CHAPTER 6

SUMMARY AND FUTURE WORKS

6.1 Summary

1. Synthesis of polyunsaturated fatty acid-rich fish oil by two-step enzymatic method consists of hydrolysis of tuna oil and selective esterification of resulting FFA. The results showed that Lipase D hydrolyzed the ester of EPA and DHA as strongly as those of the other constituent fatty acids and was suitable for the first-step hydrolysis. Lipase AK was suitable for the second-step esterification with octanol because it acted only weakly on PUFA. Combination of enzymatic hydrolysis with urea complexation has the advantage to obtain highly enriched PUFA from FFA mixture by crystallization of other fatty acids at low temperature.

2. Glycerolysis of tuna oil was investigated to produce MAG rich in PUFA using immobilized Lipase AK. The optimum conditions for MAG production were found to be 10 %w/v of tuna oil in MTBE, glycerol to tuna oil ca. 3.0:1.0 mol/mol, water added in glycerol was 4 wt% and the amount of IM-AK used was 30 wt% of tuna oil. The temperature was controlled at 45°C. Under these conditions, the yield of 24.6 wt% containing of 56.0 wt% PUFA (EPA and DHA) was obtained at 24 h. MAG were produced in good yield with high content of PUFA, especially, EPA and DHA. Thus, a suitable product or starting material for synthesis of structured triglycerides can be obtained.

3. Physicoenzymatic production of MAG containing PUFA especially EPA and DHA was investigated. A few solvent mixtures were suitable for production of MAG by using Novozym 435 as a catalyst in glycerolysis of tuna oil with glycerol. A few reaction parameters have been evaluated including solvent amount, substrate ratio, enzyme load, and temperature. The yield of MAG up to 90.8% could be achieved with suitable conditions. The temperature fractionation under different solvents was evaluated in order to produce a fraction with higher content of EPA and DHA. Temperature was a critical parameter for effective fractionation. A

fraction under 0 °C fractionation could be obtained with around 70% EPA and DHA and in a yield of around 50%. A possibility of enriching the EPA and DHA into MAG has been built.

4. Lipozyme TL IM was used for the interesterification of FOEE with MAG for synthesis of TAG rich PUFA with FOEE by three steps addition. The quadratic response model developed in this study satisfactorily expressed the content of TAG in the Lipozyme TL IM-catalyzed interesterification with regard to reaction time (T_e), reaction temperature (T_i), substrate molar ratio (S_r), and reaction vacuum (V_c) in the batch system. The R^2 (0.92) and ANOVA indicate that the model well represented the real relationship of reaction parameters and the response. The optimal reaction conditions for the production of TAG rich PUFA by Lipozyme TL IM-catalyzed interesterification between FOEE and MAG were found to be S_r, 6.0/1.0 mol/mol, V_c, 0.2 mbar, T_e, 70 °C and T_i, 15 h. At these experimental conditions, 90.2% TAG can be obtained.

6.2 Future works

1. The kinetics and thermodynamic properties of immobilized lipase-catalyzed synthesis of TAG rich in PUFA should be studied.

2. The application to used bioreactor for production of TAG rich in PUFA should be studied.