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The Enzymatic Hydrolysis of Used Frying Oil by Native Lipase

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ABSTRACT

The enzymatic hydrolysis of used frying oil catalyzed by native lipase of *Nigella sativa* seed was investigated under different conditions to elucidate the role of different process parameters on the reaction rate. Data fitted the first-order rate equation.

Index Entries: Used frying oil; *Nigella sativa* seed; lipase; hydrolysis; kinetic.

INTRODUCTION

At present, high-pressure and high-temperature processes for the industrial production of fatty acids and glycerol are commonly used. These high-energy processes induce polymerization and color development, and require subsequent purification, such as distillation of fatty acids for many uses. These processes are energy intensive and give rise to a variety of undesirable side reactions. For example, highly unsaturated fatty acids can polymerize, and if the temperature rises above 505 K, anhydrates form that, with continued heating, decompose to yield ketones and hydrocarbons (*1*). It would be advantageous to develop a low-energy process that at the same time produces a colorless product. The use of lipolytic enzymes as a potential approach to achieve these goals is being actively investigated by many workers (*2,3*). A lot of lipases, such as *pancreatic, castor, candida rugosa, aspergillus niger,* and *rhizopus arrbizus,* are widely used in several syntheses (*3,4,5*). The purpose of the present study is to determine the

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kinetics of enzymatic hydrolysis of used frying oil by native lipase of *Nigella sativa* seed and elucidate the effect of process parameters, i.e., temperature, concentration of lipase, and pH, on the lipolysis.

MATERIALS AND METHODS

Materials

Nigella sativa seeds of Turkish origin were purchased locally and used as a lipolysis catalyst. The oil content of seed on moisture-free base was 22.0%, moisture, 5.6%, and the free fatty acid content of seed oil was 2.5%. The used frying oil obtained from the campus cafeteria was filtered to remove pieces of food and used directly without any special purification. The main characteristics of used oil are shown in Table 1. Other reagents were analytical-grade Merck products.

Apparatus

Lipolytic hydrolysis reactions were carried out in a three-necked flask (1000 mL) equipped with a stirrer, a temperature controller, and a sampling pipet.

Enzymatic Hydrolysis of Used Frying Oil by Native Lipase of *Nigella sativa* Seed

In all experiments, 220 g used frying oil and 220 mL distilled water (pH 6.7) were placed into the reaction flask and heated by stirring to the reaction temperature. Ground *Nigella sativa* seeds (600–1400 μm) were added to the reaction flask as lipolysis catalyst at the reaction temperature. The stirring rate was adjusted to 700 rpm.

Samples were withdrawn at predetermined time intervals and placed in a 363 K water bath for 15 min to inactivate the enzyme. Then, they were centrifuged to separate the ground seed, and the oil phase was dried using anhydrous Na_2SO_4. Acid value (AV) of the oil samples and of the blank without oil was determined by titration (*6*). The degree of hydrolysis was calculated by the following equation:

$$\text{Hydrolysis \%} = 100 \cdot (AV_2 - AV_1) / (SV - AV_1) \quad (1)$$

where AV_1 and AV_2 are acid value of samples at the initial time and time t, respectively, and SV is the saponification value of used frying oil.

At the same time, the composition of hydrolyzed oil samples was investigated by TLC-FID Iatroscan TH-10 analyzer with SIII rods (Iatron Lab. Inc., Tokyo). Complete separation of the lipid mixture was achieved by petroleum ether (bp 313–333 K):diethylether:acetic acid (70:30:2) into triglyceride (TG), fatty acid (FA), 1,3-diglyceride (1,3-DG), 1,2-diglyceride

Table 1
The Main Characteristics of Used Frying Oil

Oil characteristics	
Refractive index, n_D^{293}	1.4758
Acid value (mg KOH/g)	0.65
Saponification value (mg KOH/g)	185
Iodine value (g I_2/100 g)	125.4
Unsaponifiable matters (wt%)	1.37
Moisture (wt%)	0.17
Density, 293 K, ($g \cdot cm^{-3}$)	0.9208
Component fatty acids[a] (wt% of total fatty acids)	
Palmitic	7.9
Stearic	4.7
Oleic	26.0
Linoleic	59.1
Others[b]	2.3

[a]The fatty acid composition was determined by capilar gas chromatography under the following conditions: Column, Ultra 2 25 m×0.32 mm×0.52 μm film thickness of 5% diphenyl, 95% dimethyl polysiloxane; N_2 carrier gas at 2.5 mL/min; air flow of 374 mL/min; hydrogen flow of 27 mL/min; injection, split 50:1, 0.5 μL; injector temp., 473 K; oven temperature program, 303 K (5 min), 303–443 K (278 K/min), 443 K (5 min), 443–473 K (276 K/min), 473 K (30 min); flame ionization detector temperature, 523 K.

[b]Caprylic, 0.3%; Capric, 0.7%; Caproleic, 0.3%; Lauric, 0.2%; Myristic, 0.2%; Myristoleic, 0.4%; Palmitoleic, 0.2%.

(1,2-DG), 2-monoglyceride (2-MG), and 1-monoglyceride (1-MG). The automatic scanning of rods was performed under the following conditions: flow rate of hydrogen, 160 mL/min; flow rate of air, 2000 mL/min; scan speed, 30 s/scan. A standard mixture including TG, FA, 1,3-DG, 1,2-DG, 2-MG, and 1-MG was used for the evaluation purpose and quantitative determination following the procedure of Ranny (*7*).

These procedures were conducted to determine the optimum conditions on the enzymatic hydrolysis reaction. To investigate the optimum temperature, reactions were carried out at 303, 313, 318, 323, and 333 K with keeping seed content at 40% based on oil. The amount of seed required to achieve a degree of hydrolysis above 90% at the optimum temperature in 8-h reaction time was investigated by using 50 and 60% seed content. All reactions were conducted at pH 6.7. To investigate the effect of pH on enzymatic hydrolysis, reaction was conducted at pH 5 and 6 keeping the temperature and seed content at their optimum values. To adjust pH, disodium hydrogen phosphate (1/15 mol/L) and potassium dihydrogen phosphate (1/15 mol/L) buffer solutions (*8*) were used instead of distilled water (pH 6.7).

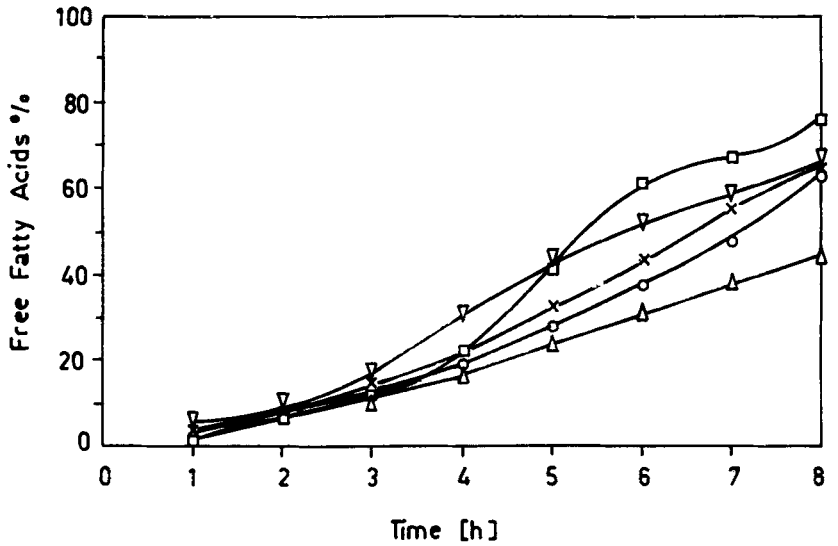

Fig. 1. The effect of temperature on the fatty acid content of hydrolyzed oil. ▽, 60°C; □, 50°C; ×, 45°C; ○, 40°C; △ 30°C.

RESULTS AND DISCUSSION

Effect of Temperature

It is observed that when the seed content is 40% based on oil, the optimum temperature for the hydrolysis of used frying oil by native lipase of *Nigella sativa* seed is 323 K. The increase in process temperature increases the degree of hydrolysis until the lipase activity is affected by high temperatures. After 8 h of reaction time, the hydrolysis degrees at 303, 313, 318, 323, and 333 K with keeping seed content at 40% were calculated as 44.31, 56.29, 62.05, 69.84, and 61.83%, respectively. At 333 K, degree of hydrolysis decreases after 6 h. This might be related mainly to the loss of lipase activity at this temperature. Usually lipases lost their activities at 323–333 K depending on the type of lipase (*9,10*). The effect of temperature on the fatty acid content of hydrolyzed oil was presented by using free fatty acid percentages determined with TLC-FID analyzer in Fig. 1. By determining initial velocities at different temperatures from Fig. 1, the Arrhenius plot was drawn in Fig. 2.

Effect of the Seed Content

The amount of ground seed as a lipolysis catalyst required to achieve a degree of hydrolysis above 90% in 8 h was determined at 323 K and found to be 60% seed. The hydrolysis degrees were calculated as 88.21 and 94.18% at 50 and 60% seed content in 8 h, respectively. The effect of the seed content on the fatty acid yields was shown by using the free fatty acid percentage determined with TLC-FID analyzer in Fig. 3.

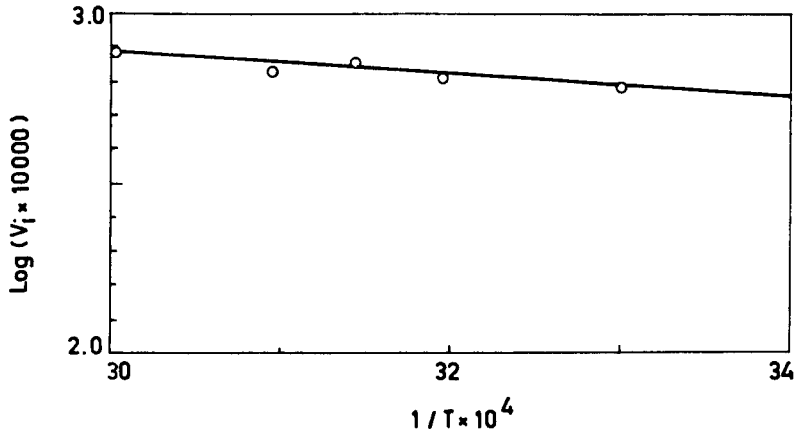

Fig. 2. The effect of temperature on the initial velocities.

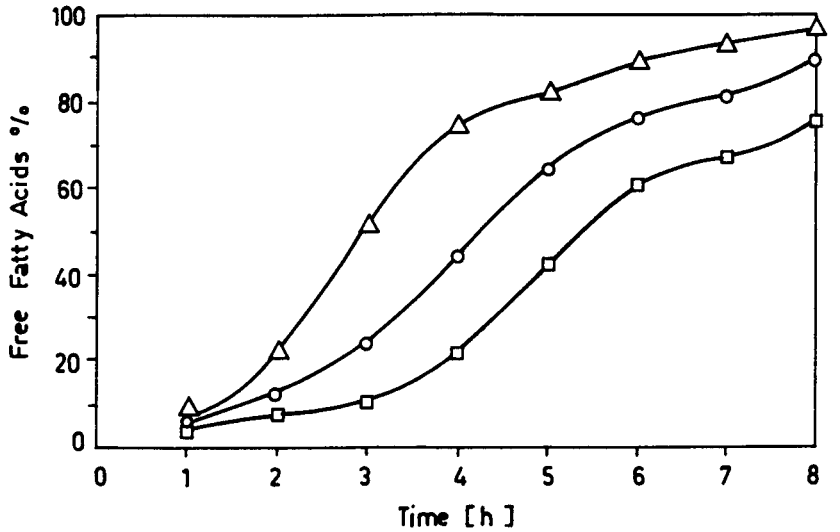

Fig. 3. The effect of seed content on the fatty acid yields. △, 60%; ○ 50%; □, 40%.

Effect of pH

For the reactions conducted with 60% seed content and at 323 K, we observed in parallel investigation that the optimum pH for the hydrolysis of olive oil by *candida rugosa* is around pH 6 (*11*). After 8 h, the hydrolysis degree was calculated as 85.57% at pH 5 and 95.38% at pH 6. The effect of pH on the fatty acid production was presented by using the free fatty acid percentage determined with TLC-FID analyzer in Fig. 4. By determining initial velocities at different pH, at 60% seed content, and at 323 K from Fig. 4, a pH profile was given in Fig. 5. The hydrolyzed oil composition from the reaction conducted with 60% seed content, at 323 K and at pH 6.0, which shows the highest hydrolysis degree, was presented by using the values determined with TLC-FID analyzer in Fig. 6.

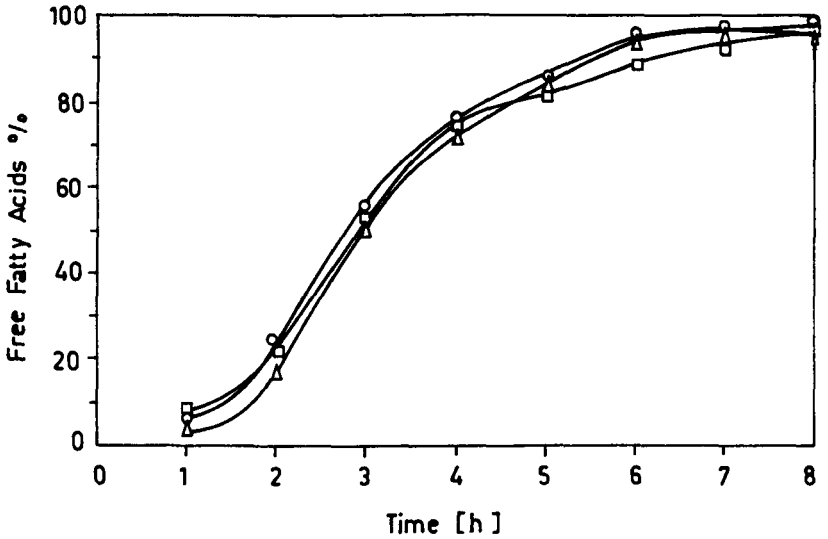

Fig. 4. The effect of pH on the fatty acid production. □, pH 6.7; ○, pH 6.0; △, pH 5.0.

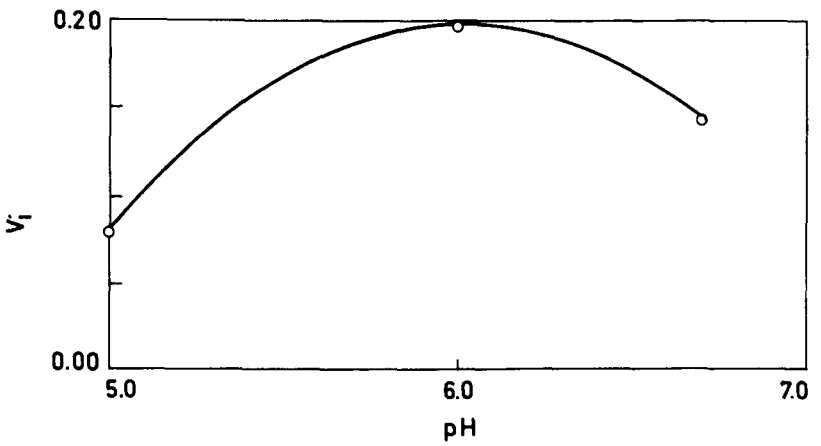

Fig. 5. The effect of pH on the initial velocities.

Kinetics

In order to correlate the experimental data, the integral method was applied. For this purpose, a differential rate equation based on the disappearance of functional groups was constructed by assuming the overall hydrolysis reaction is first order under the applied condition. Based on the above consideration, the following rate equation was used (*12*).

$$\text{Ln } C_o/C = k \cdot t \quad (2)$$

where C_o and C are the concentration of triglyceride at the beginning and at time t, respectively.

To test Eq. 2, Ln C was plotted against time. In this procedure, the weight percentage of TG was used as a concentration, since C_o/C is a ratio

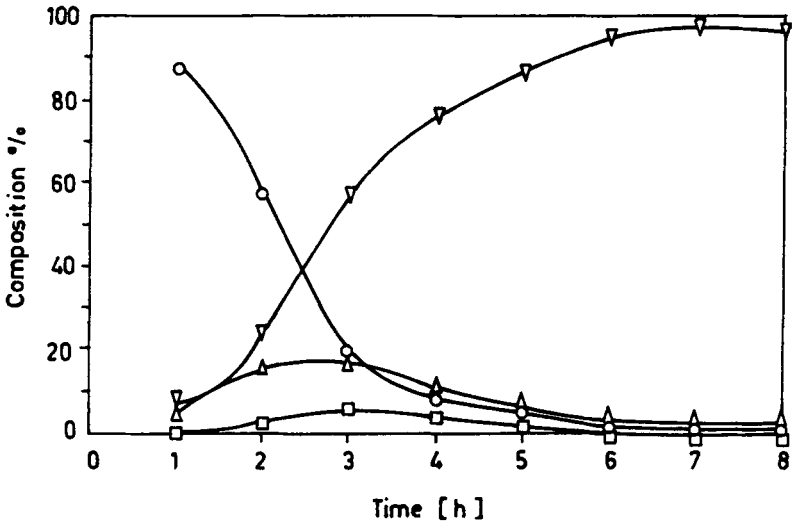

Fig. 6. The hydrolyzed oil composition from the reaction conducted at optimum temperature, seed content, and pH. ○, TG; ▽, FA; △, 1,2- and 1,3-DG; □, 1- and 2-MG.

Table 2
The Overall Rate Constants for Enzymatic Hydrolysis of Used Frying Oil by Native Lipase of *Nigella sativa* Seed (Seed Cont.: 40%, pH: 6.7)

Temperature, K	Rate constant, k, min^{-1}	Coefficient of determination, r^2	Standard error of estimate, s
303	2.210×10^{-3}	0.9828	4.639×10^{-2}
313	2.908×10^{-3}	0.9595	8.479×10^{-2}
318	4.002×10^{-3}	0.9243	1.818×10^{-1}
323	5.622×10^{-3}	0.9630	1.909×10^{-1}
333	4.427×10^{-3}	0.9875	7.915×10^{-2}
323[a]	7.550×10^{-3}	0.9717	2.044×10^{-1}
323[b]	8.966×10^{-3}	0.9664	2.654×10^{-1}
323[c]	1.225×10^{-2}	0.9858	1.845×10^{-1}
323[d]	1.190×10^{-2}	0.9854	1.817×10^{-1}

[a]Seed content: 50%, pH: 6.7.
[b]Seed content: 60%, pH: 6.7.
[c]Seed content: 60%, pH: 5.0.
[d]Seed content: 60%, pH: 6.0.

of concentration and its value is independent of units provided, so that the same units are used for both C_o and C. In fitting straight line to test experimental data, least squares approximation was applied, and in each case, the standard error of estimate (s), correlation coefficient (r), and coefficient of determination (r^2) were determined (*13*).

The effect of temperature, lipase concentration, and pH on the overall enzymatic hydrolysis reaction rate is presented in Table 2. As can be seen,

the reaction followed first-order kinetics under the applied conditions. The activation energy and frequency factor were 26.79 $kJ \cdot mol^{-1}$ and 2.50 $\times 10^2$ min^{-1} at optimum seed content and pH, respectively.

These results suggest that an increase in process temperature and the seed content will increase the rate of fatty acid formation, and the net yield of fatty acids may increase until the lipase activity is affected by high temperature. Thus, the net yield of fatty acids can be maximized, if the process of enzymatic hydrolysis by native lipase of *nigella sativa* seed is carried out at an optimum temperature, seed content, and pH degree.

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