Chapter 6

Summary

This study was carried out to investigate the characteristics of the lipid-modified lipase for interesterification of fats and oils. Enzymatic interesterification with lipid-modified lipase has much attention for food application. In this study, the modification condition and the best combination of lipases and surfactants were studied. And the kinetics of interesterification was investigated and one-parameter model was proposed. Further, to develop practical process, continuous membrane reactor system and interesterification in non-solvent system were also studied. The followings are the summary of this study.

Chapter 1

The several benefits of functional and nutritional fats, and the enzymatic interesterification to produce functional/nutritional fats were discussed. And the objectives of the research are described.

Chapter 2

Interesterification of tripalmitin and stearic acid in *n*-hexane was investigated using surfactant-modified lipases. Various kinds of lipases and surfactants were screened for high interesterification activity of the modified lipases. The modified lipase obtained from Lipase Saiken 100 (*Rhizopus japonicus*) with sorbitan monostearate as surfactant had the highest activity in the *n*-hexane system. The modified lipase activity was influenced by the lipase source, the surfactant composition and the pH and the weight ratio of surfactant to lipase used during the modification. The water content in the reaction solution has strongly influenced the enzyme activity. By repeating the lipase modification processes three times for the lipases from *R. japonicus, R. delemar and R. miehei*, total protein recoveries over the range 17-35% could be obtained. The original lipases had no interesterification activities at all, however, all modified lipases in the first process had significant interesterification activities. In the hydrolysis reactions, all modified lipases obtained from the first

process showed about 3 times higher specific activities than the original lipases. The modified lipase could be made from lipase and stearic acid, and the stearic acid-modified lipase protein was characterized using SDS-PAGE. These results suggested that the modification process was effective for not only interesterification but also for lipase purification.

Chapter 3

Simple kinetic models for the interesterification between triglycerides and fatty acids, and the interesterification between triglycerides by the modified lipase in *n*-hexane have been proposed. The models assume that the modified lipase has 1,3-positional specificity and do not distinguish among the different fatty acid residues considered in this study. The models are based on material balances of consecutive second-order reversible reactions and requires only one parameter that can easily be determined experimentally. The differential rate equations have been solved analytically to give explicit equations linking the concentrations of all possible fatty acids and triglycerides to the initial conditions and the reaction time. The models were in good agreement with experimental data for different catalyst concentrations using the same value of specific rate constant.

Chapter 4

The feasibility of using a hollow-fibre membrane reactor for the modified lipase-catalyzed interesterification reaction of triglycerides and fatty acids in a microaqueous *n*-hexane system was investigated. This modified lipase catalyzed predominantly the interesterification reaction in a stirred tank reactor as well as in a hollow-fibre membrane reactor system. The membrane reactor system was operated both batchwise and continuously. In a four-run batch experiment, biocatalyst activity loss was observed after the first run, which was probably caused due to permeation of the free surfactant through the membrane. However, there was no observed activity

losses in the subsequent three runs. The modified lipase retained its original activity for more than 70 h in continuous operation, and the membrane module system showed excellent resistance to the hydrophobic organic environment.

Chapter 5

Interesterification between medium chain fatty acid triglycerides (MCT) and long chain fatty acid triglycerides (LCT) in a non-solvent system was investigated using the modified lipase. 74% conversion was obtained after a 48 h reaction period, and the triglyceride composition was well described by the 1,3-random 2-random stochastic model. The interesterification reaction between MCT and LCT closely followed the simple kinetic model, and the change in MCT and LCT contents could be simulated using one parameter. The effects of the water activity (Aw) of modified lipase, the water content of the reaction system and the reaction temperature on the reaction rate were studied. A modified lipase Aw of 0.35 and a water content of the reaction system at 0.09 wt% showed the highest activity. Inactivation did not occur below 60°C, however, the activity decreased at temperatures over 70°C.

In this study, characterization of the lipid-modified lipases and their application to interesterification of oils and fats in *n*-hexane and non-solvent systems were carried out. Optimal modification conditions for effective modification process were identified. Furthermore, basic information for high value added lipid products was collected. These findings would be useful to develop functional oils and fats, and contribute to food industry.

The modified lipase could be utilized for food, detergent and pharmaceutical application because of its high activity, low investment and safety. However, the stability and activity were not sufficient to utilize for industries, therefore further study of the modification is required. First of all, the structure of modified lipase is required to be investigated. It was confirmed experimentally that the

interesterification activity of lipase was increased by the modification. However, how lipase structure was changed according to the modification has not been clarified. To make the modified lipase with high and stable activity, the mechanism of the modified lipase should be studied. X-ray scattering analysis method and scanning electric microscopy are seemed to be useful. According to identification of the modified lipase structure, the lipase modification technique would be improved. The modified lipase with high and stable activity or the modified lipase for specified application would be made by a new modification method.

This thesis is based on the published papers as follows.

1) Basheer, S., K. Mogi and M. Nakajima (1995) Surfactant-modified lipase for the catalysis of the interesterification of triglycerides and fatty acids, *Biotechnol. Bioeng.*, 45, 187-195

2) Basheer, S., J. B. Snape, K. Mogi and M. Nakajima (1995) Transesterification kinetics of triglycerides for a modified lipase in *n*-hexane, *J. Am. Oil Chem. Soc.*, 72, 231-237

3) Basheer, S., K. Mogi and M. Nakajima (1995) Interesterification kinetics of triglycerides and fatty acids with modified lipase in *n*-hexane, *J. Am. Oil Chem. Soc.*, 72, 511-518

4) Basheer, S., K. Mogi and M. Nakajima (1995) Development of a novel hollow-fibre membrane reactor for the interesterification of triglycerides and fatty acids using modified lipase, *Process Biochemistry*, 30, 531-536

5) Mogi, K. and M. Nakajima (1996) Selection of surfactant-modified lipases for interesterification of triglyceride and fatty acid, *J. Am. Oil Chem. Soc.*, 73, 1505-1512

6) Green, K. D., S. Ichikawa, M. Nakajima and K. Mogi (1997) Evaluation of lipid modified lipase for interesterification and hydrolysis reactions in *n*-hexane, *Food Sci. Technol. Int. Tokyo*, 3, 357-361

7) Mogi, K., M. Nakajima and S. Mukataka (1999) Surfactant modification of lipases for lipid interesterification and hydrolysis reactions, *J. Am. Oil Chem. Soc.*, 76, 1259-1264

8) Mogi, K., M. Nakajima and S. Mukataka, Transesterification reaction between medium and long chain fatty acid triglycerides using surfactant-modified Lipase, *Biotechnol. Bioeng.* (in press)