Partial Purification of *Nigella sativa* L. Seed Lipase and Its Application in Transesterification Reactions

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ABSTRACT: *Nigella sativa* L. seed lipase isolated from defatted seeds was partially purified and used as catalyst in transesterification reactions. Purification of an ammonium sulfate-precipitated sample (at 35% saturation, *Nigella PL*) by DEAE ion-exchange chromatography increased the specific activity from 13.9 to 156.7 U/mg protein. *Nigella PL* and *Nigella CPL* (the partially purified enzyme sample obtained by DEAE ion-exchange chromatography) catalyzed the transesterification of vinyl acetate with octanol, with racemic sulcatol (6-methyl-5-hepten-2-ol), and with racemic *trans*-sobrerol (*trans-p*-menth-6-ene-2,8-diol) in different organic solvents. Both activity and enantioselectivity of the enzyme samples used for these biotransformations were affected by the nature of the organic solvent.

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KEY WORDS: Enantioselectivity, enzymatic transesterification, *Nigella sativa* L. seed lipase, purification, sobrerol, sulcatol.

Lipases, which catalyze not only the hydrolysis of TAG but also their synthesis from glycerol and FFA, can be used in the production of detergents, foods, pharmaceuticals, and other synthetic organic materials (1).

Lipases can be obtained from mammals, yeast, bacteria, and higher plants. To date, most industrial lipases have been produced from fungal sources. These enzymes are usually well characterized, but lipases from higher plants generally have not yet been investigated in detail despite the fact that they may display different properties that could be exploited for industrial or technical purposes. Lipases are present in oil seeds, such as rape (*Brassica napus*), mustard (*Sinapis alba*), and cereals (2,3). Usually these enzymes are absent in dormant seed and are produced during seed germination. However, *Nigella sativa* L, also known as black cumin or fennel flower, contains active lipases even in its dormant seeds (3–5).

FA selectivity of lipases has been utilized to enrich for FA (or of their derivatives) from naturally occurring mixtures (6). More recently, lipases have been used as biocatalysts for the enzymatic resolution of racemic substances to produce pure enantiomeric drugs (7,8).

Nigella sativa L. is a member of the Ranunculaceae family (9). Its capsule-shaped fruit contains the black-colored, oval, and three-faced seeds. Owing to their aromatic nature, *Nigella sativa* L. seeds are used as condiment or spice in cooking.

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Crude and immobilized *N. sativa* L. lipases are effective biocatalysts for the hydrolysis and glycerolysis of TAG and for the esterification of FFA (5,10–12). A crude lipase sample from *N. sativa* L. seeds was immobilized by adsorption on Celite 535 from phosphate buffer solutions at pH values varying from 5.0 to 8.0 at 25°C, and pH 6 was established to be optimal for adsorption (12).

The goals of this study were to obtain crude and partially purified samples of lipase(s) and to investigate the efficiency of these enzymatic preparations for transesterification reactions in organic solvents.

EXPERIMENTAL PROCEDURES

Materials. Nigella sativa L. seeds from the Denizli region of Turkey were purchased locally. The proximate compositions of the seeds, determined according to standard AOCS methods (13), were 9.4% moisture, 4% ash, 35.4% oil, 23.3% protein, 6.7% crude fiber, and 21.2% carbohydrates.

All chemicals were purchased from Aldrich (Milwaukee, WI). The solvents used in the reaction were of analytical grade.

Determination of lipase activity and protein content. Two methods were applied for the determination of lipase activity: (i) Spectrophotometric assay. A spectrophotometric assay, similar to that employed by Bornscheuer *et al.* (14) and based on the use of *p*-nitrophenyl laurate (pNPL) as a substrate, was found to be suitable for a quick analysis of both supernatant liquids and pellets. One unit of lipase activity was defined as the amount of enzyme that liberated 1 μmol of *p*-nitrophenol/min from pNPL. (ii) pH-stat assay. A pH-stat method described by Peled and Krenz (15) was used, employing tributyrin as the substrate. One unit of lipase activity was defined as the amount of enzyme that liberated 1 μmol of butyric acid/min from tributyrin.

Protein content was determined according to the Bradford method using the Bio-Rad protein assay kit (Bio-Rad Laboratories, Milano, Italy), reading the absorbance at a wavelength of 595 nm.

Preparation of the acetonic powder. A method similar to the one developed by Afolabi *et al.* (16) was applied for the preparation of acetonic powder. Air-dried seeds (100 g) and 200 mL of cooled acetone (4°C) were homogenized in a blender for 3.5 min. The resulting mixture was transferred to a 1000-mL beaker that was cooled using a salt/ice mixture (–16°C). The container was rinsed with acetone (150 mL) and the rinsings were added to the original mixture, then stirred for 1 min. The sus-

pension containing low-density material was decanted and filtered by vacuum filtration through Schleicher & Schuell white band filter paper. The seed residue was washed twice with 200 mL acetone in the beaker, followed by filtration on the previously obtained filter cake. The grayish filter cake was transferred to a 1000-mL beaker that was cooled with a salt/ice mixture and then washed twice with 150-mL portions of acetone and twice with 100-mL portions. The combined acetone fractions were vacuum-filtered to leave a light grayish powder. The powder was then air dried and stored at 4°C. The yield of acetone powder was approximately 20% w/w from the original seeds.

A sample of 200 mg of acetone powder showed a lipase activity of 5.5 units/g using the pH-stat method.

Preparation of defatted seeds and isolation of lipase(s). To obtain the defatted seeds, four sets of extractions were conducted separately using approximately 25 g of untreated seeds. Specifically, the seeds were homogenized in a blender, then the contained oil was extracted with hexane for 6 h in a Soxhlet extraction apparatus equipped with a cooling system. The defatted seeds obtained (approximately 16 g each) were then extracted for 6 h at room temperature with different solutions: potassium phosphate buffer (10 mM, pH 7) containing 1% Triton X-100 (Triton); phosphate buffer containing 1% Triton and 10% ethylene glycol; distilled water containing 1% Triton; and distilled water containing 1% Triton and 10% ethylene glycol. The different mixtures were placed in the refrigerator overnight, then the homogenates were filtered through cheesecloth. The activities of the different supernatants and solid residues were determined.

Precipitation of lipase samples from the supernatant with ammonium sulfate. The solution obtained from the extraction of defatted seeds using water containing 1% Triton was filtered through cheesecloth and centrifuged at $15{,}000 \times g$ for 20 min. The lipase(s) in the supernatant then were precipitated by adding different amounts of solid ammonium sulfate at room temperature, with recovery of the solid residues by centrifugation at $15{,}000 \times g$ for 20 min.

Enzymatic purification by DEAE exchange chromatography. The pellets obtained at 35% ammonium sulfate saturation of the supernatant were dissolved in 5 mM potassium phosphate buffer, pH 7.5 (buffer A), and dialyzed against the same buffer overnight. The solution was loaded on a DEAE column (15 × 2.5 cm) previously equilibrated with buffer A. The column was washed with the same buffer, and the enzymes were eluted using a linear gradient according to three different protocols.

In the first protocol, the lipase sample was eluted using a two-step gradient. Flow rate was adjusted to 3 mL/min. In the first step, which lasted 2 h, the initial solution was buffer A and the final solution was buffer A containing 0.3 M NaCl (buffer B). At the end of the first gradient the column was washed for 0.5 h with buffer B, and then the second gradient was applied, moving in 2 h from buffer B to a final solution containing buffer A and 1 M NaCl (buffer C).

In the second protocol the sample was eluted in 2.5 h using a linear gradient from buffer A to buffer C containing also 0.5% Triton. The detergent was used to avoid nonspecific interactions between the lipase protein and the DEAE-resin, interactions that might be responsible for the wide spreading of activity in the eluted fractions observed when the first protocol was applied.

In the third protocol the pellets were dissolved in buffer A and directly loaded on the DEAE column without dialysis. The column was then developed as described in the second protocol.

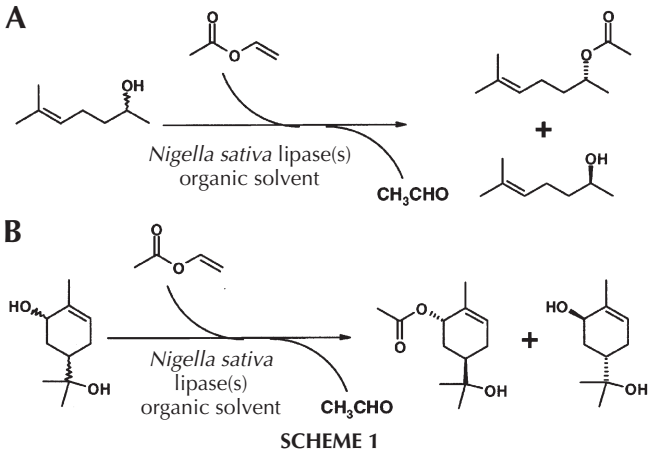
Transesterification reactions. The partially purified lipase samples were tested for their abilities to catalyze transesterification reactions in different organic solvents (17,18). Vinyl acetate was the acyl donor, and the acceptor alcohols were either *n*-octanol (to evaluate enzyme activity) or racemic sulcatol (6-methyl-5-hepten-2-ol) and racemic *trans*-sobrerol (*trans-p*-menth-6-ene-2,8-diol) (to evaluate enzyme enantioselectivity).

The enzymatic samples tested in the experiments were obtained as follows: (i) *Nigella LS* supernatant, obtained from the extraction of defatted seeds with distilled water containing 1% vol/vol Triton, was lyophilized. (ii) *Nigella PL: Nigella LS* was dissolved in potassium phosphate buffer (5 mM, pH 7.5) and the enzyme was precipitated using 35% ammonium sulfate. (iii) *Nigella CPL* sample, eluted from the DEAE-column, was lyophilized and the residual solid was dissolved in a smaller amount of the same elution buffer, dialyzed against potassium phosphate buffer (5 mM, pH 7.5), and lyophilized.

Transesterification of vinyl acetate with octanol. Initially, the transesterification of vinyl acetate with *n*-octanol was conducted in two different organic solvents (toluene and petroleum ether) to evaluate the activity of the various partially purified samples of *N. sativa* L. seed lipases. The reactions were carried out by adding 90 U lipase (evaluated with the tributyrin assay) to a solution of 30 μL *n*-octanol and 100 μL vinyl acetate in 870 μL solvent. The reaction mixtures were shaken in an orbital shaker at 250 rpm at 20°C. At scheduled times reaction samples were analyzed by capillary GC.

To investigate the influence of water activity (a_w) on the reaction, this transesterification reaction was subsequently investigated using 40 U of *Nigella PL* or of *Nigella CPL* at 40°C and 250 rpm. In this study, six different organic solvents (toluene, petroleum ether, methyl *t*-butyl ether, *t*-amyl alcohol, acetone, and acetonitrile), octanol, vinyl acetate, and *Nigella PL* and *Nigella CPL* were equilibrated in sealed flasks in the presence of molecular sieves, adjusting the a_w value to <0.1. The other reaction conditions were as above. Three of the six solvents (toluene, petroleum ether, and *t*-amyl alcohol) as well as all the other reaction components (vinyl acetate, octanol, *Nigella PL*, and *Nigella CPL*) were also equilibrated at $a_w = 0.3$ (incubation overnight in sealed flasks containing a saturated water solution of $MgCl_2$) and at $a_w = 0.8$ (incubation overnight in sealed flasks containing a saturated water solution of KCl) (19).

Transesterification reactions of vinyl acetate with racemic sulcatol and racemic trans*-sobrerol. (i) Racemic sulcatol (Scheme 1A).* Racemic sulcatol (10 μL) and vinyl acetate (100



SCHEME 1

μL) were dissolved in petroleum ether (890 μL) and subjected to the action of different preparations of *N. sativa* L. seed enzymes (approximately 350 U enzyme/10 μL racemic sulcatol), the reaction mixtures being shaken in an orbital shaker at 250 rpm at 20°C.

The degree of conversion of sulcatol into its acetate and the enantiomeric excess of the product (ee_P) were calculated using the following formulas:

$$\text{conversion} = \frac{[S]_{\text{ester}} + [R]_{\text{ester}}}{([S]_{\text{alcohol}} + [R]_{\text{alcohol}})/0.75 + [S]_{\text{ester}} + [R]_{\text{ester}})} \quad [1]$$

where $[R]_{\text{ester}}$, $[S]_{\text{ester}}$, $[R]_{\text{alcohol}}$, and $[S]_{\text{alcohol}}$ were the GC peak areas of the respective enantiomers [(*R*) or (*S*)] (see below). The value 0.75 represents the combustion ratio of sulcatol over its acetate using a FID detector.

$$ee_P = \frac{[R]_{\text{ester}} - [S]_{\text{ester}}}{[R]_{\text{ester}} + [S]_{\text{ester}}} \quad [2]$$

The enantiomeric ratio (*E*), a parameter that indicates the enantioselectivity of the enzymes in kinetic resolutions, was calculated from ee_P and the conversion degree (*c*), obtained from the same chromatographic analysis, using the following formula (20):

$$E = \ln[1 - c(1 + ee_P)]/\ln[1 - c(1 - ee_P)] \quad [3]$$

(ii) Racemic trans*-sobrerol (Scheme 1B).* In a typical experiment, racemic *trans*-sobrerol (20 mg) and vinyl acetate (100 μL) were dissolved in a solvent (900 μL) and subjected to the action of different preparations of *N. sativa* L. seed enzymes (approximately 350 U enzyme/20 mg racemic *trans*-sobrerol), the reaction mixtures being shaken in an orbital shaker at 250 rpm and at 40°C.

Chromatographic analysis of the products of transesterification of vinyl acetate with octanol. The reaction products were analyzed using a capillary methyl silicone column (HP-1 Crosslinked Methyl Silicone Gum, 20 m × 0.32 mm i.d., Hewlett-Packard). The conditions for the analysis were: 35°C (initial time 10 min) to 160°C with a heating rate of 15°C/min; H_2 was the carrier gas. The peaks were identified using standard mixtures.

Analysis of transesterification products of racemic sulcatol and racemic trans *sobrerol.* Both the conversion of sulcatol and *trans*-sobrerol into the corresponding esters and the ee_P were determined by GC using a chiral column (CP-Cyclodextrin-β-2,3,6-M-19 column, 50 m × 0.25 mm i.d., Chrompack). The conditions for the analysis of the transesterification products of racemic sulcatol were: 90°C (initial time 15 min) to 130°C with a heating rate of 1.5°C/min; H_2 was the carrier gas (21). The reaction mixtures of *trans*-sobrerol were analyzed using the same column under the following conditions: 105°C (initial time 10 min) to 140°C with a heating rate of 0.5°C/min; H_2 was the carrier gas (22).

RESULTS AND DISCUSSION

Preparation of defatted seeds and isolation of lipase samples. Supernatants were obtained from the extraction of defatted seeds using different solutions: (i) phosphate buffer/1% Triton (solution I); (ii) phosphate buffer/1% Triton/10% ethylene glycol (solution II); (iii) distilled water/1% Triton (solution III); (iv) distilled water/1% Triton/10% ethylene glycol (solution IV). All supernatants showed higher activities (determined using the pH-stat method) than the solid residues, increasing from 0.02–0.03 U/mg solid (defatted seeds) to 57.5, 63.9, 93.9, and 99.5 U/mL for solutions I, II, III, and IV, respectively. Total activities obtained using solutions I, II, III, or IV were 2731, 3035, 4599, or 4826 U, respectively. Better results were obtained with the extraction carried out in distilled water containing both Triton and glycol; however, for practical reasons, the subsequent purification experiments were carried out using solution III (no ethylene glycol added), which gave similar results. Accordingly, the supernatant obtained by extracting the defatted seeds with solution III was lyophilized and used as a catalyst for transesterification reactions (designed as *Nigella LS*).

Precipitation of lipase(s) samples from the supernatant with ammonium sulfate. Table 1 shows the activities, the total lipase activity units, the total protein contents, and the specific activities measured both in the solids and in the supernatants obtained at 35, 40, or 50% $(NH_4)_2SO_4$ saturation of solution III.

As the activities of the pellets obtained at 40 or 50% ammonium sulfate saturation were similar or only slightly higher than those obtained at 35% saturation, it was decided to use the latter concentration of $(NH_4)_2SO_4$ for the precipitation of the crude lipase(s).

Enzymatic purification by DEAE ion-exchange chromatography. The pellet samples obtained by precipitation at 35% $(NH_4)_2SO_4$ saturation were used for further purification by DEAE ion-exchange chromatography (described in the Experimental Procedures section). Total activity, total protein content, and the specific activity of the partially purified enzyme solution which was obtained using the first ion-exchange chro-

TABLE 1
Activity and Protein Content of Samples Obtained by Extraction of Defatted Seeds Using Water Containing 1% vol/vol Triton (solution III) Followed by Precipitation with Ammonium Sulfate

Procedure	Sample	Activity	Total lipase activity (units)	Total protein (mg)	Specific activity (U/mg protein)
Cheese cloth	Solid residue	0.03[a]	534	[c]	[c]
Filtration	Filtrate	65.45[b]	458	[c]	[c]
Centrifugation	Supernatant	61.24[b]	3797	583	7.0
35% $(NH_4)_2SO_4$ precipitation	Pellet	101.48[a]	1015	73	13.9
	Supernatant	2.70[b]	58	81	0.7
40% $(NH_4)_2SO_4$ precipitation	Pellet	136.60[a]	1364	78	17.4
	Supernatant	4.35[b]	87	70	1.2
50% $(NH_4)_2SO_4$ precipitation	Pellet	134.19[a]	1342	69	19.6
	Supernatant	2.44[b]	42	39	1.1

[a]U/mg solid.
[b]U/mL solution.
[c]The protein content could not be determined because of the turbidity of the filtrate.

matography protocol (see Experimental Procedures section) were determined as 2,092 U, 19.9 mg, and 105.1 U/mg protein, respectively. The eluate obtained by DEAE chromatography with the second protocol contained 134.4 mg protein and 14,776 total U, with a specific activity of 109.9 U/mg protein. The eluate obtained with the third DEAE chromatography protocol had a total activity, total protein content, and specific activity of 18,477 U, 117.9 mg, and 156.7 U/mg protein, respectively.

This third chromatographic protocol allowed an 11-fold increase of the specific activity of the ammonium sulfate precipitated enzyme that moved from an initial value of 13.9 U/mg (see Table 1) to a final value of 156.7 U/mg after the DEAE-ion exchange chromatography. However, as might be expected, the recovered material was still a complex mixture of proteins (shown by SDS-PAGE gel electrophoresis) containing more than one hydrolase (shown by native gel electrophoresis; data not shown). Nevertheless, this purification protocol (oil extraction with hexane, enzyme extraction with water containing 1% Triton, 35% $(NH_4)_2SO_4$ precipitation, DEAE ion-exchange chromatography) was repeated several times and proved to be highly reproducible in terms of the recovered total and specific activity.

Transesterification reactions. (i) Transesterification of vinyl acetate with octanol. The experimental data showed that all the enzymatic samples were able to catalyze this transesterification reaction in toluene and in petroleum ether (conversion: 99.9%, reaction time: 24 h), the initial rates being higher with the more purified samples. As shown in Figure 1, *Nigella PL* and *Nigella CPL* gave similar results. Transesterification activity was higher in petroleum ether than in toluene.

The effect of the solvent on the transesterification reactions conducted at controlled low water activity ($a_w < 0.1$) using *Nigella PL* and *Nigella CPL* is shown in Table 2. As expected, transesterification activities were higher in the solvents possessing higher log *P* values (that is, the more hydrophobic ones, where *P* represents the partition coefficient). The performances of the two enzymatic preparations, *Nigella PL* and *Nigella CPL*, were again similar, and petroleum ether was the best solvent.

The influence of different a_w values on this transesterification reaction is shown in Table 3. It is clear that an increase of a_w was accompanied by a decrease in the initial rate of transesterification. The effect was more evident with hydrophobic solvents such as toluene and petroleum ether.

(ii) Transesterification reactions of vinyl acetate with racemic sulcatol and racemic trans*-sobrerol.* For racemic sulcatol, transesterification reactions were performed in different organic solvents. As shown in Table 4, the nature of the solvent influenced enzyme enantioselectivity (21–24), best results being obtained in acetone ($E = 4.1$) (20).

The data obtained in the kinetic resolution of racemic *trans*-sobrerol are reported in Table 5. In this case too, the nature of the solvent affected the enantioselectivity, best results being obtained using neat vinyl acetate. Additionally, enzymatic activity and enantioselectivity in transesterification was increased by increasing the enzyme purity (*Nigella CPL* was better than *Nigella PL*).

The high activity of these enzymatic preparations in apolar solvents may make them suitable to catalyze selective transesterification of complex TAG mixtures. Future work will focus on this specific target as well as on the purification

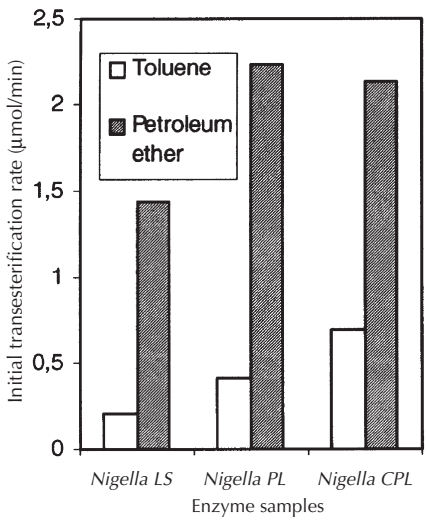


FIG. 1. Transesterification of vinyl acetate with *n*-octanol in toluene and in petroleum ether using different samples of *Nigella sativa* lipase (90 U) at 20°C.

TABLE 2
Enzymatic Transesterification[a] of Vinyl Acetate with Octanol at a_w < 0.1

Enzyme	Solvent	Log P[e]	Initial rate (μmol/min)[b]	Conversion at 0.5 h	Conversion at 24 h
Nigella PL[c]	Petroleum ether	3.50	6.95	62.4	99.8
Nigella CPL[d]	Petroleum ether		4.70	51.6	99.8
Nigella PL	Toluene	2.50	3.11	18.8	99.6
Nigella CPL	Toluene		1.35	12.9	99.99
Nigella PL	Methyl *t*-butyl ether	1.38	2.68	17.5	99.8
Nigella CPL	Methyl *t*-butyl ether		1.96	20.3	99.9
Nigella PL	*t*-Amyl alcohol	1.30	0.39	3.2	21.5
Nigella CPL	*t*-Amyl alcohol		0.44	3.2	11.2
Nigella PL	Acetone	–0.23	0.38	2.7	24.1
Nigella CPL	Acetone		0.46	3.6	35.4
Nigella PL	Acetonitrile	–0.33	0.21	2.2	34.6
Nigella CPL	Acetonitrile		0.21	2.4	55.2

[a]Reactions were performed with 30 μL *n*-octanol and 100 μL vinyl acetate in 870 μL solvent using 40 U enzyme at 40°C.
[b]Determined after 5 min. a_w, water activity.
[c]35% $(NH_4)_2SO_4$ precipitate.
[d]Sample obtained by DEAE-ion exchange chromatography.
[e]Log *P*, the logarithm of the partition coefficient of a given compound in the octanol/water two-phase system.

TABLE 3
Enzymatic Transesterification of Vinyl Acetate with Octanol at Different a_w Values[a]

Enzyme	Solvent	a_w	Initial rate (μmol/min)[b]	Conversion at 0.5 h	Conversion at 24 h
Nigella PL	Toluene	<0.1	3.11	18.8	99.6
Nigella PL	Toluene	0.3	0.92	7.3	99.8
Nigella PL	Toluene	0.8	0.07	1.1	99.1
Nigella CPL	Toluene	<0.1	1.35	12.9	99.9
Nigella CPL	Toluene	0.3	0.21	3.6	99.9
Nigella CPL	Toluene	0.8	0.13	1.4	14.3
Nigella PL	Petroleum ether	<0.1	6.95	62.4	99.8
Nigella PL	Petroleum ether	0.3	1.53	34.7	99.9
Nigella PL	Petroleum ether	0.8	0.39	5.4	85.1
Nigella CPL	Petroleum ether	<0.1	4.70	51.6	99.8
Nigella CPL	Petroleum ether	0.3	0.85	11.9	99.9
Nigella CPL	Petroleum ether	0.8	0.33	2.5	17.7
Nigella PL	*t*-Amyl alcohol	<0.1	0.39	3.2	21.5
Nigella PL	*t*-Amyl alcohol	0.3	0.45	5.0	39.9
Nigella PL	*t*-Amyl alcohol	0.8	0.24	4.1	44.9
Nigella CPL	*t*-Amyl alcohol	<0.1	0.44	3.2	11.2
Nigella CPL	*t*-Amyl alcohol	0.3	0.33	2.9	27.6
Nigella CPL	*t*-Amyl alcohol	0.8	0.04	0.8	21.3

[a]Reactions were performed with 30 μL *n*-octanol and 100 μL vinyl acetate in 870 μL solvent using 40 U enzyme at 40°C.
[b]Determined after 5 min. a_w, water activity.

TABLE 4
Enantiomeric Ratio (*E*) of Kinetic Resolution of Racemic Sulcatol Catalyzed by *Nigella PL*[a]

Solvent	Conversion (%)[b]	ee_P	*E*
Petroleum ether	10.4	0.464	2.9
Toluene	19.9	0.496	3.3
t-Butyl methyl ether	16.8	0.496	3.3
Acetone	4.3	0.602	4.1

[a]Reactions were performed with 10 μL racemic sulcatol (6-methyl-5-hepten-2-ol) and 100 μL vinyl acetate in 890 μL solvent using approximately 350 U enzyme/10 μL substrate at room temperatures.
[b]Reaction time, 2 h. ee_P, enantiomeric excess of the product.

TABLE 5
Enantiomeric Ratio (*E*) of Kinetic Resolution of Racemic *trans*-Sobrerol Catalyzed by *Nigella sativa* L. Seed Lipases (*Nigella PL* and *Nigella CPL*)[a]

Enzyme	Solvent	ee_P	Conversion[b] (%)	*E*
Nigella PL	Methyl *t*-butyl ether	15.0	23.5	1.4
Nigella PL	Vinyl acetate	15.3	15.3	1.4
Nigella PL	*t*-Amyl alcohol	49.0	5.0	3.0
Nigella CPL	Methyl *t*-butyl ether	38.7	36.9	2.8
Nigella CPL	Vinyl acetate	67.3	31.6	6.9
Nigella CPL	*t*-Amyl alcohol	68.1	7.3	5.6

[a]Reactions were performed with 20 mg racemic *trans*-sobrerol (*trans*-*p*-menth-6-ene-2,8-diol) and 100 μL vinyl acetate in 900 μL solvent using approximately 350 U enzyme/20 mg substrate at 40°C.
[b]Reaction time, 5 d. For abbreviation see Table 4.

to homogeneity of this/these lipases(s) and their biochemical characterization.

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REFERENCES

1. Malcata, F.X., Engineering of/with Lipases, Scope and Strategies, in *Engineering of/with Lipases*, edited by F.X. Malcata, Kluwer Academic, Dordrecht, 1996, pp. 1–16.
2. Huang, A.H.C., Plant Lipases, in *Lipases*, edited by B. Borgstrom, and H.L. Brokman, Elsevier Science, Amsterdam, 1990, pp. 419– 442.
3. Mukherjee, K.D., Plant Lipases in Lipid Biotransformations, in *Engineering of/with Lipases*, edited by F.X. Malcata, Kluwer Academic, Dordrecht, 1996, pp. 391–401.
4. Dandik, L., and H.A. Aksoy, The Kinetics of Hydrolysis of *Nigella sativa* (black cumin) Seed Oil Catalyzed by Native Lipase in Ground Seed, *J. Am. Oil Chem. Soc. 69*:1239–1241 (1992).
5. Dandik, L., G. Arioglu, and H.A. Aksoy, The Enzymatic Hydrolysis of Used Frying Oil by Native Lipase, *Appl. Biochem. Biotechnol. 42*:119–126 (1993).
6. Mukherjee, K.D., Lipase-Catalyzed Reactions for the Fractionation of Fatty Acids, in *Engineering of/with Lipases*, edited by F.X. Malcata, Kluwer Academic, Dordrecht, 1996, pp. 51–64.
7. Kamiya, N., M. Goto, and F. Nakashio, Surfactant-coated Lipase Suitable for the Enzymatic Resolution of Menthol as a Biocatalyst in Organic Media, *Biotechnol. Prog. 11*:270–275 (1995).
8. Sinisterra, J.V., Synthesis of Chiral Drugs Using Lipases, in *Engineering of/with Lipases*, edited by F.X. Malcata, Kluwer Academic, Dordrecht, 1996, pp. 73–101.
9. Üstün, G., S.Türkay, and A. Karaali, *Nigella sativa* Seeds: A Potential Source for Oil and Oleochemicals, in *Proceedings of the World Conference on Oilseed and Edible Oils Processing*, edited by S.S. Koseoglu, K.C. Rhee, and R.F. Wilson, AOCS Press, Champaign, 1998, Vol. 2, pp. 155–160.
10. Üstün, G., L. Kent, N. Cekin, and H. Civelekoglu, Investigation of the Technological Properties of *Nigella sativa* (black cumin) Seed Oil, *J. Am. Oil Chem. Soc. 67*:958–960 (1990).
11. Dandik, L., and H.A. Aksoy, Applications of *Nigella sativa* Seed Lipase in Oleochemical Reactions, *Enzyme Microb. Tech. 19*:277–281 (1996).
12. Akova, A., and Ustun, G., Activity and Adsorption of Lipase from *Nigella sativa* Seeds on Celite at Different pH Values, *Biotechnol. Lett. 22*:355–359 (2000).
13. AOCS, *Official Methods and Recommended Practices of the American Oil Chemists' Society,* 3rd ed., American Oil Chemists' Society, Champaign, 1971.
14. Bornscheuer U., O.W. Reif, R. Lausch, R. Freitag, T. Scheper, F.N. Kolisis, and U. Menge, Lipase of *Pseudomonas cepacia* for Biotechnological Purposes: Purification, Crystallization and Characterization, *Biochim. Biophys. Acta 1201*:55–60 (1994).
15. Peled, N., and M.C. Krenz, A New Assay of Microbial Lipases with Emulsified Trioleoyl Glycerol, *Anal. Biochem. 112*:219–222 (1981).
16. Afolabi, O.A., M.A. Ologunde, W.A. Anderson, J.S. Read, M.D. Dacosta, F.A. Epps, and F.O. Ayorinde, The Use of Lipase (acetone powder) from *Vernonia galemensis* in Fatty Acids Analysis of Seed Oils, *J. Chem. Technol. Biotechnol. 52*:41–46 (1991).
17. Klibanov, A.M., Improving Enzymes by Using Them in Organic Solvents, *Nature 409*:241–246 (2001).
18. Carrea, G., and S. Riva, Properties and Synthetic Applications of Enzymes in Organic Solvents, *Angew. Chem. Int. Ed. 39*: 2226–2254 (2000).
19. Valivety, R.H., P.J. Halling, and A.R. Macrae, Reaction Rate with Suspended Lipase Catalyst Shows Similar Dependence on Water Activity in Different Organic Solvents, *Biochim. Biophys. Acta 1118*:218–222 (1992).
20. Chen, C.S., Y. Fujimoto, G. Girdaukas, and C.J. Sih, Quantitative Analysis of Biochemical Kinetic Resolutions of Enantiomers, *J. Am. Chem. Soc. 104*:7294–7299 (1982).
21. Secundo, F., S. Riva, and G. Carrea, Effects of Medium and Reaction Conditions on the Enantioselectivity of Lipases in Organic Solvents and Possible Rationales, *Tetrahedron: Asymmetry 3*:267– 280 (1992).
22. Bovara, R., G. Carrea, L. Ferrara, and S. Riva, Resolution of (±)-*trans*-Sobrerol by Lipase PS-Catalyzed Transesterification and Effects of Organic Solvents on Enantioselectivity, *Tetrahedron: Asymmetry 2*:931–938 (1991).
23. Carrea, G., G. Ottolina, and S. Riva, Role of Solvents in the Control of Enzyme Selectivity in Organic Media, *Trends Biotechnol. 13*:63–70 (1995).
24. Wescott, C.R., and A.M. Klibanov, The Solvent Dependence of Enzyme Specificity, *Biochim. Biophys. Acta 1206*:1–9 (1994).

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