Review Article

OXIDATION AND POLYMORPHISM OF FATTY ACIDS AND NUTRITIONAL ASPECTS CONJUGATED OF LINOLEIC ACIDS

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ABSTRACT

Fats and oils are triglycerides containing one unit of glycerol with three units of fatty acids. It also contained mono- and diglycerides, phosphatides, cerebrosides, sterols, terpenes, fatty alcohols, free fatty acids, fat-soluble vitamins, and other substances. Fats and oils are recognized as essential nutrients in both human and animal diets. They provide the most concentrated source of energy and also provide essential fatty acids which are precursors for important hormones. Conjugated linoleic acid (CLA) is a group of geometrical and positional isomers of linoleic acid. In contrast to linoleic acid, double bonds in CLA are usually located at positions 9 and 11 or 10 and 12 and each double bond can be either in the cis or trans configuration. Meat and dairy products from ruminant animals are the principal natural sources of CLA in the human diet. Dietary CLA has been shown to have potent anti-carcinogenic, antiatherogenic, immune modulating and also have other biological activities. The CLA was also reported to reduce body fat content.

Key words: anti-atherogenic, anticarcinogenic, conjugated linoleic acid, Fats and oils, immune modulator

INTRODUCTION

Fats and oils are chemically "triglycerides" resulting from the combination of one unit of glycerol with three units of fatty acids. Triglycerides are the predominant component of most food fats and oils. The minor components include mono- and diglycerides, free fatty acids, phosphatides, sterols, fatty alcohols, fat-soluble vitamins, and other substances. Triglycerides are present in the form of esters of glycerol. The predominant fatty acids are saturated, unsaturated and polyunsaturated carbon chains with an even number of carbon atoms and a single carboxyl group. Edible oils also contain minor amounts of branched chain and cyclic acids. Also odd number straight chain acids are typically found in animal fats (Asif, 2011; Gardner and Kraemer, 1995). Fatty acids occurring in edible fats and oils are classified according to their degree of saturation and unsaturation of fatty acids. The melting point of saturated fatty acids increases with chain length, longer chain fatty acids are solids at normal room temperatures. Unsaturated Fatty Acids are conjugated and non-conjugated; with the bonds in a conjugated position, there is a further increase in certain types of chemical reactivity (Aydin, 2005; Aydin *et al.*, 2001). For example, fats are much more subject to oxidation and polymerization when bonds are in the conjugated position.

Nutritional and Physiological Aspects of Fats and Oils: Fats are a principal and essential constituent of the human diet along with carbohydrates and proteins. Fats are a major source of energy which supplies about 9 calories per gram. Proteins and carbohydrates each supply about 4 calories per gram. In calorie deficient situations, fats together with carbohydrates spare protein and improve growth rates. Some fatty foods are sources of fat-soluble vitamins, and the ingestion of fat improves the absorption of these vitamins (Asif, 2011; Mattson and Grundy, 1985; Riel, 1963). Fats are vital to a palatable and well rounded diet and provide the essential fatty acids. Polyunsaturated Fatty Acids like linoleic, linolenic, arachidonic, eicosapentaenoic, and docosahexaenoic acids containing respectively two, three, four, five, and six double bonds are of most interest. "Essential Fatty Acids" are the principal sources of linoleic and linolenic acids. Arachidonic acid is found in small amounts in lard, which also contains about 10% of linoleic acid.

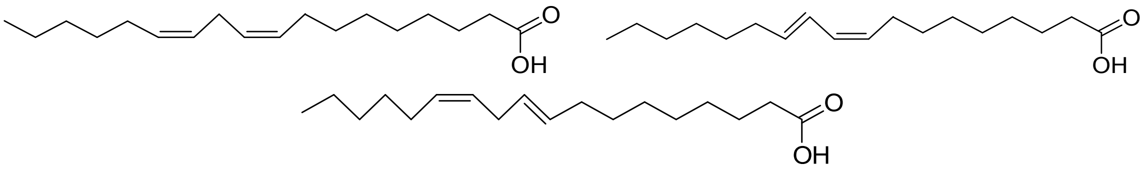

Figure 1. Structures of linoleic acid (top), Conjugated linoleic acid (middle) and linoleic acid isomer (bottom).

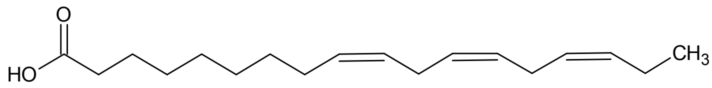

Figure 2. Structures of Alpha-linolenic acid (ALA).

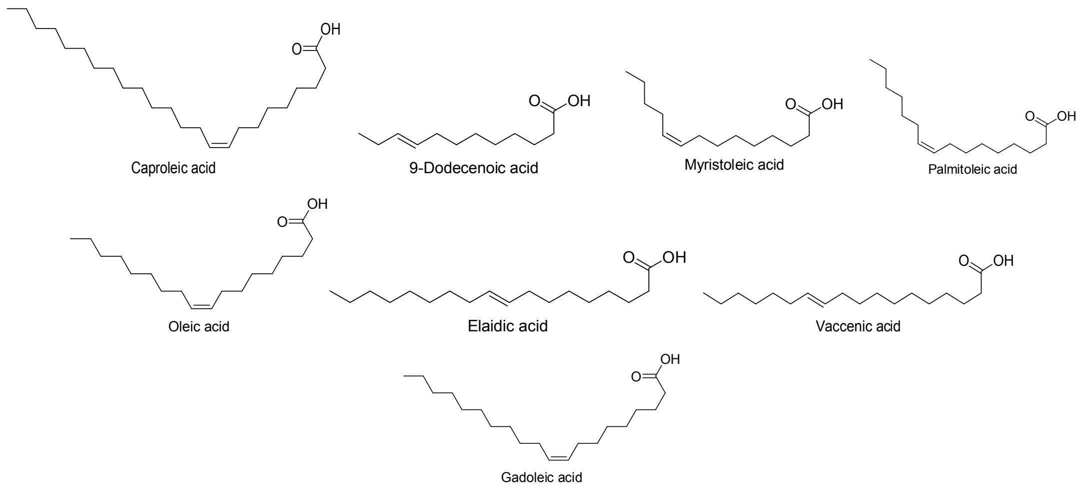

Figure 3. Structures of various mono unsaturated fatty acids.

Fish oils contain large quantities of a variety of longer chain fatty acids having three or more double bonds including eicosapentaenoic and docosahexaenoic acids (Chamruspollert and Sell, 1999; Mir *et al.*, 1999; Adlof *et al.,* 2000; Allison *et al.*, 1999).

Certain long chain PUFAs, linoleic and arachidonic acid (AA), are essential for growth and good skin and hair quality. Now linoleic and linolenic acids (LA) are termed "essential" because they cannot be synthesized by the body and must be supplied in the diet. However, AA can be synthesized by the body from dietary linoleic acid. AA is considered an essential fatty acid (EFAs) because it is an essential component of membranes and a precursor of a group of hormone like compounds called eicosanoids including prostaglandins, thromboxanes, and prostacyclins which are important in the regulation of widely diverse physiological processes. LA is also a precursor of a special group of prostaglandins. The dietary LA necessary to prevent EFAs deficiency in several animals and also in humans is 1-2% of dietary calories. In the case of LA, the requirement for humans has been estimated to be 0.5% of calories. The average intake of PUFAs (primarily linoleic acid) remain at the current level of about 7% of calories and that individual intakes not exceed 10% of calories (Chouinard *et al.*, 1999; Mensink and Katan, 1990; Miller *et al.*, 1994).

Fat level in the diet and its metabolism

Fats in the diet are often referred to as "visible" or "invisible." Visible fats are those added to the diet in foods, where as invisible fats are those that are naturally occurring in foods such as meats and dairy products.

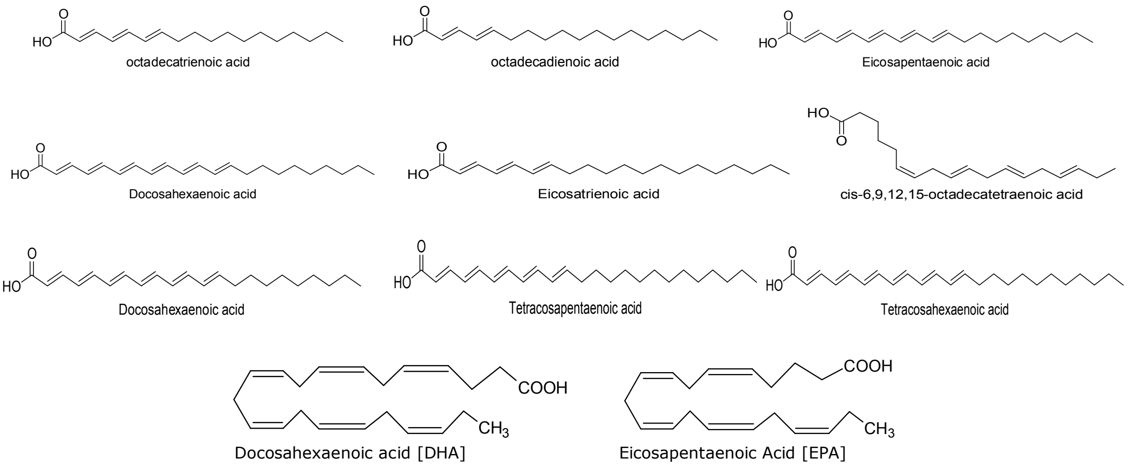

Figure 4. Structures of various polyunsaturated fatty acids.

Table I. Major saturated and saturated fatty acids

Saturated fatty acids	Molecular formula	Unsaturated fatty acids	Molecular formula
Palmitic acid	$CH_3(CH_2)_{14}COOH$	Oleic acid	$CH_3(CH_2)_7CH{=}CH\ (CH_2)_7COOH$
Stearic acid	$CH_3(CH_2)_{16}COOH$	Linoleic acid	$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH\text{-}(CH_2)_7COOH$
Arachidic acid	$CH_3(CH_2)_{18}COOH$	Linolenic acid	$CH_3CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$
		Arachidonic acid	$CH_3(CH_2)_3(CH_2CH{=}CH)_4(CH_2)_3COOH$

Table II. List of several different names for the most common *n*−3 fatty acids.

Common name	Lipid name	Chemical name
Alfa Linolenic Acid (ALA)	18:3 (*n*−3)	*all-cis*-9,12,15-octadecatrienoic acid
Eicosapentaenoic acid (EPA)	20:5 (*n*−3)	*all-cis*-5,8,11,14,17-eicosapentaenoic acid
ocosahexaenoic acid (DHA)	22:6 (*n*−3)	*all-cis*-4,7,10,13,16,19-docosahexaenoic acid

The percentage of total calories consumed, fat intake from visible and invisible sources would appear to have decreased from about 43% to about 33% of calories. It has been estimated that wastage of deep frying fats used in the food service sector may be as high as 50%; therefore, food compo-nent estimates from food "disappearance" data, particularly fat, may be overestimated. The guidelines for fat call for a total fat intake of no more than 30% of calories with a saturated fatty acid intake of no more than 10% of calories. Although, the relatively small changes in fat intake reflect the difficulty on a national scale in achieving dietary goals such as reducing fat intake. In the intestinal tract, dietary triglycerides are hydrolyzed to 2-monoglycerides and free fatty acids (FAs). There the FAs and the monoglycerides are absorbed into the cell and the bile acid is retained in the intestines. Most dietary fats are 95-100% absorbed. In the intestinal wall, the monoglycerides and free FAs are recombined to form triglycerides. These triglycerides, whether coming from the diet or from endogenous sources, are transported in the blood as lipoproteins. The triglycerides are stored in the adipose tissue. Fat is mobilized from adipose tissue into the blood as free FAs. These form a complex with blood proteins and are distributed through out the organism.

The oxidation of free FAs is a major source of energy for the body (Chin *et al*., 1994; Cook *et al*., 1998; Dugan *et al*., 1997; Eulitz *et al*., 1999; Franklin *et al.,* 1999). The three major categories of dietary fatty acids are as; *Saturated fatty acids:* Specific saturated fatty acids palmitic (principal saturated fatty acid in diet), myristic and lauric acids, whereas stearic acid and medium-chain saturated fatty acids (6 to 10 carbon atoms). *Monounsaturated fatty acids:* Monounsaturated fatty acid oleic acid (e.g., olive, canola) and Polyunsaturatedfatty acids: Polyunsaturated fatty acid linoleic and linolenic acids (e.g., sunflower, corn, soybean) fatty acids.

Physio-chemical characteristics of fats and oils

The physiochemical characteristics of a fat or oil are dependent upon the degree of unsaturation, length of the carbon chains, isomeric forms, molecular configuration and the type and extent of processing (Baumgard *et al*., 2001; Gavino *et al*., 2000; Kelly *et al*., 1998; Park *et al*., 1999; Parodi, 1977).

Degree of Unsaturation: Fats and oils are made up of triglyceride molecules which may contain both saturated and unsaturated fatty acids. Depending on the type of fatty acids combined in the molecule, triglycerides can be classified as mono-, di-, and triunsaturated. Generally, fats that are liquid at room temperature tend to be more unsaturated than those that appear to be solid. It is not necessarily true, however, that all fats which are liquid at room temperature are high in unsaturated fatty acids.

Length of Carbon Chains in Fatty Acids: The chain length of the saturated fatty acid increases, the melting point also increases. Thus, a short chain saturated fatty acid such as butyric acid has a lower melting point than saturated fatty acids with longer chains. Some of the higher molecular weight unsaturated fatty acids, such as oleic acid also have relatively low melting points. The melting properties of triglycerides are related to those of their fatty acids. This explains why coconut oil, which contains almost 90% saturated fatty acids but with a high proportion of relatively short chain low melting fatty acids, is a clear liquid at 80°F in contrast to lard which contains only about 37% saturates, most with longer chains, is semi-solid at 80ºF.

Isomeric forms of fatty acids

For a given fatty acid chain length, saturated fatty acids will have higher melting points than those that are unsaturated. The melting points of unsaturated fatty acids are profoundly affected by position and conformation of the double bonds. For example, the monounsaturated fatty acid oleic acid and its geometric isomer elaidic acid have different melting points. Oleic acid is liquid at temperatures considerably below room temperature, whereas elaidic acid is solid even at temperatures above room temperature. The presence of isomeric fatty acids contributes substantially to the semi-solid form of products. Thus, the presence of different geometric isomers of fatty acids influences the physical characteristics of the fat.

Molecular configuration of triglycerides

The molecular configuration of triglycerides can also affect the properties of a fat or oil. Melting points of fats will vary in their sharpness depending on the number of different chemical entities which are present. A simple triglyceride will have a sharp melting point. A mixture of triglycerides, as is typical of lard and most vegetable shortenings, will have a broad melting range. A mixture of several triglycerides has a lower melting point than would be predicted for the mixture based on the melting points of the individual components. The mixture will also have a broader melting range than any of its components. Monoglycerides and diglycerides have higher melting points than triglycerides with a similar fatty acid composition.

Polymorphism of fats

Solidified fats exhibit polymorphism, i.e., they can exist in several different crystalline forms, depending on the manner in which the molecules orient themselves in the solid state. The crystal forms of fats can transform from lower melting to successively higher melting modifications. The rate of transformation and the extent to which it proceeds are governed by the molecular composition and configuration of the fat, crystallization conditions, and the temperature and duration of storage. In general, fats containing diverse assortments of molecules tend to remain indefinitely in lower melting crystal forms, whereas fats containing a relatively limited assortment of molecules (such as soybean stearine) transform readily to higher melting crystal forms.

Mechanical and thermal agitation during processing and storage at elevated temperatures tend to accelerate the rate of crystal transformation. The crystal form of the fat has a marked effect on the melting point and the performance of the fat in the various applications in which it is utilized. Food technologists apply controlled polymorphic crystal formation in the preparation of household shortenings and margarines. In order to obtain desired product plasticity, functionality, and stability, the shortening or margarine must be in a crystalline form called "beta-prime" (a lower melting polymorph). Partially hydrogenated oil tends to crystallize in the "beta" crystal form (a higher melting polymorph). Beta-prime crystal formation is promoted in a oil product through inclusion of beta-prime promoting fats such as hydrogenated oils. Beta-prime is a smooth, small, fine crystal whereas beta is a large, coarse, grainy crystal. Shortenings and margarines are smooth and creamy because of the inclusion of beta-prime fats (Aro *et al.*, 1997; Fritsche *et al.*, 1999; Ip and Scimeca, 1997; Judd *et al.*, 1994; Judd *et al.*, 1998).

Oxidation of fats and oils autoxidation

The process of oxidation induced by air at room temperature referred to as "autoxidation." Ordinarily, it is a slow process which occurs only to a limited degree. In autoxidation, oxygen reacts with unsaturated fatty acids. Initially, peroxides are formed which in turn break down to hydrocarbons, ketones, aldehydes, and smaller amounts of epoxides and alcohols. Heavy metals present such as copper at low levels in fats and oils can promote autoxidation. Fats and oils often are treated with chelating agents such as citric acid to inactivate heavy metals. The autoxidation of fats and oils is the development of objectionable flavors and odors, the condition known as "oxidative rancidity." Some fats resist this change to a remarkable extent while others are more susceptible depending on the degree of unsaturation, the presence of antioxidants, and other factors. The presence of light, heat, moisture, and aerobic bacteria's, increases the rate of oxidation. It is a common practice to protect fats and oils from oxidation to preserve their acceptable flavor and shelf life. The peroxide value determination, if used judiciously, may be helpful in measuring the degree of oxidative rancidity in the fat (Kramer *et al.*, 1998; Mata *et al.*, 1992; Pariza *et al.*, 2000; Park *et al.*, 1997).

Oxidation at higher temperatures

The rate of oxidation is greatly accelerated at higher temperatures, may not follow precisely the same routes and mechanisms as the reactions at room temperatures. Thus, differences in the stability of fats and oils often become more apparent when the fats are used for frying or slow baking. The more unsaturated the fat or oil, the greater will be its susceptibility to oxidative rancidity.

Predominantly unsaturated oils such as soybean, cottonseed, or corn oil are less stable than predominantly saturated oils such as coconut oil. Methyl silicone often is added to institutional frying fats and oils to reduce oxidation tendency and foaming at elevated temperatures. Frequently, partial hydrogenation is employed in the processing of liquid vegetable oil to increase the stability of the oil. Also oxidative stability has been increased in many of the oils developed through biotechnological engineering. The stability of a fat or oil may be predicted to some degree by the oxidative stability index (Ip *et al.*, 1995; Blankson *et al.*, 2000; Ha *et al.*, 1987).

Conjugated linoleic acid

Conjugated linoleic acid (CLA) is a group of geometrical and positional isomers of linoleic acid (C18:2, cis-9, cis-12). In contrast to linoleic acid, double bonds in CLA are usually located at positions 9 and 11 or 10 and 12 and each double bond can be either in the cis or trans configuration. Meat and dairy products from ruminant animals (milk, butter, cheese, etc) are the principal natural sources of CLA in the human diet. Egg and meat products from poultry contain less CLA than ruminant products. Dietary CLA has been shown to have potent anti-carcinogenic, antiatherogenic and also have a potent immune modulating activity characterized by increased blastogenesis and macrophage killing ability in animals. In addition to these biological properties, CLA was reported to reduce body fat content and increase lean body mass in rodents (Belury *et al*., 1996; Ip *et al*., 1999; Kepler *et al*., 1966).

Conjugated linoleic acid-An Area of Current Interest: A group of isomers of the essential fatty acid linoleic acid, collectively termed "conjugated linoleic acid" (CLA), has received considerable attention in recent years because these isomers appear to have both anticarcinogenic and antiatherogenic properties and may affect body composition. CLA differs from linoleic acid by the position and geometric configuration of one of its double bonds. CLA isomers are found primarily in lipids originating from ruminant animals (beef, dairy, and lamb) and are reported to range from about 3 to 11mg/g fat (Kris-Etherton and Nicolosi. 1995; Park *et al*., 1999). Fats from non-ruminants (pork and chicken) and vegetable oils contain lower amounts of CLA ranging from 0.6 to 0.9mg/g fat. Various animal studies have indicated that CLA reduces the incidence of tumors induced by carcinogens such as dimethylbenz[a]anthracene and benzo[a]pyrene. CLA appears to be a unique anticarcinogen because it is a naturally occurring substance found primarily in food products derived from animal sources. Most other naturally occurring substances that have been demonstrated to have anticarcinogenic activity are of plant origin.

CLA is also unique because it is a fatty acid mixture and anticancer efficacy is expressed at concentrations close to human consumption levels. Animal data indicated that approximate 3.0g/d of CLA may be beneficial for humans (Asif, 2011; Lee *et al*., 1998; Liew *et al.,* 1995; Munday *et al*., 1999). Inhibition of tumor development in animals has been seen with CLA at concentrations as low as 0.1% in the diet. In addition to its anticarcinogenic properties, CLA appears to be antiatherogenic as well (Ip *et al*., 1996; Chin *et al*., 1992; Nicolosi *et al*., 1997). The studies involving rabbits or hamsters indicated that incorporation of CLA into the diets suppressed total and LDL-cholesterol and also atherosclerosis. Furthermore, dietary CLA is able to affect body composition (Ntambi *et al*., 1999; Pariza and Hargraves, 1985; Pariza, 1997; Shultz *et al*., 1992; Thompson *et al*., 1997). Diets supplemented with 0.5% CLA have been found to decrease body fat content and increase lean body mass in several species including poultry, pigs, and rodents. CLA also showed improved feed efficiency (weight gain per unit weight of food consumed) and improved immune systems. Thus, in the future CLA could be a useful additive for animal feeds. Another area of research interest is in the possibility of using dietary intake of certain plant sterols, such as sitosterol, to help reduce the risk of coronary heart disease. Sitosterols were found to be serum cholesterol lowering agents, and their mode of action due to the inhibition of cholesterol absorption during the digestive process.

The studies with humans have examined the serum cholesterol lowering ability of sterol esters incorporated into margarines. Sitostanol esters, a hydrogenated sitosterol obtained from a wood pulp byproduct, fed in margarine to mildly hypercholesterolemic person at levels ranging from 1.9-2.6g sitostanol/day. A mean reduction in plasma serum cholesterol of 10% was observed after one year. Other study used esters of sitosterol that were extracted from soybean oil and incorporated into margarine, and compared their cholesterol lowering effect directly with that of sitostanol esters. This study, using normo-cholesterolemic and mildly hyper-cholesterolemic person, found a reduction of 8-13% of plasma total and LDL cholesterol levels, and both the soybean sterols and the sitostanols were equally effective compared to the control diet (Lee *et al*., 1994; Cesano *et al*., 1998; Ip *et al*., 1994; Knekt *et al*., 1996; Willett *et al*., 1993; Zu and Schut, 1992).

CONCLUSION

The disease risk can be reduced by an overall dietary pattern that includes a high proportion unsaturated fatty acids than saturated fatty acids, limited amounts of meat, dairy products, and other high-fat foods, and a balance of caloric intake and physical activity. They also are rich in antioxidant vitamins, minerals, and phytochemicals that may play a role in reducing diseases risk. Much research continues on the role of dietary fat in relation to health. CLA, recently there are a lot of interest in enriching egg, meat and dairy products for human consumption. It is possible to change the lipid composition of food products easily, such as eggs, milk or meat, by modifying the diet of the animals. Animal data indicated that approximate 3.0g/d of CLA may be beneficial for humans. CLA is a mixture of several isomers and any isomer might have different activities or work synergistically. Currently, the c-9, t-11 CLA and t-10, c-12 CLA are the only isomers that have actually been shown to exert physiological properties. It is not known if the other CLA isomers have biological activities as well.

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