Materials and Methods

1. Materials

Lipases

Lipase PS (*Pseudomonas* sp.) was gifts from Amano Pharmaceutical Co. Ltd., Nagoya, Japan.

Carriers

The supports was Polypropylene powder EP-100 (Accurel), a gift from Akzo Nobel (Obernburg, Germany).

Raw materials

Palm olein was purchased from Morakot Industry Co. Ltd., Thailand. Glycerol was purchased from Carlo Erba Reagenti Co. Ltd.

Chemicals

Analytical grade chemicals were used for immobilized enzyme assay of lipase activity, glycrolysis and analyzed of composition.

Instruments

Spectrophotometer Model U-2000, Hitachi Koki Co., Ltd.

Microcentrifuge Model

pH meter Model

Water bath Model

Peristatic pump, Bio-Rad Laboratories

Magetic stirrer Model

TLC/FID analyzer Model

Refrigerator Model

Analytical methods

Lipase activity was assayed by the modified cupric acetate method (Lee and Rhee, 1993).

Oil composition was determined by a thin-layer chromatography flame ionization detection (TLC/FID). The course of glycerolysis was monitered by intermittent sampling (150 mg) followed by chloroform extraction. The extract was analyzed for triacylglycerol (TAG), 1,3 diacylglycerol (1,3 DAG), 1,2 diacylglycerol (1,2 DAG), MAG and free fatty acid (FFA) using a thin-layer chromatography/flame ionization detection (TLC/FID) (IATROSCAN MK5, Iatron Laboratories Inc.,Tokyo). In this experiments, percent of peak area was assumed as percent content of the

corresponding compound. Enzyme protein are determined by the Folin-Lowry method. Hydrolytic activity of the lipase is assayed by the modified cupric acetate method. One unit of hydrolytic activity is defined as the amount of the enzyme which liberates 1 μmole equivalent of palmitic acid from palm olein in 1 min at 30°C.

2. Immobilization

The support in powdered form (0.5 g) was added to 5.0 ml 0.1 M phosphate buffer pH 7.0 containing lipase approximately 100 U/ml and stirred with a magnetic bar at 100 rpm for 1 h. Afterward, 5.0 ml of 0.1 M phosphate buffer pH 7.0 was added and the suspension was filtered through a Buchner funnel. The immobilized enzyme was washed on the filter paper with another 5.0 ml of 0.1 M phosphate buffer pH 7.0 to remove unadsorbed soluble enzyme and dried in a vacuum desiccator for 8 h. For this immobilized study, the immobilized yield was calculated using the following formula:

$$\text{Immobilized yield} = \frac{\text{Total immobilized activity (U)}}{\text{Total initial soluble enzyme activity (U)}} \times 100$$

Lipase immobilization was performed in initial method as described above and varying concentration of lipase at 5, 10, 50, 100 and 150 U/ml. The immobilized activity and immobilized yield were calculated.

3. Glycerolysis

The glycerolysis experiments were carried out in batch system. The reaction mixture consisted of various enzyme, water, glycerol and palm olein concentrations in organic solvent (acetone/isooctane mixture 3:1, v/v). The temperature was controlled at 45 °C. The reaction was mixed by magnetic stirrer at 300 rpm. Samples of the reaction mixture were centrifuged to remove immobilized lipase before analysis.