CHAPTER V

SUMMARY AND CONCLUSION

The present study consisted of three phases: 1) study on lipid characteristics of fish meals, 2) preparation of fat emulsions utilizing lecithin rich in omega 3 polyunsaturated fatty acids derived from fish meal as emulsifier, 3) study the effect of the prepared fat emulsions on erythrocyte membrane lipid and fatty acid compositions. All results may be concluded as follows.

A. Study on Lipid Characteristics of Fish Meals

The chemical investigations were carried out in 4 grades of fish meal Each parameter in individual samples was analysed in 2-5 applications. The results were summarized as follows.

1. All samples of fish meal were manufactured from two plants located in the province of Rayong and kindly supplied to us by the Department of Marine Science, Faculty of Science, Chulalongkorn University. All fish meals were the production of trash fish harvested from the Gulf of Thailand and categorized according to their protein contents into grade 1-4 with their protein values ranging from 60.16-69.52 % and fat from 7.56-11.34%.

2. The average content of fat in fish meal was 12.46 g/100 g whereas lecithin was 1.43 g/100g. Considerably, the contents of fat and lecithin in grade-1 fish meal were higher than those found in three other grades.

3. Among phospholipid subclasses found in fish meal lecithins, phospholipid with choline containing, i.e. phosphatidylcholine, sphingomyelin and lysophosphatidylcholine, were majority at the average value of 68.33 mole percentage.

4. In comparison to fatty acids in triglycerides moiety, polyunsaturated fatty acids of both omega 6 and omega 3 especially docosahexaenoate were found in higher amounts in moiety of fish meal phospholipids.

5. The average actual amount of omega 3 polyunsaturated fatty acids in grade 1 fish meal was 3.3 g/100 g whereas docosahexaenoate was 2.4 g/100 g. The proportion of docosahexaenoate in crude oil extracted from grade 1 fish meal was 18.84 g/100 g fatty acids. All those three values obtained from grade 1 fish meal were markedly higher than those of other grades of fish meal.

6. Omega-3 polyenoic fatty acids especially docosahexaenoate in PL of grade 1 fish meal were as high as 410.26 and 323.55 mg/100 g fish meal. The values were markedly higher than the corresponding values found in other fish meals.

7. According to its prominent characteristics of lipids especially when the contents of lecithin and docosahexaenoate were taken priorily into consideration, grade 1 fish meal was finally selected as fish meal grade of choice for utilizing as raw material for the further experiment.

B. Preparation of Fat Emulsions Utilizing Lecithins Rich in Omega 3 Polyunsaturated Fatty Acids Derived from Fish Meal as Emulsifier

Grade 1 fish meal was selected as raw material for preparation of lecithin according to its superior property as summarized earlier. The extract of lecithin was achieved after 3 consecutive solvent extractions. Fat emulsions were obtained by blending lecithin extract with certain oil in saline solution. The lipid characteristics of the obtained emulsions were studied. All results were summarized as follows.

1. Lecithin was separated from fish meal after conventional n-hexane extraction. One step of n-hexane extraction yielded lecithin fat and lecithin contents as low as 6.86 and 0.53 g/100 g fish meal, respectively.

2. Pretreatment of fish meal with alchohol either methanol or ethanol assisted higher extractions of fat and lecithin upto 11.4-11.69 and 0.97-1.12 g/100 g fish meal, respectively. It was concluded that lecithin separation from fish meal was

much better with alcohol pretreatment of the sample prior conventional n-hexane extraction.

3. Pretreatment of sample with alcohol gave benefit to the extraction of lipids with highly polar polyunsaturated fatty acids. Extraction with n-hexane alone yielded the proportion of docosahexaenoate in total fatty acid of crude extract at the level of 13.92 but increased to 15.07-15.49 when the sample was pretreated with alcohol.

4. The separation of lecithin was achieved after lipid extract was deoiled by means of acetone treatment. It was found that the existence of acetone in lecithin extraction process made methanol slightly superior over ethanol. Highly polar unsaturated fatty acid such as omega-3 fatty acids especially docosahexaenoate were extracted remarkedly higher in solvent system with methanol pretreatment in comparison to those with ethanol pretreatment (25.78 vs 22.18 of n-3 PUFA and 17.51 vs 14.63 g/100 g total fatty acids of DHA for methanol and ethanol pretreatments, respectively).

5. Phospholipid subclasses were not affected either with any alcohol pretreatments or during acetone deoiling process. Lecithins and its subclasses especially choline-containing phospholipids were extracted simultaneously without any purturbation during being processed through three consecutive solvent extractions.

6. Phospholipid subclasses of lecithin derived from fish meal were obviously different from those of lecithins derived from vegetable source such as soya. They were relatively similar to egg yolk lecthins in term of choline containing phospholipids.

7. Despite the composition of phospholipids in fish meal and egg yolk considering as animal lecithins were relatively similar to those of plasma, they were considerably different in phosphatidylethanolamine (PE) (4 vs 9.22 vs 28.95 for PE of plasma, fish meal and egg yolk, respectively). The total choline containing phospholipids were relatively equal between fish meal and egg yolk at 68.75 and 71.05, respectively. However, those of plasma was prominently higher at the value of 94.

8. The purities of lecithins in crude extracts after solvent extraction were 27.39, 58.33 and 54.28 g/100 g crude extracts for fish meal, egg yolk and soya, respectively. The values reflected to the contamination of triglyceides or neutral lipids in the crude extracts. To obtain three different fat emulsions with similar phospholipid-triglyceride ratio, soya oil was added up into crude extracts of egg yolk and soya. The final ratio of phospholipid-triglyceride ratio of all three fat emulsions were relatively similar at the level of 0.36-0.37.

9. Three different fat emulsions with lecithin rich were prepared according to the method of mechanical dispersion. Waring blender, mixer and sonic bath were main equipments used for this purpose.

10. The composition of triglyceride in the core of three fat emulsions may be concluded as follows: fish meal derived lecithin-rich fat emulsion (FM-LRFE) had its core wholely derived from triglycerides of fish meal; egg yolk derived lecithin-rich fat emulsion (EY-LRFE) had its core derived from triglycerides of two sources, egg yolk and soya in the ratio of 1:1, wt/wt; soya derived lecithin-rich fat emulsion (SY-LRFE) had its core wholely derived from triglycerides of soya.

11. Three fat emulsions had totally different in phospholipid surfaces with the presence of similar phospholipid liposomes in the corresponding emulsions. Phospholipid surface and liposomes of FM-LRFE constituted high content of docosahexaenoate at the level of 22.16 whereas those of SY-LRFE constituted huge content of linoleate at the level of 63.8 g/100 g total phospholipid fatty acids.

C. Study the Effect of the Prepared Fat Emulsions on Erythrocyte Membrane Lipid and Fatty Acid Compositions

Red blood cells or erythrocytes were incubated at 37 °C for 1 h with three different fat emulsions in the presence of phospholipids at the concentrations either of 100, 150, 200 and 300 mg/100 ml of supernatant. The exchanges of fatty acids between emulsions/liposomes and red blood cells were studied. The alteration

of those fatty acids in red blood cells during incubations were monitored. The results may be concluded as follows.

1. It was likely that neither fat emulsions at the concentrations of phospholipids upto 300 mg/dl could affect red blood cell lipids. No alteration was observed neither in membrane contents of cholesterol nor phospholipids after incubations of red blood cells with any fat emulsions.

2. FM-LRFE induced the increments of membrane fatty acids in groups of monoenes and polyenes. Membrane docosahexaenoate increased markedly from 6.33 to 9.25 or 46% after incubation with the highest phospholipid concentration. The significant relationship between phospholipid concentrations and membrane docosahexaenoate was found (slope=0.0100, r^2=0.87). Such a relationship was found also with membrane omega 3 fatty acids (slope=0.0134 , r^2=0.87).

3. EY-LRFE induced an increment of membrane monoenes. Oleic acid in membranes was a major monoene showing its mild rise in composition from 13.33 upto 13.96. The slope of linear regression line was not steep, however, the relationship between two functions, phospholipid concentrations and the rise, was significantly close (slope=0.0024, r^2=0.91).

4. SY-LRFE with plentiful content of linoleic acid in their surface induced a marked rise of linoleic acid in the membranes. Membrane linoleate showed its jump after being incubated with SY-LRFE at the highest concentration of phospholipids from 9 upto 16.2 or 80% increased. This rise was closely related to the concentrations of phospholipids in SY-LRFE (slope=0.0240, r^2=0.93).

5. It was likely that during the incubation with FM-LRFE docosahexaenoate as a major polyunsaturated fatty acid replaced other polyunsaturated fatty acids in the family of omega 6, linoleate and arachidonate, thus induced the decrement of omega 6 fatty acids similarly in degree of change but oppositely in direction.

6. No big alteration of any fatty acids was observed during the incubation with EY-LRFE. However, omega-3 fatty acids especially docosahexaenoate

were slightly dropped. This decrement might be responsible by the increment of membrane saturated and monounsaturated fatty acids received from EY-LRFE.

7. It was likely that the marked increment of linoleic acid during the incubation of red blood cells with SY-LRFE perturbed the composition of many polyunsaturated fatty acids in membranes mainly arachidonate and docosahexaenoate, two major polyunsaturated fatty acids in erythrocyte membranes.

Implications of the Results of Our Study

The results of these three main experiments of our study provide some information on at least two different aspects: 1) animal feed industry, 2) fat emulsion for medical use. According to the former aspect, it is known that Thailand domestic fish meal production plays significant role in huge growth of aquaculture production in this country. The implication from our study to the industry of fish meal is based on the fact that fish meal is valued according to its protein content. Products utilized as protein supplements in animal and fish feeds must have nutritional or price advantages over competing alternative ingredients. Actually, separating fat from fish meal results not only in lowering fat content downward to its accepted value within specification but also in rising its protein content in proportion resulting in higher value of such fish meal. According to our application, the separation of fat from fish meal provides simultaneously of both fish oil and lecithin. Conventionally, both fat products are widely utilized in both feed and food purposes. The implication of our study might extend a possible way of utilizing fish meal fat product further in clinical and pharmeceutical purposes. This might gain a great value added to fish meal.

According to the latter aspect, fish meal lecithin was demonstrated for the first time in our study that it behaved as supplier of omega 3 polyunsaturated fatty acids to blood cells. The results from our study imply that circulating blood cells, e.g. red blood cells, platelets, white blood cells, might be manipulated for their membrane fatty acid composition by emulsion or liposomes derived from fish meal. It has been widely recognized that polyunsaturated fatty acids on membranes play significant role

on membrane function, e.g. fluidity, flexibility, aggregation, prostanoid biosynthesis etc. The polyunsaturated fatty acids in membranes might be adjusted in their composition positively for supporting those membrane functions. This is probably a promising field of research. The novel fat emulsions as well as liposomes with omega-3 polyunsaturated fatty acid rich in phospholipid surface might play, at least partly, a significant role in this aspect.