Reactions of Olive Oil and Glycerol over Immobilized Lipases

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ABSTRACT: The reaction of olive oil and glycerol over immobilized lipases was studied. For oil samples with free fatty acid (FFA) contents larger than 2%, FFA esterification and glycerolysis took place simultaneously, but the esterification reaction was faster than glycerolysis. Similar product distributions were obtained for glycerol/oil mole ratios of 3:1 and 6:1. Therefore, an excess of glycerol does not result in a significant increase in monoglyceride yield within the experimental range tested. The main reaction product at 80°C was diglyceride. No increase in monoglyceride yield was observed by lowering the reaction temperature to 10°C.

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KEY WORDS: Free fatty acids, glycerol, glycerolysis, immobilized lipases, monoglycerides, olive oil.

Monoglycerides are widely used in the food industry as emulsifiers for bakery products, margarines, dairy products, confectionery, etc. They also have important applications in the cosmetic and pharmaceutical industry for the preparation of oil/water (O/W) emulsifying systems and for improving the consistency of creams and lotions. In addition, owing to their excellent lubricant and plasticizing properties, monoglycerides are used in textile processing, production of plastics, and formulation of oils for different types of machinery. The most commonly used products are glycerol monostearate, monooleate, and monoricinoleate (1).

Mixtures that contain 40–48% monoglycerides (MG), 30–40% diglycerides (DG), 5–10% triglycerides (TG), 0.2–9% free fatty acids (FFA), and 4–8% free glycerol are generally termed monoglycerides. These mixtures have applications in food fats (margarine, ice cream, sweets, etc). Pure MG (90–97%), obtained by molecular distillation of the above mixtures, are also commercially available. The higher-purity MG are preferred for bakery uses because of their good amylase-complexing ability. Most commercial MG are produced from edible, refined, hydrogenated animal fats (tallow, lard, etc.) or from hydrogenated vegetable oils (palm, soybean, etc.). High-oleic vegetable oils can also be used as raw materials for the production of emulsifiers for liquid and low-fat margarines. Owing to the large content of monounsaturated fatty acids, improved consistency and stability is obtained and can be compared to conventional MG based on hardened fats and oils.

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Monoglycerides are produced on an industrial scale by glycerolysis of fats and oils by means of inorganic alkaline catalysts, such as sodium hydroxide. The reaction is carried out at high temperature (220–260ºC) to obtain both a high reaction rate and good miscibility between the glycerol and fat phases (2). The use of high temperature favors side reactions, resulting in a low-quality product (dark color, burnt taste). Therefore, a purification process is required to obtain food-grade MG.

Application of enzymes as catalysts for reactions in the oils and fats industry is being seriously investigated (3). Lipases have been successfully applied to synthesis of esters (4,5), hydrolysis of oils (6), and interesterification reactions (7,8). Immobilized lipases show many advantages over traditional inorganic catalysts: they have large catalytic activity under mild operating conditions; they show large selectivity to the desired product with no significant side reactions, leading to products of high purity; they are easily recovered from the reaction mixture and can be reused; and there is no contamination of the final product, saving time and cost in the purification stage.

The production of MG with lipases as catalysts can be achieved through three different pathways: (i) hydrolysis of fats and oils (9), in which the reaction should be carefully controlled to avoid complete hydrolysis; (ii) esterification of fatty acids and glycerol (10), which is not attractive from a practical point of view because a previous hydrolysis step is required to produce the fatty acid to be used as raw material; (iii) reaction of triglycerides and glycerol (glycerolysis) as a direct route to produce MG. The adoption of glycerolysis may consume some of the glycerol surplus that originates from recently developed processes, such as biodiesel production (11).

Information in the literature on the application of lipases as catalysts for glycerolysis processes is rather limited, and most studies refer to lipases in solution. In 1991, McNeill *et al.* (12) reported the glycerolysis of several fats and oils with unsupported lipases as catalysts. The optimal temperature to obtain a large yield of monoglycerides was between 30–46ºC for animal fats and 5–10ºC for vegetable oils. However, owing to the low reaction temperature, reaction times up to 5 d were required. To activate the unsupported lipases, it was necessary to dissolve a small amount of water in the glycerol phase, resulting in the production of free fatty acids by TG hydrolysis.

An important advantage of immobilized lipases over unsupported lipases is the presence of a certain amount of water "immobilized" in their structures. Because of this immobilized water, the lipase can work at an O/W interface without addition of water to the reaction mixture. This way, FFA formation from hydrolysis of TG may be reduced. In addition, immobilization gives lipases greater stability than unsupported preparations. Therefore, reactions can be carried out at higher temperatures. The aim of the present work was to study the catalytic behavior of commercial immobilized lipases for the glycerolysis of olive oil samples with different FFA contents. The effect of operating conditions (glycerol/oil mole ratio and temperature) on MG production has been analyzed.

EXPERIMENTAL PROCEDURES

Materials. Unrefined olive oil samples of different FFA contents were kindly supplied by Olibau, S.A. (Madrid, Spain). Glycerol (99+%) was purchased from Sigma-Aldrich (Quimica, Madrid, Spain). *Candida antarctica* lipase, immobilized on a macroporous acrylic resin (Novozym 435), and *Mucor miehei* lipase, immobilized on a macroporous anion exchange resin (Lipozyme IM), were supplied free of charge by Novo Nordisk Bioindustrial, S.A. (Madrid, Spain).

Procedure. Glycerolysis experiments were carried out in a stirred tank reactor, immersed in a Heto CB 8-30e thermostatic bath (Heto-Holten A/S, Allerød, Denmark) that provided an operating temperature from −30 to +110°C ± 0.05°C. Reactants were introduced in the reactor. When the reaction temperature was reached, the catalyst was added. Stirring speed was set at 600 rpm. Samples were taken at regular time intervals for analysis. After each reaction, the enzyme was separated by filtration and washed with solvent in a Soxhlet extraction apparatus. The catalytic activity of the recovered enzyme was tested to check that no deactivation had taken place during the reaction.

Analysis. FFA were determined by titration with alkali as described in AOCS Official Method Ca 5a-40 (13). Quantitative analysis of MG, DG, TG, and FFA was performed on a Hewlett-Packard 5890 Series II gas chromatograph fitted with an HP-5MS (low-bleed 5% phenyl methyl siloxane) capillary column (Hewlett-Packard, España, Madrid, Spain). Data were collected on a Hewlett-Packard 3396A integrator. Pure oleic acid, monoolein, diolein, and triolein, purchased from Sigma-Aldrich, were used for calibrations.

RESULTS AND DISCUSSION

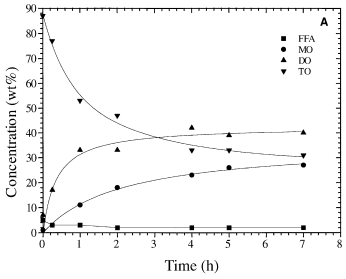
Choice of catalyst. Preliminary experiments were carried out to determine the activity of immobilized lipases for glycerolysis of olive oil. Table 1 shows product distributions (expressed as weight percentage) obtained after 3 and 7 h with Lipozyme and Novozym 435. No activity was detected for Lipozyme. Even after 7 h, the composition of the reaction mixture was identical to that of the starting oil sample. Different behavior was observed for Novozym 435, where TG were rapidly converted into DG and MG. Therefore, Novozym 435 was the catalyst chosen for further tests.

TABLE 1
Product Distribution (wt%) for the Glycerolysis of Olive Oil at 75°C over Lipozyme and Novozym 435[a]

Catalyst	Time (h)	FFA	MG	DG	TG
Olive oil	0	5	1	7	87
Novozym 435	3	2	23	27	48
Novozym 435	7	2	33	31	34
Lipozyme	3	5	1	8	86
Lipozyme	7	5	1	7	87

[a]Novozym 435 and Lipozyme, Novo Nordisk Bioindustrial, S.A. (Madrid, Spain). Abbreviations: FFA, free fatty acids; MG, monoglycerides; DG, diglycerides; TG, triglycerides.

Effect of glycerol/oil mole ratio. The results obtained for the glycerolysis of olive oil at glycerol/oil mole ratios 3:1 and 6:1 are shown in Figure 1. In both reactions, the concentration of TG decreased sharply during the first minutes to form DG and MG. The compositions of the reaction mixtures



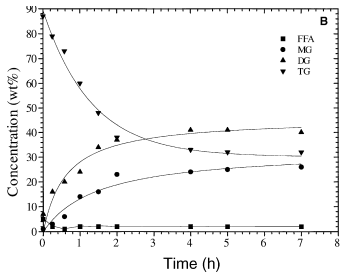


FIG. 1. Product distribution vs. time for the reaction of 5% free fatty acids (FFA) with glycerol at 75°C. (A) Glycerol/oil mole ratio 3:1; (B) glycerol/oil mole ratio 6:1. Abbreviations: MO, monoolein; DO, diolein; TO, triolein; MG, monoglycerides; DG, diglycerides; TG, triglycerides.

reached steady levels after 4 h. Final compositions were similar for both mole ratios. This indicates that, within the range of glycerol levels examined, product distribution after 7 h is not significantly affected when an excess of glycerol is used.

Esterification of FFA. Figure 1 shows a surprising decrease in FFA content at the beginning of the reaction. The initial FFA concentration of the unrefined oil was 5%, and it decreased to 2% after 30 min and remained constant thereafter. Similar results were obtained when a sample of olive oil with a higher FFA content (7.4%) was used. The final FFA concentration was 2%. Therefore, FFA esterification with glycerol and glycerolysis occur simultaneously, the esterification reaction being faster than glycerolysis. This observation contrasts with results reported for glycerolysis of fats and oils with unsupported lipases as catalysts. When the lipase is not immobilized, it is necessary to dissolve water in the glycerol phase for the reaction to occur. This results in partial hydrolysis of the TG and leads to the formation of FFA. McNeill *et al.* (12,14) observed that the FFA content at equilibrium depends on the water concentration in the glycerol phase, and even at very low water content, a small amount of FFA is synthesized. In the present work, no water was added to the reaction mixture; presumably, the necessary amount of water for the lipase to be active is "immobilized" in the structure of the catalyst. Immobilization of the lipase seems to have a beneficial effect on the reaction because not only is FFA formation avoided but also part of the FFA present in the oil is esterified. This behavior could have interesting applications in refining of vegetable oils. Deacidification of vegetable oils for edible purposes is currently carried out on an industrial scale by neutralization with a solution of sodium hydroxide. The soaps formed are separated by centrifugation, and the oil is washed with water. This process involves high energy and water treatment costs. Biorefining of vegetable oils with immobilized lipases could be an interesting alternative to conventional chemical neutralization (15). To check whether FFA could be further reduced, olive oil with 2% FFA was treated with glycerol at two different temperatures, and the results are shown in Figure 2. After 2 h, the FFA concentration had not varied significantly with respect to the initial value. FFA determination was carried out both by titration with alkali according to the AOCS method and by gas chromatography. These results indicate that partial esterification takes place for oils with FFA contents above 2%. When the FFA concentration reaches 2%, no further esterification occurs.

As shown in Figure 2, glycerolysis at 75°C resulted in a larger decrease in TG concentration than at 50°C. Similar amounts of DG were obtained for both temperatures, whereas the MG concentration at 75°C was three times larger than the value at 50°C. This indicates that the rate of glycerolysis increases with increasing temperature.

Effect of temperature. Most glycerolysis studies reported in the literature have been carried out at relatively low temperatures, in the range of 25–50°C. Therefore, long reaction times, up to 5 d, were required. Higher temperatures would lead to deactivation of the lipase. Immobilized lipases show greater stability than unsupported enzymes, making it possible to operate at higher temperatures. As expected, the rate of glycerolysis increases significantly with increasing temperature (Fig. 2). Figure 3 shows the results obtained for the glycerolysis of olive oil at 80°C. TG concentration decreased rapidly during the first 2 h, leading to DG and MG formation. Product distribution did not change significantly between 7 h and 2 d, and DG was the main component of the reaction mixture (approx. 50%). The amount of MG formed at equilibrium was only half the amount of DG. McNeill *et al.* (12) reported large MG yields (90%) for the glycerolysis of olive oil at 10°C over 4 d. On the other hand, Bornscheuer and Yamane (16) obtained 95% MG after 5 d from the glycerolysis of triolein at a reaction temperature of 25°C for the first 8 h, followed by cooling to 8°C. Therefore, low temperatures seem to favor MG production. To check whether MG yield could be improved at low temperatures, a glycerolysis reaction was carried out at 80°C for the first 4 h, and the temperature was then lowered to 10°C. The results, shown in Figure 4, revealed no significant changes in the final composition of the reaction mixture after 48 h, compared to the

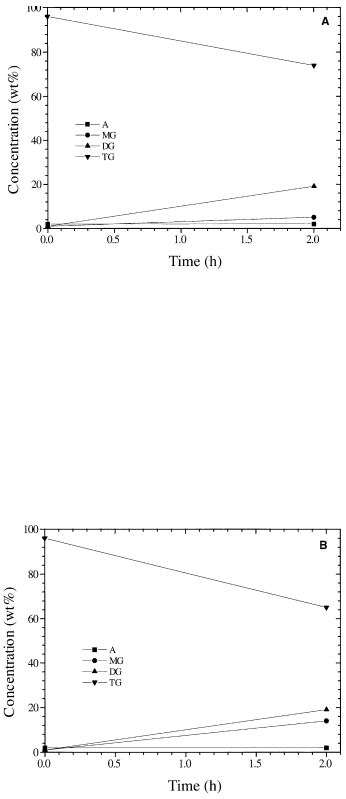


FIG. 2. Product distribution vs. time for the reaction of olive oil that contained 2% FFA with glycerol. Glycerol/oil mole ratio 1:1. (A) T = 50°C; (B) T = 75°C. For abbreviations see Figure 1.

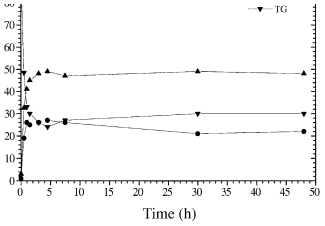


FIG. 3. Change in concentration with time for the glycerolysis of olive oil at 80°C. For abbreviations see Figure 1.

results at 80°C (Fig. 3). DG was again the major component in the reaction mixture.

A possible explanation for the different behavior observed for unsupported lipases (12,16) and immobilized lipases (present work) could be found in the immobilization support. The lipase used in the present study was immobilized on a macroporous acrylic resin. Owing to the relatively high melting point of MG (35°C for monoolein), when the temperature was lowered to 10°C, the reaction mixture became highly viscous and a paste formed. Therefore, the support pores may have been blocked, making it difficult for the reactants to have access to the active sites of the lipase. This diffusion effect can probably be minimized by using unsupported lipases, although separation of the lipase after the reaction requires complex purification operations compared to the easy removal of lipases immobilized on solid supports.

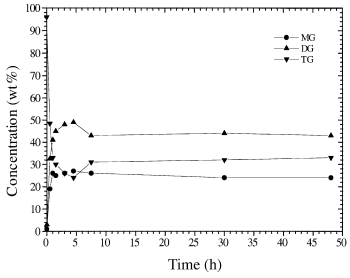


FIG. 4. Change in concentration with time for the glycerolysis of olive oil. The reaction temperature was 80°C during the first 4 h, and then it was lowered to 10°C. For abbreviations see Figure 1.

The results shown in the present study reveal the large potential of immobilized lipases as alternative catalysts for the glycerolysis of oils. The large activity of the biocatalysts observed under mild operating conditions makes it possible to obtain products of great purity because no side reactions take place. Furthermore, the solid catalyst can be easily separated at the end of the reaction and can be reused. An additional advantage of immobilized lipases over unsupported preparations is a significant reduction in FFA content for highly acidic oils.

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