Effect of Phospholipids on Enzyme-Catalyzed Transesterification of Oils

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The effect of phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylinositol (PI) on the activity of an immobilized lipase, Lipozyme, during transesterification of oils has been studied. PC concentrations less than 0.05% did not affect the initial rate of transesterification. Higher concentrations of PC as well as PE and PI-rich phospholipids at the 0.5% level caused a reduction in the initial reaction rate, but after ten hours transesterification had progressed to the same extent as the control sample. Reuse of Lipozyme for ten batch reactions showed that a PC content of less than 0.05% did not cause significant inactivation of the enzyme, but PC contents above this level caused progressive inactivation of the enzyme and the inactivation increased with PC content. The ability of phospholipids to inactivate the Lipozyme was in the order PE > PC > PI. Variation in the acyl groups of PC did not significantly affect inactivation of the enzyme. It is concluded that the phospholipid content of edible oils should be reduced below 200 ppm by degumming if unacceptable inactivation of Lipozyme is to be avoided.

KEY WORDS: Lipase, Lipozyme, phospholipids, transesterification.

Since scientists discovered that lipases remain active and can catalyze esterification and transesterification reactions under anhydrous conditions, there has been great interest in the use of lipases for selective transesterification of oils and fats. Lipase-catalyzed transesterification allows the modification of edible oils and fats to produce more valuable products, such as cocoa butter equivalents, which cannot be obtained by conventional chemical and physical modification (1). In recent years, the development of immobilized lipases has increased the possibility of industrial application of this process either in batch or continuous-flow reactors. Lipozyme, an immobilized lipase from *Mucor miehei* supported on a macroporous anion-exchange resin, has been shown to be highly promising because it has strong activity, good selectivity and a long life (1–4). Posorske *et al.* (4) reported that Lipozyme was inactivated by some minor components in the substrate oils during transesterification. This reduces the life of the enzyme and makes the process less economical.

Phospholipids are important minor components in vegetable oils. The phospholipid content of crude vegetable oils varies from 0.1% to 3.2%, although the level in well-refined vegetable oils is usually less than 40 ppm (5). Phospholipids have been shown to affect the activity of lipases. Phospholipids inhibited the hydrolysis activity of bovine pancreatic lipase and this was explained as being due to the presence of phospholipids on the surface of the emulsified triglyceride droplet and also to coating of the active sites of the enzyme (6). However, Kurashige *et al.* (7) showed that surface-active agents, such as lecithin, increased the esterification activity of pure *Pseudomonas fluorescens* lipase at ultra-micro-aqueous conditions, when the phospholipid was adsorbed, together with the enzyme onto celite. These reports prompted this study of the effect of phospholipids on Lipozyme activity during the transesterification of oils.

MATERIALS AND METHODS

Lipozyme IM 20 was donated by Novo Nordisk Bioindustries U.K., Farnham, U.K. (the enzyme activity was 28 BIU/g and its moisture content was 12%). Triolein (95%), lauric acid (99%), phosphatidylcholine (PC) (99%, Type III-S from soybean), phosphatidylinositol (PI) (50% from soybean), phosphatidylcholine (PC) (99%, dioleyl) and phosphatidylethanolamine (PE) (99%, dioleyl) were purchased from Sigma Chemical Co., Poole, U.K.

The reaction mixture contained 75 mg Lipozyme, 8 mg water, 250 mg triolein, 500 mg lauric acid, 5 mL petroleum ether (bp 100–120°C) and phospholipid (as required). The transesterification was performed in a 25-mL stoppered glass vessel at 65 ± 0.2°C with stirring at 100 rpm with a magnetic stirrer. Each batch reaction was conducted for 10 hr. Small samples were removed after 2, 4, 6 and 10 hr for analysis. Products from the batch reactions were removed by means of a syringe equipped with a filter and then the Lipozyme was twice washed back into the flask with 10 mL hexane. Product solution (*ca.* 0.2 mL) was washed with sodium hydroxide (0.05 M) to remove free fatty acids, and the acylglycerols were converted to fatty acid methyl esters with sodium methoxide (0.5 M) in methanol. Analysis of acylglycerols was by gas chromatography with a column of 15% diethylene glycol succinate on Chromosorb W AW-DMCS (100–120 mesh) at 175°C.

RESULTS AND DISCUSSION

Initial reaction. The activity of Lipozyme was unaffected by the presence of up to 500 ppm PC during the first batch reaction. However, levels of PC ≥ 0.1% or PE or PI at 0.5% concentration caused a reduction in the initial reaction rate, although after ten hours, transesterification of the fatty acids and triacylglycerols had progressed to about the same level as that of the control sample (Figs. 1 and 2). Svensson *et al.* (8) have shown that PC is transesterified by Lipozyme, and therefore it appears that this reduction in the initial rate of transesterification of triacylglycerols with fatty acids is due to competition between PC and triacylglycerols or fatty acids for an active site on the enzyme.

Operational stability tests. The presence of PC at levels less than 500 ppm did not cause significant inactivation of the enzyme during 10 repeat batch reactions. However, PC ≥0.1% reduced the enzyme activity, and the degree of inactivation increased with PC concentration (Fig. 3).

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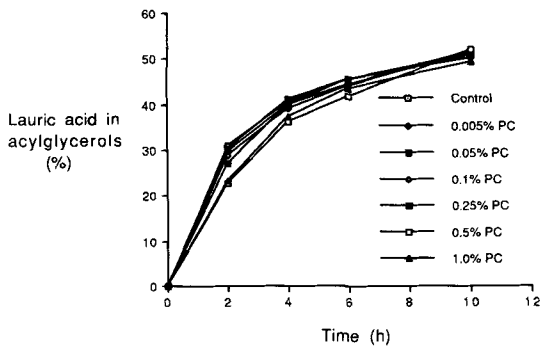

FIG. 1. Effect of PC concentration on the transesterification of triolein and lauric acid in the presence of fresh Lipozyme.

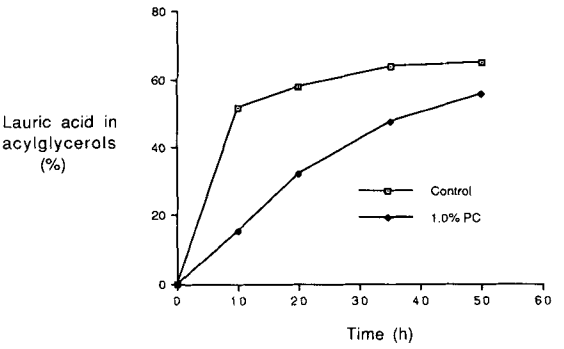

FIG. 4. Lauric acid incorporation into acylglycerols in a transesterification catalyzed by Lipozyme after 9 previous batch reactions in the presence of 0% or 1% PC.

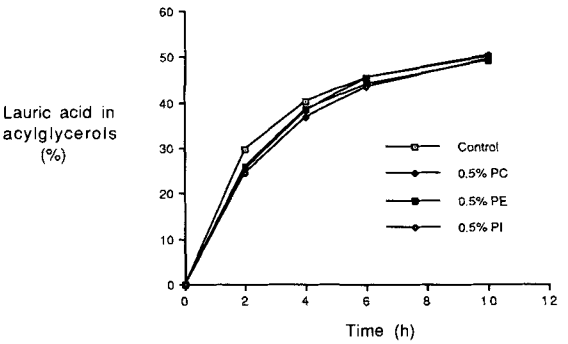

FIG. 2. Effect of PC, PE and PI on the transesterification of triolein and lauric acid in the presence of fresh Lipozyme.

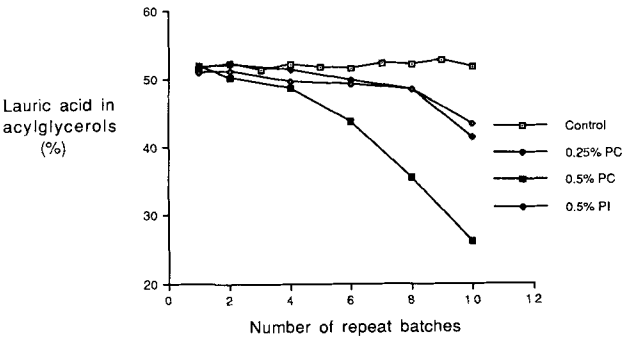

FIG. 5. Operational stability of Lipozyme in the presence of soybean PC or PI.

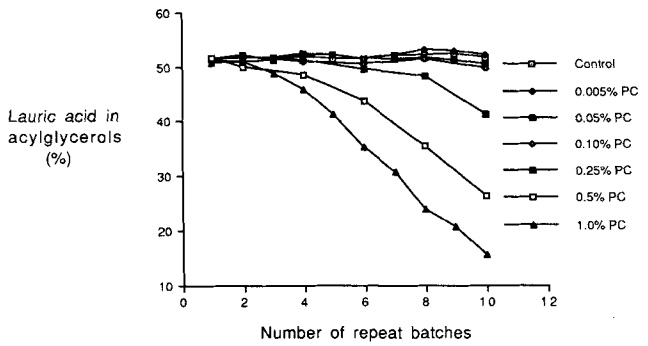

FIG. 3. Changes in lauric acid incorporation into acylglycerols in 10-hr batch reactions catalyzed by Lipozyme used repeatedly in the presence of PC.

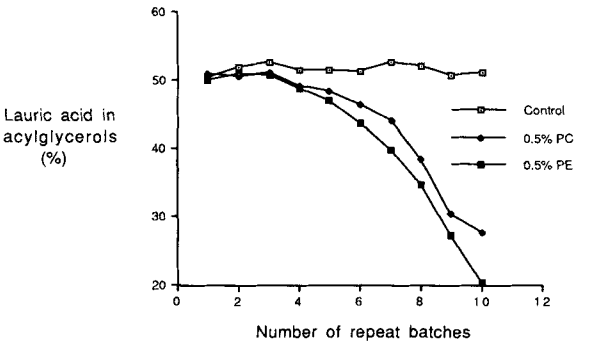

FIG. 6. Operational stability of Lipozyme in the presence of dioleyl PC and dioleyl PE.

Despite the large reduction in the extent of transesterification after the enzyme had been used for 10 batch reactions of 10 hr each in the presence of 1% PC, it was clear that the reaction continued to proceed at a moderate rate over a long period of time. After the 10th batch reaction had been allowed to proceed for 50 hr, the sample containing 1% PC had approached the degree of transesterification of the control (Fig. 4).

In order to improve the understanding of the inhibition of Lipozyme by phospholipids, the effects of PC, PI from soybean, dioleyl PC and dioleyl PE were studied. The purity of the PI was only about 50%. However, it was clear that the PI did not have a strong inhibitory effect, since the enzyme used for 10 batches in the presence of 0.5% PI was more active than the enzyme used for 10 batches in the presence of 0.25% PC (Fig. 5).

Dioleyl PE caused a bigger reduction in the operational stability of Lipozyme than dioleyl PC over 10 batch reactions (Fig. 6). Thus, the inactivation of Lipozyme by phospholipids is in the order PE > PC > PI. Furthermore, the effect of dioleyl PC is similar to that of soybean PC (compare Figs. 5 and 6), despite the

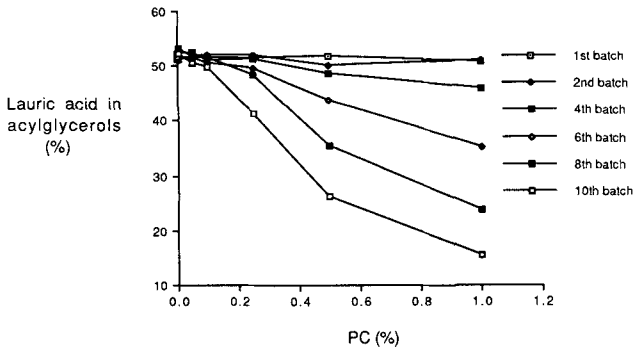

FIG. 7. Effect of PC concentration on the incorporation of lauric acid into acylglycerols in the presence of Lipozyme with repeat usage of the enzyme.

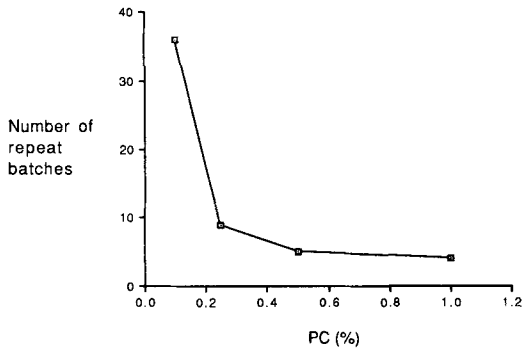

FIG. 8. Effect of PC concentration on the number of batch reactions for 10% loss of enzyme activity. (Data for 0.1% PC extrapolated from 10 batch reactions.)

fact that soybean PC is rich in linoleic and linolenic acids (9).

The inactivation of the Lipozyme clearly is progressive with time and increases with PC concentration (Fig. 7). The inactivation is not reversed by washing the enzyme with hexane, which is performed after each batch reaction. Therefore, it appears that the phospholipid is binding chemically to the enzyme, although the possibility that the phospholipid is binding to the support and affecting the enzyme indirectly cannot be ruled out. Because both PC and PE inactivate the enzyme, it appears that the phosphate group is involved in the inactivation, although the amine group of PE may also contribute to inactivation of the enzyme.

It is clearly necessary that the phospholipid content of oils must be less than 500 ppm if the enzyme is to have a long operational life. Indeed, the phospholipid content should be reduced below 200 ppm if significant inactivation is to be avoided, because even 200 ppm causes significant inactivation in 10 batches (Fig. 8). This has the consequence that oils such as soybean oil must be degummed well before they are used as substrates for transesterification with Lipozyme.

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