SUMMARY AND CONCLUSIONS

1) Crude ricebran lecithin used in the present studies contained 54% phospholipids, 8% glycolipids and 35% neutral lipids. The major fatty acids present in crude lecithin were 42.7% oleic acid, 34% linoleic acid, 17.9% palmitic acid and 4.1% stearic acid.

2) The initial enrichment step involved the de-oiling of lecithin based on the solubility of various lipids in supercritical fluid carbon dioxide. The functional variables were temperature and pressure. Response Surface Methodology has been used to optimize the parameters.

3) The total lipid solubility increased with pressures at constant temperature, but decreased with rise in temperature at constant pressure. The trials indicated that maximum solubility of total lipids was 1.0941 g/kg of CO_2 at 220 bar and 40^0C. The solubility of phospholipids was very minimal in SCF CO_2 in the pressure and temperature range studied and varied from 0.003 to 0.038 g/kg of CO_2.

4) Carbon dioxide – extracted lecithin was further fractionated with alcohol into alcohol – soluble and alcohol insoluble lecithin fractions. The alcohol soluble lecithin fractions represented mainly phosphatidyl choline and phosphatidyl ethanolamine. The alcohol insoluble fraction had phosphatidyl inositol and phosphatidyl ethanolamine.

5) Depending on the pressure, the alcohol – soluble fractions of carbon dioxide - extracted lecithin was found to contain 63% phosphatidyl choline as against 25% present in crude lecithin. The alcohol insoluble fraction of carbon dioxide extracted lecithin contained 34% phosphatidyl inositol as against 8% in crude lecithin.

6) The de-oiled soylecithin was subjected to ethanol extraction for the enrichment of phosphatidyl choline. The effect of time, temperature and ethanol to lecithin ratio were studied for the optimization of phosphatidyl choline yield using response surface methodology.

7) Phosphatidyl choline yield generally increased with increase in all the three variables. There was no significant increase in phsophatidyl choline yield beyond 6 min extraction time. ethanol to lecithin ratio was optimal at 1 to 40 and a rise in temperature of extraction increased phosphatidyl choline yield.

8) The phosphatidyl choline – rich alcohol soluble fraction was further fractionated to phsophatidyl choline and phosphatidyl ethanol amine on a silica column using ethanol as the solvent. The purity of the phosphatidyl choline (>98%) thus purified was ascertained by TLC and then further confirmed by HPLC.

9) The phosphatidyl choline used in the present study are from soy, egg and rice bran.

10) The major fatty acids found in soy phosphatidyl choline were palmitic acid (17%), stearic acid (5%), oleic acid (10%), linoleic acid (58%) and linolenic acid (8%). Egg phosphatidyl choline contains palmitic acid (36%), stearic acid (15%), oleic acid (37%) and linolenic acid (13%). Ricebran phosphatidyl choline contains palmitic acid (46%), stearic acid (5%), oleic acid (39%) and linolenic acid (10%).

11) Kinetic studies in the hydrolysis of oxidized phosphatidyl choline in soy, egg and ricebran phosphatidyl choline liposomal membranes by Crotalus adamenteus venom PLA_2 were carried out.

12) PLA_2 did not discriminate between soy, egg, ricebran phosphatidyl choline and PLPC – OOH as substrate.

13) PLA_2 had a preference to hydrolyze PLPC – OOH over soy, egg and ricebran phosphatidyl choline when more than 40 mole % of cholesterol incorporated into soy PC liposomal membrane, like wise more than 35 mole % and 30 mole % of cholesterol incorporated into egg PC and ricebran PC liposomal membranes.

14) The same results were shown when PLPC – OH is incorporated to the above membranes.

15) Cholesterol makes the liposomal membranes pack more tightly, it is reasonable to assume that definite amount of cholesterol in the membrane displaces the hydrophilic hydroperoxide moieties of PLPC – OOH and PLPC – OH to the surface interface of the liposomal membrane where PLA_2 can easily access these substrate. Amount of cholesterol required depends on the nature of fatty acids in the membrane.

16) Stimulation of PLA_2 activity by adding PLPC – OOH to liposomal membrane were carried out. The amount of fatty acids formed increased with increasing concentration of PLPC- OOH. Relative yield of PLPC – OOH is significantly greater than that of soy, egg, and ricebran PC when 2.5 and 5 mole % of PLPC – OOH when added. However the preferential hydrolysis was not observed when 10 mole % PLPC – OOH was added in all the three membrane. This suggests that the preferential hydrolysis of oxidized phospholipid occurs only at an early stage of oxidation.

17) To know the effect of cholesterol upon the thermotropic behavior of phosphatidyl cholines liposomes containing different types of fatty acids. The soy, egg and rice bran PC liposomes were investigated.

18) Two transitions are visible in these mixtures which indicate that phase separation occurs. In egg and ricebran PC liposomes phase separation is not complete, where as in soy PC liposomes the two transitions are well separated.

19) In soy PC liposomes the two transitions are well separated. Cholesterol shows a preference for species with the lowest transition temperature and its concentration to reduce the phase transitions depends on the amount of these species in the membrane i.e. 30 mole % in soy PC liposomes. Hence cholesterol is non randomly distributed in the lipid bilayer at temperatures at which phase separation occurs. Thus non random distribution is maximal in soy PC liposomes.

20) In egg and ricebran PC liposomes, phase separation is not complete. Hence non random distribution decreases.