Chapter I

Introduction
INTRODUCTION

Fats/oils are important components of human diet. Naturally occurring oils and fats are liquid or solid mixtures consisting primarily of glycerides. The oils and fats are mainly comprised of triacylglycerols (>95%) accompanied by diacylglycerols, mono acylglycerols and free fatty acids, and also contain phospholipids, free sterols and sterol esters, tocols (tocopherols and tocotrienols), triterpene alcohols, hydrocarbons and fat-soluble vitamins.

Functional role of dietary lipid in foods

Fat in the diet impart certain texture qualities, taste and palatability to the foods. Fats in food exhibit unique physical and chemical properties. Their composition, crystalline structure, melting properties and ability to associate with water and other non lipid molecules are important to their functional properties in many foods. Many vegetable oils are used food ingredients or sometimes consumed directly and they serve the following functional roles.

1. Texture which makes other ingredients less sticky.
2. Imparts flavour: for example, coconut oil, olive oil, ground oil etc
3. Function as flavor base- carrier of flavors from other ingredients
4. Serve as cooking medium; example sunflower oil, safflower oil, ground nut oil, mustard oil/rapeseed oil etc.
5. Fats and oils are an important component in most emulsions.
6. Impart palatability/ sensory satiety
7. Aid in mastication

Nutritional role of dietary lipids

Dietary fats play important role in human nutrition. Fat is the most concentrated source of energy. One gram of fat gives 9 kcal of energy, where as carbohydrates and protein provide 4 kcal/g only. Hence fat provides calorie density to the diet. Fats are essential in the diet for absorption and mobilization of fat soluble vitamins such as Vitamins A, D, E & K and fat soluble antioxidants. These vitamins are not utilized by the body if fat is not available in the diet. Thus fat works as a vehicle to carry the fat soluble vitamins, nutrients and antioxidants in the body.
Stored fats provide insulation, helps to regulate body temperature. Fat is stored in adipose tissue also provides some cushioning between organs. Vegetable oils are the only source of Essential Fatty Acids (EFAs) to the body. EFAs are the precursor for a group lipid related compounds called eicosanoids. They are like local hormones and regulate many physiological functions. Lipids act as principal components of cell membranes, maintain cellular integrity, shape, and flexibility. Lipids are needed by all cell membranes, nerve, brain, eye, heart, adrenal and thyroid cells to function. These also help to prevent or relieve symptoms of depression, facilitate the delay of memory loss and dementia (Bidlack 1998).

Classification of Lipids

Classification of lipid structures is possible based on physical properties at room temperature (oils are liquid and fats are solid), their polarity (polar and neutral lipids), and their essentiality for humans (essential and non-essential fatty acids), or their structure (simple or complex). Neutral lipids include fatty acids, alcohols, glycerides, and sterols, while polar lipids include glycerophospholipids and glyceroglycolipids. Based on structure, lipids can be classified as derived, simple, or complex (Figure 1.1). Lipids include fatty acids and alcohols, which are the building
blocks for the simple and complex lipids. Simple lipids, compose of fatty acids and alcohol components, include acylglycerols, ether acylglycerols, sterols, and their esters and wax esters. In general terms, simple lipids can be hydrolyzed to two different components, usually an alcohol and an acid. Complex lipids include glycerophospholipids (phospholipids), glyceroglycolipids (glycolipids), and sphingolipids. These structures yield three or more different compounds on hydrolysis. The fatty acids constitute the obvious starting point in lipid structures. (Akoh 2008).

**Classification of Fatty acids and their biological role**

Triacylglycerides (TGs) are formed mainly from alcohol and fatty acids combined together by ester linkage (Figure 1.2). TGs are the main sources of fatty acids. 100 g of TG will yield approximately 95 g of fatty acids. The physical and chemical characteristics and the health and nutritional effects of dietary fatty acids are influenced greatly by the kinds and proportions of the component fatty acid.

![Figure 1.2. Structure of Triacylglyceride (Neutral lipid molecule).](image)

The fatty acids that form the triglycerides (Figure 1.2) of naturally occurring oils and fats are predominantly even-numbered, straight chain, aliphatic monocarboxylic acids with chain lengths ranging from C4 to C24 (Figure 1.3). The more common fatty acids are known by trivial names such as butyric, lauric, palmitic, oleic, stearic, linoleic, linolenic.
Fatty acids are classified according to presence or absence of double bonds into
1. Saturated fatty acids (contain no double bonds).
2. Unsaturated fatty acids (contain double bonds).

In almost all naturally occurring unsaturated fatty acids, the double bonds are in the cis configuration and are typically positioned at the 3\textsuperscript{rd}, 6\textsuperscript{th} or 9\textsuperscript{th} carbon atom from the terminal methyl group.

**Saturated fatty Acids**

Fats containing single carbon-to-carbon bonds (i.e. no double bonds) are termed as saturated. Most of the saturated fatty acids (SFAs) occurring in nature have unbranched structures and even number of carbon atoms. They have the general formula R-COOH, in which the R group is a straight-chain hydrocarbon of the form CH\textsubscript{3} (CH\textsubscript{2})\textsubscript{x}. Fatty acids with 2–30 carbons do occur, but the common dietary lipids contain between 4 and 24. SFAs are the least reactive due to absence of double bonds and therefore they are more stable and have a longer shelf life than the unsaturated fatty acids. The melting point of SFAs increases with chain length. They are solid at ambient room temperature. The SFAs are further classified into 4 subclasses according to chain lengths: short, medium, long and very long.
Short chain fatty acids have between 3 and 7 carbon atoms. Butyric (4:0) and caproic (6:0) are the most important members of this group and they occur in milk, but are not found in common vegetable oils.

Medium chain fatty acids have between 8 and 13 carbon atoms. Caproic (8:0), capric (10:0) and lauric (12:0) are members of this group. Caproic and capric occur in milk and lauric in coconut and palm oils.

Long chain fatty acids have between 14 and 20 carbon atoms. Palmitic (16:0) and stearic (18:0) are the most important fatty acids of this group. Palmitic acid is the most widely occurring SFA, being present in practically every fat examined. It is present in vegetable, marine oils, milk and fat deposits of land animals. The main sources include palm oil, cottonseed oil, lard and beef tallow. Stearic acid is less common than palmitic acid, but present in most vegetable oils, though a significant component in only a few, such as cocoa butter and shea butter. It is also present in most animal fats and is the major component in the tallow of ruminant fats.

Very long chain fatty acids have 21 or more carbon atoms. Behenic (22:0) and lignoceric (24:0) are the most common very long chain fatty acids present in most dietary fats, but at very low levels of > 0.1% of total fatty acids. Peanut oil, high oleic sunflower and high oleic safflower oil contain 2 and 1.5% of 22:0 and 24:0 respectively.

Dietary saturated fatty acids are the major source of energy, and their intake should be closely adjusted to match the energy expenditure because individual SFA have different effects on plasma cholesterol levels. Lauric (C12:0), myristic (C14:0), and palmitic (C16:0) acids are reported to increase LDL-C and HDL-cholesterol (Hegsted et al., 1993; Katan et al., 1994; Clarke et al., 1997; Yu-Poth et al., 1999), which can be explained by the observed reduction in LDL receptor activity, protein, and amount of mRNA (Fernandez & West 2005). Stearic acid decreases LDL-cholesterol levels relative to other SFA (lauric, myristic, and palmitic acids) and monounsaturated fatty acids (MUFAs) (Mensink 2005). The mechanism is attributed to the high conversion rate of C18:0 to C18:1 (oleic acid) (Lichtenstein 2006). Stearic acid accounts for about a quarter of dietary saturated fats (Clarke et al., 1997).
animal study with monkeys demonstrated that polyunsaturated fatty acids (PUFAs) and MUFAs had similar effects on plasma LDL-cholesterol levels, whereas SFAs significantly raised LDL levels. Animals fed with MUFA-rich diet developed equivalent amounts of coronary artery atherosclerosis as those fed a diet rich in SFAs, but monkeys fed a PUFA-rich diet demonstrated less progression of atherosclerosis (Rudel et al., 1995). SFA intake was directly associated with CHD in several prospective epidemiological studies (Kromhout et al., 1985; Esrey et al., 1996; Kabagambe et al., 2003). Therefore, FAO/WHO dietary recommendations for intake of SFAs should not exceed 10% energy to keep cholesterol levels in a normal range and to reduce the risk of CHD.

**Unsaturated fatty Acids**

Unsaturated fatty acids can be divided into two categories: Monounsaturated fatty acid (MUFAs) and polyunsaturated fatty acids (PUFAs). Presence of double bonds in unsaturated fatty acids make them more reactive than SFAs. This reactivity increases as the number of double bonds increases. The unsaturated fatty acids are also further classified into 3 subgroups according to chain lengths.

- Short-chain unsaturated fatty acids: fatty acids with 19 or fewer carbon atoms.
- Long-chain unsaturated fatty acids: fatty acids with 20–24 carbon atoms.
- Very-long unsaturated chain fatty acids: fatty acids with 25 or more carbon atoms

**Monounsaturated fatty Acids**

More than 100 naturally occurring MUFAs have been identified. In general, they have an even number of carbon atoms, between C14 to C24, and the double bond is located mostly at the 9\textsuperscript{th} position. Common monounsaturated fatty acids are palmitoleic acid (16:1; n-7), oleic acid (18:1; n-9), cis-vaccenic acid (11c-18:1; n-7), gadoleic acid (9c-20:1; n-11), erucic acid (13c-22:1) and nervonic (15c-24:1).

**Polyunsaturated fatty acids**

Polyunsaturated fatty acids (PUFAs) contain more than one double bond in their structure. Natural PUFAs with methylene interrupted double bonds and with all cis-configuration can be divided into 12 different families ranging from double bonds
located from the \( n-1 \) to \( n-12 \) positions counted from the terminal carbon of the fatty acid chain (Gunstone 1996). Saturated fatty acids and most monounsaturated fatty acids can be synthesized in mammalian tissues from non-fat precursors like glucose or amino acids. However, mammals cannot insert double bonds between the methyl terminus and carbon number 9 in oleic acid (18:1n-9). Thus, mammals cannot convert oleic acid into linoleic acid (18:2n-6). The enzyme which catalyzes this reaction is 12-desaturase which is found in plants only. Likewise, mammals cannot convert linoleic acid into \( \alpha \)-linolenic acid (18:3n-3) due to lack of the enzyme 15-desaturase which is also found in plants only. Because these two fatty acids cannot be synthesized by mammals they are termed essential fatty acids (EFAs). Further, mammalian tissues do not contain the 15-desaturase, therefore they cannot convert n-6 to n-3 fatty acids.

**Essential fatty acids**

Linoleic acid (LA C18:2n-6), arachidonic acid (AA C20:4n-6) and \( \alpha \)-linolenic acid (ALA C18:3n-3) are considered as EFAs (Burr & Burr 1929). These fatty acids are necessary for the proper growth and development of animals and possibly humans. Till, 1960s EFAs were considered to have marginal nutritional importance for humans. Signs of clinical deficiency were first recorded in infants fed skimmed milk-based formula (Hansen et al., 1963) and neonates fed with fat-free parenteral nutrition (Caldwell et al., 1972; Paulsrud et al., 1972; Hansen et al., 1963). Dryness, desquamation, thickening of the skin and growth faltering as frequent clinical manifestations of LA deficiency in young infants. Study on infants fed with cow milk-based formulations with different proportions of LA ranging from 10 mg/kg to 800 mg/kg showed more subtle symptoms of n-3 EFA deficiency. The clinical symptoms reported include abnormalities in skin, abnormal visual function and peripheral neuropathy in subjects receiving high n-6 fatty acids and low n-3 fatty acids as part of their intravenous nutrition supply (Holman et al., 1982; Holman 1988). Humans can synthesize saturated and monounsaturated fatty acids, but they cannot synthesize the n-3 and the n-6 polyunsaturated fatty acids. The parent fatty acids of these families ALA and LA are essential fatty acids and must be obtained through the diet. The essentiality of n-6 and n-3 fatty acids for humans is best explained by the inability to introduce double bonds in positions proximal to carbon 9,
counting from the methyl terminus. Moreover, the fatty acids of these families cannot be interconverted, thereby making both n–3 and n–6 fatty acids very essential.

**Fat digestion and absorption**

Dietary fats are composed mainly of TGs containing various long chain saturated and unsaturated fatty acids as well as a small proportion of short and medium chain fatty acids. Since they are water insoluble compounds, they cannot be transferred to the enterocyte in their intact form. Therefore, the ingested TGs are emulsified and hydrolyzed to monoacylglycerols and free fatty acids prior to absorption. The digestive process is very complex and requires coordinated lingual, gastric, intestinal, biliary and pancreatic functions. Initially, the dietary fat is masticated and mixed with lingual lipase, followed by hydrolysis by gastric lipase in the stomach and then by pancreatic lipase in the small intestine.

The stomach plays a further role in fat digestion, since its churning action facilitates formation of oil-in-water emulsion. This emulsion is stabilized by phospholipids. The emulsified acylglycerols enter the duodenum where they stimulate contraction of the gall-bladder and release of bile and pancreatic juice. The bile acids attach themselves to the emulsion particles imparting a negative charge and co-attracting colipase, a protein present in pancreatic juice that binds to TG molecules. The composition of the fat entering the upper duodenum is 80% TG with the remainder consisting of partially digested hydrolysis products formed primarily by gastric lipase. The pancreatic secretion contains a sn–1(3)-specific pancreatic lipase. This enzyme acts on TGs yielding small amounts of 1,2- and 2,3-diacylglycerols as intermediates and 2-monoacylglycerols and free fatty acids as final products (Mu et al., 2005). Although, the pancreatic lipase attacks both 1 and 3 positions of TG, the relative rates of hydrolysis depend on the molecular structure of fatty acids in the TG molecules.
Figure 1.4. Absorption and transport of fatty acid in intestine (Nelson et al., 2008).

The lipid digestion products absorbed into the enterocyte are transported to the endoplasmic reticulum (Figure 1.4) in association with a fatty acid binding protein (Lehner et al., 1996). The fatty acids are activated to their CoA derivatives by acylcoenzyme A and converted to TG by either the monoacylglycerol pathway or the sn-glycero-3-phosphate pathway. The newly synthesized TGs, PLs and CEs are transported out of the enterocyte and into the bloodstream via the lymph vessels in form of chyomicrons. While in the blood stream, the TGs of the chyomicrons are hydrolyzed to free fatty acids and glycerol by lipoprotein lipase, an enzyme associated with the capillary endothelium. The fatty acids and glycerol can then pass through the capillary walls to be used by cells as energy or stored as fats in adipose tissue.
Fat metabolism

Biosynthesis of fatty acids

Fatty acid biosynthesis can take place by de novo synthesis, where in a small precursor molecule (usually the 2C acetyl group) is gradually lengthened by 2C units to give rise to 16C and 18C products by various modifications such as elongation and desaturation resulting in the long-chain fatty acids (Figure 1.5).

**Figure 1.5. Pathway of fatty acid synthesis.**

*De novo* synthesis of fatty acid

Most of the carbon for *de novo* fatty acid synthesis goes through the pyruvate pool, the end product of glycolysis. Although pyruvate is produced in the cytosol (where the animal fatty acid biosynthesis takes place), acetyl-CoA is formed mainly in mitochondria. Under conditions favouring fatty acid biosynthesis, pyruvate is transported into mitochondria where pyruvate dehydrogenase is activated. The acetyl-CoA product is combined with oxalacetate to produce citrate which leaves the mitochondria via a tricarboxylate anion carrier. Back in the cytosol, acetyl-CoA is produced by ATP citrate lyase. The NADPH needed for the reductive steps of fatty acid synthase comes from the cytosolic pentose phosphate pathway.
Fatty acid synthesis begins with condensation of acetyl-CoA with bicarbonate (catalysed by acetyl-CoA carboxylase) to form malonyl CoA. Acetyl-CoA then combines with a series of malonyl CoA to form saturated fatty acid of different carbon length of which end product is palmitic acid (16:0) (Figure 1.6). The fatty acid synthetic reaction up to this stage takes place within the fatty acid synthase complex. Once palmitic acid is released from the synthetic complex, it can be elongated to stearic acid and even higher fatty acid by further additions of acetyl groups through
the action of fatty acid elongation systems. In animal tissues, the desaturation of de novo synthesized SFAs stop with the formation of MUFA of the n–9 series. This conversion is performed by Δ9 desaturase, which is a very active in mammalian tissues, and introduces double bonds at the 9–10 position of the fatty acid chain. Oleic acid (18: Δ9 or 18:1n–9) is the main product of Δ-9 desaturation. Small amounts of 16:1 n–9 are also formed. These MUFAs may act as substrates for fatty acid elongase, which produces cis-vaccenic acid (18:1-11 or 18:1n–7) from palmitoleic acid and eicosenoic (20:1n–9), erucic (22:1n–9) and nervonic acids (24:1 n–9) from oleic acid.

Dietary fatty acids have a significant influence on the de novo synthesis of fatty acids. For some time it was thought that only PUFA suppressed fatty acid synthesis, but SFA also suppresses fatty acids synthesis (Kelley et al., 1986). It is suggested that, the dietary fatty acids, except short-chain fatty acids are absorbed through the portal vein suppress de novo fatty acid synthesis. The excess CoA derived from dietary fatty acids in the liver is probably responsible for suppressing the de novo synthesis by inhibiting both acetyl CoA carboxylase. Generally it is accepted that, little or no de novo synthesis takes place in humans when adequate or excess calories are consumed through high-fat diet. But when carbohydrates are fed in excess of energy requirement, the conversion of carbohydrates to fatty acids occurs, and this takes place in the liver. Strawford et al., 2004 have shown that free-living healthy humans have the capacity for de novo synthesis of fatty acids. The estimate is based on the incorporation of deuterium from deuterated water on the glycerol moiety of TGs that de novo synthesis contributes on an average approximately 20% of newly formed adipose TG-palmitate. However, there were considerable individual variations (12 to 36%) in de novo synthesis of fatty acids.

**Fatty acid oxidation**

Fat, stored as TGs, is the body’s most concentrated source of energy. The energy yield from the catabolism of 1 g of fat is approximately 9 kcal, when compared to 4 kcal from protein or carbohydrates. Catabolism of fat involves the oxidation of their 2 building blocks, glycerol and fatty acid chains. Most body cells, easily convert glycerol to glyceraldehyde phosphate. Glyceraldehyde is half a glucose
molecule, and the energy released from it is half that of carbohydrate and accounts approximately for 5% of fat.

Fatty acids yield energy by β-oxidation in the mitochondria. Fatty acid oxidation takes place in three stages. In the first stage oxidation fatty acids undergo oxidative removal of successive two carbon units in the form of acetyl-CoA, starting from the carboxyl end of the fatty acyl chain. In the second stage of fatty acid oxidation, the acetyl groups of acetyl-CoA are oxidized to CO₂ in the citric acid cycle, which also takes place in the mitochondrial matrix. Acetyl-CoA derived from fatty acids thus enters a final common pathway of oxidation with the acetyl-CoA derived from glucose via glycolysis and pyruvate oxidation. The first two stages of fatty acid oxidation produce the reduced electron carriers NADH and FADH₂, which in the third stage donate electrons to the mitochondrial respiratory chain, through which the electrons pass to oxygen with the concomitant phosphorylation of ADP to ATP. The energy released by fatty acid oxidation is thus conserved as ATP. Four enzyme catalyzed reactions make up the first stage of β-oxidation fatty acid (Figure 1.7). First, dehydrogenation of fatty acyl CoA produces a double bond between the α and β carbon atoms (C-2 and C-3), yielding a trans-Δ2-enoyl-CoA (the symbol Δ2 designates the position of the double bond. In the second step of the β-oxidation cycle, water is added to the double bond of the trans- Δ2-enoyl-CoA to form the L stereoisomer of β-hydroxyacyl-CoA (3-hydroxyacyl-CoA). This reaction, catalyzed by enoyl-CoA hydratase. In the third step, L-β-hydroxyacyl-CoA is dehydrogenated to form β-ketoacyl-CoA, by the action of β-hydroxyacyl-CoA dehydrogenase; NAD is the electron acceptor. This enzyme is absolutely specific for the L-stereoisomer of hydroxyacyl-CoA. The fourth and last step of the β-oxidation cycle is catalyzed by acyl-CoA acetyltransferase, more commonly called thiolase, which promotes reaction of β-ketoacyl-CoA with a molecule of free coenzyme A to split off the carboxyl-terminal two-carbon fragment of the original fatty acid as acetyl-CoA. The four steps repeated till the reach of methyl end.
Figure 1.7. β-oxidation of fatty acid

Unsaturated fatty acids are also catabolized by β-oxidation, but two additional mitochondrial enzymes an isomerase and a novel reductase are required to handle the cis-double bonds of naturally occurring fatty acids. As an example, consider the breakdown of oleic acid, an 18-carbon chain with a double bond at the 9,10-position. The reactions of β-oxidation proceed normally through three cycles, producing three molecules of acetyl-CoA and leaving the degradation product cis-Δ^3^-dodecenoyl-CoA. This intermediate is not a substrate for acyl-CoA dehydrogenase. With a double bond at the 3,4-position, it is not possible to form another double bond at the 2,3- (or β) position. This problem is solved by enoyl-CoA isomerase, an enzyme that rearranges this cis-Δ^3^ double bond to a trans-Δ^2^ double bond. This latter species can proceed through the normal route of β-oxidation. Polyunsaturated fatty acid was
oxidized by the combined action of enoyl-CoA isomerase and 2,4-dienoyl-CoA reductase, as allows re-entry of this intermediate into the β-oxidation pathway.

The fatty acid structure affects the rate of fatty acid oxidation. In general, various animal and human studies have suggested that long-chain fatty acids are oxidized more slowly and unsaturated fatty acids oxidized more rapidly than SFAs. In rats, oxidation of SFAs decreases with increasing carbon chain length (laurate > myristate > palmitate > stearate) (Leyton et al., 1987). For unsaturated fatty acids, 24-hour oxidation is in the order ALA > OA > LA > AA. DeLany et al., 2000 gave human subjects labelled fatty acids in a blended meal and found that the oxidation of ALA was higher than that of LA and OA, which had similar rates of oxidation. A recent study by McCloy et al., 2004 also found greater oxidation of ALA compared to LA, but the oxidation rate of ALA was similar to that of OA. The inconsistencies in the various tracer studies may be related to the method used to measure fatty acid oxidation and possibly also to the differences in the back-ground diets.

**Biosynthesis of eicosanoids**

The term ‘eicosanoids’ is used to denote a group of oxygenated, twenty carbon fatty acids derivatives of AA (n-6) and EPA (n-3). Cell membranes contain mainly AA compared to EPA, AA is the predominant precursor for eicosanoid biosynthesis. The carboxyl groups of AA/EPA are esterified with the hydroxyl groups of the glycerol back-bone of phospholipids or glycerides. AA/EPA Fatty acids can be mobilized/re-hydrolyzed by phospholipase A2 (PLA2). Cyclo-oxygenases (COX) convert free AA to the prostaglandin-2-series (PGs), thromboxane-2-series (TXs). There are 2 isoforms of COX namely COX-1 and COX-2.
Both are present in many normal human tissues and are up-regulated in various pathologic conditions (Zidar et al., 2009). COX-1 is a constitutive enzyme and is mainly involved in the basal production of eicosanoids in physiological conditions (In Gastric mucosa line), whereas COX-2 is inducible and overexpressed, for example, in inflammation. COX-2 is responsible for the markedly increased production of PG. Metabolism of AA by the 5-lipoxygenase (5-LOX) pathway gives rise leukotriene-4-series (LT₄-series) and to various hydroperoxy- and hydroxyl-eicosatetraenoic acid (5-HPETE and 5-HETE) derivates and lipoxin-A₄ (Figure 1.8). EPA can also be metabolised by both COX and 5-LOX leading to the formation of 3-series PGs, thromboxane A₃ (TXA₃) (Li et al., 2002).

**Figure 1.8. Biosynthesis pathway of eicosanoids (Calder 2006)**
Table 1.1. Physiological functions of eicosanoids in humans

<table>
<thead>
<tr>
<th>Eicosanoids</th>
<th>Physiological action</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGE$_2$</td>
<td>Pro-inflammatory, pro-aggregatory, suppresses immune response, promotes cell growth, proliferation, vasodilation, bronchoconstriction, mild anti-inflammatory (inhibits 5-LOX and so decreases inflammatory 4-series LTs, induces 15-LOX which promotes formation of anti-inflammatory lipoxins</td>
</tr>
<tr>
<td>PGI$_2$</td>
<td>anti-inflammatory, inhibits platelet aggregation, potent vasodilator</td>
</tr>
<tr>
<td>TXA$_2$</td>
<td>potent platelet aggregation, potent vasoconstrictor</td>
</tr>
<tr>
<td>PGD$_2$</td>
<td>inhibits platelet aggregation, vasodilation, promotion of sleep</td>
</tr>
<tr>
<td>PGF$_{2\alpha}$</td>
<td>induces smooth muscle contraction, uterine contraction</td>
</tr>
<tr>
<td>LTB$_4$</td>
<td>pro-inflammatory, causes neutrophil aggregation, neutrophil and eosinophil chemotaxis</td>
</tr>
<tr>
<td>LTC$_4$</td>
<td>pro-inflammatory, promote endothelial cell permeability, contracts smooth muscle cells, constricts peripheral airways</td>
</tr>
<tr>
<td>LTD$_4$</td>
<td>contracts smooth muscle cells, constricts peripheral airway</td>
</tr>
<tr>
<td>12-HETE</td>
<td>neutrophil chemotaxis, stimulates glucose-induced insulin sec</td>
</tr>
<tr>
<td>15-HETE</td>
<td>inhibits 5- and 12-LOX</td>
</tr>
<tr>
<td>Lipoxin A</td>
<td>superoxide anion generation, chemotaxis</td>
</tr>
<tr>
<td>Lipoxin B</td>
<td>inhibits NK cell activity</td>
</tr>
</tbody>
</table>

Table adopted from Ratnayake & Galli (2009).

The eicosanoids derived from AA are involved in a variety of physiological actions (Table 1.1). Eicosanoids are produced when cells are stimulated and they are rapidly metabolized and hence they are not accumulated in cells. Imbalance in the synthesis of eicosanoids in tissues can lead to development of certain pathological conditions.

n-3 and n-6 fatty acid derived eicosanoids have opposing effects. Eicosanoids that formed from EPA and DHA are anti-inflammatory, whereas those formed from AA are pro-inflammatory or show other disease-propagating effects. Prostaglandins PGI$_2$ and PGE$_2$, generated from AA, have pro-arrhythmic effects, whereas the EPA-
derived prostaglandins PGI₃ and PGE₃ are anti-arrhythmic. Concerning inflammatory modulation PGE₂ has both pro and anti-inflammatory effects. PGE₂ induces COX-2 and thereby increases its own synthesis and the production of the pro-inflammatory cytokine IL-6 in macrophages (Bagga et al., 2003). On the other hand, PGE₂ inhibits 5-lipoxygenase and thereby decreases the production of pro-inflammatory leukotriene 4-series species (Levy et al., 2001). PGE₂ also induces 15-lipoxygenase promoting the formation of anti-inflammatory lipoxins (Vachier et al., 2002; Gewirtz et al., 2002; Serhan et al., 2003). TXB₂, produced from AA, is a potent vasoconstrictor and platelet activator, whereas TXB₃, derived from EPA has less potent physiological effects compared to TXB₂. EPA-derived prostaglandins have been shown to inhibit TXB₂-mediated platelet aggregation and promote vasodilatation (Weber et al., 1986).

The leukotrienes LTB₄, LTC₄, LTD₄ and LTE₄ (from n-6 FA) increase vascular permeability and promote hypersensitivity (Calder et al., 2006; Fogh et al., 1989). n-3 Fatty acid-derived LTB₃ blocks biosynthesis of the highly inflammatory AA-derived LTB₄. The E-series of resolvins, oxidative derivatives of EPA, exert anti-inflammatory effects (Schwab et al., 2007; Serhan et al., 2000 & 2004). Resolvin E₁ reduces inflammation by suppressing the activation of NFκB and, thereby, blocks the synthesis of inflammatory cytokines and chemokines (Prescott, 2005). The DHA-derived D-series of resolvins, docosatrienes and neuroprotectins, were identified as anti-inflammatory and inflammation resolving (Serhan, 2002; Hong et al., 2003; Marcheselli et al., 2003).

EPA, DHA and AA are competitive substrates for enzymes involved in autacoid biosynthesis. Increased dietary consumption of EPA and DHA results in higher concentrations of these fatty acids compared to arachidonic acid in cellular phospholipids and glycerides. Fish oil supplementation (n-3 PUFAs) in the human diet decreases production of PGE₂, TXB₂, LTB₄ and 5-HETE, and accelerates production of LTB₅, LTE₅ and 5-hydroxy-EPA in inflammatory cells (Lee et al., 1985; Endres et al., 1989; Caughey et al., 1996) Thus, the n-3 and n-6 ratio in the diet strongly affects autacoid production and increased consumption of n-3 fatty acids leads to reduced synthesis of inflammatory eicosanoids from AA (n-6 FA), but elevated production of anti-inflammatory autacoids from n-3 fatty acids.
**β-oxidation and carbon recycling**

Major percentage (60–85%) of ALA is directed towards β-oxidation (Golovko et al., 2006 & 2007; Murphy et al., 2005). In the brain, about 67% of the ALA is directed toward β-oxidation (Demar et al., 2006), but other fatty acids, such as AA, only about 30% undergoes β-oxidation (Golovko & Murphy 2006; Golovko et al. 2007). For plasma-derived erucic acid (22:1 n-9), which is found at trace levels in the brain, the β-oxidation rates is similar to that of ALA (Golovko et al., 2006). However, if this fatty acid is infused directly into the brain ventricles, a much smaller percentage is subjected to β-oxidation. This suggests the ability of the brain to uniquely distinguish the metabolic fate of fatty acids coming in from the plasma as opposed to that derived directly from the cerebral spinal fluid. This notion is consistent with the very high degree of radioactivity present in the choroid plexus of rats infused with radiotracer fatty acids (Rosenberger et al., 2002; Rapoport 2001). Nonetheless, the high percentage of ALA used for β-oxidation is not exclusive for ALA as other dietary derived PUFA are β-oxidized in similar percentages. For n-6 fatty acids, about 50% are used for β-oxidation and about 65% of DHA undergoes the same fate (Poumes-Ballihaut et al., 2001). Thus, dietary ALA, similar to other dietary fatty acids, undergoes significant utilization by tissues for energy, e.g. heart and muscle, or it is recycled by tissues to be used as a carbon source for production of other fatty acids, amino acids, and sterols, e.g. brain, liver. It is important to note that carbon recycling of ALA is quite efficient and that in developing rat, this carbon is found enriched in brain saturated fatty acids and in cholesterol (Menard et al., 1998). Similarly, in developing and adult non-human primates, carbon recycling from ALA also occurs (Greiner et al., 1996), indicating that this process occurs across species. Fatty acid carbons are recycled and saturated fatty acids are formed within a 5–10 min time frame in both liver and brain (Demar Jr et al., 2006; Igarashi et al., 2007; Rapoport et al., 2007; Golovko & Murphy 2005; Murphy et al., 2008). The rapidity by which carbon recycling occurs is often poorly understated, it is generally difficult to view lipid metabolism in short span of time.
Elongation and desaturation

A pathway for the conversion of the essential fatty acids LA and ALA to long chain PUFA has been summarized in Figure 1.9. With the exception of the final reaction which results in the formation of DHA, all reactions occur in the endoplasmic reticulum. Since both n-6 and n-3 PUFA are metabolised by the same desaturation/elongation pathway, there exists a potential competition between these two families of fatty acids. The initial conversion of ALA to C18:4 n-3 by the action of Δ6-desaturase is the rate limiting reaction of the pathway. The affinity of Δ6-desaturase for ALA is greater than for LA (Moore et al., 1991). However, the typically higher concentration of LA than ALA in cellular pools results in greater conversion of n-6 PUFA. The introduction of a double bond at the C6 position is followed by the addition of C2 by elongase activity and then by desaturation by Δ5-desaturase to form EPA. Docosapentaenoic acid (C22:5 n-3, DPA n-3) is synthesised from EPA by addition at C2. The conversion of DPA n-3 to DHA has been a matter of controversy and Δ4-desaturase activity has been suggested to be the primary mechanism for DHA synthesis (Williard et al., 2001). However, there are reports strongly suggesting that synthesis of DHA involves desaturation at the Δ6 position as DPA n-3 is elongated to C24:5 n-3, which is desaturated at the Δ6 position by the action of Δ6-desaturase to form C24:6 n-3 (Kaduce et al., 2008). It is unclear whether the same enzyme is responsible for desaturation of ALA and C24:5n-3 (Kronberg et al., 2006; Maddock et al., 2006). C24:6 n-3 is translocated from the endoplasmic reticulum to the peroxisome, where the acyl chain is shorted by C2 by one cycle of the β-oxidation pathway to form DHA. DHA is then translocated back to the endoplasmic reticulum. Although the precise regulation of the translocation steps and limited β-oxidation is not known, it is possible that this represents a locus for metabolic regulation that facilitates control of DHA synthesis independently from the preceding steps of the pathway.
Evidence for ALA conversion

Studies in cell cultures

HepG2 cells (human hepatocellular liver carcinoma cells) incubated with increasing concentrations of ALA (1.8–72 μl), showed a linear increase in ALA, EPA, and DPAn-3 levels with increasing ALA concentration in the medium (Portolesi et al., 2007). In contrast to EPA and DPAn-3, the levels of DHA reached saturation at an ALA concentration of 1.8–72 μM (Portolesi et al., 2007). Despite the reduction in Δ6 desaturase expression by PUFA (LA and ALA included) there is a marked increase in accumulation of elongated and desaturated ALA and LA in these cells (Portolesi et al., 2007). This demonstrates the complexity of ALA conversion in HepG2 cells and that the perceived relationship between Δ6 desaturase expression and desaturation of ALA and LA may not be quite as simple as it conceived. This leads to the important
point that measures of gene expression by PUFA may not directly relate to enzyme activity and accumulation of products.

In cardiomyocytes studies (Bordoni et al., 1996; Hrelia et al., 1995), results were obtained equivalent to those in Hep2G cells, although results in intact rats suggest that the heart lacks the capacity to elongate ALA (Igarashi et al., 2007). This is principally because the heart lacks elongase-2 expression (Wang et al., 2005; Igarashi et al., 2007), which appears to be absolutely essential for elongating 22:5 n-3 to 24:5 n-3. However, an emerging use of kinetic analysis has shed new light on these processes and the importance of tissues in maintaining the type of long chain n-3 fatty acid in it.

The conversion of ALA to DHA in brain is particularly important because it is one of tissues with the highest DHA content, whereas brain levels of ALA, or its metabolic intermediates, EPA and DPAn-3 are very low. Studies were carried out on human neuroblastoma cell line SH-SY5Y, a model system for human neurons. ALA elongation was demonstrated by incubating these cells with 30 μM ALA for 24 h, after which EPA (330%) and DPAn-3 (430%) content in ethanolamine glycerophospholipids (EtnGpl) is increased, while DHA content increased by 10% only (Langelier et al., 2005). Another study in this cell line demonstrated that after 72 h of incubation, EPA, DPAn-3, and DHA content is increased (780%, 850%, and 65% respectively) in EtnGpl and a similar change occurs in Choline glycerophospholipids (ChoGpl) (Alessandri et al., 2008). Again, both of these studies indicate a much slower conversion of DPAn-3 to DHA, similar to what is seen in liver, yet there is little doubt that neuroblastoma cells elongate and desaturate ALA to longer chain n-3 fatty acids, including DHA. However, experiments in rat primary cortical neurons showed that these neurons were unable to elongate ALA to DHA, suggesting that the capacity of neurons to undertake these steps might be limited (Bernoud et al., 1998; Moore et al., 1991; Langelier et al., 2005). A recent study confirmed this point, and demonstrated that primary rat hippocampal neurons were able to convert ALA to DHA (Kaduce et al., 2008), suggesting that the neuronal cell type might be an important contributing factor to the ability of neurons to elongate and desaturate ALA to DHA. This is further supported by the capacity of cerebellar granule cells to
convert ALA to DHA (Moore et al., 1991). Collectively, these studies indicate that it is more difficult for neurons to elongate and desaturate ALA all the way to DHA, suggesting that astrocytes are the major source of DHA for neurons (Moore et al., 1991; Williard et al., 2001; Sinclair et al., 1994; Bernoud et al., 1998).

The cell culture studies, with a variety of tissues, including hepatic, cardiac and neural cells, indicate that these cells all have the capacity to synthesize longer chain n-3 fatty acids from ALA, including DHA in cells of liver and neural origin. In addition, these results suggest that compared with EPA and DPAn-3, the production of DHA from ALA is metabolically limited at a step beyond DPAn-3 formation. However, these differences may, in part, be due to differential tissue expression of elongase-2, an enzyme required to convert 22:5n-3 to 24:5 n-3 (Igarashi et al., 2008; Wang et al., 2005; Horton et al., 2003).

Studies in animals

Conversion of ALA into its n-3 fat PUFA derivatives has been studied in many different mammalian species including rats (Barcelo-Coblijn et al., 2003; Bowen et al., 2000) hamsters (Fiaccavento et al., 2006; Morise et al., 2004), guinea pigs (Abedin et al., 1999; Ander et al., 2004), rabbits (Abedin et al., 1999), piglets (Bazinet et al., 2003; Blank et al., 2002), baboons (Su et al., 2001), monkeys (Anderson et al., 2005) and cattle (Kronberg et al., 2006; Maddock et al., 2004; Mann et al., 2003; Ponnampalam et al., 2001; Scollan et al., 2001 & 2003; Cooper et al., 2004). Each of these studies demonstrate that ALA is rapidly converted to EPA and DPA n-3, but that conversion to DHA is significantly less, although results on the latter aspect are more controversial. It is led to hypothesis that ALA is converted to longer chain n-3 fatty acids in tissue-dependent manner and tissue-selective manner (Barceló-Coblijn et al., 2005).

In most of the studies, rats fed on ALA-enriched diets (canola, perilla, or flaxseed oil) have significantly increased ALA, EPA, DPAn-3 content in plasma, liver, heart and DPAn-3 and DHA in brain (Barceló-Coblijn et al., 2005 & 2003; Kim et al., 2001, Spady et al., 1993; Ayalew-Pervanchon et al., 2007; Abedin et al., 1999; McLennan et al., 1995). In contrast, ALA and EPA content increased in hearts from
cardiomyopathic hamsters fed on high ALA diet (Fiaccavento et al., 2006) and hypercholesterolemic rabbits (Ander et al., 2004), which was consistent with kinetic studies demonstrating that heart does not elongate [1-14C]18:3n-3 past EPA and DPA3 (Igarashi et al., 2007). The mechanisms accounting for this lack of conversion to DHA in heart appears to be due to the lack of elongase-2 expression (Igarashi et al., 2007; Wang et al., 2005), limiting the step where 22:5 n-3 is elongated to 24:5 n-3 (Sprecher 2000; Voss et al., 1991).

Evidence indicates that accumulation of DHA in tissues from ALA-fed animals is somewhat tissue-selective process. While DHA accumulation occurs in hearts (Abedin et al., 1999, McLennan et al., 1995), hepatic membranes (Kim et al., 2001) and livers (Bowen 2000; Abedin et al., 1999) of animals fed a high ALA-containing diet. However, rats fed on a 7% ALA-diet did not show significant changes in liver or heart DHA levels over 8 week period (Barcelo-Coblijn et al., 2003), which is consistent with the recent observation that heart is not capable of elongating ALA to DHA (Igarashi et al., 2008). Similar inconsistency in results for DHA accumulation brains of ALA-fed animals has also been observed between groups. So, while some of the studies observed DHA accumulation in brain (Barcelo-Coblijn et al., 2003 & 2005; Abedin et al., 1999), other studies did not detect any increase in DHA content (Bowen et al., 2000, Spady et al., 1993). Despite the heterogeneity of these models, all of the results converge to the same conclusion that ALA is converted to longer chain n-3 fatty acids, including DHA, but the conversion of ALA to DHA is dependent not only on the type of tissue, but on the individual phospholipid class examined as well. Thus, while ALA, EPA and DPAn-3 are accumulated in plasma, liver and heart tissues, brain tends to accumulate DPAn-3 and DHA (Barcelo-Coblijn et al., 2003), suggesting a tissue-selective process.

Studies on the amount of dietary ALA on its accumulation and conversion to longer chain n-3 fatty acids were studied in hamsters fed with four doses of ALA (1, 10, 20 and 40 g ALA/100 g) over 5 weeks while keeping LA constant in the diet (Morise et al., 2004). In this study, the results clearly indicated that, ALA content increased dramatically in epididymal adipose tissue and plasma cholesteryl ester (CE) fraction, but by a much lesser extent in red blood cells (RBC), heart, and plasma
phospholipid fraction. The increased ALA content in all of these lipid fractions was positively associated with increasing amounts of ALA in the diet, and therefore decreasing LA/ALA ratio or n-6/n-3 ratio. While plasma phospholipid ALA and EPA content increased with increased ALA intake, DPAn-3 and DHA content did not change. Heart ALA content increased as did EPA, but no changes in DHA were observed, consistent seen in rats fed over an 8-week-period with a 7% ALA diet (Barcelo-Coblijn et al., 2003). ALA storage in the epididymal adipose tissue is important as it represents a slow releasable pool of ALA that is utilized over time by other tissues (Fu 2000). Again, the importance of this study is that there is a linear increase in ALA and EPA in these hamsters, while that of DPAn-3 and DHA was not linear. Similar observations from several laboratories have indicated that the tissue concentrations of the long-chain n-3 polyunsaturates, particularly DHA, are lower in an ALA-based diet than one in which the preformed LCPUFA are present. For example, one study showed that rat brain and retinal DHA were greater in pups fed a diet with preformed EPA and DHA compared to pups fed diets containing only ALA. Even when the ALA intake was increased by a factor of 10 greater than the EPA/DHA levels, the retinal DHA content remained below the value obtained for retina in the preformed DHA diet and led to a diminution of the arachidonic acid (AA) content (Poumes-Ballihaut et al., 2001). Similarly, in guinea pigs, both the brain and retina DHA levels were greater when a diet containing 1% ALA and 1.8% DHA was fed relative to one with only 7.1% ALA (Barcelo-Coblijn et al., 2003). In the heart, the DHA level was over 7-fold greater in the DHA diet relative to the ALA only diet. The liver was an even more extreme case, with DHA 17-fold greater when preformed DHA was in the diet. Glial cell phospholipids of neonatal rats contained more DHA when the dams were fed a diet containing DHA than when they were fed only ALA (Su et al., 1999). When guinea pigs were fed a high level of ALA, many tissues had very substantial increases in ALA, EPA and DPAn-3 but comparatively little increase in DHA (Smith 2005). Piglets fed a diet with 1.7% of fatty acids as ALA supplemented with DHA (0.7%) had lung phospholipid DHA markedly increased compared to the control group consuming 1.7% ALA as the only dietary omega-3 (Wallace et al., 2003).
From above discussion it understood that ALA-enriched diets are able to increase tissue n-3 fatty acid content, but question arises that whether ALA rich diets can replenish the ALA deficiency? To answer this question studies were conducted on female monkeys by feeding ALA-poor diet for 2 months before conception and throughout the pregnancy. The results showed a 70–90% decrease in plasma, erythrocytes and tissue n-3 fatty acid content in the offspring (Anderson et al., 2005). For the next 3 years, these offspring fed a diet containing soybean oil (rich in LA and ALA). The recovery of tissue n-3 fatty acid levels was followed and all tissue recovered but at different rates, although the retina never fully recovered and had a content that was 84% of control. This lack of recovery of retinal DHA levels is associated with a lower functional response (Anderson et al., 2005). In rats depleted ALA was maintained for three generations, brain DHA levels were completely restored by supplementing dietary ALA within 8 weeks (Ander et al., 2004). This includes complete depletion of plasmalogen DHA levels and the restoration by dietary ALA is comparable to that by dietary DHA. These studies suggest that the brain has a unique plasticity with regards to conversion of ALA to DHA and supports the concept of a tissue-selective process for conversion of ALA to DHA.

**Studies in humans**

As in animals, there is a dose-dependent response to ALA-enriched diets in humans. High dosage of ALA feeding (40g/d) and for a prolonged period of time (42 weeks), showed a significant increase in the plasma EPA levels. In an other study consisting of 27g/d of flaxseed oil (14 g/d ALA) for 4 weeks was sufficient to significantly increase the ALA levels in serum triacylglycerols (TAG, 5.7-fold) and in CE (4-fold), while EPA levels were slightly increased, and no changes in DHA content (Schwab et al., 2007). Similar results were obtained when patients with an atherogenic lipoprotein phenotype were treated with 15 g ALA/d for 12 weeks. The treatment led to an increase in ALA, EPA levels in plasma and RBC, although there were no changes in DHA (Wilkinson et al., 2005). A dietary intake of flaxseed oil (3 g ALA/d) for 12 weeks lead to an increase in plasma EPA levels (60%), DPAn-3 (25%), with no changes in DHA. A study in Japan tested efficacy of long term intake of ALA in elderly subjects (Ezaki et al., 1999). In this study, daily intake of ALA was increased to 3 g by substituting perilla (55–60% ALA) for soy oil (7% ALA). At 3
months, there was increase in plasma ALA and EPA, without any change DHA. At 10 months, DHA increased by 21%, and then returned to baseline 3 months after being switched back to soy oil. A study in India showed considerable increases of EPA, n–3 DPA and DHA in plasma, but not in platelets by partially substituting canola oil (10% ALA) for sunflower oil (0.1% ALA) or peanut oil (0.1% ALA) to obtain cooking oils with 25–40% LA and 4% ALA (Ghafoorunissa et al., 2002). On the basis of increased long-chain n–3 PUFA, the authors estimated that 0.75% energy (2.2 g) as ALA may be required to increase plasma PL levels of long-chain n–3 PUFA to the same extent as 0.1% energy (0.3 g) from preformed n–3 long-chain PUFA of fish oil. However, dietary ALA (35 mg/d for 3 months) did not alter plasma TAG and total cholesterol, but did result in higher EPA content in lipoprotein fatty acids. However, this effect was seen in subjects with a high PUFA/saturated fatty acid ratio, suggesting that this ratio may be an important factor in elongation and desaturation of ALA. Four weeks consumption of ALA-enriched diet (flaxseed oil) and a spread consisting of flaxseed oil and butter (2:1), ALA and EPA content was increased in mononuclear phospholipids (Caughey et al., 1996). All the above studies suggest that, blood levels of DHA can be improved by long term intakes of vegetable oils containing ALA and less amount of LA. The results of the Indian study suggests that it is not essential to have a large increase in ALA intake, but a modest increase leads to significant improvement in the long-chain n–3 PUFA status. This observation is very important for vegetarians and for those who do not include fish in their regular diets for various reasons.

Despite the fact that all of these studies suggest that humans can convert ALA into longer chain fatty acids, there are some concerns regarding the efficiency of ALA conversion (Burdge et al., 2002; Pawlosky et al., 2001), which in humans seems to be lower than in other mammals such as baboons (Burdge et al., 2002; Su et al., 1999). However, it is important to suggest that it is difficult to address ALA conversion at the tissue levels, and the conversion of ALA to longer chain n-3 fatty acids in humans is still a subject of debate (Burdge et al., 2005; Brenna et al., 2009).
Influence of LA/ALA ratio on LCPUFA metabolism

The ratio of LA to ALA is considered as one of the factors that influence conversion of ALA to long chain n-3 fatty acids. A number studies were undertaken to establish the optimal ratio of LA to ALA that leads to maximal tissue DHA accumulation (Barcelo-Coblijn et al., 2003; Yehuda et al., 1999; Blank et al., 2002). By varying the LA to ALA ratios from 0.5:1 to 10:1, a significant effect on plasma and brain DHA levels were observed, but there was apparently some degree of complexity in this process (Blank et al., 2002). The highest levels of DHA were not found in diets containing the highest ALA content, but rather maximal DHA accumulation occurs when the LA to ALA ratio was within the range of 2:1–4:1, a similar optimal ratio was obtained by other groups (Blank et al., 2002; Yehuda et al., 1999). However, a recent study on hamster revealed that when the level of LA is held constant at 2% of total energy, the maximum incorporation of DHA occurred in liver and plasma at a ratio of 1:2 of LA:ALA (Aziz et al., 2008).

Extending these studies to examine gene expression, the same non-linear effect of the LA to ALA ratio was observed in gene expression of brain in rats (Barcelo-Coblijn et al., 2003). Collectively, these studies demonstrate that the ratio of LA to ALA is critical for elongation and desaturation of ALA to longer chain fatty acids. This process is undoubtedly controlled in part by gene expression, which is also subject to the same non-linear relationship to dietary ALA intake. These non-linear effects of dietary ALA levels on its conversion to longer chain n-3 fatty acids may account for the difference in ALA conversion to DHA among various studies. The FAO/WHO 1994 joint expert consultation report recommended that the relative amounts of LA and ALA in the diet should be between 5:2 and 10:1 (FAO/WHO, 1994).

Isotope studies of in vivo ALA and EPA metabolism

Early studies of in vivo ALA metabolism in rodents indicated that conversion of ALA through EPA and to DHA does occur (Dhopeshwarkar et al., 1976). Subsequently, stable isotope labeled fatty acids were employed to demonstrate elongation and desaturation of ALA to EPA and DHA in human subjects (Dhopeshwarkar et al., 1976). This was followed by several studies in human infants.
where metabolism of ALA to EPA and DHA was evident (Alessandri et al., 2008; Sinclair et al., 1994), later reports showing a downward trend in conversion efficiency with gestational age at birth in preterm infants (Bowen et al., 2000). The whole body natural isotope tracer approach reported that an average of 42% of DHA is biosynthesized from ALA in 1-month-old infants consuming formulas with 0.64% w/w DHA. This drops to 11% by 3 months of age, and 7% at 7 months of age (MacDonald-Wicks et al., 2004). Adult metabolism of stable isotope labeled fatty acids in vivo have clearly shown metabolism of ALA to EPA with decreasing amounts of DPAn-3 and DHA (Cho et al., 1999; Kim et al., 2001; Ander et al., 2004) with little or no DHA formed in young men (Blank et al., 2002; Cunnane et al., 1997). It has been proposed that women have a greater activity of elongation/desaturation than men and the finding has been confirmed by Anderson et al., 2005. These findings are consistent with higher plasma DHA concentrations in women compared to that of men (Mann et al., 2003; Ponnampalam et al., 2001). The extent of conversion of ALA to DHA is influenced by the dietary long-chain PUFA content (Cho et al., 1999; Kim et al., 2001; Ayalew-Pervanchon et al., 2007; Ander et al., 2004; Anderson et al., 2005; Scollan et al., 2003). ALA metabolism to EPA and DHA has been observed in humans of all ages from premature infants (Innis et al., 2002; Sinclair et al 1994; Scollan et al., 2003) to adults in their sixth decade (Cleland et al., 2005; Ayalew-Pervanchon et al., 2007 Ander et al., 2004; Scollan et al., 2001). DHA biosynthesis may be impaired in disease states such as retinitis pigmentosa (Cooper et al., 1997) and is altered by smoking (Scollan et al., 2001).

Consumption 1200 mg ALA per day by a human would result in 2.4–3.6 mg of DHA per day in the brain. This is important as the human adult brain is reported to use about 4.6 mg of DHA per day (Rapoport et al., 2007; Umhau et al., 2009). However, the human brain requires 17.8 mg of AA per day (Rapoport et al., 2007), which is significantly greater than its requirement for DHA. In addition, the half-life of DHA in the human brain is 773 days as estimated using positron emission tomography (PET) to assess brain DHA uptake parameters (Rapoport et al., 2007; Umhau et al., 2009). Again, this is in stark contrast to AA which has an estimated half-life of 147 days using the same technique (Rapoport et al., 2007). Thus, the low daily requirement of the human brain for DHA and the capacity of the brain to retain DHA
as demonstrated by its long half-life, suggests that the 2.4–3.6 mg of DHA produced by the rodent (DeMar et al., 2005) and non-human primate (Su et al., 2001; Su et al., 1999) brain is indicative of the brain’s capacity to make an adequate supply of DHA in the presence of sufficient dietary ALA (1200 mg of ALA per day). That said, the liver itself will also more than adequately convert ALA to DHA at a rate that is 6–10 times that of the brain, indicating that the liver is the primary source of non-dietary DHA in mammals. Recent whole-body synthesis experiments using [U-13C] ALA further substantiate this point as the total daily synthesis of longer chain n-3 fatty acids is 8.4 μmol of EPA per day, 6.31 μmol of DPAn-3 per day, and 9.8 μmol of DHA per day (Gao et al., 2009). This production of DHA is greater than 30 times the required needs of the brain, again indicating that the liver can provide more than adequate DHA to meet the needs of the brain and the other organ systems for DHA.

In summary, the work by Rapoport and colleagues agrees with many of the earlier studies using stable isotope methods and demonstrates that the liver is the primary tissue in which dietary ALA is converted to DHA. The rapid conversion and packaging into TAG destined for export by the liver as well as its plasticity to increase the conversion of ALA to DHA under low n-3 conditions, indicates that this organ system is fully capable of providing ample DHA when adequate ALA is consumed. While the brain is not the major source of DHA, it has the capacity to nearly meet its daily needs for DHA through the conversion of plasma-derived ALA. This coupled with the long half-life for DHA in human brain provides the brain a degree of protection when liver function may be limited or when n-3 deprivation is on-going.

**Dietary recommendations of fats and fatty acid intake**

Joint FAO/WHO Expert Consultation on Fats and Fatty Acids in Human Nutrition, held in Geneva, Switzerland, November 10–14, 2008. FAO/WHO recommendations by expert on fats and fatty acid intake are summarized in the following table (table 1.2).
Table 1.2. Dietary recommendations of fats and fatty acid intake by FAO/WHO

<table>
<thead>
<tr>
<th>Total Fat (%E)</th>
<th>ADMR</th>
<th>20-35</th>
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<tr>
<td></td>
<td>Maximum level</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>Minimum level</td>
<td>15</td>
</tr>
<tr>
<td>Saturated fatty acid (SFAs) (%E)</td>
<td>Maximum level</td>
<td>10</td>
</tr>
<tr>
<td>Monounsaturated fatty acid (MUFAs) (%E)</td>
<td>ADMR</td>
<td>By differencea, b</td>
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<tr>
<td>Total Polyunsaturated fatty acid (Total PUFAs) (%E)</td>
<td>ADMR (LA+ALA+EPA+DHA)</td>
<td>6-11</td>
</tr>
<tr>
<td></td>
<td>Maximum level</td>
<td>11</td>
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<tr>
<td></td>
<td>Min level (To prevent deficiency)</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Min level (to prevent chronic diseases)</td>
<td>6</td>
</tr>
<tr>
<td>n-6 PUFAs</td>
<td>ADMR (LA)</td>
<td>2.5-9</td>
</tr>
<tr>
<td></td>
<td>Average LA requirement</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Individual LA levels</td>
<td>2.5</td>
</tr>
<tr>
<td>n-3 PUFAs (%E)</td>
<td>ADMR (n-3c), %E</td>
<td>0.5-2</td>
</tr>
<tr>
<td></td>
<td>Min requirement (ALA), %E</td>
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<tr>
<td></td>
<td>ADMR (EPA+DHA), g/day</td>
<td>0.250-2d</td>
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<tr>
<td></td>
<td>Maximum level, g/day</td>
<td>3</td>
</tr>
<tr>
<td>Trans fatty acid (TFAs) (E%)</td>
<td>Upper limit (from rumints &amp; industrially Produced)</td>
<td>1</td>
</tr>
</tbody>
</table>

aTotal fat [%E] = SFAs [%E] + PUFAs [%E] + TFAs [%E]; bcan amount up to 15–20%E; cALA + n-3 long-chain PUFAs; dfor secondary prevention; eincluding supplements. AMDR = acceptable macronutrient distribution range; E=energy; EPA= eicosapentaenoic acid; Max level = maximum level; Min level = minimum level.

**Dietary enrichment with n-3 PUFA**

n-3 fatty acids have been associated with many health benefits relating to heart health, brain function, eye health, joint health, mood, behavior, cancer, diabetes, skin disorders, pregnancy, and lactation. Interest in delivery of omega-3 fatty acids into foods has primarily been driven by consumer awareness of the health benefits of omega-3 fatty acids and an increased consumer demand for foods fortified with omega-3 fatty acids that have desirable nutritional, sensory, and functional attributes. Oxidation affects the nutritional quality and safety of the oil to consumers. The fishy taste and odour associated with omega-3 oils from marine sources is another hurdle for incorporation of omega-3 fish oils into food products. The development of oxidative rancidity of omega-3 oils is a factor that has limited its use in foods. The biggest challenge to the omega-3 oil manufacturers and suppliers is overcoming the development of undesirable taste and odours in the end products and the difficulties in
using the oil as an ingredient in food and beverage applications. As the oxidation of unsaturated fatty acids is an autocatalytic process, unsaturated oils used for incorporation into foods must be of the highest quality practically achievable in order to minimize the development of off-flavors. This means that the oils should be extracted from raw materials of good quality and the bulk oils should be refined, stabilized, packaged, and stored under appropriate conditions, which minimize exposure to factors that promote oxidation such as air, oxygen, and light antioxidants may be added to protect the oil from oxidation. Synthetic antioxidants, such as tertiary butyl hydroquinone (TBHQ), butylated hydroxylanisole (BHA) and butylated hydroxytoluene (BHT), singly or as mixtures, have been used in fish oils. The push toward natural ingredients has resulted in natural antioxidants such as tocopherols, ascorbic acid, carotenoids, rosemary extracts and ascorbyl palmitate as more effective antioxidants for retarding oxidation in fish oil. Even with the addition of antioxidants, unprotected oils can still oxidize very rapidly once they are exposed to a food environment.

Recent advances in the area of “functional foods and nutraceuticals” have resulted in the generation of a variety of food products with enhanced n-3 fatty acid contents. This technology primarily utilizes the incorporation of either fish or flaxseed products in livestock feed, resulting in accumulation of these fatty acids in tissues and other animal products. This can be considered a significant achievement for fulfilling current recommendations of increasing intake of n-3 fatty acid. Other technologies include the manipulation of genetic make up of higher plants and animals to create transgenic creatures with enhanced abilities for synthesizing n-3 fatty acids. Newer technologies and processing techniques minimize oxidation, and in some instances mask flavor and smell for improved palatability. Following some of strategies applied to enrich n-3 PUFA in food system is discussed briefly.

1. Designer eggs

The designer food approach has been explored widely using egg in providing various essential nutrients to the humans. Over the past decade, significant efforts have been made to produce high quality designer eggs which are rich in n-3 fatty acids, including DHA. However, increased levels of these PUFA in eggs may result in
decreased shelf-life of these products as they become more susceptible to oxidation due to the presence of long-chain PUFA (Van Elswyk 1997; Leeson et al., 1998). To overcome this deficiency, further enrichment with vitamin E, beta carotene, and antioxidant minerals have been considered (Leeson et al., 1998; Grune et al., 2001). Thus, these new products have been considered to provide health benefits beyond their general food application. Incorporation of flaxseed and or flaxseed oil in hens’ feed has resulted in significantly elevated levels of both ALA and DHA in egg yolks (Scheideler & Froning 1996). However, this increase in DHA may not be sufficient to generate a good source of dietary DHA needed for the prevention of human diseases. Further investigation led to incorporation of fish oil or other fish products in the hens’ feed. This strategy resulted in a product with lower n-6:n-3 fatty acid ratio which was close to the current recommendation of intake of these essential fatty acids. Despite this achievement, fishy taste still remains in eggs. To reduce this off-flavor, the addition of antioxidant vitamins was used. However, high levels of vitamin E did not reduce the fishy taste of the eggs. On the other hand, a diet containing lower levels of flaxseed (up to 5%) or high quality menhaden oil (up to 1.5%) significantly reduced the fishy taste. Aymond & Elswyk (1995) reported that the addition of 15% ground flaxseed to the diet of hens produced DHA content of 1.5% in yolk similar to menhaden oil supplementation (Marshall et al., 1994). This indicates that hens are able to efficiently convert ALA to DHA. It is of interest that both of these two lines of n-3 fatty acid-enriched eggs showed comparable thiobarbituric acid reactive substances (TBARS) values which were not significantly different from those of ordinary eggs (Marshall et al., 1994). Another approach was the inclusion of dried DHA-enriched marine micro-algal products into hens’ feed, this strategy resulted in higher contents of DHA in yolk compared to the supplementation of feed with menhaden oil (Herber & VanElswyk 1996).

2. In meat and poultry

Inclusion of n-3 fatty acid containing materials such as fish and linseed in animals’ feed can increase the content of n-3 fatty acids in their meat. Several studies have reported that feeding lambs with diets supplemented with either fish meal, fish oil, or protected tuna oil had a significant increased EPA and DHA contents in their meat (Ponnampalam et al., 2001; Kitessa et al., 2004). A similar observation was
reported in pigs and steers after feeding animals with fish product-supplemented diets (Mandell et al., 1997; Scollan et al., 2001). Like fish oil/meal, flaxseed oil markedly increased the ALA content of pork meat with a high level of EPA in intramuscular fat (Leskanich et al., 1997). Other studies reported that linseed oil is more efficient in increasing the n-3 fatty acid contents of muscle tissues in pigs compared to that of flaxseed meal (Cherian & Sim 1995). Rey et al., 2001 reported that a low level of dietary flaxseed oil (0.5%) is sufficient to markedly increase intramuscular n-3 fatty acid concentrations, including DHA in pigs. Kouba et al., 2003 reported significant increases in total n-3 fatty acids and decreases in the ratio of n-6:n-3 fatty acids in meat of pigs fed with a diet containing 60 g/kg of crushed flaxseed for 100 days. Higher levels of n-3 fatty acids in these meat products were not associated with increased indicates of lipid oxidation as compared to controls. Enser et al., 2000 reported increased levels of both EPA and DHA in pork meat and liver (10 to 20 times) following flaxseed feeding in pigs. In one study, long term (65 days) feeding of flaxseed (3% w/w) was associated with increased levels of DHA in pork meat. Dietary flaxseed oil resulted in a significant reduction in the ratio of n-6:n-3 fatty acids ratio in pork tenderloin muscle due to increases in the levels of n-3 fatty acids (Hoz et al., 2003). Addition of vitamin E to flaxseed oil-rich diets markedly reduced lipid oxidation in n-3 fatty acid rich meat samples (Hoz et al., 2003).

Several approaches have been engaged to increase the content of n-3 fatty acids in poultry meat without compromising the optimal sensory quality. These strategies include the addition of tocopherol to fish oil, blends of fish oil with vegetable or seed oil rich in ALA, or replacement of fish oil with flaxseed oil prior to slaughter. In this regard, Bou et al., 2005 reported that feeding broiler chicken with either fish oil or flaxseed oil resulted in increased levels of n-3 fatty acids in mixed raw red and white chicken meat with skin. Similar observations supporting the notion of enrichment of chicken meat with either flaxseed oil or fish oil were also reported by other laboratories (Lopez-Ferrer et al., 2001). However, the consumer acceptability of meat produced by birds on flaxseed oil was better than that for meat from fish oil-fed birds (Lopez-Ferrer et al., 1999).
3. Milk and dairy products

Milk produced by animals on grass based diets contain more n-3 fatty acids as compared to milk from maize fed animals (Chilliard et al., 2004; Chilliard et al., 2003; Chilliard et al., 2000). This is due to high contents of ALA in fresh grass. However, whole flaxseed supplementation resulted in higher milk production as well as higher content of n-3 fatty acids along with reduced n-6:n-3 fatty acid ratio in cows’ milk compared to controls (Petit et al., 2004). Other studies reported that the intake of 200–400 g/day of ALA from extruded rapeseeds and/or flaxseeds significantly increased ALA levels in cow milk fat (Focant et al., 1998). Encapsulated ALA resulted in even higher levels of milk ALA from cows that received 410 g protected flaxseed oil/day (Goodridge & Ingalls 1998). The addition of extruded flaxseed into the diet of cows resulted in a 2-fold increase in their milk content of ALA, as compared to controls (Ponter et al., 2006). This effect appeared as early as 1 week after dietary intervention and was consistently seen in weekly milk samples up to two months. Supplementation of grazing dairy cows with rumen-protected tuna oil resulted in 3-fold higher concentrations of total n-3 fatty acids, including DHA and EPA in their milk (Kitessa et al., 2004). These changes reduced the atherosclerotic index of milk by 17% without noticeable effects on consumer acceptability of the products taste and smell. Feeding cows with protected fish oil resulted in reduced milk yield, milk protein contents, proportion of short chain fatty acids, stearic acid, and oleic acid while increasing n-3 fatty acid levels in the milk, compared to controls (Lacasse et al., 2002). These changes in milk composition were accompanied by an increased peroxide index and lower consumer acceptability, as judged by a taste panel evaluation. In addition, it should be noted that the transfer of EPA and DHA from diets to milk in cows is limited to a high degree of saturation in the rumen. Feeding goats with either flaxseed oil or flaxseed meal significantly increased the milk contents of total fat and the proportion of ALA (Chilliard et al., 2003; Chilliard et al., 2000).

4. Designer oils

The age of true designer plant oils has arrived. Using the tools of biotechnology, it is now possible to modify the fatty acid content of oilseed plants to change the relative abundance of individual fatty acids in seed oil for health purposes.
or to produce nutritional fatty acids which are not normally found in crop plant (Damude et al., 2007).

Constitutive expression of delta-9 elongase pathway was reported using genes from the microalgae Isochrysis galbana and Euglena gracilis, the fungus Mortierella alpina in the model plant Arabidopsis (Qi et al., 2004). Individual pathway genes (delta-9 elongase, delta-8 desaturase, delta-5 desaturase) were each linked to a Ca35S promoter and resulting EPA contents as high as 3.0% and AA contents up to 6.6% were produced in Arabidopsis leaves (Abbadi et al., 2004).

In soybean studies were conducted for extensive characterization of multiple seed specific promoters and LCPUFA biosynthetic genes from different microbial sources, optimization of promoter-gene cassette combinations and orientations. Using this approach, soybean seeds with an EPA content as high as 19.5% were produced with virtually no AA. The low AA content was attributed to the use of the S. diclina delta-17 desaturase. Additionally, the DHA precursor, DPA was found in high EPA lines at an abundance of about 4%, a result of the additional activity of the M.alpine delta-6 elongase towards the delta-5 fatty acid EPA (Kinney et al., 2011).

Nevertheless, key challenges remain in obtaining plant based oil that is substantially similar to other commercially available fish oils. The first target will be to produce an EPA plus DHA plant oil with DHA comprising at least 10% of the total fatty acids, close to that of some marine oils. Although the highest abundance of DHA reported so far is less than 4%, current technology will allow for this abundance to be increased around threefold while maintaining EPA abundance in the 10–15% range and this will provide an effective marine oils substitute. The promising use of plant seed oils modified by biotechnology to produce n-3 long chain fatty acids will provide a readily available source of these important fatty acids in the future, overcoming the problems associated with obtaining n-3 LCPUFA from declining ocean fish supplies (Damude et al., 2007).

**Garden cress seed oil – As an alternative source of ALA**

*Lepidium sativum* L. commonly called garden cress belongs to the family Brassicaceae (Table 1.3). It is an annual erect herbaceous plant, cultivated all over the world. The plant is native to Southwest Asia (Persia) and it spread to many centuries ago to western Europe. Garden cress is an age old plant, as it was recorded by the
philological trace of its names in different Indo-European languages. These include the Persian word *turehtezuk*, the Greek *kardamon*, the Latin *nasturtium* and Arabic *tuffa'* and *hurf*. Xenophon (400 BC) mentioned that the Persians used to eat this plant even before bread was known. It was also familiar to the Egyptians and was very much appreciated by the Greeks and Romans, who were very fond of banquets rich in spices and spicy salads (Bermejo & León 1994). Garden cress's main use was always as an aromatic and slightly pungent plant. It was not only in antiquity but also in the Middle Ages it enjoyed considerable prestige on royal tables. The young leaves were used for salads. The ancient Spartans ate them with bread. This use still continues and it is eaten along with bread and butter or with bread to which lemon, vinegar or sugar is added. It is mainly used nowadays in the seedling stage, the succulent hypocotyls being added to salads and as a garnish and decoration for dishes. The roots, seeds and leaves have been used as a spicy condiment (Bermejo & León 1994).

Garden cress is an annual, erect, herbaceous plant, grow up to height of 50 cm. The basal leaves have long petioles and are lyrate-pinnatifid, the caulinar leaves are laciniate-pinnate while the upper leaves are entire (Figure 1.10). The inflorescences are in dense racemes. The flowers have white or slightly pink petals, measuring 2 mm. The siliquae measure 5 to 6 × 4 mm, elliptical, elate from the upper half and glabrous. Cress is a plant that is well suited to all soils and climates, although it does not tolerate frosts. In temperate conditions, it has a rapid growth rate (Bermejo & León 1994).

Table 1.3. Botanical classification of garden cress plant

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sub kingdom</td>
<td>Tracheobionta</td>
</tr>
<tr>
<td>Sub division</td>
<td>Spermatophyta</td>
</tr>
<tr>
<td>Division</td>
<td>Mangoliophyta</td>
</tr>
<tr>
<td>Class</td>
<td>Mangoliopsida</td>
</tr>
<tr>
<td>Sub class</td>
<td>Dilleniidae</td>
</tr>
<tr>
<td>Order</td>
<td>Capparales</td>
</tr>
<tr>
<td>Family</td>
<td>Brassicaceae</td>
</tr>
<tr>
<td>Genus</td>
<td><em>Lepidium</em></td>
</tr>
<tr>
<td>Species</td>
<td><em>L.sativum</em></td>
</tr>
</tbody>
</table>
Figure 1.10. Garden cress plant

Figure 1.11. Garden cress seeds
In India the seeds are harvested for food and medicinal purposes. It is also known as common cress, land cress and Haliv in India (Table 1.4). It figures prominently in Indian Materia Medica with Sanskrit name Chandrasura (Nadkarni 1954). The seeds of garden cress claimed to possess varied medicinal properties like galactogogue, aperient, diuretic, alterative, tonic, demulcent, aphrodisiac, carminative and emmenagogue. Mucilage of the seeds allays the irritation of the mucous coat of intestines. Seeds are also useful in hiccups, dysentery, diarrhea and skin diseases caused by impurities and toxins in blood and chronic enlargements of spleen (Nadkarni 1954). Pharmacological study of the seeds indicates the presence of cardioactive substance and is shown to have probable action through adrenergic mechanisms (Vohora 1977). The aqueous extract of L. sativum seeds has been reported to exhibit a potent hypoglycaemic activity in normal and streptozotocin induced diabetic rats (Eddouks 2005) as well as an antihypertensive effect when studied in both normotensive and spontaneously hypertensive rats. According to ethnopharmacological survey carried out in the north central region of Morocco, seeds were used for the treatment of hypertension and renal disease (Jouad et al., 2001). The high protein, fat, dietary fibre, calcium, phosphorous and iron contents in this seed bring out its high nutritive value which may be making it useful in post pregnancy diets in India (Figure 1.12) (Gokavi et al., 2004).

<table>
<thead>
<tr>
<th>Language</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assamese</td>
<td>Halim-Shak</td>
</tr>
<tr>
<td>Bengali</td>
<td>Halim-Shak</td>
</tr>
<tr>
<td>Gujarati</td>
<td>Asaliya</td>
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<tr>
<td>Hindi</td>
<td>Aselio, Halim</td>
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<tr>
<td>Kannada</td>
<td>Allibija, Kurthike</td>
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<tr>
<td>Marathi</td>
<td>Alhiv, Aliv</td>
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<tr>
<td>Punjabi</td>
<td>Halon, Tezak</td>
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<tr>
<td>Tamil</td>
<td>Ali, Ariverai</td>
</tr>
<tr>
<td>Telugu</td>
<td>Adiyalu</td>
</tr>
<tr>
<td>Sanskrit</td>
<td>Chandrasura</td>
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<tr>
<td>Urdu</td>
<td>Halim</td>
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</table>
The seeds resemble some of the oil seeds morphologically with the dicotyledonous endosperm accounting to 80–85% of the seed matter, whereas the seed coat and the embryo account for 12–17% and 2–3% of the seeds, respectively. While the seed coat is of red or brown redcolored, the endosperm has yellow color. Seed bears 21.5-23% oil (Diwakar et al., 2008; Moser et al., 2009). Fatty acid profile of oil shows 30-34% of ALA. (Diwakar et al., 2008). oil has one of the higest amount of tocopherols(1799 mg/kg oil) and phytosterols(14.33 mg/gm) (Moser et al., 2009). Bioavilabality studies on ALA from garden cress oil in albino rats, showed that ALA is absorbed, incorporated in the tissues and metabolized to long chain fatty acids- EPA and DHA.

Despite its great medicinal and nutritional value, garden cress has not received the attention it deserves. The United Nation Organization’s FAO has classified garden cress is one of the underutilized or neglected (cultural suppression) crop among age old crops. (Bermejo & León 1994). In India, garden cress is grown mainly for the sake of seeds. The seeds are used in many ayurvedic and traditional medicine preprations.
Aim and scope of the present work

Polyunsaturated fatty acid (PUFA) comprise the parent essential fatty acids (EFAs) namely, linoleic acid (LA 18:2n-6) and α-linolenic acid (ALA 18:3n-3). These fatty acids are very much essential for human development and health. EFAs cannot synthesized denovo in human and therefore need to be consumed or supplemented as part of the diet. LA and ALA are precursor molecules from which the rest of the n-6 or n-3 fatty acid family fatty acids i.e. AA, EPA and DHA are synthesized through a series of elongation and desaturation reactions.

In recent years, human dietary lipids intake has shifted more towards PUFAs due to their cholesterol lowering effect compared to saturated lipids. The increased consumption of vegetable oils (sunflower, corn oil, safflower oil, soybean oil) rich in n-6 PUFA has shifted the n-6 to n-3 PUFA ratio to ~50:1 instead of a recommended ratio of 10:1 or 2:1. Studies indicated that intake of high n-6 PUFAs in our diet has shifted the physiological status to one that is pro-thrombotic and pro-aggregatory, characterized by increases in blood viscosity, vasospasm, and vasoconstriction and decreases in bleeding time. Further, the deficiency of n-3 PUFAs has been implicated in inflammatory diseases viz., atopic dermatitis, rheumatoid arthritis, asthma, ulcerative colitis and cancer. However, sufficient intake of n-3 PUFAs alters membrane fluidity, down-regulates inflammatory genes, lipid synthesis and stimulates fatty acid degradation.

A survey on Indian dietary fatty acid pattern, in both rural and urban populations showed that linoleic acid (LA) levels from adequate to too high levels due to consumption of vegetable oils rich in n-6 fatty acids. Majority of Indians (31%) are vegetarians and among non-vegetarians fish consumption is limited due to geographic and economic reasons. Therefore, there is a need to moderate n-6 PUFA and increase n-3 PUFA and long chain polyunsaturated fatty acids (LCPUFAs) in Indian vegetarian diet.

Garden cress seeds contain 21.5-23% oil and one of rich and new source of ALA (32-34%) and also contains highest amount of tocopherols and phyotosterol. It has balanced ratio of MUFA:PUFA. Garden cress seed oil is quite stable compared to
ALA rich flaxseed oil. Garden cress oil can be considered as potential, alternative, underutilized seed oil rich in ALA and it can be exploited to increase n-3 fatty acids in oils and food products. The present study is aimed to utilize the garden cress seed oil as a new source of health oil by blending with vegetable oils and supplementation in food products.

**Objectives**

1. Isolation and characterization of bioactive compounds including phenolics, phytosterols, tocopherols from Garden cress seeds and oil.

2. Safety evaluation of Garden cress seed oil and blended oils in experimental rats.

3. Microencapsulation of bioactive compounds of Garden cress seeds/oil and supplementation in food products.