Castor Bean Lipase as a Biocatalyst in the Esterification of Fatty Acids to Glycerol

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ABSTRACT: Castor bean lipase was investigated as biocatalyst in the esterification of fatty acids to glycerol. For this purpose, pressed seeds were pretreated with phosphate–citrate buffer solution at optimal preincubation time and pH and used as a lipase source in esterification of fatty acids with glycerol. The effect of process parameters in the esterification, i.e., molar ratios of reactants, temperature, water content of glycerol and concentration of lipase, were determined by using pretreated castor bean.
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KEY WORDS: Castor bean, esterification, lipase, lipase activity, oleic acid.

Esters of glycerol with fatty acids (FA) occur naturally in fats and oils, and they are widely used for edible and industrial purposes. Modification of fats and oils and production of glycerides from glycerol and FA promise to produce new oil and fat compounds that possess new properties in comparison to the original materials. Conventional esterification of glycerol to produce mono- (MG), di- (DG), and triglycerides (TG) by chemical catalysts requires high temperatures and leads to dark-colored products and undesired by-products (1). Enzymes to catalyze these reactions are superior to conventional chemical methods owing to mild reaction conditions, high catalytic efficiency, and inherent selectivity, which results in much purer products. The use of lipolytic enzymes to catalyze the esterification reaction has been investigated by many workers (2–8). Lipases are present at high activity in reserve tissue of many oilseed plants (9). Some lipases only catalyze the hydrolysis reaction of fats and oils while others show catalytic activity on both the hydrolysis and esterification reactions. In contrast to most oilseeds that develop lipase activity during seed germination, castor bean contains an active triacylglycerol lipase even in the dormant seed (10). Lipase preparations from castor bean have been used to hydrolyze fats for the preparation of FA (11–14).

In this study, development of the catalytic activity of castor bean for the esterification of FA with glycerol was studied, and the effects of process parameters, such as reactant ratio, temperature, concentration of lipase, and water content of glycerol, were investigated.

EXPERIMENTAL PROCEDURES

Materials. Castor beans of Turkish origin were obtained from Adana Agriculture Faculty (Adana, Turkey). The oil content of seeds was 45%, and the moisture level was 5.8%. Glycerol and oleic acid were purchased from Alemdar Chemical Co. (Istanbul, Turkey). FA and glycerol were used directly without further purification. The acid value of FA was 202.47, and the mixture contained 6.1% palmitic, 1.97% stearic, 79.2% oleic, 9.6% linoleic, and 3.04% other acids ($C_{8:0}$, $C_{10:0}$, $C_{12:0}$, $C_{14:0}$, $C_{16:1}$). All other chemicals used were analytical grade (Merck, Darmstadt, Germany). Buffer solution was prepared with 0.2 M disodium phosphate and 0.1 M citric acid stock solution (15).

Apparatus. Enzymatic esterification reactions were carried out in a three-necked flask (250 mL), equipped with a sampling pipette and a temperature controller. The stirring rate was adjusted to 1100 rpm.

The data presented are the averages of duplicate determinations, which varied by no more than 0.1%.

Preparation of pretreated castor bean lipase. In all experiments, decorticated castor beans were ground in a mortar and pressed with a laboratory-type Carver hydraulic press (Fred S. Carver Inc., Wabash, IN) at ambient temperature under the following pressure program: 69×10^6 N m^{-2} (15 min), 103.5×10^6 N m^{-2} (20 min), 138×10^6 N m^{-2} (20 min). The pressed residue obtained was kept in phosphate–citrate buffer solution at different pH values and different time periods at 4°C. The suspension was vacuum-filtered and dried at ambient temperature for 24 h and used as the lipase source.

Enzymatic esterification of glycerol with FA. For the esterification reactions conducted at stoichiometric molar ratio of glycerol/FA, 5.5 g glycerol and 50 g FA were placed into the reaction flask and heated to the reaction temperature with stirring. In subsequent experiments, in which the effect of molar ratio of glycerol/FA was investigated, the total weight was always kept constant as 55 g. The pretreated beans were added to the flask as a catalyst. The coproduced water was allowed to freely evaporate. For this purpose, the reaction flask was left uncovered at all times. Samples 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 lipase source, and the oil phase was transferred

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into a centrifuge tube that contained distilled water to separate the glycerol phases and recentrifuged.The oil phase was dried with anhydrous Na_2SO_4. The composition of the ester product was investigated with a thin-layer chromatograph–flame-ionization detector (TLC–FID) Iatroscan TH-10 analyzer with SIII rods (Iatron Lab. Inc., Tokyo, Japan). Complete separation of the lipid mixture was achieved by petroleum ether (bp 40–60°C)/diethyl ether/acetic acid (70:30:2) into TG, FA, 1,3-DG, 1,2-DG, 1-MG, and 2-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 of TG, FA, 1,3-DG, 1,2-DG, 1-MG, and 2-MG was used for evaluation and quantitative determination by following the procedure of Ranny (16). These procedures were conducted to determine whether lipase of the pretreated castor beans would catalyze the esterification of FA with glycerol and the optimal conditions for the enzymatic esterification reaction.

RESULTS AND DISCUSSION

Effect of preincubation time. To determine the effect of preincubation time, pressed castor beans (12 g) were kept in phosphate–citrate buffer solution (200 mL) at pH 5 for different time periods (2, 4, 6, 8, and 16 h) and then used as lipase source in the esterification reaction conducted at a stoichiometric molar ratio of glycerol/FA (1:3). The lipase source content was kept at 20% based on total weight, and temperature was 35°C during 24-h reaction period. The product compositions as a function of preincubation time are shown in Figure 1. As can be seen, when seeds were preincubated in buffer solution for 4 h, the conversion of FA to glycerides was greatest. This product contained 23.82% TG, 30.71% DG, 7.98% MG, and 37.49% FA.

Effect of preincubation pH. To investigate the effect of preincubation pH, pressed seeds were kept in buffer solution at different pH values (3, 4, 5, and 6) for 4 h, then used as a lipase source in the esterification reaction, conducted under the same conditions as described above. The product composition as a function of preincubation pH is shown in Figure 2. The ester product with the highest TG content (30.93%) was observed for preincubated seed at pH 4. This product contained 30.95% TG, 31.18% DG, 2.98% MG, and 34.89% FA. All enzymes have an optimal pH range for activity. This might be related mainly to the change in the ionization state of amino acid side chains that are essential for the catalytic activity of the enzyme (17,18).

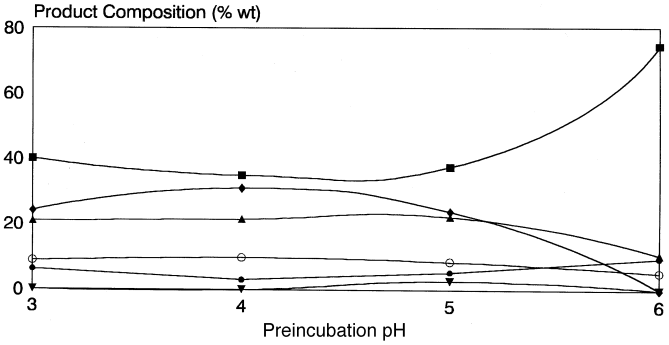

FIG. 2. The effect of preincubation pH on product composition (temperature: 35°C; pretreated seed content: 20%; glycerol/FA: 1:3; preincubation time: 4 h, reaction time: 24 h). ◆: TG, ■: FA, ▲: 1,3-DG, ○: 1,2-DG, ▼: 1-MG, ●: 2-MG. See Figure 1 for abbreviations.

When unpretreated seeds were used directly as lipase source under the same esterification conditions, TG production was not observed, and the MG content was only 2%. The esterification product from the reaction conducted with pretreated seeds that were preincubated at pH 4 for 4 h showed the highest TG synthesis. For these reasons, pretreated seeds incubated under these conditions were used as the lipase source in the esterification reactions below to determine optimal reaction conditions.

Effect of pretreated seed content. To determine the effect of pretreated seed content, reactions were conducted at stoichiometric glycerol/FA acid molar ratio and 35°C with pretreated seed content at 10, 20, 30, and 40% based on total weight. The results are presented in Figure 3. The TG content was increased by increasing pretreated seed content up to

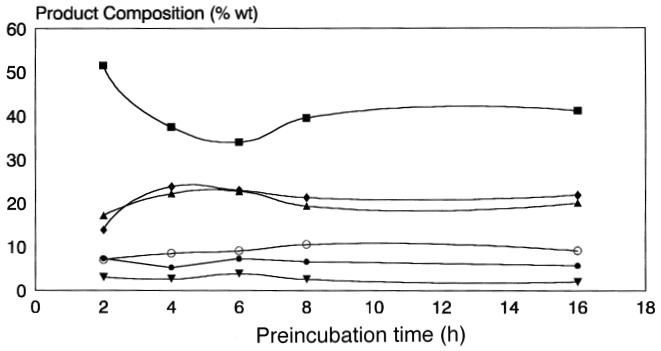

FIG. 1. The effect of preincubation time on product composition [temperature: 35°C; pretreated seed content: 20%; glycerol/fatty acid (FA): 1:3; preincubation pH: 5; reaction time: 24 h]. ◆: Triglyceride (TG); ■: FA; ▲: 1,3-diglyceride (DG); ○: 1,2-DG; ▼: 1-monoglyceride (MG); ●: 2-MG.

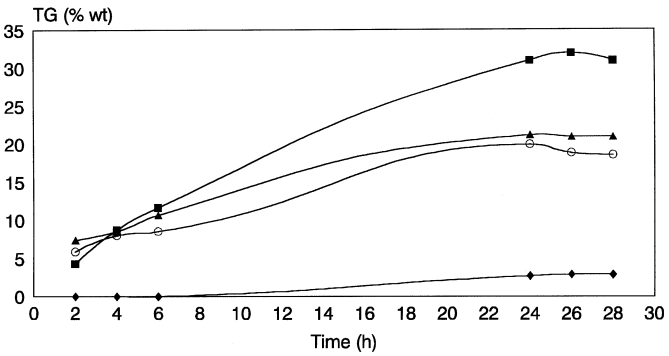

FIG. 3. Comparison of the TG contents of the products that were produced with different lipase content as a function of reaction time (temperature: 35°C; glycerol/FA: 1:3; preincubation time: 4 h; preincubation pH: 4; ◆: 10%; ■: 20%; ▲: 30%; ○: 40%. See Figure 1 for abbreviations.

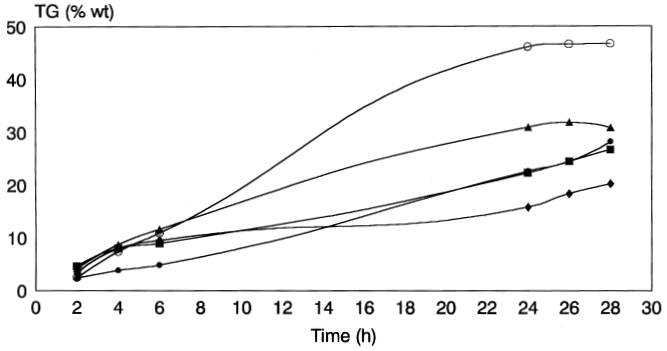

FIG. 4. Comparison of the TG contents of the products that were produced with different temperatures as a function of reaction time (pretreated seed content: 20%; glycerol/FA: 1:3; preincubation time: 4 h; preincubation pH: 4). ◆: 25°C, ■: 30°C, ▲: 35°C, ○:40°C, ●: 45°C. See Figure 1 for abbreviations.

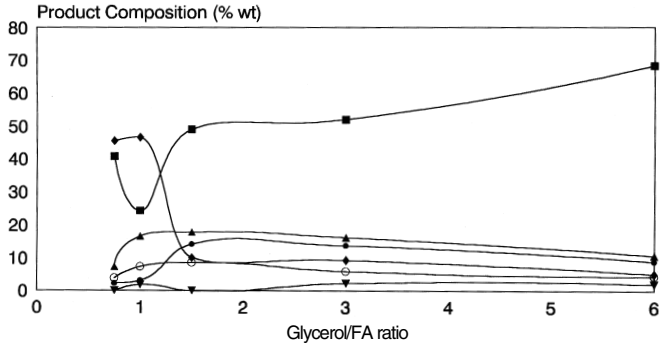

FIG. 5. Comparison of product composition as a function of glycerol/FA ratio (temperature: 40°C; preincubation time: 4 h; preincubation pH: 4; pretreated seed content: 20%; reaction time: 28 h). ◆: TG, ■: FA, ▲: 1,3-DG, ○: 1,2-DG, ▼: 1-MG, ●: 2-MG. See Figure 1 for abbreviations.

20%, then it decreased. This might be related to the excess solid material in the reaction medium causing difficulty in stirring the reaction medium. The best glyceride synthesis was observed with the reaction at 20% pretreated seed content. This product contained 30.93% TG, 31.18% DG, 2.98% MG, and 34.89% FA.

Effect of temperature. The effect of temperature was investigated at a stoichiometric glycerol/FA molar ratio and at 20% pretreated seed content while keeping temperature at 25, 30, 35, 40, or 45°C. When the reactions were conducted at 40°C, we observed the highest TG content (46.66%). This ester product contained 23.99% DG, 4.97% MG, and 24.37% FA. The results are shown in Figure 4. The increase in reaction temperature increased FA conversion until lipase activity is affected by high temperature. When the reaction was conducted at 45°C, FA conversion was decreased. This might be related to the loss of lipase activity at this temperature. The temperature dependence of enzyme-catalyzed reactions exhibits an optimum, and the optimum lies generally between 40 and 60°C (19).

Effect of glycerol/FA ratio. To determine the effect of molar ratio, reactions were conducted at ratios of 0.75, 1.0, 1.5, 3.0, and 6.0 of glycerol/FA (1.0 is equal to stoichiometric ratio) while keeping pretreated seed content and temperature at their optimal values during the 28-h reaction period. The product composition as a function of glycerol/FA molar ratio and the effect of glycerol/FA molar ratio on TG synthesis as a function of reaction time are shown in Figures 5 and 6, respectively. The stoichiometric ratio was the optimal ratio for the esterification product with the highest triolein synthesis (46.66%). This ester product contained 23.99% DG, 4.97% MG, and 24.37% FA. At a glycerol/FA ratio between 1 and 1.5, TG yield decreased with increasing DG and MG content, and at ratios above 1.5, TG, DG, and MG yields remained constant with increasing FA content. These results suggest that the desired glyceride composition would be obtained at different molar ratios of glycerol to FA. Similar results were also observed by other researchers (20,21).

Effect of water content. For the investigation of the effect of water content, the reactions were conducted at the determined optimal conditions with glycerol that contained 13, 25, and 35% water based on weight. The results are presented in Figure 7. The highest TG (52.37%) synthesis was obtained with glycerol having 13% water. When the water content of glycerol was above 13%, TG content decreased. Similar results were observed by other researchers (22).

All these results suggest that pretreated castor bean could be used as a catalyst in esterification of FA with glycerol, and that the net yield of TG can be maximized if the process of enzymatic esterification by pretreated castor bean lipase is carried out at optimal temperature, seed content, glycerol/FA molar ratio, and water content.

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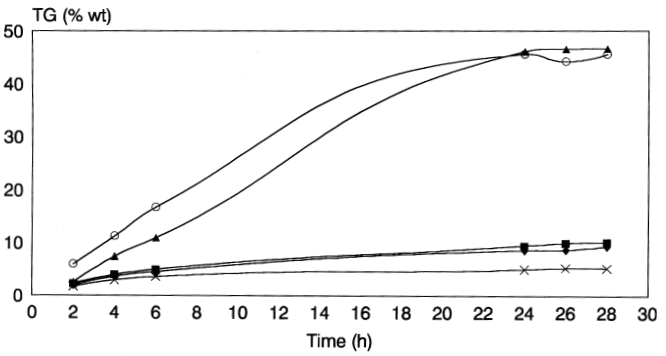

FIG. 6. The effect of glycerol/FA molar ratio on the synthesis of TG as a function of reaction time (temperature: 40°C; preincubation time: 4 h; preincubation pH: 4; pretreated seed content: 20%). ◆: 1:1, ■: 1:2, ▲: 1:3, ○: 1:4, ×: 2:1. See Figure 1 for abbreviations.

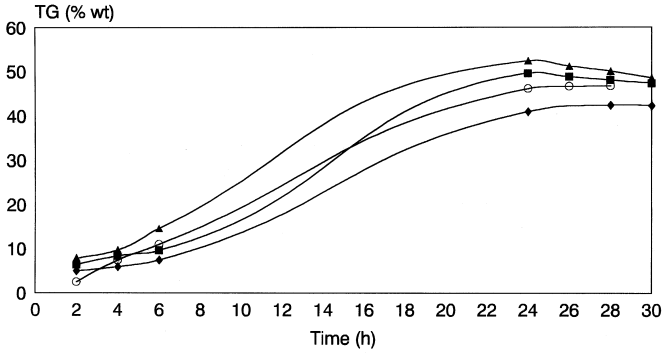

FIG. 7. The effect of water content of glycerol on the synthesis of TG as a function of reaction time (temperature: 40°C; glycerol/FA: 1:3; pretreated seed content: 20%; preincubation time: 4 h; preincubation pH: 4). ◆: 35%, ■: 25%, ▲: 13%, ○: anhydrous. See Figure 1 for abbreviations.

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