Solvent-Free Glycerolysis Catalyzed by Acetone Powder of *Nigella sativa* Seed Lipase

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ABSTRACT: The solvent-free glycerolysis of used sunflower oil catalyzed by acetone powder of *Nigella sativa* seeds was investigated. The highest partial acylglycerols yield was obtained at 60°C. The glycerolysis reactions, conducted at molar ratios of 1:1, 2:1, and 3:1 of oil to glycerol keeping the acetone powder content at 30% based on oil weight and the temperature at 60°C, approached equilibrium after 2 h. The highest partial acylglycerol content of the products was 66% (1:1 molar ratio) and 60% (2:1 molar ratio).

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KEY WORDS: Acetone powder, diacylglycerol, glycerolysis, lipase, monoacylglycerol, *Nigella sativa* seed.

Partial acylglycerols are used as emulsifiers in food and pharmaceutical industries (1,2). Addition of diacylglycerols (DG) into triacylglycerols (TG) makes oil more hydrophilic. DG is also used in the food industry as an estranger oil to separate materials from molds easily and as an adjuster of fat crystals (2). The widely used commercial process for the production of partial acylglycerols is glycerolysis of oils that consists of heating the glycerol with oils or fats at temperatures greater than 220°C in the presence of an alkaline catalyst (3–6). Enzyme-catalyzed glycerolysis reactions are superior to these conventional chemical glycerolysis methods because enzymes are catalytically active at relatively low temperatures.

The use of lipase enzymes to catalyze the glycerolysis reactions is being actively investigated by many workers. Enzymatic glycerolysis reactions were carried out using solvent or in solvent-free systems and in aqueous and nonaqueous microemulsions (2–6). McNeill *et al.* (3) developed the solvent-free glycerolysis of fats and oils giving a high yield of monoacylglycerols (MG) and showed that the yield of MG is strongly influenced by the reaction temperature and lipase type. The yield of DG prepared by the reaction of hydrogenated beef tallow and glycerol in the presence of a *Pseudomonas* lipase depended strongly on the reaction temperature and glycerol-to-TG oil molar ratio (2). The investigated commercially available lipases (E.C. 3.1.1.3) were nonspecific TG lipases such as *Pseudomonas* sp. and *Chromobacterium viscosum* and 1,3-specific lipases such as *Rhizopus niveus, R. delamer,* and *Mucor miehei* (3–7). Lipases are present in oil seeds and cereal grains (8,9). In the earlier literature, lipase preparations and acetone powder obtained from oil seeds such as castor bean, rape, and *Nigella sativa* seeds were used as biocatalysts for hydrolysis and esterification of lipids and esterification of oleic acid with methanol and glycerol (9–16). The catalytic effect of native lipase of *N. sativa* seeds in the hydrolysis and esterification reactions was first investigated by us (12–15). Ground and pressed *N. sativa* seeds can be used as a lipase source for the oleochemical reactions. Acetone powder of *N. sativa* seed catalyzes the oil hydrolysis. Hassanien and Mukherjee (10) observed that acetone powder from seedlings has a lipase activity as high as the undialyzed crude lipase preparation. The preparation of acetone powder is simple, making it quite suitable for technical use.

The aim of this present work was first to investigate the possible application of acetone powder of *N. sativa* seed for glycerolysis of TG oils. However, we want to show the usability of a waste oil "used sunflower oil" for the production of partial acylglycerols.

EXPERIMENTAL PROCEDURES

Materials. Nigella sativa seeds of Turkish origin were purchased from an herbal shop in Istanbul, Turkey. The oil content of seed in dry basis was 41.6%, and moisture content was 6.7%. The vegetable oil "used sunflower oil," which was used twice for frying of vegetables like potato, pepper and eggplant, was obtained from the campus cafeteria, filtered and used directly without any special purification. The characteristics of the oil were as follows: acid value: 2.32; iodine value: 117.82; and saponification value: 194. The oil consisted of 9.5% palmitic, 5.6% stearic, 31.1% oleic, and 53.8% linoleic acids. All other chemicals were analytical grade (Merck, Darmstadt, Germany).

Experimental setup. Glycerolysis reactions were carried out in a three-necked flask (250 mL) with mechanical stirring. Lipase preparation "Acetone powder" of *N. sativa* seeds was prepared in a blender (Braun Type MX82, Frankfurt, Germany). A laboratory-type minor centrifuge BA-6297 (MSE, London, England) was used for separation of glycerolysis product from unreacted glycerol and lipase source.

Preparation of acetone powder. Acetone powder was precipitated from original *N. sativa* seeds or from the seeds homogenized with buffer solution. For the preparation of ace-

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TABLE 1
The Change of the Product Composition of Glycerolysis Catalyzed by Acetone Powder of Original *Nigella sativa* Seed as a Function of Reaction Time [40°C, oil/glycerol (96%) molar ratio: 1:2, 30% acetone powder][a]

Reaction time (h)	Composition (wt%)					
	TG	FA	1,3-DG	1,2-DG	1-MG	2-MG
0	82.3	6.7	6.8	2.2	1.9	0
1	68.7	9.8	9.0	4.5	3.0	4.9
2	66.9	6.8	7.3	7.5	6.2	5.3
3	65.4	8.0	13.1	4.4	5.1	4.0
4	62.4	12.2	11.2	5.3	4.8	3.8
5	63.2	12.4	12.0	5.7	4.1	2.6
8	62.1	10.4	12.2	5.9	5.8	3.5

[a]TG, triacylglycerol; FA, fatty acid; DG, diacylglycerol; MG, monoacylglycerol.

tone powder of original seeds, a method similar to that developed by Afolabi *et al.* (16) was applied. For the preparation of acetone powder from seeds pretreated with buffer solution, original seeds (100 g) and buffer solution (pH 6, 100 mL) were homogenized in the blender for 3.5 min at ambient temperature. The homogenate was vacuum-filtered. The pH value of buffer solution did not change during the homogenization. The solid residue was stored at 4°C for 16 h and then blended with 200 mL cooled acetone (4°C) in the blender for 1.5 min for the precipitation of acetone powder using the method of Afolabi *et al.* (16). The acetone powder yields of original and pretreated seeds were 12.8 and 22.6%, respectively. The buffer solution was prepared with stock solutions "potassium dihydrogen phosphate" (1:15 M) and "disodium hydrogen phosphate" (1:15 M) (17).

Glycerolysis reactions. Oil and glycerol at different molar ratios were added to a three-necked flask (250 mL) and heated to the reaction temperature. The stirring rate was adjusted to 500 rpm. At the reaction temperature, the acetone powder was added to the mixture. Unless otherwise stated, acetone powder used as catalyst was precipitated from seed pretreated with buffer. Approximately 2-mL samples that consisted of acetone powder, unreacted glycerol, and glycerolysis product were withdrawn at selected time intervals and placed in a 90°C water bath for 15 min to inactivate the enzyme. The samples were centrifuged to separate the oil phase from the acetone powder. Then the oil phase was transferred into a centrifuge tube containing distilled water in equal volume of the oil phase, mixed, and recentrifuged. Next, the aqueous phase was separated. Washing of glycerol was repeated twice. Oil phase was dried adding the anhydrous Na_2SO_4 until the oil phase was clear. The oil phase was not dissolved in a solvent for the separation of glycerol. The amount of anhydrous Na_2CO_3 was not measured. The glycerolysis product was investigated for TG, 1,3-DG, 1,2-DG, 1-MG, 2-MG, and fatty acids (FA) by thin-layer chromatography–flame-ionization detection Iatroscan TH-10 analyzer with SIII rods (Iatron Lab. Inc., Tokyo, Japan). Samples (1 μL) (0.1 g/10 mL chloroform) were applied to the rod followed by development in petroleum ether (b.p.: 40–60°C)/diethyl ether/acetic acid (70:30:2) solvent. The rods were dried and scanned as described in our previous study (12).

TABLE 2
The Change of the Product Composition of Glycerolysis Catalyzed by Acetone Powder of *N. sativa* Seed Pretreated with Buffer Solution (pH: 6) as a Function of Reaction Time [40°C, oil/glycerol (96%) molar ratio: 1:2, 30% acetone powder][a]

Reaction time (h)	Composition (wt%)					
	TG	FA	1,3-DG	1,2-DG	1-MG	2-MG
0	85.4	5.2	5.7	2.2	—	1.4
1	69.1	11.0	11.6	5.8	2.5	—
2	59.3	13.5	12.4	6.7	4.6	3.5
3	54.9	14.5	14.6	7.2	5.9	2.8
5	48.2	15.8	17.4	7.3	7.8	3.4
6	42.2	19.4	18.3	8.2	8.7	3.2
8	35.9	20	19.1	8.8	11.7	4.5

[a]See Table 1 for abbreviations.

RESULTS AND DISCUSSION

These procedures were conducted to determine whether *N. sativa* seed lipase "Acetone powder" would catalyze the glycerolysis reaction and the optimal conditions for the production of partial acylglycerols. Two sets of experiments were carried out. To investigate the catalytic activity of acetone powder, the glycerolysis reaction was conducted at 40°C, 1/2 oil/glycerol (96%) molar ratio keeping the acetone powder content at 30% based on oil weight. In the second set of experiments, to develop the possible applications of acetone powder in the glycerolysis reactions, the effect of process parameters such as temperature, oil/glycerol molar ratio, and water content of glycerol on the reaction was investigated.

Catalytic effect of lipase preparations. Enzymes are amphoteric molecules containing acidic and basic groups, situated mainly on the surface, with charges varying with the pH of their environment—thus affecting the total net charge of the enzyme, the distribution of charge on its exterior surface, and the reactivity of the catalytically active groups (18). Therefore, acetone powder of *N. sativa* seed was precipitated either from original or pretreated seeds with phosphate buffer solution of pH 6, which was determined as optimal pH for the hydrolysis reaction in our previous study (12). Tables 1 and 2 show the change in the composition of glycerolysis product during the reactions catalyzed with acetone powder in different types. As expected, pretreatment of seeds with buffer solution increased the catalytic activity of acetone powder and after 8 h glycerolysis product consisted of 35.9% TG, 20% FA, 19.1% 1,3-DG, 8.8% 1,2-DG, 11.7% 1-MG, and 4.5% 2-MG.

Effect of temperature. To investigate the effect of temperature, the reaction was conducted at 40, 50, 60, and 70°C at the oil/glycerol (96%) molar ratio of 1:2 and 30% acetone powder content based on oil weight. The effect of temperature on the composition of glycerolysis product as a function of reaction time is shown in Figure 1. As can be seen, the increase in process temperature increased the conversion of TG until the

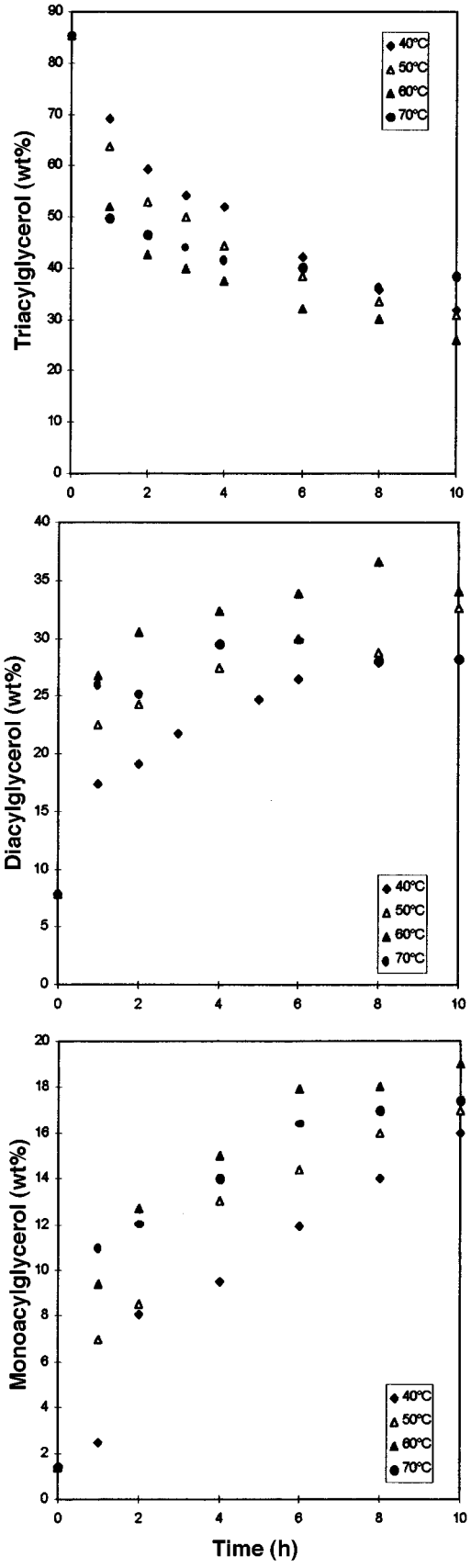

FIG. 1. The effect of temperature on the composition of glycerolysis product.

TABLE 3
Product Composition of the Glycerolysis of Used Oil Using 30% Acetone Powder Based on Oil Weight at 60°C After 4 h Reaction Time[a]

Oil/glycerol molar ratio	Composition (wt%)					
	TG	FA	1,3-DG	1,2-DG	1-MG	2-MG
1:3	55.1	10.7	13.7	7.9	7.1	5.5
1:2	37.4	15.3	23.4	9.0	11.9	3.0
1:1	14.3	19.8	23.3	10.4	21.4	10.8
2:1	14.8	24.8	22.6	8.8	16.6	12.4
3:1	17.3	26.8	21.4	8.2	14.1	12.1

[a]See Table 1 for abbreviations.

lipase activity was affected by high temperature. The optimal temperature was 60°C with the highest DG and MG yields. At 70°C the initial rates of the disappearance of TG and the appearance of total DG and MG were as high as at 60°C. After 2-h reaction, the conversion rate of TG decreased, because the lipase activity decreased at 70°C. In our previous studies, where we investigated hydrolysis of used sunflower oil after usage twice for frying of vegetables and esterification of glycerol with FA catalyzed by native lipase of *N. sativa* seed, loss of lipase activity was observed above 50 and 55°C (12,13).

Effect of oil/glycerol molar ratio. The reactions were carried out with glycerol of 96% at oil/glycerol molar ratios ranging from 1:3 to 3:1 at 60°C with a reaction time of 10 h. At oil/glycerol molar ratios of 1:1, 2:1 and 3:1, the reaction approached equilibrium after 2 h. The product composition at 4 h after glycerolysis reaction as a function of oil/glycerol molar ratio is shown in Table 3. TG content of the product decreased from 55.1 to 14.3% when the oil/glycerol molar ratio increased from 1:3 to 1:1. The highest DG (33.7%) and MG (32.2%) yields were obtained at the molar ratio of 1:1. DG content of the products changed from 21.6 to 33.7%, whereas MG content increased from 12.6 to 32.2% when the molar ratio increased from 1:3 to 1:1. Increase of the molar ratio from 1:1 to 3:1 decreased the DG and MG contents of the product. FA contents of the products were high (10.7–26.8%).

Effect of acetone powder content. The effect of acetone powder content was investigated at 1:1 and 2:1 molar ratios at 60°C, keeping the acetone powder content 20, 25, and 30%. The change of the TG content of the product as a function of the reaction time is shown in Figure 2. As can be seen, the increase of the acetone powder increased the rate of the TG conversion. In the reactions conducted at 1:1 molar ratio, 30% acetone powder and 2:1 molar ratio, 25% acetone powder, the initial rates of TG conversion and partial acylglycerol formation were similar. After 4 h reaction time, total partial acylglycerol contents of the products were 65 and 66%.

Effect of water content. The effect of water content on the glycerolysis reaction was investigated using glycerol of 92 and 96% and 25% acetone powder at 2:1 molar ratio. The highest DG and MG contents and the highest TG conversion rate were observed in the reaction conducted with glycerol of 96% (Fig. 3). The MG and DG yields were 32.6%.

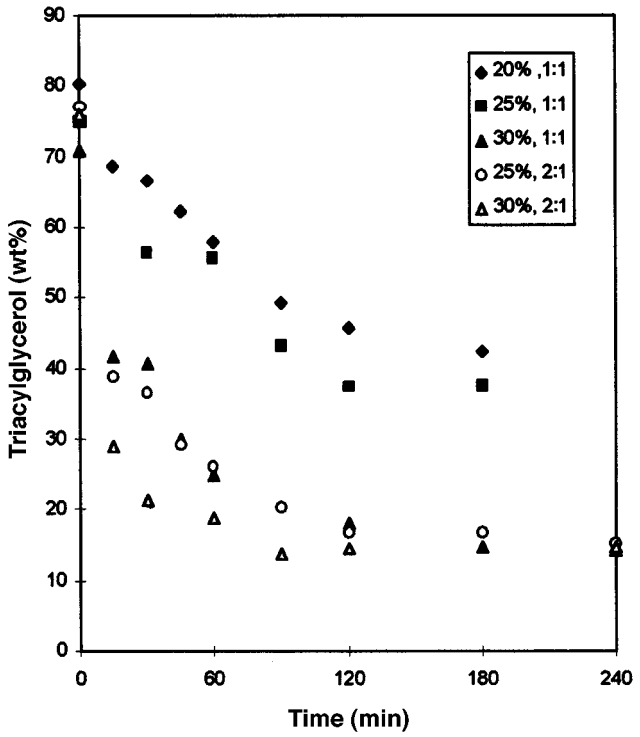

FIG. 2. The effect of acetone powder content on the glycerolysis reaction at oil/glycerol ratios of 1:1 and 2:1.

In our previous studies where we observed various reactions including ester hydrolysis, esterification of oleic acid with glycerol and methanol can be catalyzed by native lipase of *N. sativa* seed, which seems to be a nonspecific lipase (14). This work shows that acetone powder of *N. sativa* seed catalyzes the glycerolysis of vegetable oils. A comparison of our results with the commercial glycerolysis processes and the solvent-free enzyme-catalyzed glycerolysis reactions that are conducted at above the critical temperatures of oils from the literature shows that the yield of DG and MG is as high as that of commercial glycerolysis processes. As known, the yield of MG, which are manufactured by the glycerolysis reaction of fats and oils at temperatures greater than 220°C in the presence of an inorganic catalyst, is 30–40%. However, in the case of reacting several TG oils with glycerol using lipase as catalyst at the temperatures higher than the critical temperatures of oils, *ca.* 30% MG was produced at equilibrium (4).

The highest MG yield determined in this study (32.6%) shows that MG yield of the reaction catalyzed by acetone powder of *N. sativa* seed can be higher when the reaction is conducted below the critical temperature of oil. As known, at the critical temperature the solubility of MG in the reaction mixture is low, causing the crystallization of MG to shift the equilibrium toward synthesis of more MG.

The yield of 1,3-DG is *ca.* 2.5 times of 1,2-DG and the 1-MG yield is 1.5 times of 2-MG, supporting the fact that the *N. sativa* seed lipase is nonspecific under these investigated reaction conditions.

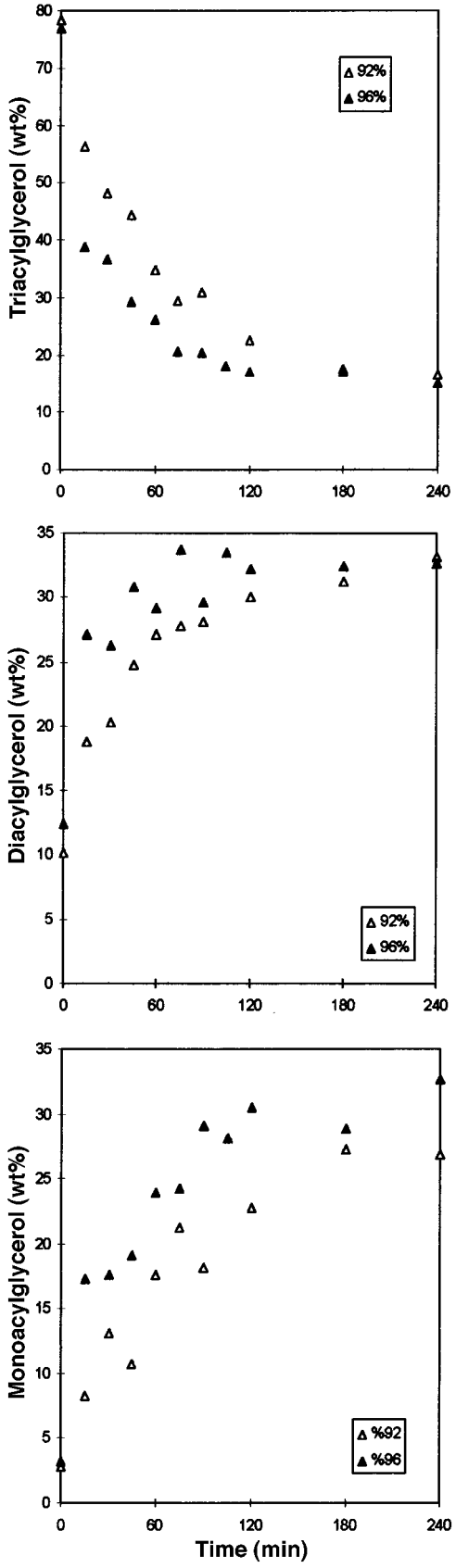

FIG. 3. The effect of water content of 8% (92% glycerol) and 4% (96% glycerol) on the composition of product.

Further experiments will be carried out to study the glycerolysis catalyzed by acetone powder using different solvents. The nature of solvent may have an effect on substrate specificity, as well as on the activity. Acetone powder of *N. sativa* seed may be a possible alternative to the commercially produced lipases.

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