The Kinetics of Hydrolysis of *Nigella sativa* (Black Cumin) Seed Oil Catalyzed by Native Lipase in Ground Seed

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Kinetics of the lipolysis of *Nigella sativa* oil catalyzed by native lipase in crushed seed were studied between 20 and 90°C. Data fitted the pseudo first-order rate equation at 20, 30 and 40°C; and the pseudo second-order equation at 50, 60 and 70°C, but neither equation fit at 80 and 90°C. Lipolysis approximated first-order with respect to water.

KEY WORDS: Hydrolysis, kinetic, lipase, *Nigella sativa*.

Nigella sativa (black cumin) seeds are used for edible and medical purposes. *N. sativa* seed contains 30–35% oil. The presence of lipase in the seed results in enzymatic hydrolysis at ambient temperature during harvesting, handling and processing of oil (1). Therefore, the seed oil often has a free fatty acid content of 40% or higher.

Many investigators have studied the enzymatic hydrolysis of fats and oils as an attractive alternative to the currently used high-pressure and high-temperature processes for the industrial production of fatty acids (2–5). In all the research, lipases of different origin were studied, and the conditions to reach a given high acid value were determined. Lipolysis by native lipases was not employed, and the subject was not investigated in view of the reaction kinetics. However, the hydrolysis of tallow, coconut and olive oil by lipase from *Candida rugosa* showed that these reactions could be approximated by first-order kinetics (6,7).

The purpose of the present study is to develop rate equations for enzymatic hydrolysis of *N. sativa* seed oil by native lipase at several temperatures and moisture contents and, hence, to elucidate the role of process parameters on the reaction rate.

EXPERIMENTAL PROCEDURES

Materials. N. sativa seeds of Turkish origin were purchased locally. The oil content of the seed on a moisture-free basis was 22%, moisture was 5.6%. The free fatty acid content of the seed oil was 2.5%. All the chemicals used for experiments were of analytical grade (Merck, Darmstadt, Germany).

Experimental set-up. Enzymatic hydrolysis by native lipase was carried out in a closed Pyrex petri dish with an i.d. of 14 cm, a height of 2.4 cm and a wall thickness of 0.2 cm.

Enzymatic hydrolysis of Nigella sativa *seed oil in ground seed.* The petri dish was heated in an oven to the desired temperature, filled with ground seeds (600–1400 μm) that had been crushed in a blender and then kept at constant temperature for 8 h. The samples were withdrawn at the predetermined time intervals and pressed immediately with a laboratory-type Carver hydraulic press (Fred S. Carver Inc., Wabash, IN) under 69×10^6 N·m^{-2} (Pa) pressure at ambient temperature; the oil obtained within the first minute was pipetted for analysis. Then 0.1 g oil was added to 10 mL chloroform and analyzed by thin-layer chromatography-flame ionization detection (TLC-FID) analyzer (Iatron Laboratories Inc., Tokyo, Japan). At the same time, the change in the moisture content of the seeds was determined. These procedures were conducted at temperatures between 20 and 90°C with increments of 10°C.

To examine the effect of water content on enzymatic hydrolysis, ground seeds were mixed with water to average moisture contents of 5.00, 9.23 and 14.32%. All reactions were conducted at 50°C.

Analysis of sample. The oil samples (0.1 g oil/10 mL $CHCl_3$, 1 μL) were quantitatively analyzed by the Iatroscan TH-10 TLC-FID analyzer with Chromarod S III rods (Iatron Laboratories) under the following conditions: flow rate of hydrogen, 160 mL/min; flow rate of air, 2000 mL/min; scan speed, 30 s/scan.

Separation of lipid by petroleum benzine/diethyl ether/acetic acid (70:30:2) into triglyceride (TG), fatty acid (FA), 1,3-diglyceride (1,3-DG), 1,2-diglyceride (1,2-DG), 2-monoglyceride (2-MG) and 1-monoglyceride (1-MG) was done according to the procedures of Tanaka (8) and Ranný (9).

RESULTS AND DISCUSSION

The integral method was used to correlate the experimental data. For this purpose, a differential rate equation based on the disappearance of functional groups was constructed by assuming that the reaction was pseudo first-order under the applied conditions. The hydrolysis of triglycerides is a consecutive and reversible reaction. The reaction can be driven to completion by the addition of one of the reactants in excess, or by removal of one of the products.

By assuming that the lipolysis reaction is pseudo first-order with respect to the TG, the following rate equation is obtained:

$$k' \cdot t = \mathrm{Ln}[TG]_o/\mathrm{Ln}[TG] \quad [1]$$

where $[TG]_o$ and [TG] are the concentrations of TG at the initial time and at time t, respectively;

$$k' = k \cdot [W]^n \quad [2]$$

where k′ is the overall pseudo rate constant, k is the overall rate constant or velocity, [W] is the average concentration of water and n is the reaction order with respect to water.

Based on the above considerations, the rate data from the lipolysis reaction in ground seeds would fit with Equation 1. To test this rate equation, Ln[TG] was plotted against time. In this procedure, the weight percentage of TG was used as concentration because $[TG]_o/[TG]$ is a concentration ratio and its value is independent of units, provided that the same units are used for both $[TG]_o$ and [TG]. Least-square approximation was applied, in fitting a straight line to the experimental data, and in each case the standard error of estimate(s), correlation coefficient (r) and coefficient of determination (r^2) were determined.

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As can be seen from Figure 1, the reactions at 20, 30 and 40°C followed pseudo first-order kinetics. On the other hand, for the reactions conducted at 50, 60 and 70°C, there was a decrease in the correlation coefficient and coefficient of determination for the pseudo first-order kinetic model. The coefficient of determination is especially important because the value of $r^2 \times 100$ represents the percentage of original uncertainty as explained by the linear model (10). Figure 2 shows that the reaction at these temperatures fitted the pseudo second-order reaction kinetic model better (shown in Equation 3).

$$k' \cdot t = 1/[TG] - 1/[TG]_o \quad [3]$$

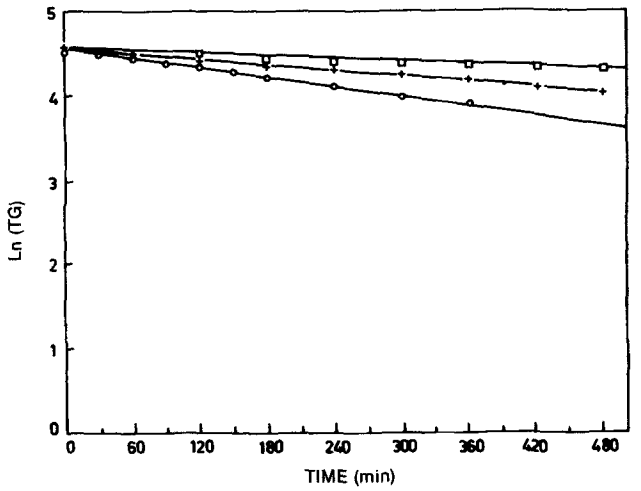

FIG. 1. Determination of the reaction order in the enzymatic hydrolysis of *Nigella sativa* seed oil. □, 20°C; s (standard error of estimate) = 1.9629×10^{-2}, r^2 (coefficient of determination) = 0.9546; +, 30°C; s = 2.2568×10^{-2}, r^2 = 0.9867; ○, 40°C; s = 2.2463×10^{-2}, r^2 = 0.9909.

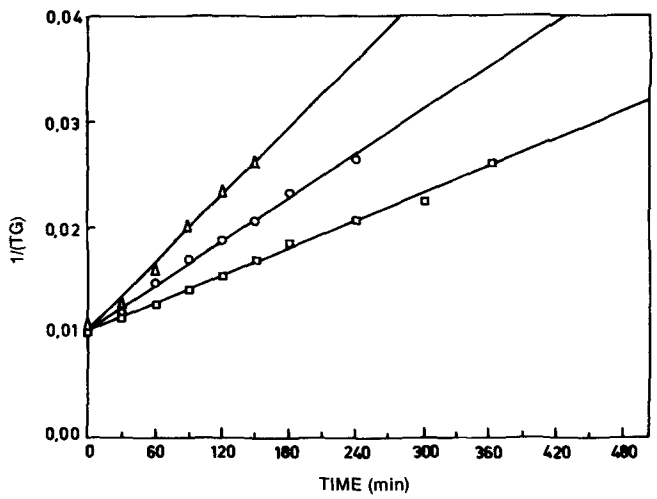

FIG. 2. Determination of the reaction order in the enzymatic hydrolysis of *Nigella sativa* seed oil. □, 50°C; s (standard error of estimate) = 4.1993×10^{-4}, r^2 (coefficient of determination) = 0.9941; ○, 60°C; s = 4.0880×10^{-4}, r^2 = 0.9953; △, 70°C; s = 6.4425×10^{-4}, r^2 = 0.9916.

In view of the difficulty of establishing the concentration in mole per liter at the applied conditions, the TG concentration is expressed as weight percentage.

For the reactions conducted at 80 and 90°C, there was an increase in triglyceride concentration after 3 and 4 h (see Fig. 3), and the data did not fit the pseudo first- or the pseudo second-order reaction rate equation. This might be related mainly to the loss of lipase activity and loss of water from the seed at these temperatures. The water content of seeds varied from 5.6 to 2.76% at 80°C and from 5.6 to 1.72% at 90°C. At these ratios, the equilibrium conversion is much lower and reverse reactions are much faster, because the excess water is less than twice that of theory.

The overall pseudo rate constants determined from the slopes of the straight lines shown in Figures 1 and 2 are presented in Table 1. As mentioned before, the reactions followed pseudo second-order kinetics at 50, 60 and 70°C. This may be due to the change of the reaction mechanism with decreases of lipase activity and water content at these temperatures. Usually, lipases lose their activities at 50–60°C, depending on the type of lipase (11). The change in the reaction order with temperature and the

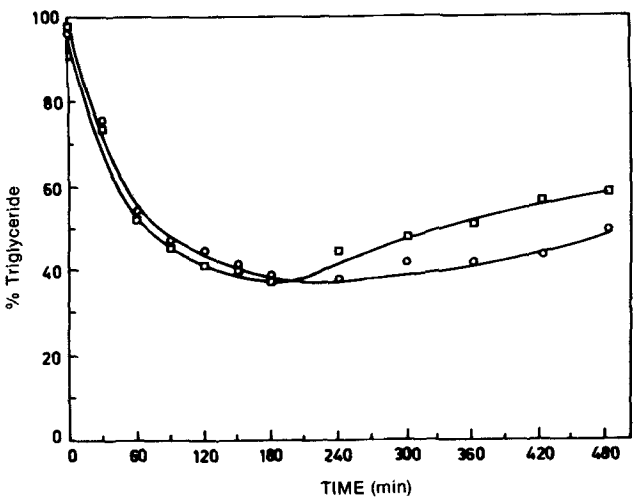

FIG. 3. Change of triglyceride content during the enzymatic hydrolysis of *Nigella sativa* seed oil conducted at 80°C and 90°C. ○, 80°C; □, 90°C.

TABLE 1

The Overall Pseudo Rate Constants for the Enzymatic Hydrolysis of *Nigella sativa* Seed Oil in Ground Seed

Temperature (°C)	Average water concentration (wt%)	Pseudo rate constant,[a] k' $(min)^{-1}$
20	5.60	5.55×10^{-4}
30	5.29	1.13×10^{-3}
40	5.33	1.97×10^{-3}
50	5.00	4.35×10^{-5}
60	4.86	6.99×10^{-5}
70	4.49	1.07×10^{-4}

[a]The units of pseudo rate constants for the enzymatic hydrolysis reactions conducted at 50, 60 and 70°C are $(wt\%)^{-1} \cdot (min)^{-1}$.

extent of the reaction also was observed by others. Freedman *et al.* (12) explained previously that lowering the molar ratio of alcohol to vegetable oil in the transesterification reaction of soybean oil caused a change in the forward reaction order and reduced the conversion. The forward reaction for a 6:1 molar ratio of alcohol to oil followed second-order kinetics, whereas it appeared to be pseudo first-order at a 30:1 molar ratio. Smith and co-workers (13) explained that the esterification of rosin with pentaerythritol in concentrated solution followed second-order kinetics at 260°C, whereas it appeared to be third-order at 300°C. In addition, data from the reaction carried out at 280°C could be fitted to either of the second- or third-order rate equations (13).

To predict the reaction order with respect to the water content, the value of k′ at 50°C was determined at three different concentration levels of water (Fig. 4). The k′ values were 4.35×10^{-5}, 9.15×10^{-5} and 1.48×10^{-4} at an average water content of 5.00, 9.23 and 14.32%, respectively. Equation 2 can be rewritten as follows:

$$\text{Ln } k' = \text{Ln } k + n \cdot \text{Ln } [W] \qquad [4]$$

Lipolysis in ground seed was approximately first-order in water (n ≅1). This result can be used to understand the effect of water concentration on the net yield of fatty acids by lipolysis. A similar approach was applied in the kinetic study of oxirane cleavage in epoxidized soybean oil, which was modelled as first-order with respect to the epoxide concentration, and second-order with respect to the acetic acid concentration (14).

The activation energy and the frequency factor were 12.00 kcal·mol^{-1} and 9.21×10^4 min^{-1} for the first-order reactions, and 10.98 kcal·mol^{-1} and 2.32×10^2 (wt%)$^{-1}$·(min)$^{-1}$ for the second-order reactions, respectively.

These results suggest that an increase in process temperature will increase the rate of fatty acid formation, and the net yield of fatty acids may increase unless the lipase activity and the moisture content are unaffected by high temperatures. Thus, the net yield of fatty acids can be maximized if the lipolysis process in ground seed is carried out at optimum temperature and water concentration.

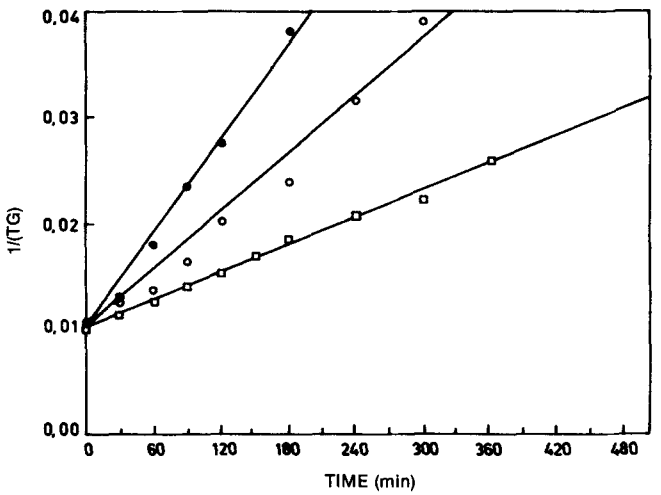

FIG. 4. Determination of the overall pseudo rate constant, k′ at different concentrations of water at 50°C. □, 5%; s (standard error of estimate) = 4.1993 × 10^{-4}, r^2 (coefficient of determination) = 0.9941; ○, 10%; s = 1.6951 × 10^{-3}, r^2 = 0.9834; ●, 15%; s = 1.1654 × 10^{-3}, r^2 = 0.9896.

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