CHAPTER III

RESULTS

Characteristics of Lipids in Fish Meals

A. Preliminary Selection of Raw Materials

At the earliest step of the experiment several raw materials: crude fat wastes obtained from tuna canning manufacturers, refined tuna oils and fish meals, were sampling for proximate fat analysis as well as for lecithin determination according to the procedures described earlier. The results showed that crude oil extracted from fish meals had lecithin contents as high as 11-15 g lecithins/100 g crude oil or 10-100 times higher comparing with 1-2 and 0.0-0.1 g lecithins/100 g of respective crude fat wastes and refined tuna oils (data not shown as Table). The results provided sufficient informations for the reason to select fish meal as raw materials for this experiment.

B. Contents of Fat and Lecithin

Fish meal samples utilized in this experiment like other available fish meals are categorized to be different grades with different prices according to their protein contents. As shown in **Table 1**, protein contents of fish meal samples used in our study range between 60-70 g/100 g samples. Fat contents of grades 1 and 2 are much higher than those of grades 3 and 4. According to the manufacturers, the results were obtained by means of proximate analysis employing petroleum ether for fat extraction. This procedure yielded an average fat content in all fish meal samples of 9.48 g/100 g samples (data not shown). However, the extraction of crude fat from the same samples at FORC by the modification procedure of Bligh and Dyer (1959) employing dichloromethane-methanol as extraction solvent system yielded 30% higher

Table 1 Characteristics of proximate ingredients of fish meals used in the experiment

Grade	Manufacturer	Moisture	Protein	Fat	Ash
1	Seri Baanpe	6.39	69.52	11.05	14.98
2	Seri Baanpe	4.12	68.58	11.34	13.14
3	SPP. FEED	8.32	63.20	7.96	17.86
4	SPP. FEED	7.16	60.16	7.56	19.85

The results are average of two determinations. (values are g/100g)
Data obtained from the manufacturers.

(12.46 g/100 g sample shown in **Table 2**). This can be explained by the advantage of absolute lipid extraction by means of chloroform-methanol as described elsewhere (Folch et al., 1957). Replacing chloroform by dichloromethane in any procedure does not provide less benefit (Hamilton and Hamilton, 1992).

As shown in **Table 2**, fish meals employed in the experiment provided fat and lecithin contents as much as 11-14 and 1-2 g/100 g samples, respectively. The lecithin concentrated upto the ranges of 8.86-14.30 g/100 g when its content in crude fat extracted from the corresponding fish meals is considered.

C. Content and Composition of Fatty Acids

1. Total Fatty Acids in Crude Fats

Table 3 shows the actual concentrations of individual and total fatty acids present in crude oils extracted from fish meal. Considering total fatty acids from the addition of individual fatty acids, grade 1 fish meal contains higher amount than any other grades ($p<0.05$). This advantage is extended to the content of fatty acid of interest, DHA, which grade 1 ranks the highest (2,411 in comparison to 2,009, 1,698 and 1,704 mg/100 g of respective grades 2, 3 and 4 fish meals). When the composition of fatty acids in crude fats are considered, grade 1 fish meal still shows its highest DHA content of 18.8 g/100 g total fatty acids comparing with 15.9-16.7 for those of other grades (**Table 4**). According to **Tables 3** and **4**, grade 1 thus expresses its prominent characteristics of having DHA at highest in amount as well as in proportion.

2. Fatty Acid Moieties of Triglyceride and Phospholipid

The fractions of triglycerides or triacylglycerols (TG) and phospholipids (PL), the latter so-called in this experiment as lecithin, were isolated from crude fats by chromatographic technique of one-dimentional TLC as described in the text. The actual concentrations of individual and total fatty acids derived from TG are shown in

Table 2 Contents of fat and lecithin in grade 1-4 fish meals and lecithin content in crude oil determined as described in the text (values are g/100g)

Grade	Fish Meal		Crude Oil's
	Fat	Lecithin	Lecithin
1	13.91	1.99	14.30
2	13.12	1.64	12.54
3	11.58	1.11	9.58
4	11.23	0.99	8.86
Average	12.46 ± 1.27	1.43 ± 0.47	11.32 ± 2.55

The results in grades 1-4 are average of two determinations.

The average values are expressed as Mean ± S.D.

Table 3 Contents of individual and total fatty acids, expressed as mg/100g fish meals, in crude oils extracted from grade 1-4 fish meals

Fatty acid	Fish Meal			
	Grade 1	Grade 2	Grade 3	Grade 4
C12:0	18.57 ± 0.73	13.32 ± 0.63	20.20 ± 1.61	21.06 ± 1.61
C14:0	697.01 ± 21.95	701.55 ± 15.59	652.61 ± 12.55	630.15 ± 29.59
C14:1	32.05 ± 0.81	31.97 ± 0.59	27.01 ± 0.91	32.94 ± 1.35
C16:0	3644.76 ± 63.63	3722.61 ± 59.61	2595.02 ± 33.14	2753.78 ± 66.22
C16:1n-7	743.27 ± 19.76	713.17 ± 10.82	694.84 ± 11.73	671.12 ± 19.60
C18:0	1479.70 ± 18.67	1460.31 ± 15.77	790.87 ± 6.11	840.79 ± 9.64
C18:1n-9	1569.25 ± 21.94	1532.89 ± 18.34	1404.67 ± 14.97	1393.95 ± 20.87
C18:2n-6	180.70 ± 1.90	173.34 ± 2.43	178.86 ± 19.15	176.38 ± 1.74
C18:3n-3	76.41 ± 1.54	71.43 ± 0.81	97.89 ± 1.40	103.79 ± 2.24
C20:0	99.55 ± 2.28	104.64 ± 4.85	51.39 ± 1.84	58.29 ± 2.86
C20:1n-9	77.66 ± 3.66	69.03 ± 4.00	366.20 ± 43.96	249.71 ± 4.79
C20:4n-6	412.03 ± 9.58	314.02 ± 9.97	238.76 ± 16.96	179.38 ± 2.17
C20:5n-3	648.57 ± 15.30	561.72 ± 13.94	850.23 ± 12.62	711.20 ± 17.91
C22:5n-3	201.89 ± 6.51	145.48 ± 9.97	175.65 ± 4.80	136.72 ± 6.57
C22:6n-3	2411.49 ± 57.94	2009.54 ± 59.20	1698.52 ± 3.98	1704.13 ± 64.81
Others	504.30 ± 30.53	445.38 ± 8.09	865.21 ± 16.57	704.59 ± 29.94
Total FA (g/100g)	12.80 ± 0.30^{a}	12.07 ± 0.25^{b}	10.65 ± 0.21^{c}	10.33 ± 0.29^{c}

The results of individual and total fatty acids are expressed as Mean ± S.D. of five determinations.

The different letters in the same row shown as a, b, c are significant differences ($p<0.05$)

Table 4 Fatty acid composition of crude oil of grade 1-4 fish meals, expressed as g/100g total fatty acids

Fatty acid	Fish Meal			
	Grade 1	Grade 2	Grade 3	Grade 4
C12:0	0.15 ± 0.01	0.11 ± 0.01	0.19 ± 0.01	0.20 ± 0.02
C14:0	5.45 ± 0.17	5.81 ± 0.13	6.10 ± 0.09	6.08 ± 0.29
C14:1	0.25 ± 0.01	0.26 ± 0.00	0.25 ± 0.01	0.32 ± 0.01
C16:0	28.48 ± 0.50	30.84 ± 0.49	24.25 ± 0.15	26.56 ± 0.64
C16:1n-7	5.81 ± 0.15	5.91 ± 0.09	6.49 ± 0.08	6.47 ± 0.18
C18:0	11.56 ± 0.15	12.10 ± 0.13	7.39 ± 0.03	8.11 ± 0.07
C18:1n-9	12.26 ± 0.17	12.70 ± 0.15	13.18 ± 0.14	13.49 ± 0.20
C18:2n-6	1.41 ± 0.01	1.44 ± 0.02	1.67 ± 0.18	1.70 ± 0.01
C18:3n-3	0.60 ± 0.01	0.59 ± 0.01	0.91 ± 0.01	1.00 ± 0.02
C20:0	0.78 ± 0.02	0.87 ± 0.04	0.48 ± 0.01	0.56 ± 0.03
C20:1n-9	0.61 ± 0.03	0.57 ± 0.03	3.42 ± 0.40	2.41 ± 0.04
C20:4n-6	3.22 ± 0.07	2.60 ± 0.08	2.18 ± 0.02	1.74 ± 0.03
C20:5n-3	5.07 ± 0.12	4.65 ± 0.12	7.94 ± 0.12	6.86 ± 0.15
C22:5n-3	1.58 ± 0.05	1.21 ± 0.08	1.64 ± 0.05	1.32 ± 0.07
C22:6n-3	18.84 ± 0.45	16.65 ± 0.49	15.87 ± 0.15	16.43 ± 0.60
Others	3.94 ± 0.24	3.69 ± 0.07	8.12 ± 0.16	6.82 ± 0.29

The results of individual fatty acids are expressed as Mean ± S.D. of five determinations.

Table 5. The total concentrations of TG fatty acids (TG-FA) in grades 1 and 2 are significantly higher than those of grades 3 and 4 ($p<0.05$). The results imply the high DHA contents present in grades 1 and 2 as well. When profile or composition of TG-FA are considered as shown in **Table 6**, percentage of DHA present in TG of grades 1 and 2 are still higher than those in TG of the remaining grades (14.8-16.4 vs 11.4-13.9). This also reflects to the quality of oil extracted from fish meals. Actually, Thai fish meals provide DHA rich fish oil and are probably selected alternatively as raw material for fish oil manufacturing.

Table 7 shows the actual amount of individual and total fatty acids found in PL fraction separated from fish meals' crude fats. The results are likely that the total contents of PL-FA relate to the quality of fish meal. Grade 1 has the highest PL-FA content (1.46 g/100 g fish meal) whereas the other grades show the figures in order from 2 to 4 (1.20 to 0.73). The significant differences at the level of $p<0.05$ are found in the order of 1-4. Actual DHA content of PL fraction also shows the similar order and grade 1 still contains the highest DHA in PL fraction. As shown in **Table 8,** the percentage values of DHA in PL-FA still confirm the prominent characteristics of lipids in fish meals provided by Thai manufacturers that they are rich source of DHA which present in both TG and PL fractions.

Focussing into the degree of unsaturation, **Table 9** shows that crude oils extracted from fish meals employed in the experiment constitute equal amounts of saturated and unsaturated fatty acids (5089 vs 5766 mg/100 g fish meals, the latter figure obtained from the addition of monoenes and polyenes). Omega-3 PUFA are the major polyenes in fish meal's crude oil (2901 of n-3 vs 463 of n-6). Again, grade 1 contains the highest polyenes especially n-3 PUFA in comparison to those of other grades ($p<0.05$). Similar to those of crude oil fats as mentioned earlier, saturated fatty acids and unsaturated fatty acids of TG-PL (**Table 10**) as well as of PL-FA (**Table 11**) in every grades of fish meals are in equal amounts. Still, grade 1 contains the highest amount of polyenes especially n-3 PUFA in comparison to those of other grades (**Tables 10** and **11**).

Table 12 shows degree of unsaturation of fatty acids found in TG, PL and crude oil. One can notice that average percentage of polyenoic fatty acids

Table 5 Contents of individual and total fatty acid, expressed as mg/100g fish meals, in triglyceride (TG-FA) of grade 1-4 fish meals

Fatty acid	Fish Meal			
	Grade 1	Grade 2	Grade 3	Grade 4
C12:0	19.52 ± 3.85	16.01 ± 3.92	23.86 ± 3.07	15.50 ± 4.68
C14:0	781.82 ± 53.75	734.42 ± 33.89	746.09 ± 49.33	708.74 ± 42.06
C14:1	32.69 ± 0.60	31.25 ± 0.77	27.74 ± 1.53	31.66 ± 1.35
C16:0	3432.38 ± 114.49	3450.60 ± 48.50	2462.41 ± 82.12	2461.09 ± 24.07
C16:1n-7	792.46 ± 21.12	707.45 ± 22.26	761.58 ± 49.15	730.98 ± 42.47
C18:0	1199.47 ± 115.63	1294.19 ± 65.71	703.05 ± 46.07	667.71 ± 30.03
C18:1n-9	1425.36 ± 101.56	1453.66 ± 30.37	1376.69 ± 57.17	1376.57 ± 42.63
C18:2n-6	170.58 ± 8.01	155.31 ± 5.02	160.59 ± 5.45	182.40 ± 11.20
C18:3n-3	81.68 ± 15.86	65.65 ± 6.93	95.23 ± 8.00	112.01 ± 15.23
C20:0	98.40 ± 20.47	109.62 ± 14.66	54.48 ± 7.89	61.04 ± 6.48
C20:1n-9	73.86 ± 12.44	72.31 ± 8.66	455.12 ± 54.52	346.23 ± 36.92
C20:4n-6	258.75 ± 10.17	242.54 ± 2.99	181.18 ± 12.22	131.26 ± 2.28
C20:5n-3	561.08 ± 106.92	462.51 ± 30.48	714.35 ± 74.02	665.08 ± 94.80
C22:5n-3	185.53 ± 24.61	140.20 ± 9.88	167.61 ± 19.94	150.08 ± 9.05
C22:6n-3	1852.17 ± 135.84	1607.42 ± 101.06	1138.10 ± 77.43	1351.42 ± 28.84
Others	358.25 ± 8.03	353.86 ± 22.59	878.89 ± 104.61	736.21 ± 44.57
Total FA (g/100g)	11.32 ± 0.80[a]	10.90 ± 0.43[a,b]	9.95 ± 0.70[b]	9.73 ± 0.54[b]

The results of individual and total fatty acids are expressed as Mean ± S.D. of three determinations.

The different letters in the same row shown as a, b are significant differences ($p<0.05$).

Table 6 Fatty acid composition of triglyceride of grade 1-4 fish meals, expressed as g/100g total fatty acids

Fatty acid	Fish Meal			
	Grade 1	Grade 2	Grade 3	Grade 4
C12:0	0.17±0.03	0.15±0.04	0.24±0.03	0.16±0.05
C14:0	6.90±0.47	6.74±0.31	7.50±0.50	7.29±0.43
C14:1	0.29±0.01	0.29±0.01	0.28±0.02	0.33±0.01
C16:0	30.31±1.01	31.67±0.45	24.76±0.83	25.30±0.25
C16:1n-7	7.00±0.19	6.49±0.20	7.66±0.49	7.51±0.44
C18:0	10.59±1.02	11.88±0.60	7.07±0.46	6.86±0.31
C18:1n-9	12.59±0.90	13.34±0.28	13.84±0.57	14.15±0.44
C18:2n-6	1.51±0.07	1.43±0.05	1.61±0.05	1.88±0.12
C18:3n-3	0.72±0.14	0.60±0.06	0.96±0.08	1.15±0.16
C20:0	0.87±0.18	1.01±0.13	0.55±0.08	0.63±0.07
C20:1n-9	0.65±0.11	0.66±0.08	4.58±0.55	3.56±0.38
C20:4n-6	2.28±0.09	2.23±0.03	1.82±0.12	1.35±0.02
C20:5n-3	4.95±0.94	4.24±0.28	7.18±0.74	6.84±0.97
C22:5n-3	1.64±0.22	1.29±0.93	1.69±0.20	1.54±0.09
C22:6n-3	16.36±1.20	14.75±0.93	11.44±0.78	13.89±0.30
Others	3.16±0.07	3.25±0.21	8.84±1.05	7.57±0.46

The results of individual fatty acids are expressed as Mean ± S.D. of three determinations.

Table 7 Contents of individual and total fatty acid, expressed as mg/100g fish meals, in phospholipid (PL-FA) of grade 1-4 fish meals

Fatty acid	Fish Meal			
	Grade 1	Grade 2	Grade 3	Grade 4
C12:0	0.84 ± 0.22	0.77 ± 0.14	0.44 ± 0.10	0.68 ± 0.18
C14:0	35.95 ± 0.38	39.61 ± 2.04	23.12 ± 1.22	25.46 ± 5.08
C14:1	2.25 ± 0.31	2.15 ± 0.24	1.22 ± 0.01	1.71 ± 0.41
C16:0	401.14 ± 9.83	359.11 ± 7.32	222.39 ± 1.37	211.18 ± 11.49
C16:1n-7	48.77 ± 0.43	43.90 ± 1.87	27.52 ± 0.82	28.86 ± 2.44
C18:0	188.99 ± 7.86	166.05 ± 1.42	77.51 ± 1.91	75.46 ± 2.87
C18:1n-9	179.09 ± 1.72	137.08 ± 1.09	100.11 ± 1.60	90.78 ± 1.65
C18:2n-6	15.41 ± 0.47	13.33 ± 0.08	9.65 ± 0.13	9.63 ± 0.22
C18:3n-3	3.39 ± 0.21	3.98 ± 0.16	3.60 ± 0.27	3.57 ± 0.07
C20:0	9.07 ± 0.30	8.18 ± 0.32	3.05 ± 0.10	3.87 ± 0.22
C20:1n-9	7.47 ± 1.86	6.90 ± 1.78	8.61 ± 1.25	8.38 ± 0.62
C20:4n-6	70.28 ± 1.16	38.79 ± 1.26	22.64 ± 1.04	18.37 ± 1.02
C20:5n-3	64.84 ± 0.96	51.69 ± 0.67	58.50 ± 0.36	43.50 ± 2.02
C22:5n-3	18.47 ± 1.13	12.09 ± 0.27	10.61 ± 0.31	7.32 ± 0.29
C22:6n-3	323.55 ± 6.24	234.80 ± 8.90	183.40 ± 1.81	149.69 ± 10.01
Others	90.49 ± 4.53	78.58 ± 7.04	57.64 ± 2.08	51.52 ± 4.37
Total FA (g/100g)	1.46 ± 0.04[a]	1.20 ± 0.03[b]	0.81 ± 0.01[c]	0.73 ± 0.04[d]

The results of individual and total fatty acids are expressed as Mean ± S.D. of three determinations.

The different letters in the same row shown as a, b, c, d are significant differences ($p<0.05$).

Table 8 Fatty acid composition of phospholipid of grade 1-4 fish meals, expressed as g/100g total fatty acids

Fatty acid	Fish Meal			
	Grade 1	Grade 2	Grade 3	Grade 4
C12:0	0.06 ± 0.02	0.06 ± 0.01	0.05 ± 0.01	0.09 ± 0.02
C14:0	2.46 ± 0.03	3.31 ± 0.17	2.85 ± 0.15	3.49 ± 0.70
C14:1	0.15 ± 0.02	0.18 ± 0.02	0.15 ± 0.00	0.23 ± 0.06
C16:0	27.48 ± 0.67	30.00 ± 0.61	27.46 ± 0.17	28.93 ± 1.57
C16:1n-7	3.34 ± 0.03	3.67 ± 0.16	3.40 ± 0.10	3.95 ± 0.33
C18:0	12.94 ± 0.54	13.87 ± 0.12	9.57 ± 0.24	10.34 ± 0.39
C18:1n-9	12.27 ± 0.12	11.45 ± 0.09	12.48 ± 0.12	12.44 ± 0.23
C18:2n-6	1.06 ± 0.03	1.11 ± 0.01	1.19 ± 0.02	1.32 ± 0.03
C18:3n-3	0.23 ± 0.01	0.33 ± 0.01	0.44 ± 0.03	0.49 ± 0.01
C20:0	0.62 ± 0.02	0.68 ± 0.03	0.38 ± 0.01	0.53 ± 0.03
C20:1n-9	0.51 ± 0.13	0.58 ± 0.15	1.06 ± 0.15	1.15 ± 0.08
C20:4n-6	4.81 ± 0.08	3.24 ± 0.11	2.79 ± 0.13	2.52 ± 0.14
C20:5n-3	4.44 ± 0.07	4.32 ± 0.06	7.22 ± 0.04	5.96 ± 0.28
C22:5n-3	1.27 ± 0.08	1.01 ± 0.02	1.31 ± 0.04	1.00 ± 0.04
C22:6n-3	22.16 ± 0.43	19.62 ± 0.74	22.64 ± 0.22	20.51 ± 1.37
Others	6.20 ± 0.31	6.56 ± 0.16	7.12 ± 0.26	7.06 ± 0.69

The results of individual fatty acids are expressed as Mean ± S.D. of three determinations.

Table 9 Saturated, Monoenes and Polyenes fatty acids in crude oil of grade 1-4 fish meals (mg/100g fish meals)

Fatty acid	Fish Meal				Average
	Grade 1	Grade2	Grade 3	Grade 4	
Saturated	5939.58 ± 85.26^{a}	6002.42 ± 61.87^{a}	4110.09 ± 52.48^{c}	4304.07 ± 92.33^{b}	5089.04 ± 888.06
Monoenes	2422.23 ± 30.56^{b}	2347.06 ± 31.59^{c}	2492.72 ± 52.89^{a}	2347.71 ± 32.90^{c}	2402.43 ± 71.44
Polyenes	3931.09 ± 81.89^{a}	3275.54 ± 87.66^{b}	3239.91 ± 22.62^{b}	3011.61 ± 89.28^{c}	3364.54 ± 350.67
n-3	3338.36 ± 74.01^{a}	2788.18 ± 77.72^{b}	2822.29 ± 12.30^{b}	2655.84 ± 87.66^{c}	2901.17 ± 269.10
n-6	592.73 ± 10.47^{a}	487.36 ± 10.62^{b}	417.62 ± 25.11^{c}	355.76 ± 2.71^{d}	463.37 ± 89.22
n-3/n-6	5.63 ± 0.09^{c}	5.72 ± 0.06^{c}	6.78 ± 0.41^{b}	7.46 ± 0.22^{a}	6.40 ± 0.80

The results are expressed as Mean ± S.D. of five determinations.

The different letters in the same row shown as a, b, c, d are significant differences ($p<0.05$).

Table 10 Saturated, Monoenes and Polyenes fatty acids in triglyceride of grade 1-4 fish meals (mg/100g fish meals)

Fatty acid	Fish Meal				Average
	Grade 1	Grade 2	Grade 3	Grade 4	
Saturated	5531.59 ± 192.79^{a}	5604.84 ± 124.51^{a}	3989.91 ± 118.48^{b}	3914.08 ± 45.43^{b}	4289.96 ± 1319.77
Monoenes	2324.37 ± 101.31^{c}	2264.67 ± 15.87^{c}	2621.14 ± 86.04^{a}	2485.45 ± 70.86^{b}	2311.77 ± 597.76
Polyenes	3109.79 ± 288.10^{a}	2673.62 ± 108.93^{b}	2457.06 ± 175.94^{b}	2592.26 ± 107.86^{b}	2505.70 ± 686.20
n-3	2680.46 ± 272.66^{a}	2275.78 ± 109.07^{b}	2115.29 ± 162.80^{b}	2278.59 ± 103.50^{b}	2166.95 ± 596.20
n-6	429.33 ± 15.58^{a}	397.85 ± 5.25^{b}	341.77 ± 16.88^{c}	313.67 ± 10.19^{d}	338.75 ± 95.73
n-3/n-6	6.23 ± 0.42^{b}	5.72 ± 0.29^{b}	6.19 ± 0.33^{b}	7.27 ± 0.31^{a}	6.07 ± 1.65

The results are expressed as Mean ± S.D. of three determinations.

The different letters in the same row shown as a, b, c, d are significant differences ($p<0.05$).

Table 11 Saturated, Monoenes and Polyenes fatty acids in phospholipis of grade 1-4 fish meals (mg/100g fish meals)

Fatty acid	Fish Meal				Average
	Grade1	Grade2	Grade3	Grade4	
Saturated	636.00 ± 4.68^{a}	573.72 ± 8.92^{b}	326.51 ± 0.88^{c}	316.66 ± 14.97^{c}	472.34 ± 143.83
Monoenes	237.58 ± 1.46^{a}	190.03 ± 1.29^{b}	137.46 ± 1.17^{c}	129.73 ± 1.73^{d}	176.12 ± 43.97
Polyenes	495.94 ± 7.28^{a}	354.67 ± 10.55^{b}	288.39 ± 2.12^{c}	232.09 ± 13.15^{d}	346.40 ± 101.14
n-3	410.26 ± 5.94^{a}	302.55 ± 9.33^{b}	256.10 ± 1.75^{c}	204.09 ± 12.16^{d}	295.73 ± 78.34
n-6	85.68 ± 1.44^{a}	52.12 ± 1.34^{b}	32.29 ± 1.01^{c}	28.00 ± 1.18^{d}	50.67 ± 23.10
n-3/n-6	4.79 ± 0.03^{d}	5.80 ± 0.08^{c}	7.94 ± 0.25^{a}	7.29 ± 0.26^{b}	6.36 ± 1.23

The results are expressed as Mean ± S.D. of three determinations.

The different letters in the same row shown as a, b, c, d are significant differences ($p<0.05$).

Table 12 Saturated, Monoenes and Polyenes fatty acids in Crude oil, triglycerides and phospholipids of grade 1-4 fish meal (g/100g total fatty acids)

Fatty acid	Fish meal				Average
	Grade 1	Grade 2	Grade 3	Grade 4	
Crude oil					
Saturated	46.41 ± 0.67	49.73 ± 0.51	38.58 ± 0.49	41.66 ± 0.89	44.10 ± 4.45
Monoenes	18.93 ± 0.24	19.44 ± 0.26	23.40 ± 0.50	22.72 ± 0.32	21.12 ± 2.04
Polyenes	30.72 ± 0.64	27.14 ± 0.73	30.41 ± 0.21	29.15 ± 0.86	29.35 ± 1.60
n-3	26.09 ± 0.58	23.10 ± 0.64	26.49 ± 0.12	25.71 ± 0.85	25.35 ± 1.50
n-6	4.63 ± 0.08	4.04 ± 0.09	3.92 ± 0.24	3.44 ± 0.03	4.01 ± 0.46
n-3/n-6	5.63 ± 0.09	5.72 ± 0.06	6.78 ± 0.41	7.46 ± 0.22	6.40 ± 0.82
Triglyceride					
Saturated	48.85 ± 1.70	51.43 ± 1.14	40.11 ± 1.19	40.24 ± 0.47	43.91 ± 5.19
Monoenes	20.53 ± 0.89	20.73 ± 0.15	26.35 ± 0.86	25.55 ± 0.73	23.97 ± 2.78
Polyenes	27.46 ± 2.54	24.54 ± 1.00	24.70 ± 1.77	26.65 ± 1.11	25.79 ± 2.11
n-3	23.67 ± 2.41	20.88 ± 1.00	21.27 ± 1.64	23.42 ± 1.06	22.32 ± 2.05
n-6	3.79 ± 0.14	3.65 ± 0.05	3.44 ± 0.17	3.22 ± 0.10	3.48 ± 0.25
n-3/n-6	6.23 ± 0.42	5.72 ± 0.29	6.19 ± 0.33	7.27 ± 0.31	6.45 ± 0.69
Phospholipid					
Saturated	43.56 ± 0.32	47.93 ± 0.75	40.31 ± 0.11	43.38 ± 2.05	44.02 ± 2.98
Monoenes	16.27 ± 0.10	15.88 ± 0.11	16.97 ± 0.14	17.77 ± 0.24	16.71 ± 0.79
Polyenes	33.97 ± 0.50	29.63 ± 0.88	35.60 ± 0.26	31.79 ± 1.80	32.56 ± 2.53
n-3	28.10 ± 0.41	25.28 ± 0.78	31.62 ± 0.22	27.96 ± 1.67	28.01 ± 2.44
n-6	5.87 ± 0.10	4.35 ± 0.11	3.99 ± 0.12	3.84 ± 0.16	4.55 ± 0.86
n-3/n-6	4.79 ± 0.03	5.80 ± 0.08	7.94 ± 0.25	7.29 ± 0.26	6.36 ± 1.27

The results of fatty acids in crude oil are expressed as Mean ± S.D. of five determinations whereas fatty acids in triglycerides and phospholipids are Mean ± S.D. of three determinations.

especially those in n-3 group present majorily in PL or lecithin fractions (32.56 vs 25.79 for polyenes and 28.01 vs 22.32 for n-3 in PL-FA and TG-FA, respectively).

D. Polyenes and Phospholipids

As mentioned earlier, PL is rich source of polyene especially n-3 PUFA. **Figure 5** compares the proportion of unsaturated fatty acids found in PL and TG fractions. The results confirm that PL has markedly lower content of MUFA ($p<0.05$) but significant higher content of both n-3 and n-6 polyenoic fatty acids ($p<0.05$). The result also shows that n-3 PUFA are major polyenes in fat of marine animals. The difference between the contents of n-3 and n-6 obtained from fish meals used in the study range 5-6 times. In comparison to other n-3 PUFAs, DHA dominates in moieties of both TG and PL. Obviously, DHA of PL is found much higher than that of TG. This confirms the fact that DHA of marine fish distributes favorably in PL.

Figure 6 shows the ratios of total and major polyenoic fatty acids in n-3 and n-6 of each fish meal samples. As described earlier, grade 1 has the benefits of high contents of fat and lecithin as well as proportions of DHA in both moieties of TG and PL in comparison to those of three other grades. When the ratios of n-3/n-6 and DHA/AA are considered, grade 1 ranks the lowest ($p<0.05$). When the ratio of DHA and LA is calculated, however, grade 1 shows the highest. Both DHA and LA are major fatty acids found in respective marine fish and vegetable sources thus play crucial role in fatty acid exchanges during intravenous infusion of fat emulsions with their constituents derived from either origins.

Table 13 shows the composition or subclasses of lecithin obtained from individual sample of fish meal. Noticeably, lecithin of fish meals comprises majorily of choline containing PL, for instances, PC and SM. The average total choline PL is 68.33 mole% whereas PC is found as the major choline PL and share approximately half of the content. The result confirms that fish meal PL is a good source of choline which is known as brain food as will be described later.

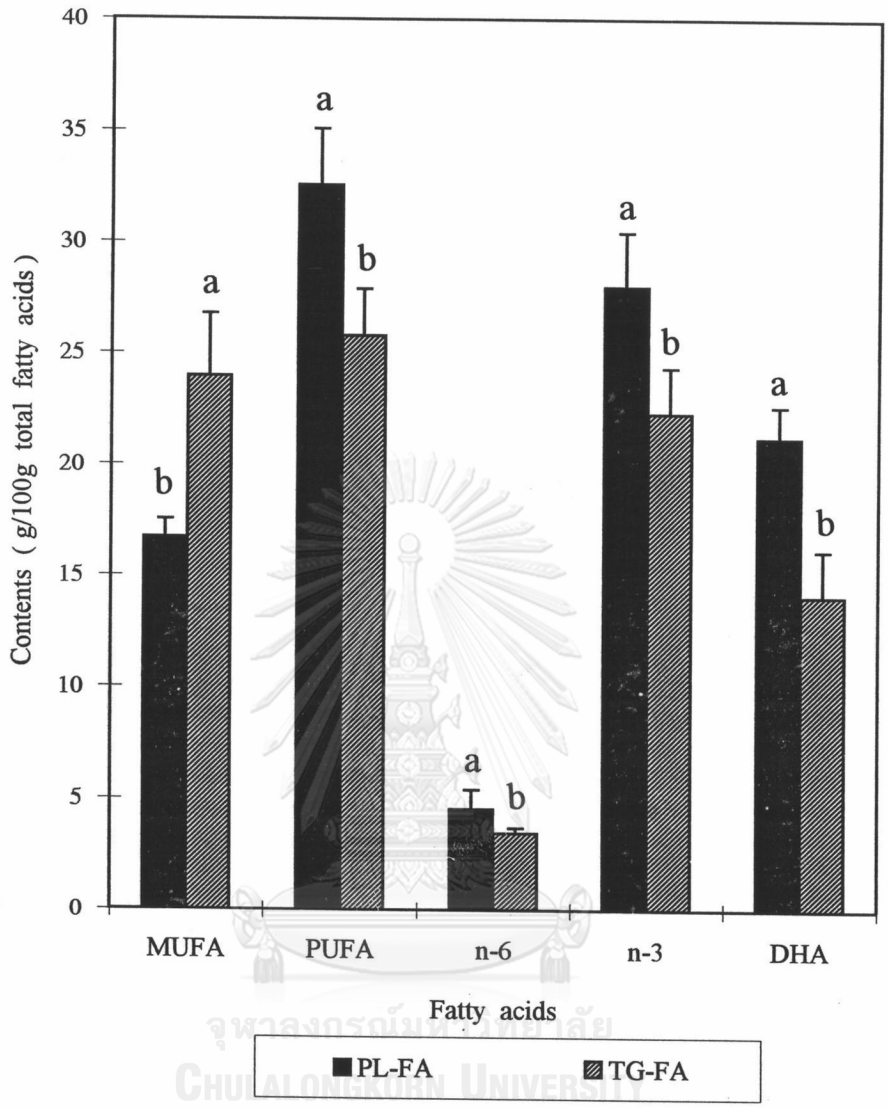

Figure 5 Comparison of fatty acid content in percentage between unsaturated fatty acids: MUFA, PUFA, n-6 PUFA, n-3 PUFA and DHA, expressed as Mean+S.D., in phospholipids (PL-FA) and those in triglycerides (TG-FA) of fish meal. Each values were mean of four grades of fish meal. The different letters shown as a,b are significant differences ($p<0.05$).

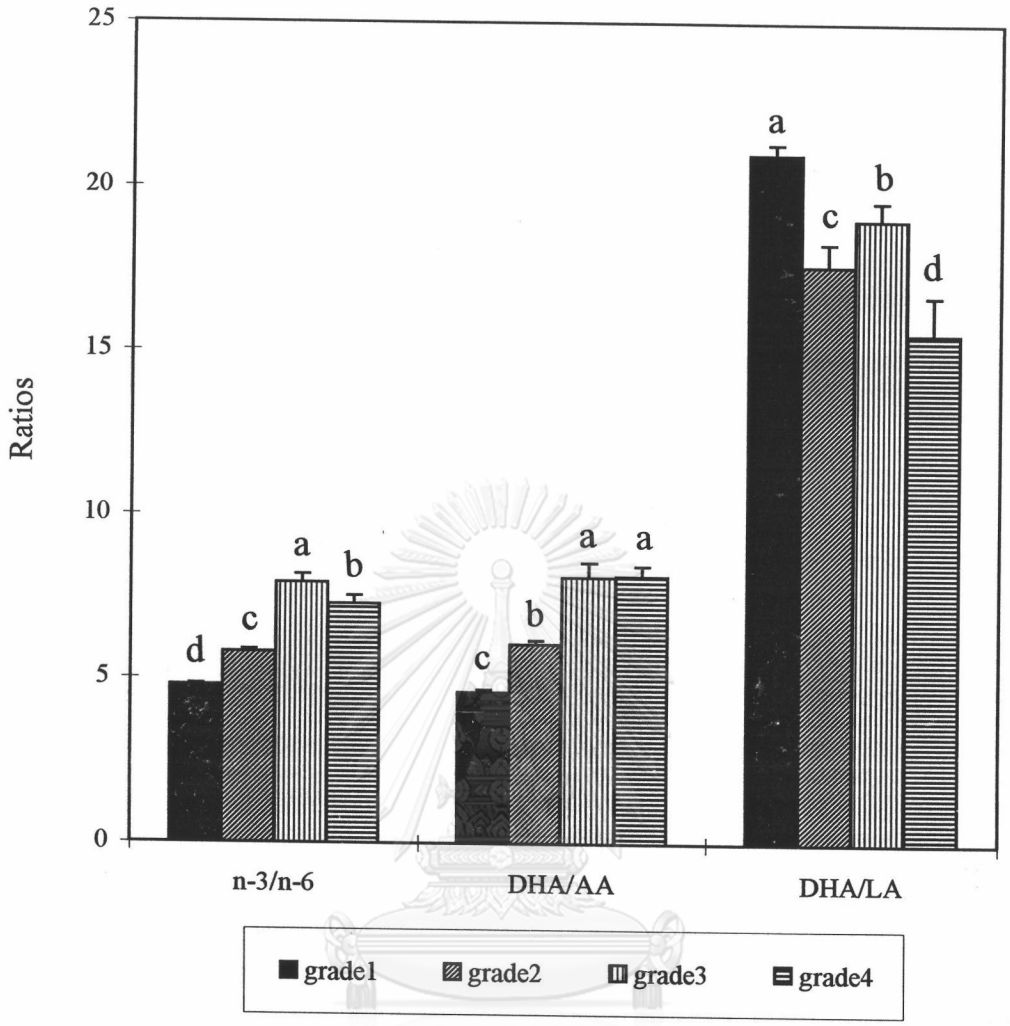

Figure 6 Ratios of n-3/n-6, DHA/AA and DHA/LA in PL-FA, comparing among grade 1-4 fish meals. The different letters shown as a,b are significant differences ($p<0.05$).

Table 13 Phospholipid subclasses of fish oil from grade 1-4 fish meals extracted by dichloromethane:methanol (mole/100moles total phospholipids)

PL-subclasses	Grade				Average
	1	2	3	4	
PA	2.83	2.40	1.18	1.91	2.08 ± 0.71
PE	10.14	8.50	9.94	9.69	9.57 ± 0.74
PC	51.15	48.15	48.00	52.32	49.91 ± 2.17
PS+PI	5.78	3.38	5.91	6.04	5.28 ± 1.27
SM	15.36	12.40	15.84	14.04	14.41 ± 1.54
LPC	3.82	8.90	3.34	0.00	4.02 ± 3.67
Others	10.92	16.27	15.76	16.00	14.74 ± 2.55
Total choline (PC+SM+LPC)	70.33	69.45	67.18	66.36	68.33 ± 1.87

The results are average of two determiantions.

E. Selection of Fish Meal

The results of this section as described above demonstrates that grade 1 fish meal has prominent characteristics of lipids over any other grades. The contents of fat, lecithin as well as n-3 PUFA of grade 1 are impressive to be anonymously selected as raw material for further study of the experiment.

Extraction of Lecithin

A. Study on the Effect of Alcohol Pretreatment

The efficiency of organic solvent like n-hexane conventionally employed for extracting fat and lecithin in food industrial process was studied. As shown in **Figure** 7, fat yielded after extracting sample with n-hexane alone is significantly lesser than those yielded after alcohol pretreatment of sample with either ethanol or methanol and followed by extracting with n-hexane ($p<0.05$). The data of fat content shown as histogram from the leftmost column to the rightmost of top figure (**Figure 7 a**) were as follows: 6.86±0.13, 11.40±0.22 and 11.69±0.23 g of fat/100 g fish meal for those obtained from n-hexane alone, those from n-hexane pretreated with ethanol and with methanol, respectively. Lecithin content found in the corresponding crude fats extracted as described earlier are different in a same means (0.53±0.02, 0.97±0.04 and 1.12±0.08 g lecithin/100 g fish meal for n-hexane and those pretreated with ethanol and methanol, respectively, $p<0.05$). The data verify the advantage of alcohol pretreatment of samples before conventional n-hexane extraction of lecithin.

The actual concentrations of lecithin present in crude fat extracted by n-hexane alone in comparison to those pretreated with either ethanol or methanol are considered in **Figure 8**. The top figure (a) shows lecithin concentrations before-whereas the bottom figure (b) shows the concentrations after the exclusion of neutral lipids exclusively TG by flushing extraction solvent system with acetone. Pretreatment sample with methanol before consequently extracting with n-hexane yields lecithin higher in comparison to extracting with n-hexane with or without pretreating with ethanol (**Figure 8 a**, $p<0.05$). After reducing neutral lipids from

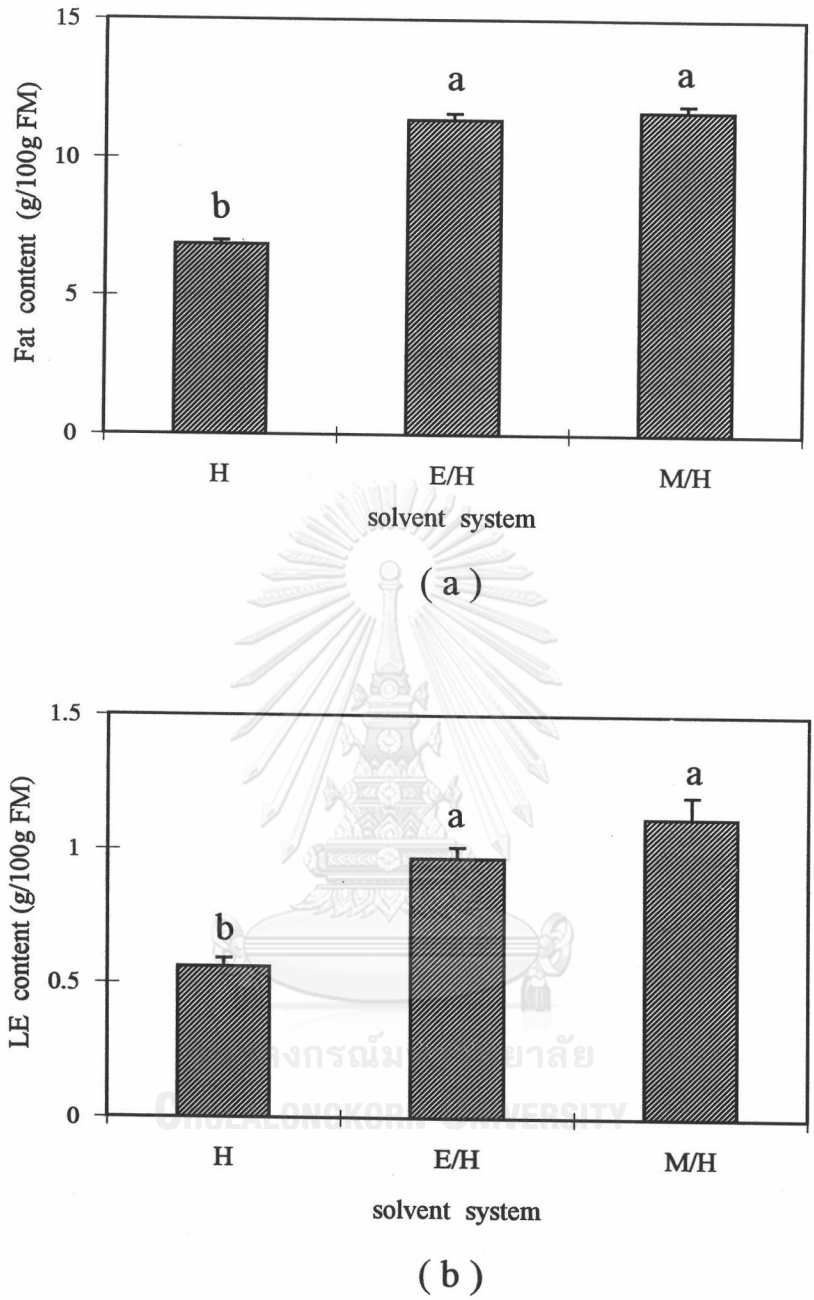

Figure 7 Fat (top figure, a) and lecithin (bottom, b) contents yielded from fish meal after extracting with n-hexane alone (leftmost column) and with pretreating with ethanol (centered) or methanol (rightmost) before extracting with n-hexane. Results were expressed as g/100g fish meal. Abbreviations: H, hexane; E, ethanol; M, methanol.

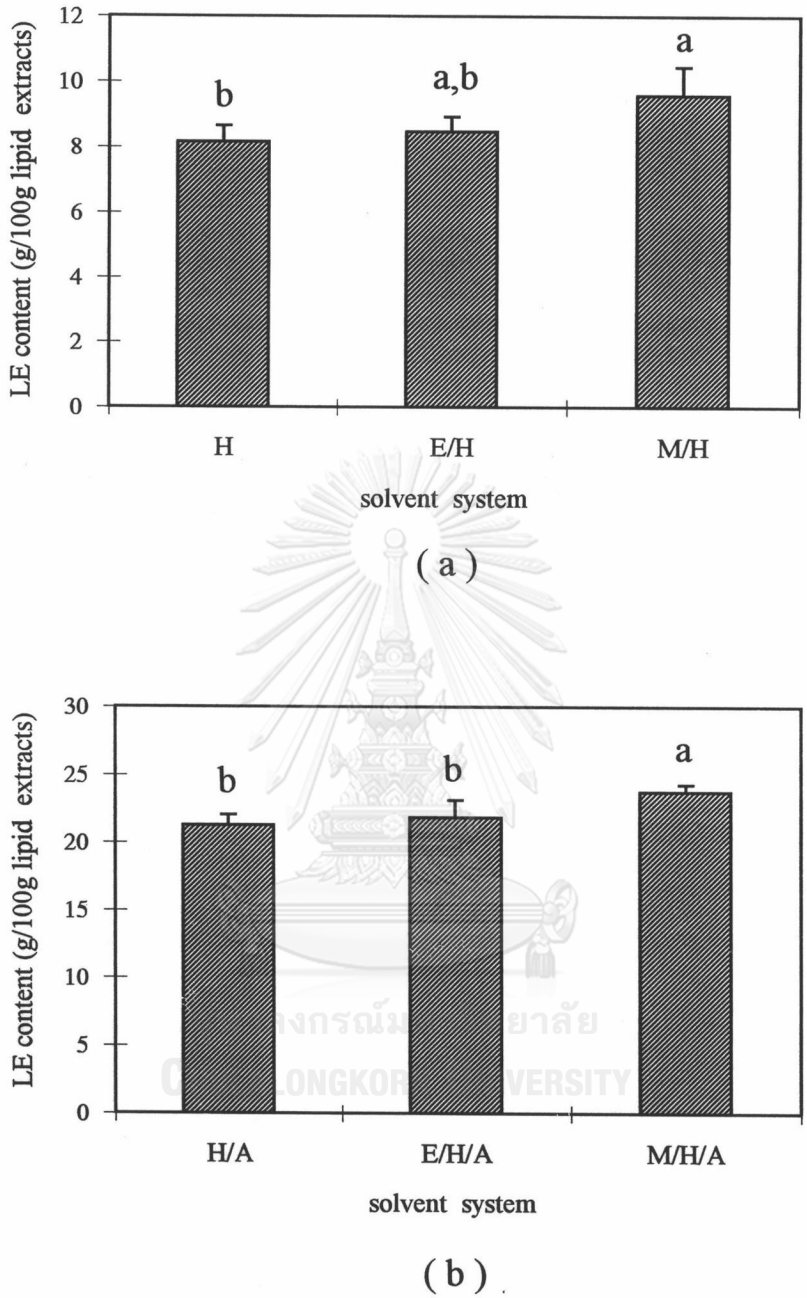

Figure 8 Contents of lecithin in lipid extracts obtained from extracting fish meal with hexane alone or with hexane pretreated either with ethanol or methanol before deoiling (top figure, a) and after deoiling with acetone (bottom figure, b). Results were expressed in g lecithin/100 g lipid extracts. Abbreviations: H, hexane; E, ethanol; M, methanol; A, acetone.

crude fats by means of acetone, lecithin contents in pooled organic solvent mixture are subsequently concentrated. Pretreatment of sample with methanol yields lecithin content higher in acetone insoluble fractions in comparison to those obtained from the extraction with n-hexane with and without ethanol pretreatment (**Figure 8 b**, $p<0.05$). Superior to ethanol, the obtained result confirms the benefit of pretreatment procedure with methanol before conventional n-hexane crude fat extraction.

B. Lipid Characteristics during the Extraction Process

1. Composition of Fatty Acids

Tables 14-16 demonstrate the effects of solvent extraction system on the composition of fatty acids of crude oil, acetone soluble (neutral lipids exclusively TG) and acetone insoluble fractions (polar lipids exclusively lecithins), respectively. The treatments of either ethanol or methanol prior (**Tables 14**) and after (**Table 15**) neutral lipid extraction with acetone give slight and non-significant effect on fatty acid composition of crude oil and acetone soluble fraction, respectively. However, the similar treatment after neutral lipid extraction with acetone is likely to give some alternation on n-3 PUFA especially DHA (**Table 16**). Total amount of polyenes especially n-3 PUFA expressed as g/100 g fatty acids in acetone insoluble fraction is likely to rise with methanol pretreatment (**Table 17**). The significant differences of above-mentioned polyenes are vividly clear by histograms as shown in **Figure 9**. Preparation of lecithin by means of n-hexane-acetone extraction with methanol pretreatment demonstrates the benefit of higher polyenes proportion especially n-3 PUFA ($p<0.05$). No difference is observed in n-6 PUFA. Among n-3 polyenes, DHA shows significantly and prominently high (**Figure 9**). This result confirms the superiority of methanol pretreatment over ethanol pretreatment procedure for the preparation of lecithin from animal origin especially fish meals. Hence, the preparation of lecithin in our experiment follows the procedure of two stepwise n-hexane-acetone extraction with methanol pretreatment.

Table 14 Fatty acid composition, expressed as g/100g total fatty acids of crude oil extracted by solvents system with and without alcohol pretreatment before excluding out neutral fats by treating with acetone

Fatty acid	Solvent systems		
	H	E/H	M/H
C12:0	0.19 ± 0.01	0.17 ± 0.01	0.18 ± 0.01
C14:0	6.96 ± 0.22	6.26 ± 0.11	6.36 ± 0.15
C14:1	0.26 ± 0.01	0.24 ± 0.00	0.24 ± 0.01
C16:0	24.14 ± 0.65	24.21 ± 0.07	26.15 ± 0.27
C16:1n-7	7.08 ± 0.19	6.58 ± 0.05	6.62 ± 0.04
C18:0	7.06 ± 0.11	7.08 ± 0.07	7.56 ± 0.14
C18:1n-9	13.66 ± 0.29	13.13 ± 0.22	13.35 ± 0.18
C18:2n-6	1.61 ± 0.03	1.53 ± 0.01	1.62 ± 0.02
C18:3n-3	0.93 ± 0.02	0.88 ± 0.01	0.88 ± 0.02
C20:0	0.51 ± 0.02	0.45 ± 0.03	0.41 ± 0.07
C20:1n-9	3.82 ± 0.08	3.85 ± 0.12	3.14 ± 0.03
C20:4n-6	1.98 ± 0.04	2.15 ± 0.04	2.13 ± 0.02
C20:5n-3	7.82 ± 0.12	7.89 ± 0.19	7.78 ± 0.07
C22:5n-3	1.64 ± 0.10	1.67 ± 0.04	1.55 ± 0.06
C22:6n-3	13.92 ± 0.53	15.49 ± 0.08	15.07 ± 0.35
Others	8.41 ± 0.51	8.41 ± 0.48	6.95 ± 0.19

The results of individual fatty acids are expressed as Mean ± S.D. of three determinations.

Abbreviations: H, hexane; E, ethanol; M, methanol.

Table 15 Fatty acid composition, expressed as g/100g total fatty acids found in acetone soluble fraction (neutral lipid fraction) after extracting by three consecutive solvents, hexane, alcohol and acetone

Fatty acid	Solvent systems		
	H/A	E/H/A	M/H/A
C12:0	0.19 ± 0.01	0.20 ± 0.01	0.19 ± 0.01
C14:0	6.95 ± 0.11	6.88 ± 0.19	6.89 ± 0.20
C14:1	0.26 ± 0.01	0.26 ± 0.00	0.26 ± 0.00
C16:0	24.07 ± 0.09	24.78 ± 0.29	24.77 ± 0.24
C16:1n-7	7.22 ± 0.02	7.03 ± 0.12	7.08 ± 0.09
C18:0	6.91 ± 0.06	6.83 ± 0.04	6.87 ± 0.10
C18:1n-9	13.70 ± 0.30	13.16 ± 0.05	13.18 ± 0.33
C18:2n-6	1.61 ± 0.02	1.58 ± 0.01	1.63 ± 0.05
C18:3n-3	0.95 ± 0.01	0.88 ± 0.00	0.94 ± 0.03
C20:0	0.50 ± 0.06	0.45 ± 0.01	0.45 ± 0.01
C20:1n-9	4.28 ± 0.01	3.36 ± 0.05	3.40 ± 0.06
C20:4n-6	2.01 ± 0.03	2.09 ± 0.07	2.12 ± 0.05
C20:5n-3	7.91 ± 0.09	8.06 ± 0.03	8.14 ± 0.07
C22:5n-3	1.55 ± 0.07	1.61 ± 0.02	1.59 ± 0.07
C22:6n-3	13.69 ± 0.25	15.16 ± 0.28	14.89 ± 0.38
Others	8.19 ± 0.33	7.66 ± 0.21	7.61 ± 0.12

The results of individual fatty acids are expressed as Mean ± S.D. of three determinations.

Abbreviations: H, hexane; E, ethanol; M, methanol; A, acetone.

Table 16 Fatty acid composition, expressed as g/100g total fatty acids found in acetone insoluble fraction (lecithin fraction) after extracting by three consecutive solvents, hexane, alcohol and acetone

Fatty acid	Solvent systems		
	H/A	E/H/A	M/H/A
C12:0	0.19 ± 0.01	0.13 ± 0.01	0.11 ± 0.01
C14:0	6.37 ± 0.11	4.66 ± 0.10	4.74 ± 0.05
C14:1	0.25 ± 0.01	0.21 ± 0.01	0.20 ± 0.00
C16:0	25.33 ± 0.29	29.43 ± 0.40	28.47 ± 0.16
C16:1n-7	6.55 ± 0.03	4.70 ± 0.07	4.75 ± 0.03
C18:0	8.32 ± 0.08	10.53 ± 0.15	9.52 ± 0.03
C18:1n-9	13.87 ± 0.22	13.36 ± 0.14	12.81 ± 0.15
C18:2n-6	1.56 ± 0.01	1.35 ± 0.02	1.39 ± 0.09
C18:3n-3	0.78 ± 0.02	0.57 ± 0.02	0.59 ± 0.01
C20:0	0.51 ± 0.03	0.55 ± 0.05	0.50 ± 0.02
C20:1n-9	3.41 ± 0.08	1.79 ± 0.17	2.31 ± 0.00
C20:4n-6	1.99 ± 0.18	2.21 ± 0.04	2.30 ± 0.01
C20:5n-3	7.16 ± 0.19	6.04 ± 0.09	6.42 ± 0.11
C22:5n-3	1.64 ± 0.11	0.94 ± 0.10	1.26 ± 0.02
C22:6n-3	14.08 ± 0.29	14.63 ± 0.26	17.51 ± 0.27
Others	8.00 ± 0.13	8.90 ± 0.42	7.11 ± 0.13

The results of individual fatty acids are expressed as Mean ± S.D.of three determinations.

Abbreviations: H, hexane; E, ethanol; M, methanol; A, acetone.

Table 17 Saturated, Monoenoic and Polyenoic fatty acids, expressed as g/100g total fatty acids, of crude oil, acetone soluble and acetone insoluble fractions after extracting by three consecutive solvents, hexane, alcohol and acetone

Fatty acid	Solvent system		
	H/A	E/H/A	M/H/A
Crude oil			
Total amount (g/100g FM)	6.86 ± 0.12	11.40 ± 0.22	11.69 ± 0.23
Saturated	38.87 ± 0.81	38.17 ± 0.23	40.67 ± 0.47
Monoenes	24.82 ± 0.40	23.81 ± 0.23	23.36 ± 0.21
Polyenes	27.90 ± 0.69	29.61 ± 0.23	29.02 ± 0.44
n-3	24.31 ± 0.68	25.93 ± 0.18	25.28 ± 0.42
n-6	3.59 ± 0.04	3.68 ± 0.05	3.74 ± 0.03
n-3/n-6	6.77 ± 0.18	7.07 ± 0.04	6.76 ± 0.08
Acetone soluble			
Total amount (g/100g FM)	5.62 ± 0.11	9.05 ± 0.17	6.67 ± 0.13
Saturated	38.63 ± 0.10	39.14 ± 0.43	39.17 ± 0.37
Monoenes	25.47 ± 0.30	23.81 ± 0.13	23.92 ± 0.28
Polyenes	27.72 ± 0.39	29.39 ± 0.36	29.31 ± 0.34
n-3	24.10 ± 0.34	25.71 ± 0.31	25.56 ± 0.42
n-6	3.62 ± 0.05	3.68 ± 0.07	3.75 ± 0.08
n-3/n-6	6.66 ± 0.02	6.99 ± 0.11	6.83 ± 0.25
Acetone insoluble			
Total amount (g/100g FM)	1.25 ± 0.02	2.36 ± 0.05	5.02 ± 0.10
Saturated	40.72 ± 0.30	45.30 ± 0.51	43.34 ± 0.24
Monoenes	24.08 ± 0.20	20.06 ± 0.09	20.08 ± 0.14
Polyenes	27.20 ± 0.54	25.74 ± 0.35	29.47 ± 0.30
n-3	23.65 ± 0.42	22.18 ± 0.36	25.78 ± 0.29
n-6	3.55 ± 0.19	3.56 ± 0.02	3.69 ± 0.09
n-3/n-6	6.67 ± 0.31	6.23 ± 0.11	7.00 ± 0.20

The results of individual fatty acids are expressed as Mean ± S.D. of three determinations.

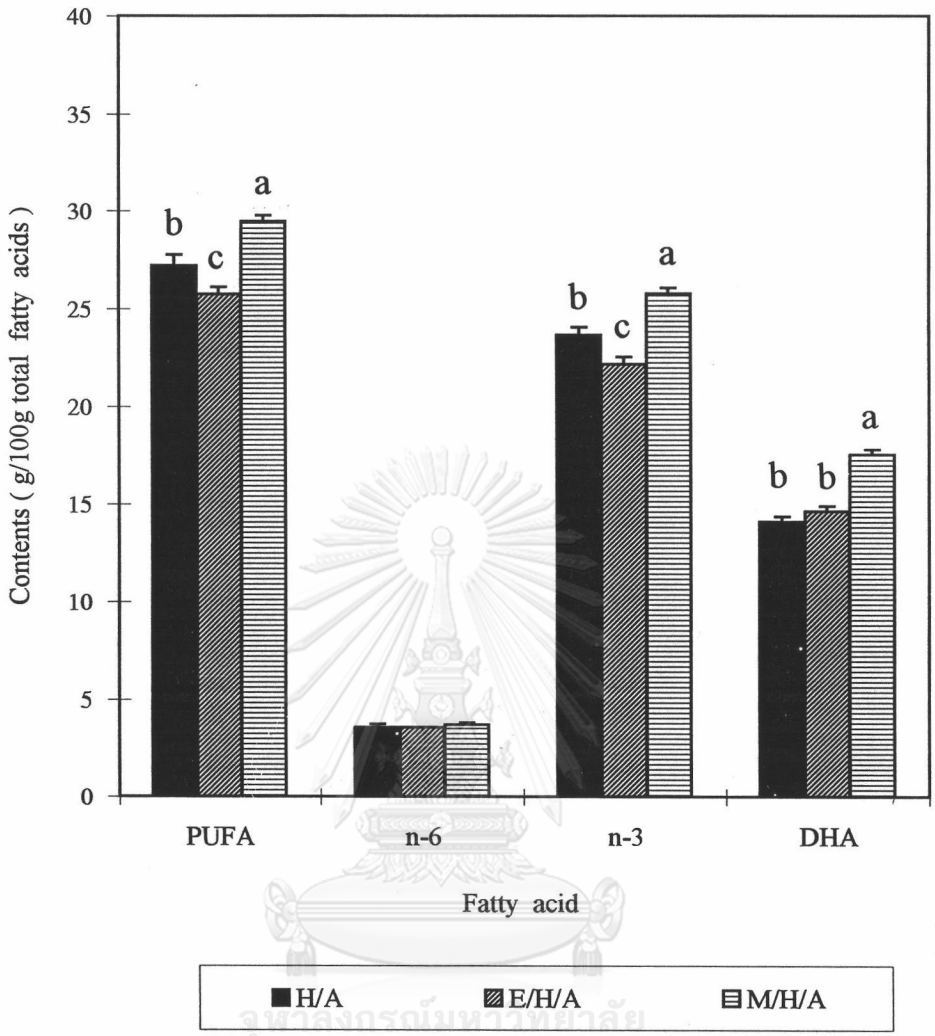

Figure 9 Comparison of fatty acid content in percentage between unsaturated fatty acids; PUFA, n-6 PUFA, n-3 PUFA and DHA found in three acetone insoluble fractions according to the extraction procedures described in the text. Abbreviation: H, hexane; E, ethanol; M, methanol; A, acetone.

2. Phospholipid Subclasses

During the extraction process PL subclasses were monitored as shown in **Table 18**. One can see that lecithin is barely affected throughout the process. Mild alternation of subclasses is observed. At the end of the process or after treatment with acetone (shown as M/H/A in the rightmost column) total choline-containing PL reaches to 69 mole% which is not different from that of the original (value of 70.4 as shown in the leftmost column). No marked alteration is seen in any PL subclasses. The result demonstrates that the extraction process with three consecutive organic solvent system, methanol/n-hexane/acetone, does not affect the composition of PL in the obtained lecithin. The major PL subclass in fish meals is PC comprising half of the content. Choline PL constitutes nearly 70 mole% and make fish meal lecithin a good source of choline.

Table 19 exhibits the comparison between PL subclasses of our prepared fish meal lecithin with other lecithins eg., those of plasma, egg yolk and soya. Two latter lecithins are conventionally employed as emulsifiers for the preparation of commercial fat emulsions. Fish meal lecithin has its PL composition not markedly different from plasma PL. Both PL's constitute mainly of PC and SM leading to the high choline content of 70-90 mole%. Lecithin derived from egg yolk has composition of PL subclasses close to plasma but different from those derived from soya. Among all lecithins, fish meal provides lecithin mostly similar in its composition to plasma PL. The similarity is demonstrated in the ratios of SM/PC and PE/PC. Ratios of SM/PC and PE/PC of plasma are respective 0.22 and 0.05 and likely similar to those of fish meal with the values of 0.36 and 0.18, respectively. Egg yolk lecithin provides SM/PC as low as 0.02 whereas its PE/PC ratio is 0.42 as high. The most difference from plasma in both ratios are existed in soya with the values of 0.0 and 0.53 for SM/PC and PE/PC ratios, respectively.

Table 18 Phospholipid subclasses of lecithin changed during the extraction process by methanol (M), n-hexane (H) and acetone (A) in comparison to original lecithin (mole/100 moles total phospholipids)

Subclass	Original	M	M/H	M/H/A
PC	51.2	50.4	51.2	51.0
SM	15.4	25.9	16.1	17.8
LPC	3.8	0.6	0.4	0.2
PE	10.1	7.2	7.5	9.4
PA	2.8	1.6	2.4	2.1
Others	16.7	14.3	22.3	19.6
Total choline (PC+SM+LPC)	70.4	76.9	67.7	69.0

The results are average of two determinations.

Table 19 Phospholipid subclasses of plasma, fish meal, egg yolk and soya (mole/100 moles total phospholipids)

PL-subclasses	Plasma	Fish meal	Egg yolk	Soya
PA	-	2.38	-	14.80
PE	4.00	9.22	28.95	22.03
PC	73.00	50.60	68.47	41.44
PS+PI	2.00	8.70	-	7.16
SM	16.00	17.98	1.51	-
LPC	5.00	0.17	1.07	6.81
Others	-	10.95	-	7.79
Total choline (PC+SM+LPC)	94.00	68.75	71.05	48.25
SM/PC	0.22	0.36	0.02	-
PE/PC	0.05	0.18	0.42	0.53

The results are average of two determinations.

Preparations of Lecithin-Rich Fat Emulsions

A. Composition of Lipids in Emulsions

Three fat emulsions with high content of lecithins derived from either fish meal, egg yolk or soya were prepared in the present study. **Table 20** shows the contents of PL in crude lecithins employed for the preparation of each fat emulsion. Among three crude lecithins, fish meal lecithin has lowest PL content of 27.4 g/100 g whereas the contents of PL in crude lecithins derived from egg yolk and soya have the values reaching half of the crude contents at 58.33 and 54.28 g/100 g, respectively. The results exhibit the contamination of TG in the crude lecithins prepared in this experiment. Actually, nearly three-fourth of weight content of fish meal lecithin are TG. The limitation of its PL/TG ratio thus defined the composition of PL and TG in all three fat emulsions used in the experiment to be 1:3 (w/w).

As shown in **Table 21**, three fat emulsions with lecithin rich prepared in the experiment according to the dispersion procedure have their ratio between surface of lecithin and core of TG approximately similar. The emulsions so-called FM-LRFE, EY-LRFE and SY-LRFE with lecithins derived from respective fish meal, egg yolk and soya have their PL/TG ratio (LE/TG) ranging narrowly between 0.36-0.37. As mentioned earlier, those three fat emulsions have ratio of PL and TG physically similar, however, they are different chemically in the composition of their fatty acid in moieties of both surface and core lipids as shown in **Tables 22-24**. The content of DHA in the LE fraction (surface) of FM-LRFE is approximately 35% as much higher than that found in TG fraction (core) (22.16 vs 16.36 as shown in **Table 22**). Total n-3 FA of the surface is also nearly 20% higher than that of the core (28.10 vs 23.67). By contrast, EY-LRFE shows its rich of n-6 FA's especially LA and AA (C20:4n-6) in the surface (**Table 23**) whereas SY-LRFE exhibits as much as high content of LA in the surface to be 63.8% (**Table 24**). Subsequently, the ratio of n-3/n-6 of FM-LRFE's surface lipids shown in **Table 22** is much greater than those of EY-LRFE in **Table 23** and of SY-LRFE in **Table 24** (4.79 vs 0.10 and 0.11).

Table 20 Phospholipid contents of crude lecithins prepared from fish meal, egg yolk and soya employed in the experiment

Crude lecithins	PL contents
	g/100g crude lecithins
Fish meal	27.39 ± 0.67
Egg yolk	58.33 ± 0.69
Soya	54.28 ± 1.72

The results are expressed as Mean ± S.D. of three determinations.

Table 21 Contents of Lecithins (LE) and Triglycerides (TG) in emulsion: FM-LRFE, EY-LRFE and SY-LRFE

Emulsion	Emulsions'		LE/TG
	LE surface	TG core	
	g/dl emulsion		
FM-LRFE	1.16	3.22	0.36
EY-LRFE	1.15	3.23	0.36
SY-LRFE	1.21	3.17	0.37

The results are average of two determinations.

FM-LRFE is fish meal-derived lecithin-riched fat emulsion whereas EY-LRFE and SY-LRFE are those derived from egg yolk and soya, respectively.

Table 22 Fatty acid compositions in whole mixtures, TG fraction and LE fraction of FM-LRFE (g/100 g total fatty acids)

Fatty acid	FM-LRFE		
	Whole mixture	TG fraction	LE fraction
C14:0	5.45 ± 0.17	6.90 ± 0.47	2.46 ± 0.03
C14:1	0.25 ± 0.01	0.29 ± 0.01	0.15 ± 0.02
C16:0	28.48 ± 0.50	30.31 ± 1.01	27.48 ± 0.67
C16:1n-7	5.81 ± 0.15	7.00 ± 0.19	3.34 ± 0.03
C18:0	11.56 ± 0.15	10.59 ± 1.02	12.94 ± 0.54
C18:1n-9	12.26 ± 0.17	12.59 ± 0.90	12.27 ± 0.12
C18:2n-6	1.41 ± 0.01	1.51 ± 0.07	1.06 ± 0.03
C18:3n-3	0.60 ± 0.01	0.72 ± 0.14	0.23 ± 0.01
C20:0	0.78 ± 0.06	0.87 ± 0.18	0.62 ± 0.02
C20:4n-6	3.22 ± 0.07	2.28 ± 0.09	4.81 ± 0.08
C20:5n-3	4.82 ± 0.12	4.95 ± 0.94	4.44 ± 0.07
C22:5n-3	1.58 ± 0.05	1.64 ± 0.22	1.27 ± 0.08
C22:6n-3	18.84 ± 0.45	16.36 ± 1.20	22.16 ± 0.43
Sum n-3	25.84 ± 0.58	23.67 ± 2.41	28.10 ± 0.41
Sum n-6	4.63 ± 0.08	3.79 ± 0.14	5.87 ± 0.10
n-3/n-6	5.58 ± 0.09	6.23 ± 0.42	4.79 ± 0.03

The results are expressed as Mean ± S.D. of three determinations.

Table 23 Fatty acid compositions in whole mixtures, TG fraction and LE fraction of EY-LRFE (g/100 g total fatty acids)

Fatty acid	EY-LRFE		
	Whole mixture	TG fraction	LE fraction
C14:0	0.18 ± 0.01	0.19 ± 0.01	0.13 ± 0.01
C16:0	19.90 ± 0.19	16.33 ± 0.24	30.61 ± 0.04
C16:1n-7	1.15 ± 0.02	1.18 ± 0.02	1.07 ± 0.01
C18:0	6.84 ± 0.09	4.05 ± 0.09	15.19 ± 0.09
C18:1n-9	33.28 ± 0.37	34.80 ± 0.46	28.70 ± 0.09
C18:2n-6	32.24 ± 0.34	38.06 ± 0.44	14.78 ± 0.05
C18:3n-3	3.27 ± 0.02	4.36 ± 0.08	-
C20:4n-6	1.38 ± 0.01	-	5.52 ± 0.02
C22:6n-3	0.51 ± 0.00	-	2.05 ± 0.14
Sum n-3	3.78 ± 0.02	4.36 ± 0.08	2.05 ± 0.14
Sum n-6	33.62 ± 0.38	38.06 ± 0.46	20.30 ± 0.07
n-3/n-6	0.11 ± 0.01	0.11 ± 0.02	0.10 ± 0.01

The results are expressed as Means ± S.D. of three determinations.

Table 24 Fatty acid compositions in whole mixtures, TG fraction and LE fraction of SY-LRFE (g/100 g total fatty acids)

Fatty acid	SY-LRFE		
	Whole mixture	TG fraction	LE fraction
C14:0	0.06 ± 0.00	0.06 ± 0.00	0.07 ± 0.00
C16:0	13.44 ± 0.16	11.77 ± 0.18	18.45 ± 0.10
C16:1n-7	0.12 ± 0.00	0.11 ± 0.00	0.15 ± 0.00
C18:0	3.37 ± 0.03	3.47 ± 0.05	3.07 ± 0.00
C18:1n-9	22.09 ± 0.31	26.90 ± 0.40	7.67 ± 0.04
C18:2n-6	54.56 ± 0.24	51.48 ± 0.31	63.80 ± 0.04
C18:3n-3	6.36 ± 0.10	6.21 ± 0.12	6.80 ± 0.03
Sum n-3	6.36 ± 0.10	6.21 ± 0.12	6.80 ± 0.03
Sum n-6	54.56 ± 0.24	51.48 ± 0.31	63.80 ± 0.04
n-3/n-6	0.11 ± 0.00	0.12 ± 0.00	0.11 ± 0.00

The results are expressed as Means ± S.D. of three determinations.

B. PUFA in Fat Emulsions' Surface

Figure 10 compares some PUFA's present in the lecithin surfaces of three emulsions: DHA, total n-3 FA's and total n-6 FA's. The solid column represents fatty acids in the surface of FM-LRFE whereas the striped and the horizontal striped columns represent those of EY-LRFE and SY-LRFE, respectively. One can see the highest content of DHA found in FM-LRFE's surface whereas small amount is found in EY-LRFE's surface but none in SY-LRFE's. Again, the result confirms that fish meal is the rich source of DHA-containing lecithins which exhibits predominantly at the surface of the prepared emulsion as previously described in **Table 22**. The differences between n-3 PUFA as well as DHA present in the surface of FM-LRFE in comparison to other two emulsions, EY-LRFE and SY-LRFE, are obviously impressive and statistically significant ($p<0.05$). However, when n-6 PUFA contents in the emulsions' surface are considered, FM-LRFE, by contrast, expresses its lowest value whereas SY-LRFE surface contains majorily of n-6 PUFA exclusively LA to the value of 65 g/100 g total PL-FA.

Study on the Effect of Fat Emulsions on Erythrocyte Lipids

A. Membrane Cholesterol and Phospholipids

After 1 h incubation of RBC with each LRFE, major membrane lipids, cholesterol and PL, were monitored for their alterations. **Figure 11** demonstrates the values of cholesterol-PL mole ratio of RBC before (the leftmost column) and after the incubation with FM-LRFE, EY-LRFE and SY-LRFE as shown in the left-centered, the right-centered and the rightmost columns, respectively. No significant change of membrane cholesterol-PL ratio was found after the incubation of RBC with any fat emulsions. The results verify that our prepared fat emulsions give no effect to the RBC membrane lipids even at high PL concentration of 300 mg/dl of incubation mixture.

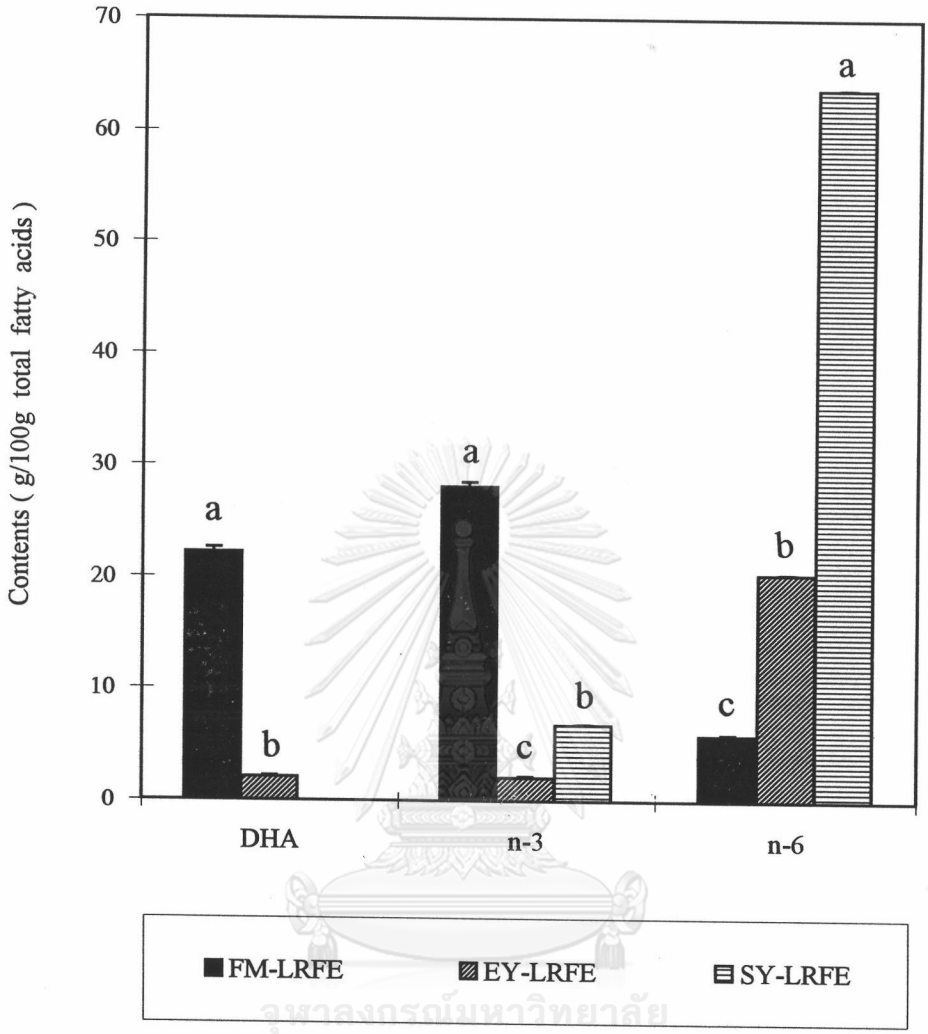

Figure 10 Comparison of fatty acid content in percentage between DHA, n-3 PUFA and n-6 PUFA found in LE surface of FM-LRFE, EY-LRFE and SY-LRFE.

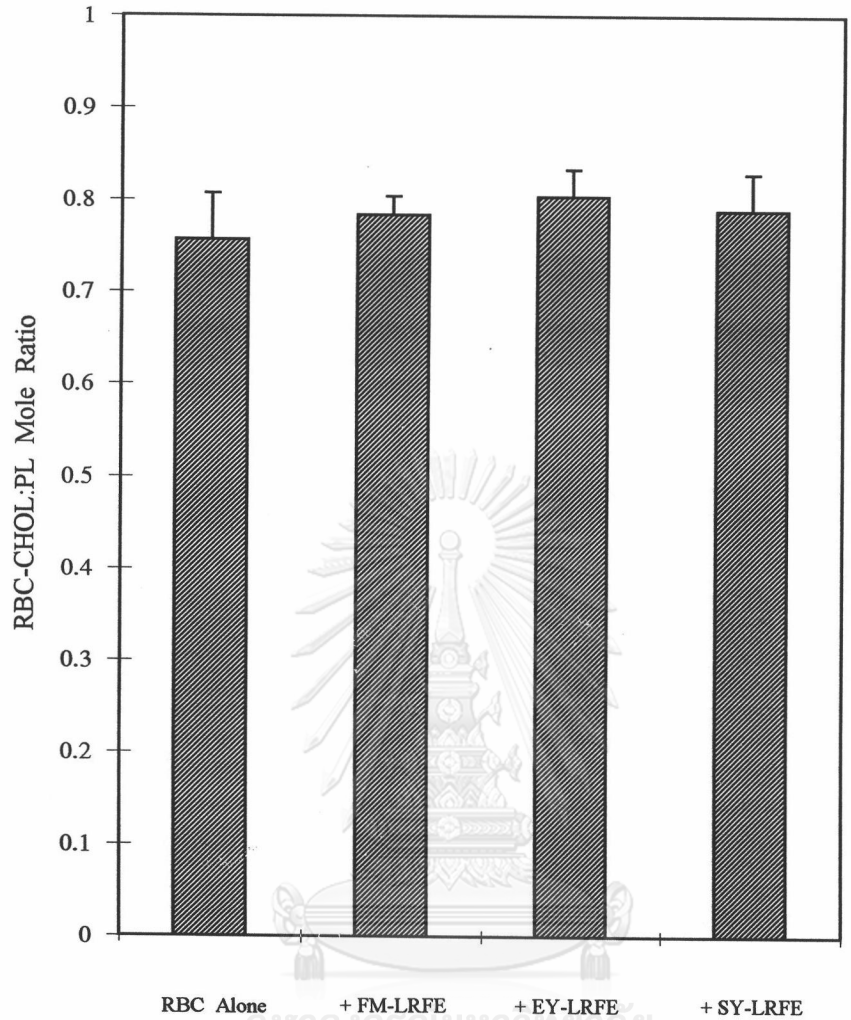

Figure 11 CHOL:PL mole ratio of RBC after their 1 h incubation with FM-LRFE, EY-LRFE and SY-LRFE at the concentration of 300 mg lecithins/dl in comparison to those of normal RBC (leftmost column). No significant alteration of membrane lipids was observed after incubation of RBC with any emulsions.

In the present study, the effect of LRFEs on actual contents of either cholesterol and PL of RBC membranes was not demonstrated. Preliminarily, the expression of membrane lipid concentrations as mg per ml packed cell was monitored. However, it was found that the slight degree of hemolyses observed after the incubation of RBC with fat emulsions caused the inaccuracy assessment of packed cell volume as well as total cell counts. Expressing results as ratio of major membrane lipids as demonstrated in the present study is then advantageous and all errors of actual concentration are then corrected (Dahlan et al., 1992a, 1992b).

B. Effect on RBC' Fatty Acids

Fatty acid profiles of erythrocytes are known to closely correspond to the pattern found in membrane phospholipids because erythrocytes lack organelles and have only a plasma membrane (Dahlan, 1989).

1. Total Fatty Acids

Tables 25-30 show the profiles of RBC fatty acids before and after incubating with various concentrations of either FM-LRFE, EY-LRFE and SY-LRFE. **Table 25** demonstrates the alteration of individual fatty acids of RBC after incubating with FM-LRFE. There are at least three individual RBC fatty acids obviously affected by fatty acids of FM-LRFE, ie. C18:1n-9, LA and DHA. The significant differences are expressed in **Table 26** which one can observe the marked rise of monoenes ($p<0.05$). Polyenes of n-3 show their expansion in composition from 6.8 upto 10.78 at FM-LRFE concentration of 300 mg PL/dl incubation mixture ($p<0.05$). Contrarily, n-6 polyenes dropped from 20.2 before the incubation to 16.57 after incubating with the highest concentration of FM-LRFE ($p<0.05$). Omega-3 polyene expansion is higher than n-6 polyene drop. The consequent rise of n-3/n-6 ratio is then observed ($p<0.05$).

Individual fatty acid profiles of RBC before and after incubation with EY-LRFE are shown in **Table 27**. The results are likely to demonstrate the

Table 25 Fatty acid profiles in g/100g total RBC-FA of erythrocytes (RBC) after the incubation with FM-LRFE for 1 h at 37°C in the presence of lecithin at the concentrations of 0, 100, 150, 200 and 300 mg/dl

RBC-FA	FM-LRFE				
	0 mg/dl	100 mg/dl	150 mg/dl	200 mg/dl	300 mg/dl
C14:0	0.28 ± 0.03	0.99 ± 0.04	1.00 ± 0.06	1.03 ± 0.06	1.18 ± 0.04
C16:0	21.02 ± 0.94	24.04 ± 0.47	23.66 ± 0.55	23.24 ± 0.55	23.39 ± 0.33
C16:1n-7	0.25 ± 0.09	1.66 ± 0.04	1.66 ± 0.12	2.08 ± 0.11	2.17 ± 0.06
C18:0	16.23 ± 0.10	16.13 ± 0.24	15.75 ± 0.14	14.97 ± 0.19	14.57 ± 0.18
C18:1n-9	11.85 ± 0.14	12.81 ± 0.11	12.73 ± 0.09	13.62 ± 0.08	13.80 ± 0.16
C18:2n-6	8.37 ± 0.23	6.47 ± 0.10	6.31 ± 0.23	6.81 ± 0.21	6.79 ± 0.14
C18:3n-3	0.07 ± 0.01	0.13 ± 0.01	0.14 ± 0.01	0.14 ± 0.01	0.16 ± 0.01
C20:0	0.22 ± 0.13	0.39 ± 0.02	0.40 ± 0.02	0.46 ± 0.02	0.46 ± 0.03
C20:4n-6	11.83 ± 0.13	9.31 ± 0.07	9.46 ± 0.31	9.71 ± 0.22	9.78 ± 0.23
C20:5n-3	0.41 ± 0.05	1.01 ± 0.01	1.25 ± 0.06	1.18 ± 0.02	1.37 ± 0.04
C22:6n-3	6.33 ± 0.18	7.82 ± 0.23	8.69 ± 0.35	9.08 ± 0.19	9.25 ± 0.16
C24:0	4.67 ± 0.10	3.30 ± 0.17	3.19 ± 0.33	3.66 ± 0.35	3.54 ± 0.20
Others	18.45 ± 1.15	15.93 ± 0.39	15.77 ± 0.37	14.03 ± 0.36	13.55 ± 0.58

The results are expressed as Mean ± S.D. of five determinations.

Table 26 Composition of Saturated, and Unsaturated fatty acid in g/100g total RBC-FA of erythrocytes (RBC) after the incubation with FM-LRFE for 1 h at 37°C in the presence of lecithin at the concentrations of 0, 100, 150, 200 and 300 mg/dl

RBC-FA	FM-LRFE				
	0 mg/dl	100 mg/dl	150 mg/dl	200 mg/dl	300 mg/dl
Saturated	42.43 ± 0.88^{c}	44.85 ± 0.40^{a}	43.99 ± 0.71^{b}	43.36 ± 0.34^{b,c}	43.13 ± 0.22^{b,c}
Monoenes	12.11 ± 0.22^{c}	14.46 ± 0.13^{b}	14.39 ± 0.18^{b}	15.70 ± 0.14^{a}	15.97 ± 0.10^{a}
Polyenes	27.01 ± 0.28^{a}	24.75 ± 0.21^{c}	25.85 ± 0.63^{b}	26.91 ± 0.49^{a,b}	27.35 ± 0.54^{a}
n-3	6.80 ± 0.20^{d}	8.96 ± 0.18^{c}	10.07 ± 0.39^{b}	10.39 ± 0.17^{a}	10.78 ± 0.19^{a}
n-6	20.20 ± 0.28^{a}	15.79 ± 0.14^{c}	15.77 ± 0.52^{c}	16.52 ± 0.39^{b}	16.57 ± 0.36^{b}
n-3/n-6	0.34 ± 0.01^{c}	0.57 ± 0.02^{b}	0.64 ± 0.03^{a}	0.63 ± 0.01^{a}	0.65 ± 0.01^{a}

The results are expressed as Mean ± S.D. of five determinations.

The different letters in the same row shown as a, b, c, d are significant differences ($p<0.05$).

Table 27 Fatty acid profiles in g/100g total RBC-FA of erythrocytes (RBC) after the incubation with EY-LRFE for 1 h at 37°C in the presence of lecithin at the concentrations of 0, 100, 150, 200 and 300 mg/dl

RBC-FA	EY-LRFE				
	0 mg/dl	100 mg/dl	150 mg/dl	200 mg/dl	300 mg/dl
C14:0	0.18 ± 0.01	0.21 ± 0.01	0.19 ± 0.04	0.21 ± 0.01	0.22 ± 0.02
C16:0	23.83 ± 0.61	24.25 ± 0.45	22.91 ± 0.59	24.86 ± 0.35	24.89 ± 0.88
C16:1n-7	0.33 ± 0.09	0.33 ± 0.02	0.48 ± 0.27	0.40 ± 0.04	0.36 ± 0.04
C18:0	15.74 ± 0.31	15.76 ± 0.34	16.24 ± 0.17	16.03 ± 0.17	16.53 ± 0.25
C18:1n-9	13.33 ± 0.31	13.43 ± 0.29	13.38 ± 0.40	13.77 ± 0.25	13.96 ± 0.34
C18:2n-6	7.97 ± 0.15	8.24 ± 0.12	7.93 ± 0.44	8.23 ± 0.17	8.53 ± 0.38
C20:0	0.26 ± 0.02	0.26 ± 0.03	0.27 ± 0.05	0.28 ± 0.04	0.31 ± 0.06
C20:4n-6	10.67 ± 0.22	10.79 ± 0.24	10.95 ± 0.56	11.11 ± 0.18	10.32 ± 0.25
C20:5n-3	1.73 ± 0.15	1.84 ± 0.05	1.34 ± 0.33	1.82 ± 0.08	1.69 ± 0.31
C22:6n-3	5.19 ± 0.24	5.26 ± 0.45	5.67 ± 0.39	5.12 ± 0.31	4.57 ± 0.38
C24:0	5.44 ± 0.29	5.29 ± 0.24	4.76 ± 0.70	5.05 ± 0.33	4.53 ± 0.88
Others	15.30 ± 1.20	14.44 ± 1.30	15.88 ± 1.30	13.12 ± 0.21	14.08 ± 1.00

The results are expressed as Mean ± S.D. of five determinations.

maintenance of membrane fatty acid composition. However, total saturated fatty acids was found to rise significantly at the concentrations of 200 and 300 mg PL/dl (**Table 28**, $p<0.05$). Surprisingly, polyenes which show their consistency after the incubation at any concentrations of EY-LRFE reduce significantly in their n-3 FA fraction at the concentration of 300 mg PL ($p<0.05$). This reflects to the fact that egg yolk lecithin is still deficient in n-3 FA.

Table 29 demonstrates the alteration of RBC -FA composition after the incubation with SY-LRFE at various concentrations. The significant changes are clearly observed in **Table 30**. The results show that saturated and monoenoic fatty acids were not much affected by SY-LRFE. The obvious alterations are observed in polyenoic fatty acids in both groups of n-3 and n-6. As shown in **Table 30** one can see the mark decrement of total n-3 FA's especially DHA (from 5.76 before the incubation to 4.39 at the concentration of 300 mg PL, **Table 29**). Omega-6 fatty acid shows its significant increment in relation to the contents of PL in incubation mixture (**Table 30**). This is due to the fact that SY-LRFE contains n-6 FA especiall LA in their PL surface as much higher proportion in comparison to RBC (64 vs 9, for SY-LRFE's surface shown in **Table 24** and RBC shown in **Table 29**, respectively).

2. Individual RBC Fatty Acids

The effect of fat emulsion particles and liposomes on RBC membranes was elucidated when the alterations of each individual fatty acid during the incubation of RBC with LRFE's were calculated as percentage of relative membrane FA changed as shown in **Figures 12-14**. Incubating RBC with FM-LRFE, the seven major membrane fatty acids were markedly affected and changed in their contribution (**Figure 12**). Three fatty acids demonstrated their increments: DHA, palmitic acid (C16:0) and oleic acid (C18:1). Among them, DHA exhibited its mark increment whereas C16:0 and C18:1 rose with lesser extent. In the mean time, four fatty acids decreased, two were saturated: C18:0 and C24:0, other two were polyenes: LA and AA. Among them, C18:0 showed its gradual drop whereas those of three fatty acids reached the plateau at the lowest concentration of PL at 100 mg/dl incubation mixture.

Table 28 Composition of Saturated, and Unsaturated fatty acid in g/100g total RBC-FA of erythrocytes (RBC) after the incubation with EY-LRFE for 1 h at 37°C in the presence of lecithin at the concentrations of 0, 100, 150, 200 and 300 mg/dl

RBC-FA	EY-LRFE				
	0 mg/dl	100 mg/dl	150 mg/dl	200 mg/dl	300 mg/dl
Saturated	45.46 ± 0.53^{b}	45.67 ± 0.27^{b}	44.36 ± 0.55^{c}	46.43 ± 0.47^{a}	46.49 ± 0.25^{a}
Monoenes	13.67 ± 0.26^{b}	13.77 ± 0.29^{b}	13.86 ± 0.57^{a,b}	14.17 ± 0.23^{a,b}	14.32 ± 0.34^{a}
Polyenes	25.57 ± 0.53^{a,b}	26.12 ± 0.24^{a}	25.90 ± 0.88^{a,b}	26.28 ± 0.30^{a}	25.11 ± 0.77^{b}
n-3	6.93 ± 0.35^{a}	7.09 ± 0.43^{a}	7.02 ± 0.50^{a}	6.94 ± 0.29^{a}	6.26 ± 0.61^{b}
n-6	18.64 ± 0.28	19.02 ± 0.34	18.89 ± 0.98	19.34 ± 0.33	18.85 ± 0.60
n-3/n-6	0.37 ± 0.02	0.37 ± 0.03	0.37 ± 0.04	0.36 ± 0.02	0.33 ± 0.04

The results are expressed as Mean ± S.D. of five determinations.

The different letters in the same row shown as a, b, c, are significant differences ($p<0.05$).

Table 29 Fatty acid profiles in g/100g total RBC-FA of erythrocytes (RBC) after the incubation with SY-LRFE for 1 h at 37°C in the presence of lecithin at the concentrations of 0, 100, 150, 200 and 300 mg/dl

RBC-FA	SY-LRFE				
	0 mg/dl	100 mg/dl	150 mg/dl	200 mg/dl	300 mg/dl
C14:0	0.25 ± 0.07	0.22 ± 0.02	0.21 ± 0.02	0.21 ± 0.02	0.20 ± 0.01
C16:0	22.70 ± 0.22	23.54 ± 0.73	23.13 ± 0.43	24.99 ± 1.30	24.32 ± 0.32
C16:1n-7	0.20 ± 0.03	0.18 ± 0.00	0.18 ± 0.01	0.20 ± 0.01	0.19 ± 0.01
C18:0	15.02 ± 0.10	14.22 ± 0.18	13.96 ± 0.13	14.08 ± 0.18	13.73 ± 0.16
C18:1n-9	12.80 ± 0.11	13.04 ± 0.27	13.07 ± 0.15	12.77 ± 0.26	12.58 ± 0.09
C18:2n-6	9.00 ± 0.20	12.88 ± 0.11	13.80 ± 0.15	15.25 ± 0.42	16.22 ± 0.30
C18:3n-3	0.05 ± 0.01	0.48 ± 0.03	0.59 ± 0.02	0.77 ± 0.04	0.87 ± 0.03
C20:0	0.28 ± 0.03	0.31 ± 0.01	0.31 ± 0.03	0.26 ± 0.04	0.29 ± 0.01
C20:4n-6	12.70 ± 0.40	11.23 ± 0.19	10.87 ± 0.30	9.95 ± 0.21	10.08 ± 0.19
C20:5n-3	0.36 ± 0.03	0.30 ± 0.05	0.28 ± 0.04	0.23 ± 0.03	0.25 ± 0.01
C22:6n-3	5.76 ± 0.14	4.94 ± 0.27	4.82 ± 0.07	4.09 ± 0.36	4.39 ± 0.23
C24:0	3.36 ± 0.14	3.24 ± 0.15	2.97 ± 0.41	2.47 ± 0.51	2.64 ± 0.23
Others	17.53 ± 0.88	15.42 ± 0.86	15.81 ± 0.70	14.72 ± 1.17	14.25 ± 0.21

The results are expressed as Mean ± S.D. of five determinations.

Table 30 Composition of Saturated, and Unsaturated fatty acid in g/100g total RBC-FA of erythrocytes (RBC) after the incubation with SY-LRFE for 1 h at 37°C in the presence of lecithin at the concentrations of 0,100, 150, 200 and 300 mg/dl

RBC-FA	SY-LRFE				
	0 mg/dl	100 mg/dl	150 mg/dl	200 mg/dl	300 mg/dl
Saturated	41.60 ± 0.38^{a}	41.53 ± 0.73^{a}	40.58 ± 0.14^{b}	42.02 ± 0.97^{a}	41.18 ± 0.33^{a,b}
Monoenes	13.01 ± 0.08^{a,b}	13.23 ± 0.27^{a}	13.25 ± 0.16^{a}	12.97 ± 0.27^{a,b}	12.77 ± 0.10^{b}
Polyenes	27.86 ± 0.72^{c}	29.83 ± 0.41^{b}	30.37 ± 0.49^{b}	30.29 ± 0.27^{b}	31.80 ± 0.20^{a}
n-3	6.17 ± 0.12^{a}	5.72 ± 0.34^{b}	5.70 ± 0.10^{b}	5.09 ± 0.35^{c}	5.50 ± 0.21^{b}
n-6	21.70 ± 0.60^{d}	24.11 ± 0.21^{c}	24.67 ± 0.42^{b,c}	25.20 ± 0.27^{b}	26.29 ± 0.17^{a}
n-3/n-6	0.28 ± 0.00^{a}	0.24 ± 0.01^{b}	0.23 ± 0.00^{b}	0.20 ± 0.02^{c}	0.21 ± 0.01^{c}

The results are expressed as Mean ± S.D. of five determinations.

The different letters in the same row shown as a, b, c, d are significant differences ($p<0.05$).

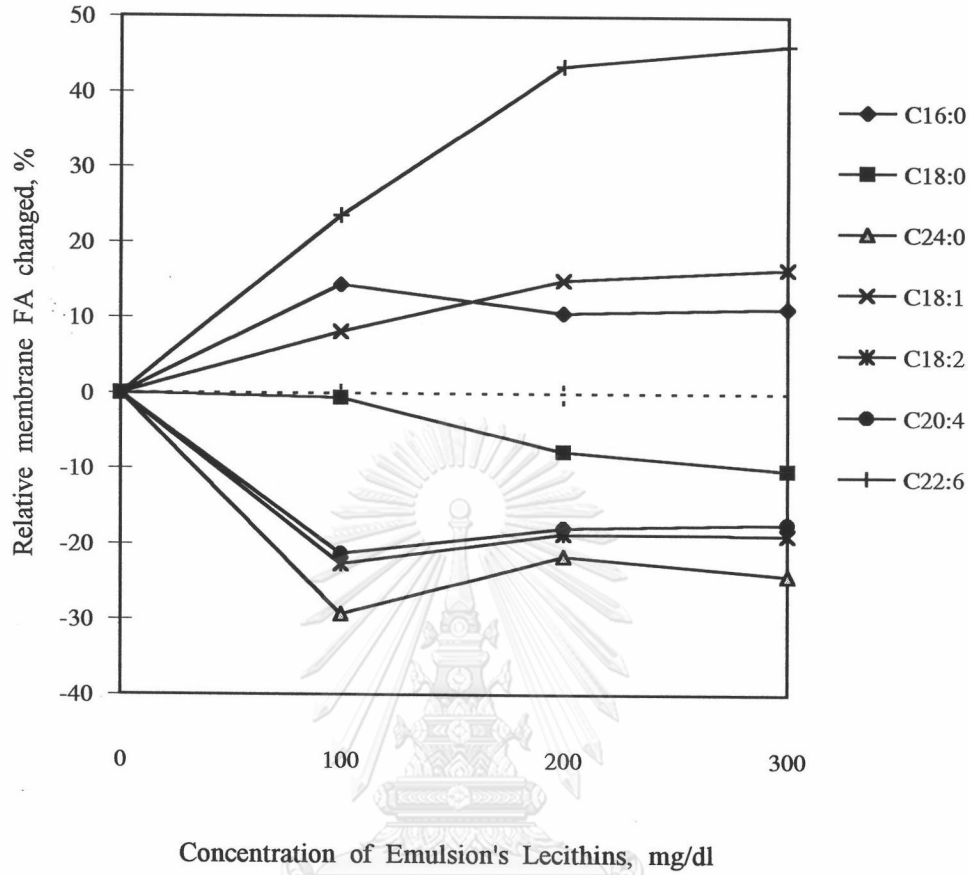

Figure 12 The mean values of relative individual membrane fatty acid changed in percentage after the incubation of red cells with FM-LRFE at the concentrations of 0, 100, 200 and 300 mg lecithins/dl. The individual values was calculated as described in the method.

After the incubation of RBC with EY-LRFE, seven major RBC fatty acids were slightly affected as seen in **Figure 13**. The steady decrement of C24:0 was probably responsible by the rise of two saturated fatty acids, C16:0 and C18:0. Noticeably, slight drop of C22:6 showed its affected by the incubation with EY-LRFE. **Figure 14** shows the results of seven major RBC fatty acids affected by SY-LRFE. membrane LA rose markedly reaching the relative value of approximately 80%. Rationably, the hugh amount of LA present in SY-LRFE's surface was responsible for this rise in membrane. The losses of membrane fatty acids were found in five major fatty acids especially DHA and AA. The replacement of LA for DHA and AA at sn-2 position of membrane PL was probably the answer of this exchanges.

The alterations of membrane fatty acids after incubation with three fat emulsions are summarized in **Table 31**. Incubating with SY-LRFE induced the marked increment of polyenes upto 3.84% whereas FM-LRFE and EY-LRFE affected much less (0.34 and -0.46, respectively, $p<0.05$). Focussing in polyenes, FM-LRFE induced the marked increase of n-3 FA upto +3.97% with DHA contributed upto 74% (2.93 in 3.97) whereas no alteration was observed with EY-LRFE and SY-LRFE. Meanwhile, marked decrease of n-6 FA was found in FM-LRFE group in contrary to the result observed in SY-LRFE which shows its rise upto 4.56% exclusively LA ($p<0.05$). Differently from two former groups, EY-LRFE shows consistency of n-6 FA. All results of n-3 FA and n-6 FA as described earlier lead to higher ratio of n-3/n-6 of FM-LRFE in comparison to those of EY-LRFE and SY-LRFE.

C. RBC-FA Changed at Various PL Concentrations

The questions of whether the concentrations of PL in incubation mixture affect the alteration of individual membrane fatty acids and how they affect are answered by considering the values of slope and coefficient of determination as shown in **Table 32**. Both values were obtained from linear regression of equation. SFA was not affected by the concentration of PL in any emulsions. The contents of PL-FA in FM-LRFE as well as in EY-LRFE influenced obviously the alteration of monoenes as expressed by coefficient of determination, r^2, with the values of

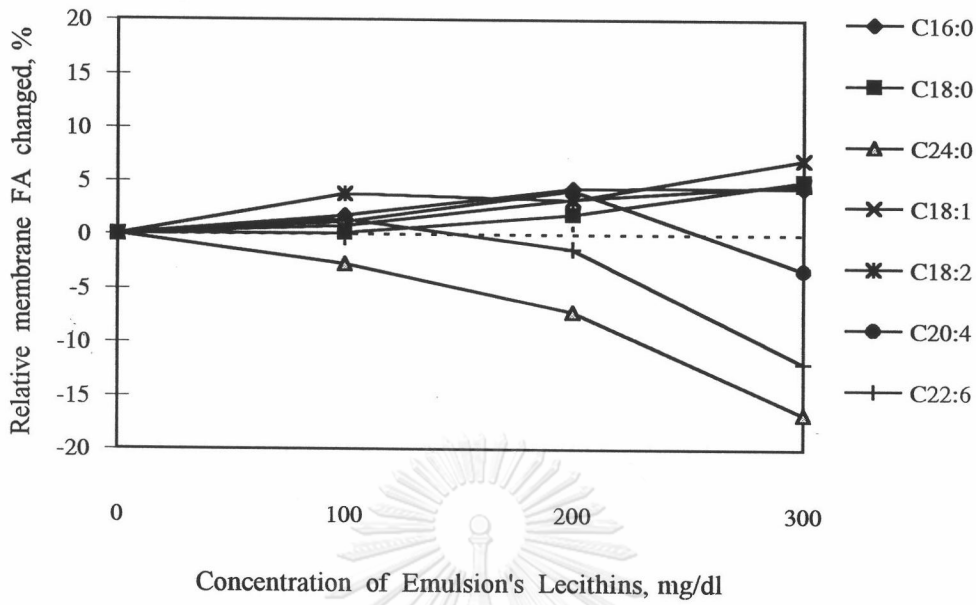

Figure 13 The mean values of relative individual membrane fatty acid changed in percentage after the incubation of red cells with EY-LRFE at the concentrations of 0, 100, 200 and 300 mg lecithins/dl. The individual values was calculated as described in the method.

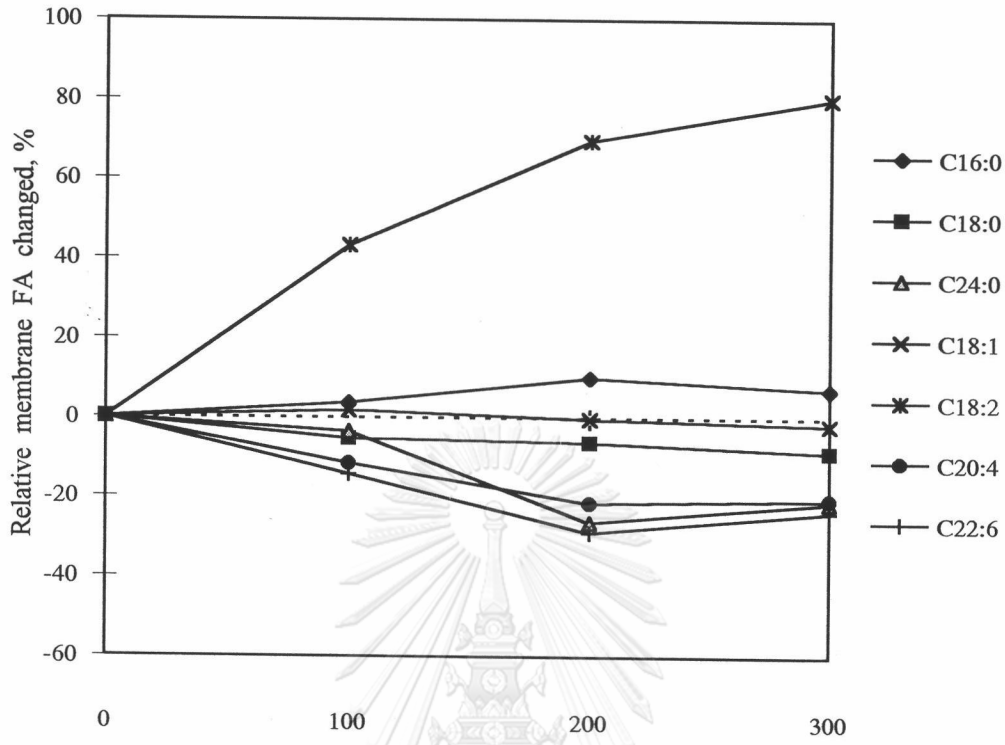

Figure 14 The mean values of relative individual membrane fatty acid changed in percentage after the incubation of red cells with SY-LRFE at the concentrations of 0, 100, 200 and 300 mg lecithins/dl. The individual values was calculated as described in the method.

Table 31 Changes of erythrocyte fatty acids (RBC-FA) after the incubation with FM-LRFE, EY-LRFE and SY-LRFE for 1 h at 37°C in the presence of lecithin at the concentrations of 300 mg/dl

RBC-FA	FM-LRFE	EY-LRFE	SY-LRFE
SFA	+0.70 ± 0.33[a]	+1.02 ± 0.28[a]	-0.26 ± 0.05[b]
MUFA	+3.88 ± 0.37[a]	+0.65 ± 0.33[b]	-0.22 ± 0.03[c]
PUFA	+0.34 ± 0.03[b]	-0.46 ± 0.05[c]	+3.84 ± 0.90[a]
n-3	+3.97 ± 0.28[a]	-0.67 ± 0.26[b]	-0.73 ± 0.36[b]
n-6	-3.62 ± 0.61[c]	+0.21 ± 0.02[b]	+4.56 ± 0.60[a]
n-3/n-6	+0.31 ± 0.02[a]	-0.04 ± 0.01[b]	-0.07 ± 0.01[c]
DHA	+2.93 ± 0.24[a]	-0.63 ± 0.23[b]	-1.43 ± 0.42[c]
LA	-1.58 ± 0.41[c]	+0.55 ± 0.23[b]	+7.22 ± 0.27[a]
AA	-2.06 ± 0.20[b]	-0.34 ± 0.05[a]	-2.65 ± 0.61[b]

The figures obtained by substracting the percentage values of RBC-FA at 300 mgPL/dl from those at 0 mgPL/dl.

The results are expressed as Mean ± S.D. of five determinations.

The different letters in the same row shown as a, b, c, are significant differences ($p<0.05$)

Table 32 Values of slope and coefficient of determination, r^2 obtained from the regression equations of percentages of fatty acids before and after the incubation at various concentrations of either FM-LRFE, EY-LRFE and SY-LRFE

Fatty acid	FM-LRFE		EY-LRFE		SY-LRFE	
	Slope	r^2	Slope	r^2	Slope	r^2
SFA	0.0006	0.0056	0.0038	0.2463	-0.0008	0.0255
MUFA	0.0128	0.8814	0.0024	0.9087	-0.0010	0.3025
PUFA	0.0032	0.1110	-0.0012	0.0854	0.0123	0.9349
n-3	0.0134	0.8688	-0.0022	0.5195	-0.0026	0.5686
n-6	-0.0102	0.3787	0.0010	0.1692	0.0149	0.9470
n-3/n-6	0.0010	0.7236	-0.0001	0.7042	-0.0003	0.8054
DHA	0.0100	0.8727	-0.0020	0.3222	-0.0050	0.7622
LA	-0.0044	0.3583	0.0017	0.5929	0.0240	0.9266
AA	-0.0058	0.3892	-0.0007	0.0738	-0.0091	0.8529
DHA/LA	0.0019	0.6870	-0.0004	0.3663	-0.0012	0.8030

Data obtained from the calculation as described in the text.

respective 0.88 and 0.91. The relationship for PUFA was found only in SY-LRFE at r^2 of 0.94 as high.

Considering two major essential PUFA, the relationship between concentration of PL and n-3 FA was found in FM-LRFE at the r^2 value of 0.89 whereas the relationship with n-6 FA was in SY-LRFE at r^2 of 0.95. The n-3/n-6 ratio of three groups of incubation shows their r^2 values ranging from 0.7-0.8. While DHA of RBC rose by the influence of PL content in FM-LRFE (slope = +0.01, r^2 = 0.87) it dropped by the influence of those in SY-LRFE (slope = -0.005, r^2 = 0.76). The relationship was also observed among the major n-6 FA's, eg. LA and AA with the values of 0.93 and 0.85, respectively. The replacement of DHA by LA is also observed indirectly as DHA/LA ratio with the value as high as 0.80.