PREPARATION AND EVALUATION OF BLENDED AND INTERESTERIFIED OILS

A thesis submitted to the

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Doctor of Philosophy

in

Biochemistry

by

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DECLARATION

I hereby declare that the thesis entitled "**PREPARATION AND EVALUATION OF BLENDED AND INTERESTERIFIED OILS**" submitted to the University of Mysore for the award of the degree of **DOCTOR OF PHILOSOPHY in BIOCHEMISTRY** is the result of research work carried out by me under the guidance of **Dr. B.R. Lokesh**, head, Department of Lipid Science & Traditional Foods, Central Food Technological research Institute, Mysore during the period 2003-2009.

I further declare that these results have not been submitted for any other degree or fellowship.

Date:

Place: Mysore

(ANITHA NAGARAJU)

Dr. B.R. Lokesh
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Department of Lipid Science & Traditional Foods

CERTIFICATE

This is to certify that the thesis entitled "**PREPARATION AND EVALUATION OF BLENDED AND INTERESTERIFIED OILS**" submitted by Mss. **ANITHA NAGARAJU** to the University of Mysore for the award of the degree of **DOCTOR OF PHILOSOPHY in Biochemistry** is the result of research work carried out by her in the department of Lipid Science & Traditional Foods, CFTRI, Mysore under my guidance during the period of 2003-2009.

Date:
Place: Mysore

(B.R. Lokesh)

(Guide)

Dedicated to…….

My Father & brothers

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ANITHA NAGARAJU

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Abbreviations and units

ω	Omega
(B)	Blend
(I)	Interesterified
°C	Degree Celsius
µg	Microgram
µl	Micro litre
ACAT	Acyl Co A: cholesterol acyl transferase
bw	body weight
Cal	Calories
CAT	Catalase
CHD	Coronary heart disease
CO	Coconut oil
CVD	Coronary Vascular disease
DSC	Differential scanning calorimetry
FER	Food efficiency ratio
FFA	Free fatty acid
g	Gram
GC	Gas chromatography
GNO	Ground nut oil
GPx	Glutathione peroxidase
h	Hours (s)
HDL	High density lipoprotein
ICMR	Indian Council of Medical Research
LA	Linoleic acid
LCAT	lecithin: cholesterol acyltransferase
LCFA	long Chain Fatty acid
LCPUFA	long chain poly unsaturated fatty acid
LDL	Low density lipoprotein
LNA	Linolenic acid
MAG	Monoacylglycerol
MCT	Medium chain triglyceride
MDA	Malondialdehyde
mg	Milligram

mins	Minutes
ml	Milliliter
MUFA	Monounsaturated fatty acid
ND	Not detected
ng	Nanogram
nm	Nanometer
OD	Optical density
OLO	Olive oil
P/S	Polyunsaturated fatty acid/Saturated fatty acid
PBS	Phosphate buffer saline
PUFA	Polyunsaturated fatty acid
PV	Peroxide value
SD	Standard deviation
SFA	Saturated fatty acid
SFC	Solid fat content
SL	Structured lipid
SOD	Superoxide dismutase
TBA	2-Thiobarbituric acid
TBARS	Thiobarbituric acid reactive substances
TCA	Trichloroacetic acid
TG	Triglyceride
v/v	Volume/volume
VLDL	Very low density lipoprotein
w/v	Weight/Volume
α	Alpha
β	Beta
γ	Gamma
δ	Delta

Thesis title: Preparation and Evaluation of Blended and Interesterified Oils.

Vegetable oils are part of traditional diets all over the world. Triglycerides make up the majority of the lipids in edible fats/oils. The fatty acid composition of the edible oils has been one of the main focuses in defining nutritional value of dietary fats. Nutritional properties of dietary lipids are largely influenced by the nature of their constituent fatty acids, degree of unsaturation, distribution of fatty acid on triglyceride back bone and the composition of non-glyceride fraction of oil.

Certain saturated fatty acids play an important role in increasing plasma cholesterol concentrations and determining the risk of coronary heart disease. In contrast, mono unsaturated fatty acids and polyunsaturated fatty acids have been shown to decrease the plasma cholesterol concentration in various clinical studies and various animal models.
The majority of the reported adverse effects of fats and oils on health are related to their excessive consumption. The growing awareness on the impact of dietary saturated fats on cardiac problems is resulting in the changes in the types of the oils used in our diet. All the negative effects attributed to fats is slowly leading to great concerns and possible elimination of fat from diet. However, oils and fats are essential part of balanced diet, as they provide numerous components needed for maintaining our health.

According to the Indian council of medical research, the desirable proportions of saturated, mono and polyunsaturated fatty acids in the dietary fats should be in the proportion of about 1:1:1. According to the American heart association, the optimum intake of fat for an adult is 30% of total calorie and the ratio of saturated/monounsaturated/polyunsaturated fatty acid 1:1:1, which was also endorsed by studies in India. Naturally available oils are unique in its composition. Careful analysis of edible oils available in India indicates an imbalance in their composition and is not in tune with recommendations made by nutritionists. Some of the commonly used edible oils in India are Coconut oil, Ground nut oil, Sunflower oil, Mustard oil.

Coconut oil is characterized by high levels of medium chain fatty acids, mainly lauric acid and small amounts of capric, caprylic and caproic acid. It is deficient in essential fatty acids which needs to be supplemented. This can be achieved by blending Coconut oil with oil like ground nut oil containing essential fatty acids. Olive oil which is consumed in

mediterranian regions is endowed with many health benefits due to the presence of phenolics. However olive oil is not in the main stream of edible oils in India due to its limited availability and high cost. Blending of olive oil with coconut oil may be a way of introducing it in Indian market at affordable cost and providing health benefits of olive oil in Indian population. However blending of two oils may not provide all the required benefits. They may need to be further modified by way of rearranging fatty acids in the triacylglycerol molecules of blended oil. Hence modification of oils and fats is one of the requirements in food processing industry that demands novel economic and green technologies. In this respect, tailor made vegetable oils with nutritionally beneficial structural triacylglycerols and altered physicochemical properties have a potential in the future market. Structured lipids are tailor made fats and oils with improved nutritional or physical properties. Recent Investigators have demonstrated that structured lipids have a desirable metabolic actions superior to the physically modified oils with identical composition but not subjected to interesterification reactions.

In majority of the experiments reported in the literature, the oils containing medium chain triglycerides are interesterified with polyunsaturated fatty acid containing oils. Structured lipids of this type have been reported to have beneficial effects on a range of metabolic parameters including immune function, nitrogen balance and improved lipid clearance from the blood stream. This interesting observation need to be exploited to develop oils which can provide superior health benefits.

To provide balanced edible oils to Indian population, the present study aims at the preparation and evaluation of coconut oil which is blended and interesterified with unsaturated oils like ground nut oil or olive oil.

The thesis is organized in the following manner.

Introduction

In this section a brief review of existing literature on dietary fats, modification of fats by enzymatic interesterification reactions, Preparation of Structured lipids, and metabolic effect of structured lipids is presented. This also highlights the aim and scope of the present investigation.

Materials and Methods

The source of chemical used and experimental methods employed in the present investigation is detailed.

Chapter 1

Preparation of Blend and Interesterified oils and Effect of modified oils on physicochemical properties

Coconut oil (CO), Ground nut oil (GNO) and Virgin olive oil (OLO) were purchased from local market. The quality of these oils were monitored by determining the peroxide value, free fatty acid content and minor constituents. The fatty acid composition of the individual oils were determined by GC. CO was blended with GNO at appropriate levels to get equal proportions of saturated:monounsaturated:polyunsaturated fatty acid. CO and OLO combination was also prepared to get equal proportions of saturated to total unsaturated fatty acid. Blended oils were subjected to interesterification using immobilized lipase enzyme *Rhizomucor meihie* at predetermined temperature with varying concentrations of enzyme for the different time period. Triglyceride profile of the blend and interesterified oil was analysed using reverse phase HPLC. The changes in the triglyceride molecular species was monitored by HPLC Refractive index detector (RID). Fatty acid compositon of newly formed triglyceride species in interesterified oils were determined by GC. Change in the solid fat content of the native, blend and interesterified oils were evaluated by Differential Scanning Calorimetry (DSC). Changes in the thermal behaviour and crystallization behavior of Native, Blend and Interesterified oils were determined.

The results indicated that blending of two selected oils at appropriate levels can provide an oil with desired ratios of saturated:monounsaturated:polyunsaturated fatty acids or desired ratios of saturated: total unsaturated fatty acids. Further interesterification of blended oils with lipase under optimized conditions did not had any adverse effect on the quality and the content of minor constituents of the oils. Fatty acid composition of the interesterified oils were similar to that found in blended oils. Interesterification of oils however resulted in the redistribution of the fatty acids among the triglycerides resulting in few changes in the molecular species of triglycerides in the oil. These changes in triglyceride molecular species altered properties of oils measured in terms of solid fat content, enthalpy and crystallization behaviour of triglycerides measured by DSC.

Chapter 2

The Effect of Blend and Interesterified oils on serum and Tissue lipids

Feeding male Wistar rats were fed with modified oils at 10% level. The diet was prepared according to the AIN-76 diet formula. The rats were fed for 60 days with diet containing native, blend and interesterified oils. The animals had free access to food and water throughout the study.

After feeding for 60 days, rats were fasted overnight and sacrificed under ether anesthesia. The blood was drawn by cardiac puncture and serum was isolated by centrifugation. Liver was also collected, washed, blotted and used for analysis of lipids. The lipid was extracted from the serum and Total cholesterol, phospholipids and triglyceride levels were estimated by methods described in chapter II. The fatty acid methyl esters of the serum and liver lipids were prepared. The fatty acid composition of the samples was determined using Gas Liquid Chromatography.

A Significant reduction in serum cholesterol and TG were observed in rats fed a diet containing CO:GNO and CO: OLO blends as compared to those given CO containing diets. The hypocholesterolemic effects of blended oils were further enhanced when subjected to interesterification reaction and fed to rats. Feeding rats with CO blends containing GNO or OLO had marginal effect on liver cholesterol and triglyceride levels compared to rats given CO containing diets. However, when these blended oils were subjected to interesterification reactions and fed to rats a significant reduction in hepatic cholesterol levels were observed compared to rats given blends of CO with GNO or OLO combinations. There were no differences in the liver triglyceride levels in rats fed blended and interesterified oils in liver.

These studies indicated that it is possible to reduce the atherogenic potentials of a saturated fat like coconut oil by blending with an unsaturated fat such as ground nut oil or olive oil to balance the proportions of saturated to unsaturated fatty acids. Their hypolipidemic effects can be further enhanced by subjecting these blended oils to enzyme catalysed transesterification reaction.

Chapter 3

Effect of Blend and Interesterified oils on Antioxidant enzymes and LDL oxidation

The intake of particular type of dietary fat affects the antioxidant profiles of the body and susceptibility to undergo oxidation. Hence, effect of feeding modified fats on the lipid peroxidation and antioxidant enzymes were studied.

Feeding rats with a diet contained Blend and interesterified oils of CO:GNO and CO:OLO enhanced the hepatic antioxidant enzyme activities such as Superoxide dismutase, Catalase and Glutathione peroxidase as compared to the rats given CO alone. The lipid peroxidation in rats given CO:GNO and CO:OLO were found to be lower than that observed in rats given GNO alone. The susceptibility of LDL to oxidation in rats given diets containing CO:GNO and CO:OLO was found to be lower than that found in control animals.

These studies indicated that though the blend and interesterified oils had higher levels of unsaturated fatty acids compared to CO and thereby increased lipid peroxidation, a complimentary mechanism was observed in the form of elevated levels of antioxidant enzymes in the liver.

Chapter 4

Platelet Aggregation and Erythrocyte Membrane bound enzymes in rats fed Blend and interesterified oils

Changes in the fatty acid composition of plasma and membrane lipids can modulate the platelet function. Hence the effect of modified fats on fatty acid composition on platelet aggregation were studied. After aggregation of platelets, malondialdehyde released was analysed. The effect of feeding modified oils on membrane bound enzymes like Na^{+} K^{+} ATPase and Ca^{+2} Mg^{+2} ATPase in erythrocyte ghost cells were also determined.

It was noticed that Platelet aggregation was decreased in response to ADP and Collagen in rats fed diet containing CO:GNO and those fed CO:OLO blends compared to the rats fed CO. In addition to extent of aggregation, the rate of platelet aggregation was also decreased in rats given blend and interesterified oils.

These studies suggests that fatty acids plays a significant role in platelet aggregation.

Blend and interesterified oils with higher levels of unsaturated fatty acids lowered the rate of ADP and Collagen induced platelet aggregation. These modified oils also enhanced the activities of erythrocyte membrane bound enzymes like Na^{+} K^{+} ATPase and Ca^{+2} Mg^{+2} ATPase.

General Discussion and Summary

The overall findings with relevance to stated objectives of the investigation is discussed in this chapter. A brief summary of the major findings are also covered at the end of the discussion. In conclusion, the present study showed that fatty acid composition of oils can be modified by blending two selected oils at appropriate levels to get a oil with balanced fatty acid composition. Lipase interesterification process did not had any adverse effect on the quality parameters and the content of minor constituents of these blended oils. However the triglyceride molecular species were altered in interesterified oils. Nutritional evaluation indicated that modified oils beneficially modulated the risk factors for atherosclerosis, antioxidant defense mechanism and platelet aggregation. Hence the blend and interesterified oils with balanced fatty acid composition may help in better utilization of oils in promoting health.

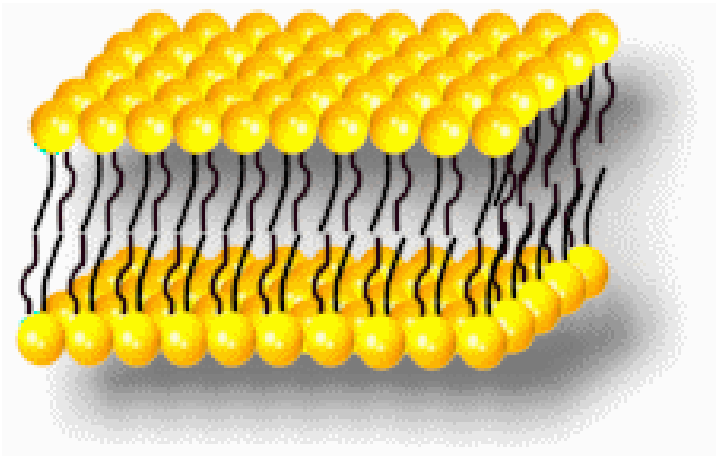
Bibliography

The published papers relevant to current investigation is given in this section.

Anitha Nagaraju
(Student)

B. R. Lokesh
(Guide)

General Introduction



NUTRITION is the science of foods, the nutrients and other substances there in; their action, interaction, and balance in relationship to health and disease; the process by which an individual ingests, absorbs, transports and utilizes nutrients and disposes their end products. In addition, nutrition must be with social, economic, cultural and psychological implications of food and eating (Robinson, 1966). Food has been the primary concerns of humankind in its physical environment through out all recorded history.

NUTRIENTS are the constituents in food that must be supplied to the body in suitable amounts. These include Proteins and the amino acids of which they are composed, fats and fatty acids, carbohydrates, minerals, Vitamins and Water.

NUTRITIONAL CARE is the application of science and art of human nutrition in helping people select and obtain food for the primary purpose of nourishing their bodies in health or in disease, through out the life cycle. This participation may be in single or combined functions: in feeding groups involving food selection and management; in extending the knowledge of food and nutrition principle; in teaching these principles for application according to particular situations; and in dietary counseling (Committee on Goals of life time education of the Dieticians, 1964).

Vegetable oils and fats are part of traditional diets all over the world. The application of fats and waxes, as illuminants, in cosmetics, in medicines and as lubricants, dates back to before our earliest records of civilized man.

Dietary fat is a concentrated source of energy which gives 9 kcals/g where as carbohydrates and proteins give 4 kcals/g each. The higher energy yield from fat is due to higher carbon to hydrogen ratio or in other words they are highly reduced (Widdowson, 1987).

Fats have been recognized as a separate category of food stuffs since prehistoric times. Dietary fat has always generated more interest as well as controversy than any other nutrient. Though Edible oils and fats are essential nutrients, growing public health and fitness awareness and advances in nutrition research have raised a debate on the pros and cons of various dietary fats and oils. Often fats and oils have an unhealthy connation for human growth

and health. The lipids and lipid related compounds play essential roles in disease prevention and growth. In the past, edible oil quality was mainly defined by organoleptic parameters such as taste, odor and color.

The effects of dietary fats generally reflect the collective influences of multiple fatty acids in the diet or food. The perception of which foods, nutrients and supplements are "healthy" is often being amended as new scientific data is presented and simplified for the consumers. What was once perceived as a healthy diet is often no longer considered as such and vice versa.

LIPIDS

No exact definition of lipids actually exists. Christie (Christie, 1982) defines lipids as "a wide variety of natural products including fatty acids and their derivatives, steroids, terpenes, carotenoids and bile acids which have in common ready solubility in organic solvents such as diethyl ether, hexane, benzene, chloroform or methanol".

Kates (1986) defines the lipids as "those substances which are (a) insoluble in water; (b) soluble in organic solvents such as chloroform ether and benzene; (c) contain long chain hydrocarbon groups in their molecules".

Gurr and James (1971) point out that the standard definition for lipids includes "a chemically heterogenous group of substances, having in common the property of insolubility in water, but solubility in non-polar solvents such as chloroform, hydrocarbons or alcohols".

Classification of lipids is based on physical properties at room temperature (Oils are liquid and fats are solid at room temperature). Their polarity (**polar and neutral lipids**), 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. The derived lipids include fatty acids and alcohols, which are the building blocks for the simple and complex lipids. Simple lipids, composed of fatty acids and alcohol components, include acylglycerols,

ether acylglycerols, sterols and their esters and wax esters. Complex lipids include glycerophospholipids (phospholipids), glyceroglycolipids (glycolipids), and sphingolipids.
Lipids and Lipid related compounds play essential roles in disease prevention and growth.

1. Fat provides calorie density to the diet.

2. Fats are essential in the diets for the absorption and the mobilization of fat soluble vitamins such as Vitamin A, Vitamin E and fat soluble antioxidants. Thus fat works as a vehicle to carry the fat-soluble vitamins, nutrients and antioxidants in the body.

3. Vegetable oils are the only sources of essential fatty acids (EFA) to the body, which the body can not synthesise and need to be supplied through the diet. Essential fatty acids are the precursors for a group of chemically related compounds, known as prostaglandins that are synthesized in the body from EFA.

4. Fat in the diet imparts some textural qualities, taste and palatability to the food. Fats and oils are integral lubricants of foods in two ways; through use as release agents as a part of the cooking process and as a lubricant during the mastication (chewing).

LIPID CLASSES

Fatty Acids

1. Saturated fatty acids

The saturated fatty acids contain no double bonds in the carbon chain of these fatty acids. Saturated fatty acids (SFA) mainly comprise of butyric (C4:0), caproic (C6:0), capryilic (C8:0), capric (C10:0) termed as short chain fatty acids (SCFA), lauric (C12:0) as medium chain fatty acids (MCFA) and myristic (C14:0), palmitic (C16:0) and stearic (C18:0) acids are the major long chain fatty acids (LCFA). Other minor LCFA of dietary origin are arachidic (C20:0), behnic (C22:0), lignoceric (C24:0), cerotic (C26:0) and montanic acids (C28:0).

2. Unsaturated fatty acids

The fatty acids with one or more double bonds are generally classified as unsaturated fatty acids. These may be further subdivided according to the degree of unsaturation.

a. Monounsaturated fatty acids

The most common monounsaturated fatty acid is oleic acid (18:1), although more than 100 monounsaturated fatty acids have been identified in nature. The most common double bond position in monoenes is Δ^9. Certain families of plants have been shown to accumulate unusual fatty acids. For example, *Eranthis* seed oil contains Δ^5 monoenes and non-methylene interrupted polyunsaturated fatty acids containing Δ^5 bonds (Aizetmuller, 1996). Erucic acid (22:1) is found at high levels (40-50%) in Cruciferae such as rapeseed and mustard seed. Canola and rapeseed oil that is low in erucic acid (< 3% 22:1) have been developed.

b. Polyunsaturated fatty acids

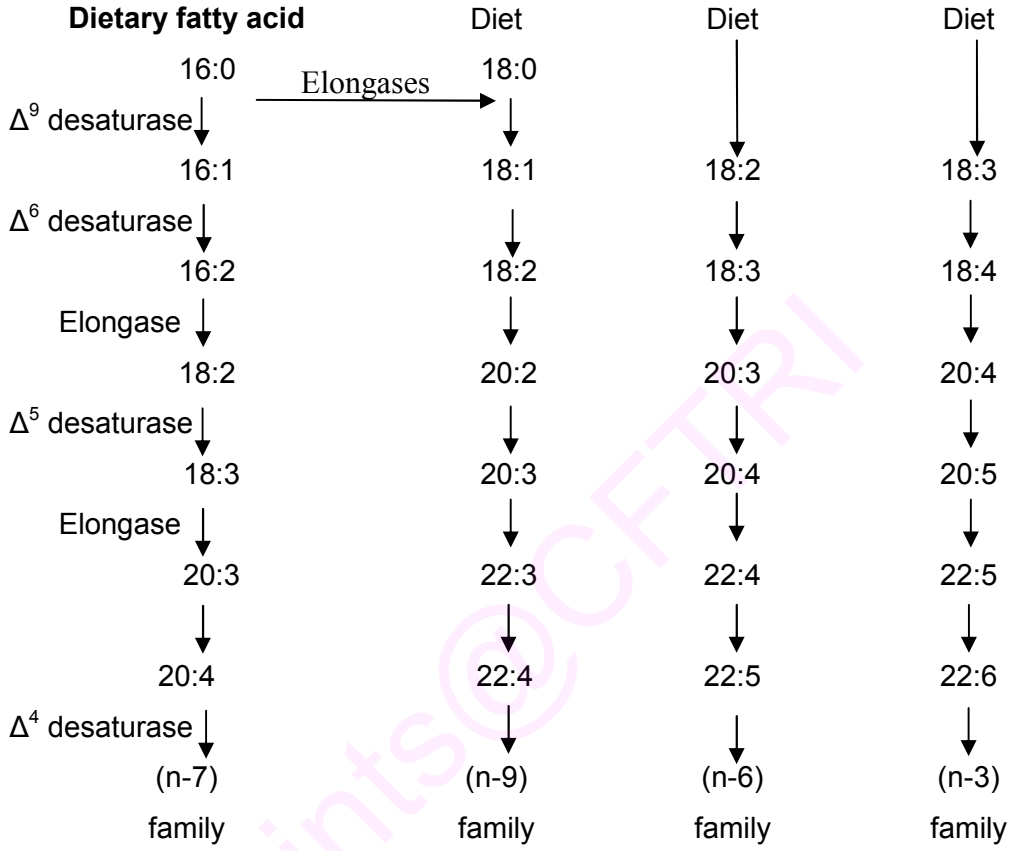
The unsaturated fatty acids with more than one double bond are classified as polyunsaturated fatty acids (PUFA). PUFA can be subdivided in to two groups referred as n-6 PUFA and n-3 PUFA. SFA and MUFA can be synthesized in the body and thus they are not dietary essentials. The parent acids of the two PUFA groups are linoleic (LA, n-6) and alpha-linolenic (ALNA, n-3) acid. These can not be synthesized in the human body but have structural and functional roles in all cells. They are therefore dietary essential fatty acids.

Not all PUFAs are essential fatty acids. Plants are able to synthesise de novo and interconvert n-3 and n-6 via desaturases with specificity in the Δ^{12} and Δ^{15} positions. Animals have Δ^5, Δ^6 and Δ^9 desaturase enzymes and are unable to synthesise the n-3 and n-6 PUFA de novo. They are found in abundance in the chloroplast membranes of plants, in certain vegetable oils and in the tissues of plant eating animals (Nettleton, 1991). The most common of the n-6 fatty acids in our diets is 18:2 n-6 and often considered the parent of n-6 family. The essentiality of n-6 fatty acids has been known since the late 1920s. Symptoms of essential fatty acid deficiency include fatigue, skin problems, weakened immune functions, gastrointestinal disorders, heart and circulatory problems, growth retardation, and sterility (Belzung et al., 1998).

Linoleic acid is a member of the family of n-6 fatty acids and alpha-linolenic acids is an n-3 fatty acid. Other n-6 fatty acids can be manufactured in the body using linoleic acid as the substrate. These include gamma-linolenic acid (GLA), dihomo-gamma-linolenic acid (DHGLA), and arachidonic acid (AA). Similarly, other omega-3 fatty acids manufactured in the body using alpha-linolenic acid as a starting point include eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These essential fatty acids can be metabolized in to longer, more unsaturated products (Holman, 1968). This process involves sequential desaturation (adding double bonds) and chain elongation (adding carbon atoms).

The main metabolite of the n-6 series is arachidonic acid (20:4 n-6, AA), whereas eicosapentaenoic acid (20:5 n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA) are the main metabolites of the n-3 series (Holman, 1968). Essential fatty acids are involved in energy production, the transfer of oxygen from the air to the blood stream, and the manufacture of hemoglobin. They are also involved in growth, cell division, and nerve function. EFA are found in high concentration in the brain and are essential for normal nerve impulse transmission and brain function (Salvati et al., 1993). Among the significant components of cell membranes are the phospholipids, which contain fatty acids. The types of fatty acids in the diet determine the type of fatty acids that are available for cell membranes. A phospholipid made from saturated fat has a different structure and is less fluid than one that incorporates an essential fatty acid.

Fig. 1. Biosynthesis of fatty acids



Source : Jenkins and Atwal (1995).

3. fatty acids: These are unsaturated fatty acid that contains double bond geometry in the *trans* configuration. Nomenclature differs only from normal *cis* fatty acids in the configuration of the double bonds. The main origins of *trans* fatty acids in our diet are deodorized oils and partially hydrogenated oils. *Trans* fatty acids are also formed by some bacteria primarily under anaerobic conditions. It is believed that the formation of *trans* fatty acids in bacterial cell membranes is an adaptation response to decrease membrane fluidity, perhaps as a reaction to elevated temperature or stress from solvents or other lipophilic compounds that affect membrane fluidity.

Foods contain fatty acids mainly as a component of, triglycerides, phospholipids, glycolipids and cholesteryl esters.

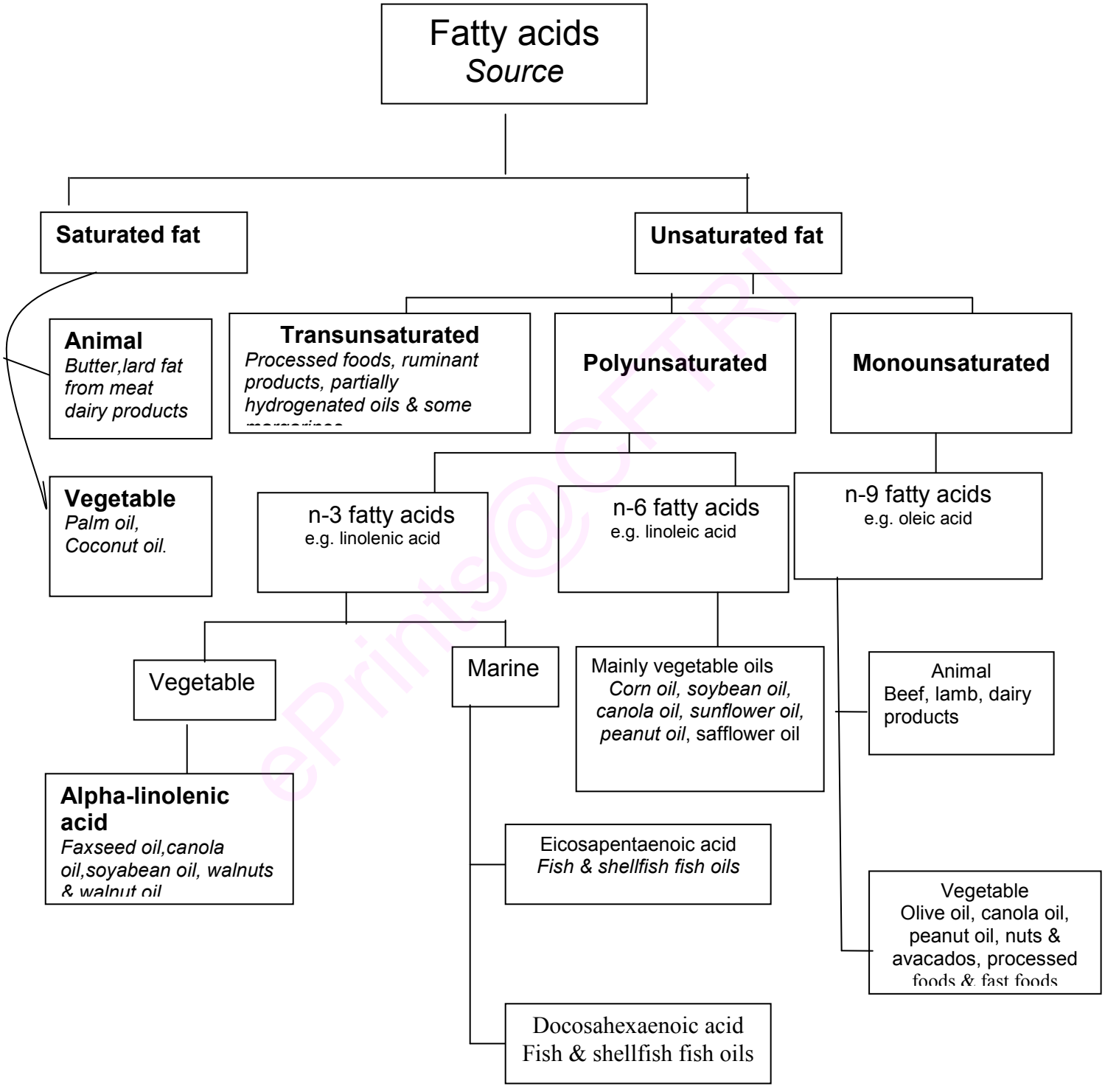
Dietary fatty acids modulate risk factors for atherosclerosis, thrombosis and syndrome X through several independent mechanisms.

Fatty acids have four major functions.

1. Fatty acids are building blocks for phospholipids and glycolipids.
2. Many proteins are modified by the covalent attachment of the fatty acids, which target them to membrane locations.
3. Fatty acids are fuel molecules. They are stored as triglycerides, which are uncharged esters of fatty acids with glycerol. Fatty acids mobilized from triglycerides are oxidized to meet the needs of a cell or organism.
4. Fatty acid derivatives serve as hormones and intracellular messengers.

Although SFA as a group raise total and LDL cholesterol (atherogenic), the individual SFA have different effects. Palmitic acid is less hypercholesterolemic than myristic acid. Oleic acid lowers plasma cholesterol levels almost as effectively as linoleic acid, but it does not reduce HDL cholesterol as is usually observed with high dietary levels of linoleic acid. *Trans* fatty acids which are formed during hydrogenation of vegetable oils increased LDL cholesterol and reduce HDL cholesterol and therefore have even greater atherogenic effects than SFA.

Fig 2. Fatty acid categories.



Source: Elaine et al., 2006

DIGESTION, ABSORPTION AND TRANSPORT OF LIPIDS

The majority of the digestion and absorption of dietary TG occur when the food has reached the small intestine where fats are exposed to bile and pancreatic juice. As most TG can not be absorbed by the intestinal epithelial cells, transport of TG requires further metabolism in the gut. The pancreatic lipase preferentially hydrolyses fatty acid in the sn-1 and sn-3 position of the TG leaving 2-monoacylglycerol (2-MAG) that, due to its polar (glycerol) and nonpolar (FA) moieties, functions as an emulsifying agent (Entressangles et al., 1961). The lipolysis product including FFA, MAG and DAG are solubilised together with phospholipids and cholesterol by lysophospholipids and by bile salts into micelles and thus absorbed (Small, 1991 and Bracco, 1994). Because the rate of hydrolysis at the sn-2 position of the glyceride is very slow, the FA in the sn-2 position remain intact as 2-MAG during digestion and absorption. MAG readily form mixed micelles and are subsequently absorbed, but free FA have variable incorporation into mixed micelles (Wilson et al., 1971).

Digestion and absorption of long chain saturated fatty acid occurs less readily than for shorter chain or unsaturated FA. Unsaturated FA and 2-MAG require lower concentrations of bile salts to achieve emulsification in to micellar form (Wilson et al., 1971 and Lien 1994). Many studies have indicated that the content of long chain SFA and the arrangement of long chain SFA are the major factors that determine digestibility of fats. In particular, the positional specificity of pancreatic lipase has been indicated to be an advantage for the absorption of the SFA located at sn-2 positions.

Fat absorbed from the diet and lipids synthesized by the liver and adipose tissue are transported to the various tissues and organs for utilization and storage. Lipoproteins are major vehicular system for transport of lipids in the body.

Lipoproteins are globular, high molecular weight particles, which are complex aggregates of lipid and protein molecules. A lipoprotein consists of a hydrophobic core that mainly contains triglyceridels and cholesterylesters, and a polar, hydrophilic coat composed of phospholipids, unesterified cholesterol, and specific apolipoproteins. This protects the hydrophobic core from the aqueous surroundings and allows transport of large amounts of cholesterol and triglycerides through the blood vessels.

Lipoproteins are a heterogeneous group, which can be divided in to five major classes: chylomicrons derived from the intestinal absorption of triglyceride; very low density lipoproteins (VLDL or pre β-lipoproteins), derived from the liver. Intermediate density lipoproteins (IDL), low density lipoproteins, (LDL, or β-lipoproteins) and high density lipoproteins (HDL or α-lipoproteins). Each class has a characteristic lipid and apolipoprotein composition, size and density, while each apolipoprotein has its own specific metabolic functions help to transport lipids in an aqueous environment of the blood plasma. The protein moiety of lipoproteins is known as an **apolipoprotein** or **apoprotein**, constitute nearly 60% of some HDL and as little as 1% in chylomicrons. One or more apolipoproteins (proteins or polypeptides) are present in each lipoprotein. The major apolipoprotein of HDL is designated as apo A. The main apolipoprotein of LDL is apo B, which is found also in VLDL and chylomicrons. However apo B of chylomicrons (B-48) is smaller than apo B of LDL or VLDL (B-100). B-48 is synthesized in the intestine and B-100 in the liver. These apolipoproteins confer stability and also specificity on the particles, allowing them to be recognized by specific receptors on cell surfaces in different tissues and organs. The amphiphilic phospholipids also confer stability to lipoproteins.

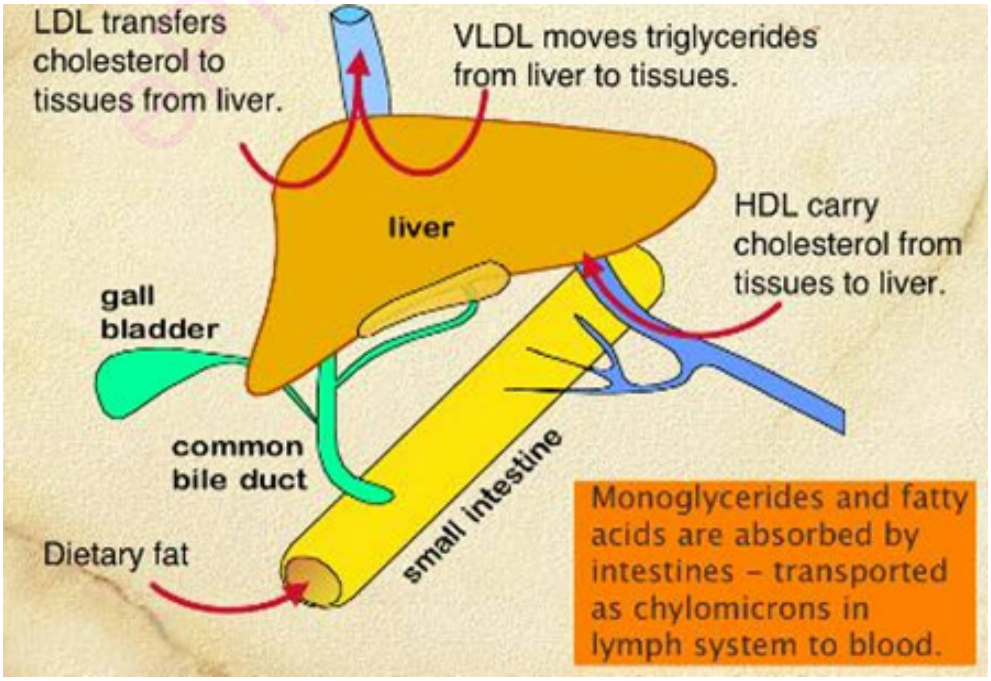
The chylomicrons which are assembled in the intestinal mucosa, keep exogenous triglycerides and cholesterol suspended in aqueous solution. These lipoproteins are released in to intestinal lymph and transported through lymphatic vessels. In the blood vessels, chylomicrons are converted to cholesterol enriched chylomicron remnants by the action of lipoprotein lipase in muscle and adipose tissue. Chylomicrons, therefore, function to deliver dietary triglycerides to muscle and adipose tissue and dietary cholesterol to liver.

Very low density lipoproteins (VLDL) which are synthesized in liver as lipid transport vehicles are also degraded by lipoprotein lipase. The VLDL remnants appear in circulation first as intermediate density lipoproteins (IDL) and then as low density lipoproteins (LDL). In the transformation of VLDL to LDL, all its apoproteins except apo B-100 are removed and much of their cholesterol is esterified by HDL-associated enzyme lecithin: cholesterol acyltransferase (LCAT). The enzyme transfers a fatty acid molecule from sn-2 of lecithin to cholesterol with concomitant formation of lysolecithin.

Low density lipoproteins (LDL) formed from VLDL is then taken up by liver via LDL receptor, a cell surface transmembrane glycoprotein which specifically binds both apo B-100 and apo E. Thus, LDL uptake is receptor mediated endocytosis. In the liver cells, the LDL's apo B-100 is rapidly degraded to its component amino acids. The cholesterol esters are hydrolysed by a lysosomal lipase to yield cholesterol, which is subsequently incorporated in to the cell membrane. Any excess intracellular cholesterol is re-esterified for storage within the cell through the action of acyl CoA: cholesterol acyltransferase (ACAT), mainly with oleic acid. LDL is involved in distributing cholesterol to various tissues.

High density lipoprotein (HDL) has essentially the opposite function of LDL. It removes cholesterol from various tissues. HDL is assembled in the plasma from components largely obtained through the degradation of other lipoproteins. HDL removes cholesterol from cell surface membranes and converts it into cholesterol esters through the action of lecithin: cholesterol acyltransferase (LCAT). HDL is thus functioning as cholesterol scavenger and getting involved in reverse cholesterol transport. HDL brings cholesterol from various tissues to liver which is the only organ capable of disposing significant quantities of cholesterol as bile acids. HDL associated cholesterol is therefore called 'Good cholesterol'

Fig. 3 Transport of lipoproteins



DIETARY FATS AND OILS

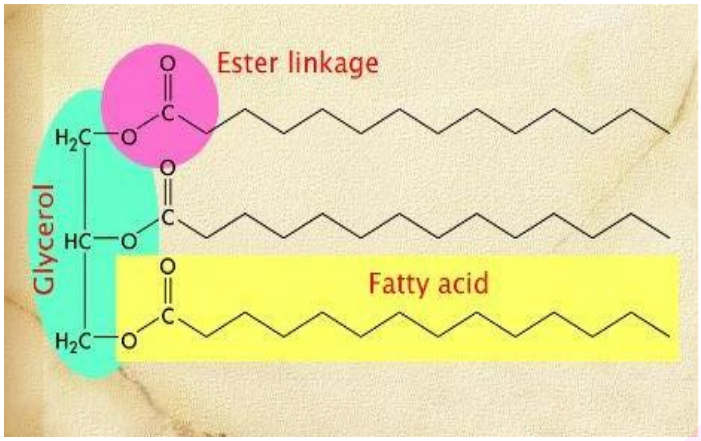
Vegetable oils are part of traditional diets all over the world. Next to food grains, Oil seeds constitute the second most important agricultural crop in our country. India is a major oilseed producing as well as oil consuming country.

There is an increasing interest in knowing the relation between dietary fat intake and health. Dietary fats and oils have long been recognized as a macronutrient with a primary function to provide a concentrated source of energy for human metabolic processes. The composition of edible oils and fats is very complex. Numerous components such as mono-, di-, and triglycerides (TGs), free fatty acids (FFAs), Phospholipids, Pigmented compounds and Waxes, as well as several nutritionally beneficial bioactive compounds are present in oils.

Nature has put oil/fat in almost each and every item of the food. Human beings have been taking oil/fat from day one as he set foot on this earth millions of years ago. He must have received fat from the first sip of mother's milk and later from food of animal/ vegetable origin. Fats and oils are either saturated or unsaturated; unsaturated fats can be either monounsaturated or polyunsaturated. Saturated fats, which come mainly from animal sources, increase serum cholesterol levels. Coconut and palm are two examples of vegetable oils which provide saturated fats. Monounsaturated fats are known to help in reducing the levels of LDL (bad) cholesterol without lowering the good HDL cholesterol.

Until recently, the fatty acid composition of the edible oils has been one of the main criteria for defining their nutritional value. Several studies have demonstrated a correlation between dietary fatty acids and their benefits on health. Substitution of saturated fatty acids with monounsaturated or polyunsaturated fatty acids (PUFAs) remains the best nutritional intervention for reducing the risk factors for cardiovascular disease. Triglycerides, which are basically fatty acids bound to a glycerol molecule, make up 96-99% of the dietary fats we consume. The remaining 1-4%, termed as unsaponifiable matter, includes the fat soluble minor components in the oil or fat.

Fig. 4 The structure of triglyceride



Edible oils and fats provide essential nutrients. However, growing awareness in public health, fitness and advances in nutrition research have raised few questions on the pros and cons of various dietary fats and oils.

Some concerns on the use of oils/fats are:

1. It is assumed that certain saturated fats raise cholesterol where as polyunsaturated fats reduce serum cholesterol levels.
2. Trans Isomers of the fatty acids produced during the hydrogenation and high-temperature used during refining of oils are harmful to health.
3. The refining processes, both chemical as well as physical take away natural vitamins and antioxidants present in raw oil and leave behind oils devoid of nutraceuticals or those containing undesirable substances.
4. Oxygenated polymers and cyclic products formed during high temperature processing such as during repeated frying may give rise to substances which can be carcinogenic.

Fat remains a highly debated topic because of concerns over its associations with the incidence of some chronic conditions including coronary artery disease, diabetes, cancer and obesity.

PLASMA CHOLESTEROL LIPOPROTEINS and CORONARY HEART DISEASE (CHD).

All animal food products contain some cholesterol. Dietary cholesterol seems to increase blood cholesterol when consumed in large amounts in particular with saturated fats. People differ in their ability to handle dietary cholesterol and also cholesterol which our body synthesizes endogenously for various functions. In the absence of dietary Cholesterol, the liver can still manufacture enough cholesterol to meet the body needs.

Cholesterol is a isoprene polymer present in eukaryotic cells. Humans synthesise 1-4 g of cholesterol daily, total amount of cholesterol in the human body ranging from 100-150g of which 10-12 g are constantly present in the blood. Cholesterol is an important structural component of cell membranes and is also a precursor of bile acids and steroid hormones such as progesterone, testosterone, estradiol, cortisol, and vitamin D (Dempsey, 1974). Cholesterol and fatty acids in the cell membrane are the primary determinants of the membrane fluidity, which in turn controls a number of events at the cell surface, including enzymatic activities such as phospholipase and cyclooxygenase. Free cholesterol is the component of membrane but cholesterol ester does not takes part in the membrane assembly because the 3-hydroxyl group is necessary for hydrogen bonding with membrane phospholipids. However in the serum, 60-70% of cholesterol is in the esterified form. Because of its hydrophobicity it is carried in aqueous environment of blood as lipoproteins. Liver is the main organ involved in cholesterol metabolism. A large body of evidence supports a direct relationship between total serum cholesterol and the risk of coronary heart disease (CHD).

LDL and HDL have different effects on the risk for CHD. High concentrations of LDL are atherogenic, while high levels of HDL are negatively associated with the risk of coronary heart disease. Since LDL carries most of the plasma cholesterol it is also a good index for the risk of CHD. However, some individuals have high total cholesterol concentrations as a result of high HDL levels. Therefore, the total cholesterol to HDL cholesterol ratio might be the most efficient predictor for the risk of CHD (Bruce et al., 1994). Also, high levels of triglycerides, which are in the fasting conditions mainly found in the VLDLs, are positively related to the risk of CHD (Griffin et al., 1994).

It is well accepted that even small changes in blood lipid profiles can have important benefits for public health. A decrease in TC is associated with a reduction in the risk of CHD for all age-groups (Law et al. 1994). In general, a 1% decrease in TC is associated with a 1–2% reduction in risk of CHD. Furthermore, a 1% decrease in LDL-cholesterol has been estimated to reduce the risk of major coronary events by 1.7% (Pedersen et al. 1998).

Association of Lp(a) and CHD was first observed in 1974. Studies have shown that Serum Lipoprotein (a) [LP(a)] concentration is an independent risk factor for coronary atherosclerosis **(**Md. Ali et al., 2008), cerebrovascular and peripheral vascular diseases (Genest, 1992 and Scanu, 1991). Lp(a) is a cholesteryl-ester-rich lipoprotein of unknown function formed by the covalent disulfide linkage of apolipoprotein (a) (apo(a) to apolipoprotein B (apo B) of low density lipoprotein cholesterol (LDL-c). Lp(a) is a genetically determined lipoprotein molecule. Its constituent, low density lipoprotein cholesterol participates in the process of atherosclerosis, High levels of Lp(a) (> 30 mg/dl) appear to increase the risk of premature CHD.

Oxidized LDL-c and Lp(a) accumulate in excessive amounts in macrophages ('foam cells') forming fatty streak. Intact Lp (a) deposition has been demonstrated in the arterial wall and venous grafts and atherosclerotic plaques. The relationship between dietary lipids and CHD is further complicated by the fact that the quality and quantity of dietary fats also influence platelet aggregation and vascular reactivity through their effect on the synthesis of eicosanoids in platelets and endothelial cells of arterial wall. High intake of SFA increases platelet aggregation and blood pressure. Lipoprotein (a) may affect atherosclerosis and thrombosis mainly by binding to fibrin and attenuating the fibrin-enhanced plasminogen activation. Tissue factor-complex initiates coagulation by activating factor X and factor IX leading in the presence of calcium to the generation of thrombin. Lipid lowering treatment with statins stabilizes atheromatous plaque and has antithrombotic effects. Therefore there are links between lipids and the haemostatic mechanisms which affect atherosclerotic, vasomotor and thrombotic components of ischemic heart disease (Dimitris et al., 2002)

HUMAN DIETARY LIPIDS REQUIREMENTS:

Since dietary lipids exhibited profound effects on risk factors for CHD, much attention has been paid in the last few decades on the quality and quantity of oils and fats consumed in our diet. There are two aspects to lipid requirements in the human diet. These are qualitative and quantitative.

Firstly, certain lipids are needed for good health. Essential fatty acids and fat soluble vitamins are good examples. Secondly, it is usually considered that, the normal diet should supply 25-30% of the total calories as fat (Jones, 1974). Such lipids in reasonable amounts also usually make food more palatabe.

Dietary sources of fat are of two kinds: 'invisible' fat (present as an integral part of food items) and 'visible' fat. milk, egg, meat, fish, nuts, oilseeds, cereals and millets are important sources of invisible fat. Cereal and millet contain only 2-3 percent of invisible fat. However being staple items in Indian diets, they contribute significantly to total fat intake. From the data-base generated on fatty acid composition in Indian foods, National Nutrition Monitoring Bureau surveys estimated that the daily intake of invisible fat amounts to 7% calories in rural and 13% in urban population.

Several factors determine the intake of fat by Indian population. Fat intake is income dependent and there are regional preferences in both the quality and type of the fat consumed. Vegetable oil used in cooking constitutes about 80% of visible fat consumption. Nutrient allowances for Indians were recommended by an expert committee of the Indian council of medical Research in 1988 (ICMR, 1992) states that dietary fat should supply at least 15 % calories for normal adults and the requirement of fat in the form of invisible (10 % calories) and visible (20g/day or 9% calories) has been recommended. According to the American Heart Association, the optimum intake of fat for an adult should be 30% of total caloric intake. Therefore for an adult man consuming 2000 calories in the diet should be getting about 600 calories or 60-65 g of total fat per day. Typical intakes in Europe and North America are between 80 and 150g/day which represent 30-40% of calories from the dietary fat.

Requirements of Essential fatty acid (EFA).

Essential fatty acids are necessary to prevent the deficiency symptoms and are utilized as precursors of prostaglandins, thromboxanes and leukotrienes (Samuelsson et al., 1975; 1978). EFA deficiency leads to a decrease in body weight, changes in skin permeability and in capillary integrity, dermatitis, impaired wound healing. EFA deficiency is easily recognized because tissue fatty acids of the n-6 group are partly replaced by those of n-9 group. In particular, arachidonic acid is reduced and eicosatrienoic acid (Cis-5, 7, 11-20:3) is increased. Dietary guidelines for lipids were originally introduced to deal with deficiencies, but today countries develop guidelines in an effort to prevent chronic diseases such as heart disease, obesity, and diabetes.

In India, while chronic energy deficiency associated with low fat intake continues to be widely prevalent, the deaths attributed to CHD have also increased parallelly (Reddy, 1993; 1999). During the past 15 years India adopted several strategies to increase the production of edible oil-seeds and oils. Indian government is also importing oil to meet the growing demands of India's increasing population. It has now become necessary to educate the public about the optimum quantity of fat needed in the daily diets to maintain positive health.

Krawczyk (2001) has summarized the dietary guidelines around the world. Diet modifications recommended for the general population for the prevention of heart disease, cancer and diabetes include reductions in total dietary fat and cholesterol, replacement of saturated fat with polyunsaturated fats of both n-3 and n-6 types.

Vegetable oils rich in polyunsaturated fats had the ability to reduce total cholesterol, a risk factor for CHD. Such findings favored use of PUFA rich oils such as Sunflower oil and Safflower oil. In due course, evidence began to accumulate establishing that although polyunsaturated fats were effective in reducing LDL-cholesterol, very high content of polyunsaturated fats had the undesirable effect of reducing HDL cholesterol as well. Since the oils with high levels of polyunsaturated fats oxidize faster than those with MUFA or SFA, the excessive intake of PUFA enriched oils could impair the ability of the anti-oxidants system to control free radicals and thereby enhance the risk of certain cancers, cataracts, rheumatoid arthritis, parkinson's disease and contribute to the ageing problem.

In view of the specific effects of each fatty acid on lipoprotein and eicosanoid metabolism, platelet vascular homeostasis and inflammatory response, it is important to avoid excess intake of any fatty acid. Most of the recent recommendations (BNF Task Force, 1992; FAO/WHO, 1994; Simopoulos et al., 1999) for optimal health suggest the following range of intake of fatty acids (percentage of total energy, en%): total fat 15-30; SFA 7-8; LA 3-6; ALNA 1-2 and the ratios of PUFA/SFA and LA/ALNA (n-6/n-3) between 0.8-1.0 and 5-10 respectively.

The World Health Organization (WHO) recommends polyunsaturated fatty acid to saturated fatty acid (PUFA/ (SFA) ratio of 0.8-1.0 and linoleic acid (n-6) alpha linolenic acid (n-3) ratio of 5-10 in the diet. The American Heart Association now recommends the use of oils having an equal proportion of Saturated, Monounsaturated and Polyunsaturated fats.

The most recent recommendation according to the National Research Council, Washington DC are 10% saturates, 12 to 13% for monounsaturates, not more than 7-8% for Polyunsaturates. It is recommended that the intake of polyunsaturated fat should be accompanied by Vitamin E, Vitamin C or Carotene consumption to help prevent lipid peroxidation to which PUFA are vulnerable.

QUALITY OF FAT IN THE DIET

The quality of oil and the source of invisible fat and type determine the intake of different fatty acids in the total diet. In Indian diets, the vegetable oil used in cooking represents major visible fat consumed.

Visible fat

Visible fats are those that are extracted from oilseeds (vegetable oils) or from milk (butter and ghee). The visible fats are largely triglycerides. The common fatty acids found in visible fats are palmitic, oleic and LA. The composition of fatty acids in different fats varies widely (Table 1). The percentage of LA in safflower, sunflower, soyabean, corn and cottonseed is more than 50% of the total fatty acids, while sesame, rice bran, groundnut and mustard oils contains 20-40% LA. Palm oil contains about 10% LA. Coconut and palm kernel oil contains a high proportion of SFA of short and medium chain length. Rapeseed, mustard and soyabean oil

contains appreciable amounts of LNA. Rapeseed, mustard oil contains large quantities of erucic acid. Vanaspathi contains long chain-saturated fatty acid and about 55% trans fatty acids.

Table 1. Approximate fatty acid compositions of visible fats (g/100g)

Oil	SFA	MUFA	LA	LNA	LA/LNA
Coconut	89	7	2	<0.5	4
Palm Kernel	82	15	2	<0.5	4
Red Palm	50	40	9	<0.5	18
Palm	45	44	9	<0.5	20
Olive	13	76	10	<0.5	20
Groundnut	24	50	25	<0.5	20
Sesame	15	42	42	1	42
Rice bran	22	41	35	1.5	23
Cotton seed	21	25	52	1	52
Corn	12	32	55	1	55
Sunflower	13	27	60	<0.5	120
Safflower	13	17	70	<0.5	140
Soybean	15	27	53	5	11
Ghee	65	32	2	0.5	4

SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, LA: Linoleic acid, LNA: Linolenic acid **(**Ghafoorunissa, 1994**).**

Invisible fat

Every food contains fat as an integral component. Invisible fats are tightly bound component of food matrix which are present in common dietary items like cereals, pulses, condiments which are not coming under the category of oil seeds. Cereals and pulses are the bulk items of our diet which contribute substantially to fat intake. A daily intake of about 300-500 g of cereals and millets alone provides about 8-12 g invisible fat. The invisible fats of plant foods are good sources of oleic, LA and LNA, Legumes and green leafy vegetables contain high proportions

of LNA. The invisible fat from cereals and pulses contribute as much as 7% of energy in adult that provide 2400 Kcal. In average Indian diets, the visible and invisible fat together contributes about 15% energy in low income groups. A daily Indian diet which provides 2400 Kcal contains about 40g of fat a day, out of which 25g will be from invisible fat and 15g from visible fat.

The content of invisible fat in many food materials have a relatively high LA (rice 50%, wheat 55%, bengalgram 65% and redgram 55%) and a low content (about 3%) of ALA, while spices have a particularly high content of ALA (5.4%) (Table 2). As a result, the average Indian diet is predominantly based on staple cereals (rice and wheat) accounting for 75-80% of dietary energy and some pulses, including the diets of poor income groups which carry sufficient LA levels and a proportion of ALA as well.

Table 2. Invisible fat and fatty acids in plant foods in g/100g of the food.

Food	Fat	SFA	MUFA	LA	LNA	LA/LNA
Cereals	1.7	0.4	0.4	0.5	0.01	50
Wheat	2.9	0.5	0.3	1.0	0.17	6
Maize	4.8	0.8	1.1	2.2	0.05	44
Jowar	3.3	0.6	1.0	1.5	0.05	30
Ragi	1.5	0.3	0.7	0.3	0.05	6
Bajra	5.5	1.2	1.2	2.2	0.13	17
Legumes						
Black gram	1.7	0.3	0.2	0.1	0.7	0.2
Rajmah	2.2	0.4	0.2	0.5	0.7	0.7
Green gram	1.7	0.5	0.05	0.6	0.2	3
Red gram	2.2	0.5	0.1	1.0	0.1	10
Lentil	2.0	50.3	0.4	0.8	0.16	5
Bengal gram	6.0	0.7	1.7	1.2	2.7	1.8
Peas	2.1	0.3	0.4	0.8	0.15	5
Soybean	20	2.8	5.4	10.4	1.4	7
Vegetables						
Green leafy	0.4	0.09	0.025	0.04	0.15	0.3
Others	0.2	0.05	0.016	0.06	0.03	2
Nuts						
Coconut	40	36	3.2	0.6	-	-
Ground nut	40	8.8	21	10	0.2	50
Sesame	40	6.0	18	16	0.4	40
Mustard	40	2.0	5	5	3.5	1.4
Almond	56	25	19	8	0.2	40

SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, LA: Linoleic acid, LNA: Linolenic acid. (Ghafoorunissa, 1996).

MINOR CONSTITUENTS OF OILS.

In addition to the constituent fatty acids, minor components in the non-glyceride fraction contribute to their nutritional health effects. These minor constituents are unique to each oil. All the vegetable oils contain tocopherols, their levels are by and large related to the percentage of PUFA in the oil. It inhibits LDL-oxidation (Liu et al., 2004), reduce atherogenic effects (Wilson et al., 1978) and prevent ischemic heart disease (Gey et al., 1991). Palm oil and ricebran oil have tocotrienols, in addition to tocopherols. Ricebran oil contains oryzanol. γ–oryzanol, esters of ferulic acid is uniquely present in rice bran oil and has hypocholesterolemic activity by suppressing the HMG Co-A reductase (Ha et al., 2005). Sesame oil contains lignans (sesamin, sesamolin and sesamol) which is a potent and specific inhibitor of Δ-5 desaturase (Shimizu et al., 1991) and it has hypocholesterolemic properties (Chen et al., 2005a). It is a precursor of enterolactone (Penalvo et al., 2005), which reduces the risk of acute coronary events (Varharanta et al., 1999). Sesamolin, metabolic product of sesamin inhibits lipid peroxidation (Kang et al., 1998). Olive oil, in particular the "extra virgin" type, has a high content of phenolic components, which are shown to be powerful antioxidants (Visioli et al., 2002). Olive oil phenols have greater antioxidant potency than other components of vegetable oils (Wiseman et al., 1996). High phenolic olive oil reduced LDL peroxidation in hypercholesterolemic postmenopausal women (Oubina et al., 2001). Tocopherols and tocotrienols protect the cells from free radical damage (Shichiri et al., 2007). Sesame lignans also have antioxidant properties. Oryzanol and sesamin have hypocholesterolemic effects (Lim et al., 2007).

FAT INTAKE IN INDIA

Regional differences in the dietary habit is common across India. The nature of the edible oil used varies from one part of the country to the other: groundnut oil in the west and South, coconut oil in Kerala, rape seed oil/mustard seed oil from Punjab to West Bengal and safflower oil in parts of North Karnataka and Southern Maharashtra. This results in variation in quantity and fatty acids composition of total fat consumed by population in the diet.

Indian diets are usually cereal-pulse based. Vegetable oils are used as cooking fat which is the major source of the visible fat. Diet surveys conducted by National Nutrition Monitoring Bureau (NNMB) in 10 states of India (NIN-Hyderabad, 1999) show that daily intake of visible

fats vary from 3g to 20g/day consumption unit in rural India; in many states as much as quarter of population may not have any visible fat at all in their diets. The total quality of invisible fat depends on the kind of diet eaten. The diets of even poor people which consist predominantly of cereals and have little or no visible fat contain significant quantities of invisible fat.

COCONUT OIL

Coconut palms, *Cocos nucifera* L., source for CO and an important fat used in southern part of India. *Copra* is the trade name for the dried coconut meat or kernel. The first step in making copra is to remove the husk from the mature nuts, the nut is then opened for drying to produce copra. Coconut oil is extracted from the copra by pressing, solvent extraction, and other procedures Sharp-melting fats of CO leave a clean, cool, nongreasy sensation on the palate. More than 90% of the coconut oil fatty acids are saturated, which accounts for its excellent oxidative stability. It is characterized by high levels of short and medium chain fatty acids (C 8:0-C 12:0). It finds application in infant formulae, parenteral and enteral nutrition and in food products for fat malabsorption cases (Sales et al., 1998). More recently, lauric acid has been recognized for its unique properties, which are related to its antivirus, antibacterial, and antiprotozoal functions.

Medium chain fatty acids are transported via the portal system and are rapidly oxidized in the liver to provide quick energy. It is not stored in the tissues (Bach and Babyan, 1982). CO has very low levels of PUFA. Since it has very low levels of essential fatty acids, long term consumption of CO as the sole source of fat in the diet may lead to the essential fatty acid deficiency. CO has been considered as an atherogenic fat.

GROUNDNUT OIL

Arachis hypogaea L., commonly known as peanut or groundnut because the seed develops underground. It is known to have been cultivated as early as 2000 to 3000 B.C and is a legume native to South America. GNO is the most important edible oil in India, accounting for nearly 60% of the total indigenous edible oil supply. It contains oleic acid and linoleic acid and is used for culinary purposes. Ground nut oil differs from most other vegetable oils in that it contains up to 6% of long chain saturated fatty acids, arachidic, behnic and lignoceric. In the triglycerides of GNO palmitic and stearic acid are found predominantly in the sn-1 and sn-3

positions, the long chain (C-20 to C-24) fatty acids are located almost exclusively in the sn-3 position, and the sn-2 position is high in unsaturated fatty acids (Sanders, 2002). The triglyceride content of peanut oil is generally in the range of 96%. Several studies have shown that peanuts contain resveratrol, a phytochemical also found in red wine that has been linked to lower risk of heart disease (Haumann, 1998). Commercial peanut varieties have been developed that incorporate a high-oleic fatty acid trait. In recent studies with human subjects, it was found that high-oleic peanut oil produced positive benefits in the blood lipids, including a reduction in LDL cholesterol and triglyceride levels but did not alter the beneficial high-density lipoprotein (HDL) cholesterol levels (Ramesh et al., 2006).

OLIVE OIL *(Olea europaea)*

Virgin Olive oil *(Olea europaea* Linn*)* is one of the oldest known vegetable oils and one of the few oils that can be consumed in its crude form (unrefined) (Boskou, 1996). It is valued for its unique aroma and flavour, and long shelf life (Kiritsakis, 1998). Today, its biological, nutritional and healthful benefits effects are universally acknowledged (Visioli et al., 1998). It has been shown that consumption of olive oil lowers the incidence of coronary heart disease and some forms of cancer. The lower incidence of coronary heart disease and cancer of prostate and colon in Greece, Italy and Spain is attributed to the consumption of olive oil in the Mediterranian diet.

The proximate composition of the olive oils varies widely depending on fruit variety, degree of fruit ripeness, environmental conditions, geographical region and conditions used for processing and storage.

Abundance of oleic acid, a monounsaturated fatty acid, is the feature that sets olive oil apart from other vegetable oils. Oleic acid (18:1 n-9) ranges from 56-84% of total fatty acids, while linoleic acid (18:2 n-6) is present in concentrations between 3 and 21%. In addition to triglycerides and free fatty acids, olive oil contains a variety of non-saponifiable compounds that add up to 1-2% of the oil and are important for its stability, unique flavour and taste.

Olive oil as a highly monounsaturated oil, is resistant to oxidation. The complex phenolic compounds, tocopherols and other natural antioxidants present in the olive oil prevent the lipid

oxidation. The aroma and the flavour compounds as well as the chlorophyll and pheophytin pigments of olive oil fecilitate the absorption of the natural antioxidants, which protect the body tissues from oxidation. Three groups of phenolic compounds have been identified and quantified in olive oil. These are the simple phenols (Hydroxytyrosol and tyrosol), the secoiridoids oleuropein, the aglycone of ligstroside and their respective decarboxylated dialdehyde derivatives) and lignans [(+) -1-acetoxypinoresinol and (+)-pinoresinol)].

The flavanoid polyphenols in olive oil have been shown to have a host of beneficial effects on humans from healing sunburn to lowering cholesterol, bood pressure, and risk of coronary disease.

Numerous studies have shown that these phenols are potent inhibitors of LDL oxidation (Visioli et al., 1995 and Weinbrenner et al., 2004). In particular hydroxytyrosol, one of the major phenolic constituent in olive oil, has been reported to reduce the risk of coronary heart disease and atherosclerosis (Gonzalez-Santiago et al., 2006). It has also been shown that hydroxytyrosol inhibits lipoxygenase (Petroni et al., 1997 and Rocio, 1999), inhibits the platelet aggregation (Petroni, 1995). Squalene a component in olive oil is known to inhibit the activity of HMG-CoA reductase, the key enzyme of cholesterol synthesis.

MODIFICATION OF OILS AND FATS

Careful analysis of oils consumed in India shows imbalance in their fatty acid composition. No single fat or oil is completely satisfactory for all purposes such as cooking, processing, preparation of foods with desirable nutritional and biological activity. Among the dietary factors which can affect the plasma cholesterol level, the amount of dietary cholesterol and amount and degree of saturation of fatty acids are perhaps more important. A significant relationship between dietary fat, plasma lipid levels and coronary heart disease is reported by many epidemiologists world wide. Many investigators have reported that PUFA decrease and SFA increase the plasma total cholesterol and low density lipoprotein levels in humans as well as in animals (Arja et al., 2008). However monounsaturated fatty acids such as oleic acid were considered to have no effect on plasma cholesterol level (Keys, 1957). Hence PUFA/SFA (P/S) ratio has frequently been used in predicting the cholesterolemic effect of dietary fat. Diets with higher P/S ratios were found to have hypocholesterolemic effect than those with

lower P/S ratios (Chang et al., 2004). Even though increasing dietary P/S ratio has been recommended by earlier investigators for reducing risk factors for CVD, a high P/S ratio of diet may enhance oxidative stress because PUFAs are highly susceptible to oxidation. However, studies by Mattson and Grundy (Mattson and Grundy, 1985) and other investigators have reported that when MUFA rich safflower oil or olive oil is supplemented to the diet or replaced by SFA, serum cholesterol and LDL-cholesterol levels decrease but HDL does not show any decrease in humans hence MUFA may be better than PUFA in preventing or treating atherosclerosis. Recently, Chang *et al* also have reported that not only P/S ratio is important but P+M/S ratio is also important in controlling the serum and hepatic lipids in rats (Chang et al., 2004). Therefore, P/S ratio as a criterion for nutritional assessment of fats is insufficient because of the presence of monounsaturated fatty acids in significant quantities in all oils, which cannot be ignored. A more elaborate criterion for the nutritional assessment of fats incorporating monounsaturated fatty acids such as a ratio of saturated fatty acids to monounsaturated fatty acids to polyunsaturated fatty acids (SFA:MUFA:PUFA) may serve the purpose more efficiently.

Monounsaturated fatty acids being in-between the saturated fatty acids and polyunsaturated fatty acids, apart from supplying energy, also have a cushioning effect on the negative qualities of the saturated fatty acids and polyunsaturated fatty acids. Such an SFA:MUFA:PUFA ratio of 1:1:1 would seem to be appropriate in the dietary fats. Indian Council of Medical Research recommended that the saturated fatty acid (SFA): monounsaturated fatty acid (MUFA): poly unsaturated fatty acid (PUFA) in the oils be in equal proportions and the fat in that diet may provide 20-25% of energy (Ghafoorunissa, 1998). These guidelines are in tune with the ones promoted by the American Heart Association (Folig-cawley, 2006).

There is no single oil in nature with a balanced fatty acid composition of SFA:MUFA:PUFA ratio 1:1:1. A simple and alternative method to achieve this is the modification of oils by physical mixture of different oils or to develop structured lipids from different oils. This can be achieved by blending two different oils in a right proportion and modifying oils by interesterification reaction.

METHODS AVAILABLE FOR MODIFICATION OF OILS AND FATS

Hydrogenation, fractionation and interesterification are three processes available to food manufacturers to tailor the physical and chemical properties of food lipids (Sreenivasan 1978 and Young, 1985). At present, roughly one-third of all edible fats and oils in the world are hydrogenated, whereas ~10% of oils are either fractionated or interesterified (Haumann 1994). The remaining portion is consumed as such.

FRACTIONATION

Fractionation is a physical separation of components of oils which is based on the crystallization behaviour of individual triglycerides (Deffense, 1985). Winterisation is the most common type of fractionation used in the fats and oils industry. This technology is often used to fractionate cotton seed and palm oil into olein (liquid, oleic acid-rich) and stearin (solid at room temperature, stearic acid-rich) fractions.

HYDROGENATION

Hydrogenation is a chemical process leading to the saturation of double bonds to harden fats for use as margarine and shortening base stocks. The primary objective of hydrogenation is to modify the melting properties of oils. This is accomplished by converting a certain number the unsaturated double bonds in the liquid oil to saturated bonds by reaction with hydrogen using a metal catalyst. Nickel is the most frequently used catalyst for this process. Since the hydrogenation process is not entirely selective, several side reactions can also occur during the process, which create both geometric and positional isomers of fatty acids. The hydrogenation can create isomers of double bonds leading to formation of *Trans* isomer. While natural oils contain mostly *Cis* isomer.

BIOTECHNOLOGICAL APPROACHES

Biotechnology tools presents new alternative to manipulating lipids. Three rapidly expanding areas of lipid biotechnology are

1. Genetic engineering of oil seeds and oil bearing plants for improved agronomic

properties, altered fatty acid and lipid composition.

2. Use of microorganisms for the production of oils and fats from the non-lipid and lipid containing carbon sources. Biotransformation of fats and oils into fat based value-added products using whole microbial cells or enzymes derived from them are also feasible.
3. Interesterification: It is a process which results in fatty acid redistribution within and among the triglyceride molecules, which can lead to the substantial changes in lipid functionality. The chemical composition of a fat partly dictates its physical and functional properties (Akubor et al., 2004). The chemical nature of lipids is dependent on fatty acid structure and distribution on the glycerol backbone. Fatty acids vary in chain length and in the number, position, and configuration of double bonds (Nawar, 1996).

BLENDING OF OILS

Blending of oils is the admixing of two or more oils having different fatty acid composition. Blending of oils and fats has acquired great importance as an alternative to hydrogenation for suitable modification of oils and fats to get tailor-made or specialty fats.

To ensure optimal health benefits, the use of more than one oil is recommended. Blending vegetable oils of different origin or their combination with animal fats (e.g., with milk fat) is justified for technological, nutritional and economic reasons. The different vegetable oils of known fatty acid compositions can be combined in appropriate ratios to obtain products of improved composition and better functional properties. Government of India has permitted admixture of any two edible vegetable oils (PFA Act, 1954) in which the minimum proportion of any one oil has to be 20% or more. The selection of appropriate oils and their proportions for blending determines the nutritional value and functional properties of blended oils. Use of more than one source of oil gives an additional advantage of providing greater variety of minor components present in oils.

Digestion and absorption of fats depends on the melting point of the glyceride as a whole rather than the melting point of the individual fatty acids. It is here that interesterification comes in to the picture. By directed interesterification reaction preferably enzymatic interesterification

(Bhattacharya and Bhattacharya, 2000) blended product of desired melting point and consistency can be obtained.

ESTERIFICATION AND INTERESTERIFICATION.

The interchange of fatty acids between two different fats and oils is called interesterification. The esterification reaction between hydroxyl-rich moieties (glycerol, propylene glycol, polypropylene glycol, and sucrose) and fats or fatty acids are frequently applied to produce emulsifiers, such as monoglycerides, propylene glycol monoglycerides, polyglycol esters.

There are three types of reactions associated with interesterification: these are acidolysis (Fatty acid-TG), glycerolysis (glycerol-TG) and transesterification (TG-TG) (Malcata, 1990.and Chaplin and Bucke, 1990). Interesterification reactions have been performed since the mid-1800s. There are two types of interesterification presently in use for modifying oils and fats, they are chemical modification and enzymatic modification. In chemical modification, metal alcoholate catalysts are usually employed. Where as Enzymatic modifications rely on the use of random or regiospecific (1, 3- or 2-specific) and fatty-acid specific lipases as catalysts.

CHEMICAL INTERESTERIFICATION

Chemical interesterification has been industrially adopted in the food industry since 1940s, to improve the spreadability and baking properties of the lard (Lars et al., 1961 and Theodore et al., 1961). In 1970s, there was renewed interest in this process, particularly as a hydrogenation replacement for the manufacture of zero-trans margarines. There are two types of chemical interesterification-random and directed. Interesterification reactions performed at the temperatures above the melting point of the highest-melting TG component in the mixture results in a complete randomization of fatty acids among all available TGs. If, however, the interesterification is carried out at temperatures below the melting point of the highest-melting TG component (usually a trisaturated TG), the end result will be a mixture enriched with the component of highest melting point. As the trisaturated TG is produced, it will crystallize and separate out from solution. This will push the equilibrium of the reaction towards increased production of the trisaturated TG. In this respect, directed interesterification can be thought of as a combination of interesterification and fractionation.

Chemical interesterification is used industrially to produce fats and oils used in margarines, shortenings, and confectionary (Petrauskaite, 1998). Newer applications of chemical interesterification include the production of low calorie fat substitutes such as Salatrim and Olestra. Salatrim/Benefat consists of chemically interesterified mixtures of short chain and long chain fatty acid triglycerides (Smith et al., 1994). Chemically catalysed interesterification is not selective. Therefore it is sometimes called as randomization process.

Drawbacks of Chemical esterification:

1. Employment of drastic reaction conditions like high temperature and pressure.
2. Non-specific esterification leading to the formation of many products.
3. Tedious workout procedures.
4. Employment of toxic solvents or catalysts.

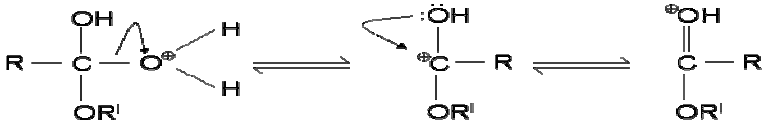
Fig 5. Mechanism of Chemical esterification (Morrison and Boyd, 1992)

Step 1:

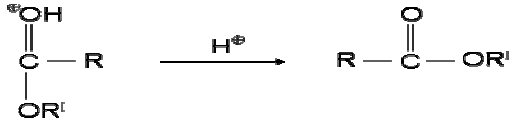
Step 2:

Step 3:

Step 4:



Step 5:



Mechanism of chemical esterification

Fig. 5 shows the mechanism of chemical esterification.

1. The carbonyl group is protonated by the H^+ of the acid catalyst.
2. The more electropositive carbonyl carbon is attacked by the nucleophile, the alcohol molecule.
3. The proton is transferred to one of the hydroxyl groups.
4. The hydroxyl group leaves as a molecule of water giving a protonated ester.

The resonance stabilizes the protonated ester by the loss of a proton.

ENZYMATIC INTERESTERIFICATION

Interesterification can also be carried out using enzymes such as lipase. Even though randomization mediated by chemical catalysis is a mature technology, it is not a popular process. Therefore, lipase catalysed interesterification is favored by the industry as the means to produce various base fats for formulations. Rearrangement of the fatty acid positions of TG molecules of fats and oils through interesterification process can alter the initial physical properties of the oils and can lead to the formation of the new products. An important feature is that the lipase-catalysed interesterified fat product retains the fatty acids in the *sn-2*-position intact (Sil Roy, 1993). We know very little about the nutritional properties of the Interesterified fats and it is possible that this process has unintended effects too. Professor Sanders and his research group have begun the process of characterizing the nutritional properties of the interesterified fats. The available results suggests that interesterified fats might be better for the cardiovascular system than either partially hydrogenated fats or unmodified fats as they have less effects on the blood triglyceride response and the state of activation of blood coagulation factor VII (Sundram, et al., 2007).

Advantages of Enzymaic Interesterification.

The most useful property of lipases is their regio-and stereo specificity, which results in the products with defined, predictable products with desired chemical composition and structure than those obtained by chemical catalysis. Potential advantages of using enzymes over chemical procedures may be found in the specificity of the enzymes and the mild reaction conditions under which enzymes operate (Gunstone, 1989). Enzymes form products that are more easily purified and produce less waste, and thus make it easier to meet the

environmental requirements. Chemical catalysts randomize fatty acids in triglyceride mixtures and do not lead to the formation of specialty products with desired physicochemical properties (Vulfson, 1993). The specificities of lipase have classically been divided in to five major types: lipid class, positional, fatty acid, stereochemical and combinations there of (Malcata et al, 1992). Enzymes have higher turn over numbers and are well suited for the production of chiral compounds important to the pharmaceutical industry.

Transesterification using sn-1,3 specific lipase results in SL products with fatty acid at the sn-2 fatty acids remaining intact. This is significant from the nutritional point of view because 2-MGs produced by pancreatic lipase digestion are the main carriers of fatty acids through the intestinal wall. Fatty acids esterified at the sn-2 position are therefore more efficiently absorbed than those at the Sn-1 and Sn-3 positions Short or medium chain fatty acids at the 2-position of glycerol are more stable than those linked at the 1-and 3-positions. The energy saved and minimizations of thermal degradation of fatty acids are probably among the greatest attractions in replacing the current chemical technology with enzyme biotechnology for modified oils (Vulfson. 1993).

STRUCTURED LIPIDS

Even though one can balance the fatty acid composition to desired proportions by blending of suitable oils, it may not always provide desired physico-chemical (Kurashge. et al., 1993) or nutritional properties (Marangoni and Rousseau, 1998). In blended oils, the characteristics of triglyceride molecules from individual oil is retained as such. They follow the original digestion, absorption and metabolic pathways and physiological effects even when they are presented as mixtures. By directed interesterification reaction preferably enzymatic interesterification (Bhattacharya and Bhattacharya, 1992) blended product of desired melting point and consistency can be obtained.

Structured lipids (SLs) are the lipids that have been reconstructed from the native state of natural fats and oils to change the position of the fatty acids to give special functionality or nutritional properties. Structured lipids are often referred to as new generation of fats that can be considered as “nutraceuticals” as they can provide medical or health benefits, including the potential for the prevention and/or the treatment of the disease (Kennedy, 1991). Sometimes

they are referred to as **functional foods** or **functional lipids**. With the ability to combine the beneficial characteristics of component fatty acid in to one triglyceride (TG) molecule, lipid modification enhances the usefulness of fats and oils for nutrition and health applications.

SLs have been synthesized to target specific metabolic effects or to improve physical characteristics of fats and oils. Considerable advances have been made over the past 10 years in designing structured lipids, especially in getting stereospecific location of fatty acid (Kim and Yoon, 2003). There are varieties of structured lipids such as structured triglycerides, diglycerides, monoglycerides and non-glycerol based fat.

Enzymatic mediated structured lipids formation result in specific placement of the fatty acids on the glycerol backbone. Thus the structured lipids can combine the unique characters of the each fatty acid, such as melting behaviour, digestion, absorption and metabolism and can increase their application in foods, nutrition and therapeutics. SL can be manipulated to improve their physical characteristics such as melting points. SL is texturally important in the manufacture of plastic fats such as margarines, modified butters, and shortenings. Caprenin, a structured lipid produced by Procter and Gamble company (Cincinnati, OH) consists of C8:0-C10:0- C22:0 it has the physical properties of cocoa butter but only about half of the calories. Benefat, originally produced as SALATRIM, consists of short chain (C2:0-C4:0) and long chain (C 18:0) fatty acids. Both products can be used as cocoa butter substitutes. Currently they are manufactured through a chemical interesterification process. Because of the low caloric value of SCFAs and the partial absorption of the stearic acid on SALATRIM, this product was projected as a low calorie fat substitute. The caloric content of the Caprenin and the Benefat is about 5 kcal/g (Vs. 9Kcal/g for a regular TG). These SLs can also be manipulated for nutritive and therapeutic use.

LIPASES

Lipases are the enzymes whose main function is to hydrolyse of glyceride ester bonds. These enzymes are also termed as acylglycerol acylases or acylhydrolases or lipases. They also act on substrates other than acylglycerols. Lipases can be regarded as transferases since the fatty acid released is transferred to water or some other compound with a free hydroxyl group or related moiety. The EC nomenclature for lipase is EC 3.1.1.3 (IUPAC-IUB, 1979). Their role

is to initiate glyceride metabolism and are exploited for generating energy from lipid stores.

CLASSIFICATION OF LIPASES

Lipases can be classified in to three broad categories based on their ability to hydrolyse glyceride esters (Hass et al., 1991).

1. Those which cleave only the terminal positions of triglycerides, are termed 1, 3-specific. Many lipases from microbial source come under this category.
2. Non-specific lipases hydrolyse both primary and secondary esters.
3. The third group of lipases is positionally non-specific but exhibits fatty acid selectivity, cleaving the ester bond of a particular type of fatty acid. For example, lipase from *Geotrichum candidum* is known to preferentially hydrolyse the esters of 9, 10-unsaturated fatty acids (Iwai and Tsujisaka, 1984; Baillargeon, 1990; Baillargeon and Mc Carthy, 1991).

STRUCTURE OF LIPASES

Rhizomucor miehei lipase (molecular weight-29 KDa) was the first lipolytic enzyme for which a high resolution structure was determined by X-ray crystallography (Brady et al., 1990). This development was followed by high resolution structures for the lipase from the human pancreas (Winkler et al., 1990). Though lipases vary largely in sizes, many of them share common features. The lipases are found to contain Ser-His-Asp/Glu catalytic triad reminiscent of the serine proteases (Blow, 1990) which is buried beneath surface loops and is not accessible to the substrate. Another common feature is the basic folding pattern in their catalytic domains (David, et al., 1992). This fold called α/β-hydrolase fold", is also found in many esterases and other hydrolytic enzymes. Lipases encompass a wide range of molecular weights ranging from 19 KDa (cutinase) to 60 KDa *(G. candidum).* All lipases, except pancreatic lipase, are single domain proteins. The catalytic domains are formed from parallel β sheet backbones and a number of α helices is present in all the lipases (David et al., 1992). However there are minor differences in individual lipases like presence of extra long and a short helix in lipase from *Rhizomucor* family.

Reactions catalysed by lipases.

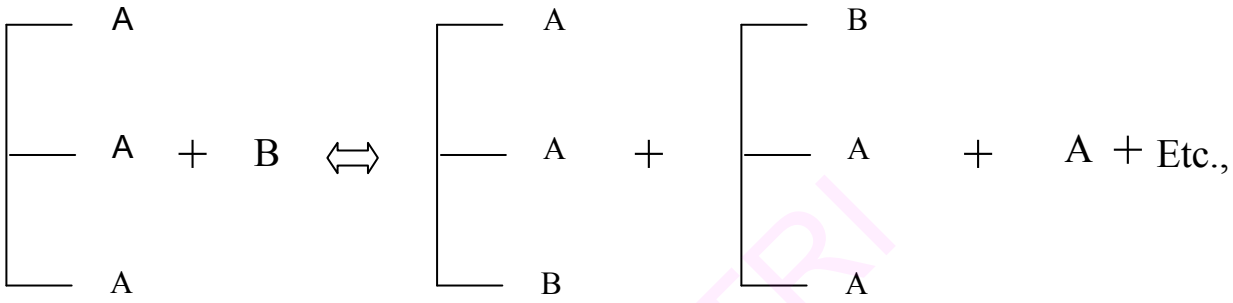
Although lipases are termed as acylglycerol hydrolases, they are far more versatile in their action. The range of substrates with which they react and also the range of reactions they catalyse are far more than any other enzyme studied till date (Rogalska et al., 1990).

Lipases catalyse three types of reactions

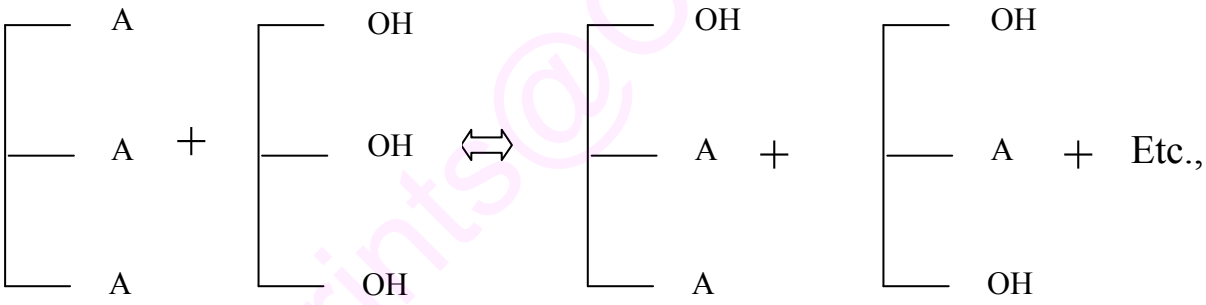
1. **Hydrolysis:** In aqueous media when there is large excess of water, ester hydrolysis is the dominant reaction.
2. **Esterification:** esterification becomes predominant if reactions are carried out in anhydrous solvents.
3. **Transesterification:** In transesterification reactions, acid moiety of an ester is exchanged with another one. If the acyl donor is a free acid, the reaction is called acidolysis and if the acyl donor is an ester, the reaction is called interesterification. In alcoholysis, the nucleophile alcohol acts as an acceptor.

Fig. 6 INTERESTERIFICATION

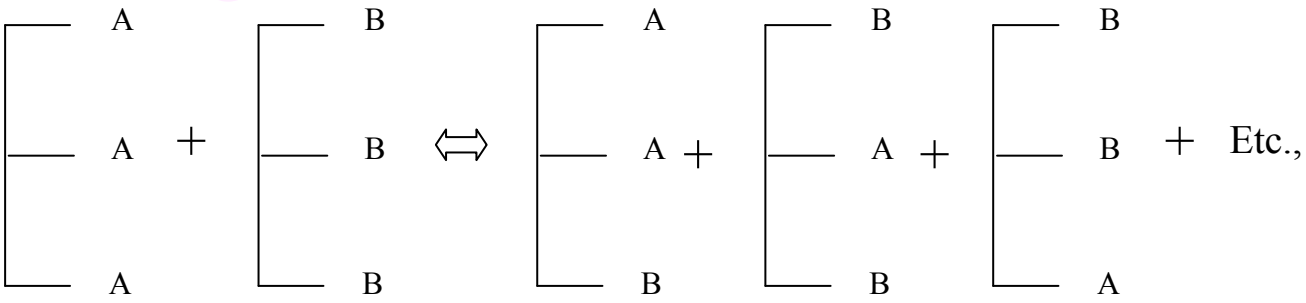
Acidolysis



Glycerolysis



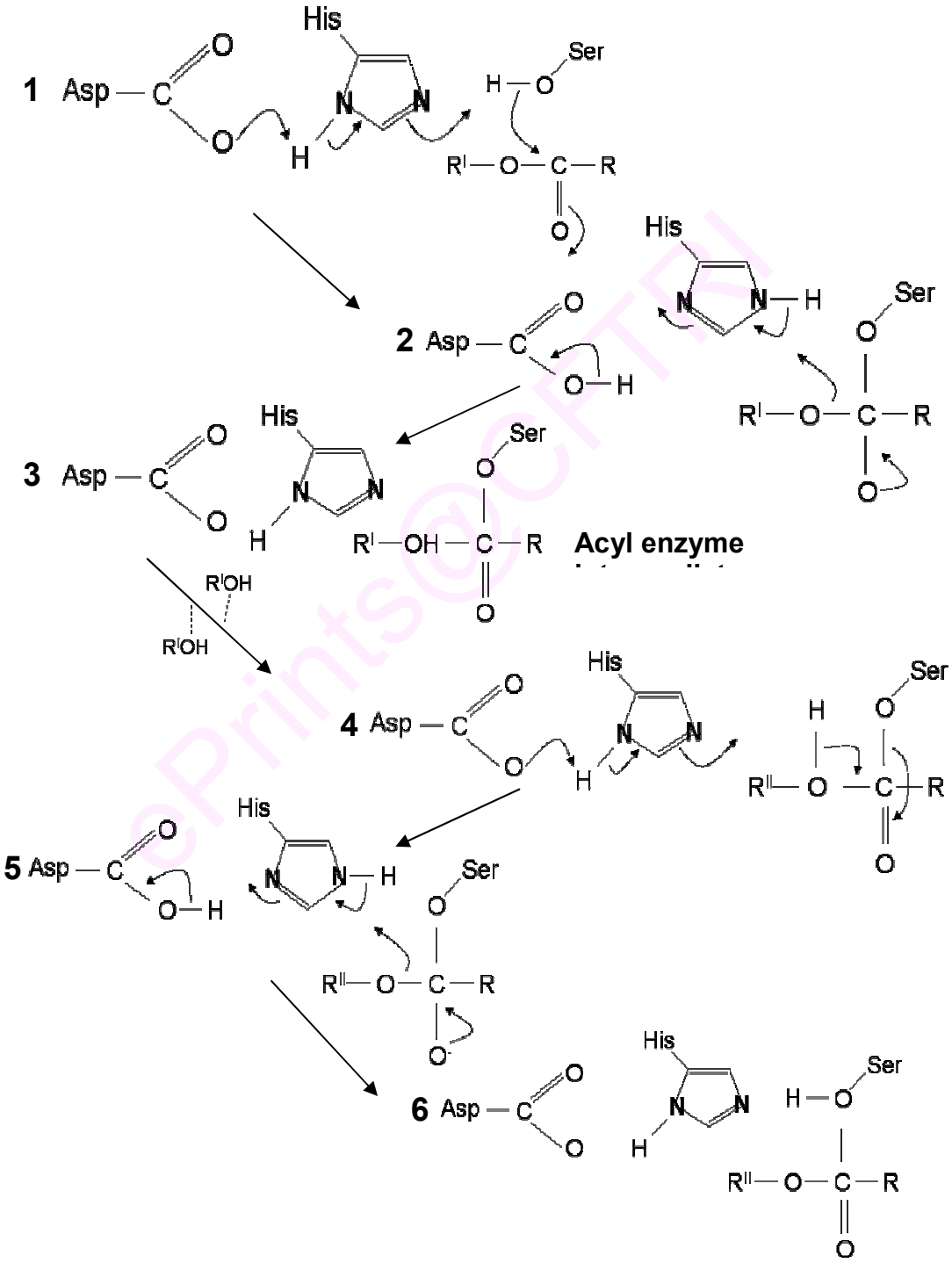
Transesterification



MODE OF ACTION OF LIPASES

Lipases act at the oil-water interface of heterogenous reaction systems. This property makes them well suited for reactions in hydrophobic media. Lipases differ from esterases in the involvement of a lipid-water interface in the catalytic process (Brockman, 1984). Some regions of lipase molecular structure responsible for the catalytic action are presumed to be different from those of ordinary enzymes that act on water-soluble substrates in the heterogeneous medium (Iwai and Tsujisaka, 1984). Because lipases work at substrate-water interfaces, a large area of the interface between the water-immiscible reaction phase and the aqueous phase that contains the catalyst is necessary to obtain reasonable rates of interesterification. Theoritical interpretations of the activation of lipase by interfaces can be divided into two groups: those assuming that the lipase undergoes a change to an activated form upon contact with an oil-water interface. The first interpretation assumes higher concentrations of the substrate near the interface rather than in the bulk of the oil; and the second involves the existence of separate absorption and catalytic sites for the lipase such that the lipase becomes catalytically active only after biding to the interface.

Fig 7. Mode of action of lipases (catalytic mechanism for lipase-mediated enzymatic interesterification). (Marangoni and Rousseau, 1995)



Mechanism of lipase mediated esterification catalysis

Lipases can also carry out esterification reactions if the substrates are provides in non aqueous medium. In case of *Rhizomucor miehei* lipase, the catalytic triad consists of Asp 203, His 257 and Ser 144. In the case of porcine pancreas lipase, the triad consists of Asp 176, His 263 and Ser 152.

1. Electron density on the primary hydroxyl group of serine is increased by the combined relay type action of Asp 203 and His 257. This allows nucleophilic attack of Ser 144 hydroxyl group on the carbonyl group of the acid moiety to form the tetrahedral intermediate. The tetrahedral intermediate contains a negative charge on the oxygen of the acid group. In case of *Rhizomucor miehei* lipase, this is stabilized by hydrogen bonding to amino acid residues, Ser 82 and Leu 145, which constitute the oxyanion hole.

2. The tetrahedral intermediate loses water, leading to the formation of acyl-enzyme complex where the acyl group is covalently attached to Ser 144.

3. In the next step, the acyl-enzyme complex is attacked by nucleophile like alcohol (R'-OH) to form the second tetrahedral intermediate. The nucleophile can also be any other compound like hydrogen peroxide, ammonia (Sheldon et al., 1994) and amines (Gotor et al., 1991).

4. Finally, the ester is released and the enzyme reverts back its original state (Fig. 7).

Immobilisation

Although enzymes have been used for several years to modify the structure and composition of food lipids they have only recently become available for large scale use in industry. The progress in genetics and in process technology may now enable the industry to offer enzymes with improved properties at reduced cost (Vulfson, 1993). For lipases to be economically useful in the industry, enzyme immobilization is necessary. This will enable reuse of enzymes and to facilitate the continuous processes.

Lipases are active at the oil-water interphase. To obtain high rates of reaction, the reaction systems should have a large area of interphase. This can be achieved by immobilization of the enzyme. Immobilisation refers to the localization or confinement of enzymes during a process that allows ready separation of the substrate from the product for reuse of the enzyme. The advantages of immobilizing lipases are repetitive use of a given batch of enzymes, better process control, enhanced stability, enzyme-free products (Frank, 1990), increased stability of polar substrates, shifting of thermodynamics equillibria to favour ester synthesis over hydrolysis, reduction in water dependent side reactions such as hydrolysis, thermal stability of enzymes, elimination of microbial contamination, potential to be used directly in chemical process (Chibata, 1978).

Immobilisation often causes drastic changes in the apparent measured parameters of the enzyme-catalysed reactions. The maximum velocity of reactions, Michaelis-menten constant, temperature and pH optima and effect of inhibitors may all change for an immobilized enzyme compared to the enzyme in the free form. When enzyme is immobilized, the degree and nature of this change will depend not only on the immobilization method used, but also on the enzymatic reaction.

Immobilisation of lipases has been performed by various methods such as adsorption, entrapment and covalent binding, using different supports. For optimum activity, immobilization of enzyme on supports must involve reactions of proper strength between the enzyme and support. While very strong interactions result in loss of activity, weaker interactions lead to poor stability. Thus operational stability of an immobilized matrix depends on the choice of support and the method of immobilization. The materials tested as solid supports for

immobilization of lipase include ion exchange resins (DEAE-Sephadex A-50 or Amberlite IRA94) (Yang and Rhee, 1992), Silica gel (Sonnet et al 1994) and microporous polypropylene (Vitro et al., 1994). In other cases, lipases have been immobilized by entrapping them in gels of photo-cross-linkable resins (Yang and Chen 1994).

LIPASES IN MODIFICATION OF FATS AND OILS.

Immobilised lipases can be used to impart new and improved physical and nutritional properties to triglycerides. Enzymatic interesterification can improve structuring and/or melting behaviour of fats for application in products such as spreads, nondairy creams and confectionaries. The synthesis of structured triglycerides for cocoa butter substitutes (Yankah and Akoh, 2000) MLM lipids (triglycerides with medium chain length at sn-1 and sn-3 and a long chain at sn-2) employ 1,3 selective lipase. The synthesis of SLs enriched in PUFA (Xu et al., 2000) or oleic acid (Yu and Hammond, 2000) require fatty acid specific lipase. Interesterification has been applied to edible oil to randomize the fatty acid distribution in triglycerides to prevent phase separation in fats such as lard and to generate hard butters by interesterifying fully hydrogenated oils and coconut oils. Interesterification of a blend of palm stearin and coconut oil was catalysed by an immobilized Thermomyces lanuginose lipase for production of margarine fats (Zhang et al., 2001). This type of triglyceride rearrangement is also used to modify the crystallization behaviour and melting points of the solid fats. Another potential application of lipase is the synthesis of Zero-trans margarines (Marangoni and Rousseau, 1995). Pal et al., have modified butter stearin fraction by blending and interesterification with liquid oils like sunflower and soyabean oil using *Mucor miehei* lipase to offer nutritionally important fat products with enriched content of essential fatty acids like C 18:2 and C 18:3. These fat products have desirable properties that can be used in making mélange spread fat with polyunsaturated fatty acids (Pal et al., 2001).

APPLICATIONS OF LIPASE CATALYSED ESTERIFICATION REACTIONS

Lipases find wide applications in the preparation of many commercially important esters.
Lipase catalysed interesterification is often preferred over chemical interesterification for specificity (Akoh and SISTA, 1995; Ray and Bhattacharya 1995).

SLs incorporating MCFA (Capric,Caprylic,Caproic) are being used in the medical fields as a fat

for nutritional feeding. Sandoz nutrition (Inform 1990) launched a product called IMPACT containing SLs made from a high lauric acid oil and a high linoleic acid oil which is beneficial for patients who have or at risks of developing hyper metabolism-induced immune suppression.

Caprenin, a structured lipid produced by Procter and Gamble company (Cincinnati,OH) consists of C8:0- C10:0. It has the physical properties of cocoa butter but provides only about half of the calories. Benefat, originally produced as SALATRIM, consists of short chain (C2:0-C4:0) and long chain (C18:0) fatty acids. Both products can be used as cocoa butter substitutes. Because of the low caloric value of SCFAs and the partial absorption of stearic acid on SALATRIM, this product has strong potential for use as a low calorie fat substitute. The caloric content of the Caprenin and the Benefat is about 5 Kcal/g (Vs. 9 kcal/g for regular TG). Unilever scientists launched a product called Betapol, a SL made by reacting tripalmitin with unsaturated fatty acid of human milk (Quinland and Moore 1993). These SLs can also be manipulated for nutritive and therapeutic use. Captex 810D is a reduced calorie SLs produced by Abitic corporation (Columbus, Ohio, USA). Akoh et al., (1998) found that a Captex diet resulted in increased heat production and altered energy metabolism in obese Zucker rats. McKenna and others also reported improved absorption of 18:2 (n-6) when captex was administered to cystic fibrosis patients (McKenna, 1985). Another reduced calorie SL containing oleic (C18:0) and behenic (C22:0) acids, Bohenin, was designed to slow or prevent fat bloom (fat crystallization) formation in chocolate products. It promotes the formation of stable crystals and provides 5 Kcal per/g. Thus interesterified fats provide range of products with desired physical properties, improved nutrition and therapeutic advantages.

AIM AND SCOPE OF THE STUDY

Vegetable oils are part of traditional diets all over the world. They provide energy, essential fatty acids, substrates for forming biological membranes, eicosanoids, are carriers for fat-soluble Vitamins (A, D, E and K) and anti-oxidants. The fatty acid composition of edible oils has been one of the main focuses in defining the nutritional value of dietary fats. Dietary fatty acids play a crucial role in modulating plasma cholesterol concentrations and modifying the risk for coronary heart disease (Temme, 1996). Coronary Heart Disease is progressively increasing in Indian population and projected to be the number one killer in the next decade (Gupta et al., 2002) Regional difference in the dietary habit in general and type of fat consumption in particular is observed in India. Groundnut oil, sunflower, mustard oil, coconut oil, sesame oil are few of the vegetable oils consumed in India.

Coconut oil is consumed in Kerala and costal Karnataka regions of India. Groundnut oil is consumed in central and southern part of India. Olive oil is a main component of Mediterranean diet. In India there is no oil equivalent to olive oil, and imported olive oils are expensive. The high cost of olive oil makes it beyond the reach of common man. Olive oil contains high levels of monounsaturated fatty acid. Studies have suggested that the glyceride and nonglyceride fractions of olive oil provides cardio protective effects. CO is characterized by high levels of medium chain fatty acids, mainly lauric acid and small amounts of capric, caprylic and caproic acid. Therefore a combination of CO with olive oil may provide benefits of these two oils at an affordable cost.

To get the optimum benefits from the oils, the Indian Council Of Medical Research recommended the intake of fat by an adult at 25-30% and the proportions of saturated, oleic and polyunsaturated fatty acids to be in the ratio of about 1:1:1 (ICMR, 1992). American Heart Association also recommends 30% total calorie from fat and the ratio of Saturated/ Monounsaturated / Polyunsaturated fatty acid at 1:1:1 (AHA, 1990).

Careful analysis of edible oils available in India indicates an imbalance in their composition and is not in tune with recommendations of the expert committee. This can however be

overcome by blending two selected oils in appropriate proportions. Recently Government of India has legally allowed blending of any two vegetable oils in which one of the oils should be present to a minimum of 20%.

Apart from the fatty acid composition of a particular TG, the positional distribution among sn-1,-2 and sn-3 in the glycerol backbone determines their absorption and nutritional quality (Edward Hunter, 2001). In this respect, tailored vegetable oils with nutritionally superior benefits combined with altered physicochemical properties may be preferred in future by the consumers. Structured lipids prepared by interesterification reactions are tailor-made fats and oils with improved nutritional or physical properties.

Numerous investigations have demonstrated that SL have different metabolic actions compared to physical mixtures of oils used for blending (Quinland and Moore, 1993). This interesting observation need to be exploited to develop oils which can provide superior health benefits.

To provide balanced edible oils to Indian population, the present study aims at the preparation and evaluation of the blended and interesterified oils using Coconut oil and unsaturated oils like Ground nut oil, and Olive oil. Protocols were developed for blending CO with GNO in which the saturated, monounsaturated and polyunsaturated levels were balanced to give approximately 1:1:1 proportion and CO:OLO in which the proportion of SFA:total Unsaturated fatty acids are balanced to give 1:1 ratio. They were further subjected to lipase catalyzed interesterification reactions.

The changes in the physicochemical properties of the blend and interesterified oils were monitored by Differential Scanning Calorimetry. Finger printing of the triglycerides of modified oils were carried out using HPLC. The effect of these modified oils on growth and lipid profile were studied in rats and compared with those given native oils. Influence of these blended and interesterified oils on the activities of enzymes involved in the antioxidant defense systems, susceptibility of LDL to oxidation, platelet aggregation was also monitored. These studies provide an insight in to physicochemical and nutritional changes that were brought about by modifying the oils using lipase catalysed interesterification reactions.

Materials & Methods

MATERIAL

Refined coconut oil, groundnut oil and extra virgin olive oil were obtained from a local super market. Lipozyme IM-60 was gift from NOVO Nordisk Bioindustrial Inc., (Danbury, CT, USA). Boron triflouride in methanol, triolein, zeolite, sodium metaperiodate, NADPH, 5,5'-dithio bis (2-nitrobenzoic acid), collagen, adenosine diphosphate, trizma base, bovine serum albumin, dipalmitoyl phosphatidyl choline, caprylic acid (10:0), lauric (12:0), myristic (14:0), oleic (18:1), linoleic (18:2), linolenic (18:3), thiobarbituric acid, glutathione, glutathione reductase, hydrogen peroxide, NADPH, 1-chloro 2, 4 dinitrobenzene were purchased from sigma chemical Co, (St. Louis, MO, USA). Heparin and manganese chloride, EDTA were obtained from Sisco Research Laboratory (Mumbai, India). Choline chloride, DL-methionine, tocopherol acetate and all vitamins were obtained from Hi-media Laboratories, Mumbai, India. Calcium phosphate, potassium citrate monohydrate, potassium sulphate, magnesium oxide, manganese carbonate, ferric citrate and sodium selenite were from Loba Chemie, Mumbai, India. Fatty acid standards were obtained from Sigma Chemical Co (St. Louis, MO, USA). Casein was purchased from Nimesh Corporation, Mumbai, India. All solvents were of analytical grade and distilled before use. 1, 1, 3, 3 tetramethoxy propane was purchased from Fluka (Switzerland). Phosphotungstic acid and cadmium acetate were purchased from Qualigens, Mumbai, India. HPLC grade hexane, isopropanol, methanol, butanol, acetonitrile, benzene were obtained from E. Merck, Mumbai, India.

METHODS:

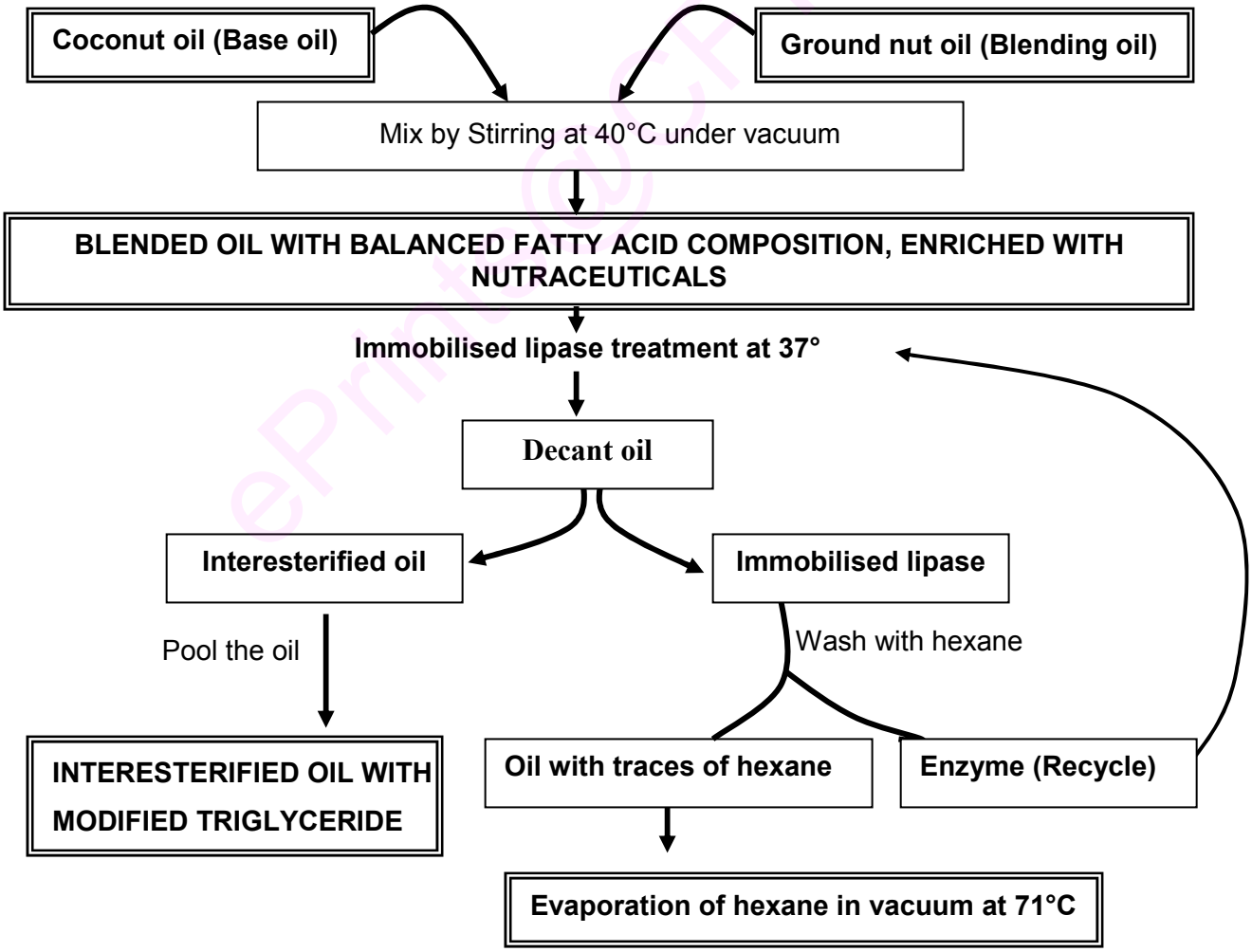
Blending of Oils.

Fatty acid composition of individual oils were determined by gas chromatography. Blends of two oils were prepared by mixing appropriate amounts of individual oils so that the proportion of saturated: monounsaturated: polyunsaturated was approximately 33:33:33% in CO: GNO combination and approximately 50:50% of SFA: Total unsaturated fatty acids for CO: OLO combinations. The blended oils were mixed by stirring after flushing with nitrogen at 40°C for 1 hr. The mixing efficiency was followed by periodically withdrawing the aliquots and determining the fatty acid composition of resulting blend and comparing it with theoretical values.

Interesterification reaction.

The blended oils were subjected to interesterification reaction with 0.5% immobilized lipase lipozyme IM-60 (specific activity 6.2±0.2 µmole/mg protein) from *Mucormiehei* at 37°C. Incubations were carried out in an orbital –shaking incubator at a rotary speed of 150 rpm for periods ranging from 1 hour to 96 hours. After the incubation period, the oil was decanted, the immobilized enzyme was washed using hexane to collect the traces of oil entrapped and hexane was removed under vacuum using flash evaporator. This oil was added back to decanted oil.

Scheme 1: PROTOCOL FOR THE PREPARATION OF MODIFIED OIL



Fatty acid analysis.

Fatty acid composition of oils, dietary lipids, serum, liver were analysed as methyl esters by gas chromatography (Fisons, fitted with FID) using fused silica capillary column 25m x 0.25 mm. The methyl esters of fatty acids were prepared using BF_3/MeOH as described by Morrison and Smith (1963). The operating conditions were Column temp 120°C, injection temp 230°C and detector temp 240°C. The column temp was programmed to rise at 10°C/ min and the final temp was 220°C. Nitrogen gas 1ml/ min was used as carrier. Individual fatty acids were identified by comparing with retention times of standard fatty acids.

Determination of peroxide value of oils.

Peroxide value (PV) was determined by AOCS official method (1990). Five gram of oil is weighed in to a conical flask and 30 ml of (3:2) acetic acid: chloroform mixture is added along with 0.5 ml of potassium iodide and kept closed for a minute followed by the addition of 25ml of distilled water; 0.2 ml of starch was added and titrated against standardized 0.1 N sodium thiosulphate. PV of the oil is calculated as mill equivalents of oxygen per thousand gram of the oil sample, using the formula PV= Volume of sodium thiosulphate consumed × N × 1000/ weight of the oil.

Determination of free fatty acid.

Free fatty acid (FFA) was determined as described in official methods and recommended practices by AOCS (1989). Five gram of the oil was taken in pre-weighed conical flask and to this 30 ml of the alcohol, which was neutralized with phenolphthalein indicator was added and the contents are heated to boiling pint. This was titrated in hot condition against 0.1 N NaOH. The percentage of free fatty acid in most of the oils was expressed as percent oleic acid. However, for coconut oil it was expressed as percent lauric acid. Free fatty acids of oleic % = ml of alkali × 28.2/ wt. of the sample.

Free fatty acids as lauric % = ml of alkali × 20.0 / wt. of the sample

Determination of tocopherols.

1 gram of oil was taken and saponified by refluxing with 4ml of pyragallol ethanolic solution, followed by addition of 1 ml of KOH and refluxed with ethanol for 3 mins. The unsaponifiable matter was then extracted with diethyl ether. The ether extracts were washed with Benzene and hexane and evaporated under vacuum at 40°C. The tocopherol extract was then dissolved in Heptane. The tocopherols in heptane was reacted with a mixture of ferric chloride and 2,2' bipyridyl in ethanolic solution which gives a characteristic red color which was read at 520nm. The tocopherols were quantitated using α-tocopherol as reference standard (Joshi and Desai, 1952).

Determination of tocopherols by HPLC method

Tocopherol content in the oil was analyzed by HPLC (Shimadzu Corp., Tokyo, Japan) using a Phenomenex C18 column (250 x 4.60mm, 5µ). The mobile phase used to separate tocopherol and tocotrienols isomers was prepared using acetonitrile, methanol, isopropyl alcohol and 1% acetic acid, at 45:45:5:5 (v:v:v:v) in pump A and 25:70:5:0 (v:v:v:v) in pump B and time programmed as described by Chen and Bergman (2005). The isomers of tocopherols and tocotrienols were monitored at 298 nm.

Determination of Total polyphenols.

The Folin-Ciocalteau reagent assay was used to determine the total phenolic content (Mosca et al., 2000). A sample aliquot of 100 µl was added to 900 µl of water, 0.5 ml of Folin-Ciocalteau reagent previously diluted with distilled water (1:2 v/v) and 1 ml of 10% sodium carbonate solution in distilled water, mixed in a cyclomixer. The absorbance was measured at 675 nm after incubation for 60 mins at room temperature. Gallic acid was used as standard for calibration curve.

Determination of Phenolic acids by HPLC method

The potential active components in sample extracts were characterized by HPLC (model LC-10A. Shimadzu Corporation, Tokyo, Japan) analysis on a reverse phase Shimpak C_{18} column (4.6 × 250 mm) using a diode array UV-detector (operating at 280 nm). A solvent system consisting of water:acetic acid:methanol (isocratic; 80:5:15, v/v/v) was used as mobile phase at a flow rate of 1 ml/min.

Fingerprinting of triglyceride molecular species.

Triglyceride profile of the oils were analysed using a non-aqueous reverse phase HPLC (Shimadzu liquid chromatograph, LC-10AD), equipped with commercially packed C18 column. Sample was loaded in 20µl acetone and then eluted with acetone: acetonitrile (65:35 v/v) at a flow rate of 1ml/min. The molecular species of triglycerides were monitored using a refractive index detector (Swe et al., 1995).

Differential Scanning Calorimetry.

The melting and crystallization profiles of blend and interesterified oils were determined using a Differential Scanning Calorimetry (DSC 30, Zurich, Switzerland). The instrument was provided with auto accessory connected to liquid nitrogen to cool the DSC cell at the required rate. The DSC was calibrated with indium, lead and tin as reference standards by the supported thermal software attached to computer with the hard current in the cell. Sample of 5-10 mg of triglycerides was placed in an aluminum pan and hermitically sealed. An empty pan served as reference. The samples were initially subjected to tempering by heating rapidly (10°C/min) from room temperature to 80^0 C and holding at that temperature for 5 min to destroy the crystal memory. The samples were then cooled at the rate of 10°C /s to -80°C and holding at this temperature for 5 mins. Heating and Cooling thermograms were recorded and used for determination of temperature for onset, completion, melting and crystallisation range and also for enthalpy of melt and crystallization. The percentage solid fat content (SFC) was determined from the melting profiles.

NUTRITIONAL STUDIES

Experimental animals.

Male Wistar rats 45±3g [OUTB-wistar, IND-cft (2c)] were grouped by random distribution (n=5 animals per group). They were placed in individual cages in an approved animal house facility and fed fresh diets daily. The animals had free access to food and water throughout the study. The experimental protocol was approved by the institutional animal ethical committee.

Diet composition.

The rats were grouped by random distribution and fed AIN-76 (American Institute of Nutrition) purified diets (Anon 1977). The basal composition of the AIN-76 purified diets is given table 1 Native oils, blend and interesterified oils as indicated were used as a source of fat in the diet. Fresh diets (20g/day) were given to rats daily for a period of 60 days. Food intake by animals was monitored daily. Animals had free access to food and water throughout the study.

Table 1: Composition of AIN-76 purified diet

Ingredients	g/Kg
Sucrose	600
Casein	200
Fat	100
Cellulose	50
AIN-76 vitamin mix[1]	10
AIN-76 mineral mix[2]	35
Choline chloride	2
Methionine	3

[1]100g vitamin mix contained 60mg thiamine hydrochloride, 60 mg riboflavin, 70mg of pyridoxine hydrochloride, 300 mg of nicotinic acid, 160mg D-calcium pantothenate, 20mg folic acid, 2mg D-biotine, 0.1mg cyanocobalamine, 40,000/IU vitamine A (retinyl acetate) 5,000/IU vitamine E (tocopherol acetate). 0.25 mg cholecalciferol, 0.5mg menadione and made to 100g with sucrose.

[2]100g mineral mix contained 50g calcium phosphate, 7.4g sodium chloride, 22g potassium citrate monohydrate, 5.2g potassium sulphate, 2.4g magnesium oxide, 0.35g manganese carbonate, 0.6g ferric citrate, 0.001g sodium selinite 0.16g Zinc carbonate, 0.03g cupric carbonate (55%Cu), 0.001g potassium iodate and 0.0213g potassium chromate and made up to 100g with sucrose.

SERUM AND LIVER LIPID ANALYSIS

Isolation of serum

After 60 days on specified diets, rats were fasted overnight and sacrificed under ether anesthesia. Blood was drawn by cardiac puncture and kept at 4°C for 2h. Serum was separated by centrifugation at 700g for 10 mins in a table top refrigerated centrifuge and serum was stored at-20°C till analysed. Liver was removed and rinsed in ice-cold saline, blotted, weighed and stored at -20°C until analyzed.

Total lipid extraction

Serum: Lipids from serum was extracted by the method of Bligh and dyer (Bligh and Dyer, 1959). 1 ml of serum was taken and 3.75 ml 1:2 (v/v) chloroform: methanol was added and vortexed well for 15 mins. Then 1.25 ml of chloroform was added and vortexed well. Finally 1.25 ml distilled water was added vortexed well for 1 mins. Centrifuged at 1000 RPM in table-top centrifuge for 5 mins at room temperature to give a two-phase system (aqueous top, organic bottom).Bottom organic phase was recovered by using Pasteur pipette.

Liver – total lipids from liver tissue was extracted by Folch method (Folch et al., 1957) One gram of tissue was homogenized with 1.0mL of 0.74% potassium chloride in a potter Elvehjem homogenizer. To the homogenate, 20ml of chloroform: methanol (2:1 v/v) was added and homogenized for 2mins. The mixture was left overnight and filtered through whatman no.1 filter paper. 3ml of 0.74% potassium chloride was added and mixed well by vortexing. The solution was allowed to stand at room temperature. The upper aqueous layer was removed carefully and then lower phase was washed with 3ml of 0.74% potassium chloride and then twice with 3m of chloroform: methanol: water (3:48:47 v/v) mixture. The chloroform layer was used for lipid analysis.

Total cholesterol estimation.

Serum and liver cholesterol levels were quantitated by the method of Searcy and Bergquist (1960). An aliquot from the chloroform extract was dried under a stream of nitrogen followed by the addition of 1.5ml of ferric chloride–acetic acid reagent. The reagent was prepared by diluting the stock containing 504mg cholesterol/ml anhydrous $FeCl_3$ in 10ml glacial acetic acid to 1:100 dilution with glacial acetic acid. After mixing thoroughly, it was left at room temperature for 15 mins. 1ml of concentrated sulphuric acid was added, mixed immediately on a vortex mixer and left at room temperature in dark for 45 mins. The optical density of the clear solution was measured in the spectrophotometer (Shimadzu 160A model) at 540 nm. The cholesterol level in biological sample sample was estimated from the standard curve generated with Anal R cholesterol (30-150microgram).

HDL cholesterol estimation.

To 0.5ml of serum, 25µl of heparin (5,000 units/ml) was added, followed by addition of 25µl of 2M manganese chloride (3.969g/10ml). The solution was vortexed and kept at 4°C night (Warnick and Albers, 1978). This was then centrifuged at 1100g for 20 mins. Cholesterol in HDL fraction was measured as described earlier after extracting the supernatant with 3ml of acetone: alcohol (1:1 v/v).

LDL+VLDL Cholesterol estimation.

The precipitate obtained from serum after adding heparin and manganese chloride contained LDL+ VLDL. The precipitate was dissolved in 0.5ml of saline and cholesterol was extracted in 3ml acetone: alcohol (1:1 v/v) and cholesterol was estimated as described earlier.

Phospholipid estimation.

Phospholipids were analysed by ferrous ammonium thiocyanate method (Stewart, 1980) using dipalmitoylphosphatidyl choline (10 to 100µg) as reference standard. The lipid extract in chloroform was evaporated under a stream of nitrogen and then dissolved in 2ml of chloroform. Two ml of ferrous ammonium thiocyanate was added and vortexed for 1 mins.

Following the phase separation, absorbance of chloroform phase was measured at 488 nm in Shimadzu 160 A spectrophotometer.

Triglyceride estimation.

Triglycerides were estimated by the method of Fletcher (1968) using triolein as reference standard (30-300µg). The lipid extract in chloroform was evaporated under a stream of nitrogen and then dissolved in 4ml of isopropanol. Two grams of zeolite was added, mixed well and centrifuged. Supernatent was transferred to another test tube, saponified with 0.6ml of 5% potassium hydroxide in isopropanol: water (40:60 v/v) at 60^0 C for 15 mins. After cooling, 1ml of sodium metaperiodate prepared from the stock solution of 0.025M in 1N acetic acid (Sodium metaperiodate (12ml)and 20 ml of isopropanol and made up to 100ml 1N acetic acid) was added, mixed and 0.5ml of acetyl acetone was added, stoppered and incubated at 50^0 C for 30 mins. After cooling to room temperature, the color intensity was read at 405nm in shimadzu 160 A spectrophotometer.

Estimation of lipid peroxidation in liver homogenates by TBARS method.

1 gram of liver was homogenized in 10ml of 0.15M potassium chloride, in a teflon homogenizer. The homogenate was filtered through cheesecloth and used for assay. Liver homogenates (4mg protein) in 0.15m potassium chloride, 0.025M Tris-hydrochloride buffer pH 7.5, 2mM adenosine diphosphate and 10 µM ferrous sulphate were incubated at 37°C for 5 mins. The reaction was initiated by adding 0.1 mM ascorbic acid and incubated at 37°C for 30 mins. The final volume of the reaction mixture was 1ml. The reaction was terminated using 2ml of thiobarbituric acid (0.375% thiobarbituric acid, 15% trichloroacetic acid in 0.2N hydrochloric acid) containing 10µM butylated hydroxyl anisole. Samples were heated for 15 mins in a boiling water bath. Malondialdehyde (MDA) formed was measured at 535mm and quantitated using an extinction coefficient of $1.56x10^{-5}$ cm^{-1}. The lipid peroxides were expressed as nmoles of MDA/mg protein. Appropriate blank samples were included for measurements (Buege and Aust 1989).

Enzyme activities.

Superoxide dismutase activity was measured by the inhibiton of epinephrine and monitored at 430nm (Hara and Irwin, 1972). One unit of superoxide dismutase was defined as the amount required to inhibit epinephrine oxidation by 50%. Catalase activity was assayed according to the method of Abei (1984) by following the decomposition of hydrogen peroxide at 240nm. Glutathione peroxidase activity was determined by NADPH oxidation in a coupled reaction system consisting oxidized glutathione (Tappel, 1978). Glutathione transferase was measured with 1-chloro 2, 4-dinitrobenzene as the substrate (Hoilg et al., 1974). The enzyme activity is expressed as μmoles of CDNB-GSH conjugates formed by per mins per mg protein. All spectrophotometic measurements were carried out in a Shimadzu ultraviolet spectrophotometer with 1.0 ml quartz cuvettes. Specific activities were expressed as μmol/min per mg protein.

Platelet aggregation.

Blood was collected in heparinised tubes and platelet rich plasma (PRP) was separated from erythrocytes and leucocytes. Washed platelets were prepared as described by Brunaer and Huestis (1993) and suspended in tyrodes buffer. Platelets (40,000 x 10 cells/μl) were incubated for 15 mins 37°C prior to use. Platelet aggregation measurements were performed using a Chrono-log dual channel aggregometer (Denmark). 450μl of platelet suspension was stirred at 1000 rpm at 37°C and 10 μl of ADP (25 μM) or 15 μl of collagen (5mg/10ml 0.1 N acetic acid) was added and aggregation followed for atleast 5mins which was recorded using aggregometer. Platelet aggregation was quantitated as described by Niranjan and Krishnakantha (2000).

Estimation of malondialdehyde (MDA) formed in platelets.

Platelets after aggregation (0.45ml) were transferred to an eppendorf tube containing BHT in ethanol plus 0.1 ml of 100% TCA in 3 N HCl and centrifuged at 10,000 rpm for 10 mins. From this 0.5 ml of supernatant was taken and mixed with 0.1 ml of TBA reagent (0.12 M TBA in 0.26 M Tris-HCl). The contents were left in boiling water bath for 30 mins and colour generated was measured at 532 nm. The malondialdehyde (MDA) was calculated by using following equation-

Amount of MDA formed = (Absorbance/156) × (Total volume/mg of protein/ml)

MDA was expressed in nmoles of MDA formed /mg protein/hr (Maguire and Csona-Khalifah, 1987).

Na^+ K^+ ATPase.

The activity of Na^+ K^+ ATPase in RBC membrane was assayed as described by Savitha and Panneerselvam (2006). 1ml reaction mixture contained final concentrations of 5mM ATP, 100mM NaCl, 20mM KCl, 5mM $MgCl_2$, 0.2 M Tris buffer (pH 7.4) and 30-50µg of enzyme protein. The total ATPase activity was measured with Na/K^+ and Mg^{2+} present in the reaction mixtures. The Mg^{2+} ATPase was measured in the presence of 1mM ouabain, a specific inhibitor of Na^+/ K^+ ATPase enzyme. The difference between Total ATpase and Na^+/ K^+ ATPase was used for calculating ouabain insensitive ATpase. The incubation was carried out at 37°C for 20mins. The reaction was terminated by the addition of 0.5 ml of 5% TCA. Thirty mins after addition of 1ml reagent containing 1% (w/v) ammonium hepta molybdate, 40 mg/ml $FeSO_4$ and 1.15 N H_2SO_4 to each tube, the released phosphate was estimated at 690 nm and quantitated using KH_2PO_4 (1-10 ng) as reference standard. The enzyme activity is expressed as µmoles of phosphate liberated/hr/mg protein.

$Ca^{+2}Mg^{+2}$ ATPase.

The ATPase activity of RBC membrane was determined by measuring the inorganic phosphate liberated from the hydrolysis of ATP. The reaction medium contained 0.2 M Tris-HCl buffer pH 7.4, 5mM $MgCl_2$, 4mM ATP and 25-40µg of protein as an enzyme source. The reaction was carried out at 37°C for 20mins then stopped by addition of 5% trichloro acetic

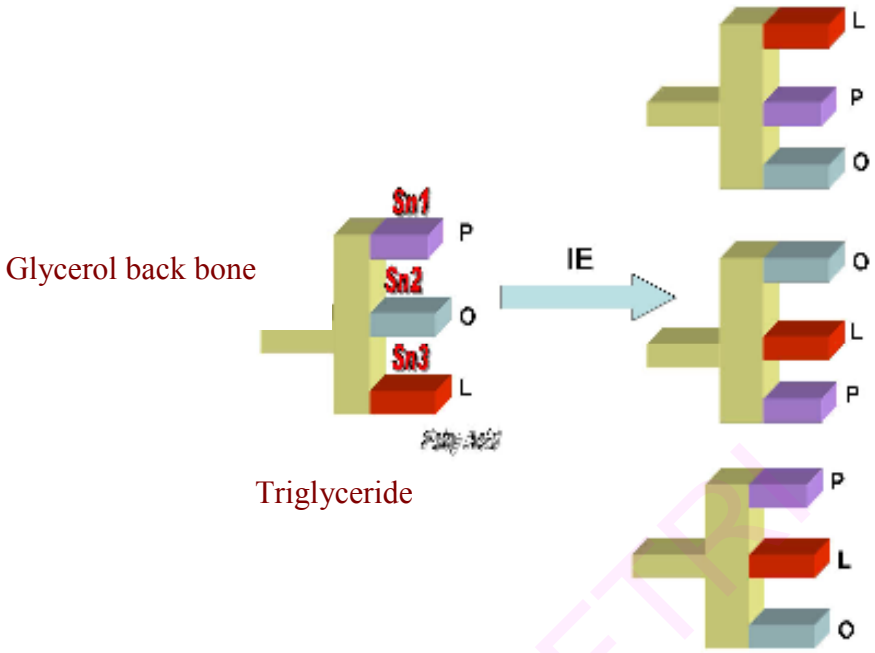
acid. Thirty mins after addition of 1ml reagent containing 1% (w/v) ammonium hepta molybdate, 40 mg/ml $FeSO_4$ and 1.15 N H_2SO_4 to each tube, the released phosphate was measured using a spectrophotometer. The total ATPase activity was measured with Ca^{2+} and Mg^{2+} present in the reaction mixtures while Mg^{2+} ATPase was measured in the presence of 0.5mM EGTA. Ca^{2+} activity was obtained by subtracting Mg^{2+} activity from Total ATPase activity (Savitha and Panneerselvam, 2006).

Protein estimation.

Protein was estimated by the method of Lowry et al. (1951) using bovine serum albumin as reference standard.

STATISTICAL ANALYSIS.

Data was expressed as mean ± SD. Analysis of variance was employed to evaluate the difference between the groups. A difference of $p<0.05$ was considered significant (Fisher, 1970).



Chapter 1

Preparation of Blend and Interesterified Oils and Effect of Blending and Interesterification on Physicochemical Properties of Oils

INTRODUCTION

The types of the fatty acids in a TG molecule play an important role in the nutritional and functional properties of the fats and oils. Studies have suggested that SFA raise TC, LDL and that PUFA lower them. SFA exert differential effects on the LDL/HDL ratio. Fats rich in myristic and palmitic acid (e.g., milk fat, coconut oil and palm kernel oil) raise LDL the most. Stearic acid (18:0) is neutral in its effect on blood cholesterol when consumed in natural fats. The linoleic acid has the greatest impact on regulating the LDL/HDL ratio, whereas linolenic acid and its longer chain derivatives have a major influence over the clotting mechanism. Diets enriched in 18:3(n-3) or 22:6(n-3) have been shown to exert a significant anti-CHD effect on humans in clinical and epidemiological studies. However the excess intake of PUFA may be harmful as they are vulnerable to oxidation and hence the P/S ratio as a criterion for nutritional assessment of fats is not adequate. MUFA is present at different levels in almost all oils. Apart from supplying energy they also have cushioning effect on individual fatty acids and has prompted oil technologists & nutritionists to search for an ideal oil which has the SFA:MUFA:PUFA ratio of 1:1:1. According to American Heart Association, the optimum intake of fat for an adult is 30% of total calorie and the ratio of Saturated / Monounsaturated/Polyunsaturated fatty acid as 1:1:1 (American Heart Association: 1990), which was also endorsed by studies in India (ICMR, 1989). Japanese ministry of Health and Welfare has slightly modified this ratio to 1.1:1:1, World Health Organization recommends intake of fats with PUFA/SFA ratio of 0.8 to 1.0 and linoleic (omega 6) to α-linolenic (omega 3) ratio of 5-10 in the diet (FAO/WHO Report of a joint expert consultation, 1994).

Each oil is unique in its fatty acid composition and also in its minor components. The fatty acid composition of various vegetable oils commonly used in India indicates a wide variation in their composition. The right combination of fatty acid in triglyceride of oils and fats as suggested by the nutritionists cannot be always met from a single natural fats and oils. Balancing of fat is traditionally carried out by physical mixing of two oils containing fatty acids of different chain lengths to meet specific requirements (Adolph, 1999, & Rubin et al., 2000). Physical mixtures of medium chain triglycerides and long chain triglycerides have proven useful in enteral and parenteral nutrition (Adolph M, 1999). Blending of edible oils has now been accepted by Government of India and currently blends of two edible Vegetable oils are permitted in which the proportion of any one oil has to be no less than 20% (Indian standard Blended edible vegetable oils- specification BIS, IS: 14309, ICS 67.200.10 1995 & Padmavathy, 2001). In

Blended oils, the structure of triglyceride molecules from individual oil is not altered and the original digestion, absorption and physiological effects of these triglycerides remain unaltered even when they are presented as mixtures with other oils. Therefore it is not clear whether blended oils can provide all the perceived benefits when two oils of different composition are mixed. This drawback may be overcome by rearranging the fatty acids in the triglyceride molecules using chemical or enzymatic interesterification process. Studies on the effects of different dietary lipid emulsions have demonstrated significant advantages with the interesterified fats compared with the physical mixture of the oils and fats. Lipases can catalyse the modification of fats and oils either by transesterification or interesterification reaction under appropriate conditions, which in turn may overcome differences in physical properties of individual triglycerides in blended oils (Casimir, 1997).

Every oil or fat has characteristic fatty acid (FA) and triglyceride (TG) profiles, which are unique to the oil. The principal variation in FA composition of oils and fats is the chain length and degree of unsaturation of the component FA. This variation in FA composition can influence the bioavailability of individual fatty acids and digestibility of oils and fats in infants and adults (Willis et al., 1998). Analysis of the TG composition of an oil or fat requires a method of separating their complex mixtures in to their individual components or in to simpler mixtures that contain only a few TG each. The complex mixtures of TG from oils and fats have been usually analyzed by reverse phase HPLC (Ruiz-Sala, 1996). The finger printing of TG composition in oil contains structural information, which reflects on its physical as well as nutritional properties.

The physical characteristics of a fat are determined by the nature of the TG species it contains. Oils containing TG of higher-melting fatty acids tend to show cloudiness or remain as solid at room temperature while oils containing unsaturated FA will be in liquid form. Transesterification between a fat and oil typically involves exchange of fatty acid residues between the native triglycerides and is accompanied by a concomitant change in the properties of the original oil. The randomization of fatty acids with different degrees of unsaturation may also affect the physical nature of the triglycerides in the oil.

TG in individual oils influences the thermal profiles of oils and fats (Md Ali, 1994). Thermal behaviour of oils and fats can be used to elucidate their physical and chemical properties. Oils

and fats can exhibit extremely complex thermal behaviour (Tan and Che man, 2000), which will be highly dependent on its overall detailed chemical composition. Oils and fats do not have specific melting and crystallization temperatures. Rather they show melting/crystallization profiles. In light of this constraint, investigations were directed towards understanding basic information about the relationship between the chemical composition and thermal profile of different edible oils. It is well known that melting and crystallization behaviours of edible oils and fats are two important properties for giving right functionality to many prepared food products. The thermal properties are the counterparts of the TG profile in edible oils and fats (Breitschuh, 1996). Crystallization of liquid oil results in a volume contraction and a positive exothermic heat effect. Melting of fat results in volume expansion and a negative (endothermic) heat effect. Differential scanning calorimetry (DSC) is the most widely used technique for characterizing the thermodynamic properties of edible oils and fats (Tan and Che. 2000).

In the present study blends of CO:GNO were prepared in which the saturated, monounsaturated and polyunsaturated fatty acid levels were balanced to give approximately 1:1:1 proportion. Another blend CO:OLO were prepared in which the proportion of SFA:Unsaturated fatty acids are balanced to give 1:1 ratio. They were further subjected to lipase catalyzed interesterification reactions. Changes in the TG profile were monitored by reverse phase HPLC (RP-HPLC) analyses. The selected TG species were further evaluated using GC. Melting profiles and Solid fat contents of the blend and interesterified fats were determined by DSC to assess the changes introduced due to interesterification reaction. These studies indicated differences between the blended oils and interesterified oils in TG molecular species even though both of them may contain similar fatty acid composition.

RESULTS

Fatty acid composition of oils

The fatty acid compositions of the native oils are given in Table 1.1. In CO, 93% of the total fatty acids are saturated. The proportion of SFA:MUFA:PUFA in this oil is 93:6:1 (Table 1.1). GNO contains high amounts of oleic acid and linoleic acids and the proportion of SFA:MUFA:PUFA in this oil was found to be 21:45:33. Olive oil is highly enriched in oleic acid and SFA:MUFA:PUFA proportion in this oil to be 16:74:10. CO was blended with GNO at appropriate levels to get approximately similar proportions of saturated, monounsaturated and polyunsaturated fatty acids (Table 1.2). Since both CO and OLO had very little PUFA, it was not possible to obtain the combinations having high levels of PUFA with CO and OLO. Hence the proportion was adjusted to provide approximately similar amounts of Saturated to total Unsaturated fatty acids. Most of the unsaturation in this combination was provided by monounsaturated fatty acid namely oleic acid. When these blended oils were subjected to interesterification reactions, there were no significant differences in the fatty acid composition between blended and interesterified oils (Table 1.2).

Table 1.1 Fatty acid (%) composition of the Native oils.

Fatty acids (%)	CO	GNO	OLO
8:0	8.4	ND	ND
10:0	5.7	ND	ND
12:0	48.3	ND	ND
14:0	20.1	ND	ND
16:0	7.9	14.9	13.0
18:0	2.8	4.9	3.4
18:1	5.8	45.4	74.1
18:2	1.0	33.4	9.4
20:0	ND	1.3	ND
SFA	93.0	21.0	16.0
MUFA	6.0	45.0	74.0
PUFA	1.0	33.0	9.0
P/S ratio	0.01	1.5	0.63
SFA:MUFA:PUFA	93:6:1	21:45:33	16:74:10

Values are mean of triplicate samples. ND,not detected

Table 1.2 Fatty acid (%) composition of the blend and interesterified oils.

Fatty acids (%)	CO-GNO (B)	CO-GNO (I)	CO-OLO (B)	CO-OLO (I)
8:0	ND	ND	1.2	1.0
10:0	ND	ND	2.0	3.0
12:0	10.1	9.9	19.6	18.2
14:0	4.9	4.2	8.5	8.3
16:0	13.7	13.2	11.3	10.5
18:0	4.2	3.9	1.3	1.7
18:1	36.2	37.4	49.8	51.0
18:2	30.7	31.1	6.0	6.3
20:0	ND	ND	ND	ND
SFA	33.0	31.0	44.0	43.0
MUFA	36.0	38.0	50.0	51
PUFA	31.0	31.0	6.0	6.0
P/S ratio	0.94	1.0	0.13	0.14
SFA:MUFA:PUFA	33:36:31	32:37:31	45:49:6	43:51:6

Values are mean of triplicate samples. ND,not detected. B, Blended oils; I, Interesterified oils.

Quality parameters of the blend and Interesterified oils.

The quality of the oils used in the study were monitored by determining the peroxide (PV), free fatty acid (FFA), tocopherols, polyphenols in the native, blend and interesterified oils (Table 1.3 & 1.4).

Table 1.3 Determination of Peroxide value, Free fatty acid content and minor constituents of Native Oils.

Parameters	CO	GNO	OLO
Peroxide Value (Milli equivalents of O_2/kg oil)	< 0.1	2.6±0.6	8.5±0.8
Free fatty acid (%)	< 0.1	0.4±0.1	0.5±0.04
Tocopherol (ppm)	54.2±4.0	517±53	268±58.7
Total phenols (ppm)	nil	nil	290±32

are mean ± SD of 5 determinations.

Table 1.4 Determination of Peroxide value, Free fatty acid content and minor constituents of Blended and Interesterified oils.

Parameters	CO-GNO (B)	CO-GNO (I)	CO-OLO (B)	CO-OLO (I)
Peroxide Value (Milli equivalents of O_2/kg oil)	3.1±0.5	3.0±0.4	7.5± 0.6	7.7± 0.5
Free fatty acid (%)	0.5± 0.03	1.2±0.04	0.6± 0.02	1.4±0.01
Tocopherol (ppm)	424±23.4	400±45.2	151±11.4	148±20
Total phenols (ppm)	nil	nil	170±28	165±16

Values are mean ± SD of 5 determinations

CO has only 54 ppm tocopherol while OLO had 4 times more tocopherol and GNO had 10 times more tocopherol. After blending, 8 times more tocopherol was available in CO:GNO and 3 times more in CO:OLO blended oils. Interesterification reaction kept these minor constituents intact. PV and FFA of these oils were in the acceptable range of the PFA specifications.

The total phenolic content in OLO and its blends determined by Folin-Ciocalteu colorimetric method is presented in Table 1.3 & 1.4. The high content of phenolic compounds imparts stability to the oil. OLO had 290 ppm phenolic acid and 165-170 ppm of phenols were present in CO: OLO blend and interesterified oils.

Tocopherol composition of oils

α-tocopherol was found to be the major tocopherol in OLO 81.3%, GNO contains 44.3% β + γ tocopherol and 48.6% α tocopherol. CO:GNO blend had 42.1.% of β + γ & 46.5% α tocopherol. 75.2% of α tocopherol was present in CO:OLO blended oil (Table 1.5).

Table 1.5 Tocopherol composition of oils

	Tocopherols (%)			Tocotrienols (%)		
	α	β+γ	δ	α	β+γ	δ
CO	11.7	ND	ND	60.8	17.6	ND
GNO	48.6	44.3	3.2	ND	ND	ND
OLO	81.3	12.9	2.4	7.5	ND	6.5
CO:GNO(B)	46.5	42.1	3.6	ND	ND	ND
CO:GNO(I)	45.9	42.9	2.9	ND	ND	ND
CO:OLO(B)	75.2	13.2	ND	ND	ND	ND
CO:OLO(I)	74.8	14.1	ND	ND	ND	ND

ND, Not detected; B, blend; I, interesterified.

Phenolic acid composition of Oils.

The major phenolic compounds identified in olive oil were hydroxytyrosol and tyrosol, which represent 75% of the total phenolics. This result was similar to a previous report by Lee (2006). It has been shown that hydroxytyrosol is the principal phenolic antioxidant in olive oil (Papadopoulos & Boskou, 1991).

Finger printing of triglyceride molecular species of oils.

The stereospecific structure of triglycerides influences their biochemical, physical properties (Small, 1991 and Bracco, 1994). In order to monitor changes introduced in the oils during blending and interesterification reactions, the TG species were separated by HPLC.

The trilglyceride molecular species of the native, blended and interesterified oils are shown in the Table 1.6. Triglyceride molecules were identified by correlating the Theoretical carbon number (TCN) and retention time of TG standards. Three standards LLL, OOO and PPP were

used as retention time references to correlate the TCN values with the TG retention times (Bland,1991). To positively characterize the composition of TG molecule separated by HPLC, eluted fraction was collected. Each TG molecule was saponified and fatty acids were esterified to its fatty acid methyl esters (FAME) and injected to GC. The fatty acid methyl esters were identified based on comparison of retention time with FAME standards.

Fingerprinting of TG species of CO:GNO and CO:OLO blend and interesterified oils by HPLC is shown in Fig 1.1 (a) and 1.1 (b). Major triglycerides of CO were CCLa (11.9%), CLaLa (17.3%), LaLaLa (21.7%), LaLaM (16.1%), LaMM (10.8%) and LaMP (6.4%). In GNO, LLL (6.5%), OLL (16.1%), PLL (9.2%), OOL (18.8%), POL (16.5%), PPL (4.6%), OOO (11.6%), POO/SOL (15.4%) were found to be the major TGs. In OLO 6 TG molecules were identified as OOL (13.6%), POL (6.1%), OOO (47.8%), POO/SOL (23.9%), PPO (3.6%) & SOO (5.0%). The major triglycerides in OLO were trisaturated (UUU 61.4%) and monosaturated (SUU 35%) – (Table 1.7). These trilglyceride molecular species were comparable with those reported in the earlier reports (Tan and Che Man, 2000). It was observed that blended oils have combination of TGs found in individual oils used for mixing. While in the interesterified oils there were alterations in some of the TG species and also some new species were observed.

Blending of CO with GNO decreased the proportion of the trisaturated (SSS) triglycerides by 79% compared to native CO and resulted in the emergence of 44% triunsaturated (UUU) triglycerides and 33% of monosaturated glycerides (SUU) ((Table 1.7). Blending did not alter the structure of the triglyceride species of the parent oils, but their amounts varied according to the ratio of oils used for the blending. Interesterification of the blended oils resulted in the increase or decrease in the quantity of existing trilglyceride molecules and/or the emergence of new triglyceride molecules. Interesterification of CO:GNO blended oil resulted in increase of peak corresponding to PLL (19%) , OOL (7%) and POL by 5.3% and decrease in the peaks OLL, OOO and OLS/POO by 3.6%, 15.7% and 13.6% respectively (Fig. 1.1a). The new triglyceride species emerging in the CO:GNO interesterified oil were LaMO (0.9%) and MOL (0.6%).

Blend of CO:OLO showed a 64% decrease in the trisaturated triglycerides compared to native CO and increase in the monosaturated triglycerides (SSU) by 34%. Interesterification of CO:OLO resulted in the decrease of CCLa, CLaLa, LaLaLa, LaLaM, OOO by 23, 8, 8.4, 15.8 and 2.6 % respectively, LaLaO, LaMM, LaMO, LLO/LaMP and POL increased by 34.6, 18.4,

36, 51.8 and 27.2% (Fig 1.1b). Interesterification of blended oils resulted in a decrease in SSS (8%) and increase in UUU (31%).There was also formation new TG species LLL (0.7%) in the interesterified oils compared to the blended oil.

Table 1.6 Triglyceride molecular species of native, blend and Interesterified oils.

	CO	GNO	OLO	CO:GNO(B)	CO:GNO(I)	CO:OLO(B)	CO:OLO(I)
CaCC	1.2	ND	ND	ND	ND	ND	ND
CaCLa	3.5	ND	ND	0.7	ND	1.2	1.5
CCLa	11.9	ND	ND	2.8	2.8	5.6	4.3
CLaLa	17.3	ND	ND	3.8	3.4	7.4	6.8
LaLaLa	21.7	ND	ND	4.3	3.7	8.3	7.6
LaLaM	16.1	ND	ND	3.2	3.4	6.3	5.3
LaLaO	1.6	ND	ND	0.2	ND	2.6	3.5
LLL	ND	6.5	ND	4.9	5.4	ND	0.7 new
LaMM	10.8	ND	ND	3.9	4.3	3.8	4.5
LaMO	2.2	ND	ND	ND	0.9 new	2.5	3.4
LLO/LaMP	6.4	16.1	ND	14.1	13.6	2.7	4.1
LaOO	1.6	ND	ND	ND	ND	ND	ND
PLL	ND	9.2	ND	7.4	8.8	ND	ND
MOL	ND	ND	ND	ND	0.6 new	ND	ND
MMO/LaPO	ND	ND	ND	ND	ND	ND	ND
LOO/LaPP	2.2	18.8	13.6	15.6	16.7	8.7	8.9
PLO	0.9	16.5	6.1	13.2	13.9	4.4	5.6
PLP	ND	4.6	ND	3.7	3.3	ND	ND
MPO	0.8	ND	ND	ND	ND	ND	ND
OOO	0.2	11.6	47.8	9.5	8.0	26.3	25.6
OLS/POO	ND	15.4	23.9	12.5	10.8	14.5	13.3
POP	0.9	1.3	3.6	ND	ND	2.3	1.8
PPP	ND	ND	ND	ND	ND	ND	ND
OOS	0.4	ND	5.0	ND	ND	3.4	3.2
POS	ND	ND	ND	ND	ND	ND	ND
SOS	ND	ND	ND	ND	ND	ND	ND

ND, Not detected; B, blend; I, interesterified; Ca, caprylic; C, capric; La, lauric; M, myristic; P, palmitic; S, stearic; O, oleic; L, linoleic.

Table 1.7 Distribution of Trisaturated, Monounsaturated and Triunsaturated triglycerides in Native, blend and interesterified oils.

TG distribution (%)	CO	GNO	OLO	CO:GNO (B)	CO:GNO (I)	CO:OLO (B)	CO:OLO (I)
UUU	0.2	53.0	61.4	44.1	43.7	37.7	49.3
SUU	3.7	41.1	35	33.1	34.1	22.3	22.1
SSU	5.5	5.9	3.6	3.9	4.5	7.4	9.4
SSS	91.1	0	0	18.7	17.6	32.6	30.0

TG, triglyceride; U, unsaturated; S, saturated; B, blend; I, interesterified;

Figure 1.1(a). HPLC separation of triglycerides of blend and interesterified oils.

HPLC of CO: GNO (B) and CO: GNO (I) oils using C18 Column and refractive index detector with a mobile phase of acetone: acetonitrile (65:35) isocratic mixture at a flow rate of 1ml/ min, Numbered peaks are compared in Table 1.

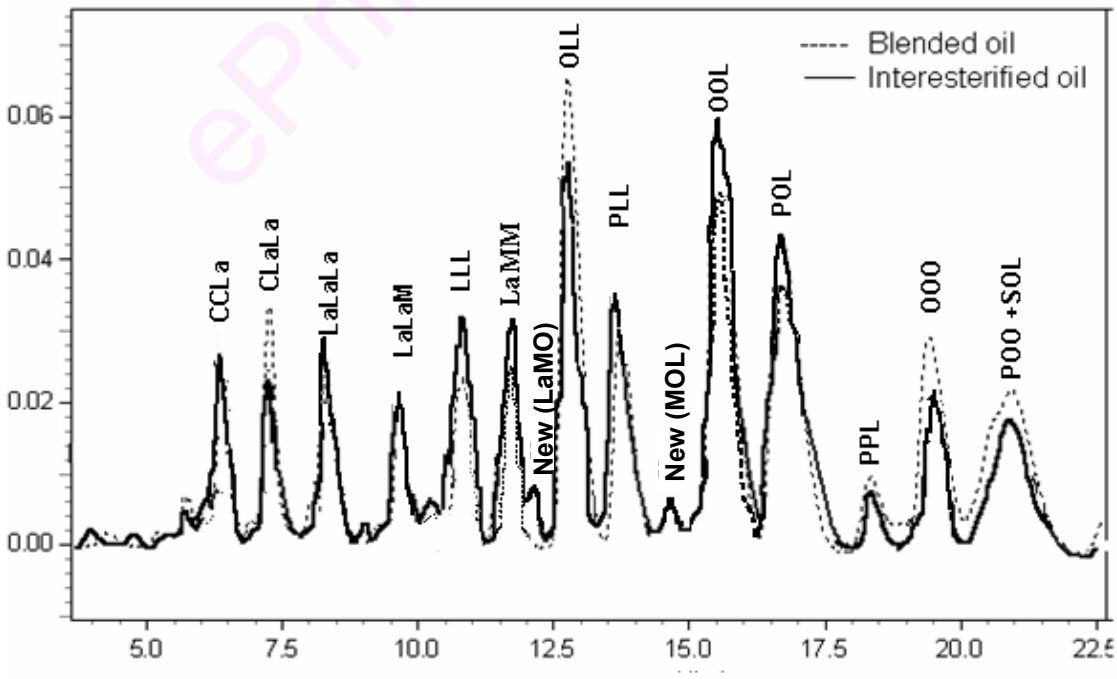
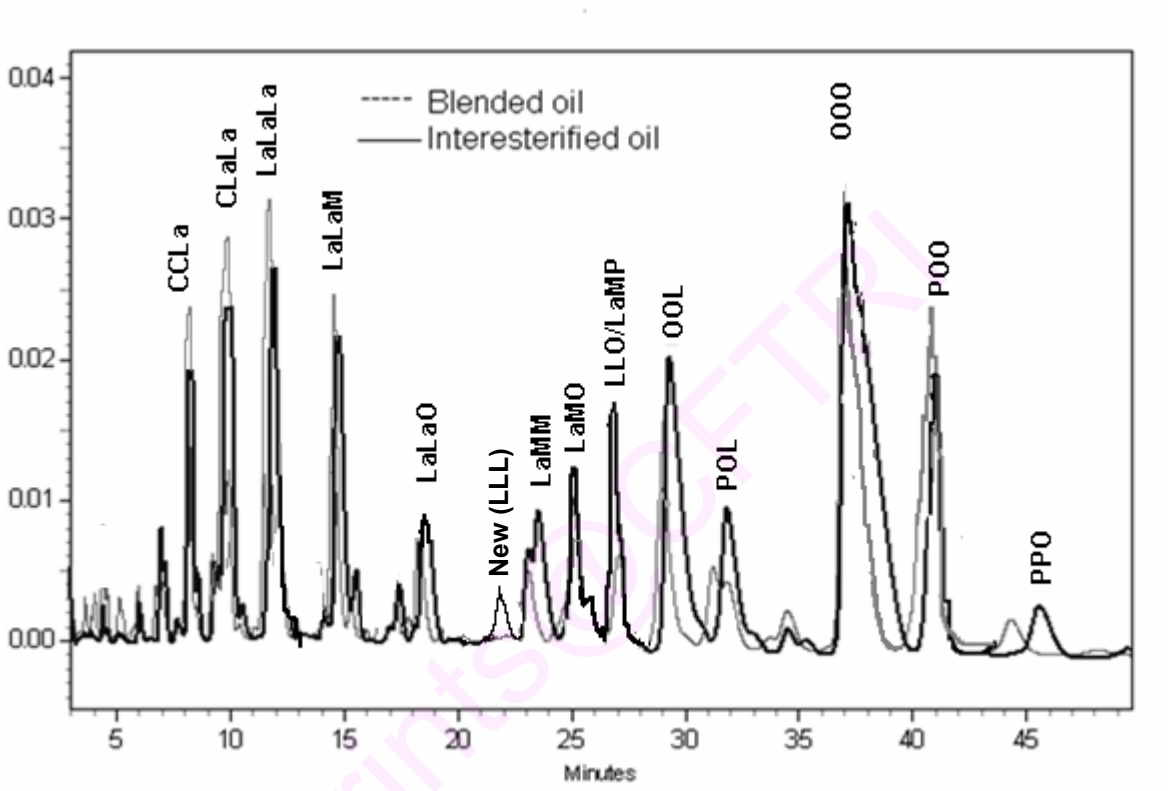


Figure 1.1(b) HPLC separation of triglycerides of blend and interesterified oils.

HPLC of CO: OLO (B) and CO: OLO (I) oils using C18 Column and refractive index detector with a mobile phase of acetone: acetonitrile (65:35) isocratic mixture at a flow rate of 1ml/ min, Numbered peaks are compared in Table 1.1.



Thermal profiles.

Melting profiles of native, blended and interesterified oils.

The thermal profiles of CO showed a single endothermic melting peak in the range of 16-24.6°C (Fig 1.2a) showing homogeneous nature of triglycerides, which account for more than 80% (Table 1.7) triglycerides in CO. The solid fat content (SFC) of CO showed 33.1% solids at 20°C (Fig 1.3a) but at 25°C the solid content was only 1% indicating the triglycerides of CO is completely melting in between 20-25°C. GNO showed two endothermic peaks at temperatures ranging from -27.47°C to -20.02°C and 6.76°C to 9.82°C (Table 1.8) revealing the existence of heterogenous triglycerides with different unsaturation. GNO showed 87% of solids melted below 0°C and there were no solids at 20°C (Fig 1.3a), indicating that there are no high melting triglycerides in the oil. The melting endotherms of OLO showed two endothermic peaks at temperatures ranging from -28.82°C to 6.11°C.

The blended oil containing CO:GNO showed 3 endothermic peaks at different temperatures, the low melting peak at -29°C and the high melting peak at 16°C (Fig 1. 2b). Blending of CO with GNO resulted in the decrease of solid fat content from 33.1% to 0.98% at 20°C compared to that of CO alone and Interesterification of this blended oil resulted in further lowering of melting temperature with the endothermic peak being observed in the range of -37°C and 15.5°C. There were no solids found at 20°C in CO:GNO interesterified fat.

The CO:OLO blended oil showed 2 endothermic peaks at -11.14°C and 27.24°C (Fig 1.2c). Interesterified oil showed peaks in the range of -26 °C to 23°C and 26.56 to 28.05°C. The SFC decreased to 3.6 % and 3.28% at 20°C in blend and interesterified oils compared to the CO (Fig 1.3c).

Fig 1.2(a) DSC Thermograms of native oils

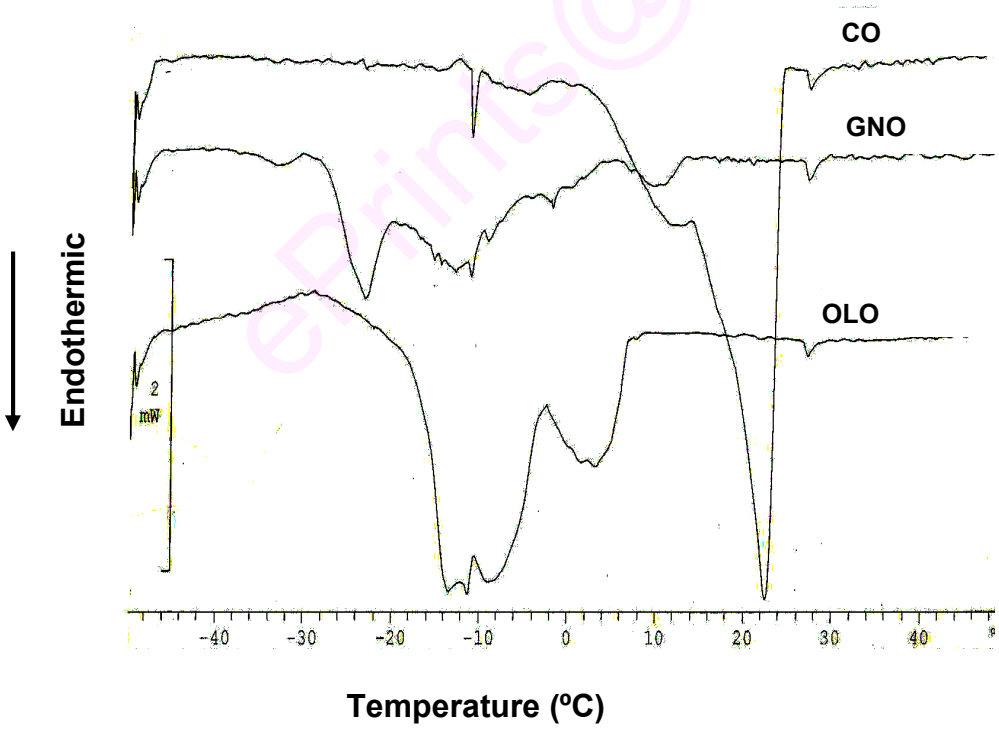


Fig 1.2(b) DSC Thermograms of CO:GNO (B) and (I) oils

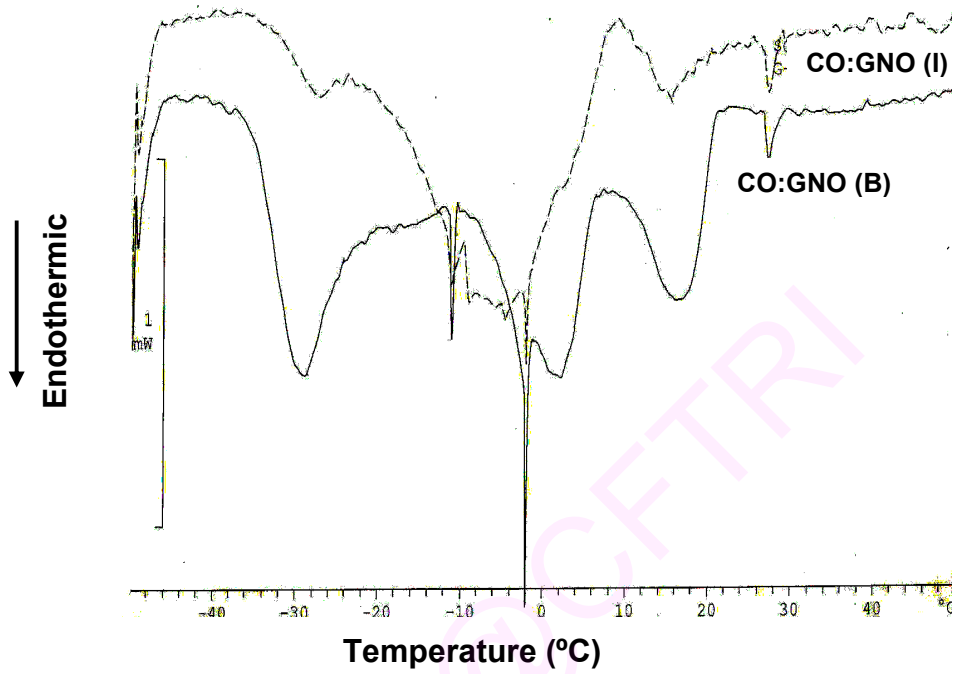


Fig 1.2(c) DSC Thermograms of CO:OLO (B) and (I) oils

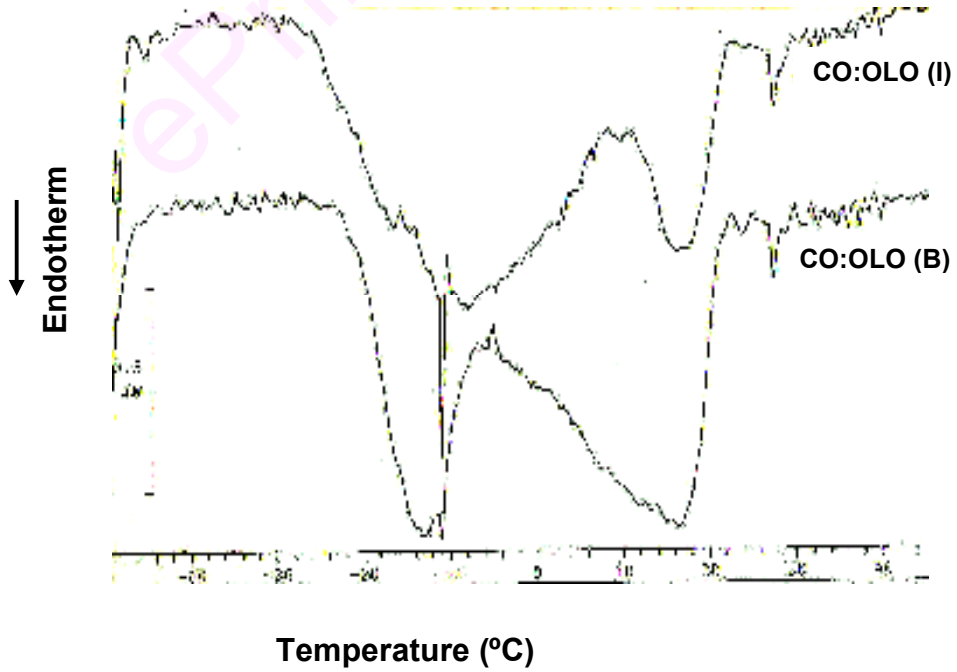


Fig 1.3(a) Melting profiles of Native oils.

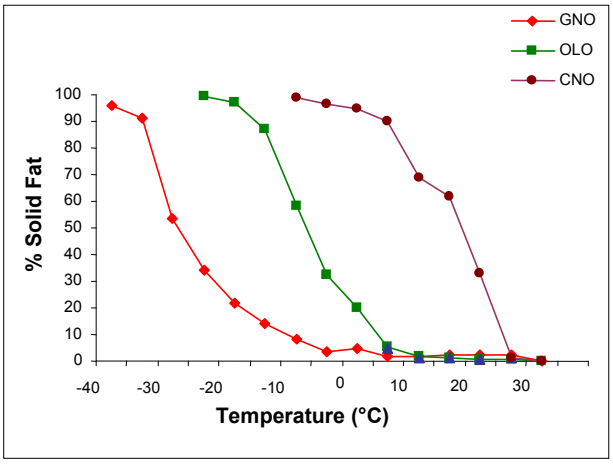


Fig 1.3(b) Melting profiles of CO:GNO (B) and (I) oils.

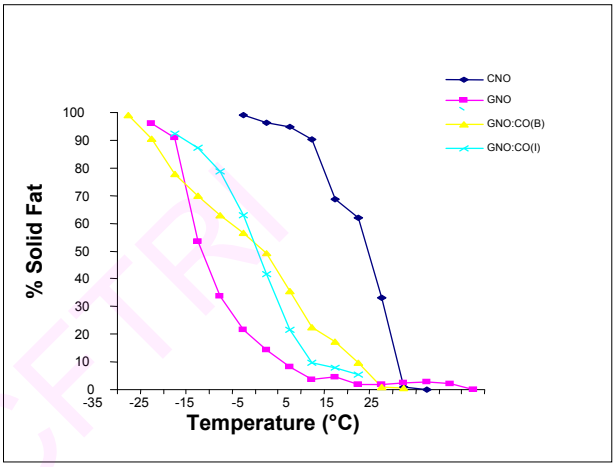
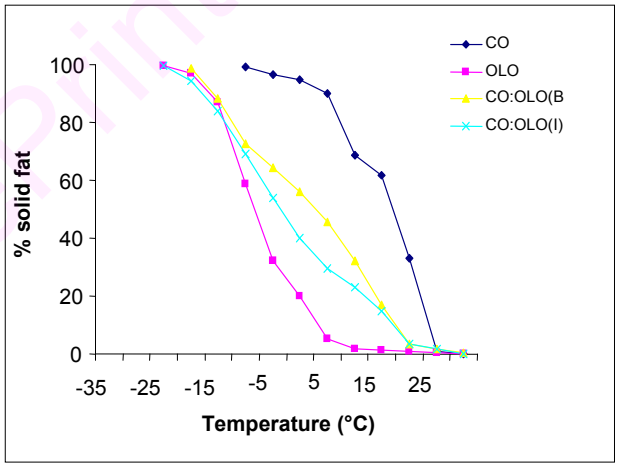


Fig 1.3(c) Melting profiles of CO:OLO (B) and (I) oils.



Crystallisation behaviour of native, blend and interesterified oils.

Cooling curves of oils show the temperature at which the triglyceride species start crystallizing. The crystallization of CO triglycerides showed a single exothermic peak at 0.97°C, while that of GNO showed two exothermic peaks at -2.57°C and -13.5°C, respectively, OLO showed exothermic peak at -20.5 and -47.7°C(Table 1.9).

CO:GNO blends showed shifting of crystallisation temperature to -9.87°C and -23.17°C, respectively while the interesterified oil showed a single exothermic peak at -32.98°C (Fig 1.4a). The blended oils of CO:OLO (Fig 1.4b) showed exothermic peaks at -4.95 and -19.87°C and interesterified oils showed at -7.68 and -22.3°C (Table 1.9).

Table No. 1.8 Peak temperature and enthalpy of native, blended and interesterified oils during melting.

Samples	Peak 1				Peak 2				Peak 3			
	Onset [°C]	Peak [°C]	End set [°C]	ΔH [J/g]	Onset [°C]	Peak [°C]	End set [°C]	ΔH [J/g]	Onset [°C]	Peak [°C]	End set [°C]	ΔH [J/g]
CO	ND	ND	ND	ND	16.9	22.94	24.57	75.49	ND	ND	ND	ND
GNO	-27.47	-23.26	-20.02	-33.51	6.76	9.54	9.82	ND	ND	ND	ND	ND
OLO	-28.82	-13.40	6.11	-52.05	26.56	27.09	28.0	-0.23	ND	ND	ND	ND
CO:GNO (B)	-38.0	-2.14	23.0	-52.84	26.64	27.2	28.21	-0.36	ND	ND	ND	ND
CO:GNO (I)	-37.0	5.0	9.0	-45.7	10.0	15.5	26.0	3.7	25	27	29	0.46
CO:OLO (B)	-20.98	-11.14	-3.82	-47.21	26.56	27.24	28.05	-11.7	ND	ND	ND	ND
CO:OLO (I)	-26.0	-11.23	23.0	-53.06	26.54	27.22	28.27	-0.79	ND	ND	ND	ND

ND, Not detected; B, blend; I, Interesterified.

Table No 1.9. Crystallisation behaviour of native, blended and interesterified oils.

Samples	Peak 1				Peak 2				Peak 3			
	Onset [°C]	Peak [°C]	End set [°C]	ΔH [J/g]	Onset [°C]	Peak [°C]	End set [°C]	ΔH [J/g]	Onset [°C]	Peak [°C]	End set [°C]	ΔH [J/g]
CO	5.52	0.97	-3.90	60.68	ND	ND	ND	ND	ND	ND	ND	ND
GNO	-1.61	-2.57	-5.11	1.90	-12.95	-13.5	-14.17	0.26	ND	ND	ND	ND
OLO	-16.98	-20.5	-25.19	2.78	-43.27	-47.6	-52.90	15.74	ND	ND	ND	ND
CO:GNO (B)	-5.81	-9.87	-16.89	4.05	-19.70	-23.1	-27.67	9.06	ND	ND	ND	ND
CO:GNO (I)	ND	ND	ND	ND	-28.13	-32.9	-49.78	12.95	ND	ND	ND	ND
CO:OLO (B)	-0.83	-4.95	-9.74	4.77	-15.54	-19.8	-24.17	18.96	-25.03	-27.8	-31.85	3.49
CO:OLO (I)	-4.23	-7.68	-12.72	5.81	-19.55	-22.3	-27.07	24.56	ND	ND	ND	ND

ND, not detected; B, blend; I, Interesterified.

Fig 1.4(a) Crystallisation exotherms of CO:GNO (B) and (I) oils

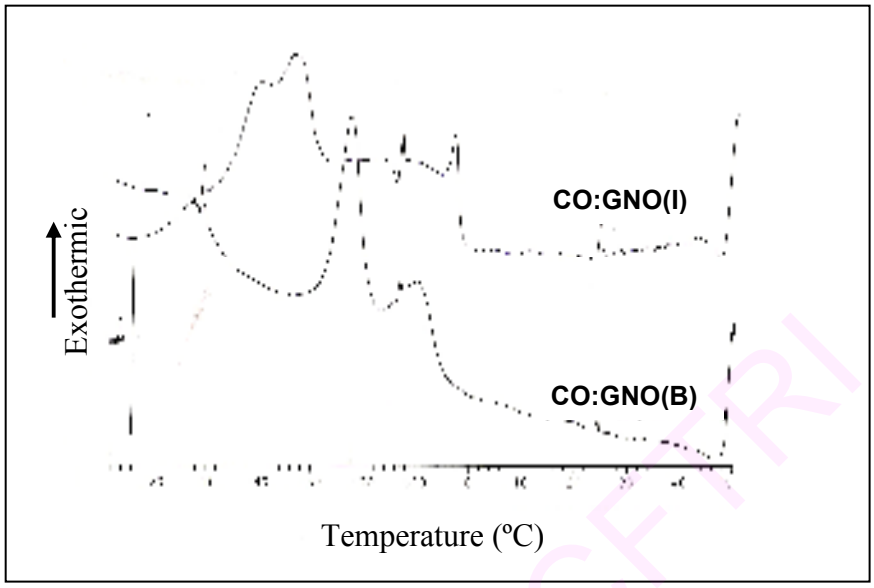
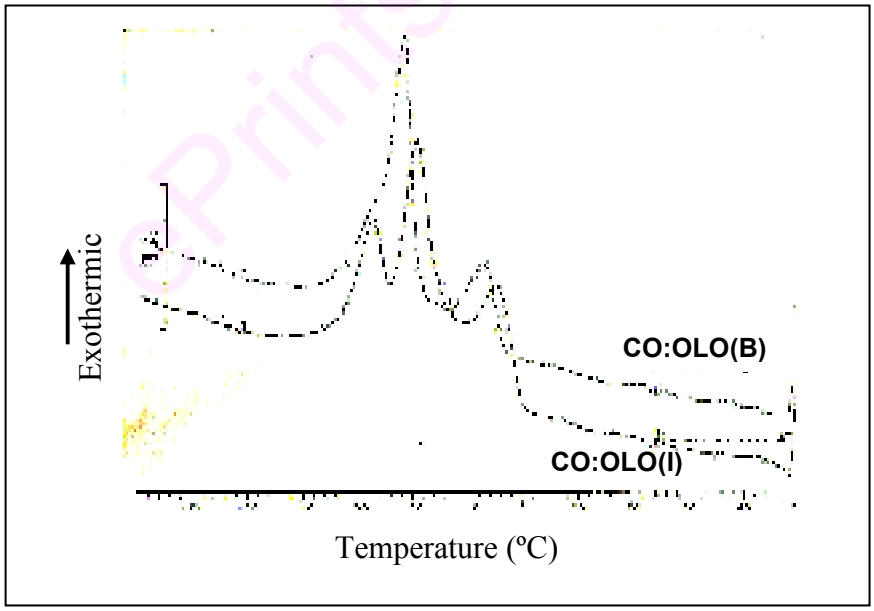


Fig 1.4(b) Crystallisation exotherms of CO:OLO (B) and (I) oils.



DISCUSSION

CO is considered to be atherogenic because of the presence of high amount of saturated fatty acids. The saturated fatty acid solidifies at room temperature. Blending of vegetable oils gives the manufacturer greater flexibility to tailor the products to accomplish specific functional properties or satisfy nutritional requirements (Chen et al., 2007). However, blending does not result in the chemical modification of the TG composition. SLs have emerged as the preferred alternative to physical mixtures to meet the particular requirements although both products provide the identical fat content.

The present investigation is aimed at preparing blends of CO with GNO and OLO, which provides medium chain triglycerides from coconut oil and PUFA from GNO or MUFA from OLO. The combinations were prepared to get approximately equal proportions of saturated to unsaturated fatty acids as has been recommended by nutritionists for balanced lipids. Further, these blended oils were subjected to interesterification reaction so as to rearrange the fatty acids in the TG molecule, which may alter its physical as well as nutritional properties. Such modifications help in developing specific products using altered fats.

Many products have been developed in the last decades, such as low-fat butter products, fat substitutes, and zero-calorie fats. In the early 1980s, blends of butter and vegetable oil products appeared in the US market. Blending butterfat with vegetable oils that are liquid at refrigerator temperature can lead to spreads that harmonize nutrition and offer desirable organoleptic attributes and lowered overall costs of production (Rousseau et al., 1998).

Overall fatty acid composition and their positions in the triglycerides altered the physical properties like melting behaviour of the fat (Juliana, 2003). A single TG can also show multiple melting behaviour due to the presence of different crystal forms (Polymorphism) (Lopez, 2005). This is one of the major factors influencing the melting point of fats and other important properties such as softening point, solid fat content, plasticity, and brittleness. Polymorphic forms are represented by melting endotherms in the DSC scan. TG generally occur in three main crystal forms, namely α, β' and β in order of their increasing melting temperature and stability (DeMan, 1992). In our study the melting curves showed features to indicate the complex nature of the triglyceride in oil samples. The polymorphic behavior of oils and fats are quite complex since the oils are mixtures of different triglycerides with variable degree of

unsaturation. The efficiency of the interplanar packing of hydrocarbon chains, which is high in TG with uniform fatty acid chain lengths, is reduced when TG containing fatty acids of different chain lengths are mixed.

CO showed a single endothermic peak in the range of 16-24.6°C showing homogenous nature of triglycerides. The melting endotherms of GNO showed two endothermic peaks at -23.26°C and 9.54°C. OLO showed endothermic peak at 11.69 and 8.25°C indicating the existence of heterogeneous triglycerides with different unsaturation (Table 1.9).

Interesterification brought down the melting point of the triglyceride species found in respective blends. The melting profile of CO:GNO blended and interesterified oils showed two endothermic peaks shifting to lower temperatures compared to that observed in CO alone. Blending resulted in a decrease in the trisaturated triglyceride (Table 1.7), due to which a lowering of the melting temperature was observed compared to the native CO. Interesterification of this blended oil resulted in further lowering of melting temperature. Interesterification of the CO:GNO blend resulted in a decrease in trisaturated triacylglycerols (SSS, 6%).

When CO was blended with OLO, two endothermic peaks were observed in the range of -20.98 to -3.82 °C and 17 °C. Interesterification resulted in shifting of the onset of low melting peak to -11.77 °C to -10.24°C. This may be due to the decrease in the trisaturated triglycerides in (SSS 8%) Interesterified oil. Solid fat content (SFC) is responsible for many important characters of fat like physical appearance, organoleptic properties and spreadability (Reena, 2001). SFC also influences the melting properties indicating the behaviour of a fat at different temperatures. Plasticity or consistency of an edible oil product depends on the amount of solids present (Laia, 2000). According to Deman (1992). SFC at 25 °C influences the plasticity while that between 33 °C and 38°C influences mouth feel.

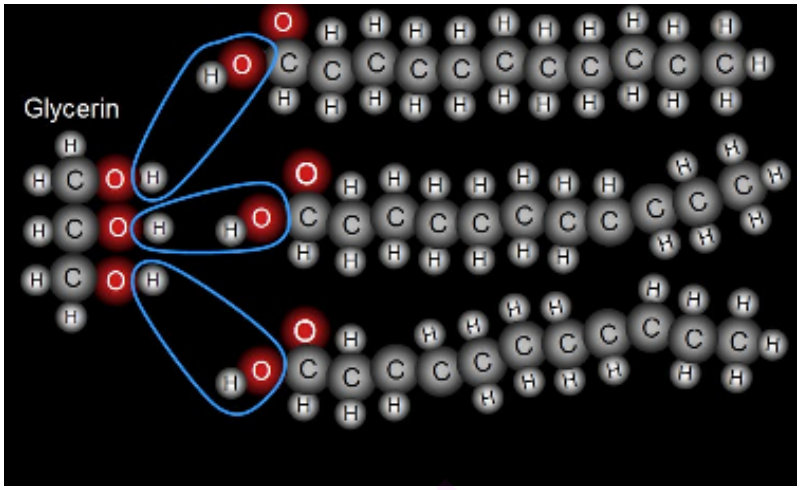
In the present study, it was observed that there is a change in SFC in structured lipids prepared from CO. The SFC of CO showed 33.1% solids at 20°C, while GNO showed no solids at 20°C. In case of OLO 0.8 % solids were present at 20°C. The blended oil containing CO:GNO showed endothermic peak shifting to lower temperature compared to that observed with CO. This is due to the blending of saturated CO with unsaturated GNO. The solid fat

content of CO:GNO was 0.98% at 20°C and in the interesterified fat there were no solids present at 20°C.

Blending of CO:OLO resulted in the decrease of solid fat content to 3.6 % and interesterification further decreased the SFC to 3.28 %. CO triglycerides are primarily rich in saturated fatty acids of medium chain length and were replaced by unsaturated fatty acids which had lower melting temperature range and hence showed lower SFC. These results were in agreement with those observed by Ribeiro et al., (2009) where interesterified blends of soyabean and fully hydrogenated soybean oil displayed reduced SFC and melting profiles as compared with the original blends.

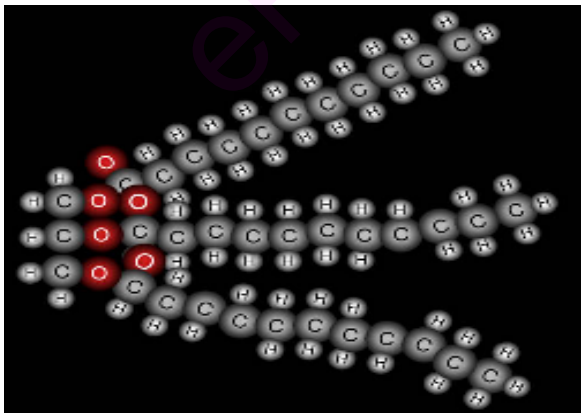
The cooling thermogram of CO shows single peak at 0.97°C. GNO showed two peaks at -2.57 and -13.51 °C (Table 1.10). The cooling thermograms of OLO also showed two peaks at -20.52 and -47.7°C. Blending of CO with GNO resulted in shifting of crystallization temperature to -5.81 °C and -23.17 °C. Interesterified oil prepared from this blend showed only one peak at -32.98 °C. When CO was blended with OLO, three peaks observed, and the triglyceride molecules in CO:OLO blend crystallized at higher temperatures compared to the triglycerides of the native CO. The interesterified oil from this blend showed two peaks at higher temperatures. This indicates that interesterified oil is more homogeneous than the corresponding blended oils.

In conclusion, these results indicate that treatment of blended oils with lipase catalysed interesterification process did not have any adverse effect on the quality parameters and the content of minor constituents of the oils. Blending of oils retained the TG structure found in the parent oils but interesterification resulted in the redistribution of the fatty acids among the triglycerides. This altered the physical properties of the triglycerides. These alterations in triglyceride molecular species of oils may have an impact on the nutritional properties. These are being evaluated.



Chapter 2

Effect of Blend and Interesterified Oils on Serum and Tissue Lipids



INTRODUCTION

The dietary saturated fat increases plasma total and LDL-Cholesterol concentration while PUFA have lowering effect on both these parameters (Muller et al., 2003). In addition, dietary MUFA has also been shown to be hypocholesterolemic (Mattson and Grundy, 1985). The saturated fatty acids lauric, myristic and palmitic acids elevate serum cholesterol and LDL levels. Stearic acid has neutral effect on serum cholesterol and LDL levels. Linoleic acid (polyunsaturated) moderately reduces serum cholesterol and LDL levels. Oleic acid (monounsaturated) also appears to marginally reduce LDL but raises HDL (Gill et al., 2003). Dietary cholesterol elevates serum cholesterol and LDL levels, but the extent of increase is highly variable. It is generally agreed that an individual response to dietary fat can be best evaluated by measurement of serum total cholesterol, low density lipoprotein-cholesterol and high density lipoprotein (HDL-C). The dietary fat which lowers Cholesterol and LDL/HDL ratios are being considered ideal. These types of findings on the effects of individual fatty acids has prompted Oil technologists and Nutritionists to search for an oil which has an ideal S:M:P levels ratio of 1:1:1. Such a combination is also recommended by World health organization (FAO/WHO, 1994). The distribution of type and quantity of fatty acids in a TG molecule is unique to each fat (Emken et al., 2004). The fat with a desired fatty acid concentration can not be obtained from the single natural oil. Hence there is a need to modify the available oils to produce SLs containing fatty acids in desired proportions. This can be achieved by blending of oils or by interesterification reactions.

Rearrangement of fatty acid on the glycerol moiety of TG change the physical (Willis et al., 1998) and nutritional properties of the oil (Akoh, 1995). The new triglyceride molecules formed in the interesterified oil may be absorbed differently than the original TG from the native oil or in the blend (Kennedy, 1991). Studies evaluating the metabolic effects of different lipid emulsions have demonstrated advantages of structured triglycerides compared with physical mixture of the same oils (Kennedy, 1991).

As explained in the previous chapters we have prepared the blends and interesterified oils of CO with GNO and OLO. CO:GNO combination had the saturated, monounsaturated and

polyunsaturated fatty acid levels in approximately 1:1:1 proportion. In CO:OLO the proportion of SFA:Unsaturated fatty acids are balanced to give 1:1 ratio.

The present study was carried out to verify whether feeding these modified oils of CO:GNO and CO:OLO exhibit any beneficial effects on the serum and liver lipid profiles in rats as compared to that observed in the rats given CO alone containing high levels of saturated fatty acids.

RESULTS

Fatty acid composition of the diet

In CO, 93% of the total fatty acids are saturated. The proportion of SFA:MUFA:PUFA in this oil is 93:6:1 (Table 2.1). GNO contains high amounts of oleic acid and linoleic acids and the proportion of SFA:MUFA:PUFA in this oil was found to be 22:45:33. Olive oil has high content of oleic acid and SFA:MUFA:PUFA proportion in this oil was 16:74:10. Therefore, none of these oils has ideal combinations of SFA:MUFA:PUFA for a balanced composition of fatty acids. The purpose of this study was to obtain oil which has an SFA:MUFA:PUFA in proportions of approximately 33:33:33%. Accordingly, CO and GNO were blended in such a way to get approximately equal proportions of saturated, monounsaturated and polyunsaturated fatty acids (Table 2.1). These blended oils were subjected to interesterification reactions as described in methods. There were no significant differences in the fatty acid composition between blended and Interesterified oils (Table 2.1). The diets containing these modified oils were fed to rats for a period of 60 days.

The amount of diet consumed by rats fed on different dietary lipids was comparable (Table 2.2). There was no change in the food efficiency ratio and body weight gained by rats fed different diets. No significant changes were observed in the weights of liver, heart and brain among the rats fed different diets containing blend and interesterified oils (Table.2.2).

Table 2.1 Fatty acid (%) composition of the dietary fats.

Fatty acids (%)	CO	GNO	OLO	GNO:CO (B)	GNO:CO (I)	CO:OLO (B)	CO:OLO (I)
8:0	8.4	ND	ND	ND	ND	1.2	1.0
10:0	5.7	ND	ND	ND	ND	2.0	3.0
12:0	48.3	ND	ND	10.1	9.9	19.6	18.2
14:0	20.1	ND	ND	4.9	4.2	8.5	8.3
16:0	7.9	14.9	13.0	13.7	13.2	11.3	10.5
18:0	2.8	5.9	3.4	4.2	3.9	1.3	1.7
18:1	5.8	45.4	74.1	36.2	37.4	48.5	51.0
18:2	1.0	33.4	9.4	30.7	31.1	6.0	6.3
20:0	ND	1.3	ND	ND	ND	ND	ND
SFA	93.0	22.0	16.0	33.0	31.0	45.0	43.0
MUFA	6.0	45.0	74.0	36.0	38.0	49.0	51.0
PUFA	1.0	33.0	10.0	31.0	31.0	6.0	6.0
P/S ratio	0.01	1.5	0.63	0.94	1.0	0.13	0.14
SFAMUFA:PUFA	93:6:1	22:45:33	16:74:10	33:36:31	32:37:31	45:49:6	43:51:6

Values are mean of triplicate samples. ND,not detected; B, Blended oils; I, Interesterified oils

Table 2.2 Growth and Organ weights of the rats fed native, blend and interesterified oils.

Parameters	CO	GNO	OLO	CO:GNO (B)	CO:GNO (I)	CO:OLO (B)	CO:OLO (I)
Food intake (g/d/rat)	12.5±0.7[a]	11.9± 0.7[a]	13.5± 1.3[a]	12.6± 0.6[a]	12.8± 0.7[a]	12.6± 0.4[a]	12.4± 1.0[a]
FBW (g)**	234±17.8[a]	221± 22.3[a]	255± 35.1[a]	227± 17.5[a]	244±19.7[a]	226± 22.2[a]	246± 26.2[a]
FER	0.32 ±0.01[a]	0.34 ± 0.02[a]	0.31 ± 0.30[a]	0.30 ± 0.01[a]	0.32 ± 0.01[a]	0.36 ± 0.02[a]	0.31± 0.01[a]
Liver weight*	3.20± 0.3[a]	3.35±0.3[a]	3.20± 0.2[a]	3.10± 0.3[a]	3.20± 0.35[a]	3.20± 0.2[a]	3.20 ± 0.2[a]
Heart weight*	0.27± 0.04[a]	0.25±0.02[a]	0.30± 0.03[a]	0.28± 0.01[a]	0.28± 0.02[a]	0.27± 0.02[a]	0.24± 0.05[a]
Brain weight*	0.51± 0.01[a]	0.59±0.05[a]	0.5± 0.05[a]	0.6 ± 0.06[a]	0.57± 0.07[a]	0.55± 0.05[a]	0.58± 0.05[a]

*(g/100g body weight). **FBW final Body weight at the time of sacrifice (g), Values are mean ± SD (n=5 rats). FER (Food efficiency ratio) measured as Food intake/Gain in body weight. Values not sharing a common superscript within a row are statistically significant ($p<0.05$)

Serum lipid profile

Rats fed diets containing CO had significantly higher levels of Cholesterol and TG compared to those rats fed with GNO or OLO (Table 2.3).

The serum cholesterol was lowered by 24% in the rats fed with GNO compared to those given CO. There was a marginal decrease in the cholesterol level in the rats fed CO: GNO blends which was not significant but it was significantly decreased by 27% in the rats given interesterified oils of the same combination. No significant changes were observed in the HDL-cholesterol levels in different groups. LDL+VLDL cholesterol levels in the animals fed with GNO was lowered by 40% in comparison to the groups fed with the CO. The LDL cholesterol level was decreased by 36% in the animals fed with the CO:GNO blended oil, and decreased by 38% in the rats fed with the interesterified oil in comparison with rats given CO diet. Similarly TG levels were decreased by 14%, 18% and 26% in rats given GNO, CO:GNO blends and CO:GNO interesterified oils compared to the groups fed with the CO. Phospholipids level was also decreased by 22%, 15% and 15% in groups fed with the GNO, CO: GNO blend and interesterified oils. These studies indicated that CO:GNO combinations in the interesterified oils could exhibit better hypocholesterolemic effects compared to the blended oils (Table 2.4).

In the group fed with OLO, CO:OLO blend and the interesterified oils, Serum cholesterol levels decreased by 21%, 13% and 25% respectively, compared to the CO fed groups (Table 2.3). VLDL+LDL-cholesterol was also significantly reduced by 33% and 22% and 32% in rats fed native OLO, blend and interesterified oils compared to the groups fed with CO. There was no difference in the level of HDL-cholesterol in any of these groups. TG levels were decreased by 32%, 13% and 16%. Phospholipids levels were decreased by 16%, 23% and 26% respectively in rats given OLO, CO:OLO blend CO:OLO interesterified oils as compared to those given CO containing diets (Table 2.5).

Table 2.3. Serum lipid profile (mg/dl)of Rats fed Native oils.

Parameters	CO	GNO	OLO
Total cholesterol	91.3 ± 3.6[a]	69.5 ± 4.7[b]	72.3 ± 3.5[b]
HDL-cholesterol	22.7 ± 4.2[a]	28.6 ± 7.9[a]	26.3 ±6.0[a]
VLDL+LDL Cholesterol	68.6 ± 4.5[a]	40.9 ± 5.8[b]	46.0 ± 6.0[b]
Triglyceride	177.0 ±2.0[a]	152.0 ± 1.2[b]	120 ± 5.2[c]
Phospholipids	107.6 ±9.7[a]	83.8 ± 5.7[b]	90.6 ± 4.9[b]

Values show the mean ± S.D (n=5 rats). Values in the same row with common superscript letters are not significantly different at P<0.05.

Table 2.4. Serum lipid profile (mg/dl) of rats fed CO:GNO Blend and Interesterified oils.

Parameters	CO	GNO	CO: GNO (B)	CO:GNO (I)
Total cholesterol	91.3 ± 3.6[a]	69.5 ± 4.7[b]	86.1 ± 1.8[a]	67.1 ± 1.6[b]
HDL-Cholesterol	22.7 ± 4.2[a]	28.6 ± 7.9[a]	27.3 ± 3.0[a]	30.0 ± 6.3[a]
VLDL+LDL Cholesterol	68.6 ± 4.5[a]	40.9 ± 5.8[b]	58.8 ± 2.8[c]	37.1 ± 4.3[b]
Triglyceride	177.0 ±2.0[a]	152.0 ± 1.2[b]	146 ± 3.0[b]	131 ± 3.0[c]
Phospholipids	107.6 ±9.7[a]	83.8 ± 5.7[b]	90.6 ± 5.1[b]	91.3 ± 4.3[b]

Values show the mean ± S.D (n= 5 rats). Values in the same row with common superscript letters are not significantly different at P<0.05. B, Blended oils; I, Interesterified oils

Table 2.5. Serum lipid profiles (mg/dl of Rats fed CO:OLO Blend and Interesterified oils

Parameters	CO	OLO	CO: OLO (B)	CO:OLO (I)
Total cholesterol	91.3 ± 3.6^{a}	72.3 ± 3.5^{bc}	79.9 ±6.5^{b}	68.5 ± 2.0^{c}
HDL-cholesterol	22.7 ± 4.2^{a}	26.3 ±6.0^{a}	26.2 ± 4.2^{a}	21.7 ± 2.1^{a}
VLDL+LDL Cholesterol	68.6 ± 4.5^{a}	46.0 ± 6.0^{b}	53.7 ± 3.5^{c}	46.8 ± 2.6^{b}
Triglyceride	177.0 ±2.0^{a}	120 ± 5.2^{b}	154 ± 9.4^{c}	149 ± 1.4^{c}
Phospholipids	107.6 ±9.7^{a}	90.6 ± 4.9^{b}	82.7 ± 4.5^{b}	79.2 ± 2.7^{b}

Values show the mean ± S.D (n= 5 rats). Values in the same row with common superscript letters are not significantly different at P<0.05. B, Blended oils; I, Interesterified oils.

Serum fatty acid Composition.

The fatty acid composition in the serum lipids of rats fed blend and interesterified oils are given in Table 2.6 and 2.7. The level of 18:2 was increased by 2.2 to 2.5 folds in group fed with CO:GNO in blend or interesterified form when compared to the rats fed with CO. However the

level of 18:1 was similar in different groups. There was no significant change in the level of 20:4 (Table 2.6).

In the groups fed with the CO:OLO blend and interesterified oils, the level of 18:1 was increased by 42% in the blend and 45% in interesterified group compared to CO fed rats. There was no significant change in the level of 18:2 and 20:4 compared to those given CO containing diets (Table 2.7).

Table 2.6. Fatty acid composition (%) of the serum in the group fed with CO, GNO, CO: GNO (B) and CO:GNO (I) oils.

Fatty acid	CO	GNO	CO: GNO (B)	CO: GNO (I)
12:0	2.0 ± 1.0[a]	ND	0.6 ± 0.04[b]	0.4 ± 0.1[b]
14:0	3.0 ± 1.5	ND	ND	ND
16:0	27.0±2.1[a]	19.9±0.1[b]	26.0 ± 0.3[a]	24.0 ± 3.5[a]
16:1	4.1 ± 0.9[a]	3.0 ± 1.2[a]	4.2 ± 0.2[a]	3.3 ± 1.7[a]
18:0	11.8±1.5[a]	14.2± 2.5[a]	8.5 ± 1.0[b]	7.8 ± 1.2[b]
18:1	31.6±0.1[a]	32.9± 1.7[a]	29.3 ± 2.4[a]	31.7 ± 1.5[a]
18:2	7.1 ±0.7[a]	16.3±1.2[b]	15.9 ± 1.8[b]	17.5 ± 2.7[b]
20:4	12.7±1.3[a]	14.8± 2.7[a]	14.3 ± 2.2[a]	14.6 ± 2.6[a]

Values show the mean ± S.D of 5 rats. Values in the same row with common superscript letters are not significantly different at P<0.05. B,Blended oils; I, Interesterified oils , ND,not detected

Table 2.7. The fatty acid composition (%) of the serum in the group fed with CO, OLO, CO:OLO (B) and CO:OLO (I) oils.

Fatty acid	CO	OLO	CO: OLO (B)	CO: OLO (I)
12:0	2.0 ± 1.0[a]	ND	ND	ND
14:0	3.0 ± 1.5	ND	ND	ND
16:0	27.0±2.1[a]	26. 6±1.2[a]	28.3±2.8[a]	26.2±1.5[a]
16:1	4.1 ± 0.9[a]	4.0±0.02[a]	3.8±0.2[a]	4.0±1.7[a]
18:0	11.8±1.5[a]	7.9±0.07[c]	6.7±0.4[b]	9.1±0.8[a]
18:1	31.6±0.1[a]	44.1±2.8[b]	45.0±1.2[b]	46.3±0.7[b]
18:2	7.1 ±0.7[a]	8.2±0.9[a]	8.0±0.5[a]	6.2±0.5[a]
20:4	12.7±1.3[a]	10.1±2.5[a]	8.4±3.7[a]	9.4±3.5[a]

Values show the mean ± S.D of 5 rats. Values in the same row with common superscript letters are not significantly different at P<0.05. B,lended oils; I,Interesterified oils , ND,not detected.

Liver lipid profile

The total cholesterol levels in the liver of rats fed with GNO were decreased by 15% compared to the rats fed with CO (Table 2.8). The rats fed with CO:GNO blend had 11% lower levels of cholesterol compared to the CO fed groups. Cholesterol level was decreased by 21% in the groups fed with interesterified oils. The Triglyceride level was decreased by 26% in the rats fed with GNO compared to the group fed with CO. The rats fed with CO:GNO blended oils and interesterified oils had similar levels of triglycerides as that found in GNO fed groups. Phospholipid levels remained similar in these rats.

Cholesterol level was decreased by 23% in olive oil fed groups compared to rats given CO diet (Table 2.9). In the rats given CO: OLO blends the hepatic cholesterol levels were similar to that found in rats given CO alone, but in the rats given interesterified oils the cholesterol levels were decreased by 32% compared to the rats given CO diet. TG level was decreased by 26% in the group fed with olive oil compared to the CO fed groups. The TG levels were decreased by 17-18% in the rats given CO: OLO as blends or after interesterification. No significant changes were observed with respect to phospholipids in different groups.

Table 2.8. Liver lipid profiles (mg/g tissue) of Rats fed CO, GNO, CO: GNO (B) and CO: GNO (I) oils.

Parameters	CO	GNO	CO: GNO (B)	CO: GNO (I)
Total cholesterol	7.3 ± 0.4^{a}	6.2 ± 0.5^{b}	6.5 ± 0.7^{ab}	5.8 ± 0.4b^{c}
Triglyceride	21.0 ± 1.0^{a}	15.6 ± 2.0^{b}	15.5 ± 2.4^{b}	15.6± 2.0^{b}
Phospholipids	15.2 ± 1.6^{a}	14.8 ± 1.7^{a}	14.6 ± 2.5^{a}	14.3±1.5^{a}

Values show the mean ± S.D (n= 5 rats). Values in the same row with common superscript letters are not significantly different at P<0.05. B,Blended oils; I, Interesterified oils.

Table 2.9 Liver lipid profile (mg/g tissue) of the Rats fed with CO, OLO, CO:OLO (B) and CO:OLO (I) groups.

Parameters	CO	OLO	CO:OLO (B)	CO:OLO (I)
Total cholesterol	7.3 ± 0.4[a]	5.6 ± 0.5[b]	7.1 ± 0.6[a]	5.0 ± 0.6[b]
Triglyceride	21.0 ± 1.0[a]	15.5 ±2.9[b]	17.5 ± 1.9[b]	17.3 ±1.3[b]
Phospholipids	15.2 ± 1.6[a]	14.1 ± 2.46[a]	13.8 ± 2.45[a]	12.9±2.36[a]

Values show the mean ± S.D of 5 rats. Values in the same row with common superscript letters are not significantly different at P<0.05. B, Blended oils; I, Interesterified oils

Liver fatty acid profile

The level of 18:2 was increased by 2 to 2.2 folds in rats fed GNO, CO:GNO blended oils and CO:GNO interesterified oils when compared to the rats fed with CO (Table 2.10) indicating the improved EFA status in animals when GNO was given as a dietary fat. However 20:4 levels remained unchanged. The level of 18:1 was increased by 21% in the groups of rats fed with CO:OLO as interesterified oils. No change in PUFA was observed in hepatic lipids (Table 2.11).

Table 2.10 Fatty acid (%) composition of the liver in the group fed CO, GNO, CO:GNO blend and Interesterified groups.

Fatty acid	CO	GNO	CO:GNO (B)	CO:GNO (I)
12:0	1.8±0.04	ND	ND	ND
14:0	2.3±0.6	ND	ND	ND
16:0	26.5±3.2[a]	22.3±3.6[a]	22.0±4.3[a]	24±3.5[a]
16:1	5.1±0.4[a]	4.8±0.4[a]	4.4±0.7[a]	5.0±0.2[a]
18:0	8.1±1.4[a]	8.8±2.0[a]	10.5±2.5[a]	9.9±0.7[a]
18:1	34.9±0.6[a]	35.6±1.9[a]	34.7±0.5[a]	35.3±1.2[a]
18:2	7.9±1.6[a]	17.5±0.7[b]	15.8±0.9[b]	15.1±2.7[b]
20:4	13.3±1.7[a]	11.4±2.6[a]	12.3±1.6[a]	12.1±1.3[a]

Values show the mean ± S.D of 5 rats. Values in the same row with common superscript letters are not significantly different at P<0.05. B, Blended oils; I, Interesterified oils. ND-Not detected,

Table 2.11 Fatty acid (%) composition of the liver in the group fed CO, OLO, CO: OLO blend and interesterified groups.

Fatty acid	CO	OLO	CO:OLO (B)	CO:OLO (I)
12:0	1.8±0.04	ND	ND	ND
14:0	2.3±0.6	ND	ND	ND
16:0	26.5±3.2[a]	25.6±0.5[a]	28.0±1.7[a]	27.0±1.2[a]
16:1	5.1±0.4[a]	4.8±0.9[a]	5.1±0.3[a]	4.0±0.4[a]
18:0	8.1±1.4[a]	9.2±0.9[a]	10.0±0.7[a]	10.0±0.7[a]
18:1	34.9±0.6[a]	42.2±0.5[b]	38.3±2.8[a]	42.2±3.1[b]
18:2	7.9±1.6[a]	8.9±1.4[a]	7.5±0.7[a]	6.5±1.5[a]
20:4	13.3±1.7[a]	9.4±3.1[a]	10.9±2.2[a]	9.65±2.2[a]

Values show the mean ± S.D of 5 rats. Values in the same row with common superscript letters are not significantly different at P<0.05. B, Blended oils; I, Interesterified oils. ND,Not detected,

DISCUSSION

CO is one of the major edible oil in southern regions of India. It has high levels of medium chain triglycerides but poor in essential fatty acids. GNO which is also one of the major edible oil in India has balanced amounts of unsaturated fatty acids in terms of oleic and linoleic acid. Olive oil is extensively used by Mediterranean population and has been attributed to be the main reason for low incidence of cardiovascular diseases in these regions (Covas, 2007). Olive oil is not in the main stream of edible oils in India. However olive oil is rich in oleic acid and also a good source for phenolics, which provides a high stability to the oil. In India, more than 80% of edible oils are used for frying dishes. Hence oil which are stable to frying conditions and also provide health benefits like olive oil will be ideal for a country like India. However, high cost of olive oil restricts its utility in India.

The objective of this study was to determine the effect of blend and interesterified oils of CO:GNO which contained approximately equal amounts of SFA:MUFA:PUFA and CO:OLO (where the proportion of SFA to total unsaturated fatty acid is balanced) on serum and hepatic lipid profile of rats in comparison with rats given CO. The effects of blended oils were also compared with that of interesterified oils whose fatty acid composition is similar with that of blended oils. CO:GNO oil blends showed tendency towards lowering serum cholesterol as compared CO alone, but it was not as effective in its hypocholesterolemic activity as compared with CO:GNO combinations which was subjected to interesterification. Significant reduction in serum cholesterol and TG were observed when CO:OLO blends were used. The cholesterol lowering effects were further enhanced when CO:OLO were subjected to interesterification.

Studies have been carried out to explore the importance of the SFA:MUFA:PUFA balance in regulating Total cholesterol and the LDL/HDL ratio. Comparison between the effects of consuming olive oil and various fat blends in cynomolgus monkeys has revealed that, the intake of fat which is high in MUFA at the expense of PUFA and SFA does not counter the effect of dietary cholesterol and leads to an increased LDL/HDL ratio relative to consumption of oil which has a balanced SFA:MUFA:PUFA intake (Pronczuk and Hayes, 1999). Thus fat

blends with balanced SFA:MUFA:PUFA ratio provide a better lipidemic effect than olive oil alone.

Schwandt *et al.,*(1982) noticed that by switching P/S ratio of dietary fat from 0.3 to 1.0 in the diet of 30 normolipidemic men caused a significant decrease in TC and LDL without affecting HDL. This resulted in significant improvement in the LDL/HDL ratio.

Numerous studies have shown that SL have a unique metabolism and exhibit better benefits when compared to the blended oils having similar fatty acid composition (Lee et al., 2000; Zheng et al., 2007). Wilson et al., investigated the effect of structured triacylglycerols containing long chain fatty acid and short chain fatty acid on serum lipid levels in Hamsters. Hamsters fed structured TG had much lower blood cholesterol levels and a reduction in the accumulation of cholesterol in aorta compared to the hamsters fed control diet (Wilson et al., 2006).

Lee et al., have demonstrated that Conjugated linoleic acid incorporated in olive oil by enzymatic acidolysis reactions exhibited better antiatherosclerotic properties compared to native olive oil in mice (Lee et al, 2005). The supplementation of SL in the diet showed improved serum lipid profile and reduced hepatic Acylcholesterol acyltransferase activity. The atherosclerotic lesion was not observed in the aortic sinus of the mice fed SL as compared to ones given lard and olive oil diet (Lee et al., 2005 and Chambrier et al., 1999) conducted a study comparing the effect of physical mixtures of fats and structured lipid on post operative patients. Normal hepatic functions were observed in post operative patients given the SL, but disturbances in the hepatic functions were observed when physical mixtures of same fat were given to the patients. The plasma TG levels remained unaltered when SL were given but was significantly increased when physical mixture of the fat was given to the patients.

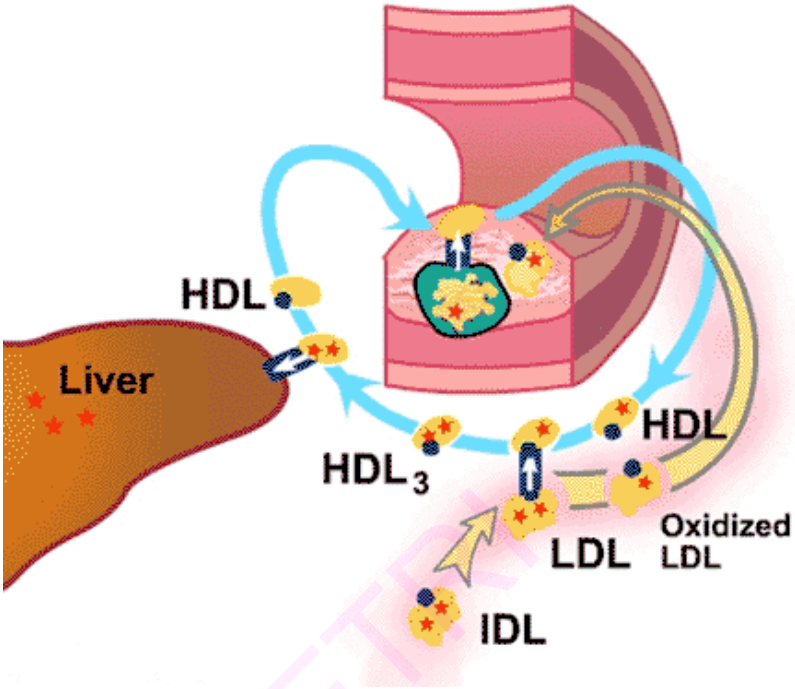
In another study patients receiving the SL prepared from CO and Soya bean oil were compared to those patients given long chain triacylglycerols (Rubin et al., 2000). No alteration of the liver function occurred in any of the patients treated with SL emulsion where as two of

the patients receiving long chain triacylglycerol emulsion developed abnormal liver function, which resolved after switching to the SL emulsion (Rubin et al., 2000). Patrick et al. showed that the digestion and transport of tocopherol and retinol is more efficient when rats were given randomly interesterified SL compared with the rats given physical mixture of fats. This effect was observed in rats with small bowel dysfunction (Patrick et al., 2001). Thus SL provide better vehicle to deliver key essential fatty acids and potentiate the absorption of fat soluble vitamins and other lipid-soluble compounds in normal and in malabsorptive conditions.

Starrup and Hoy (Starrup and Hoy, 2000) compared the lymphatic transport of SL, comprised of rapeseed oil and decanoic acid, a randomized fat, and a physical mixture, in normal rats and rats with malabsorption disorders for fats. All the three lipids compared in the study contained similar fatty acid profile. Rapeseed oil was used as the control. The recoveries of oleic acid and linoleic acid in the serum of rats given SL were higher than those given fat with a random distribution of fatty acids and control oils demonstrating the improved hydrolysis and absorption of fatty acid from the SL compared with native oils (Starrup and Hoy, 2000). Jensen., et al (1994) measured the absorption of medium chain fatty acids when given as SL or when administered in the form of a physical blend of lipids with similar fatty acid composition. The amount of medium chain fatty acids absorbed in the lymph was 2.6 fold higher from SL as compared to that from its physical mixture. Lien et al. showed that SL prepared from CO and palm oil had better absorption rates compared to their blends (Lien et al., 1993). These studies indicate that the absorption and transport of fatty acids in SL are regulated differently than that of the blended oils with similar fatty acid composition. Our studies indicated that though CO: GNO oil blends showed tendency towards lowering serum cholesterol in rats as compared to the rats given CO alone, it was not as effective in its hypocholesterolemic activity as compared with CO: GNO combinations which was subjected to interesterification. However a significant reduction in serum cholesterol and TG were observed when CO: OLO blends were used. These effects were further enhanced when CO: OLO were subjected to interesterification.

Feeding CO blends with GNO or OLO had marginal effect on liver cholesterol levels compared to control animals receiving coconut oil alone. However, when these blended oils were subjected to interesterification reactions and fed to rats, a significant reduction in hepatic cholesterol levels were observed compared to rats given CO or CO blends with GNO or OLO combinations. Rats given blended oils as well as the interesterified oils showed lower hepatic triacylglycerol levels compared to the rats receiving coconut oil alone in the diet. However there were no differences in the hypotriglyceridemic effects exhibited by blended and interesterified oils in liver.

In conclusion, these studies indicated that it is possible to reduce atherogenic potentials of a saturated fat like coconut oil by blending with an unsaturated fat such as groundnut oil or olive oil to balance the proportions of saturated to unsaturated fatty acids. Their hypolipedemic effects can be further enhanced by subjecting these blended oils to enzyme catalysed transesterification reactions.



Chapter 3

Effect of Blend and Interesterified Oils on Antioxidant Enzymes and LDL Oxidation

INTRODUCTION

Oxidative stress is one of the causative factors that has a bearing on the pathogenesis of atherosclerosis. This stress results from the imbalance between the production of oxygen free radicals and the effectiveness of the antioxidant defense system to counter the free radicals. Dietary fats are shown to affect the fatty acid composition, lipid peroxidation and antioxidant defense systems in the body (Scaccini et al., 1992). The polyunsaturated fatty acid content of the diet has been suggested to be a major factor in changing the lipid peroxide content of the tissues (Nalbone et al., 1989). Lipid peroxidation has been implicated in the modification of DNA, protein and membrane structure, formation of age pigment and in the deposition of plaques associated with low-density lipoprotein modification. Many of these effects are initiated by Reactive oxygen Species (ROS) such as super-oxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^\cdot$). These are the products of normal metabolism in the body but can become deleterious if produced in uncontrolled fashion. Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) defend the host against the damaging effects of free radicals (Thomas et al., 1994). Studies have suggested that PUFAs are susceptible to lipid peroxidation and the ratio of PUFA to SFA in the diet determines the susceptibility of the LDL to lipid peroxidation (Buckingham, 1992). The fatty acid composition and the level of antioxidants influence the susceptibility of the LDL to oxidation, which is crucial in atherosclerosis (Thomas et al., 1994). Relatively large elevations in the dietary P/S ratio have been shown to increase the requirement for Vitamin E with respect to number of biological parameters and to reduce the susceptibility of tissues to lipid peroxidation. Nutritionists have recommended an intake of diets with P/S ratio of approximately 1.0 as part of multifunctional diet programme (Singman et al., 1989).

As shown in previous chapter, consumption of Blend and Interesterified oils of CO:GNO with P/S ratio of approximately 1 and CO:OLO blends with unsaturated to saturated fatty acid ratio of approximately 1.0 significantly decreased serum and liver lipids. Since the blending of oils altered the P/S ratio in the diets compared to the individual parent oils used for blending it may affect lipid peroxidation and antioxidant defense mechanism. The present investigation was carried out to examine the effect of feeding blended and interesterified oils consisting of

CO:GNO and CO:OLO on lipid peroxidation, hepatic antioxidant enzymes and on LDL oxidation in rats.

RESULTS

Lipid peroxide and Antioxidant enzyme activities in liver.

The lipid peroxides in GNO fed animals were found to be 38% higher than in rats given CO enriched diets (Table 3.1). The rats given CO:GNO blended oils had 31% higher levels of lipid peroxides while those given CO:GNO oil blends after subjecting them to interesterification reactions had 21% higher levels of lipid peroxides as compared to the rats given CO enriched diets. The specific activities of antioxidant enzymes SOD, Catalase, Glutathione peroxidase and Glutathione transferase were found to be higher by 20%, 16%, 4% and 20% respectively in GNO fed animals as compared to those given CO enriched diets. The corresponding increase in the specific activities of these antioxidant enzymes were 31%, 39%, 17% and 26% respectively in animals given CO:GNO blends compared to those given CO diet alone. When rats were given a diet containing interesterified fats of CO:GNO, the SOD, Catalase, Glutathione peroxidase and Glutathione transferase activities were enhanced by 28%, 41%, 20% and 31% respectively compared to rats given CO diet (Table 3.1).

The animals fed OLO had 21% higher levels of lipid peroxides compared to those given CO. Animals given CO:OLO blend and CO:OLO interesterified fat showed 17% and 10% higher levels of lipid peroxides when compared to those given CO. The antioxidant enzymes SOD, Catalase, Glutathione Peroxidase and Glutathione transferase were elevated by 15%, 29%, 7% and 5% respectively in rats given OLO compared to those given CO. The elevated enzyme activity was observed in rats given blended or interesterified oils of CO:OLO combination. The corresponding increase in the rats given blended oils were 34%, 43%, 27% and 23% respectively while that given interesterified fat showed an increase of 38%, 50%, 28%, 26% respectively in these antioxidant enzyme activities (Table 3.2).

Table 3.1 Effect of dietary fat CO:GNO (B) and CO:GNO (I) on hepatic lipid peroxides and antioxidant enzymes.

Parameters	CO	GNO	CO:GNO(B)	CO:GNO(I)
LPO (nmoles/mgprotein)	2.9±0.2^{a}	4.0±0.3^{b}	3.8±0.3^{b}	3.5±0.3^{b}
SOD (Units/min/mg)	37.2±0.2^{a}	44.5±2.0^{b}	48.7±3.9^{b}	47.5±1.7^{b}
CAT (µmoles/min/mg protein)	49.6±2.3^{a}	57.7±2.3^{b}	68.0±2.0^{c}	69.1±1.9^{c}
GPx (µmoles/min/mg protein)	69.5±1.6^{a}	72.5±1.1^{b}	81.0±0.3^{c}	83.1±1.5^{c}
GST (µmoles/min/mg protein)	47±2.5^{a}	56.5±2.4^{b}	59.0±2.5^{bc}	61.5±1.2^{c}

Values are the mean ± S.D of 5 rats. Values in the same row with common superscript letters are not significantly different at $P<0.05$. LPO,lipid peroxidation, SOD,superoxide dismutase, CAT,catalase, GPx,glutathione peroxidase, GST,glutathione Transferase, B, Blended oil; I,Interesterified oil.

Table 3.2 Effect of dietary fat on hepatic lipid peroxides and antioxidant enzymes in rats fed CO:OLO (B) and CO:OLO (I) oils.

Parameters	CO	OLO	CO:OLO (B)	CO:OLO (I)
LPO (nmoles/mgprotein)	2.9±0.2[a]	3.5±0.3[b]	3.4±0.2[b]	3.2±0.3[b]
SOD (Units/min/mg)	37.2±0.2[a]	42.7±1.7[b]	50.0±1.8[c]	51.2±2.2[c]
CAT (µmoles/min/mg protein)	49.6±2.3[a]	63.8±1.8[b]	71.0±2.1[c]	74.2±2.6[c]
GPx (µmoles/min/mg protein)	69.5±1.6[a]	74.0±0.6[b]	88.5±1.3[c]	89.2±1.2[c]
GST (µmoles/min/mg protein)	47±2.5[a]	49.7±1.3[a]	58.0±2.8[b]	59.0±3.6[b]

Values show the mean ± S.D of 5 rats. Values in the same row with common superscript letters are not significantly different at P<0.05. LPO,lipid peroxidation, SOD,superoxide dismutase, CAT,catalase, GPx,glutathione peroxidase, GST,glutathione Transferase, B, Blended oil; I, Interesterified oil.

Effect on LDL oxidation.

The oxidizability of lipoproteins is dependent on the amount of unsaturated fatty acids, cholesterol and antioxidant levels.

Extensive oxidation of LDL was observed when it was incubated with Cu^{2+} (10 µM) at 37°C for 6 h. The copper induced LDL oxidation in rats fed GNO, CO:GNO blend and interesterified oils was found to be higher by 22%, 9% and 4% in comparison to that observed in rats fed CO (Table 3.3). In rats given OLO, CO:OLO blend and interesterified oils, the LDL oxidation was reduced by 11%, 26% and 29% respectively compared to those given CO containing diets. Therefore while GNO in the diet enhances the susceptibility of LDL to oxidation, OLO in the diet decreased susceptibility of LDL to oxidation (Table 3.4).

Table 3.3. Thiobarbituric acid-reactive substances (TBARS) formed during oxidation of LDL in rats fed CO:GNO (B) and CO:GNO(I) oils.

	CO	GNO (nmoles/mg protein)	CO:GNO (B)	CO:GNO (I)
LDL	8.1±1.2[a]	9.5±2.0[b]	8.8±2.2[b]	8.3±3.1[b]
LDL + Cu^{2+}	25.8±1.7[a]	31.4±3.1[c]	28.2±2.0[b]	26.9±3.7[ab]

Values show the mean ± S.D of 5 rats. Values in the same row with common superscript letters are not significantly different at P<0.05. B, Blended oils; I, Interesterified oils.

Table 3.4. Thiobarbituric acid-reactive substances (TBARS) formed during oxidation of LDL in rats fed CO:OLO (B) and CO:OLO (I).

	CO	OLO	CO:OLO(B)	CO:OLO(I)
	(nmoles/mg protein)			
LDL	8.1±1.2[a]	7.4±1.5[b]	7.0±1.1[b]	7.2±1.4[b]
LDL + Cu^{2+}	25.8±1.7[a]	23.0±2.0[a]	19.0±2.2[b]	18.2±3.2[b]

V

alues show the mean ± S.D of 5 rats. Values in the same row with common superscript letters are not significantly different at P<0.05. B, Blended oils; I, Interesterified oils.

Effect of dietary and liver P/S ratio on LPO in rats fed Native, Blend and Interesterified oils.

P/S ratios in the diet influence the lipid peroxidation. P/S ratio of CO (0.03) enriched diet was significantly lower than that of GNO (1.2) and OLO containing diets (0.4). CO:GNO blend and interesterified oils had P/S ratio of 0.73 and 0.76. The CO:OLO blend and interesterified oils had P/S ratio of 0.16 and 0.13. The LPO in the liver increased with the increase in the P/S ratio of the dietary fats (Fig 3.1a). Similarly, with the increase in the P/S ratio of the liver lipids the level of LPO increased in the liver (Fig 3.1b).

Fig 3.1(a). P/S ratio of Dietary Lipids

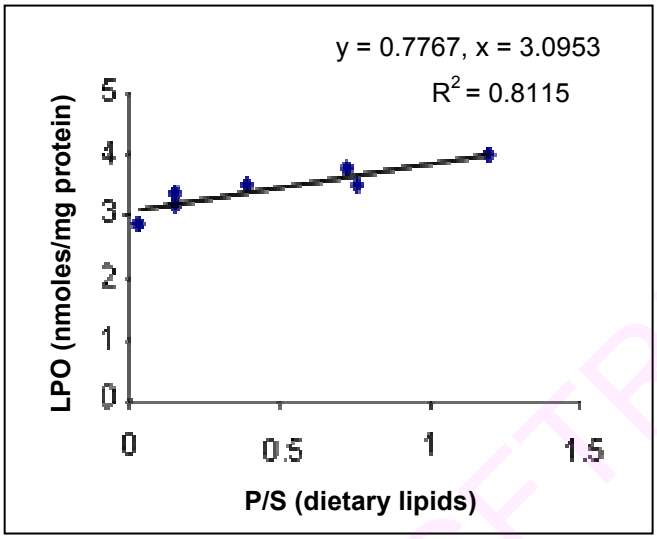
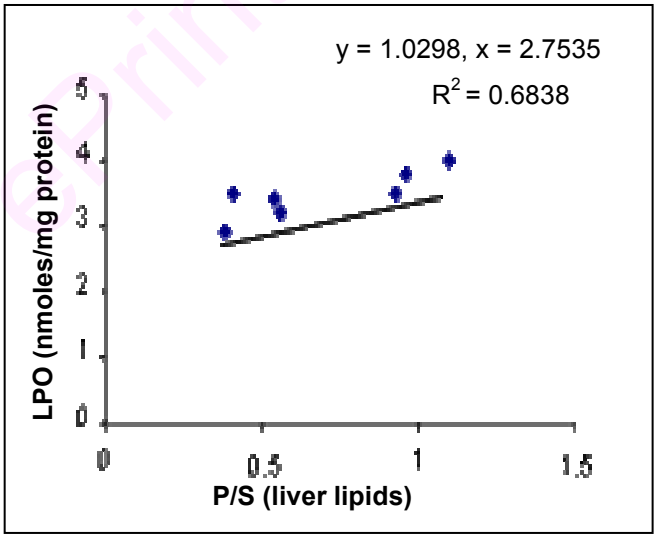


Fig 3.1(b). P/S ratio of Dietary Lipids



DISCUSSION

The objective of this study is to evaluate the effect of dietary lipids which contain approximately equal amounts of SFA:MUFA:PUFA or equal amounts of SFA:total unsaturated fatty acids on lipid peroxidation and antioxidant enzyme activities in rat liver. The PUFA containing lipids are known to be more prone to lipid peroxidation. The susceptibility of the fatty acid to lipid peroxidation increases in proportion to its degree of unsaturation (Cortinas et al., 2005). However the extent of lipid peroxidation may also be regulated by antioxidant molecules as well as antioxidant enzymes.

In the present investigation effects of dietary fatty acids on tissue lipid peroxidation and the levels of protective enzyme activities were monitored after feeding the blended and interesterified oils. An increase in the liver lipid peroxides were observed in the rats fed GNO, CO:GNO blend and CO:GNO interesterified oils compared to rats fed CO. A marginal increase in the LPO levels were also observed in rats given OLO, CO:OLO blend or CO:OLO interesterified oils. GNO and OLO and their blends with CO also enhanced PUFA levels in hepatic tissues. There was a significant correlation between P/S ratio of dietary lipids and levels of LPO (Fig 3.1a) and weaker correlation between P/S ratio of liver lipids and LPO in hepatic tissues (Fig 3.1b).

The change in the dietary lipids also influenced the activities of the antioxidant enzymes involved in scavenging the oxygen free radicals which initiates lipid peroxidation. These antioxidant enzymes protect tissues from oxidative injury by lowering lipid peroxidation. The activity of SOD was increased significantly in the rats fed GNO and the blended oils containing CO:GNO. Similar results were obtained when the blended oils of CO:GNO were subjected to interesterification and fed to rats. Similar changes were also observed when the rats were given a diet containing blend or interesterified oils containing CO with OLO. These studies indicated that though the blending of CO with GNO or OLO enhances the unsaturation index of resulting oil and thereby increase LPO in rats, but there is a simultaneous increase in antioxidant enzyme activities which may control to a certain extent of LPO levels which was increased by higher unsaturation of lipids in the diet. Therefore the antioxidant enzymes seem to adjust its levels to protect higher unsaturation of the lipids provided in the diet.

Lipid peroxidation is also involved in the oxidative modification of low density lipoprotein (Esterbauer et al., 1992). Oxidized LDL plays an important role in atherosclerosis. Diets enriched in linoleate increase the content of linoleic acid in LDL lipids which increase the susceptibility of LDL to oxidation. Studies have also shown that MUFA-rich diet renders LDL less prone to oxidation (Bonanome et al., 1992). Earlier studies have implicated that the phenolic compounds in OLO as one of the factors responsible for reducing the susceptibility of LDL to oxidation. (Covas et al, 2000). LDL in Hypercholesterolemic patients fed a diet containing oleic acid rich olive oil showed increased resistance to oxidation (Baroni et al., 1999). Our present study has shown that the LDL oxidation catalysed by Cu^{2+} was reduced in rats fed OLO or CO:OLO blend or CO:OLO interesterified oil. This is in agreement with the protective effects of OLO or its phenolics on LDL oxidation (Terner et al., 2005).

OLO is a rich source of phenolics such as Hydroxytyrosol, tyrosol, Oleuropein aglycon and decarboxymethyl oleuropein aglycon. It has been shown that hydroxytyrosol is the most active antioxidant compound in virgin olive oil (Papadopoulos and Boskou, 1991). Laponite et al (2005) investigated the effect of Mediterranean foods (rich in phenolics) on circulating oxidized-LDL levels in healthy women. The Mediterranean diets significantly decreased oxidized-LDL levels by 11.3% after 12 week of nutritional intervention.

Julio et al., 2001, compared the effect of Virgin olive oil and High oleic sunflower oil on antioxidant status in rabbits. Despite similarity in the fatty acid compositions of the oils, the animals fed high oleic sunflower oil showed higher peroxidative stress measured in terms of hydroperoxide content and the TBARS. The groups fed olive oil showed lower TBARS and hydroperoxides, higher tocopherols and ubiquinone content. The olive oil fed groups showed highest activity of Glutathione Peroxidase. These studies indicated that in addition to fatty acid composition of the dietary lipids, the minor constituents such as polyphenols present in olive oil can greatly influence antioxidant status in animals. Our results are in agreement with these findings.

In addition to polyphenols, Vitamin E also plays a beneficial role in reducing the lipid peroxidation levels. α-tocopherol is the active form of Vitamin E. Supplementation of diets with α-tocopherol has been found to reduce the susceptibility of LDL particles to oxidation in healthy, diabetic and dyslipidemic subjects (Lonn et al., 2002; Wen et al., 1999). α-tocopherol can inhibit highly reactive lipid peroxyl and alkoxyl radicals, which promote the lipid peroxidation (Abadu et al., 2004). α-tocopherol may affect the assembly of NADPH-oxidase responsible for reactive oxygen species production (Munteanu et al., 2004).

Recently, Nevin and Rajmohan, studied the effect of Virgin Coconut oil (VCO) on the antioxidant enzyme activities and lipid peroxidation level in rats (Nevin and Rajmohan, 2006). VCO had higher levels of unsaponifiable components containing Vitamin E and polyphenols than refined CO. The LPO levels and the conjugated dienes were significantly lower in the heart, liver and kidney of rats fed VCO compared to the rats fed refined CO. Refined CO had similar fatty acid composition as that of VCO. Virgin Coconut oil also significantly increased the antioxidant enzyme activities and reduced the lipid peroxide content (Nevin and Rajmohan, 2006). VCO with higer polyphenol content also rendered LDL less prone to oxidation (Nevin Rajmohan, 2004). These studies again indicated that the minor constituents in an oil may have an influence on antioxidant system in the body. Blending of oils thus provide an opportunity to enrich the native oils with useful minor constituents.

In conclusion, the present study show that the blend and interesterified oils of CO:GNO and CO:OLO enhanced the antioxidant enzyme activities and reduced the susceptibility of LDL to oxidation. Hence oils with balanced fatty acid composition and also containing minor components may beneficially modulate antioxidant status in the body.

Chapter 4

Platelet Aggregation and Erythrocyte Membrane Bound Enzymes in Rats Fed Blend and Interesterified Oils

INTRODUCTION

Platelets are tiny corpuscular cells found in the blood along with erythrocytes. They are continuously surveying the inner layers of intact blood vessels and play an important role in haemostasis. Whenever there is any break or damage in the blood vessels, platelets are exposed to the damaged tissue resulting in a cascade of events like shape change, secretion and aggregation leading to the formation of a precise haemostatic plug. If these synergistic interactions take place in vivo in uncontrolled fashion, it results in the blockage of arteries and reduction in blood supply to essential organs causing cardiovascular or cerebrovascular complications. Blood platelets have been recognized to play a role in thrombogenesis. It is assumed that lipid signaling is strongly involved in platelet activation, including various phospholipase pathways and a relevant arachidonic acid cascade leading to pro-aggregator prostanoids PGG_2/H_2 and thromboxane A_2. Increased level of platelet aggregation is regarded as a risk factor for atherosclerosis (Ross, 1986) and for the clinical recurrence of coronary heart disease (Thaulow, 1991). The physiological function of platelets is to maintain vascular integrity and arrest bleeding. Platelets take part in thrombosis by adhering to the exposed sub endothelial connective tissue on the site of injury. The adhesion is followed by platelet activation, which results in aggregation and secretion of regulatory compounds (Hunter et al., 2000).

Dietary lipids are thought to influence the development of cardiovascular disease via number of processes involving the haemostatic system and platelet function. (Miller 1997 & Dutta-Roy, 1994). Change in the dietary lipids provides a non-pharmocological means of altering eicosanoid synthesis and may in turn influence the aggregability of platelets. The prostanoids of central concern for platelet function are thromboxane A_2 that has proaggregatory activity and prostacyclin I_2 that has antiaggregatory activity. Changes in the fatty acid composition of plasma and membrane lipids can modulate the cellular eicosanoid metabolism and potentially alter a number of membrane functions relevant to atherothrombogenesis (Kinsella, 1990).

An increased level of platelet aggregation is regarded as a risk factor for atherosclerosis (Thalow, 1991). Platelets are activated only if an external stimulus, interacting with platelet

surface glycoproteins or glycolipids, is able to transduce its signal inside the platelet. All the stimuli finally activate the same fibrinogen receptor (GP IIb-IIIa) which changes its conformation and binds fibrinogen, or fibronectin. Even though the mechanism of GP IIb-IIIa activation is still obscure, it involves signaling pathways in both directions across the plasma membrane. Thus the metabolism and FA composition of platelet phospholipids are emphasized in thrombus formation.

Attempts to identify the specific lipids affecting the haemostatic system suggest that not only total fat content but also the types of fat in the diet may be influence the system (Miller, 1997). Renaud et al., showed that there is significant correlation between the saturated fatty acid content of the diet and platelet aggregability (Renaud, 1981). The cholesterol lowering properties of linoleic acid (18:2 n-6) have been known for many years, but its effect on haemostasis is less well documented. Saturated fatty acids are atherogenic and favor platelet aggregation, decreasing prostacyclin production and increasing thromboxane production. They can thus be considered prothrombotic substances (Siess et al., 1980). Polyunsaturated fatty acids of omega 3 series reduce platelet activity and the thrombogenic capacity of the arterial wall (De La Cruz et al., 1997).

(Mattson, 1985 & Barradas, 1990). The principal fatty acid of olive oil, oleic acid (18:1 n-9), is an effective hypocholesterolemic agent and has been considered to be potentially useful in the prevention of cardiovascular disease. Being a very good source of phenols olive oil has been shown to have an inhibitory effect on platelet function and thromboxane synthesis (Petroni, 1995).

Studies have also shown that the presence of other minor compounds in oils could change platelet activation (Oubina, 2001). High oleic sunflower oil is rich in tocopherols, whereas palmolein is rich in tocotrienols (Sanchez-Muniz, 1998) and both of them may give protection against cardiovascular disease because of their antioxidant activity (Petroni, 1994) and inhibition of platelet aggregation (Qureshi, 1991).

Activity of membrane bound enzymes is influenced by membrane lipids. Electrolytes play an important role in the maintenance of structure and function of a living cell. The presence of two cation transport pumps in the erythrocytes and other cell membranes has been recognized. $Ca^{2+}Mg^{2+}$-ATPase and $Na^{+}K^{+}$-ATPase catalyze the hydrolysis of ATP that is coupled to the active transport of Ca^{2+} $Mg^{2+}$and Na^{+} K^{+} across the cell membrane. Ion pumps use a large percentage of the total cellular energy, e.g., Na^{+} K^{+}-ATPase is responsible for 5–40% of total ATP turnover depending on cell type (Clausen, 1986) Maintenance of the cation gradient by these ATPase enzymes is of fundamental importance in the control of hydration, volume, nutrient uptake and fluidity of cells. The normal activity of Na^{+} K^{+}–ATPase that generates the transmembranous Na^{+} K^{+} gradient is essential for the contractibility and excitability properties of muscle and nerve tissue. $Ca^{2+}Mg^{2+}$-ATPase is a reflection of energy-dependent calcium transport across the cell membrane. The enzyme is present in all the tissues which are capable of transporting univalent cations against an electrochemical gradient. Reports suggest that Na^{+} K^{+} ATPase and Ca^{2+} Mg^{2+} ATPase of platelets and erythrocytes in hypertensive patients have lower activity resulting in defective transport mechanism (Touyz, 1992). Small change in fatty acid composition of cell membrane phosphatidylcholine causes change in the Na^{+} transport system. (Engelman, 1990). Impairment of Ca^{2+} Mg^{2+}-ATPase could lead to accumulation of intracellular calcium (Bennett, 1985). Therefore changes in membrane lipid composition will have a bearing on the functions associated with it. To evaluate this, the effect of feeding blended oils and interesterified oils on platelet and erythrocyte membrane fatty acid composition was studies in rats. Further, the platelet aggregation and membrane bound enzymes in erythrocytes were evaluated as influenced by feeding blend and interesterified oils.

RESULTS

Fatty acid composition of the platelets

The dietary lipids influenced the fatty acid composition of platelets. In the rats fed CO, linoleic acid content was found to be 4.2 % and arachidonic acid 7.2%. In GNO fed animals the linoleic acid content was found to be 10.6 % and arachidonic acid was found to be 16.7 %. In rats given a diet containing CO:GNO blends the linoleic acid and arachidonic acid levels in platelet lipids were 8.1%, 14.7% respectively and in rats fed CO:GNO interesterified oils it was found to be 7.6 and 13.3 % respectively. In OLO fed animals the linoleic acid levels was 5.6% and arachidonic acid levels was at 9.2 %. The linoleic and arachidonic acid level in rats given CO:OLO blends were found to be 4.9% and 8.5% respectively. The oleic acid content was however found to be enhanced by 19% as compared to that found in rats given CO diet (Table 4.1). The oleic, linoleic, arachidonic acid in rat platelets fed CO:OLO diet which was subjected to interesterification was found to be 37.7%, 5.1% and 8.8% respectively. These values are similar to those found in rats given blended oils of CO:OLO in the diet (Table 4.1).

Table 4.1. Fatty acid composition (%) platelets of rats fed native, blend and interesterified fats

Fatty acid	CO	GNO	OLO	CO: GNO (B)	CO:GNO (I)	CO:OLO (B)	CO: OLO (I)
12:0	6.5±1.1^{a}	ND	ND	1.1±1.8^{a}	1.8±0.8^{a}	1.5±0.3^{a}	1.3±0.2^{a}
16:0	29.3±0.4^{a}	25.2±0.4^{b}	26.6±0.5^{c}	27.8±0.4^{a}	28.5±0.3^{a}	27.3±0.6^{d}	28.1±0.6^{d}
16:1	6.5±1.1^{a}	1.6±0.1^{a}	1.4±0.5^{a}	1.8±0.4^{a}	1.7±0.3^{a}	1.9±0.1^{a}	2.1±0.3^{a}
18:0	14.1±0.3^{a}	16.2±0.1^{b}	14.6±0.3^{c}	16.8±0.8^{c}	17.8±0.4^{b}	18.1±0.2^{b}	17.1±0.2^{b}
18:1	31.4±0.2^{a}	29.6±1.3^{b}	42.5±2.2^{c}	29.5±2.6^{b}	28.8±1.3^{b}	37.5±0.1^{c}	37.7±1.1^{c}
18:2	4.2±0.5^{a}	10.6±0.7^{b}	5.6±0.3^{c}	8.1±0.6^{b}	7.6±0.1^{b}	4.9±0.5^{c}	5.1±0.2^{c}
20:4	7.2±2.1^{a}	16.7±1.9^{b}	9.2±2.1^{c}	14.7±2.3^{b}	13.3±1.7^{b}	8.5±0.1^{c}	8.8±2.8^{c}

Values are ± SD of 5 rats. Values not sharing a common superscript within a row are statistically significant $p<0.05$

Platelet aggregation in rats fed Native, Blend and Interesterified oils.

The percent of ADP induced platelet aggregation was decreased in the rats fed CO:GNO blend and Interesterified oils by 22% and 19% as compared to that found in rats given CO diet. The collagen induced aggregation was reduced by 28% and 34% compared to the CO fed groups. The rate of ADP induced aggregation in CO:GNO blend and interesterified oil fed rats was lowered by 28% & 34% as compared to that found in rats given CO diet . The rate of Collagen induced aggregation was lowered by 21% and 27% compared to the CO fed groups (Table 4.2). Similarly in rats given CO:OLO blend and interesterified oils the percent of ADP and Collagen induced platelet aggregation was decreased by 38%, 44% & 41% 45% respectively compared to the CO fed groups. The rate of aggregation ADP and Collagen induced platelet aggregation was decreased by 50%, 52% & 48%, 51% respectively in comparison with the rats fed CO containing diets (Table 4.2).

Tables 4.2 Platelet aggregation in rats fed Native, Blend and Interesterified oils.

Parameters	CO	GNO	OLO	CO:GNO (B)	CO:GNO (I)	CO:OLO (B)	CO:OLO (I)
				ADP induced aggregation			
Percent aggregation	68.0±4.0^{a}	50.0±0.8^{b}	45.0±2.1^{d}	53.0±1.5^{c}	55.0±3.2^{c}	42.0±2.6^{d}	38.0±1.7^{e}
Rate of aggregation	11.9±0.3^{a}	6.8±0.4^{b}	5.3±0.5^{c}	8.3±0.4^{d}	7.9±0.5^{d}	5.9±0.5^{c}	5.7±0.6^{c}
				Collagen induced aggregation			
Percent aggregation	64.0±3.1^{a}	48.0±1.5^{b}	40.0±0.5^{c}	46.0±0.7^{b}	42.0±1.4^{c}	38.0±1.1^{d}	35.0±2.6^{d}
Rate of aggregation	9.2±0.2^{a}	5.3±0.3^{b}	4.5±0.4^{c}	7.2±0.5^{d}	6.7±0.2^{d}	4.8±0.3^{c}	4.5±0.4^{c}

Values are ± SD of 5 rats. Values not sharing a common superscript within a row are statistically significant $p<0.05$

Lipid peroxides level in platelets of rats fed Native, Blend and Interesterified oils.

The change in the dietary lipids influenced the lipid peroxide levels in platelets. Malondialdehyde in platelets which was aggregated using ADP were increased by 2 and 1.4 folds in GNO and OLO fed animals respectively compared to those found in animals fed CO. In rats fed CO:GNO blended or interesterified oils the lipid peroxides were found to be 1.9 and 1.6 fold higher compared to those given CO containing diets. In rats fed CO:OLO blended or interesterified oils lipid peroxides levels were increased by 1.5 and 1.3 folds. Similar changes in malondialdehyde levels were observed in platelets which were aggregated using collagen. The LPO was increased by 1.9 and 1.6 folds in rats fed GNO and OLO respectively. In rats given CO:GNO blend or interesterified oils the lipid peroxide levels were increased by 1.7 and 1.5 folds. In CO:OLO B and Interesterified oils LPO was increased by 1.3 folds compared to the rats given CO containing diets (Table 4.3).

Lipid peroxides level in erythrocyte membrane of rats fed native, blend and interesterified oils.

Alteration in dietary fat affected the lipid peroxides level in erythrocyte membranes. The lipid peroxides in erythrocyte membrane of GNO fed animals were found to be 52% higher than that in rats given CO enriched diets. The erythrocytes of rats given blended oils of CO:GNO had 45% higher levels of lipid peroxides while those given CO:GNO interesterified oil had 35% higher levels of lipid peroxides as compared to the rats given CO enriched diets. The animals fed OLO had 29% higher levels of lipid peroxides compared to those given CO. Animals given CO:OLO blend and CO:OLO interesterified fat showed 21% and 17% higher levels of lipid peroxides in erythrocytes when compared to those given CO (Table 4.3).

Table 4.3. Lipid peroxidation in Platelets and erythrocyte membrane of rats fed Native, Blend and Interesterified oils

	CO	GNO	OLO	CO:GNO (B)	CO:GNO (I)	CO:OLO (B)	CO:OLO (I)
LPO levels in platelets (nmoles of MDA formed/ 1.5 X 10^8 cells)							
ADP induced	1.5±0.2^{a}	3.1±0.3^{b}	2.3±0.3^{c}	2.8±0.2^{b}	2.5±0.2^{c}	2.2±0.2^{c}	2.0±0.3^{c}
Collagen induced	1.2±0.2^{a}	2.3±0.1^{b}	1.9±0.1^{b}	2.0±0.1^{b}	1.8±0.2^{c}	1.6±0.1^{c}	1.5±0.2^{c}
LPO levels in Erythrocyte membranes (nmoles of MDA formed/mg protein)							
Collagen induced	2.0±0.1^{a}	3.1± 0.1^{cd}	2.6± 0.1^{b}	2.9±0.1^{c}	2.7±0.1^{c}	2.5 ±0.1^{b}	2.4± 0.1^{b}

Values are ± SD of 5 rats. Values not sharing a common superscript within a row are statistically significant $p<0.05$

Influence of Native, Blend and Interesterifed oils on Na^+ K^+ ATPase and Ca^{2+} Mg^{2+} ATPase activities.

The effect of blend and interesterified oils on the Na^+ K^+-ATPase activity of erythrocytes in rats is given in (Table 4.4). The activity of ouabain sensitive enzyme was significantly increased by 23% and 17% in GNO and OLO fed groups compared to that found in CO fed groups. The rats fed CO:GNO blend and interesterified oils showed 11% and 19% increase in the activity of this enzyme as compared to that found in CO fed groups. Similarly the activity of Ca^{2+} $Mg2^+$-ATPase was increased by 17% and 9.5% and 11% in rats given GNO, CO:GNO blend and CO:GNO interesterified groups as compared to those given CO containing diets.

In rats given OLO, CO:OLO blend and interesterified oils the activity of ouabain sensitive Na^+ K^+-ATPase was increased by17%, 35%, 33% respectively as compared to that found in CO fed animals. The activity of Ca^{2+} $Mg2^+$-ATPase was increased by 7.5%, 7.2% and 8.1% respectively as compared to the CO fed groups (Table 4.4).

Table 4.4 Na^+ K^+ ATPase and Ca^{2+} Mg^{2+} ATPase activities in Erythrocyte membranes of rats fed native, blend and interesterified oils.

CO	GNO	OLO	CO: GNO (B)	CO:GNO (I)	CO:OLO (B)	CO: OLO (I)
Na^+ K^+ ATPase activity (nmole Pi mg^{-1} protein h^{-1})						
241±1.5[a]	291±2.7[d]	278±3.4[c]	259±3.3[b]	265±2.7[b]	289±3.7[d]	285±3.1[d]
$Ca^{2+}Mg^{2+}$ATPase activity (nmole Pi mg^{-1} protein h^{-1})						
431±5.1[a]	486±3.4[d]	463±6.1[b]	472±3.7[c]	478±4.3[c]	462±5.7[b]	466±3.4[b]

Values are ± SD of 5 rats. Values not sharing a common superscript within a row are statistically significant $p<0.05$

DISCUSSION

Our aim in this part of the investigation was to assess the influence of dietary fats comprising of CO:GNO and CO:OLO blend and interesterified oils on the fatty acid composition and aggregation of platelets. The activities of membrane-bound enzymes in erythrocytes was also monitored. This was compared against the rats fed CO which is rich in saturated fatty acids.

Saturated fats promote arterial thrombus formation, while linoleic acid is reported to be antihrombotic (Hornstra, 2001). We found a significant effect of dietary lipids on ADP and collagen-induced platelet aggregation. Platelet aggregation was decreased in rats fed GNO, CO:GNO blend and Interesterified oils compared to the rats fed CO. Rate of aggregation was also decreased in rats given OLO, CO:OLO blend and CO:OLO interesterified oils.

Several reports are available in the literature showing the link between platelet aggregation and the type of dietary fat/oil consumed. When platelet aggregation was measured in heparin treated circulating arterial blood, the spontaneous aggregation was found to be higher in groups given coconut-oil compared to the ones given sunflower seed oil. Soya, rich in polyunsaturated fatty acids such as linoleic acid, reduces the platelet aggregation and hence lowered the risk for thrombosis. MUFA rich diets reduce platelet aggregation compared to those given SFA diet (Sirtori et al., 1986 & Smith et al., 2003).

The change in the dietary lipids also influenced the lipid peroxide level in the platelets after subjecting them to aggregation with ADP or Collagen. An increase in the lipid peroxides were observed in the rats fed GNO, CO:GNO blend and CO:GNO interesterified oils. Similar changes were observed when the rats were given a diet containing blend or Interesterified oils containing CO with OLO. This finding suggests that fatty acids play a significant role in platelet aggregation.

The principal fatty acid of olive oil, oleic acid (18:1 n-9), is an effective hypocholesterolemic agent and has been considered to be potentially useful in the prevention of cardiovascular disease. In addition to fatty acid composition, the minor components present in this oil also affect platelet aggregation. Olive oil was shown to have an inhibitory effect on platelet and

thromboxane synthesis (Giuseppina, et al., 2003). Olive oil particularly its polar lipid fraction, is rich in Platelet activating factor antagonists in comparison with other seed oils. And these platelet- activating factor (PAF) antagonists exert significant antiatherosclerotic activity in rabbits (Karantonis et al., 2006). The effect of lipoproteins on PGE_2, and TxB_2 generation in cultured endothelial cells was lowered after the ingestion of virgin olive oil which was enriched in unsaponifiable fraction compared to the stripped olive oil or high oleic sunflower oil (Perna, 2006). Petroni et al. found that 2-(3,4-dihydroxyphenyl)-ethanol (DHPE) components in the phenolic fraction of olive oil can inhibit platelet function and eicosanoid formation in vitro (Petroni, 1995). Hydroxytyrosol was proven to inhibit in vitro platelet aggregation and the accumulation of proaggregating agent thromboxane in human serum, (Petroni, 1995 and Kohyama, 1997). Hydroxytyrosol was also been found to inhibit human platelet reactivity (Giuseppina et al., 2003). These studies indicated that in addition to fatty acid composition of the dietary lipids, the minor constituents such as polyphenols present in olive oil can also influence platelet aggregation. It has also been demonstrated that dietary supplementation of polyunsaturated fatty acids or certain components of monounsaturated fatty acids reduces platelet aggregation mainly through metabolic competition between the different prostanoid pathways, which reduces thromboxane A synthesis and increases other prostaglandins with a weaker ability to favour aggregation (Murakami et al, 1999). Thus platelet aggregation was influenced by both fatty acid compositions as well as minor constituents present in the oil.

Fatty acid composition in the dietary fat can also regulate the activity of membrane-bound enzymes which are considered crucial for membrane function. Reduction in membrane fluidity has a direct relation to deficiency of essential fatty acids in the diet (Alam and Alam, 1986). This in turn could impair the function of membrane ion channels and ultimately influence electrophysiological properties of the membranes.

Membrane-bound enzymes are sensitive indices of altered cellular environment. Vajreswari *et al.,* (1992) conducted an experiment to study the relationship between fatty acid composition of the dietary fat and its influence on erythrocyte membrane (EM) lipid composition and the activities of membrane-bound enzymes, nutritionally adequate diets containing 20% groundnut, coconut, safflower or mustard oil were fed to weanling CFY rats for 4 months.

Activities of membrane-bound enzymes such as Na^+, K^+ ATPase, Mg^{2+} ATPase, Ca^{2+} Mg^{2+} ATPase, and acetylcholinesterase were assayed. The activities of all membrane-bound enzymes, except Mg^{2+}ATPase, and sialic acid content were higher in the mustard oil fed group than in the rest of the groups. Cholesterol to phospholipid molar ratio was similar in all the groups. However, safflower and mustard oil fed groups had increased cholesterol content and a higher degree of unsaturation in the membrane fatty acid composition.

The higher membrane fatty acid unsaturation in the safflower oil fed group was principally due to linoleic (18:2) and arachidonic (20:4) acids, while in the mustard oil fed group it was mainly due to oleic (18:1), eicosaenoic (20:1), erucic (22:1), and linoleic (18:2) acids. These results suggest a relationship between the quality of dietary fat, erythrocyte fatty acyl composition, and the activities of membrane-bound enzymes. (Vajreswari,.1992). Similarly our results have shown that the activity of ouabain-sensitive Na^+ K^+-ATPase in erythrocyte membrane was increased in GNO and OLO fed groups. The Ca^{2+} Mg^{2+}-ATPase activity was also significantly increased in the erythrocyte membranes of rats fed GNO and OLO as compared to the rats fed with CO. Similar results were also observed in rats given blend and interesterified oils consisting of CO:GNO and CO:OLO.

In summary our study show that rats given unsaturated oils or Blend and interesterified oils with higher levels of unsaturated fatty acids lowered the rate of ADP and Collagen induced platelet aggregation compared to the rats given CO indicating the importance of dietary fats in influencing platelet aggregation. The blend and interesterified oils also enhanced the erythrocyte membrane bound enzyme activities compared to that found in the rats given a saturated fat like coconut oil.

General Discussion & Summary

DISCUSSION

Oils and fats play a very important role in human diet, besides providing calories they are also the source of essential fatty acids, vitamins (A,D,E and K). However, these days fat is perceived to be the 'baddie' of all the nutrients, linked to obesity, cardiovascular disease (CVD) and cancer. TGs constitute the major contributor for energy of the dietary lipids. The fatty acyl groups in TGs may vary in chain length from C2 to C24 and from saturated fatty acids to unsaturated fatty acids. Evidence are accumulating that both the overall fatty acid profile and the intramolecular structure of dietary fats are important when considering the nutritional value of a given fat. The growing calorie consciousness among the present day population and the increasing incidences of cardiac problems have created the great concern about the types of the fats and oils used in our diet. Choosing the right type of fat is more important than reducing the amount of fat. The four main categories of fatty acids, that is saturated, monounsaturated, polyunsaturated and *trans* fatty acids (SAFA, MUFA, PUFA and TFA, respectively) have different effects on the balance of cholesterol-carrying lipoproteins in the blood (Mensink, 2003).

Diets containing a high proportion of saturated fatty acids elevate plasma concentrations of total and LDL-Cholesterol levels by decreasing LDL receptor-mediated catabolism (Mann, 2002 and Nicolosi 1990). Because the increased LDL cholesterol concentration represents an increase in risk for coronary heart disease (Steinberg and Witztum, 1990), recommendations have been made to reduce intake of dietary saturated fatty acids and to increase consumption of PUFAs and MUFAs (AHA 1990). However, not all SFA affect total cholesterol and LDL-cholesterol concentrations in the same manner. The medium chain fatty acids (C6-C10) enter the portal system and directly transported to liver where it is metabolized in carnitine independent pathway to generate quick energy and it gives low calorie in comparison to LCSFA. MCFA have immense medical and nutritional importance. Stearic acid has neutral effect on plasma cholesterol concentrations which has been proposed to be a result of the conversion of stearic acid in the body to oleic acid (Grundy, 1990). Lauric acid increases HDL-cholesterol and palmitic and stearic acid do not affect HDL-cholesterol (Mensink et al. 2003). The biological effects of MUFA depend on whether the MUFA are in the *cis* or *trans* configuration. *Cis*-MUFA are relatively neutral in relation to their effects on LDL and HDL

(Mensink, 2005), but *trans*-MUFA have been shown to increase LDL and decrease HDL (Mensink, 2005). *Trans*-MUFA also increase plasma levels of lipoprotein (a) (Tine Tholstrup and Samir, 2004) and triglycerides (Mensink, 1992) and may reduce endothelial function (de Roos, 2003). Sufficient amounts of linoleic and α-linolenic acid are necessary for growth and development and for maintaining health. Numerous metabolic studies have shown that linoleic acid has a lowering effect on total cholesterol and LDL-cholesterol (Harris, 2008), while other beneficial effects include improvement in platelet function (Michac, 2008) and anti-arrhythmic effects (Jing, 1996). Some of their metabolites serve vital structural functions in membrane lipids and others exert hormone-like activities. n-3 PUFA have wide-ranging biological effects. They have been shown to lower triglycerides (Kris-Etherton, 2002). PUFA is also associated with a reduced inflammatory response (Zhao, 2004). A recent study showed that higher intakes of α-linolenic or linoleic acid were inversely correlated with the occurrence of coronary artery disease (Djousse 2001). According to Hu, et al., (Hu, 1999) replacing 5% of the energy of SFAs by unsaturated fatty acids results in a 43% decrease in CHD.

Dietary fatty acids regulate plasma LDL-C levels by affecting LDL receptor activity, protein, and mRNA abundance (Maria Luz and Kristy L, 2005). Cholesterol-raising SFAs (12:0, 14:0, 16:0) decrease LDL receptor activity, protein, and mRNA abundance, while unsaturated fatty acids increase these variables Shaomei Yu-Poth (2005) demonstrated that SFA (palmitic acid) markedly decreased LDL receptor protein levels and PUFA (linoleic acid) had increased LDL receptor levels in pigs fed a diet containing 0.25% cholesterol, compared to pigs fed a low-fat, cholesterol-free diet. These distinct effects of dietary fatty acids were accompanied by parallel changes in LDL receptor mRNA levels. These data provide strong evidence for an independent and positive effect of PUFAs on the regulation of LDL receptor expression. Dietary modification of hepatocyte membrane fluidity may be one way in which diets high in PUFAs affect LDL receptor activity differently than diets enriched in SFAs. Support for this suggestion comes from in vitro (Kuo et al.,1990) and in vivo studies (Maria Luz, 2005), which showed significant alterations in LDL binding to the LDL receptor as a result of changes in membrane fluidity. It has also been suggested that dietary fatty acids can directly influence the number of receptors available for uptake of circulating LDL by specifically affecting LDL receptor synthesis. In vitro binding studies demonstrated that alterations in LDL uptake

associated with dietary fatty acid composition can be attributed to changes in LDL maximal binding, an indicator of receptor number (Fernandez et al., 1989). It has been suggested that dietary fatty acids and cholesterol regulate hepatic LDL receptor activity via cholesteryl ester and free cholesterol regulatory pools. These cholesterol regulatory pools are affected by ACAT, the rate-limiting enzyme of cholesterol esterification. SFAs suppress this enzyme, which may result in a greater proportion of cholesterol remaining in the regulatory pool. An increase in hepatic cholesteryl ester is negatively correlated with LDL receptor activity in hamsters (Spady et al., 1993).

High concentrations of total and LDL-cholesterol and low concentrations of HDL-cholesterol are established risk factors of coronary disease. Concern has also been raised that excessive PUFA consumption could be detrimental if it decreases HDL cholesterol (Grundy and Denke, 1990). Unsaturated fatty acids are particularly susceptible to oxidative modification, and the extent of this is increased as the degree of unsaturation (number of double bonds) increases. Excessive intake of PUFA may change Permeability of the Cellular membranes. They may also lead to early ageing of cells particularly skin cells. An Imbalance may be caused between different prostaglandins when excess of PUFA is consumed.

Since dietary fat with high PUFA may be as harmful as fat with low PUFA content, a balance between PUFA and SFA in the dietary fats need to be maintained.

The oxidisability of fatty acids increases with the number of double bonds. It is noted that monounsaturated fatty acid is favoured over polyunsaturated fatty acids because of its oxidative stability and neutral cholesterolemic property. Similarly saturated fatty acids of medium chains are favoured with the additional beneficial property of easier metabolism for energy. Oleic acid is favoured to replace certain polyunsaturated acids, e.g linoleic acid, because of its favourable influence on oxidative stress, proinflammatory response. Diets with MUFA-rich oils have been shown to produce LDL-cholesterol that is more resistant to oxidation than diets with PUFA-rich oils (Castro, 2000). Hence the proportions of different fatty acids in the dietary fat rather than the percentage of energy they supply is of primary importance for maintaining the level of serum cholesterol. Studies on the effects of high fat vs

low fat diets on serum lipoprotein levels varied widely with polyunsaturated/saturated fatty acid (P/S) ratio (Nelson, 1995). Raising the dietary P/S ratio has been recommended for the prevention of CVD. However, a high P/S ratio diet enhances oxidative stress because PUFA are highly susceptible to lipid peroxidation. Kang et al., demonstrated that a P/S ratio of 1.0-1.5 in the diet is a favourable range to reduce the risk of CVD (Kang et al., 2003).

However, P/S ratio alone is unsuitable to predict the change of plasma cholesterol level, because of the presence of MUFA in significant quantities in almost all the oils. Apart from supplying energy MUFA also have cushioning effect on negative qualities of SFA and PUFA. Hence it is considered that not only P/S ratio is important but P+M/S ratio is also important in controlling the serum and hepatic lipids. Chang & Huang. (1999) examined the effects of various P/S ratio Vs P + M/S ratio on lipid metabolism. When the P + M/S ratio was below 3, the change in the P/S ratios did not affect the levels of plasma total and lipoprotein cholesterol and TG. When the P/S ratio was fixed at 1 or 4, the diet of higher P + M/S ratio resulted in greater accumulation of liver cholesterol, VLDL-C and LDL cholesterol. High MUFA content resulted in greater accumulation of liver cholesterol.

The proper balance in fatty acid intake becomes exceedingly important for generating the optimal LDL/HDL ratio. As reported by Sundram *et al.*, (2007) a controlled intake of 18:2 PUFA is required for the reduction in LDL without lowering HDL. Thus, the approximately equal balance of SFA: MUFA: PUFA (1:1.3:1) as recommended by NCEP and AHA is a very important basic consideration at any fat intake level for maintaining the best LDL/HDL ratio. Schaefer *et al.* have demonstrated that a high PUFA diet (P/S 2.0) decreases both LDL and HDL in normolipidemic and hyperlipidemic subjects. Weisweiler *et al.* (1985) showed that a balance between SFA and PUFA is important to maintain HDL and lower LDL to improve the LDL/HDL ratio. The same decrease in LDL-C obtained with very high PUFA can be achieved if the SFA : MUFA : PUFA ratio is balanced, but this balanced approach does not depress HDL. These type of findings about the effect of individual fatty acids has prompted oil technologists and nutritionists to search for the ideal fat with S:M:P ratio of 1:1:1 recommended by world health organisation.

Studies on lipid lowering diets have focused on the total content of SFA, MUFA and PUFA. However, the distribution of these fatty acids on the triglyceride (TG) molecule and the molecular TG species generated by this stereospecificity are characteristic for various TGs. In the TG molecule, fatty acids are esterified to three stereospecific positions on the glycerol backbone. The positions occupied by these fatty acids are numbered relative to their stereospecificity or stereospecific numbering (*sn*) as *sn*-1, *sn*-2 and *sn*-3. The physical and nutritional properties of dietary lipids are largely influenced by the nature of their constituent fatty acids, their chain length, degree of unsaturation, geometry of their double bonds (*cis and trans*), the distribution of fatty acids on the triglyceride backbone and the composition of non glyceride fraction. Hypercholesterolemic animal fats such as lard and bovine milk fat have a saturated fatty acid at the *sn*-2 position whereas most vegetable oils contains unsaturated fatty acids at this position and saturated fatty acids at *sn*-1 and *sn*-3 positions of the glycerol molecule. There is ample evidence to support the assertion that the nature of fatty acids and their positions in the glycerol backbone play a key role in the metabolism of triglycerides (Kubow, 1996).

In clinical applications, randomization provides energy-rich substrates for parenteral, enteral and infant feeding that are well-absorbed (Hayes, 2001). For instance, the randomization of medium-chain fatty acids in TG structures upon digestion will yield fatty acids released from *sn*-1 and *sn*-3 fatty acids, which will be absorbed directly into portal circulation (Mu & Hoy. 2004). This application is used in the development of enteral products benefiting patients with fat malabsorption disorders (Stein, 1999). Structured lipid emulsions used in parenteral feeding of catabolically stressed patients are said to promote whole body fat oxidation and improve nitrogen balance (Pitkanen, 1991 and Lindgren, 2001).

More recently, there has been a renewed interest in the nontriglyceride components of dietary oils. Vitamin E is generally known as an antioxidant protecting biological membranes from free radicals in human body and is a generic term including 8 isomers: alpha (α)-, beta (β)-, gamma (γ)-, and delta (δ)- tocopherols (T) and tocotrienols (T3). In the Vitamin E groups, α-tocopherol has been considered to be the most active form. However, recent research has suggested tocotrienol to be a better antioxidant with anticancer activity. α-Tocopherol is believed to be a

gene regulator, causing up-regulation of mRNA or protein synthesis that could be the result of effects on gene transcription, mRNA stability, protein translation, protein stability and post-translational events (Azzi, 2003). Tocotrienols, analogs of tocopherol are known to decrease HMG-CoA reductase activities in vitro. Extra-virgin olive oil contains a considerable amount of phenolic compounds, e.g., hydroxytyrosol and tyrosol that are responsible for its unique taste and for its high stability. Olive oil phenolics are powerful antioxidants, both in vitro and in vivo. Further, they exert other potent biologic activities, such as anti-inflammatory and antithrombotic effects, that could partially account for the observed healthful and antiatherosclerotic effects of the Mediterranean diet. A diet enriched in virgin olive oil can reduce the sensitivity of platelets to aggregation, decreasing thromboxane B_2 levels in plasma (Fransisco et al., 2006).

The edible oils found in nature seldom contain the right combination and distribution of fatty acids on their TGs and minor constituents as recommended by nutritionists.

Coconut oil is a tropical oil widely used for edible as well as non edible purpose. CO is a saturated fat, lauric and myristic acids constituting the major fatty acids. The oil also contains about 10% of higher saturated acids, namely, palmitic and stearic. The content of linoleic acid, the essential fatty acid, is low (<2%). Over many decades coconut oil is perceived to have negative effect on health due to its saturated fat content. Modern research has shown that not all saturated fats are alike. Coconut oil is unique in its structural make-up due to its medium chain fatty acids - the closest to those found in human breast milk that nature provides. They are the reason why coconut oil is used extensively in baby formula and also in sports drinks and energy bars. The medium chain fatty acids in coconut oil are more easily digested than fats found in other oils. This is because they are processed directly in the liver and immediately converted into energy. Therefore there is less strain on the liver, pancreas and digestive system and, being easily digested, they also tend to improve the absorption of other nutrients. However, unlike other vegetable oils which are rich in unsaturated fatty acids, CO has negligible levels of PUFA, leading to EFA deficiency when fed as the sole source of fat in the diet.

GNO is the most important edible oil in India, accounting for nearly 60% of the total indigenous edible oil supply. It differs from most other vegetable oils in that it contains up to 6% of long chain saturated fatty acids, arachidic, behenic and lignoceric. These fatty acids are always present in the sn-3 position of the TG. It contains oleic acid and linoleic acid. GNO is used for frying purposes.

Olive oil is the primary source of fat in the Mediterranean diet which is perceived to be the reason for low mortality from cardiovascular disease in mediterranean population (Covas, 2007). The beneficial effects of olive oil on CHD risk factors are often attributed to its high levels of monounsaturated fatty acids (MUFA). Olive oil is a functional food which besides having a high level of MUFA contains other minor components with biological properties (Covas, 2006). Phenols and phospholipids in Virgin olive oil (Keceli & Gordon, 2001; Weinbrenner et al., 2004) not only provide the sensorial quality of the oil but also have an antioxidant effect. Their ability to bind the metals present in the oil and their ability as radical scavengers is also well known. It has also been reported that some of the phospholipids found in vegetable oils display important biological activity and have proved useful in the prevention of certain diseases like arteriosclerosis (Antonopoulou & Karantonis, 2002). The complex-forming capacity of polyphenols and different metals has already been reported in other food samples, for example, Fe, Cu and Zn in wine (Irina Karadjovaa, 2002) and Al in tea samples.

To ensure optimal health benefits to Indians, the use of more than one oil is recommended. Use of more than one source of oil gives an additional advantage of providing a greater variety of minor components present in oils. Oil blending (physical mixing of oils), to achieve a desirable fatty acid balance, or use of more than one oil seem to be a judicious step for getting the balanced oil. Physical mixtures of medium chain triglycerides and long chain triglycerides have proven useful in enteral and parenteral nutrition (Adolph, 1999). However, in blended oils the triglyceride species of individual oils remain without modification and follow their original digestion, absorption, metabolic pathways and physiological effects (Akoh 1995). But structured lipids deviate from this. Many new and interesting ideas for employing biotechnology to produce important structured lipids have been investigated. In this endeavour, lipases occupy the place of prominence and carry both position specific and

acylgroup specific modifications, thus help in desired modification of fats and oils. (Malcata et al., 1990). The most useful properties of lipases are their regiospecificity and stereospecificty, which result in products with better and more predictable chemical composition and structure than those obtained by chemical catalysis.

When designing SLs with particular structure, it is possible to modify the properties of TGs, thereby improving the nutritional and pharmaceutical properties of TGs. Based on this perspective, much attention has been directed to the synthesis of SLs. The synthesis of lipids of particular structure require specific modifications at the desired positions in the glycerol backbone. Although chemical interesterification catalysed by metal alkoxides is simple and inexpensive, it is not capable of selectively modifying specific positions due to the random nature of the reactions. In contrast, the reactions catalysed by *sn*-1,3 specific lipases are more promising for positionally specific modification of lipids.

Lipases can be obtained either from animals, plants or microbes. However, microbial lipases have gained importance owing to their multifold properties, easy extraction procedures and unlimited supply. There are good number of microbial lipases available commercially. The most potential 1, 3 selective lipase is from *mucor miehei*. Besides this, lipases from *Rhizomucor*, Penicillium and Candida spp. are also regioselective and are used for specific reactions (Macrae and Hammond et al., 1985; Rogalska et al., 1993). Microbial lipases are often preferred for modifications as they are thermostable, without co-lipase requirements, and of different specifications (Eigtved: London 1992).

Lipases are manufactured by fermentation of selected micro organisms followed by a purification process. The natural function of lipase is to catalyse the hydrolysis of fats. However, if the water level is reduced, some lipases will continue to catalyse the hydrolytic reactions but at a certain level interesterification begins to dominate over hydrolysis. Immobilised lipases can be used to impart new and improved physical and nutritional properties of triglycerides. In relation to physical properties, enzymatic interesterification can improve structuring and/or melting behaviour of fats for application in the products such as spreads, non-dairy creams and confectionaries. The use of such triglycerides in food products

can be used to improve organoleptic properties (for example, to reduce waxiness or to impart cool melting), to reduce the total saturated fat content of a product, and , in some cases, can provide the technical edge necessary to develop a new product. Specific functional triglycerides also can be made which at low levels can improve the processing or storage properties of the product.

Among reports of lipase-catalysed synthesis of SLs, most of them deal with MLM-type SLs, in which MCFA, having 6-10 carbons are attached to *sn*-1, 3 positions and LCFAs, having more than 12 carbons at sn-2 position. The interest in MLM-type SLs might be related to so-called medium-chain TG (MCT) comprising MCFA, which is useful for clinical purpose as rapid energy source for patients suffering from malabsorption of lipids (Babayan, 1987). Since the MCT itself does not contain essential FAs, supplementary addition of EFAs to MCT is necessary. A simple mixture of MCT and long chain TG containing essential fatty acid however does not provide enough absorbable FAs. Mammalian pancreatic lipases hydrolyse the ester linkages at the *sn*-1 and 3 positions with a preference for MCFAs over LCFAs (Yang, 1990). The resulting *sn*-2 MGs are better absorbable forms of FAs through the intestinal mucosa. Therefore, LCFAs located at the *sn*-2 position of MLM type SLs are expected to be well absorbed, bringing the idea to 'build in EFAs in to MCT molecule' (Christensen et al., 1995). The idea was extended to an alternative concept to use MLM-type SLs as effective carriers of LCFAs, especially bio-active FAs such as PUFAs. This concept might be related to drug delivery technology including so-called 'DG prodrugs', which are 1,3-diacyl-*sn*-glycerols with various drugs attached to glycerol's *sn*-2 position (El Kihel, 1996).

In the present study blend and interesterified oils were prepared by controlled mixing of CO with GNO. Enzymatic interesterifications were carried out using immobilised lipase from *Rhizomucor miehei* as biocatalyst. In the blend of CO with GNO the proportion of SFA:MUFA:PUFA were balanced to approximately 33% each. The interesterification reaction did not alter the total fatty acid composition compared to the blended oils. Similarly blend and interesterified oils of CO: OLO combination were also prepared. Since both the oils lack in PUFA only the ratio of SFA: total unsaturated fatty acids was balanced to approximately 1:1. In this combination most of the unsaturation is provided by MUFA which is considered to be more

stable. In this investigation the change in the physical and nutritional properties of these blend and interesterified oils with balanced fatty acid composition were studied.

The types of fatty acids in the triglycerides determine the physical and properties of oils and fats. Fats with high contents of saturated fatty acids like CO are generally solid at room temperature. In the present study the native, blend and interesterified oils differed in their thermal properties and solid fat content. CO contains about 92% saturated fatty acids, and it has a high solid fat content and a high melting point as compared to the blend and interesterified oils.

There is evidence that the specific structure of triglycerides may influence their effects on experimental atherosclerosis (Lee et al., 2005). SL produced with Conjugated linoleic acid (CLA) mixture and olive oil by lipase-catalyzed acidolysis were investigated on early atherosclerosis in hyperlipidemic C57BL/6J mice. The atherosclerotic parameters including serum lipid profiles and enzyme activities such as acyl CoA:cholesterol acyltransferase (ACAT) in liver and lipoprotein-associated phospholipase A2 (Lp-PLA2) in serum were studied. Additionally, the fatty acid composition was analyzed to determine whether SL affected the fatty acid profile of hepatic phospholipids. This study suggested antiatherogenic effect of the SL and is in good agreement with similar studies carried out using dietary CLA fed rabbit, hamsters, and mice (Lee et al., 2005).

The effects of structured triglycerides containing one long chain fatty acid (oleic acid, C18:1) and one short chain saturated fatty acid (caprylic acid, 8:0) on lipid levels in serum, liver and aortic cholesterol, and fecal neutral sterol excretion were reported in male Golden Syrian hamsters fed a hypercholesterolemic diet consisting of 89.9% commercial ration to which 10% coconut oil and 0.1% cholesterol (w/w) were added.

The hamsters fed the structured triglyceride oils had lower blood cholesterol levels and lower aortic accumulation of cholesterol (Wilson, 2006a). In the present study the Coconut oil fed rats showed higher cholesterol levels when compared to those fed oils rich in unsaturated fatty acids blended oils of CO:GNO and CO:OLO showed tendency towards lowering serum

cholesterol as compared CO alone. But when these blended oils were subjected to interesterification and fed to rats a significantly higher reduction in serum and hepatic cholesterol and TG were observed indicating the absorption transport and effects of fatty acids in SL are regulated differently than that of the blended oils with similar fatty acid composition.
Dietary vegetable oils can influence lipid peroxidation and antioxidant profiles of the body (Scaccini, 1992). Unsaturated fatty acids located in the cellular membranes and in blood lipids are prone to ROS attack, producing lipid peroxides. Phenolic compounds of OLO showed more potent antioxidant activity in both chemical assays and in prolonging the lag phase of LDL oxidation. Cell culture experiments suggested that the olive oil phenolics induce a significant reduction in the secretion of intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 (and a trend towards a reduced secretion of monocyte chemoattractant protein-1), and protect against cytotoxic effects of hydrogen peroxide and oxidized LDL (Turner, 2005). Oxidized low-density lipoprotein (LDL) is thought to be more damaging to the arterial wall than native LDL cholesterol (Navab, 1997). The susceptibility of LDL cholesterol to oxidation depends not only on its fatty content but also on its antioxidants (for example, vitamin E and polyphenols).

Olive oil containing phenolics showed more antioxidant effect on low density lipoprotein oxidation than refined olive oil (Fito, 2000). Hussein, et al (2007) studied the effects of different types of dietary fats (monosaturated fatty acids present in olive oil; polyunsaturated fatty acids present in fish oil, and saturated fatty acids present in butter) on the hepatic lipid content and oxidative stress in rat liver with experimental non-alcoholic fatty liver disease. Their study clearly indicated that rats on diet supplemented with olive oil, but not fish oil or butter fat, store less triglycerides in their liver and prevent the occurrence of dietary-induced severe hepatic steatosis (Hussein, 2007). Oxidation of low density lipoproteins (LDL) appears to occur predominantly in arterial intima in microdomains sequestered from antioxidants of plasma. Therefore phenolic compounds which are able to bind LDL are good drug candidates for the effective prevention of lipid peroxidation and atherosclerotic processes, These results provide evidence that phenolic compounds bound to LDL are likely to protect LDL from oxidation (Covas, 2000). Short-term consumption of olive oil decreased plasma oxidized LDL, 8-oxo-dG

in mitochondrial DNA, and MDA in urine, and increased HDL-C and GSH-Px, in a dose-dependent manner with the phenolic content of the olive oil administered.

The mechanisms by which phenolic compounds present in olive oil can protect lipids and DNA are related to the abilities of the phenolics to counteract both metal-and radical-dependent oxidation (Fito, 2000) and to act as chelating agents, thus depressing the superoxide-driven reactions and breaking the chain-like propagation of the lipid peroxides (Visioli, 1995). In ex vivo studies, olive oil phenolic compounds showed greater antioxidant capacity against LDL oxidation than α-tocopherol (Kellie and Peter, 2002). Ingestion of virgin olive oil increased the vitamin E and phenolic content of the LDL lipoproteins (Gimeno, 2002). Aviram and Eias (Aviram, 1993) showed that dietary olive oil reduced LDL uptake by macrophages, and decreased susceptibility of the lipoprotein to undergo lipid peroxidation, after 1 wk of olive oil diet. In addition to HDL-C, as an antioxidant defense mechanism, increase in the antioxidant enzyme GSH-Px and increase in glutathione-related enzyme activity was reported in humans after virgin olive oil consumption (Fito, 2002). Recently, phenolic compounds bound to human LDL have been shown to increase in a dose-dependent manner with the phenolic content of the olive oil administered (Covas MI, 2006). Reduced glutathione is a major mechanism for cellular protection against oxidative stress. Protection by extra-virgin olive oil against oxidative stress occurs primarily through a direct antioxidant effect as well as through an indirect mechanism that involves greater expression and activity of certain enzymes with antioxidant activities (Oliveras-Lopez, 2008).

All these studies show that olive oil is more than a monounsaturated fat. The polyphenol content of an olive oil can account for further benefits on HDL cholesterol levels and oxidative damage in addition to those from its monounsaturated fatty acid content.

We observed that the blend and interesterified oils of CO:GNO and CO:OLO enhanced the antioxidant enzyme activities. We also observed the reduced susceptibility of LDL to oxidation in rats fed blend and interesterified oils containing olive oil. These effects may be due to the nature of fatty acid composition in blend and interesterified oils in addition to its antioxidant compounds in nonglyceride fractions.

Dietary fat is known to influence the variables of blood coagulation and fibrinolysis associated with vascular disease (Hunter, 2000). Saturated fatty acids are atherogenic and favor platelet aggregation, decreasing prostacyclin production and increasing thromboxane production. They are thus considered prothrombotic substances (Siess, 1980). Polyunsaturated fatty acids reduce platelet activity and the thrombogenic capacity of the arterial wall (De La Cruz, 1997).

The increase of PAF levels and activity in blood, which occurs both in pathological inflammatory conditions and during oxidative stress through LDL and membrane phospholipid oxidation, is believed to be of crucial importance for the initiation of atherosclerosis. PAF antagonists isolated from OLO inhibited the activity of PAF (Karantonis, 2002). Priora et al., investigated the effect of extra virgin olive oil on platelet aggregation and plasma concentrations of homocysteine (Hcy) redox forms in rats in relation to the minor polar compound concentration of extra virgin olive oil and they found that minor polar compounds in extra virgin olive oil inhibit platelet aggregation and reduce the plasma homocysteine concentration (Priora, 2008). Dell'Agli confirmed that Olive oil extracts and part of its phenolic constituents inhibit platelet aggregation; cAMP-Phosphodiesterase inhibition is another mechanism through which olive oil phenols inhibit platelet aggregation. (Dell'Agli , 2008).

We studied the aggregability of the platelets in response to agonists like ADP and Collagen in experimental animals. We also studied the effect of modified oils on the activities of erythrocyte membrane bound enzymes. Our results showed a significant reduction in the platelet aggregation in rats fed CO:GNO blend and Interesterified oils compared to the rats fed CO. Rate of aggregation was also decreased in rats given CO:OLO blend and CO:OLO interesterified oils. This may be due to the increase in the unsaturated fatty acid level in blend and interesterified oils compared to the native coconut oil. The activities of ouabain sensitive Na^+ K^+-ATPase and Ca^{2+} Mg^{2+}-ATPase enzymes were significantly increased in rats fed blend and interesterified oils suggesting a relationship between the quality of dietary fat and the activities of membrane-bound enzymes.

In conclusion, the present study showed that fatty acid composition of oils can be modified by blending two selected oils at appropriate levels to get a oil with balanced fatty acid composition. Treatment of blended oils with lipase interesterification process did not had any adverse effect on the quality parameters and the content of minor constituents of the oils. However the triglyceride molecular species were altered in interesterified oils. Nutritional evaluation indicated that modified oils beneficially modulated the risk factors for atherosclerosis, antioxidant defense mechanism and platelet aggregation. Hence the blend and interesterified oils with balanced fatty acid composition may help in better utilization of oils in promoting health.

The beneficial characteristics of component fatty acids in SLs may find better applications in dietary intervention programmes to reduce risk factors for cardiovascular diseases. Our study provides some evidence that oils with balanced fatty acid composition and enriched in minor compound contents like polyphenol and α-tocopherol may provide benefits by reducing risk factors for cardiovascular diseases.

SUMMARY

The fatty acid composition of various vegetable oils commonly used in India indicates a wide variation. Each oil has unique fatty acid composition and also they are unique in minor components present in them. The ideal combination of fatty acid in triglyceride of oils and fats suggested by the nutritionists is in the ratio of 1:1:1 for SFA:MUFA:PUFA or SFA:total unsaturated fatty acid in the ratio of 1:1. This cannot be always achieved from a single natural fats and oils. Blending and Interesterification of oils using lipase enzyme is viable option to obtain oil with balanced fatty acid composition. Keeping this objective, the present investigation was undertaken to develop protocols for preparing blend and interesterified oils. The effect of these modified oils as a source of dietary fats on serum and tissue lipids, antioxidant systems were evaluated. The major findings of this investigations are as follows.

1. Ground nut oil containing oleic and linoleic acid and Olive oil rich in oleic acid were selected to blend with the Coconut oil which contains saturated fatty acid to an extent of about 92%. The blended oils were then subjected to interesterification reaction by lipase under standardized conditions.

2. Interesterification reaction did not alter the fatty acid composition and the quality of the oils compared to that found in blended oils.

3. Peroxide value, free fatty acid content and minor constituent levels of Blended and Interesterified oils were comparable.

4. Blending of CO with GNO resulted in enrichment of tocopherol in the blended oil.

5. The CO:OLO blend and interesterified oils contained phenolics contributed by contributed by olive oil and tyrosol and hydroxytyrosol were found to be the major phenolics.

6. Blended oils retained the original triglyceride molecules from the individual oils while the interesterification reaction resulted in the rearrangement of the fatty acids in some of the triglycerides and resulted in the emergence of new triglyceride molecule as assessed by HPLC.

7. Blending and interesterification altered the thermal properties of the oils as determined by DSC.

8. Blending of CO with GNO or OLO resulted in the decrease of solid fat content compared to that found in CO. Interesterification of these blends resulted in further decrease of the solid fat content.

9. Nutritional evaluation of blend and interesterified oils in rats indicated that feeding of modified oils to rats lowered cholesterol levels in serum and hepatic tissues compared to the rats fed CO containing diets. The hypocholesterolemic effects of interesterified oils were found to be significantly higher than that observed with blended oils.

10. GNO and OLO and their blends with CO enhanced PUFA levels in hepatic tissues. An increase in the liver lipid peroxides was also observed in the rats fed CO:GNO and CO:OLO blend and interesterified oils, compared to rats fed CO.

11. There was a significant correlation between the P/S ratio of dietary lipids and levels of LPO and a weaker correlation between P/S ratio of liver lipids and LPO in hepatic tissues.

12. The antioxidant enzyme activities were enhanced in the hepatic tissues when rats were fed a diet containing blend or Interesterified oils as compared to the rats fed CO containing diets.

13. Susceptibility of LDL oxidation catalysed by Cu^{2+} was reduced in rats fed CO:OLO blend or interesterified oil.

14. Platelet aggregation was decreased in response to ADP and Collagen in rats fed diet containing CO:GNO and those fed CO:OLO blends compared to the rats fed CO. In addition to extent of aggregation, the rate of platelet aggregation was also decreased in rats given blend and interesterified oils.

15. Activities of Na^+ K^+-ATPase and Ca^{2+} Mg^{2+}-ATPase in erythrocyte membrane were enhanced in the rats fed a diet containing blend and interesterified oils.

These studies indicate that oils with balanced fatty acid composition can be obtained by blending suitable oils at appropriate levels. Interesterification of blended oils by lipase alters the arrangement of the fatty acids in the TG. The physical properties like melting, crystallisation behaviour and solid fat content were altered following interesterification as a result of rearrangement of fatty acids in TG molecules. When rats were fed a diet containing the blend and interesterified oils there was a lowering of the serum and tissue lipids, enhancement in the antioxidant defence system and a decrease in platelet aggregation thus exhibiting a beneficial influence on cardiovascular function.

Thus the atherogenic potentiale of saturated fats like CO can be significantly reduced by appropriately blending with oil such as GNO or OLO which contains higher amounts of unsaturated fatty acids. Further, rearrangement of fatty acids in TG molecules by lipase catalysed interesterification enhances hypocholesterolemic effects of blended oil. The molecular mechanisms for the beneficial effects of blended oils and interesterified oils need further attention.

References

A Report of the expert Group of the Indian Council of Medical Research. 1992. New Delhi, India: 34-38.

Abadu N, Miller JJ and Levinson SS. 2004. *Clin Chimica Acta*. 339: 11-25.

Abei H. 1984. *Methods Enzymol*. 105: 121-126.

Adolph M. 1999. *Ann Nutr Metab*. 4: 1-13.

AMA Council of Scientific Affairs 1983. *JAMA*. 250: 1873-1879.

Aizetmuller K. 1996. *Lipids.* 31: 201-205.

Akoh CC and Sista RV. 1995. *J Food Lipids.* 2; 231-238.

Akoh CC, Long KD, Flatt WP, Rose BS and Martin R. 1998. *J Nutr Biochem*. 9: 267-275.

Akoh CC. 1995. *Crit Rev Food Sci Nutr*. 35: 405-430.

Akubor Peter I, Badifu, Gabriel IO. **2004**. *Internl. Jour Food Sci Technol.* 39: 223-229.

Alam SQ and Alam BS. 1986. *J Nutr.* 116:1620-1630.

American Heart Association: 1990. 81: 1721-1733.

American oil chemists' society official method. 1990. *AOCS Official Method.* 8-87.

American oil chemists' society, 1989. *Official Method Cd* 14-61,

Anonymous 1977. *J Nutr.* 107*:* 1340-1348.

Antonopoulou S and Karantonis HC. 2002. *J Liq Chromatogr Related Technol*. 25: 771–779.

Arja E, Vanessa DFM, Riserus U and David EL. 2008. *Prog Lipid Res.* 47:172-187.

Aviram M and Eias K. 1993. *Annu Nutr Metab*. 37: 75-84.

Azzi A, Gysina R, Kempnáa P, Ricciarellib R, Villacortaa L, Visariusa T and Zingg MJ. 2003. *Mol Aspects Med.* 24: 325-336.

Babayan VK. 1987. *Lipids.* 22: 417-.421.

Bach AC and Babyan VK. 1982. *Am J Clin Nutr.* 36: 950-962.

Baillargeon MW and Mc Carthy SG. 1991. *Lipids*. 26: 831-836.

Baillargeon MW. 1990. *Lipids.* 25: 841-848.

Baroni SS, Amelio M, Sangiorgi Z, Gaddi A and Battino M. 1999. *Free Radic Biol Med.* 13: 590-600.

Barradas MA, Christofides JA, Jeremy J, Mikhailidis DP, Fry DE and Dandona P. 1990. *Nutr Res.* 10: 403-411.

Belzung C, Leguisquet AM, Barreau S, Doelion-Vacassal S and Chalon S. 1998. *J Nutr.* 128: 1537-1542.

Bennett V. 1985. *Annu Rev Biochem.* 54: 273-304.

Bhattacharya S and Bhattacharya DK. 1992. *J Oil Technol Assoc India.* 24: 19-22.

Bhattacharya S and Bhattacharya DK. **2000.** *Eur J Lipid Sci Technol.* 102: 323-328.

Bland JM, Conkerton EJ and Abraham G. 1991. *J Am Oil Chem Soc.* 68: 840–843.

Bligh EG and Dyer WJ. 1959. *Can J Biochem Physiol*. 37: 911-917.

Blow D. **1990**. *Nature*. 343: 694-695.

Bonanome A, Pagnan A, Biffaniti S, Oppertuno A, Sorgato F, Dorella M, Maiorino M and Ursini F. 1992. *Arterioscler Thromb*. 12: 529-33.

Boskou D. 1996. Olive oil: chemistry and technology (85-127). Champaign. IL: AOCS press

Bracco U. 1994. *Am J Clin Nutr*. 60: 1002–1009.

Brady L, Brzozowski AM, Derewenda ZS, Dodson GG, Tolley S, Turkenburg JP, Christernsen LH, Jensen B, Nashkov L, Thim L and Menge V. 1990. *Nature*. 343: 767-770.

Breitschuh B and Windhab J. 1996. *J Am Oil Chem Soc.* 73: 1603–1610.

British Nutrition Foundation. 1992. The Report of the British Nutrition Foundation's Task Force. Chapman & Hall, London.

Brockman HL. 1984. General features of lipolysis: Reaction scheme, interfacial structure and experimental approaches. In: Lipases (B. Borgstrom and H.L. Brockman, eds.) Elsevier, Amsterdam. 3-46

Bruce K, Henry G and Gonzalo G. 1994. *Ann Intern Med.* 121: 641-647.

Brunaer LS and Huestis WH. 1993. *Biochem Biophys Acta.* 1152: 109-118.

Buckingham KW. 1992. *Free Radic Biol Med*. 13: 341-390.

Buege JA and Aust SD. 1989. *Methods Enzymol.* 52: 302-310.

Casimir CA. 1997. *Lipid Technol.* 9: 61-66.

Castro P, Miranda JL, Gomez P, Escalante DM, Segura FL, Martin A, Fuentes F, Blanco A, Ordovas JM and Jimenez FP. 2000. *Eur J Clin Nutr.* 54: 61-67.

Chambrier C, Guiraud M, Gibault JP, Labrosse H and Bouletreau P. 1999. *Nutrition*.15: 274-277.

Chang NW, Wu CT, Chen FN and Huang PC. 2004. *Nutr Res*. 24: 73-83.

Chang NW and Huang PC. 1999. *Atherosclerosis*. 142: 185-191.

Chaplin MF and Bucke C. 1990. Fundamentals of enzyme kinetics. M. F. Chaplin, In Enzyme Technology. Cambridge University Press, 1-40.

Chen CW, Chong CL, Ghazali HM and Lai OM. 2007. *Food Chem*. 100: 178–191.

Chen MH and Bergman CJ. 2005. *J Food Compos Anal.* 18: 139-151.

Chen PR, Chien KL, Su TC, Chang CJ, Liu TL, Cheng H and Tsai C. 2005. *Nutr Res.* 25: 559-567.

Chibata I. 1978. *Hindustan Antibiot Bull*. 20: 58-67.

Christensen MS, Mullertz A and Hoy CE. 1995. *Lipids.* 30: 521-526.

Christie WW. 1982. *Lipid Analysis*. Isolation, identification and structural analysis of lipids. 2nd ed., Pergamon Press, Oxford.

Clausen T. 1986. *Physiol Rev*. 66: 542 -580.

Committee on Goals of life time education for Dietetics.1969. *Am Diet Assoc.* 54: 92-94.

Cortinas L, Fito M, lamuels-Raventos RM, Sebastia N, de la Torre-Noronat C and Marrugat RM. 2005. *Poultry Sci.* 84: 48-55.

Covas MI, Fito M, Lamuela-Raventos RM, Sebastia N, de la Torre-Boronat C, Marrugat J. 2000. *Int J Clin Pharmacol Res*. 20: 49-54.

Covas MI, Ruiz-Gutierrez V, de la Torre R, Kafatos A, Lamuela-Raventos RM and Osada J. 2006. *Nutr Rev*. 64: 20-30.

Covas MI. 2007. *Pharmacol Res*. 55: 175-186.

David L. Ollis, Eong Cheah, Miroslaw Cygler, Bauke Dijkstra, Felix Frolow, Sybille M. Franken, Michal Harel, S. Jamse Remington, Israel Silman, Joseph Schrag, Joel L. Sussman, Koen H.G. Verschueren and Adrian Goldman. **1992**. *Protein Eng.* 5: 197-211.

De La Cruz JP, Martin-Romero M, Carmona JA, Villalobos MA, and Sanchez de la Cuesta F. 1997. *Thromb Res*. 87: 141-149.

de Roos NM, Schouten EG and Katan MB. 2003. *Eur J Med Res*. 20: 355-357.

Deffense E. 1985. *J Am Oil Chem Soc.* 70: 1193-1201.

Dell'Agli M, Maschi O, Galli GV, Fagnani R, Dal Cero E, Caruso D and Bosisio E. 2008. *Br J Nutr.* 99: 945-951.

DeMan JM. 1992. Fats and oils: chemistry, physics and applications. In Encyclopedia of Food Science and Technology, edited by Y.H.. Hui, Wiley, New York. 823-824.

Dempsey ME. 1974. *Annu Rev Biochem*. 43: 967-969.

Dimitris T, Graham D, John A, Costas T, Christodoulos S and Pavios T. 2002. *Intl J Cardiol*. 86: 239-247.

Djousse L, Pankow JS, Eckfeldt JH, Folsom AR, Hopkins PN, Province MA, Hong Y and Ellison RC. 2001. *Am J Clin Nutr*. 74: 612-619.

Dutta-Roy AK. 1994. *Prostaglandins Leukot Essent Fatty Acids.* 51: 385-399.

Edward Hunter J. 2001. *Lipids.* 36: 655-668.

Eigtved P. 1992. Enzymes and lipid modification. In: Advances in Applied lipid research. Ed. F.B. Padley, 1-64

Kihel El, Bourass LJ, Richomme P, Petit JY and Letourneux Y. 1996. *Drug Res.* 46: 1040–1044.

Elaine WT Chong, Andrew JS and Robyn HG. 2006. *Clin Exp Ophthalmo*. I34: 464-471

Emken EA, Adlof RO, Duval SM, Shane JM, Walker PM and Becker C. 2004. *Lipids.* 39: 1-9.

Engelman B, Op den Kamp JA and Roelofsen B. 1990. *Am J Physiol*. 258: 682-691.

Entressangles B, Pasero L, Savary P, Sarada L and Dasnelle P. 1961. *J Am Oil chem Soc.* 68: 171-176.

Esterbauer H, Gerbicki J, Puhl H and Jurgens G. 1992. *Free Radic Bio Med.* 13*:* 341-390.

FAO/WHO. 1994. Report of a joint expert consultation. Rome 1-73.

Fernandez ML and McNamara DJ. 1989. *Metabolism*. 38: 1094-1102.

Fisher RA. 1970. Statistical methods for Research workers.14th edition. Oliver and Boya, Edinberg, London.

Fito M, Covas MI, Lamuela-Raventós RM, Vila J, de la Torre C and Marrugat. 2000. *J Med Clin.* 115: 166-169.

Fito M, Gimeno E, Covas MI, Miró E, López-Sabater Mdel C, Farré M, de TR and Marrugat J. 2002. *Lipids*. 37: 245-351.

Fletcher MJ. 1968. *Clin Chim Acta.* 22: 303-307.

Folch J, Lee M and Solane-Stanley GH. 1957. *J Biol Chem*. 226: 497-509.

Folig-cawley JJ, Dwyer JT, Saltzman E, McCullough M, Troy LM and Jacques PE. 2006. The 2005. *J Nutr* 136: 2908-2915.

Francisco PJ, Javier DL, Pablo PM, Fernando LS, Francisco F, Begoña C, Aquiles L and José LM. 2006. *Public Health Nutr.* 9: 1083-1088.

Frank W, Welsh Ross E, Williams and Kathryn HD. 1990. *J Food Sci*. 55: 1679-1682.

Genest JJ, Martin-Munley JR, McNamara JM, Jenner OJ, Myers RH, Silberman SR, Wilson PW, Salem DN and Schaefer EJ. 1992. *Circulation.* 85: 2025-2033.

Gey KF, Puska P, Jordan P and Moser UK. 1991. *Am J Clin Nutr.* 53: 326-334.

Ghafoorunissa. 1994. *Nat Med J India.* 7: 270-275.

Ghafoorunissa. 1996. *Lipids.* 31: 287-291.

Ghafoorunissa. 1998. *Indian J Med Res.* 108: 191-202.

Gill JM, Brown JC, Caslake MJ, Wright DM, Cooney J, Bedford D, Hughes DA, Stanley JC and Packard C. 2003. *Am J Clin Nutr*. 78: 47-56.

Gimeno E, Fito M, Lamuela-Raventos RM, Castellote AI, Covas M, Farre M, de La Torre-Boronat MC and Lopez-Sabater MC. 2002. *Eur J Clin Nutr*. 56: 114-120.

Giuseppina IT, Anna RT, Matteo F, Carolina M and Marcella G. 2003. *J Nutr*. 133: 2532-2536.

Gonzalez-Santiago M, Martin-Bautista E, Carrero JJ, Fonolla J, Baro L, Bartolome MV, Gil-Loyzaga P and Lopez-Huertas E. 2006. *Atherosclerosis*. 188: 35-42.

Gotor V, Briever R, Gonzalez C and Rebelledo F. 1991. *Tetrahedron*. 47: 9207-9214.

Griffin BA, Freeman DJ, Tait GW, Thomson J, Caslake MJ, Packard CJ and Shepherd J. 1994. *Atherosclerosis*. 106: 241-253.

Grundy SM and Denke MA. 1990. *J Lipid Res*. 31: 1149-1172.

Gunstone FD. 1989. *Biochem Soc Trans*. 17: 1141-1142.

Gupta R, Gupta VP, Sarna M, Bhatnagar S, Thanvi J, Sharma V, Singh AK, Gupta JB and Kaul V. 2002. *Indian Heart J*. 54: 59-66.

Gurr MI and James AT. 1971. Lipid Biochemistry and Introduction. Cornell University press, Ithaca, NY, 93-96.

Ha T, Han S, Kim S, Kim I, Lee H and Kim H. 2005. *Nutr Res.* 25: 597-606.

Hara PM and Irwin F. 1972. *J Biol Chem*, 217: 3170-3175.

Harris WS. 2008. *Prostaglandin Leukot Essent Fatty Acids*. 79: 169-171.

Hass P, Joerger RD, Hue YH and Khachatours GC (eds) 1991. VCH publishers, Germany, In: Food Biotechnol. 112-116.

Haumann BF. 1994. *INFORM.* 5: 668-678.

Haumann BF. 1998. *INFORM.* 9: 746-752.

HaumanYoung FVK. 1985. *J Am Oil Chem Soc*. 62: 372-376.

Hayes KC. 2001. *Curr Opin Lipidol*. 12: 55-60.

Hoilg WH, Pabst MJ and Jakoly WB. 1974. *J Biol Chem. 249*: 7130-7139.

Holman R. 1968. *Prog chem Fats Other Lipids*. 9: 611-680.

HornstraG. 2001. *J Nutr Health Aging.* 5: 160–166.

Hu FB, Stampfer MJ, Manson JE, Ascherio A, Colditz GA, Speizer FE, Hennekens CH and Willett WC. 1999. *Am J Clin Nutr*. 70: 1001-1008.

Hunter KA, Crosbie LC, Weir A, Miller GJ and Dutta-Roy AK. 2000. *J Nutr Biochem*. 11: 408-416.

Hussein O, Grosovski M, Lasri E, Svalb S, Ravid U and Assy N. 2007. *World J Gastroenterol.* 13: 361-368.

Indian standard Blended edible veg oils- 1995. specification BIS, IS: 14309, ICS 67.200.10

Irina Karadjovaa, Belgin Izgib and Seref Gucer. 2002. *Spectrochimica Acta* Part B: *Atomic Spectroscopy*. 57: 581-590.

Iwai, M and Tsujisaka, Y. 1984. Fungal lipases. In: Lipases. Borgstorm B and Brockman HL (eds) Elsevier, NY, USA. 443-446.

Jenkins KJ and Atwal AS. 1995. Flavonoids increase tissue essential fatty acids in vitamin E-deficient chicks. *J Nutr Biochem*. 6: 97-103.

Jensen GL, McGravey N, Tarasenzewski R, Wixon SK, Seidner DL, Pai T, Yeh YY, Lee TW and De Michele SJ. 1994. *Am J Clin Nutr*. 60: 518- 524.

Jing X Kang and Alexander Leaf. 1996. *Lipids*. 31: 977–982.

Jones RJ. 1974. *J Am Oil Chem Soc*. 51: 251-254.

Joshi PN and Desai RG. 1952. *Indian J Med Res.* 40: 277-287.

Juliana N, Rodrigues and Luiz A and Gioielli. 2003. *Food Res Int.* 36: 149-159.

Kang MJ, Lee EK and Lee SS. 2003. *Kor J Nutr.* 36: 245–254.

Karantonis HC, Antonopoulou S and Demopoulos CA. 2002. Antithrombotic lipid minor constituents from vegetable oils. *J Agric Food Chem*. 50: 1150-1160.

Karantonis HC, Antonopoulou S and Perrea DN. 2006. *Nutr Metab Cardiovasc Dis*. 16: 174–185.

Kates M. 1986. Techniques of Lipidology: Isolation, Analysis and Identification of Lipids. Elsevier, New York.

Keceli T and Gordon MH. 2001. *J Sci Food Agric*. 81: 1391-1396.

Kellie LT and Peter JHl. 2002. *J Nutr Biochem*. 13: 636–644.

Kennedy JP. 1991. *Food Tech*. 45: 76-83.

Keys A, Anderson JT and Grande F. 1957. *Lancet*. 2: 959-966.

Kim, E and Yoon, S. 2003. *Food. Sci. Biotechnol*, 12: 721-726.

Kinsella JE.1990. *J Parenteral Enteral Nutr.* 14: 200-217.

Kiritsakis AK. 1998. In Olive oil handbook (110-125). champain, IL: AOCS press.

Kohyama N, Nagata T, Fujimoto S and Sekiya K. 1997. *Biosci Biotech Biochem*. 61: 347-350.

Krawczyk T. 2001. *INFORM.*12: 132-140.

Kris-Etherton PM, Hecker KD and Bonanome A. 2002. *Am J Med*. 113: 71-88.

Kubow S. 1996. *Nutr Biochem.* 7: 530-541.

Kuo P, Weinfeld M and Loscalzo J. 1990. *Biochemistry*. 29: 6626–6632.

Kurashige J, Matsuzaki N and Takahasi H. 1993. *J Am Oil Chem Soc*. 70: 849-852.

Laiaa OM, Ghazalia HM, France Cho and Chong CL. 2000. *Food Chem.* 71: 173-179.

Laponite A, Goulet J, Couillard C, Lamarche B and Lemieux S. 2005. *J Nutr.* 135: 410-415.

Lars H, Wiedermann TJ, Weiss Glen AJ and Karl FM. 1961. *J Am Oil Chem Soc*. 38: 389-395.

Law MR, Wald NJ and Thompson SG. 1994. *Br Med J.* 308: 367-373.

Lee JH, Cho KH, Lee KT and Kim MR. 2005. *J Agric Food Chem*. 53: 7295 -7301.

Lee JH, Lee KT, Akoh CC, Chung SK, and Kim MR. 2006. *J Agric Food Chem.* 54: 5416-5421.

Lee KT, Casimir CA. Willium PF and Lee JH. 2000. *J Agric Food Chem*. 48: 5696-5701.

Lien EL, Yuhas RJ, Boyle FG and Tomarelli RM. 1993. *J Nutr*. 123: 1859-1867.

Lien EL. 1994. *J Pediatr.* 25: 62-68.

Lim JS, Adachi Y, Takahashi Y and Ide T. 2007. *Br J Nutr.* 97: 85-95.

Lindgren BF, Ruokonen E, Magnusson-Borg K and Takala J. 2001. *Clin Nutr*. 20:43-48.

Liu C, Chiang T, Kuo C, Lii C, Ou C, Wei Y and Chen H. 2004. *Nutr Res*. 24: 361-371.

Lonn E, Yusuf S, Hoog Werf B, Pogue J, Yi Q and Zinman B. 2002. *Diabetes Care*. 25: 1919-1927.

Lopez C, Lavigne F, Lesieur P, Keller G and Ollivon M. 2005. *J Dairy Sci*. 84: 2402-2412.

Lowry OH, Rosebrough NJ, Farr AL and Randall RJ. 1951. *J Biol Chem.* 193: 265-275.

Macrae AR and Hammond RC. 1985. *Biotechnol Genet Eng Rev.* 3: 193–217.

Maguire MH and C sona-Khalifah L. 1987. *Biochim Biophys Acta*. 921: 426-36.

Malcata FX, Reyes HR, Garcia HS, Hill CG and Amudson CH. 1990. *J Am Oil Chem Soc*. 67: 890-910.

Malcata FX, Reyes HR, Hugo S, Garcia C, Hill G Jr and Clyde HA. 1992. *Enzyme Microb Technol*. 14: 426-446.

Mann JI. 2002. *Lancet.* 7: 783-789.

Marangoni AG and Rousseau D. 1995. *Trends Food Sci Technol*. 6: 329-335.

Marangoni AG and Rousseau D. 1998. *Food Res Int*. 31: 595-599.

Maria Luz Fernandez and Kristy L West. 2005. *J Nutr*. 135: 2075–2078.

Mattson FH and Grundy SM. 1985. *J Lipid Res*. 26:194 –202.

McKenna MC, Hubbard VS and Bieri JG. 1985. *J Pediatr Gastroenterol Nutr*. 4: 45-51.

Md. Ali B, Vahab R, Gholamreza D, Alireza A, Soheil S, Seyed HA, Reza H and Leila Poorgholi. 2008. *Clin Biochem.* 41: 117-120.

Md.Ali AR and Dimick PS. 1994. *J Am Oil Chem Soc.* 71: 299–302.

Mensink RP, Zock PL, Katan MB and Hornstra G. 1992. *J Lipid Res*. 33: 1493-1501.

Mensink RP, Zock PL, Kester AD and Katan MB. 2003. *Am J Clin Nutr*. 77: 1146-55.

Mensink RP. 2005. *Lipids.* 40: 1201-1205.

Michac R and Mozaffarian D. 2008. *Prostaglandin Leukot Essent Fatty Acids*. 79: 147-152.

Miller GJ. 1997. Dietary fatty acids and blood coagulation. *Prostaglandin Leukot Essent Fatty Acids.* 57: 389–394.

Morrison and Boyd. 1992. Organic Chemistry, 6th ed.

Morrison MR and Smith M. 1963. *J Lipid Res*. 5: 600-608.

Mosca L, De Marco C, Visioli F and Cannella C. 2000. *J Food Sci Agr*. 10: 63-68.

Mu H and Hoy CK. 2004. *Prog Lipid Res*. 43:105-133.

Muller H, Lindman AS, Brantscter AL and Pederson JI. 2003. *J Nutr*. 133: 78-83.

Munteanu A, Zing JM and Azzi A. 2004. *J Cell Mol Med*. 8: 59-76.

Murakami M, Kambe T, Shimbara S and Kudo I. 1999. *J Biol Chem*. 274: 3103-3115.

Nalbone G, Leonardi J, Termine E, Portugal H, Pauli A and Lafont H. 1989. *Lipids*. 24: 179-186.

National Nutrition Monitoring Bureau. *National Institute of nutrition, Hyderabad*, 1999: 1-12.

Navab M, Hama-Levy S, Van Lenten BJ, Fonarow GC, Cardinez CJ, Castellani LW, Brennan ML, Lusis AJ, Fogelman AM and La Du BN. 1997. *J Clin Invest*. 99: 2005-2019.

Nawar WW. 1996. Chemistry. In Balleys's Industrial oil and fat products. 5th ed. Y.H. Heu. Ed. wiley, Toranto.397.

Nelson GJ, Schmidt PC and Kelley DS. 1995. *Lipids.* 30: 969-976.

Nettleton JA. 1991. *J Am Diet Assoc*. 91: 331-337.

Nevin KG and Rajamohan T. 2006. *Food Chem*. 99: 260-266.

Nevin KG and Raj Mohan T. 2004. *Clin Biochem*. 37: 830-835.

Scowcroft WR. 1990. 1990. *INFORM.* 1: 945-951.

Nicolosi RJ, Stucchi AF, Kowala MC, Hennessy LK, Hegsted DM and Schaefer EJ. 1990. *Arteriosclerosis* 10: 119-128.

Niranjan TG and Krishnakantha TP. 2000. *Nutr Res.* 20: 1125-1138.

Oliveras-López MJ, Berná G, Carneiro EM, López-García de la Serrana H, Martín F and López MC. 2008. *J Nutr*. 138:1074-1078.

Oubina P, Sanchez-Muniz FJ, Rodenas S and Cuesta C. 2001. *Br J Nutr.* 85: 41-47.

Padmavathy A, Siddhu A and Sundararaj. 2001. *J Oil Technol Assoc India*. 33. 113-114.

Pal PK, Bhattacharya DK and Ghosh S. 2001. *J Am Oil Chem Soc*. 72: 239-243.

Papadopoulos G and Boskou D. 1991. *J Am Oil Chem Soc*. 68: 669-671.

Patrick T, Theresa L and Stephen J. 2001. *J Nutr*. 131: 2157-2163.

Pedersen TR, Olsson AG, Faergeman O. 1998. *Circulation*. 97: 1453-1460.

Penalvo JL, Heinonen S, Aura A and Aldercreutz H. 2005. *J Nutr.* 135: 1056-1062.

Perna JS, Cabello-Moruno R and Ruiz-Gutierrez V. 2006. *J Nutr Biochem.* 17: 429-425.

Petrauskaite V, De Greyt W, Kellens M and Huyghebaert A. 1998. *J Am Oil Chem Soc.* 75: 489–493.

Petroni A, Blasevich M, Papini N, Salami M, Sala A and Galli C. 1997. *Thromb Res*. 87: 315-322.

Petroni A, Blasevich M, Salami M, Papini N, Montedoro GF and Galli C. 1995. *Thromb Res*. 78:151–160.

Petroni A, Blasevich M, Salami M, Papini N, Servili M, Montedoro GF and Galli C. 1994. *World Rev Nutr Diet.* 75: 169-172.

Pitkanen O, Takala J, Pöyhönen M and Kari A. 1991. *Clin Nutr*. 10: 258-65.

Prevention of Food Adultration Act 1954. Blending of vegetable oil: Narayana PS, Hussain N, editors. Hyderabad: Asia law House, 1997; A. 17.24 GSR No. 91 (E) p200

Priora R, Summa D, Frosali S, Margaritis A, Di Giuseppe D, Lapucci C, Ieri F, Pulcinelli FM, Romani A, Franconi F and Di Simplicio P. 2008. *J Nutr*. 138: 36-41.

Pronczuk A and Hayes KC. 1999. *FASEB*. 13: 561-565.

Quinland P and Moore S. 1993. *INFORM.* 4: 580-585.

Qureshi AA, Qureshi N, Hasler-Rapacz JO, Weber FE, Chaudhary V, Crenshaw TD, Gapor A, Ong AS, Chong YH, Peterson D and Rapaez J. 1991. *Am J Clin Nutr*. 53: 1042-1046.

Ramesh B, Saravanan R, Pugalendi and Yale KV. 2006. *J Biol Med*. 79: 9-17

Ray S and Bhattacharya DK. 1995. *J Am Oil.Chem Soc*. 72: 327-330.

Reddy KS. 1993. Cardiovascular diseases in India. *World Health stat*. 46: 101-107.

Reddy KS. 1999. *Prev Med*. 29: 119-123.

Reena R, Sankar KU, Sambaiah K and Lokesh BR. 2001. *Eur Food Res Technol.* 212: 334-343.

Renaud S, Morazain R and Godsey F. 1981. *Br Heart J*. 46: 562-570.

Ribeiro APB, Grimaldia R, Gioiellib LA and Gonçalves LAG. 2009. *Food Res Int*. 42: 401-410.

Robinson WD. 1966. 'Nutrition in Medical Education', in proceedings western Hemisphere Nutrition congress-1965*, American Medical Association,* Chicago, 206-210.

Rocio de lP, Valentina RG and Robin SJ Hoult. 1999. *Biochem Pharmacol*. 57: 445-449.

Rogalska E, Cudrey C, Ferrato F and Verger R. 1993. *Chirality*. 5: 24-30.

Rogalska E, Ransac S and Verger R. 1990. *J Biol Chem*. 265: 20271-20276.

Ross R. 1986. *N Engl J Med.* 314: 488-500.

Rousseau D and Marangoni AG. 1998. *Food Res Int*. 31: 381-388.

Rubin M, Moser A, Vaser berg N, Greig F, Levy Y, Spivak H, Ziv Y and Lelcuk S. 2000. *Nutrition*. 16: 95-100.

Ruiz-Sala P, Hierro MTG, Martinez-Castro I and Santa-Maria G. 1996. *J Am Oil Chem Soc*. 73: 223–293.

Sales TR, Torres HO, Couto CM and Carvalho EB. 1998. *Nutrition*. 14: 508-512.

Salvati S, Cambegei LY, Sorcinni M, Olivieri A and Di Base A. 1993. *Lipids*. 28: 1075-1078.

Samuelsson B, Goldyne M, Granström E, Hamberg M, Hammarström S and Malmsten C. 1978. *Annu Rev Biochem*. 47: 997-1029.

Samuelsson B, Granström E, Green K, Hamberg M and Hammarström S. 1975. *Annu Rev Biochem*. 44: 669-695.

Sanchez-Muniz FJ, Oubina P, Benedi J, Rodenas S and Cuesta C. 1998. *J Am Oil Chem Soc.* 75: 217-223.

Sanders T. 2002. Groundnut (peanut) oil, in vegetable oils in food technology, Gunstone FD. Ed., Corn Press, Boca Raton, FL, p. 236

Savitha and Panneerselvam. 2006. *Mech Ageing Dev.* 127: 349-355.

Scaccini C, Nardini M, d' aquino M, Gentili V, di Felice M and Tomassi G. 1992. *J Lipid Res*. 33: 627-633.

Scanu AM, Lawn RM and Berg K.1991. *Ann Intern Med*. 115: 209-218.

Schwandt P, Janetschek P and Weisweiler P. 1982. *Atherosclerosis*. 44: 9–17.

Searcy RL and Bergquist LM. 1960. *Clin Chim Acta*. 5: 192-199.

Shaomei YP, Dezhong Y, Penny MKE, Guixiang Z and Terry DE, 2005. *J Nutr*. 135: 2541-2545.

Sheldon RA, Dezoete MC, Kock-V Dalen AC and Van Rantwijk F. 1994. *Biocatalysis.* 10: 307-316.

Shichiri M, Takanezawa Y, Uchida K, Tamai H and Arai H. 2007. *Brain Res.* 1182: 106-115.

Shimizu S, Akimmoto K, Shinmen Y, Kawashima H, Sugano M and Yamada H. 1991. *Lipids*. 26: 512-516.

Siess W, Roth P, Scherer B, Kurzmann I, Böhlig B and Weber PC.1980. *Lancet.* 1: 441-444.

Sil Roy S and Bhattacharyya DK. 1993. *J Am Oil Chem Soc*. 70: 1293-1294.

Simopoulos AP, Leaf A and Salem N. 1999. *Am J Clin Nutr*. 70: 560-569.

Singman HS, Berman SN, Cowell C, Malansky E and Archer M. 1989. *Am J Clin Nutr*. 37: 1183-1191.

Sirtori CR, Tremoli E, Gatti E, Montanari G, Sirtori M, Colli S, Gianfranceschi G, Maderna P, Dentone CZ, Testolin G and Galli C. 1986. *Am J Clin Nutr*. 44: 635-664.

Small DM. 1991. *Annu Rev Nutr.* 11: 413–434.

Smith, RE Finley, JW and Leveille. GA. 1994. *J Agric Food Chem*. 42: 432-434.

Smith RD, Kelly CN, Fielding BA, Hauton D, Silva KD, Nydahl MC, Miller GJ, Williams CM. 2003. *Br J Nutr.* 90: 597-606.

Sonnet PE, McNeill GP and Jun W. 1994. J *Am Oil Chem Soc*. 71: 1421-1423.

Spady DK, Woollett LA and Dietschy JM. 1993. *Annu Rev Nutr*. 13: 355-381

Sreenivasan B. 1978. *J Am Oil Chem Soc*. 55: 796-805.

Starrup EM and Hoy CE. 2000. *J Nutr*. 130: 2802-2808.

Stein J. 1999. *Int J Colorectal Dis*. 14:79-85.

Steinberg D and Witztum JL. 1990. *JAMA.* 264: 3047-3052.

Stewart JCM. 1980. *Anal Biochem*. 104: 10-14.

Sundram K, Karupaiah T and Hayes KC. 2007. *Nutr Metab*. 4: 1743-7075.

Swe PZ, Cheman YB and Ghazali H.M. 1995. *J Am Oil Chem Soc*. 72: 343-347.

Tan CP and Che Man YB. 2000. *J Am Oil Chem Soc*. 77: 143-155.

Tappel AL. 1978. *Methods Enzymol*. 52: 506-513.

Temme E, Mensik RP and Hornstra G. 1996 *Am J Clin Nutr*. 63: 897-903.

Terner R, Etienne N, Alonso MG, de Pascual, Teresa S, Minihane AM, Weinberg PD and Rimbach G. 2005. *Int J Vitamin and Nutr Res*. 75: 61-70.

Thaulow E, Erikssen J, Sandvik L, Stomorken H and Cohn PF. 1991. *Circulation*. 84: 613-617.

Theodore J, Weiss, Glen A, Jacobson and Lars HW. 1961. *J Am Oil Chem Soc*. 38: 396-399.

Thomas MJ, Thornburg T, Manning J, Hooper K and Rudel LL. 1994. *Biochemistry*. 33: 1828-1834.

Tholstrup T and Samir Samman. 2004. *J Nutr*. 134: 2550-2555.

Touyz RM, Milne FJ, Reinach SG and Engelman B, 1992. *Clin Exp Hypertens*. 14: 1189-209.

Turner R, Etienne N, Alonso MG, Pascual-Teresa S. de Minihane AM, Weinberg PD and Rimbach G. 2005. *Int J Vitam Nutr Res*. 75: 61-70.

Vajreswari A and Narayanareddy K. 1992. *Metabolism*. 4: 352-358.

Varharanta M, Voytilainen S, Lakka TA, Van der Lee M, Adlercreutz H and Salonen JT. 1999. *Lancet*. 354: 2112-2115.

Visioli F, Bellemo G and Galli C. 1998. *Biochem Biophys Res commun*. 247: 60-64

Visioli F, Bellemo G, Montedoro G and Galli C. 1995. *Atherosclerosis.* 117: 25-32.

Visioli F, Poli A and Galli C. 2002. *Med Res Rev.* 22: 65-75.

Visioli, F and Galli C. 1998. *J Agr Food Chem*. 46: 4292-4296.

Vitro MD, Agud I, Montero S, Blanco A, Solozabal R, Lascaray J.M, Llama MJ, Serra JL, Landeta LC and De Renobales M. 1994. Enzyme *Microb Technol.* 16: 61-65.

Vulfson EN. 1993. *Trends Food Sci Technol*. 4: 209-215.

Warnick GR and Albers JJ. 1978. *J Lipid Res*. 19: 65-76,

Weinbrenner T, Fito M, de la Torre R, Saez G.T, Rijken P, Tormos, C, Coolen S. Albaladejo MF, Abanades S, Schroder H, Marrugat J and Covas MI. 2004. *J Nutr*. 134: 2314-2321.

Weisweiler P, Janetschek P and Schwandt P. 1985. *Metabolism*. 34: 83–87.

Wen Y, Killalea S, Norris LA, Cooke T and Feely J. 1999. *Eur J Clin Invest*. 29: 1027-1034.

Widdowson EM. 1987. *Am J Clin Nutr*. 45: 898-904.

Willis WM, Lencki RW and Marangoni AG. 1998. *Crit Rev Food Sci Nutr*. 38: 639-674.

Wilson F, Salee VL and Dietschy JM .1971. *Science.* 174: 1031-1033.

Wilson RB, Middleton CC and Sun GY. 1978. *J Nutr.* 108: 1858-1867.

Wilson TA, David Kritchvesky, Timothy K and Nicolsi RJ. 2006a. *Biochim Biophys Acta*. 1761: 345-349.

Wilson TR, Nicolosi B, Woolfrey D and Kritchevsky. 2006. *J Nutr Biochem*. 18: 105-112.

Winkler FK, D'arcy A and Hunziker W. 1990. *Nature*. 343: 771-774.

Wiseman SA, Mathot JN, de Fouw, NJ and Tijburg, LB. 1996. *Atherosclerosis*. 120: 15-23.

Xu X, Balchen, Jonsson SG and Alder-Niessen J. 2000. *J Am Oil Chem Soc.* 77: 1035-1041.

Yang BK and Chen JP. 1994. *Food Sci*. 59: 424-427.

Yang D and Rhee JS. 1992. *Biotechnol Bioeng*. 40: 748-750.

Yang LY, Kuksis A and Myher JJ, 1990. *J lipid Res.* 31: 137-147.

Yankah, VV and Akoh CC. 2000. *J Am Oil Chem Soc.* 77: 495-500.

Yu L and Hammond E.G. 2000. *J Am Oil Chem Soc*. 77: 917-924.

Zhang H, Xu X, Nilsson J, Mu H, Adler-Nissen J and Hoy C.E. 2001. *J Am Oil Chem Soc.* 78: 57-64.

Zhao G, Etherton TD, Martin KR, West SG, Gillies PJ and Kris-Etherton PM. 2004. *J Nutr*. 134: 2991-2997.

Zheng WM, Dong Liu L, Kun L, Zhang YJ and Hong DJ. 2007. *Colloids Surf B.* 54: 25-32.

Publications

Anitha Nagaraju and Belur R Lokesh. 2008. Rats fed blended oils containing coconut oil with groundnut oil or olive oil showed an enhanced activity of hepatic antioxidant enzymes and a reduction in LDL oxidation. *Food Chemistry*. 108(3), 950-957.

Anitha Nagaraju and Belur R Lokesh. 2007. Interesterified coconut oil blends with groundnut oil or olive oil exhibit greater hypocholesterolemic effects compared with their respective physical blends in rats. *Nutrition Research*. 27(9), 580-586.