CHAPTER ONE

INTRODUCTION

1.1 GENERAL INTRODUCTION

1.1.1 Palm Oil Industry in Malaysia

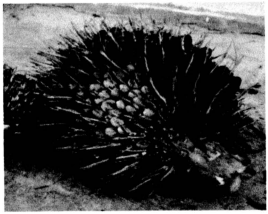
The oil palm was first introduced into Malaysia in 1875 and commercially planted in 1917 (Imam, 1984). The oil palm cultivation received much attention under the national agricultural diversification program which embarked in 1961. Palm oil has become one of the major earners of the country, producing a record of 10.8 million tonnes in the year 2001. This makes Malaysia the largest producer and exporter of palm oil in the world. The palm oil production is expected to increase to 11 million tonnes by 2005.

Due to favourable global demand for palm oil, palm planting will be boosted by an average 270,000 ha per annum worldwide during the next 20 years. However, there are some land limitations (primarily in Peninsular Malaysia) as well as shortage of labour in the plantation sector in Malaysia. Intensified efforts for mechanization, wherever possible, is needed to reduce cost and labour making it economically feasible. Palm planting is expected to further expand to about 3.9 million ha on the average of the next five years 2016-2020 (Thomas, 1998).

1.1.2 Botany of the Oil Palm

The commercially available oil palm, *Elaeis guineensis* is a monocyledon in the Palmae family. The genus *Elaeis* is one of over 220 genera in the palms family and three species are recognized within the genus, viz. *E. guineensis, E. oleifera* and *E. odora* (Paranjothy and Rao, 1984).

E. guineensis originated from West Africa and is believed to have natural habitat such as riverbanks and swamps, with minimal competition from faster growing rainforest species. It has three main fruit forms (or more loosely, three varieties) viz. *pisifera*, *dura* and *tenera*. *Tenera*, a hybrid between *dura* and *pisifera* is the best in term of oil yield and is most popular form for cultivation (Hartley, 1977).



Elaeis guineesis (variety *tenera*)

Oil palm is having a single stem with the trunk functions mainly in transportation of water and nutrients from the roots to the leaves, and transportation of photosynthesis products from leaves to the roots (Holtum, 1955). The meristematic growing point located at the apex of the stem produces leaves and inflorescence primodia. The mature

leaf often called a frond which takes about two years to develop from the primodia. It may be up to 8 m in length and has up to 300 leaflets inserted in two divergent ranks on either side of the rachis or central stalk. The fronds are routinely removed prior to harvesting of fruit bunches. The frond bases which remain on the stem reveal a striking spiral arrangement, "leaf-handed" and "right-handed" are equally common.

The oil palm has an adventitious root system. Primary roots, 6-10 mm in diameter, arise from the base of the stem. The secondary roots, 2-4 mm in diameter arise from the primaries carrying the tertiary roots, 0.7-1.2 mm in diameter. Quaternary roots, 0.1-0.5 mm in diameter, arise from ternaries.

The fruit of the oil palm is drupe, which is borne on the large compact bunch. It consists of a pericarp, made up of exocarp (or skin), mesocarp and endocarp (or shell), surrounding usually one kernel. Different fruit forms can be classified from the thickness of the shell, the presence/absence of fibre ring and other features of the fruits. Two types of oils, which posses different physical and chemical characteristics can be obtained from the oil palm fruits. Red palm oil can be extracted from the mesocarp whereas the light yellowish palm kernel oil is extracted from the kernel.

Male and female flowers are borne on the same tree but on separate inflorescence. They exude an odour of aniseed at anthesis. It has been long believed that the oil palm was wind-pollinated but it being primarily insect-pollinated is now firmly established (Syed,

1979) with the insect possibly being attracted by the aniseed odour. *Thrips hawaiinensis* was the chief pollinating insect in most estates to achieve better yield until the introduction of *Elaeidobius kamerunicus* in 1981. The fertilized flowers develop into ripe oil-rich fruits in about 20 weeks.

1.1.3 Extraction of Palm Oil

Extraction of crude palm oil involves a few steps in order to maximise the recovery of the oil from the fruits. These include sterilisation, stripping, digestion, oil extraction and clarification or purification. The ripe fruit bunches are harvested and transported to the mill as soon as possible for oil extraction in order to minimise the formation of free fatty acid which resulted in poor oil quality. The fresh fruit bunches (FFB) are sterilised by steam in cages (2-5 tonnes capacity) to stop the lipolytic enzymes activity and as well as to facilitate the stripping of fruitlets from the bunch. The steams also soften the mesocarp for oil extraction. The sterilised fruit was then stripped from the bunch. The fruits were then transferred to the digester where they were reheated to loosen the pericarp from the nut. Meanwhile, the stalks are normally passed to an incinerator to produce ash rich in potash that can be used as fertiliser. The digested fruits are finally extracted for its oil by screw presses. After pressing, the nuts and the fibre cake were separated from the oil-rich portion. The impurities found in the oil-rich portion were removed in the clarification tank by using either "gravity settling" or direct centrifugation process". The oil recovered from either decanter (direct centrifugation) or the settling tank (gravity settling) was passed to a centrifuge and then to the vacuum dryer. The final oil from both systems is pumped from the clarification plant to storage tanks (Ong *et al.*, 1987).

1.1.4 Constituents of Crude Palm Oil

Crude palm oil (CPO) consists mainly of triacylglycerols which were made up from a range of fatty acids. Triacylglycerols constitute the major component with a small proportion of di- and monoacylglycerols. Crude palm oil also contains other minor constituents, i.e. the free fatty acids and non-glycerides components such as carotenoids, vitamin E, sterols, phospholipids, glycolipids, hydrocarbons, inorganic compounds, fibre and dirt, water and some degradation products formed from the naturally occuring components. This chemical composition determines the chemical and physical characteristic of palm oil.

(a) Triacylglycerols

Crude palm oil normally consists of more than 90% triacylglycerols. Triacylglycerols are fatty triesters of glycerol. Large numbers of different triacylglycerols are present in palm oil as a result of different fatty acids being attached to the glycerol molecule. The fatty acids can be saturated or unsaturated type. The fatty acids chains in the triacylglycerols are almost exclusively even-numbered, mainly consisting of 16 and 18 carbon atoms. The saturated fatty acids are mainly palmitic acid (hexadecanoic acid, C16: 0) and stearic acid (octadecanoic acid, C18: 0) whereas the main unsaturated fatty acids are oleic acid (cis-9-octadecenoic acid, C18: 1), linoleic acid (cis, cis-9, 12-octadecadienoic acid, C18: 2) and linolenic acid (all cis-9, 12, 15-octadecatrienoic acid, C18: 3) (Gee, 1985). The unsaturated fatty acids appear to be preferentially occupying carbon-2 of the molecule. Triunsaturated triacylglycerols are liquids at room temperature (28°C) whereas the trisaturated are solid. The combination of triunsaturated, trisaturated and mixed ones results in the semisolid characteristic of palm

oil. Crude palm oil can be fractionated into liquid and solid fraction which are called olein and stearin respectively. Olein and stearin have different chemical as well as physical properties.

(b) Monoacylglycerols and Diacylglycerols

These otherwise commonly known as partial acylglycerols may occur naturally in palm oil or obtainable from partial hydrolysis of triacylglycerols. The flesh of palm fruits contains very active lipases which are released in over-ripe fruits or when the fruit is bruised during harvesting. Monoacylglycerols (MG) are normally present in trace quantity whereas the amount of diacylglycerols (DG) can be as high as 8% of the oil. The asymmetric 1,2-diacylglycerols predominant in oil from freshly harvested fruit but the 1,3 isomers predominate in commercial oils (Ong *et al.*, 1987; Gee, 1985).

Table 1.1: Fatty Acid Composition (%) of Malaysian Palm Oil

Fatty acid	Range	Mean
C12:0	0-1.0	0.2
C14:0	0.9-1.5	1.1
C16:0	41.8-46.8	44.0
C16:1	0.1-0.3	0.1
C18:0	4.2-5.1	4.5
C18:1	37.3-40.8	39.2
C18:2	9.1-11.0	10.1
C18:3	0-0.6	0.4
C20:0	0.2-0.7	0.4

Table 1.2: Triacylglycerols in Crude Palm Oil

Acylglycerols Type	Composition (%)
Trisaturated	10.2
Disaturated	48.0
Monosaturated	34.6
Triunsaturated	6.8

(c) Free Fatty Acids

Crude palm oil contains 3-5% free fatty acids (FFA) which are formed from hydrolysis of triacylglycerols, possibly by enzymatic and /or microbial action. Free fatty acids are the cause of many problems such as poor oxidative stability and difficulties in refining. Thus, the fresh fruit bunches are processed as soon as possible after harvesting. Free fatty acids found in crude palm oil are mainly palmitic acids, oleic acids and linoleic acids. They are recovered as by-product in physical refining process and are commercially available as palm free fatty acids distillate (PFAD).

(d) Carotenoids

Crude palm oil is the richest source of carotenoids in terms of retinol equivalents (Choo, 1987; Choo *et al.*, 1989; Choo, 1995). The carotenoids content of crude palm oil in Malaysia varies between 500-700ppm. Carotenoids are naturally occuring C-40 plant pigments. Their conjugated double bonds constitute of chromophore which is responsible for its light absorbing characteristic. All carotenoids exhibit cis-trans isomerisation owing to the double bond in the molecule. In nature, carotenoids are predominantly all-trans isomers, but naturally occuring cis- and poly-cis isomers have

been isolated. There are two groups of carotenoids; hydrocarbons (carotenes) and oxygenated carotenoids (xanthophyll).

The biogenesis of carotenoids are similar in higher plants, algae, bacteria and fungi which starts from mevalonic acid being converted to the universal C5 biological isoprene precursor, isopentenyl pyrophosphate (IPP) (Davies, 1976; Goodwin, 1976; Spurgeon and Porter, 1980). Phytoene is formed following isomerisation, a series of condensation reaction and dimerisation of carbon 20 intermediate. The sequential desaturation of phytoene to lycopene involves a series of dehydrogenation alternatively to the left and the right of the central phytoene to produce phytofluene, ζ-carotene, neurosporene, and lycopene successively. Cyclisation can take place in the neurosporene at one end to produce α-zeacarotene and β-zeacarotene. Further cyclisation of these carotenes at another end will produce other cyclised carotenes such as ε-carotene, α-carotene and β-carotene.

(e) Tocopherols and Tocotrienols

Tocopherols and tocotrienols are found in crude palm oil at 600-1000 ppm levels (Goh *et al.*, 1985; Tan, 1986; Gapor and Berger, 1982). Four tocopherols, which differ in the number of methyl groups and have a saturated side chain together with four corresponding tocotrienols, which have three isoprenoid double bonds in the side chain were found in crude palm oil. Palm oil is unique among the vegetable oils in having high content of tocotrienols (α, γ and δ) which account for about 80% of the total vitamin E content.

Table 1.3: Carotenes Composition (%) of Crude Palm Oil

Carotene	Percentage (%)
Phytoene	1.27
Phytofluene	0.06
cis-β-Carotene	0.68
β-Carotene	56.02
α-Carotene	35.16
cis-α-Carotene	2.49
ζ-Carotene	0.69
γ-Carotene	0.33
δ-Carotene	0.83
Neurosporene	0.29
β-Zeacarotene	0.74
α-Zeacarotene	0.23
Lycopene	1.30
Total Carotenes (ppm)	500-700

The biosynthesis of vitamin E in plants still remains obscure to date. The most striking fact is that there are at least two distinct sites of synthesis, one for α-T in the chloroplast and another for non-α-T in extrachloroplastic organelles. Two possible pathways leading to the biosynthesis of α-T are the tocopherols route and the tocotrienols route (Grams *et al.*, 1970; Threlfall *et al.*, 1971; Botham and Pennock, 1971). The tocopherols route involves the homogentisic acids and the phytyl side chain to produce precursor, namely δ-T directly. According to this scheme, α-T is synthesized from δ-T via two successive methylation reactions with δ-T as intermediate. The key distinction between the tocopherols route and tocotrienols route is the geranylgeranyl (rather than a

phytyl) side chain is added to the homogentisic acid to produce precursor, i.e. δ-T_3 . The methylation of δ-T_3 will yield β-T_3 or γ-T_3 and further methylation yield α-T_3. The final stage of the synthesis is the hydrogenation of the side chain to give α-T. According to this hypothesis, α-, γ-, and δ-T were formed by hydrogenation of the tocotrienols intermediate. Later in 1983, Pennock showed the tocotrienols route also responsible to the biosynthesis of unsaturated tocotrienols (Scheme 2.1). Besides derivation from non-α-tocopherols, α-T also can be formed directly from other intermediary precursor.

Vitamin E is known to possess antioxidant properties which is able to scavenge free radicals. It functions as antioxidant to protect unsaturated lipids from free radicals peroxidation occur particularly in biomembranes (Olcott, 1943; Sonnatag, 1979). Besides being chain-breaking antioxidants, medicinal values of tocotrienols have been found in recent years; for instance, α-tocotrienols has been shown to be anti-cholesterogenic by inhibiting the biosynthesis of cholesterol (Qureshi *et al.*, 1986) and γ-tocotrienols possess anti-thrombosis effect which can prevent aggregation of blood platelets (Machlin, 1980).

Table 1.4: Vitamin E Composition (%) of Crude Palm Oil

Vitamin E	Percentage (%)
α-T	22
α-T_3	20
γ-T_3	46
δ-T_3	12
Total (ppm)	600-1000

(f) Sterols

Sterols are tetracyclic compounds with generally 27-29 carbon atoms. They make up a sizeable portion of the unsaponifiable matter in the oil. Sterols are found at 326-527 ppm levels in crude palm oil. Among the sterols that present in crude palm oil are mainly β-sitosterol (74%), campesterol (14%), stigmasterol (8%) and cholesterol (1%) is in trace amounts. The presence of minor sterols, e.g. Δ^5-avenasterol, Δ^7-stigmasterol and Δ^7-avenasterol at 0-25 ppm had been reported (Choo, 1987). The sterol level is further reduced during the refining process. Recent findings revealed that the sterols are present not only in the free form but also as esters and glycosides (Kurt, 2000). The sterols and their esters do not seem to serve any useful function in the oil neither do they have any detrimental effect. These sterols if recovered will have potential uses in the pharmaceutical industry for conversion into steroid derivatives.

(g) Squalene

Crude palm oil contains 200-350 ppm of squalene (Choo, 1987). Squalene is a colourless, acyclic and hexaunsaturated hydrocarbon with 30 carbon atoms and found mostly in vegetable and animal fats. It is a precursor to biosynthesis of the sterol biosynthesis and exhibits antioxidant activity (Rao and Achaya, 1968).

(h) Polar Lipids

Phospholipids and glycolipids are the polar lipids of palm oil, with the former receiving considerable attention because of the suspected deleterious effect of phosphorous on oil quality. Both types of lipids constitute the important part of cellular membranes; they possess unique structures containing both lipophilic and hydrophilic functionality. The

concentration of phospholipids found in crude palm oil is 20-80 ppm and the major components are phosphatidylcholine, phosphatidylethanolamine, phosphatidic acid and phosphatidylinositol. Most of the phosphorous determined in crude palm oil was in fact inorganic phosphate rather than phospholipids. Preliminary studies indicate that the content of glycolipids in crude palm oil is at 1000-3000 ppm and the major components are monogalactosyl diglycerides, digalactosyl diglycerides and esterified steryl glycosides (Kurt, 2000). During the refining process, polar lipids are almost completely removed through washing, phosphoric acid treatment and absorption by clays or earths.

1.2 PHOSPHOLIPIDS

1.2.1 Introduction

Phospholipids (PL) are complex lipid that is usually divided into glycerophospholipid 1 and sphingophospholipid 2, based on the alcohol present. Glycerophospholipid are constructed from glycerol moiety substituted with one or two acyl or alkyl chain. In addition to fatty acids, they contain phosphoric acid and nitrogenous bases such as choline whereas sphingophospholipid 2 is the corresponding compound with sphingosine as the residue. Phospholipids with one or two hydrolysed acyl group are termed lysophospholipid 3 (Gobley, 1850).

In 1811, Vauquelin was the first to describe the substance containing organically bound phosphorous obtained from animal brain. Later in 1846, Gobley successfully isolated phosphatidylcholine (PC) and named it "lecithin", the Greek word *lekitos* meaning egg yolk from which it was isolated. From the hydrolysis products, Gobley identified the

phosphoric acid and fatty acid mixture. However, the nitrogenous base was identified by Diaknow in 1867 and Strecker in 1868 independently.

After years of scientific researches and investigations on this phosphorous-containing compounds, many other compounds have been identified. Phosphatidylethanolamine (PE) was isolated from brain tissue and named "cephalin" by Thudichum in 1884. He thought that the alcohol-insoluble fraction obtained was a pure compound but later found out it to be a mixture of lecithin and cephalin. Then, Folch and Schneider (1942) proved that phosphatidylserine (PS) was also found in the cephalin fraction extracted from the ox's brain.

Phospholipids seem to be of universal occurrence in living organism. As constituents of cell walls and active participant in metabolic processes they appear to be essential of life. Phospholipids of vegetable origin differs from animal material by the fatty acid composition. In palm oil, only the glycerophospholipids can be found. Phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol (PI) and phosphatidylglycerol (PG), presents as main component in palm oil. Minor components are phosphatidic acid (PA), diphosphatidylglycerol (DPG) and lysophosphatidylethanolamine (LPE) and trace amount of lysophosphatidylcholine (LPC) and phosphatidylserine (PS) (Goh *et al*, 1982).

1.2.2 Structure

Although the basic structure of the phospholipids have been known for years, it is only recently that the problem of the stereochemical configuration of native lecithin has been assigned (Baer, 1956; Baer *et al.*, 1956) This is the result of the unambiguous synthesis

of various L-α-phospholipids followed by their comparison with native phospholipids. Hydrolysis of phosphatidylcholine and phosphatidylethanolamine result in both α- and β-glycerophosphoric acid and this is the fact that was formally considered as evidence for both α- and β-phospholipids. However, Baer *et al.* (1956) reported that the hydrolysis of synthetic L-α phospholipids also yield α- and β-glycerophosphoric acids which closely resemble those reported for natural phospholipids. Thus, the concept of the present of α- and β- phospholipids in nature in no longer tenable. (Baer, 1956; Baer *et al*, 1956).

Table 1.5: Phospholipids Composition (%) of Palm Oil

Phospholipid	Percentage / %
Phosphatidylcholine	36
Phosphatidylethanolamine	24
Phosphatidylinositol	22
Phosphatidylglycerol	9
Diphosphatidylglycerol	4
Phosphatidic acid	3
Lysohosphatidylethanolamine	2
Phosphatidylserine	trace
Lysophosphatidylcholine	trace

Native phospholipids generally contain both saturated and unsaturated fatty acid residue, but saturated and unsaturated phospholipids has been isolated. For example, a saturated phospholipids occurs in mammalian lung, spleen and brain; and an unsaturated phospholipids in yeast. 'Mixed' phospholipids with an saturated fatty acid

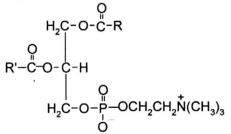
in the α-position and a unsaturated fatty acid in the β-position are found in the liver lipids of many species. (Hanahan, 1951).

1.2.3 Nomenclature

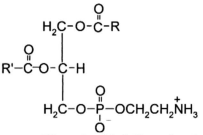
As noted above, all the glycerophospholipids found in the nature are formally derived from *sn*-glycerol-3-phosphate. In the fully systematic system of nomenclature (Ratledge, 1986), diacylglycerophospholipids are named formally as phosphodiester derivatives of 1,2-diacyl-*sn*-glycero-3-phosphate, whereas the acyl group are named according to the IUPAC system. For example, dipalmitoylphosphatidylcholine is designated as 1,2-dihexadecanoyl-*sn*-glycero-3-phosphocholine, 2-palmitoylphosphotidylcholine is designated as 2-hexadecanoyl-*sn*-glycero-3-phosphocholine, which clearly states the components and the linkage found in that particular molecule clearly.

Various trivial nomenclatures exist to define diacylglycerophospholipids. The term 'phosphatidyl' used without qualification generally implies a 1,2-diacyl-*sn*-glycero-3-phosphate- moiety. Thus, 'phosphatidylcholine' and 'phosphatidylethanolamine' are in fact the names of phospholipids classes. Under this nomenclature system, the prefix 'lyso-' is used to describe mono-deacylated derivatives of phosphatidyl compounds, e.g. lysophosphatidylcholine.

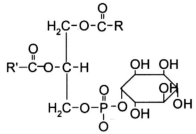
Figure 1.1: Structures of Phosholipids in Crude Palm Oil



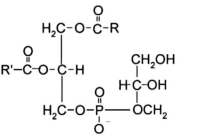
Phosphatidylcholine



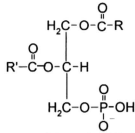
Phosphatidylethanolamine



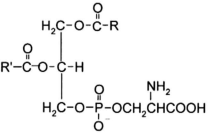
Phosphatidylinositol



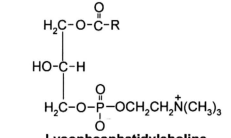
Phosphatidylglycerol



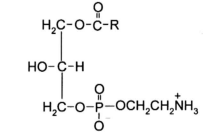
Phosphatidic acid



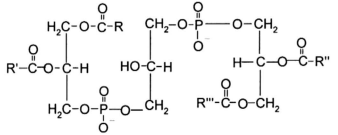
Phosphatidylserine



Lysophosphatidylcholine



Lysophosphatidylethanolamine



Diphosphatidylglycerol

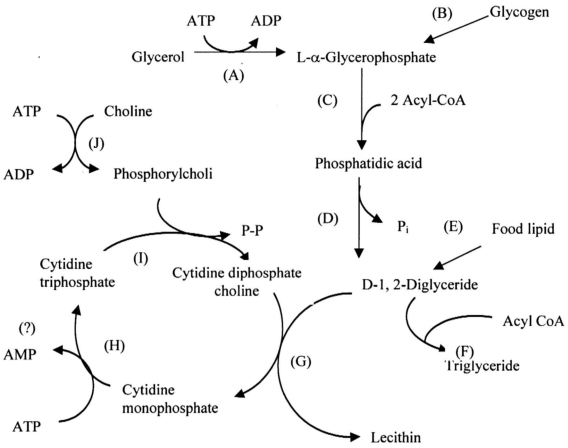
Most older systems of nomenclature used for phospholipids offer no special advantage over those described above, and their use is not recommended. Term such as 'lecithin' and 'cephalin' was originally applied to describe crude lipid fraction extracted from particular tissues and are not appropriate for use to describe pure lipid classes.

1.2.4 Biosynthesis

The impressive progress that has been made in elucidating the mechanisms of the biosynthesis for the phospholipids is to reveal the involvement of phosphorylated derivatives and cytidine nucleotides. The general pattern of synthesis (Kenneth, 1993) is outlined by the biosynthesis of the phosphatidylcholine. It will be convenient to deal with each step in the order given.

In 1953, Kennedy showed that the rate of incorporation of $^{32}P_i$ into the phospholipids of rat liver particles which simultaneously carry out oxidative phosphorylation was stimulated by addition of free glycerol and that L-α-glycerophosphate (L-α-GP) was an obligatory intermediate in the process. This indicates that phosphorylation of glycerol was the first step in the biosynthesis of phospholipids (reaction A). Then, phosphatidic acids (PA) was formed from L-α-GP by condensation of L-α-GP with two molecules of acyl-coenzyme A in the presence of phosphoglycerol trasacylase (reaction C). The details of the reaction are not yet worked out. It is not known, for example, which hydroxyl group is first esterified or even whether both groups are esterified simultaneously. Soon after the formation of phosphatidic acid, it turned to D-1, 2-diacylglycerols. The phosphorylation of glycerol appears to be a necessary activation to enable the acyl residues to be attached. The final step in the biosynthesis, the incorporation of phosphorylcholine, is carried out by enzyme phosphorylcholine

Scheme 1.1: Biosynthesis Pathway of Phospholipids andTriacylglycerols



Enzymes involved:

(A) Glycerokinase
(B) Normal glycolytic pathway plus 1-α-glycerophosphate dehydrogenase
(C) Phosphoglycerol transacylase (? phosphatidic acid synthetase)
(D) Phosphatidic acid phosphatase
(E) Pancreatic lipase
(F) Diglyceride transacylase
(G) Phosphorylcholine-glyceride transferase
(H) Unnamed
(I) Phosphorylcholine-cytidyl transferase
(J) Choline kinase

glyceride transferase which reversibly catalyses reaction (G) in which phosphocholine is transferred from Cytidine diphosphate choline (CDP-choline) to D-1,2-diacylglycerol. Particulate preparations containing this enzyme have been obtained both from rat and chicken liver and from yeast, which suggest that the same basic mechanism of phospholipids biosynthesis is widespread in nature. Other phospholipids components seem likely to be synthesised via the same scheme in which phosphorylcholine and CDP-choline are replaced by the related intermediate, e.g. phosphorylethanolamine and CDP-ethanolamine exist for the biosynthesis of phosphatidylethanolamine.

Generally it is believed that phospholipids and triacylglycerols biosynthesis have an initial common pathway (reaction A to D) involving the formation of phosphatidic acid and D-1,2-diacylglycerol. The incorporation of fatty acid into triacylglycerol can be catalysed by lipase. This is an exchange reaction and does not require an external energy supply. However, the *de novo* syntheses of triacylglycerol from L-α-GP and fatty acids require ATP and CoA. These observations are consistent with the concept above.

1.2.5 Properties of Phospholipids

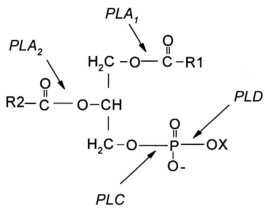
Phospholipids are functionally active component of biological membranes and their properties are based on their chemical structure. The chemical bonds in phospholipids are of three types only, C-C bonds, ester bonds and phosphoester bonds. Phospholipids can be regarded as asymmetric phosphoric acid diesters (Preobrazhenskii and Evstigneeva, 1976).

Phospholipids easily undergo hydrolytic splitting in acidic and alkaline media. Alkaline hydrolysis of phospholipids leads initially to the formation of fatty acids and glycerolphosphate. The latter are apt to undergo further hydrolysis to give a cyclic phosphate and to produce a (1:1) mixture of 2- and 3-glycerophosphate. In an acidic medium the equilibrium between 2- and 3-phosphate is shifted to 3-phosphate (Kenneth, 1993).

Under physiological conditions, the ester bonds in phospholipids are easily digested by relevant enzymes (Chapman, 1969). There are four types of phospholipases that react with phospholipids with high regio- and stereoselectivity. These are phospholipases A_1 (PLA_1), A_2 (PLA_2), C (PLC) and D (PLD). Their activities are showed in figure 1.2 below.

The stability of phospholipids depends strongly on the nature of the acyl and alkyl residues. Phospholipids carrying unsaturated bonds are prone to undergo nonenzymatic reaction of autooxidation and photooxygenation resulting in hydroperoxide formation with concomitant acyl migration and stereomutation of double bonds.

Figure 1.2: Activity of Phospholipases on Phospholipids



1.2.6 Applications of Phospholipids

The unique structure of phospholipids which consists of both hydrophobic fatty acid hydrocarbon and hydrophilic polar head made it deserve a wide variety of applications. The primary usage of phospholipids is as emulsifier in food. The hydrophilic-lipophilic balance (HLB) roughly indicates an emulsifier's preference for oil or water (Rudiger, 1982). For other food uses, it softens and retains moisture, reduces viscosity, stabilizes and disperses. In baking, phospholipids complexes with gluten proteins as dough conditioner and acts as wetting agent. Phospholipids also used as a release agent through its role as surfactant. Phospholipids' edible nature make it suitable for use in both cooked products (pan release) and release of food products from conveyor belts in commercial operations.

Phospholipids act in a wide range of human metabolic processes as well: fat absorption, cholesterol metabolism, nerve function, biosynthesis of plostaglandins and others. The retail dietary supplement has been widely available in the form of capsules or granules which usually contain less than 35% phosphatidylcholine. Beyond its functional characteristics, phospholipids can serve as active ingredient in pharmaceuticals by having an effect on biochemical functions, or as adjuvant due their physical-chemical properties. As adjuvant, phospholipids are used as emulsifiers for intravenous infusion solutions, known as parenteral solutions. Phospholipids also used to produce liposome that carries moisturizer, vitamin and fragrance (Micheal, 2001). Liposome can be used to deliver drug directly to the targeted site in human body and lessen the negative effects associated with drug by covering the toxic drug until it is delivered to the site of infection.

1.2.7 Recovery of Phospholipids

Phospholipids are extracted as co-product of the vegetable oil refining process, where the phosphorous-containing compounds are removed to improve oil quality. Soybean oil containing ca. 3% of phospholipids is the main source of this compound. Recovery of phospholipids and marketing of its many modified forms have become a part of the economics of soybean processing. Besides this, solvent extraction of liquid egg yolk has been effectively applied to recover the desired egg yolk lecithin (Michael, 1989). The newly enzymatic degumming which involves the use of phospholipase A_2 enzyme to hydrolyse the nonhydratable phospholipids to lysophospholipids might ease the production. The lysophospholipid can be hydrated with water and thus removed from the oil by centrifugation. The crude phospholipids mixture obtained is then subjected to a series of processes, namely fractionation, purification and modification to fulfil certain purposes.