Introduction

Chapter 1

1. Introduction

Day by day awareness is increasing about the healthy food. People have started paying more attention towards their diet; most importantly fats and fatty acids. Few decades back emphasis on low fat, low energy diets has provided a major stimulus for development of products with low calories, low fat or fat substitutes. Though certain fats contribute to disorders like heart problems and obesity, not all fats are detrimental. Recent researches suggest that there is a need to consider the quantity as well as the quality of fat in our diet. Nutritional importance of omega-3 (n-3) polyunsaturated fatty acids (PUFAs) over n-6 PUFAs is now a day well accepted. Proper balanced intake of fats may provide number of health benefits (Simopoulos, 2008). Disruption in proper PUFA intake and metabolism (i.e. higher n-6: n-3 ratio in the diet) is associated with poor retinal/brain development, cognitive deficiency, coronary artery disease, hypertension, diabetes, inflammatory disorders, and cancer.

1.1 Fats and lipids

Lipids are derivatives of fatty acids and biosynthetically or functionally related compounds. Naturally all the important fats and oils of animal and plant origin consist of triacylglycerols (triglycerides) with structural formula as shown in figure 1.1, where R', R", R"' represent same or different fatty acyl chains. Diacyl and monoacyl-glycerides are termed collectively "partial glycerides". They are rarely present at trace levels in animal and plant tissues (Gunstone, *et al.*, 2007).

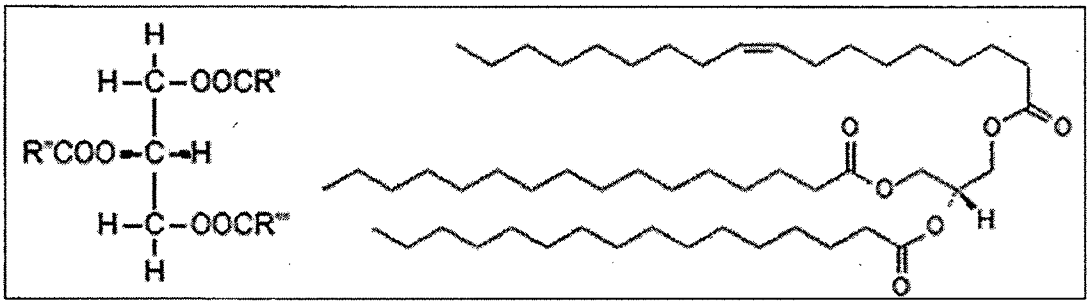

Figure 1.1 Structure of fats

1.2 Fatty acids and their classification

The fatty acids (FAs) are synthesized by chain elongation of an acetyl-CoA with malonyl-CoA (or methylmalonyl-CoA) groups that may contain a cyclic functionality and/or are substituted with heteroatoms (Fahy *et al.*, 2005). Free or unesterified fatty acids are ubiquitous in minor components of all living tissues (Gunstone *et al.*, 2007). They are classified into different groups by considering number of properties like degree of saturation, chain length, orientation of carbon chains on the sides of double bond, position of the first double bond within the acyl chain etc. Natural fatty acids usually contain 4 to 28 carbons (Most of the fatty acids in complex lipids contain 14 to 22 carbons). Depending upon chain length they are classified as short chain (2-6 carbons), medium chain (8-14 carbons) and long chain (16 carbons onwards) fatty acids.

1.2.1 Saturation Characteristics

Fatty acids are commonly classified as either saturated, monounsaturated, or polyunsaturated, based on the number of double bonds exist in their molecular structure. A 'saturated' fatty acid has the maximum possible number of hydrogen atoms attached to every carbon atom. The most abundant saturated fatty acid in nature is hexadecanoic or palmitic acid, (C16:0); purified from Palm oil and named after it. Palm oil contains mainly palmitate (43%–46%) and oleate (C18:0) (36%–41%) (Gunstone, 2002). A fatty acid with one double bond is said to be 'monounsaturated.' Common monounsaturated fatty acids (MUFAs) are palmitoleic acid (C16:1) and oleic acid (C18:1). Palmitoleic acid with 16 carbon atoms has the first double bond occurring at 7 carbon atoms away from the methyl group. Oleic acid has 18 carbon atoms with the first double bond occurring 9 carbon atoms away from the methyl group. Fatty acids having more than one double bond are called 'polyunsaturated fatty acids (PUFAs)'.

1.2.2 *cis* and *trans* orientations

Unsaturated fatty acids (MUFAs and PUFAs) are further classified as '*cis*' and '*trans*' fatty acids. In the '*cis*' configuration the hydrogen atoms at a double bond are positioned on the same side of the carbon chain and chains extend on the same side of the double bond (Figure 1.2). In fact, most of the naturally occurring fatty acids are of this type. On the other hand *trans* fatty acids have the carbon chain extends from opposite sides of the double bond. Usually *trans* fatty acids are the product of food processing. They are found in partially hydrogenated fats, processed foods, meat, beef, dairy products etc. (Kris-Etherton *et al.*, 2012; Roe *et al.*, 2013). They are responsible for increase in Low Density Lipoprotein (LDL) cholesterol in blood and hence responsible for increase in risk of heart disorders and decrease in High Density Lipoprotein (HDL) cholesterol levels (Stender and Dyerberg, 2003). Vaccenic acid is a naturally occurring *trans* fatty acid found in the fat of ruminants and in dairy products such as milk, butter, and yogurt (Lawson *et al.*, 2001).

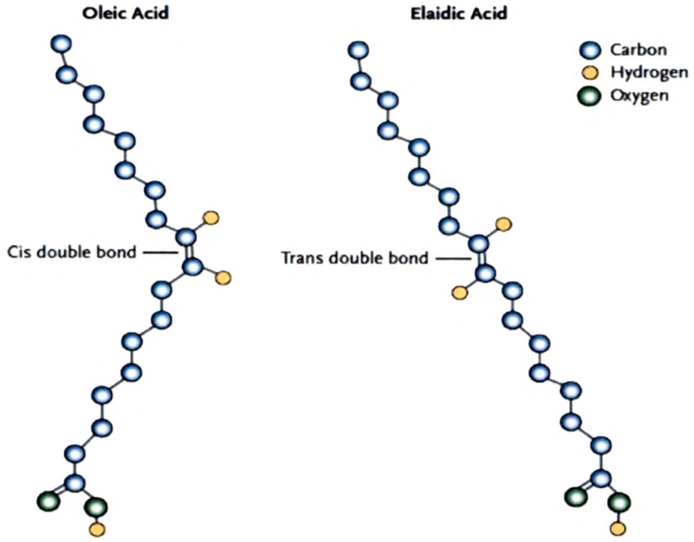

Figure 1.2 *cis* and *trans* conformation of oleic acid. The '*cis*' configuration the hydrogen atoms at a double bond are positioned on the same side of the carbon chain and chains extend on the same side of the double bond while *trans* fatty acids have the carbon chain extends from opposite sides of the double bond (Mozaffarian *et al.*, 2006)

1.2.3 Position of the double bonds

1.2.3.1 PUFAs with divinylmethane pattern

Most of the PUFAs show a divinylmethane pattern (−C−C=C−C−C=C−) in distribution of double bonds. These fatty acids have 2 or more *cis* double bonds that are separated from each other by a single methylene bridge (-CH_2- unit). In PUFAs, the carbon on the methyl group of a fatty acid is called the omega (n) carbon while that on the carboxyl end is called the delta (Δ) carbon. PUFAs are further classified as omega-3 (n-3), omega-6 (n-6) and omega-9 (n-9) fatty acids named according to the position of the closest double-bond to the methyl group. In n-3 fatty acids first double bond towards methyl group is at C3 position, which is at C6 position in n-6 fatty acids and at C9 in n-9 fatty acids. Out of these n-3 and n-6 are the major groups of PUFAs while n-9 type of unsaturated fatty acids mainly consists of MUFAs like oleic acid, ecoisanoic acid etc. Representative fatty acid structure of n-3 and n-6 PUFAs are as shown in figure 1.3.

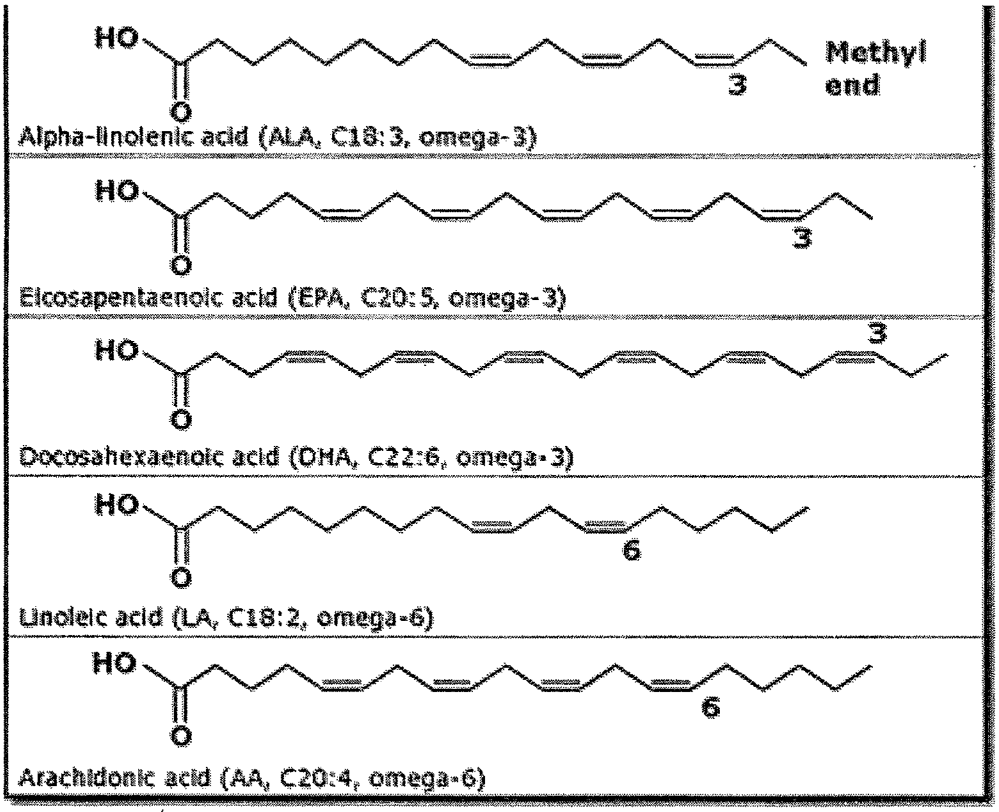

Figure 1.3 Structures of n-3 and n-6 PUFAs. n-3 fatty acids have an extra double at omega-3 position than that of n-6 fatty acids.

The PUFAs can be represented by a structural formula CX: y (n-Z); where, X stands for number of carbon atoms in the acyl chain; y is number of double bonds and n-Z indicates that the first double bond occurs at Z^{th} carbon from omega end e.g. α-Linolenic Acid (ALA): C18:2 n-3; Linoleic Acid (LA): C18:2 n-6; and Oleic acid: C18:1 n-9. LA and ALA act as precursors for n-6 and n-3 PUFAs.

1.2.3.2 Conjugated fatty acids

There is a small group of unsaturated fatty acids with two or more conjugated double bonds (-C=C-C=C-). The conjugation of the double bonds is considered as an intermediate step in unsaturated fatty acid peroxidation. Thus, conjugated LA (CLA, a collective term used to designate a mixture of positional and geometric isomers of LA) is the first step in LA peroxidation (Gunstone *et al.*, 2007). The two double bonds in CLA are primarily at position 9 and 11, and 10 and 12 from the carboxyl end along the acyl chain. There can also be geometric changes (*cis* or *trans* configuration). Eight different CLA isomers of LA have been identified. Of these isomers, the cis-9, trans-11 form is believed to be the most common natural form with biological activity. The major CLA isomer found in natural products is "rumenic acid" (Kramer *et al.*, 1998). Food products from grass-fed ruminants (e.g. mutton and beef) are good sources of CLA, and contain much more of it than those from grain-fed animals (Aldai *et al.*, 2011).

1.2.4 Nutritional classification of fatty acids

Depending upon nutritional requirements and biosynthesis, fatty acids are classified as essential and non-essential fatty acids. Essential fatty acids are not synthesized by animals hence should be obtained from the diet (Simopoulos, 2008). Linoleic and α-Linolenic acid are the two essential fatty acid. Non-essential fatty acids constitute all the other fatty acids synthesized by animals.

1.2.4.1 Linoleic acid and α-linolenic acid as essential fatty acids

Two distinct families of essential fatty acids exist in human body i.e. omega-3 (n-3) family and omega-6 (n-6). Omega-3 PUFAs family is derived from the essential fatty acid ALA and omega-6 PUFAs family is from LA. Both LA and ALA cannot be synthesized in our body from their precursor oleic acid (C18:1) due to lack of activities required for desaturation at n-3 and n-6 positions (Arterburn *et al.*, 2006; Simopoulos, 2008). The physiologically active n-3 PUFAs belong to eicosapentaenoic acid (EPA; C20:5 n-3) and docosahexaenoic acid (DHA; C22:6 n-3) while n-6 category are dihomo gamma linolenic acid (DGLA; C20:3 n-6) and arachidonic acid (AA; C20:4 n-6).

A very small amount of ALA is converted into EPA (2%-8%) and DHA (0.2%-0.6%) in our body (reviewed in Barcelo-Coblijn and Murphy, 2009) and hence these three nutritionally essential n-3 fatty acids must be obtained from diet. EPA and DHA have their own importance in different physiological functions.

1.2.4.2 Linolenic acid (C18:2 n-6)

LA is the principal n-6 fatty acid which is not synthesized in animals but represents the most common PUFA, in plants and animal tissues. It has its closest double bond at 6th carbon atom from the methyl end; thus it is called an n-6 fatty acid. LA is the precursor of all the n-6 series formed by desaturation and elongation, firstly converted into γ-linolenic acid (GLA; C18:3 n-6) and then AA. Walnut, peanut, seeds of sunflower, grape, corn, sesame and soya contain large amounts of LA (Gunstone, 2002).

1.2.4.3 α-Linolenic Acid (C18:3 n-3)

In ALA, the double-bond closest to the methyl group is only 3 carbons away, so it is an n-3 fatty acid. ALA is the principal n-3 fatty acid; which a healthy human will convert into EPA (C20:5 n-3) (Schaeffer *et al.*, 2006), and later into DHA (C22:6 n-3). ALA is abundant in flax seed oil (35% - 40%) and is present in small quantities in walnut, soybean and canola oil (Gunstone, 2002). In fish oil, PUFAs of the n-3 series, especially EPA and/or DHA, are found in greater abundance while ALA is present in relatively less amount. Most of commonly found fatty acids are listed in table.1.1 along with their common name, structure and molecular weight (Gunstone *et al.*, 2007).

Table 1.1 Types of fatty acids

Systematic name	Trivial name	Formula	Mol.wt.
n-6 PUFAs			
9,12-octadecadienoic	Linoleic acid (LA)	18:2 (n-6)	280.4
6,9,12-octadecatrienoic	γ- linolenic acid (GLA)	18:3 (n-6)	278.4
8,11,14-eicosatrienoic	dihomo- γ-linolenic acid	20:3 (n-6)	306.5
5,8,11,14-eicosatetraenoic	Arachidonic acid (AA)	20:4 (n-6)	304.5
7,10,13,16-docosatetraenoic	Adrenic acid	22:4 (n-6)	332.6
4,7,10,13,16-docosapentaenoic	Osbond acid	22:5 (n-6)	330.6
n-3 PUFAs			
9,12,15-octadecatrienoic	α-Linolenic acid (ALA)	18:3 (n-3)	278.4
6,9,12,15-octadecatetraenoic	Stearidonic acid (SDA)	18:4 (n-3)	276.4
8,11,14,17-eicosatetraenoic	ETA	20:4 (n-3)	304.5
5,8,11,14,17-eicosapentaenoic	Timnodonic acid (EPA)	20:5 (n-3)	302.5
7,10,13,16,19-docosapentaenoic	Clupanodonic acid (DPA)	22:5 (n-3)	330.6
4,7,10,13,16,19-docosahexaenoic	Cervonic acid (DHA)	22:6 (n-3)	328.6
n-9 Unsaturated fatty acids			
9-octadecenoic acid	Oleic acid	18:1 (n-9)	282.46
11-eicosenoic acid	Eicosenoic acid	20:1 (n-9)	310.5
5,8,11-eicosatrienoic	Mead acid (ETE)	20:3 (n-9)	306.5
13-docosenoic acid	Erucic acid (EA)	22:1 (n-9)	338.6
Conjugated fatty acids			
9Z,11E-octadeca-9,11-dienoic acid	Remenic acid (RA) cis-9, trans-11 18:2 acid	18:2 (n-7)	280.44
8E,10E,12Z-octadecatrienoic acid	α-Calendic acid	18:3 (n-6)	278.43