Lipase-Catalyzed Synthesis of Ferulate Esters

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ABSTRACT: Four commercially available esterases were screened for their ability to esterify ferulic acid (4-hydroxy-3-methoxy cinnamate). Novozym® 435 was found to be the only one of those screened to convert ferulic acid to ethyl and octyl ferulate at 20 and 14% yields, respectively. The highest percentage conversion was obtained using a 1:1 mole ratio of alcohol to ferulic acid in stirred batch reactions in anhydrous 2-methyl-2-propanol at 60°C using one equivalent (wt/wt based on ferulic acid) of Novozym 435. Increased water content and a higher alcohol/ethyl ferulate ratio had adverse effects on the lipase-catalyzed esterification. The Novozym 435 activity was tested in less polar solvents (anhydrous toluene and hexane) by monitoring the alcoholysis of ethyl ferulate with 1-octanol, which resulted in a 50% yield of octyl ferulate. The alcoholysis was improved to 83% by applying a 16 mm Hg vacuum for 5 min every 24 h to remove the ethanol co-product. The optimal alcoholysis parameters were applied to the alcoholysis of ethyl ferulate with monoolein and the transesterification with triolein. The transesterification of ethyl ferulate with triolein in anhydrous toluene produced a combined 44% yield of ferulyl monoolein and ferulyl diolein, a 20% greater yield than that obtained for alcoholysis using monoolein. The highest yield, 77%, of ferulyl monoolein and ferulyl diolein was achieved using a threefold excess of neat triolein. The lipase-catalyzed transesterification of ethyl ferulate with triolein appears to be a technically feasible route to ferulyl-substituted acylglycerols, which are potentially useful sunscreen ingredients.

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KEY WORDS: Alcoholysis, cinnamates, ethyl ferulate, lipase, monoolein, Novozym® 435, transesterification, triacylglycerols, triolein.

There is a growing interest in modifying fats and oils to form structured lipids with specific properties for nutritional and pharmaceutical applications. Recent reviews have outlined the strategies for synthesizing tailor-made fats and oils and their desired properties (1,2). These strategies have included blending, distillation, fractionation, hydrogenation, interesterification with chemical catalysts, and, more recently, interesterification with biocatalysts. Chemical interesterifications of triacylglycerols (TAG) for industrial applications are typically performed using inorganic catalysts at elevated temperatures (200–250°C) (3). Enzymatic interesterifications, however, offer the advantages of milder reaction conditions, a wider variety of synthetic substrates, and regioselective specificity toward the acyl groups of the TAG (4). The industrial uses of lipase-catalyzed reactions are not limited to nutritional and pharmaceutical applications, but also include the synthesis of soaps and surfactants, improvement in lumber processing, leather manufacturing, and flavor production in the dairy and related industries (3,4). This work examines the technical feasibility of using lipase-catalyzed reactions to synthesize structured lipids with properties suitable for use as possible sunscreen reagents.

Sunscreens function either as ultraviolet (UV) filters or blocks. UV blocks, such as TiO, ZnO, and derivatives of other metal oxides, form a physical barrier that scatters UV light (5). These UV blocks offer the most comprehensive sunscreen protection, blocking the full spectrum of UVA (400–320 nm) and UVB (320–290 nm) light. The particulate nature of these formulations, however, often leaves noticeable residues when applied to the skin, which are cosmetically unacceptable to the consumer. The most commonly used sunscreens are UV filters, which are typically organic compounds incorporated (2–15%) into topical formulations (6,7). A disadvantage of UV filters is that each organic compound has a limited range of maximal UV absorptivity, rendering each reagent better suited for either UVA or UVB protection, but not both. The advantage of the UV-filtering molecules, however, is that they can be engineered to provide sunscreens with desirable physical appearance, solubility, and water-resistant properties (7).

Although no longer used today, benzyl cinnamate formulated as an emulsion with benzyl salicylate was used as a sunscreen as early as 1928 (6). Today, cinnamic acid derivatives are the most widely used UVB-absorbing chemicals in sunscreen formulations, with four derivatives approved for use in the United States and 17 approved for use in Europe (6). The cinnamates' unsaturated C=C bond adjacent to the aromatic ring allows for a continuous, conjugated π-system throughout the molecule. An electron can be delocalized throughout the π-system by photoexcitation with energy corresponding to ~305 nm. Most common cinnamic acids and short-chain esters are water soluble, limiting their usefulness as waterproof sunscreens. Cinnamic acid derivatives therefore have been designed with long-chain hydrocarbons (e.g., octyl-*p*-methoxy cinnamate), which renders them water-in-

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soluble and suitable for waterproof sunscreens. The $–OCH_3$ group of octyl-*p*-methoxy cinnamate acts as an electron-releasing group to improve the electron excitation process (6).

In this work, we report a lipase-catalyzed route to ferulyl-substituted TAG. Ethyl ferulate (EF), the ethyl ester of ferulic acid (FA; 4-hydroxy-3-methoxy cinnamate) that is widely distributed in plants, possesses a UV absorbance maximum at ~320 nm. Several lipases were screened to determine their activity toward the ferulyl compounds. The most active lipase was used to facilitate the transesterification of EF with triolein (TO). TO is a water-insoluble TAG. When combined, the ferulyl-substituted oleins provide the UV absorptivity of a cinnamate ester with the water-insoluble properties of a lipid. The lipase-catalyzed transesterifications of EF with TO could potentially lead to new sunscreen products, synthesized from natural products, while providing a value-added use for vegetable oils.

EXPERIMENTAL PROCEDURES

Enzymes. Novozym® 435 (*Candida antarctica*) with a 1–2% (w/w) water content and Lipozyme® IM (*Mucor miehei*) were from Novo Nordisk (Franklinton, NC). Chirazyme® L-1 (porcine pancreas) was from Roche Molecular Biochemicals (Indianapolis, IN). α-Chymotrypsin (bovine pancreas) attached to macroporous acrylic beads was purchased from Sigma-Aldrich (St. Louis, MO). All enzymes were used as obtained from the supplier without modification.

Chemicals. FA (Sigma-Aldrich) and ethyl 4-hydroxy-3-methoxy cinnamate (EF, Sigma-Aldrich) were kept desiccated under a static vacuum. Monoolein (MO), diolein (DO), and oleic acid (OA) were from Sigma-Aldrich and stored at –4°C. TO and ethyl oleate (EO) were from Nu-Chek-Prep (Elysian, MN) and stored at –4°C.

Anhydrous (<0.005% water) toluene and *t*-butanol (2-methyl-2-propanol) were purchased in Sure/Seal® bottles (Sigma-Aldrich). Ethanol and 1-octanol were dried over activated molecular sieves and stored under a nitrogen atmosphere. Methanol, acetone, acetonitrile, glacial acetic acid, and 1-butanol were high-performance liquid chromatography (HPLC) grade. All were obtained from Sigma-Aldrich. Water used for reactions was deionized and filtered through a 0.22 μm nylon filter.

General considerations. Reactions were performed under a nitrogen atmosphere using standard Schlenk line techniques in 25-mL Schlenk tubes (1.5 × 15 cm) containing a Spinplus stirbar (3/8 × 3/8 in.) from Sigma-Aldrich. The reaction temperature was controlled using a water-jacketed oil bath heated/cooled by an external circulating water bath (Precision Scientific Inc., Chicago, IL). The oil bath was mounted over a magnetic stir plate. The quantities of enzyme added to reactions represent the combined mass of the enzyme and its support.

Esterification of FA. In a 25-mL Schlenk tube, FA (97 mg, 0.5 mmol) was dissolved in 5 mL of *t*-butanol at 25, 40, or 60°C. Typically, ethanol (29 μL, 0.5 mmol) or 1-octanol (78 μL, 0.5 mmol) was added against a nitrogen flow followed by the addition of enzyme (100 mg). The reactions were allowed to stir at 25, 40, or 60°C, and 10-μL aliquots were periodically taken for analysis.

Alcoholysis of EF with 1-octanol. In a 25-mL Schlenk tube, EF (111.1 mg, 0.5 mmol) was dissolved in 5 mL of *t*-butanol or 5 mL of toluene at 60°C. 1-Octanol (78 μL, 0.5 mol) was added against a nitrogen flow followed by the enzyme (110 mg). The reactions were allowed to stir at 60°C, and 10-μL aliquots were periodically taken for analysis.

Alcoholysis of EF with MO. MO (48 mg, 0.14 mmol) was transferred from the reagent bottle by dissolving it in 2 mL of hexane and pipetting the clear colorless solution into a 25-mL Schlenk tube. The reagent bottle was washed with an additional 2 mL of hexane, which also was added to the Schlenk tube. The hexane was removed from the Schlenk tube by vacuum, leaving a clear, colorless oil that was kept *in vacuo* for an additional 15 min. EF (30 mg, 0.14 mmol) was added to the Schlenk tube, and the stirred contents were dissolved in 5 mL of toluene at 60°C. Novozym 435 (100 mg) was added, and 100-μL aliquots were periodically taken for analysis.

Transesterification of EF with TO. TO (447 mg, 0.5 mmol) was dissolved in 5 mL of toluene in a 25-mL Schlenk tube at 60°C. EF (111 mg, 0.5 mmol) was added to the reaction mixture followed by Novozym 435 (110 mg). The suspension was stirred at 60°C, and 100-μL aliquots were periodically taken for analysis.

The reaction also was performed without solvent. EF (111 mg, 0.5 mmol) was dissolved in 1.24 g of TO (1.4 mmol) at 60°C to give a clear, colorless, and viscous solution. Novozym 435 (110 mg) was added, and 20-μL aliquots were periodically taken for analysis.

HPLC analysis. Analyses were performed using a Thermo Separation Products (San Jose, CA) HPLC system consisting of a Spectra System AS3000 autosampler, a Spectra System P4000 pump, a Spectra System UV6000LP detector, an Alltech (Deerfield, IL) 500 Evaporative Light-Scattering Detector (ELSD), and a Prodigy C_8 column (5 μm, 250 × 4.6 mm; Phenomenex, Torrance, CA). Solvents were filtered using Whatman 0.45-μm nylon membrane filters (Sigma-Aldrich) and degassed using a Thermo Separation Products SCM 1000 Membrane Degasser.

For determination of FA and ferulate esters, the 10-μL aliquots taken from the esterification and alcoholysis reactions were diluted to 2 mL with 50% aqueous ethanol and filtered through 0.45 μm Gelman Acrodisc 13LC PVDF syringe filters (Sigma-Aldrich). Injections of 10 μL were eluted from the column at a flow rate of 1.5 mL/min using a binary gradient of solvent A [263 mL water, 1 mL glacial acetic acid, 11 mL 1-butanol (3%), 70 mL methanol (20%)] and solvent B [93 mL water, 1 mL glacial acetic acid, 11 mL 1-butanol (3%), and 245 mL methanol (70%)]. The elution sequence was 5 min isocratic flow of 75:25 (A/B), 2 min linear gradient to 100% (B), 18 min isocratic flow of 100% (B), 2 min linear gradient to 75:25 (A/B), followed by 3 min of isocratic elution with 75/25 (A/B). The eluate was monitored at 300 nm using the Spectra System UV6000LP detector.

For determination of TO and its derivatives, the 100- and

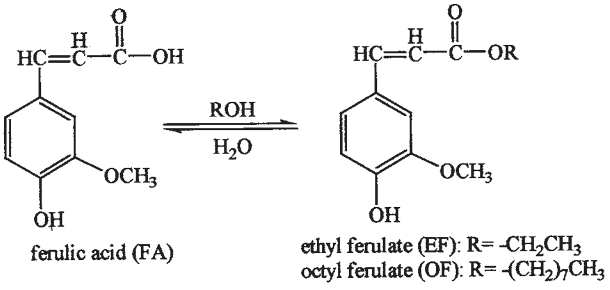
20-μL aliquots taken from the glycerolysis reactions were diluted to 1 mL with acetone and filtered through 0.45 μm Gelman Acrodisc 13LC PVDF syringe filters. Injections of 10 μL were eluted from the column at a flow rate of 1.5 mL/min using an isocratic flow of 40:60 (vol/vol) acetone (containing 1% glacial acetic acid)/acetonitrile. The eluate was monitored at 360 nm using the Spectra System UV6000LP detector followed by the Alltech 500 ELSD (nitrogen; 2.0 slpm, 70°C). The detectors were plumbed in series with a void volume resulting in a 0.1 s delay in peak retention time for species detected by both methods. It should be noted that the ELSD response is not linear and that peak intensities and areas are species dependent; therefore, the tabulated data are intended for qualitative comparisons only.

HPLC–atmospheric pressure chemical ionization–mass spectroscopy (HPLC–APCI–MS) analysis. HPLC–APCI–MS analyses were performed on samples prepared for HPLC analysis. Elution conditions were identical to those described previously except glycerolysis reaction samples were eluted using an isocratic flow of 40:60 (vol/vol) methanol (containing 1% glacial acetic acid)/acetonitrile. Mass spectral analyses were performed using a Finnigan (San Jose, CA) MAT LCQ mass spectrometer with a direct liquid APCI interface.

RESULTS AND DISCUSSION

Esterification of FA. The esterification of FA was studied as a model reaction to optimize conditions for subsequent experiments. Lipozyme IM (*M. miehei*), Chirazyme L-1 (porcine pancreas), Novozym 435 (*C. antarctica*), and α-chymotrypsin (bovine pancreas) were screened for their ability to esterify FA (Scheme 1) to produce EF or *n*-octyl ferulate (OF). Novozym 435 was found to be the only enzyme to catalyze the esterification of FA with ethanol or 1-octanol in *t*-butanol. Autoclaved samples of Novozym 435 did not facilitate the esterification of FA; therefore, the catalytic activity is attributed to the enzyme and not its acrylic support. The esterification of FA was not detected in reactions performed in hexane and toluene. Hydrophobic solvents generally support higher enzymatic activity (8–10); however, the solubility of FA was limited in these nonpolar solvents. Therefore, the esterifications were performed at 25, 40, and 60°C (manufacturer's suggested maximum temperature for Novozym 435) in *t*-butanol. The FA to EF conversion was 1, 5, and 20% at 25, 40, and 60°C, respectively, after 312 h. Figure 1 shows the effect of reaction conditions on the time course of the conversion of FA to EF or OF at 60°C in stirred batch reactions under nitrogen. At a 0.1 M concentration of substrates, equilibrium was not reached after 312 h, but the extent of conversion was greater for EF than OF throughout the time course of the reaction. The equilibria of lipase-catalyzed esterifications have been observed by Habulin *et al.* (11) to be influenced by the alcohol molar mass; For example, butanol provided a higher equilibrium conversion of OA to ester than decanol. Decreasing the concentration of reactants 10-fold to 0.01 M ethanol and FA allowed equilibrium to be achieved within 312 h, but resulted in a lower FA conversion (8%). The conversion of FA to EF after 312 h was reduced to 10 and 4% when water was added to the reaction at concentrations of 0.5 and 1.0% (vol/vol), respectively (Fig. 1). This decrease in FA conversion most likely results from a shift in equilibrium toward hydrolysis rather than any adverse effects the water may have on the enzyme (12,13). Doubling the ethanol concentration of the reaction resulted in a slightly lower conversion to EF, 18%, compared to the conversion obtained from the reaction performed with a 1:1 alcohol to FA ratio, 20% (data not shown). A lack of mass-action response has been observed previously for the Novozym 435-catalyzed esterification of glucuronic acid with cinnamic alcohol where excess alcohol did not improve the ester yield (14). The best conversion of FA to EF was obtained using a 1:1 molar ratio of 0.1 M alcohol to 0.1 M FA at 60°C; therefore, these reaction conditions were used for subsequent experiments.

Alcoholysis of EF. As discussed above, lipases typically exhibit higher activities in nonpolar solvents; however, the



SCHEME 1

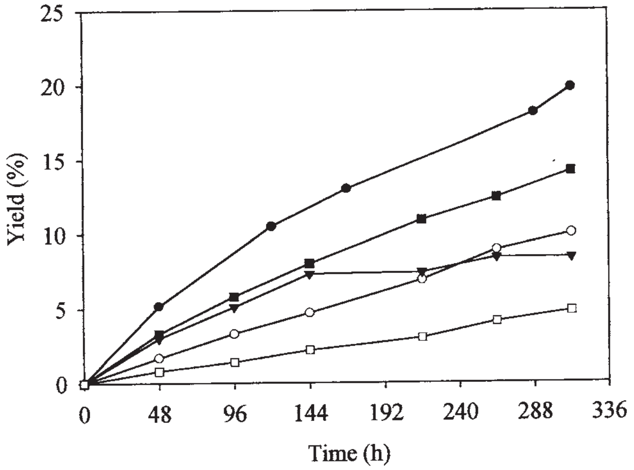


FIG. 1. Esterification of 0.1 M ferulic acid with 0.1 M ethanol or 0.1 M 1-octanol in *t*-butanol catalyzed by Novozym® 435 (100 mg) at 60°C. ●: ethyl ferulate; ■: octyl ferulate; ○: ethyl ferulate (0.5% water added to reaction medium), □: ethyl ferulate (1% water added to reaction medium); ▼: ethyl ferulate (reaction performed with 0.01 M ferulic acid and 0.01 M ethanol). Percentage yields were calculated based on total peak area of substrates and products detected by high-performance liquid chromatography analysis.

poor solubility of FA in hexane and toluene limited the study of its esterifications to using *t*-butanol as a solvent. The ability of Novozym 435 to effect the transesterification of ferulyl substrates in less-polar solvents was investigated using EF. The alcoholysis of EF with 1-octanol (1:1 molar ratio) was performed in stirred batch reactions in anhydrous toluene at 60°C. Figure 2 shows that a 50% conversion of EF to OF was achieved after 312 h. The water concentration in the reactions, based on the water content of Novozym 435 (maximum of 2% by wt) and anhydrous toluene (0.005% by vol) as obtained from the manufacturers, was 0.027 M (EF concentration was 0.10 M). Under these conditions the unwanted hydrolysis of EF to FA was observed to be <1%, and no effort was made to study enzyme performance at lower water concentrations.

A few factors may contribute to the higher conversion (50%) of EF to OF compared to the 14% conversion of FA to OF. First, the more hydrophilic solvent, *t*-butanol, used in the FA esterification may be more likely to remove essential water from the enzyme (9), lowering its activity. The addition of water to this reaction promotes hydrolysis and does not rehydrate the enzyme. Also, hydrophobic solvents are less likely to interact with the protein structure, thus preventing denaturation of the enzyme (9). Finally, the reaction is conducted at 60°C under a positive pressure of nitrogen (830 mm Hg). This may allow the ethanol formed as the alcoholysis coproduct to be driven from the reaction when the rubber stopper is removed during sampling, thus allowing the equilibrium to favor OF. To see if the removal of ethanol from the reaction medium would increase the production of OF, the same experiment was performed while applying a partial vacuum (16 mm Hg) to the reaction for 5 min every 24 h. The reaction medium refluxed slightly at 16 mm Hg and 60°C but never boiled. Figure 2 shows that a 30% increase in yield (~80% final yield) of OF was achieved after 312 h when the vacuum was applied. An alternative method used to improve the yield of OF was to increase the amount of enzyme used. Doubling the amount of Novozym 435 increased the yield of OF to 75% after 312 h (Fig. 2). Considering the cost of the enzyme, however, the application of a periodic vacuum to increase the yield of OF is preferable.

Transesterification of EF. The goal of this research was to synthesize structured lipids containing one or more substituted ferulyl moieties for possible use as ingredients in sunscreen formulations. The results discussed above provide the optimal parameters for the Novozym 435-catalyzed alcoholysis of EF. Experiments involved the reaction of EF with TO (1:1 mol ratio) catalyzed by 1 equivalent of Novozym 435 (w/w based on EF) in 5 mL of anhydrous toluene at 60°C. The reaction reached equilibrium after 72 h. The chromatogram obtained from the HPLC analysis of the reaction shows nine peaks detected by ELSD. The peaks are illustrated in Figure 3, which depicts the HPLC analysis of the transesterification performed in neat TO (discussed below). Table 1 lists the peak assignments, corresponding retention times (R_t), and relative yields of the reaction products. The peaks corresponding to EF, MO, OA, EO, DO, and TO were identified based on retention times determined from standards. This left four unidentified UV-absorbing (UVA) species with retention times of 1.8, 2.4, 2.5, and 4.4 min.

HPLC–APCI–MS was used to determine that these four reaction products were MO or DO derivatives containing ferulyl moieties. Scheme 2 shows the structures of the reactants and the UVA products of interest. The major ion corresponding to the peak at R_t = 1.8 min was 251 *m/z* and is ferulyl glycerol (FG) with a major ion value of $[M + H - H_2O]^+$. An ion

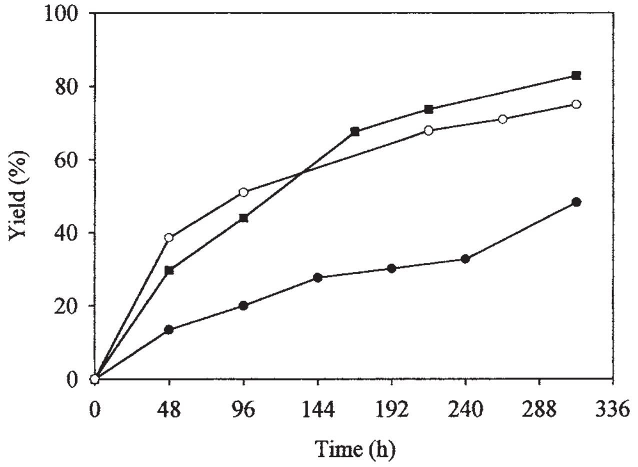


FIG. 2. Alcoholysis of 0.1 M ethyl ferulate with 0.1 M 1-octanol in anhydrous toluene catalyzed by Novozym 435 (110 mg). ●: octyl ferulate; ■ octyl ferulate (16 torr vacuum applied to reaction for 5 min every 24 h); ○: octyl ferulate (reaction performed with 220 mg of Novozym 435). Percentage yields were calculated based on total peak area of substrates and products detected by high-performance liquid chromatography analysis.

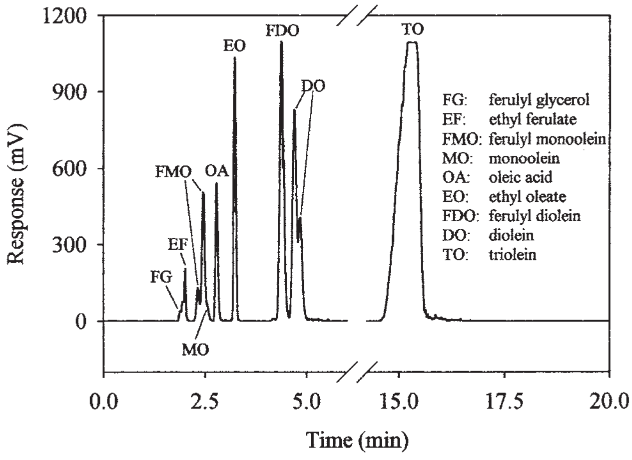


FIG. 3. High-performance liquid chromatography analysis of the Novozym® 435 (110 mg)-catalyzed transesterification of ethyl ferulate (0.5 mmol) in neat triolein (1.4 mmol) after 144 h recorded using the evaporative light-scattering detector.

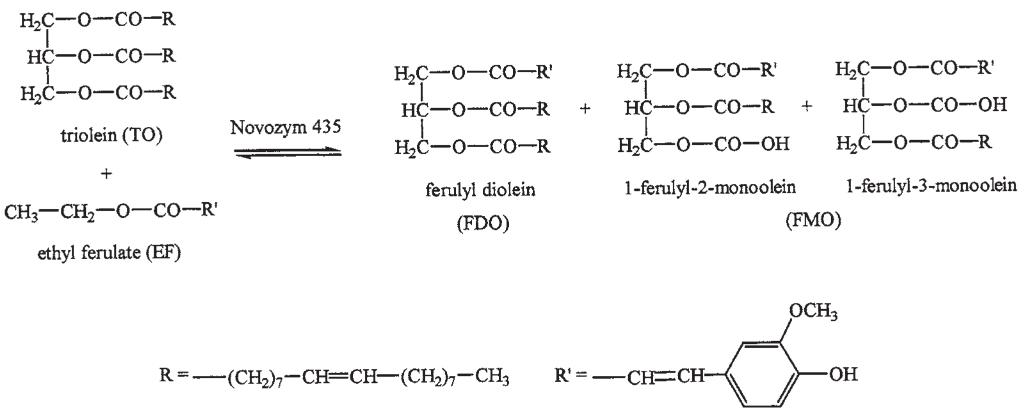
TABLE 1
Peak Assignments and Relative Yields for the Alcoholysis and Transesterification Products of Ethyl Ferulate (EF)

			Relative peak areas[a]		
R_t (min)	Peak	Major ion (m/z)[b]	MO[c]	TO[c]	TO (neat)[d]
1.8	FG	251	2.6 (44%)[e]	0.4 (19%)	— (10%)
2.0	EF	—	1.0 (32%)	1.0 (37%)	1.0 (13%)
2.4, 2.5	FMO	515	2.4 (24%)	1.3 (32%)	3.6 (29%)
2.6	MO	—	2.2	—	—
2.8	OA	—	—	0.2	2.5
3.2	EO	—	0.4	1.3	4.6
4.4	FDO	796	—(0%)	0.6 (12%)	8.1 (48%)
4.7, 4.9	DO	—	1.0	1.2	9.0
15.2	TO	—	—	4.1	40.2

[a] Peaks were obtained using the evaporative light-scattering detector, and the areas are relative for each reaction based on EF.
[b] The major ions detected by high-performance liquid chromatography–atmospheric pressure chemical ionization–mass spectroscopy were identified as follows: ferulylglycerol (FG), $[M + H - H_2O]^+$; ferulylmonoolein (FMO), $[M + H - H_2O]^+$; ferulyl diolein (FDO), $[M]^+$; monoolein, MO; oleic acid, OA; ethyl oleate, EO; diolein, DO; triolein, TO.
[c] Alcoholysis and transesterification of 0.1 M EF with 0.1 M MO and TO in 5 mL toluene catalyzed by 110 mg of Novozym® 435 for 72 h.
[d] Transesterification of 0.36 M EF in 1.4 mL TO catalyzed by 110 mg of Novozyme 435 for 144 h.
[e] Percentage yields for the ultraviolet-A (UVA) species produced in each reaction were calculated based on the total peak area recorded using the UV6000LP detector.

corresponding to diferulyl glycerol (427 m/z, $[M + H - H_2O]^+$) was not observed. The loss of H_2O from a monoacylglycerol during HPLC–APCI–MS analysis was expected and has been previously documented (15). Similarly, the peaks at R_t = 2.4 and 2.5 min possessed major ions of 515 m/z $[M + H - H_2O]^+$ and were identified as isomers of ferulyl monoolein (FMO). It is believed that the peaks at R_t = 2.4 and 2.5 min are the result of at least two FMO isomers, most likely the 1-ferulyl-3-monoolein and 1-ferulyl-2-monoolein. The formation of a third isomer, 2-ferulyl-1-monoolein, is improbable due to the size of the ferulyl moiety and the steric restrictions of the *sn*-2 position of the lipid. The last UVA species (R_t = 4.4 min) formed from the glycerolysis corresponds to a major ion of 796 m/z, $[M]^+$, and was identified as ferulyl diolein (FDO). The presence of a single peak for FDO is consistent with the formation of a single isomer, most probably 1-ferulyl-2,3-diolein.

The transesterification of EF is dependent upon the removal of oleate groups from TO to form MO and DO, which then can react with the EF-enzyme intermediate to form FMO and FDO. Novozym 435 provides enough water (0.025 M in the 5-mL toluene reactions) to promote the partial hydrolysis of TO to form MO and DO. The addition of more water to increase the formation of MO and DO was not attempted because, as shown above, additional water is deleterious in these reactions. Instead, ethanol (0.05 M) was added to the reaction in an attempt to enhance the rate of TO to MO and DO conversion, giving EO as a by-product. After 24 h with ethanol present, twice as much DO and EO formed. The amount of FMO and FDO formed after 24 h, however, was similar with or without ethanol added. When the reactions reached equilibrium (72 h), the amount of FMO, FDO, and all other products formed are comparable for both reactions. These results suggest that, although initially more DO can be made avail-



SCHEME 2

able for the glycerolysis of EF, the rate-limiting step most likely involves the formation of, or the reaction with, the EF-enzyme intermediate.

An alternative to partially hydrolyzing TO was performing the alcoholysis of EF using MO, which has available –OH groups. The reaction conditions were identical to the reaction using TO, and equilibrium was reached after 72 h. Comparing the results of the two reactions (Table 1) shows that using TO for the transesterification of EF produces 8% more FMO and 12% FDO, which is not formed in the alcoholysis reaction of EF and MO. In addition, the alcoholysis using MO forms 25% more of unwanted transesterification product FG, which lacks an oleate moiety. It was observed that the MO alcoholysis results in the formation of a significant quantity of DO after 2 h, which is slowly consumed as the reaction proceeds (data not shown). The large quantity of glycerol formed from the MO to DO conversion is then available to sequester ferulyl groups to form FG. It is reasonable to assume that a smaller quantity of glycerol is formed during TO glycerolysis, which would involve the removal of two oleate groups from the lipid. Thus, less glycerol is available to form FG. Overall, using TO for the Novozym 435-catalyzed transesterification of EF in toluene is more efficient, resulting in a 44% conversion of EF to the desired FMO and DMO products, with 56% being converted to FG or left unreacted.

If ever developed commercially, the transesterification of EF would be more attractive if the cost of the solvent, solvent waste, and residual solvent in the product could be avoided. Therefore, we decided to determine the efficiency of performing the transesterification of EF in neat TO. A sufficient quantity of TO was needed to allow the Novozym 435 to be stirred as a suspension, and it was found that 0.5 mmol EF was conveniently soluble in 1.4 mmol of TO (1.36 mL) at 60°C. The transesterification of EF in neat TO required 144 h to reach equilibrium, double the time required for the reaction performed in toluene. This is attributed to the higher viscosity of the neat TO reaction and the subsequent slower mass transfer of the EF. The relative peak areas of the species detected using the ELSD (Fig. 3) and the percent yields of FMO and FDO obtained using the UV6000LP detector are reported in Table 1. The combined yield of FMO and FDO was 77%, leaving just 23% of the EF unreacted or converted to FG. This is a 33% increase in the combined FMO and FDO yield compared to the yield obtained during the transesterification of EF with TO performed in toluene. As indicated in Table 1, the relative peak area of FG produced during the transesterification of EF in neat TO could not be calculated because an individual peak was not detected by ELSD (Fig. 3). The yield of FG (10%), however, was determined based on the peak area recorded by the UV6000LP detector. Overall, the transesterification of EF in neat TO provides the most efficient route to ferulyl-acylglycerols, producing the highest combined yield of FMO and FDO with the absence of residual solvent, but at the expense of doubling the reaction time.

One factor limiting the use of lipase-catalyzed reactions on an industrial scale is the cost of the enzyme (e.g., $2,000/kg for Novozym 435). The cost efficiency of the reaction could be greatly improved by reusing the lipase for several reactions. The catalytic stability of Novozym 435 was demonstrated by repeating the transesterification of EF with neat TO with previously used lipase. The transesterification of EF with neat TO, described above, reached equilibrium after 144 h resulting in a combined FMO and FDO yield of 77%. After equilibrium was reached, the TO was decanted, and fresh TO was added to the reaction vessel. Residual quantities of EF and reaction products from the previous reaction were determined by HPLC and subtracted from the yields obtained from the second reaction. EF was added to initiate the reaction, which stirred for an additional 120 h. HPLC analysis revealed that the second glycerolysis produced 31% FMO and 43% FDO, a combined yield of 74%. The residual amounts of EF and FG after 120 h were 17 and 9%, respectively. The slightly higher quantity of unreacted EF present at equilibrium (17%) compared to the quantity of unreacted EF present at equilibrium in the original glycerolysis (13%) is attributed to the shorter reaction time. These results indicate that Novozym 435 remains active at 60°C for weeks and is able to catalyze multiple glycerolysis reactions.

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