# Esterification of Stearic Acid with Glycerol by Lipase in Foam

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*Received June 15, 1992. In Final Form: August 13, 1992*

The enzymic synthesis of mono-, di-, and triglycerides has been studied extensively to understand the mechanism of reaction between oil-soluble fatty acid and water-soluble glycerol by the enzyme lipase in reverse micellar solutions.[1,2] The anionic double-tailed surfactant AOT [sodium bis(2-ethylhexyl) sulfosuccinate] is most frequently used to form reverse micelles. Unlike most surfactants, AOT does not require additional amphiphiles as cosurfactants for the formation of reverse micelles because of its wedge-shaped molecular geometry (3). The rate of enzymic reaction in reverse micelles depends on the surfactant concentration, the water to surfactant ratio, the temperature, and concentration of buffers as well as co- and counterions present.[4] Enzymes are proteins whose molecular weights are in the range of several thousands. Structures of several enzymes, their active sites, and substrate interactions therein have been determined by X-ray crystallography.[5] Previous studies[6,7] showed that the ratio of mono-, di-, and triglycerides formed in the reverse micelles is influenced by the pH, temperature, and reaction time. The reaction at the oil/water interface in reverse micelles yields predominantly diglyceride and a very small amount of mono- and triglycerides. It has been reported that the products in reverse micelles contain 50% diglyceride, 10% monoglyceride, and less than 3% triglyceride.[8]

However, commercial scale-up of such an enzymic synthetic process has not been reported presumably due to various limitations as the products are intended for use as food, cosmetic, or pharmaceutical ingredients. Recently several studies have been reported on the enzymic synthesis in solvent-free systems in an attempt to make the process commercially feasible. These efforts not only excluded the toxicity problem of the solvents and surfactants but also reduced many steps in the purification process. Kim et al.[9] have reported the enzymic synthesis in dispersions of capric acid and water in a surfactant-free system. The mixture of capric acid and glycerol was agitated vigorously by a magnetic stirrer in an open glass vial, and the reaction was initiated by adding the lipase into the reaction mixture. The products in this system were very similar as compared to that in the reverse micellar solution when the molar ratio of capric acid to glycerol is greater than 3.0; namely, a large amount of diglyceride and small amounts of mono- and triglyceride were obtained by the enzymic reaction.

The present paper reports the enzymic synthesis in the foam as a solvents- and surfactants-free system. Foam can provide a large interfacial area at which the synthetic reaction can occur. In this study, stearic acid supplied by Sigma Chemical Co. with purity 99% was used as the foaming agent as well as one of the reactants. Fifteen milliliters of double-distilled water and 15 mL of glycerol (Fisher Scientific Co.) were added to a glass column (4 cm diameter × 100 cm length). A 0.2-g portion of stearic acid was dissolved in 4 mL of chloroform and then added to the column. Without this process the stearic acid molecules did not dissolve in the solution. Air was blown at a high flow rate for 15 min to evaporate all chloroform before adding the enzyme because the organic solvent inhibits the activity of the enzyme.[10] A 0.2-mL portion of Lipozyme 10000 L supplied by Novo Co. was added into the column. This enzyme is a fungal lipase produced by submerged fermentation of a selected strain of *Mucor miehie*. The enzyme catalyzes the esterification of glyceride as well as enhances the foaming of solution. Air was continuously blown during the reaction to maintain a large foam volume (Figure 1). The samples were taken after the reaction time of 15 min, 30 min, 1 h, and 2 h. A 1.5-mL portion of isooctane and 2-propanol mixture (4:1 v/v) was added to each sample immediately after removal from the foam column to stop the enzymic reaction.

Normal-phase HPLC with a UV absorbance detector of 213 nm cutoff wavelength and 0.05 auf was used to analyze the product of reaction at various time intervals. The silica column (10 cm × 2.4 cm) and the mixture of isooctane and 2-propanol (96:6 (v/v)) as a mobile phase were used at room temperature. The flow rate of mobile phase was kept at 1 mL/min. The absorbance detector of the HPLC is from Spectra Physics (Model SP8450). The integrator (Model SP8880) plots raw signals from the detector and determines the presence of peaks. This signal is analyzed by Spectra Physics software LNET2 and SPMENU. The results are shown in Figure 2. In contrast to the products formed in the reverse micelles,[8] the reaction in foam produced a large amount of triglyceride (almost 20%) as well as a large amount of diglyceride (55%). The difference in the quantity of di- and triglyceride produced in the foam versus reverse micelles can be explained on the basis of the two-phase model proposed by Verger[11] for phospholipase-catalyzed hydrolysis of phospholipid monolayers because the reaction in foam also takes place at the air/water interface. In this model, the entrance of the water-soluble enzyme into the monolayer is the first step. The second step is the formation of the enzyme–substrate complex within the monolayer. In the catalytic step the product is formed and the enzyme is regenerated. If the product is water soluble, it will migrate into the aqueous phase. If the product is oil soluble, the product will remain at the air/water interface, resulting in other consecutive reactions. In case of esterification of stearic acid in foam, the molecules of stearic acid at the interface react with glycerol in water phase by the lipozyme. In a very short time period (~5 min), most of the reaction products might be diglyceride because the enzyme has specific activity for 1,3-diglyceride, but the specificity is not so strict in this esterification reaction.[12] Also the amount of diglyceride increases as the reaction time increases before the reaction reaches the equilibrium state as shown in Figure 2. With further increase in reaction time, diglyceride produced may get converted into the triglyceride because diglyceride would stay at the air/water interface in foam. In contrast, the diglyceride formed at the oil/water interface in reverse

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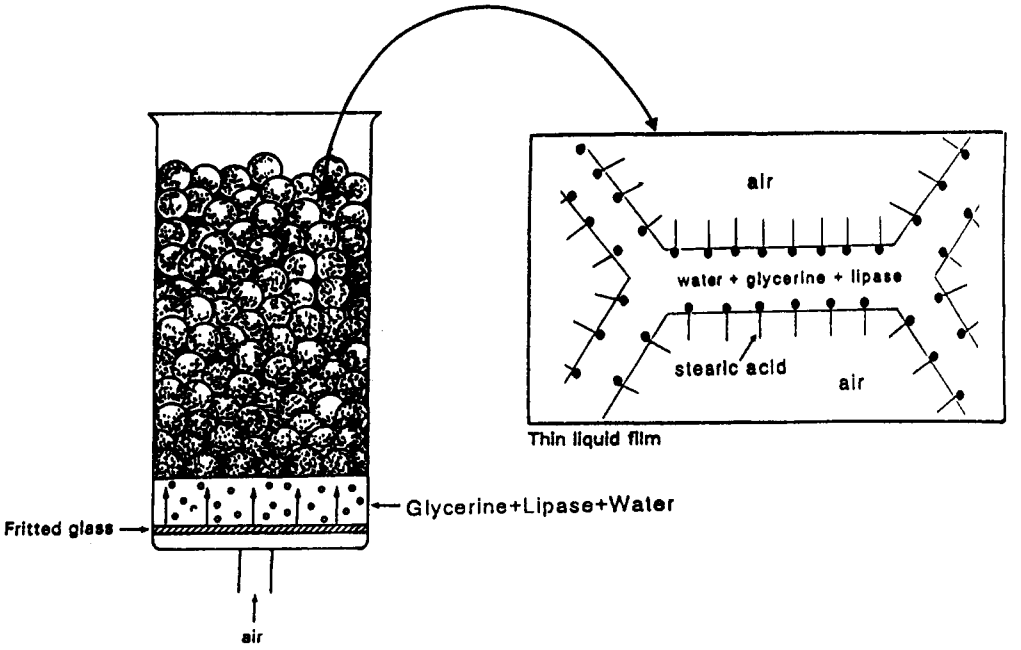


**Figure 1.** Schematic diagram of foam column and expanded representation of thin liquid film in foam.

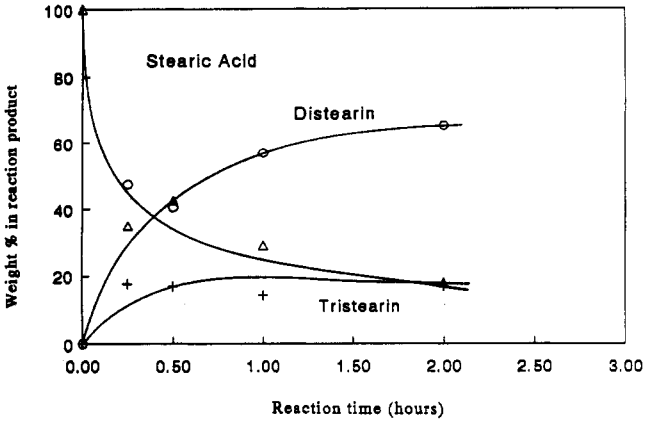


**Figure 2.** Weight % of each component in the reaction product vs reaction time.

micellar solution may partition into the oil phase. Therefore, the main product in the reverse micelles is diglyceride.

We also carried out the reaction in foam but without the addition of lipase. There was no significant change in the concentration of stearic acid after 3 h. Thus, the presence of lipase is required in foam to produce di- and triglycerides.

In summary, the esterification reactions by enzyme in foam which involves the air/water interface yielded products in different proportions as compared to that produced by the reverse micellar solutions and other solvent-free systems (e.g. dispersions). The reaction in foam produced large amounts of diglyceride (≈55 wt %) as well as triglyceride (≈20 wt %), whereas the reaction in the reverse micelles produced large amounts of diglyceride (≈50 wt %), less monoglyceride (≈10 wt %) and much less amount of triglyceride (less than 3%). The absence of monoglyceride in foam is probably due to the preferred orientation of stearic acid and the absence of surfactant at the air/water interface which favors further esterification resulting in diglycerides. It should be emphasized that the percent conversion of fatty acid into glycerides in reverse micelles is about 70%, whereas that in foam is about 80%. The reaction in foam can be employed for a large scale production of di- and triglycerides by the enzymes due to a large air/water interface at which the reaction takes place without using solvents or surfactants.

**Registry No.** Stearic acid, 57-11-4; glycerol, 56-81-5; lipase, 9001-62-1; distearin, 1323-83-7; tristearin, 555-43-1.