the Novozyme 435 catalytic activity. Surprisingly, we found that the epoxidation can be carried out in the presence of glacial acetic acid for 1 h up to the 12% conversion. The enzyme is deactivated after this reaction time. Adjustment of the reaction mixture pH to 6.0 only partially improves the catalyst lifetime.

In the last part of our experimental work, we investigated the effect of temperature, stirring speed, and type of stirrer on conversion. Comparison of slopes of linear parts of the kinetic curves at the beginning of the epoxidations in Figure 7 revealed 4.2 times faster epoxy group formation rates at 50°C than at 25°C, i.e., R_{50} = 20.77 mmol/min and R_{25} = 4.94 mmol/min. The catalytic efficiency of Novozyme 435 is significantly influenced by the stirring rate. Slow stirring at 250 rpm was insufficient to homogenize the reaction mixture properly, and the conversions are lower than at 400 rpm. This effect is evident in the case of reactions carried out at room temperature. However, high speed of rotation and high temperature lead to partial de-

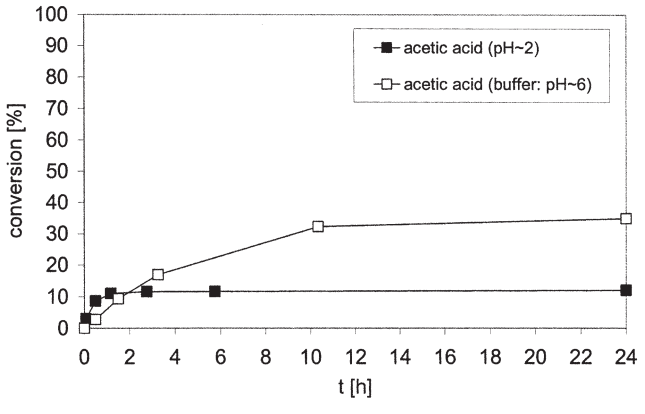

FIG. 6. Effect of reaction time on conversion with acetic acid buffered acetic acid. Conditions: H_2O_2/double bonds molar ratio = 1:1, acetic acid concentration = 1.7 wt% (related to oil), catalyst concentration = 4.0 wt% (related to oil), toluene concentration = 110 wt%, reaction temperature = 50°C.

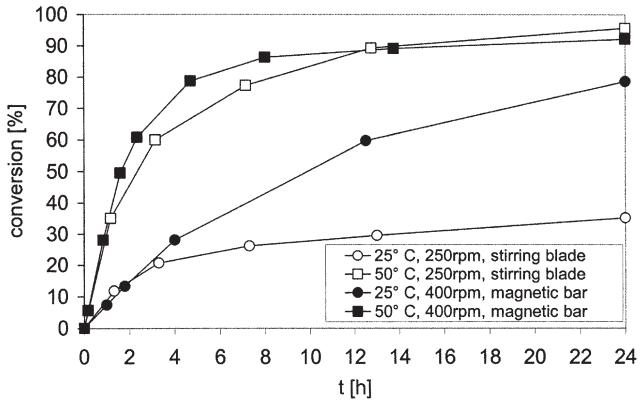

FIG. 7. Effect of reaction temperature and stirring conditions on conversion of double bonds to epoxy groups. Effect of reaction time on conversion with acetic acid buffered acetic acid. Conditions: H_2O_2/double bonds molar ratio = 1:1, oleic acid concentration = 8.0 wt% (related to oil), catalyst concentration = 4.0 wt% (related to oil), toluene concentration = 110 wt%.

struction of Novozyme 435 beads. Magnetic bars used for stirring were found to grind acrylate resin substrate to a powder, causing the decrease in catalytic activity of lipase. After finishing the syntheses at 25 and 50°C, Novozyme 435 was purified by washing in toluene, air-dried, and used in repeated epoxidations. The catalytic activity of the regenerated Novozyme 435 was strongly limited by the reaction temperature and the number of repeated runs. The regenerated enzyme dramatically loses its catalytic activity if the reaction temperature exceeds 25°C. The epoxidations carried out at room temperature affected to a tolerable degree the regenerated enzyme catalytic activity only in first four experiments. The conversion was fairly constant in this case at 73 and 72%. However, each subsequent run was accompanied by a significant decrease of the catalytic efficiency of the regenerated enzyme.

Thus, vegetable oil TG can be easily epoxidized under very mild conditions by using the enzymatic catalyst Novozyme 435. The study of optimization of the reaction conditions showed that the conversion of double bonds to epoxy groups can exceed 90% when the reaction is run at 50°C in the presence of at least 4.0 wt% catalyst, at molar ratios of hydrogen peroxide/double bonds greater than 1, in 110 wt% toluene, and with up to 25 mol% FFA. The main advantage of the enzymatic catalyst is high selectivity and the elimination of ring-opening reactions. Under certain conditions lipase catalyst may cause partial hydrolysis, leading to cleavage of up to 5.0 wt% of FFA. The epoxidation can be carried out to a conversion of over 80% with no added FFA.

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