STUDIES ON BLENDED AND INTERESTERIFIED OILS FOR HEALTH BENEFITS

A THESIS SUBMITTED TO THE

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IN

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BY

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DECLARATION

I hereby declare that the thesis entitled "**STUDIES ON BLENDED AND INTERESTERIFIED OILS FOR HEALTH BENEFITS**" submitted to the University of Mysore for the award of the degree of **DOCTOR OF PHILOSOPHY** in **BIOTECHNOLOGY** is the result of research work carried out by me under the guidance of **Dr. B. R. Lokesh**, Head, Department of Lipid Science and Traditional Foods, Central Food Technological Research Institute, Mysore, during the period 2004-2009.

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

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Dr. B. R. Lokesh
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CERTIFICATE

This is to certify that the thesis entitled "**STUDIES ON BLENDED AND INTERESTERIFIED OILS FOR HEALTH BENEFITS**" submitted by **Ms. M. B. REENA** to the University of Mysore for the award of the degree of **DOCTOR OF PHILOSOPHY** in **BIOTECHNOLOGY** is the result of research work carried out by her in the Department of Lipid Science and Traditional Foods, Central Food Technological Research Institute **(**CFTRI), Mysore, under my guidance and supervision during the period of 2004-2009.

Date:
Place: Mysore **(B. R. LOKESH)**

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M. B. Reena

Dedicated to

my Parents

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ABBREVIATIONS, SYMBOLS AND UNITS

ACAT	Acyl Co A: cholesterol acyl transferase
ADP	Adenosine diphosphate
CDNB	1-Chloro 2, 4-dinitrobenzene
CHD	Coronary heart disease
cm	Centimeter
CM	Chylomicron
cDNA	Complementary DNA
CNO	Coconut oil
CVD	Cardio vascular disease
CYP7A1	Cholesterol 7-α-hydroxylase
DAG	Diacylglycerol
DSC	Differential scanning calorimetry
DEPC	Diethylpyrocarbonate
dL	Decilitre
EC	Esterified cholesterol
ECN	Equivalent carbon number
EDTA	Ethylene diamine tetra acetic acid
EFA	Essential fatty acids
EPA	Eicosapentaenoic acid
ER	Endoplasmic reticulum
FAME	Fatty acid methyl esters
FC	Free cholesterol
FER	Food efficiency ratio
FFA	Free fatty acid
g	Gram
GC	Gas chromatography
GSH-Px	Glutathione peroxidase
GST	Glutathione transferase
h	Hour (s)
HDL	High density lipoprotein
HMG CoA	3-Hydroxy 3-methyl glutaryl-CoA
HPLC	High performance liquid chromatography
IDL	Intermediate density lipoprotein
J/g	Joule/gram
ICMR	Indian Council of Medical Research

IU	International unit
L	Litre
kcal	Kilo-calorie
Kg	Kilogram
LCAT	Lecithin- cholesterol acyl transferase
LDL	Low density lipoprotein
LDL-R	Low density lipoprotein receptor
LPO	Lipid peroxide
M	Molar concentration
MAG	Monoacylglycerol
MCT	Medium chain triglyceride
MCFA	Medium chain fatty acids
MDA	Malondialdehyde
meq	Milliequivalent
mg	Milligram
min	Minutes
mL	Millilitre
mm	Millimeter
mM	Millimolar
MOPS	3-(N-morpholino) propane sulfonic acid
mRNA	Messenger RNA
MUFA	Monounsaturated fatty acid
N	Normal
nd	Not detected
ng	Nanogram
nm	Nanometer
nmole	Nanomole
OD	Optical density
PG	Prostaglandin
Pi	Inorganic phosphate
PL	Phospholipid
pmole	Pico mole
P/S	Polyunsaturated fatty acid/saturated fatty acid
PCR	Polymerase chain reaction
PPP	Platelet poor plasma
PRP	Platelet rich plasma

PUFA	Polyunsaturated fatty acid
PV	Peroxide value
RBO	Rice bran oil
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RP	Reverse phase
rpm	Rotation per minutes
RT-PCR	Real time polymerase chain reaction
s	Second
SCAP	SREBP cleavage activating protein
SD	Standard deviation
SESO	Sesame oil
SFA	Saturated fatty acid
SFC	Solid fat content
SL	Structured lipids
S:M:P	Saturated:monounsaturated:polyunsaturated
SOD	Superoxide dismutase
SREBP	Sterol regulatory element binding protein
TAE	Tris acetate ethylene diamine tetra acetic acid
TAG	Triacylglycerol
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reactive substances
TCA	Trichloroacetic acid
TC	Total cholesterol
TCN	Theoretical carbon number
Tris	Tris (hydroxymethyl) amino methane
TX	Thromboxane
UV-VIS	Ultraviolet-visible
V	Volt
v/v	Volume/volume
VLDL	Very low density lipoprotein
wt	Weight
w/v	Weight/volume
w/w	Weight/weight
ω	Omega
(B)	Blended

(I)	Interesterified
°C	Degree Celsius
%	Percent
µ	Micrometer
µg	Microgram
µL	Microlitre
µm	Micrometer
µM	Micromolar
µmole	Micromole
α	Alpha
β	Beta
γ	Gamma
δ	Delta
6-keto $PGF_{1\alpha}$	6-Keto-prostaglandin $F_{1\alpha}$

CONTENTS

Thesis title: Studies on Blended and Interesterified Oils for Health Benefits

Cardiovascular disease (CVD) has become one of the major killer diseases in India. Dietary fat plays an important role in modulating the risk factors for CVD. Earlier it was believed that the amount of fat in the diet plays an important role in the development and progression of CVD. Studies from 1960 onwards have shown that type of fat in general and fatty acid in particular is important in determining its role in the pathogenesis of CVD. It is well established that certain saturated fatty acids in the diet increases LDL cholesterol, one of the major risk factors involved in the atherosclerotic plaque formation, where as unsaturated fatty acids exhibit hypocholesterolemic effects. However consumption of excess amount of polyunsaturated fatty acids leads to oxidative stress, if not properly balanced with antioxidants. Therefore there should be a balance in the fatty acids present in the oils for optimum health benefits. Considering the health effects of fatty acids, Indian Council of Medical Research recommended that dietary fat should ideally contain saturated:monounsaturated:polyunsaturated fatty acids in the ratio of 1:1:1.

In India, as dietary habit vary from region to region, the type of fat used for cooking is also different. Coconut oil (CNO), which contain about 90% saturated fatty acids, is the predominant dietary fat in Kerala and coastal regions of Karnataka. It contains about 2% polyunsaturated fatty acids. Sunflower oil, ground nut oil and mustard oil are other major edible oils used in this country. The other lesser known oils used include sesame oil (SESO) and rice bran oil (RBO). Sesame oil is used in southern part of India. Rice bran oil (RBO) is becoming popular in Indian market. These oils are rich in unsaturated fatty acids. Palm oil which is used by some sections of populations in India contains more saturated fatty acids. Analysis of the fatty acid composition of all these oils indicates that a single oil provided to us by nature do not have balanced amount of fatty acids as desired by nutritionists.

One of the approaches for balancing fatty acid composition in an oil is by blending i.e physical mixing of selected oils in appropriate proportions. The blending also complements the oil with minor components from individual oils some of which possess nutraceutical properties. The minor components present in the unsaponifiable matter are unique to each oil. Blending of oils has been used earlier to enhance the

oxidative and thermal stability of the oils and also to improve the nutritional quality of the oils. Even though one can balance the fatty acid composition by blending of suitable oils, it may not always result in oils with desired physicochemical or nutritional properties. This is because the physical characteristics of the individual oils may be retained in the blended oil resulting in a heterogeneous mixture. This may be overcome by rearranging the fatty acids in the TAG molecules using enzymatic or chemical interesterification process. Interesterification reaction results in the rearrangement of fatty acid molecule within and between the TAG molecules or may result in the formation of new TAG molecules which are not present in the parent and blended oil. While chemical interesterification randomizes fatty acids in the TAG molecules of oils, lipase catalyzed interesterification bring out specific changes and has become a useful tool for the production of designer lipids with improved physicochemical properties. Such lipids will have high demand in food processing industry to bring out a fat with potential health benefits.

The present investigation deals with the preparation and evaluation of modified lipids having balanced amounts of fatty acids. The modified oils prepared by blending a saturated fat CNO with unsaturated oil RBO or SESO. These were further subjected to lipase catalyzed interesterification reaction. The physicochemical characteristics, nutritional properties and influence on serum and tissue lipids of these modified lipids were evaluated by standard procedures. The thesis is organized in the following manner.

Introduction

A brief review of literature on dietary fat, metabolic effect of fats, modification of fats and interesterification reactions is presented. This section also highlights the aim and scope of the present investigation.

Materials and Methods

The source of chemicals and raw materials used in this investigation is given in this chapter. The experimental protocols to prepare modified lipids and its evaluation for physicochemical properties using AOCS methods and Differential Scanning Calorimetry (DSC) are described. The nutritional evaluation of modified

oils using rats as animal model is given. Analysis of various lipid parameters using standard procedures is also presented. The molecular methods for the expression profiling of genes is also mentioned.

Chapter I

Protocol for Blending and Interesterification: Evaluation of Physicochemical Properties

The quality of the oils was evaluated by determining the peroxide value, free fatty acid content and minor constituents. After determining the fatty acid composition of individual oils (CNO, RBO, SESO), CNO was mixed with RBO or SESO in appropriate amounts to get an oil with approximately equal proportions of saturated:monounsaturated:polyunsaturated fatty acids. These blended oils were subjected to interesterification reaction, catalyzed by immobilized lipase from *Rhizomucor miehei* in a predetermined condition. TAG molecular species in the oils were analyzed using a RP-C18 column connected to a high performance liquid chromatography equipped with a refractive index detector. Changes in the physical properties such as solid fat content, thermal and crystallization behaviour of native, blended and interesterified oils were monitored by DSC.

Blending of CNO with RBO or SESO at appropriate levels provided oil with desired ratios of saturated:monounsaturated:polyunsaturated fatty acids. The resulting oils were also enriched with nutraceuticals present in RBO or SESO. Interesterification of blended oils using lipase under optimized conditions did not show any adverse effects on the quality of the oil. Interesterification did not affect the minor constituents present in the oils. Fatty acid compositions of the interesterified oils were similar to that of blended oil. However finger printing of TAG molecules of interesterified oils monitored by HPLC showed redistribution of the fatty acids among the TAG molecules resulting in changes in specific TAG molecular species. Changes in TAG molecular species observed in interesterified oils altered the physical properties such as solid fat content, enthalpy and crystallization behaviour of TAG as monitored by DSC. Therefore it is evident that interesterification altered TAG molecular species without affecting overall fatty acid composition of oils.

Chapter II

Nutritional Evaluation of Blended and Interesterified Oils

Male Wistar rats were fed AIN-76 diet containing 10% fat from CNO, RBO, SESO, CNO+RBO(B), CNO+ SESO (B), CNO+RBO(I) or CNO+SESO(I) (B: blended; I: interesterified oil). After feeding these modified oils for a period of 60 days, rats were fasted overnight and sacrificed under ether anesthesia. After collecting blood and tissues, lipids were extracted and analyzed using standard procedures.

Feeding rats with blended oil of CNO+RBO or CNO+SESO resulted in a significant reduction in serum and liver cholesterol and TAG compared to rats given CNO. The cholesterol lowering effect of blended oil was further enhanced when oil was subjected to interesterification reaction prior to feeding. The hypocholesterolemic effects of interesterified oils were significantly higher than that observed with blended oils even though the fatty acid composition of blended and interesterified oils was similar. This indicated that both blended and interesterified oils are effective in lowering serum and tissue cholesterol.

Chapter III

Expression Profiling of Genes Involved in Cholesterol Metabolism after Feeding Modified Oils

Feeding rats with blended or interesterified oils showed hypocholesterolemic effect compared to those fed CNO. The cholesterol lowering effect of interesterified oil was significantly higher when compared to rats fed with blended oil. To assess the mechanism of hypocholesterolemic effects of blended and interesterified oils, the transcriptional profiling of proteins that regulate the cholesterol metabolism were studied. Total RNA was isolated from liver, reverse transcribed to cDNA and expression of HMG-CoA reductase, LDL receptor, cholesterol 7-α-hydroxylase (CYP7A1), and sterol regulatory element binding protein (SREBP)-2 was evaluated by Real time PCR.

The studies based on mRNA abundance for these major pathways in cholesterol homeostasis indicated that cholesterol lowering effect of blended and interesterified oils is due to a significant upregulation of hepatic LDL receptor gene

and CYP7A1. The enhanced cholesterol lowering effect observed in rats fed interesterified oil was attributed to a 2-4 fold increase in LDL receptor mRNA abundance in rats fed interesterified oils compared to those fed blended oils. These results suggest that the cholesterol lowering effect of modified oils is mediated by the increased mRNA abundance of the LDL receptor and CYP7A1.

Chapter IV

Effect of Blended and Interesterified Oils on Platelet Aggregation and Eicosanoid Production in Rats

Atherosclerosis and thrombosis are two major events responsible for cardiovascular disease. Platelet aggregation play important role in thrombosis. Platelet aggregation is significantly influenced by the prostaglandin level. Plasma and membrane fatty acid composition play an important role in modulating the platelet function. The platelet aggregation was decreased in response to ADP and Collagen in rats fed blended and interesterified oils of CNO+RBO or CNO+SESO compared to the rats fed CNO. The rate of platelet aggregation was also decreased in rats given blended and interesterified oils compared to those given CNO. Rats fed blended and interesterified oils also showed a high prostacyclin/thromboxane ratio compared to rats given CNO.

This study indicated that fatty acids play a significant role in platelet aggregation, prostacyclin and thromboxane level.

Chapter V

Effect of Blended and Interesterified Oils on Endogenous Antioxidant Enzyme System in Liver and Erythrocyte Membrane Bound Enzymes

Type of dietary fat consumed affects the antioxidant profile of the body and susceptibility to undergo oxidation. Hence, effect of feeding modified fats on the lipid peroxidation and endogenous antioxidant enzymes were studied.

The lipid peroxidation level observed in rats given blended and interesterified oils of CNO+RBO and CNO+SESO were found to be higher than those fed CNO; however it was lower than that observed in rats given RBO or SESO alone. Feeding

rats with a diet containing blended and interesterified oils of CNO+RBO and CNO+SESO enhanced the hepatic antioxidant enzyme activities such as superoxide dismutase, catalase, glutathione peroxidase and glutathione transferase, as compared to the rats given CNO alone. These studies indicated that though the blended and interesterified oils had higher levels of unsaturated fatty acids which resulted in an increase in lipid peroxidation compared to CNO, it may have been protected to some extent by elevated levels of antioxidant enzymes in the liver. These modified oils also enhanced the activities of erythrocyte membrane bound enzymes like Na^{+}/K^{+}-ATPase and Ca^{+2}/Mg^{+2}-ATPase compared to rats given CNO. Therefore changes in fatty acid composition in dietary lipids affected the activities of membrane bound enzymes.

General Discussion and Summary

The amount of fat in the diet was believed to be the deciding factor responsible for the development of CVD. But later studies established that the type of fatty acid is more important as saturated fatty acids increase cholesterol and its replacement by PUFA decrease the cholesterol level. The position of fatty acids in the TAG molecule influences the absorption and its further incorporation into lipoproteins. The fatty acids in the TAG molecule of lipoproteins affect its uptake by LDL receptor molecule, which removes the cholesterol from circulation. It has been recognized that oils and fats are heterogeneous group of TAG molecules. The distribution of fatty acids in these TAG may play an important role in hypolipidemic effects attributed to specific oils. This was addressed in the present investigation by using blended and interesterified oils having similar fatty acid composition. The results are discussed in the light of available literature on this aspect.

To summarize, blended oils were prepared by mixing appropriate amounts of CNO with RBO or SESO to get an oil with a fatty acid composition of saturated:monounsaturated:polyunsaturated fatty acids approximately in the ratio of 1:1:1. These blended oils were subjected to interesterification reaction to rearrange the fatty acids in the TAG. The physical characteristics of interesterified oil was found to be different in terms of TAG molecular species, solid fat content and crystallization behavior compared to that of blended oil of similar fatty acid composition. Nutritional evaluation of blended and interesterified oil indicated hypocholesterolemic effects in

rats when compared to rats fed CNO. The cholesterol lowering effect of interesterified oil was much higher than that observed with blended oil. The molecular mechanism involved in the hypocholesterolemic effects of oils showed that the higher cholesterol lowering effect of interesterified oils is mainly due to increased mRNA abundance for LDL receptors and CYP7A1. Platelet aggregation was lowered coinciding with high prostacyclin/thromboxane ratio in rats fed blended and interesterified oils. The activity of antioxidant enzymes in the liver were elevated in rats fed blended and interesterified oils. These results are discussed in terms of benefits to be derived from blended and interesterified oils.

References

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

M. B. Reena
(Student)

B. R. Lokesh
(Guide)

General Introduction

Materials and Methods

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Effect of Blended and Interesterified Oils on Endogenous Antioxidant Enzyme System in Liver and Erythrocyte Membrane Bound Enzymes

General Discussion and Summary

References

Belief in the medicinal power of foods is not a recent event but has been a widely accepted philosophy for generations. Hippocrates stated almost 2,500 years ago, “Let food be thy medicine and medicine be thy food”. Many of the nutrients and food components we consume as part of a diet can potentially have a positive or negative impact on health. Diet has been implicated in the etiology of diabetes, cardiovascular disease and cancer in many populations (Jew *et al*., 2009). The ultimate goal of human nutrition research is to improve the quality of life of individuals by minimizing morbidity and maximizing longevity. Diet related problems are clearly very different, and especially in developing countries there are problems associated with the rapid adoption of western style diets. Now the scientific knowledge of the nutrients that are the basis of nutrient deficiency diseases has become a key scientific objective of nutrition research to identify the role of diet in metabolic regulation (Fairweather-Tait, 2003). The importance of diet to health has become even more obvious with the realization that many of life’s modern diseases are the result of subtle but chronic metabolic imbalances related in part to diet. Diets are a part of the problem and nutrition should play a vital role in metabolic disease prevention. Metabolic balance is responsive to not simply the presence of essential nutrients at the limits of their adequacy, but also to the proportion of essential nutrients and to the abundance of nonessential components in the diet (German *et al*., 2003). As dietary strategies seek to modify metabolism for health benefits, the importance of understanding precisely how much of each nutrient is optimal leads to the necessity to redefine safety of nutrients consumed in amounts beyond those necessary for adequacy. New strategies, technologies and knowledge must be established to evaluate both the efficacy and the safety of diets designed specifically to influence metabolism.

The health of an individual and the population in general is the result of interactions between genetics and a number of environmental factors. Nutrition is an environmental factor of major importance. Investigations of the dietary patterns and health status of populations all over the world indicate a major difference among them in both dietary intake and health status (Simopoulos, 2002). Major advances have occurred over the past 15 years in the fields of both genetics and nutrition. The role of diet in promoting human health is an important area for study in the post-human

genome-sequencing era. Scientific community is able to develop and exploit post-genomic technique to deliver previously unimaginable data on nutrient requirements of individuals and long-awaited information on the relationship between diet and health (Sunde, 2001). Advances in molecular biology and genetics have facilitated the study of inherited disease at the DNA level, and the effect of dietary components on the development and progression of these diseases at the molecular level. This has led to the development of concepts of nutrigenetics (i.e. difference in the response of individuals to the same diet due to the variation in genetic makeup) and nutrigenomics (studies on the evolutionary aspects of diet and the role of nutrients in gene expression (e.g. polyunsaturated fatty acids (PUFA) suppress fatty acid synthase gene expression). Nutrigenomics could provide a framework for the development of novel foods that will be genotype dependent for the promotion of health and prevention and management of chronic diseases.

Among the dietary components, lipid has important role in control and prevention of diseases. However lipid has got maximum blame due to its role in the development and progression of chronic diseases like diabetes, cancer and cardiovascular disease. More than 95% of a dietary fat is triacylglycerol. Nutritional effect of any fat or oil is dependent upon the component fatty acid molecules in the triacylglycerol. Fatty acid molecules vary in their chain length, unsaturation and geometry of double bond. The position of fatty acids on the glycerol back bone also influences the digestion, absorption and thereby its nutritional effect.

Fatty acids

Dietary fats are important component of human diet as it provides essential fatty acids for normal growth, part of structural component, serve as precursors of substances that regulate physiological processes, are the transport vehicle for fat soluble vitamins, contribute to the palatability and flavor of food, serves as energy reservoir (provide 9 kcal/g). In nature, fats and oils occur predominantly as esters of fatty acids and glycerol. The physicochemical properties of an oil or fat are largely determined by the fatty acids that they contain and their position within the triacylglycerol (TAG) molecule. Fatty acids are distinguished by their following features:

Chain length

Fatty acids which have fewer than 8 carbon atoms are termed as short chain fatty acids. Medium chain fatty acids have 8-12 carbon atoms. Fatty acids with more than 12 carbon atoms are considered as long chain fatty acids.

Unsaturation

Based on the unsaturation, fatty acids can be classified in to saturated, monounsaturated and polyunsaturated fatty acids. Saturated fatty acids are those which do not contain any double bond in the carbon chain. Saturated fatty acids mainly comprise of butyric (C4:0), caproic (C6:0), caprylic (C8:0), capric (C10:0), lauric (C12:0), myristic (C14:0), palmitic (C16:0), stearic (C18:0), arachidic (C20:0), behenic (C22:0) and lignoceric (C24:0). Fatty acids with single double bond are called monounsaturated fatty acids. Commonly occurring monounsaturated fatty acids are palmitoleic acid (C16:1, n-7), oleic acid (C18:1, n-9), erucic acid (C22:1, n-9). Fatty acids containing two or more double bonds are termed as polyunsaturated fatty acids (PUFA). The important dietary PUFA belong to two separate families, n-6 and n-3. Linoleic (C18:2, n-6) and α-linolenic (C18:3, n-3) are considered as essential fatty acids since they are required by the body and cannot be endogenously synthesized, due to the lack of enzyme Δ^{12} and Δ^{15} desaturases. Therefore they have to be supplied through diet. Essential fatty acids (EFA) are involved in energy production, 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). These essential fatty acids differ in their structure and metabolism and hence in their biochemical and physiological properties. The long chain fatty acids synthesized from these fatty acids (Figure 1) are mainly incorporated into structural lipids.

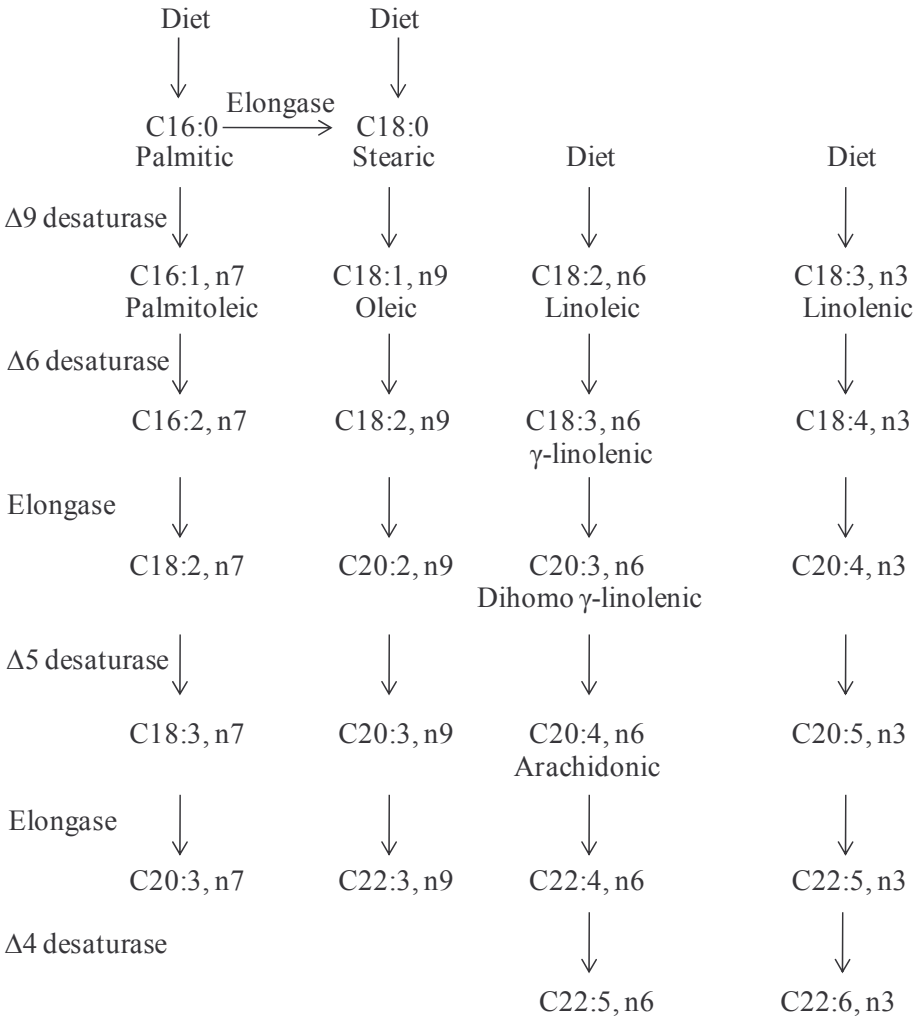


Figure 1. Biosynthesis of polyunsaturated fatty acids. (Source: Sardesai, 1992)

Geometry of double bonds

An unsaturated fatty acid with a double bond can have two possible configurations, cis or trans, depending on the relative position of the alkyl groups. Most naturally occurring unsaturated fatty acids in plants and sea foods have the cis configuration, where the alkyl group lies on the same side of the double bond. It creates a bend or kink in the molecule and cannot be stacked tightly. In trans configuration, the alkyl group positioned on either side of the double bond.

Stereospecific position

The stereospecific position of dietary fatty acid on the glycerol affect its digestion, absorption in and from the gut, their metabolism in enterocytes, incorporation into lipoproteins and subsequent metabolism, and distribution into tissues (Small, 1991).

Properties of fatty acids

The physiological behaviour of fatty acid in the diet is complicated, because of the complex chemistry of these compounds; individual fatty acids vary markedly in their physical characters, absorbability, metabolic fate, and regulatory effects due to the variation in the length of the hydrocarbon chain, number of double bonds, geometry of the double bond and also on the stereospecific position of fatty acids on the glycerol molecule (Spady *et al*., 1993). In general, fatty acids with less than 10-12 carbon atoms, 4:0, 6:0, 8:0 and 10:0 (butyric, caproic, caprylic and capric acid) have low melting points (Table 1), are readily absorbed across the gastrointestinal tract, and are not incorporated into the chylomicron particle. They are carried directly to the liver through the portal vein and rapidly oxidized to acetyl-CoA.

In contrast, longer chain saturated fatty acids, 16:0 and 18:0 (palmitic and stearic acid) have higher melting points (Table 1), and may be less well absorbed, and are incorporated into the chylomicrons particle. They eventually reach the liver, through uptake of the chylomicrons remnant and enrich the lipids present in various metabolic pools (Woollett *et al*., 1992). Dietary fats most commonly contain fatty acids with one, two or three double bonds in the cis configuration at the 9, 12 or 15 position. In general the addition of a double bond to a saturated fatty acid lowers the melting point (Table 1), of that compound and increases its absorbability. These long chain fatty acids e.g. 18:1 (Δ^9), 18:2 ($\Delta^{9, 12}$) are also incorporated into the chylomicron particle after absorption and carried to liver where they enter in various metabolic pools (Daumerie *et al*., 1992). The double bond in naturally occurring unsaturated fatty acids is usually in the cis configuration. The structural configuration of double bonds of fatty acid alters the melting point (Table 1) of the lipid which in turn affects its absorption and also its incorporation into chylomicrons particles.

Table 1. Fatty acid type and melting point

Chain length	Fatty acid	Melting point (ºC)
4:0	Butyric	-5.3
6:0	Caproic	-3.2
8:0	Caprylic	16.5
10:0	Capric	31.6
12:0	Lauric	44.8
14:0	Myristic	54.4
16:0	Palmitic	62.9
18:0	Stearic	70.1
18:1 (Δ^{9})	Oleic	16.2
18:2 ($\Delta^{9,12}$)	Linoleic	-5.0
18:2 ($\Delta^{9,11}$)	Conjugated linoleic	54.0
18:3 ($\Delta^{9,12,15}$)	α-Linolenic	-11.0
20:0	Arachidic	75.4
20:4 ($\Delta^{4,8,11,14}$)	Arachidonic	-50 (ca)
22:0	Behenic	80.0
22:1 (Δ^{9})	Erucic	33.5
24:0	Lignoceric	84.2

(Source: The Lipid Hand book. Gunstone, 2007)

The stereospecific position of fatty acid molecule influences the physicochemical properties, and thereby its digestion and absorption from the gastrointestinal tract (Small, 1991). It determines whether it is predominantly delivered to the liver or to the extra hepatic tissues. Thus the stereospecific position of a fat could markedly influence whether a particular triacylglycerol influences LDL cholesterol metabolism or not.

The types of fatty acids in the diet and their location in the TAG molecule can vary enormously. The great majority of the dietary fats contain predominantly 16:0 and 18:0 and two unsaturated fatty acids i.e. 18:1 and 18:2. Fatty acids can occupy any of three positions on the glycerol back bone; designated as sn-1, sn-2 and sn-3 ("sn" stands for "stereospecific numbering"). In general oils and fats of plant origin, such as soybean and cocoa butter contain unsaturated fatty acids in the sn-2 position and saturated fatty acids in the sn-1 and sn-3 positions. Among animal fats, bovine milk fat and lard (pork fat) contain mainly saturated fatty acids in the sn-2 position, whereas tallow contains saturated fatty acids primarily in the sn-1 and sn-3 positions (Hunter, 2001), (Table 2).

Table 2. Principal TAG species of natural fats and oils

Fat or oil	Major TAG species		
Butter fat	PPB	PPC	POP
Lard	SPO	OPL	OPO
Beef tallow	POO	POP	POS
Cocoa butter	POS	SOS	POP
Coconut oil	LaLaLa	CLaLa	CLaM
Palm oil	POP	POO	POL
Corn oil	LLL	LOL	LLP
Soybean oil	LLL	LLO	LLP
Canola oil	OOO	LOO	OOLn
Rice bran oil	PLO	OOL	POO
Sunflower oil	LLL	LLO	LLP

B- butyric acid, C- capric, La- lauric, M- myristic, P- palmitic, S- stearic, O- oleic, L-linoleic, Ln- linolenic. (Source: Small, 1991)

The stereospecific position of fatty acids in the TAG plays a major role in the functionality of fats in food products since it influence the physical properties. The analysis of a recemic mixture of TAG containing palmitic, oleic and stearic acid

shows that the location of fatty acid affects the melting behaviour of TAG even though the difference is marginal; POS: 37.5-41 ºC, OPS: 40.5-41 ºC, OSP: 41-41.5 ºC (Small, 1991). In the case of lard, the presence of palmitic acid in the sn-2 position contributes to desirable flakiness of pie crusts when lard is used as a baking shortening. In the case of cocoa butter, the unique positioning of palmitic, oleic and stearic acids in two predominant TAG forms gives cocoa butter a sharp melting point just below body temperature. The way the cocoa butter melts is one of the reasons for the pleasant eating quality of chocolate (Hunter, 2001).

Fatty acid metabolism

Fatty acids occur predominantly as esters of glycerol, i.e. triacylglycerol in natural fats of animal and plant origin. The most commonly occurring fatty acid includes myristic acid, palmitic acid, stearic acid, oleic acid and linoleic acid. The double bond in these naturally occurring unsaturated fatty acids is usually in cis configuration.

Fat is typically ingested in the form of triacylglycerol. Within the intestine this lipid is digested to free fatty acids and monoacylglycerols, which are absorbed into the enterocytes, resynthesized to triacylglycerol, incorporated into the chylomicron and secreted into the intestinal lacteals. After reaching the blood stream, much of the triacylglycerol in the core of the chylomicrons is hydrolyzed to free fatty acids which are then taken up primarily by the adipose tissue and muscle. The remnant of the chylomicron, which still contains most of the dietary cholesterol that was absorbed, is rapidly cleared from the serum by specific receptors present in the liver. The hepatocyte, therefore receives most of the cholesterol that is absorbed from the diet and in addition, becomes enriched with the specific fatty acids that were in the triacylglycerol component of the diet (Dietschy, 1997).

Plasma lipids and lipoproteins

The clinically most relevant plasma lipids are cholesterol and TAGs. Cholesterol-related discoveries serve as scientific milestones for the 20th century. Cholesterol is a structural component of cell membranes and is the precursor of steroid hormones, vitamin D and also of oxysterols and bile acids which activate nuclear hormone receptors involved in sterol metabolism. (Russell, 1999). Even

though cholesterol plays important role as structural component and functional molecule, it is one molecule which received wide attention due to its role in atherosclerosis.

Lipoproteins are spherical molecules that transport water insoluble lipids, fat soluble antioxidants and vitamins through plasma from the site of synthesis and absorption to sites of uptake. Lipoproteins have a nonpolar lipid core of TAG and cholesteryl esters with a hydrophilic surface coat containing free cholesterol, phospholipids and apolipoprotein molecules. The chylomicron and VLDL are the main TAG carrying lipoproteins. The main cholesterol carrying lipoproteins are LDL and HDL. Lipoproteins are distinguished from each other on the basis of size, density, electrophoretic mobility, lipid and protein content, composition and function (Table 3).

Table 3. Composition of human lipoprotein molecules

Lipopro-tein class	Size (nm)	Density (g/mL)	Composition (% wt)					Primary apolipo-protein
			TAG	PL	Cholesterol FC	Cholesterol EC	Protein	
CM	75-1200	0.94	80-95	3-6	1-3	2-4	1-2	A-I, A-V, B-48,C-I, C-III, E
VLDL	30-70	0.94-1.006	45-65	15-20	4-8	16-22	6-10	B-100, E, C-I, C-II, C-III
LDL	10-30	1.019-1.063	4-8	18-24	6-8	45-50	18-22	B-100
HDL	5-12	1.063-1.21	2-7	26-32	3-5	15-20	45-55	A-I, A-II, E

CM- chylomicron, VLDL- very low density lipoprotein, LDL- low density lipoprotein, HDL- high density lipoprotein, TAG- triacylglycerol, PL- phospholipids, FC- free cholesterol, EC- esterified cholesterol. (Source: The Lipid Hand book, Gunstone, 2007)

Liver plays an important role in the metabolism of two classes of lipoprotein, VLDL and LDL. The VLDL particle is synthesized in the hepatocytes and function to move triacylglycerol from the liver to the peripheral organs for utilization. A portion of the metabolized remnants of VLDL is taken back by the liver by LDL receptors. The remaining remnants are converted to LDL. The concentration of LDL cholesterol is dependent on LDL cholesterol production rate, the level of hepatic receptor activity and the affinity of the LDL particle to LDL receptor (Dietschy, 1997).

Dietary fatty acids and cardiovascular disease

Cardiovascular disease (CVD) is the leading cause of death all over the world including developing countries like India (Rajeshwari *et al*., 2005) and its etiology is multifactorial. CVD may account for at least one third of all deaths by the year 2015, replacing infectious disease as the major killer factors in India (Deedwania, 2002). Dyslipidemia such as elevated blood cholesterol, increased level of low density lipoprotein (LDL) cholesterol in the circulation, decreased high density lipoprotein cholesterol (HDL) and increases in triacylglycerol concentration in the plasma are critical for the initiation and development of atherosclerosis (Varady and Jones, 2005). Dyslipidemia rarely occur in isolation and are highly interactive, therefore, to effectively treat CVD simultaneous management of lipids as well as lipoprotein is required (Micallef and Garg, 2008). At present, these lipid imbalances are treated with pharmacological agents such as HMG-CoA reductase inhibitors (statins), bile acid sequestrants (resins), nicotinic acid and fibric acid derivatives. Even though these cholesterol lowering drugs were shown significant effect in lowering LDL cholesterol, increasing HDL cholesterol and decreasing TAG level safety concerns have surfaced due to the side effects reported for long term usage of these drugs (Okuyama *et al*., 2007). Considering the safety issues, implementation of non pharmacological therapies that beneficially modulate lipid profiles without adverse side effects would be highly advantageous. Diet and lifestyle are the two modifiable factors which influence the risk factors of cardiovascular diseases. Dietary fat modification has always been advocated for the regulation of the risk factors of CVD.

The lipid-heart hypothesis which had its root in the mid 19th century (Erkkila *et al*., 2008) had an impact in influencing public opinion about the fat consumption. It was believed that a reduction in total dietary fat intake per se can decrease risk of CVD by improving serum lipid profiles in a beneficial manner. Several lines of evidence have indicated that types of fat have a more important role in determining risk of coronary heart disease than total amount of fat in the diet. Later studies by Keys *et al*. (1965) unraveled the biological basis for the diet heart hypothesis by showing that the type of fatty acid, saturated:monounsaturated:polyunsaturated fatty acids (SFA:MUFA:PUFA) in the diet plays an important role in modulating the levels of risk factors for CVD. Work of Keys *et al.* (1965) and Hegsted *et al*. (1965) made

them to draw a conclusion by deriving predictive equations to quantify the effect of dietary cholesterol and fat on plasma cholesterol concentrations. The overall conclusions were similar even though there were minor quantitative differences between the two equations: (1) dietary cholesterol has a relatively modest plasma cholesterol raising effect; (2) dietary SFAs have a potent plasma cholesterol raising effect; (3) dietary PUFAs have a plasma cholesterol lowering effect; (4) the cholesterol raising effect of SFA is more potent than the cholesterol lowering effect of PUFA. Later it was found that the role of lipoproteins in the development of premature cardiovascular disease has increased and it became apparent to focus on the effect of specific fatty acids rather than merely looking at the broad classes based on degree of saturation. A number of studies in 1990s have been attempted to develop predictive equations taking into account the effect of dietary fatty acids on plasma total, LDL and HDL cholesterol (Kris-Etherton and Yu, 1997). Dietary TAGs may differ from each other mainly on the type of constituent fatty acids, its chain length, number, position and geometry of the double bond and also on its stereospecific position in the glycerol molecule. These differences in the type of fatty acids influence the physicochemical properties of fat thereby its digestion and absorption, which inturn differentially alter plasma lipoprotein profiles and the subsequent risk of developing CVD.

Dietary fat and cholesterol play a major role in cardiovascular disease development, mostly by modulating plasma lipoprotein concentrations. Dietary modification remains the cornerstone of CVD prevention. Studies conducted in human and animals during last 50-55 years have established that different categories of dietary fatty acids have different effects on blood lipid and lipoprotein levels (Keys *et al*., 1957; Hegsted *et al*., 1965; Katan *et al*., 1994). Unsaturated fatty acids of plant origin tend to lower total and LDL cholesterol when compared with saturated fatty acids. In contrast, saturated fatty acids found primarily in animal fats tend to raise total and LDL cholesterol when compared with unsaturated fatty acids. Despite these widely accepted generalizations, studies focussing on feeding high levels of specific saturated or unsaturated fatty acids can be difficult to explain, because fats having similar ratios of unsaturated to saturated fatty acids may have different fatty acid composition. For e.g., cocoa butter and milk fat have similar levels of saturation;

however the saturated fatty acids of cocoa butter are primarily palmitic (42.5%) and stearic acid (55%), where as milk fat contains a wider variety of saturated fatty acids including stearic (19%), palmitic (42%), myristic (16%) and medium chain and short chain fatty acids (23%). Each fatty acid differs in their physicochemical properties and metabolism as well (Hunter, 2001).

The most prevalent saturated fatty acid in the diet is palmitic acid, followed in the order of abundance by myristic, stearic and lauric acids. Not all saturated fatty acids affect total cholesterol concentrations in the same manner. Stearic acid has little effect on plasma cholesterol concentration, whereas myristic and palmitic acids have been reported to have the greatest cholesterol raising potential. Studies by Hegsted *et al*. (1993) and meta analysis by Katan *et al*. (1994) drew a conclusion for this and suggested that myristic acid may be four to six times more cholesterol raising than palmitic acid (Hegsted *et al*., 1965; Mensink and Katan, 1992). However, in edible fats, high concentrations of myristic acid are invariably associated with high concentrations of either lauric acid, such as in coconut oil, or palmitic acid as in palm oil. These associations may invalidate multiple-regression analyses through co linearity, which causes spuriously high or low regression coefficients. Later studies by Katan *et al*. (1994) using fat rich in myristic acid found that myristic acid is about 1.5 times higher in cholesterol raising compared to palmitic acid, which is much less than the factor of four to six suggested by meta-analysis (Zock *et al*., 1994). The neutral effect of stearic acid on plasma cholesterol level may be due to its poor absorbability from the intestine. Stearic acid is more insoluble than other fatty acids, and thus may be less absorbable. Tristearin resists hydrolysis in the intestine and thereby escapes absorption. Another reason is that stearic acid is rapidly converted to oleic acid in the body (Grundy and Denke, 1990). Saturated fatty acids increase LDL cholesterol concentrations by decreasing LDL receptor mediated catabolism (Nicolosi *et al*., 1990; Spady *et al*., 1983). This effect is mediated both by decreased LDL receptor mRNA expression and decreased membrane fluidity (Hennessy *et al*., 1992).

The major monounsaturated fatty acid in the diet is oleic acid. Studies show that monounsaturated fatty acids are neutral with respect to their effect on plasma total cholesterol concentrations (Hegsted *et al*., 1965; Keys *et al*., 1965).

Trans fatty acids are formed during the hydrogenation process, a process that converts vegetable oils to a semisolid state. During this process, linoleic acid is converted to stearic, oleic or elaidic acid. Studies have shown that, compared with diet enriched in linoleic or oleic acid, diet enriched in elaidic acid do not have a beneficial effect on plasma lipoprotein profile because it elevates LDL cholesterol and reduce HDL cholesterol concentrations (Lichtenstein *et al*., 1999). Studies also show that increased trans fatty acid consumption increases plasma concentrations of lipoprotein(a), an independent risk factor for coronary heart disease.

Dietary PUFAs are subclassified as n-6 and n-3, indicating the location of the first double bond from the omega end of hydrocarbon chain. The major n-6 fatty acid in the diet is linoleic acid, which serve as the precursor for arachidonic acid (20:4, n-6), which has important biological effects in the body. The other major essential fatty acid in the diet is α-linolenic acid (18:3, n-3). This fatty acid can be converted in the body to eicosapentaenoic acid, which can be further elongated and desaturated to docosahexaenoic acid (22:6, n-3) (Siguel *et al*., 1987). Linoleic acid shows hypocholesterolemic effect when substituted for dietary saturated fatty acids (Hu *et al*., 1999). Dietary arachidonic acid has little or no effect on plasma lipoprotein concentrations. n-3 Fatty acids found in fish oil especially docosahexaenoic acid, lower triacylglycerol concentrations significantly and reduces coronary heart disease risk as well. High intakes of n-3 fatty acids are associated with lower platelet aggregation and blood pressure level.

The stereospecific position of fatty acid on the TAG is important since it affects the metabolic fate of fatty acids and also how the TAGs are digested. During digestion in the gastrointestinal tract, pancreatic lipase, an enzyme specific for the sn-1 and sn-3 esters, catalyzes the formation of sn-2 monoacylglycerols and free fatty acids, that are absorbed in the small intestine. The 2-monoacylglycerols are reacylated into new triacylglycerols that enter the lymph as chylomicrons. Fatty acids released from the sn-1 and sn-3 positions often have different metabolic fates than fatty acids retained in the sn-2 position. These metabolic fates depend on the fatty acid chain length and stereospecific location in the triacylglycerol. Short chain and medium chain fatty acids can be solubilized in the aqueous phase of the intestinal contents,

where they are absorbed, bound to albumin, and transported to the liver by the portal vein. Longer chain fatty acids, such as palmitic and stearic acids have lower absorption because of higher melting points and because of their ability to form calcium soaps. Thus, fats with long chain fatty acids in the sn-1 and sn-3 positions of triacylglycerols may exhibit different absorption patterns from fats which contain these fatty acids in the sn-2 positions.

Dietary fatty acids have a considerable effect on plasma total and LDL cholesterol concentration and therefore on the risk of coronary heart disease. Dietary fatty acids have been shown to influence the circulating concentration of LDL cholesterol in animals and humans by changing hepatic LDL receptor activity, LDL cholesterol production rate or both. Dietary fatty acids alter LDL receptor activity by regulating the size of the cellular sterol regulatory pool by driving the activity of esterification enzyme acyl-CoA: cholesterol acyl transferase (ACAT). Saturated fatty acid such as palmitic acid has been shown to have inhibitory effect on ACAT which reduce the size of the cholesterol ester pool. These changes result in the decreased mRNA concentration and LDL receptor activity. In contrast, if the liver is enriched with the preferred substrate for ACAT, i.e. unsaturated fatty acids, oleic or linoleic, the cholesterol is shifted from cholesterol regulatory pool to cholesteryl ester pool and also results in increase in LDL receptor activity (Dietschy, 1998).

A systematic study using pure triacylglycerol containing single fatty acids concluded that short and medium chain saturated fatty acids are rapidly oxidized in the liver to acyl-CoA. These fatty acids do not alter the composition of the lipid pool in the liver. Long chain saturated fatty acids except stearic acid is found to inhibit cholesterol ester formation and inhibit LDL receptor activity. Monounsaturated and polyunsaturated fatty acids increases the cholesterol ester fraction in the liver, reduces the size of the regulatory pool and increases the level of hepatic LDL receptor activity.

Cholesterol homeostasis

Cholesterol plays several structural and metabolic roles that are vital to human biology. Cholesterol in the plasma membrane of the cell modulates fluidity of the membrane. In addition, cholesterol is a substrate for steroid hormones (Pasqualini,

2005). Too much cholesterol in cells, however, can have pathological consequences. This is particularly true for arterial cell wall, where accumulation of cholesterol initiates atherosclerotic plaques (Yuan *et al*., 2006). Therefore the cholesterol levels should be finely controlled, and the body relies on a complex homeostasis network to achieve this. The liver is the principal site for cholesterol homeostasis (Dietschy *et al*., 1993). Cholesterol homeostasis is controlled in mammalian cells in several stages. There are three main levels of control in the regulation of cholesterol homeostasis: cholesterol synthesis, uptake and efflux (Figure 2) (Wong *et al*., 2006).

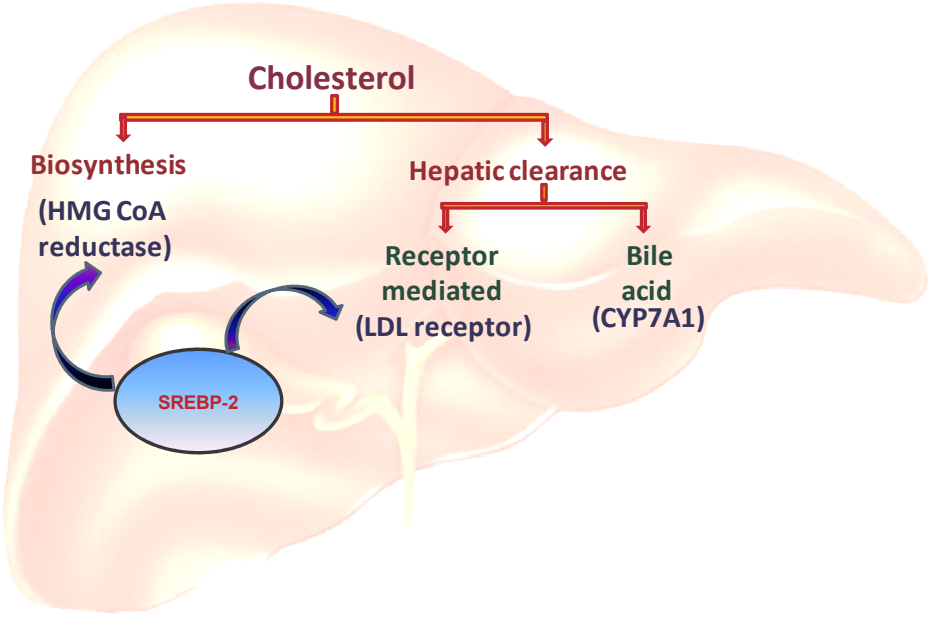


Figure 2. Cholesterol homeostasis. SREBP- sterol regulatory element binding protein, CYP7A1- cholesterol 7-α-hydroxylase.

The rate limiting step of cholesterol biosynthesis in cells is catalyzed by the enzyme HMG-CoA reductase. The supply of exogenous cholesterol to cells is regulated via the uptake of LDL by the LDL receptor. In humans, much of this control is dependent on coordinated changes in the level of mRNAs encoding enzymes involved in cholesterol biosynthetic pathway and mRNA encoding the LDL receptor. The genes encoding both HMG-CoA reductase and LDL receptor are transcribed at a relatively high rate when cells require cholesterol, while repressed in the case of excess sterol. This regulation is mediated by the sterol regulatory element (SRE)

located in the promoter regions of both HMG-CoA reductase and LDL receptor. Two proteins, sterol regulatory element binding protein (SREBP) 1 and 2 have been shown to specifically bind to SRE-1 (Hua *et al*., 1993). The transcription factor SREBP-2 regulates the expression of many genes involved in cholesterol synthesis (HMG-CoA reducatse) and uptake (LDL receptor). The precursors of SREBPs are anchored to the membrane of the endoplasmic reticulum through a tight association with SREBP cleavage activating protein (SCAP). Activation of SREBP-2 is dependent on the cholesterol status of the cell (Goldstein *et al*., 2006). Under low cholesterol conditions, SCAP escorts the SREBP precursors from the endoplasmic reticulum (ER) to the golgi apparatus where two functionally distinct proteases sequentially cleave the precursor protein releasing the nuclear SREBP in the cytoplasm (Sakai *et al*., 1998; Wang *et al*., 1994). The mature SREBPs are then translocated to the nucleus, where they bind to SRE, thus activating the transcription (Figure 3).

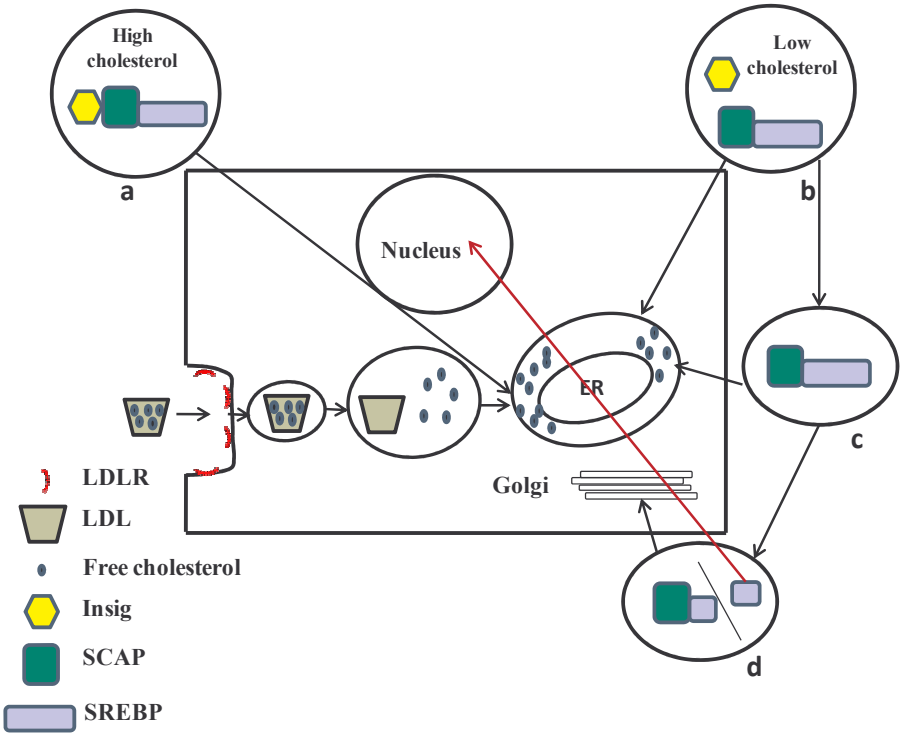


Figure 3. SREBP regulation of cholesterol. SREBP- sterol regulatory element binding protein, LDL- low density lipoprotein, LDL-R- LDL receptor, SCAP- SREBP cleavage activating protein, ER- endoplasmic reticulum. (Source: Martini and Pallotini, 2007)

The concentration of LDL in plasma is strongly influenced by the amount and the type of lipid in the diet. Studies in hamsters have shown that dietary fatty acids differentially affect circulating LDL levels primarily by altering receptor dependent LDL uptake in the liver. The concentration of LDL in plasma is determined by the rate at which LDL enters the plasma relative to the rate at which it is cleared from plasma by various tissues of the body. LDL are formed in plasma during the metabolism of VLDL, which in turn are secreted by the liver (Havel, 1984). Tissues take up LDL from plasma by two mechanisms, receptor dependent and receptor independent mechanisms. Receptor dependent mechanism involves the interaction of LDL particles with the cell surface receptors, followed by endocytosis and catabolism of the LDL particle in the lysosomal compartment (Brown and Goldstein, 1986). In humans and animals, receptor dependent mechanism account for 70-80% of total LDL turnover (Pittman *et al*., 1982) and the liver account for the major part of this. Studies in hamsters have shown that dietary fatty acids produce their differential effects on circulating LDL levels primarily by altering receptor dependent LDL uptake in the liver. Triacylglycerol containing predominantly unsaturated fatty acids accelerates the rate of receptor dependent LDL uptake in the liver as compared to those fed with TAG containing predominantly saturated fatty acids. Dietary fatty acids produce their differential effect on LDL receptor mRNA levels by altering sterol balance across the liver by altering cholesterol absorption, bile acid synthesis, or biliary cholesterol output (Horton *et al*., 1993).

Whole body cholesterol homeostasis is controlled by supply and removal pathways. The breakdown of cholesterol to bile acids in the liver is a major pathway for cholesterol elimination (Russell and Setchell, 1992; Dietschy *et al*., 1993). Stimulation of this process results in adaptive responses that promote hepatic cholesterol synthesis and LDL receptor mediated uptake. Cholesterol can be either excreted into bile directly or after conversion into bile acids. Bile acid synthesis occurs exclusively in the liver and cholesterol 7-α-hydroxylase (CYP7A1) is the first and rate-limiting enzyme of the pathway (Russell and Setchell, 1992). Conversion of cholesterol to bile acids is an irreversible and terminal process of cholesterol catabolism. Thus, the activity of CYP7A1 can have a major impact on the overall catabolism of excess cholesterol.

Many studies have shown that dietary fatty acids regulate plasma LDL cholesterol levels by affecting LDL receptor activity, protein and mRNA abundance (Fernandez and West, 2005). High activity of mRNA abundance of CYP7A1 favors lower serum cholesterol. Studies have shown that the expression of CYP7A1 is regulated by dietary fat. Feeding diet enriched with PUFA to mice showed an increase in mRNA abundance for CYP7A1 as compared with mice fed MUFA or saturated fat (Cheema *et al*., 1997).

Fatty acids and thrombosis

Uncontrolled thrombosis is another risk factor of CVD. Platelets have a central role in this process. The physiological function of platelet is to maintain vascular integrity and arrest bleeding. On the other hand, platelets are also involved in thrombosis and probably other pathological events such as inflammation and asthma (Folts *et al*., 1999). An increased level of platelet aggregation is regarded as a risk factor for atherosclerosis. Platelet interaction with damaged arterial wall is known to contribute to the development of thrombosis and atherosclerosis. Ruptured atherosclerotic plaques with platelet mediated occlusive thrombosis are known to lead to acute coronary syndromes and ischemic stroke. Platelets take part in thrombosis by adhering to the exposed subendothelial connective tissue on a site of injury. The adhesion is followed by platelet activation, which results in aggregation and secretion of regulatory compounds. Platelets are activated only if an external stimulus, interacting with platelet surface glycoprotein or glycolipids, is able to transduce its signal inside the platelet. These input stimuli can activate platelet *in vivo* and ultimately cause adhesion, clumping, aggregation and thrombus formation. These input stimuli or agonists can act independently or are synergistic with one another. The net effects of these stimuli are to raise platelet cytosolic calcium, which triggers the contraction of platelet actin and myosin fibrils, leading to platelet shape change, and the release reaction (Folts *et al*., 1999). All the stimuli finally activate the same fibrinogen receptor (GP IIb-IIIa) which changes its conformation and binds fibrinogen or fibronectin. Studies showed that fatty acids participate in intracellular signaling pathways which regulate either the activation and binding function of GP IIb-IIIa or subsequent signal transduction (Mutanen, 1997). Diet rich in saturated fatty acids

directly influence platelet and endothelial cell function by altering the fatty acid composition of these cells. These changes are accompanied by the acute effects of postprandial lipoproteins on coagulation and fibrinolytic activities which ultimately favours thrombosis. Dietary supplementation with (n-6) PUFA has been associated with a mild reduction in platelet aggregation induced by low concentrations of thrombin, prolonged platelet aggregation and reduced platelet factor, GPIIb-IIIa activity (Norday and Goodnight, 1990).

Eicosanoids

One of the important functions of dietary PUFA is to provide essential fatty acids. The requirement for EFAs such as linoleic acid is up to 3% of total calorie intake. However guidelines from agencies like Indian Council of Medical Research, American Heart Association recommends an intake of dietary polyunsaturated fatty acids at 10% of total calorie intake. This recommendation is mainly based on the cholesterol lowering property of dietary PUFA.

Eicosanoids are potent autocoids produced from arachidonic acid (20:4, n-6), eicosapentaenoic acid (20:5, n-3) and eicosatrienoic acid (20:3) by two enzymes, cyclooxygenase and lipoxygenase. Cyclooxygenase products include prostaglandins, ptostacyclins and thromboxane and that of lipoxygenase are leukotrienes and lipoxins (Mills *et al*., 2005) (Figure 4). Prostaglandins, ptostacyclins and thromboxane are together called prostanoids. Prostanoids derived from arachidonic acid are termed 2-series prostanoids, while eicosatrienoic and eicosapentaenoic acids results in the formation of 1 and 3-series prostanoids respectively. Leukotrienes derived from arachidonic acid are termed 4-series leukotrienes, while action of lipoxygenases on eicosatrienoic and eicosapentaenoic acids results in the formation of 3 and 5-series leukotrienes respectively. Eicosanoids have been implicated in several pathophysiological processes of the cardiovascular system, including vascular thrombosis, myocardial ischaemia and tissue injury as well as in sudden death. The prostacyclin (PGI_2) and thromboxane (TX) A_2, two important eicosanoids, play an important role in platelet-vessel wall interactions. TXA_2 is a vasoconstrictor and a powerful inducer of platelet aggregation and PGI_2 is a platelet antagonist (Ferretti *et al*., 1998). They are both directly derived from the cyclooxygenase pathway, from

arachidonic acid, and their ratio is considered to be a biochemical marker of risk for thrombosis (Bunting *et al*., 1983). The eicosanoids derived from n-6 and n-3 PUFAs act antagonistic to each other. TXA_3 synthesized from n-3 fatty acids is a weak aggregating agent and compared to TXA_2 produced from n-6 PUFA. PGI_3 is a potent antiaggregatory agent compared to PGI_2 (Simopoulos, 1999).

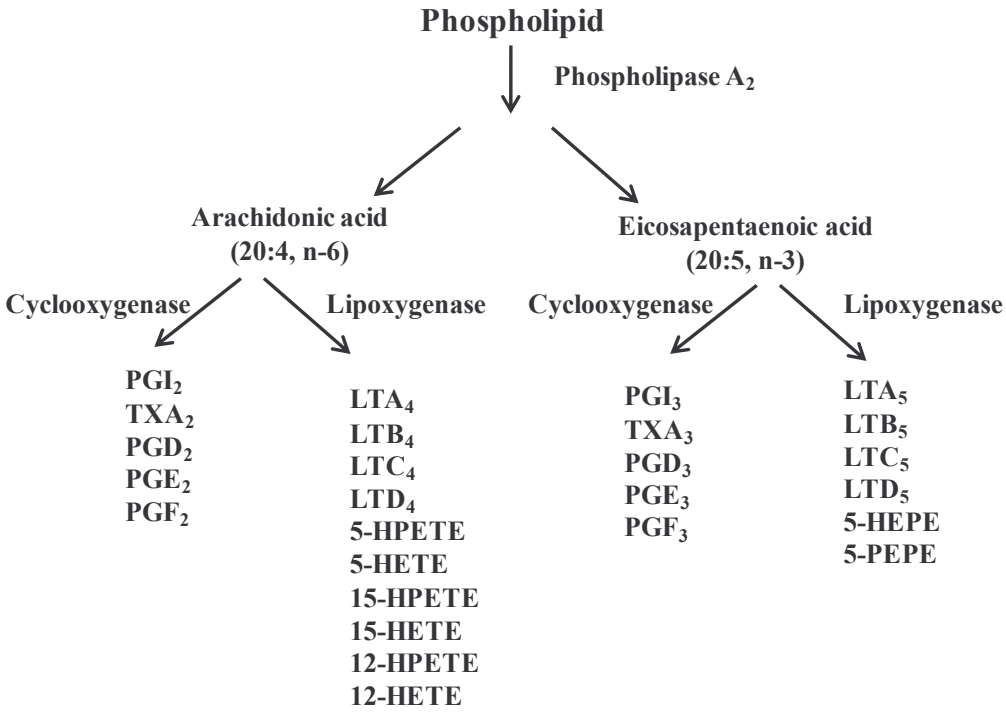


Figure 4. Synthesis eicosanoids from arachidonic acid and eicosapentaenoic acid. (Source: Mills *et al*., 2005)

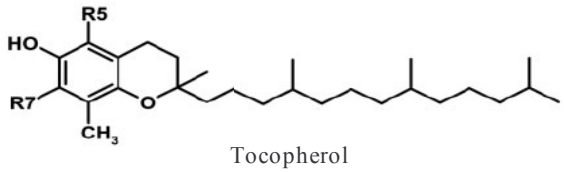
Antioxidants and CVD

The influence of antioxidants on CVD comes from the work of Steinberg (1997) who suggested that oxidatively modified LDL formed by the action of free radicals are atherogenic. Free radicals are highly reactive molecules as they contain an unbound electron. In our body they can oxidize many molecules such as lipids, especially unsaturated fatty acids. Under normal physiologic conditions, cells are protected against free radicals by endogenous enzymes (super oxide dismutase, catalase, glutathione peroxidase) and by antioxidants such as vitamin E (the main antioxidants in membranes), vitamin C and beta carotene (Halliwell, 1989). When the

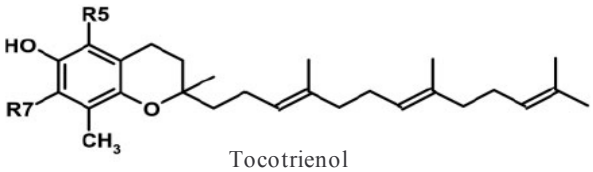
balance between the formation of free radicals and antioxidant defenses is disturbed, unsaturated fatty acids from LDL are oxidized. Oxidized LDL become atherogenic, elicits a chemotactic response which stimulates monocytes to get transformed into macrophages and subsequently leading to foam cell formation. Oxidized LDL are cytotoxic and damage endothelial cells, stimulating platelet aggregation and procoagulant activity (Henning and Chow, 1988).

Minor constituents in oils and fats as antioxidants

Apart from TAG, vegetable oils contain some minor components in their unsaponifiable fraction. These minor constituents are unique to each oil and some of them have nutraceutical properties. Tocopherols and tocotrienols are fat-soluble nutrients of vitamin E family, with a chromanol ring and a hydrophobic side chain. The tocotrienols differ from tocopherols in having three trans double bonds in the hydrophobic side chain. Individual isomers, α, β, γ, and δ of tocopherols and tocotrienols differ by the number and positions of methyl substituents on the phenolic part of the chromanol ring (Figure 5). Tocopherol is present in almost all vegetable oils. Tocotrienols are abundantly present in palm oil and rice bran oil and in small amount in coconut oil.



Tocopherol



Tocotrienol

	R5	R7
α-	CH_3	CH_3
β-	CH_3	H
γ-	H	CH_3
δ-	H	H

Figure 5. Chemical structure of tocopherol and tocotrienol. (Source: Kamal-Eldin, 2006)

Both tocopherols and tocotrienols are known for antioxidant properties and their beneficial effect on health. Studies have shown that tocotrienols possess hypocholesterolemic, antioxidant, anticancer and neuroprotective properties that are often not exhibited by tocopherols (Khanna, *et al*., 2006; Osakada *et al*., 2004). Supplementation of tocotrienols rich fraction (TRF) of palm oil in the diets of hypercholesterolemic subjects reduced serum cholesterol, prothrombotic factors like thromboxane B_2 and platelet factor IV (Qureshi *et al*., 1991a). Feeding pigs with TRF of palm oil showed antiatherogenic and antithrombotic properties (Qureshi *et al*., 1991b).

Sesame lignans; sesamin, sesamolin and sesamol (Figure 6), present in sesame oil show antioxidative, lipid lowering, anticarcinogenic and blood pressure lowering properties (Dhar *et al*., 2005). Sesamin, the lignan molecule in sesame oil is a potent and specific inhibitor of Δ-5 desaturase (Shimizu *et al*., 1991). It has hypocholesterolemic properties (Chen *et al*., 2005). 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).

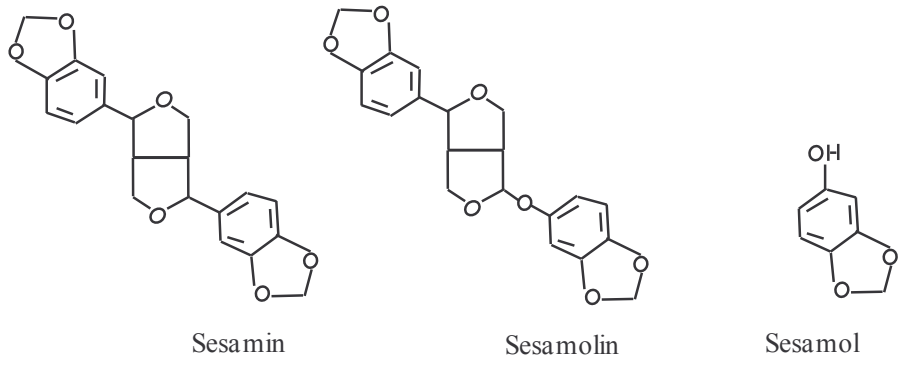


Figure 6. Chemical structure of sesamin, sesamolin and sesamol. (Source: Kamal-Eldin *et al*., 1994)

Phytosterols present in unsaponifiable fraction of oils has various health benefits. Phytosterols differ in chemical structure from cholesterol by an ethyl or methyl group in their side chain. They may be present in the oil in both free and esterified forms. They may be esterified with glucosides and ferulic acid. Ferulic acid-esterified sterols are commonly known as oryzanol (Figure 7).

γ–Oryzanol is uniquely present in RBO and has been reported to have various health benefits, including antioxidant activity and hypocholesterolemic effect (Sasaki *et al*., 1990; Seetharamaiah and Chandrasekhara, 1988) by suppressing the HMG-CoA reductase . Palm oil is a rich source of beta carotene, provitamin A, and exhibit antioxidant activity. These various studies indicate that both major and minor components of oils have an influence on our health and prevent development and progression of diseases.

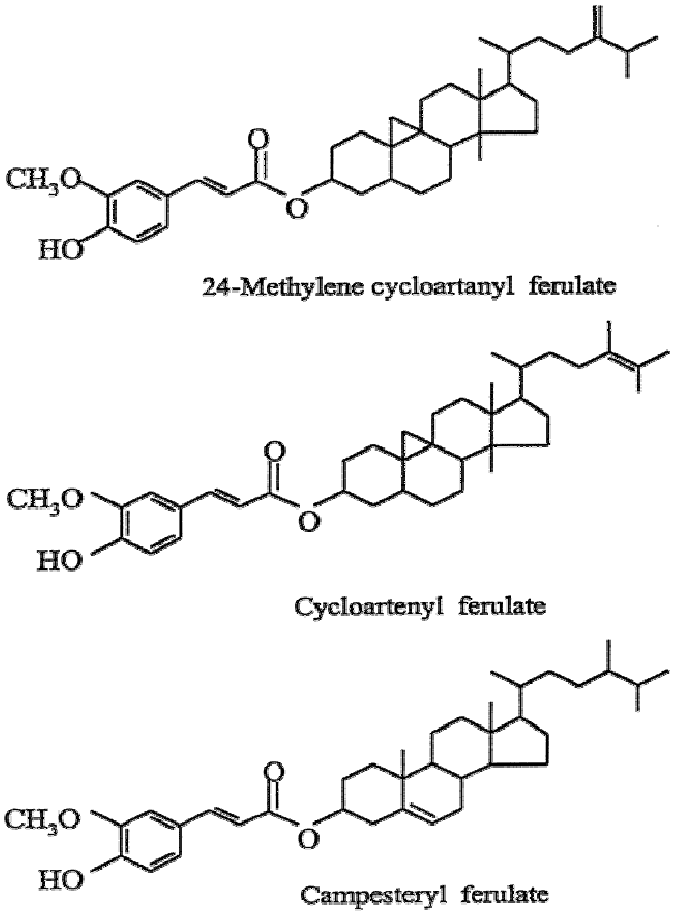


Figure 7. Chemical structure of oryzanol. (Source: Xu *et al*., 2001)

Dietary fat requirement

Fat is the nutrient with the highest energy density. It also provides essential and other functional fatty acids as well as act as carrier of fat soluble vitamins. It contributes substantially to the appearance and flavor of our food and consequently,

influences our eating behaviour. Even though fat is essential it is often blamed, due to its role in raising the risk factors of chronic diseases like CVD, diabetes obesity and cancer. However not all fats are equal in their metabolic effects. The hypocholesterolemic action of a dietary fat depends on the fatty acid composition and also to some extent on the micronutrients like tocopherols, tocotrienols, oryzanol, sesamin. Earlier guidelines focused on dietary requirements of fats but today it is for preventing the risk factors for chronic diseases. Even though the dietary requirement for essential fatty acid, linoleic fatty acid is up to 3% of total energy, American Heart Association (AHA) recommended 10% total PUFA out of 30% of calories from total fat in the diet due to its beneficial effect on reducing the risk factors of CVD. The recommendation of Indian Council of Medical Research is also in line with that of AHA (Ghafoorunissa, 1998; Achaya, 1995), which recommend intake of fat with SFA:MUFA:PUFA in the ratio of approximately 1:1:1 in a diet providing 20-25% calories from total fat (Fogli-Cawley *et al*., 2006), keeping P/S ratio at 0.8-1.0.

Fat intake in India

In India, the dietary habit, particularly fat consumption varies from region to region. Coconut oil (CNO) is the predominant dietary fat in Kerala and coastal regions of Karnataka in south India. Mustard oil is the major cooking oil in north India and the eastern part of the country. Sesame oil (SESO) is used in South India. Sunflower oil, ground nut oil is used in most parts of the country. These oils are rich in unsaturated fatty acids. Rice bran oil (RBO) which is recently introduced in Indian market is also rich in unsaturated fatty acids. Palm oil which is also available in Indian market, contain more of saturated fatty acids. Careful analysis of the fatty acid composition of all these oils (Table 4) indicate that there is no single oil which provides balanced amount of fatty acids which is in tune with the recommendations of Indian Council of Medical Research.

Table 4. Fatty acid composition of commonly used oils in India

Dietary oil	Fatty acids																				
	6:0	8:0	10:0	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	20:2	22:0	22:1	22:2	24:0	S	M	P
Sunflower	-	-	-	-	-	11.0	-	4.7	18.6	68.2	0.5	0.4	-	-	-	-	-	-	16.1	18.6	68.7
Groundnut	-	-	-	-	-	11.6	0.2	3.1	46.5	31.4	-	1.5	1.4	0.1	3.0	-	-	1.0	20.2	49.2	31.4
Mustard	-	-	-	-	0.1	2.8	0.2	1.3	23.8	14.6	7.3	0.7	12.1	0.6	0.4	34.8	0.3	1.0	6.2	71.5	22.2
Sesame	-	-	-	-	-	9.9	0.3	5.2	41.2	43.3	0.2	-	-	-	-	-	-	-	15.1	41.5	43.5
Rice bran	-	0.1	0.1	0.4	0.5	16.4	0.3	2.1	43.8	34.0	1.1	0.5	0.4	-	0.2	-	-	-	20.3	44.5	35.1
Palm	-	-	-	0.3	1.1	45.1	0.1	4.7	38.8	9.4	0.3	0.2	-	-	-	-	-	-	51.4	38.9	9.7
Coconut	0.5	8.0	6.4	48.5	17.6	8.4	-	2.5	6.5	1.5	-	0.1	-	-	-	-	-	-	92.0	6.5	1.5
Safflower	-	-	-	-	0.1	6.5	-	2.4	13.1	77.7	-	0.2	-	-	-	-	-	-	9.2	13.1	77.7
Corn	-	-	-	-	-	12.2	0.1	2.2	27.5	57.0	0.9	0.1	-	-	-	-	-	-	14.5	27.6	57.9
Soybean	-	-	-	-	0.1	11.0	0.1	4.0	23.4	53.2	7.8	0.3	-	-	0.1	-	-	-	15.5	23.5	61

S: saturated, M: monounsaturated, P: polyunsaturated fatty acids. (Source: Chow, 2002)

Coconut oil

Coconut oil is the predominant dietary fat in south India. Coconut oil is a rich natural source of medium chain fatty acids (MCFA). Saturated fatty acids account for 90% of total fatty acid in coconut oil of which 45-50% is accounted by lauric acid. Coconut oil contains very small amount of PUFA (<2%). The minor components present in coconut oil are tocopherols and tocotrienols (less than 5ppm). MCFA are transported mainly via portal system and are rapidly oxidized in the liver to provide energy faster. Medium chain fatty acids have been used for the treatment of fat malabsorption-related diseases and as significant source of energy for preterm infants (Willis and Marangoni, 1999). However the presence of high content of saturated fatty acids such as myristic and palmitic acid, which increases the LDL cholesterol level in serum, making it an atherogenic fat.

Rice bran oil

Rice bran oil has become popular in India due to health benefits it provides. Fatty acid composition of rice bran oil shows that it contain 80% unsaturated fatty acids and 20% saturated fatty acids. γ-oryzanol, ferulic acid ester is uniquely present in rice bran oil. Rice bran oil is also a rich source of tocotrienols and tocopherol. Studies on humans (Most *et al*., 2005) have shown that the cholesterol reducing property of RBO is due to the compounds present in the unsaponifiable fraction of the oil. This includes oryzanol. Oryzanol lowers the cholesterol level by suppressing cholesterol absorption and enhancing the fecal sterol excretion (Wilson *et al*., 2007; Ausman *et al*., 2005). β- and γ-isomers contribute to the major tocotrienols in RBO (Sugano and Tsuji, 1997). Studies on humans have shown that tocotrienols lower cholesterol through the inhibition of HMG-CoA reductase, the rate limiting enzyme in cholesterol biosynthesis (Kerckhoffs *et al*., 2002).

Sesame oil

Sesame oil has been used as an edible fat and is considered as a health food in Asia (Sugano and Akimoto, 1993). Sesame oil is rich in unsaturated fatty acids (>85%) and lignan molecules. Sesamin is a major lignan present in sesame oil. Sesamin is a specific inhibitor of Δ5 desaturase (Shimizu *et al*., 1991), which

catalyzes the conversion of dihomogamalinolenic acid to arachidonic acid. It also exerts hypocholesterolemic activity through the inhibition of cholesterol absorption and synthesis (Hirose *et al*., 1991). Lignans carrying a hydroxyl group, sesaminol, episesaminol and sesamolinol, exhibit antioxidant activity (Fukuda *et al*., 1985).

Modification of oils and fats

Fats contribute to the physical and functional properties of most foods and also influence sensory and nutritional aspects. The amount and type of fat present in a food has a role in determining the characteristics and consumer acceptance of that food. However fat is still the number one nutritional concern for most people in developed countries. Excessive intake of fat in the diet has been linked to certain diseases such as heart disease, cancer and obesity. It has been difficult for individual to change their dietary pattern to reduce or minimize fat intake while enjoying their favourite foods. This problem and the interest shown by consumers for alternative fats and foods which are low in calorie led to the search for the ideal fat which possesses optimum physical, functional and nutritional property. This however requires modification of oils.

Methods available for modification of oils and fats

Fractionation, hydrogenation and interesterification are three processes available to food manufacturers to tailor the physical and chemical properties of food lipids. Blending of different oils is another option available for modification of oils. 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

The melting behaviour of fat is an important property for determining its functionality in various prepared food products. Fractionation processes separate fats and oils into fractions with different melting points. Fractionation may be practiced merely to remove an undesirable component, which is the case with dewaxing and winterization process to get liquid oils that resist clouding at cool temperature. This technology is often used to fractionate palm oil into olein (liquid, oleic acid-rich) and stearin (solid at room temperature, stearic acid-rich) fractions.

Hydrogenation

Hydrogenation is one of the most important and complex chemical reactions carried out in the processing of edible oils and fats, where hydrogen gas react with the double bonds in the carbon chain of the unsaturated fatty acid. Hydrogenation of fats was developed as a result of the need to convert liquid oils to the semi–solid form for greater utility in certain food, and also to improve the oxidative and thermal stability of the fat or oil. The hydrogenation process is carried out at elevated temperature and pressure in the presence of a catalyst. The most widely used catalyst is nickel supported on an inert carrier which is removed from the fat after the hydrogenation process is completed. Hydrogenation process convert fat or oil to one with more saturated fatty acid, shift the position of the double bond to a new position, or twist the configuration of the double bond from cis to trans form, all of which increases its melting point. One of the products of hydrogenation is undesirable due to the production of trans fat, which is reported to have deleterious effects on health.

Blending

Blended oils represent a physical mixture of two or more oils. Blending of oils is used as an alternative to hydrogenation for modification of oils and fats to get an oil or fat with desired physicochemical or nutritional properties. For getting optimum 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 now permitted mixing of any two edible vegetable oils (PFA Act, 1954, amended in 2008) in which the minimum proportion of 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.

Even though one can balance the fatty acid composition by blending of oils it may not always result in oils with desired physicochemical (Anwar *et al*., 2007) or nutritional properties (Smith *et al*., 2007). This is because the physical characteristics of TAG molecular species in individual oils will be retained in a blended oil. This

may be altered by rearranging the fatty acids in the triacylglycerol molecules mediated by enzymatic or chemical interesterification process. Hence, interesterification of oils and fats has attracted the attention of technologists in recent times. Lipase catalyzed interesterification has become a useful tool for the production of designer lipids to improve the physical property such as melting profile of butter. Rearrangement of fatty acid on the glycerol moiety catalyzed by lipases may change the physical (Smith *et al*., 2007) and nutritional properties of the oil (Chu and Kung, 1998). The new triacylglycerol molecules formed in the interesterified oil may be absorbed better than the original TAG from the native oil or in the blend (Neff *et al*., 1997).

Interesterification

Interesterification is the exchange of fatty acid molecule within and between the TAG moieties which provides new lipids containing specific fatty acids for medicinal, nutritional and specialty fats for food industry. Interesterification can be further classified in to acidolysis (fatty acid-ester), glycerolysis (glycerol-ester) and transesterification (ester-ester) (Malcata *et al*., 1990) Figure 8**.** Interesterification can be carried out chemically or enzymatically.

Chemical interesterification

There are two types of chemical interesterification; random and directed. Interesterification reactions performed at the temperatures above the melting point of the highest-melting TAG component in the mixture results in a complete randomization of fatty acids among all available TAGs. If, however, the interesterification is carried out at temperatures below the melting point of the highest-melting TAG component (usually a trisaturated TAG), the end result will be a mixture enriched with the component of highest melting point. As the trisaturated TAG is produced, it will crystallize and separate out from solution. This will push the equilibrium of the reaction towards increased production of the trisaturated TAG. In this respect, directed interesterification can be thought of as a combination of interesterification and fractionation.

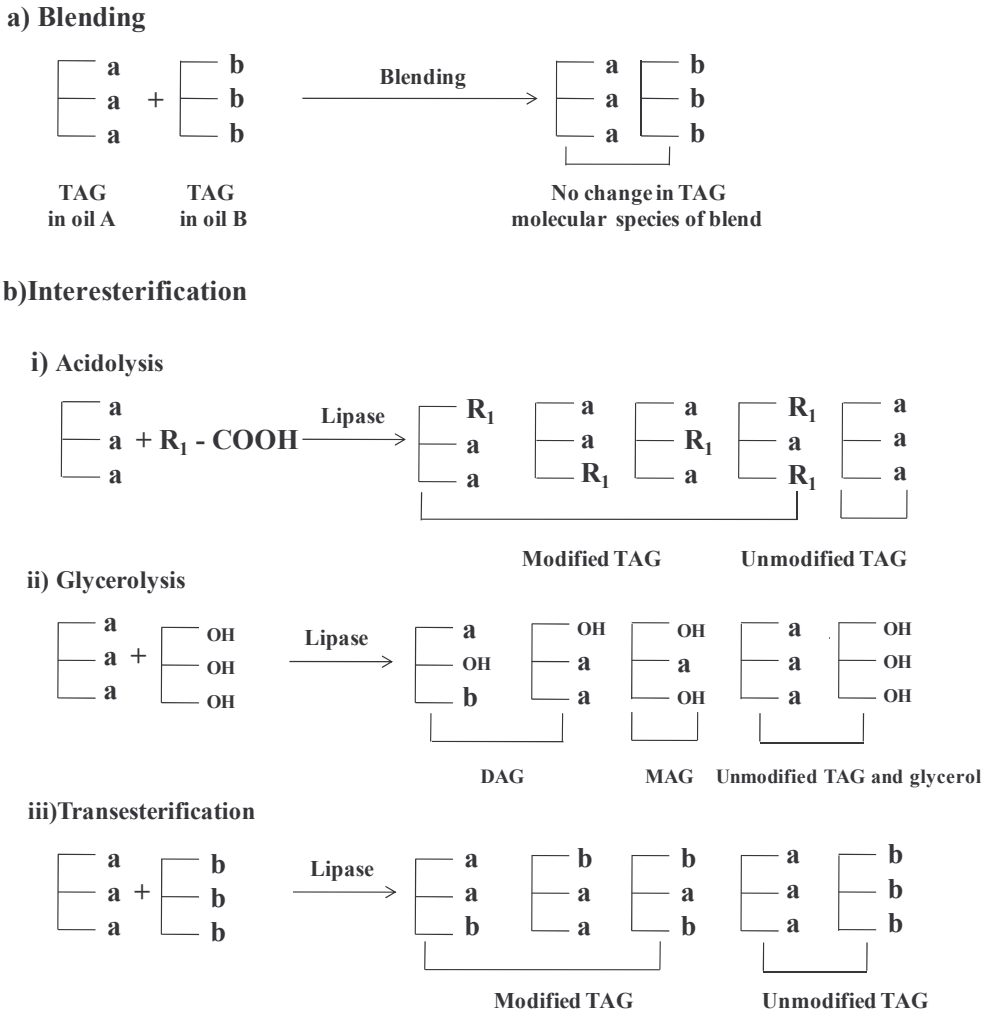


Figure 8. Modification of oils. TAG- triacylglycerol, DAG- diacylglycerol, MAG- monoacylglycerols.

The most commonly used catalyst for chemical interesterification is sodium methoxide, however other bases, acids and metals can also be used. Sodium alkylate catalysts are easy to use, inexpensive, active at relatively low temperature (50-90 ºC) and are required in small amounts for catalysis. They are extremely sensitive to moisture because water reacts with alkylate to produce the corresponding alcohol, which completely inactivate the catalyst. Free fatty acids and peroxides also impair catalyst performance and their level should be maintained as low as possible preferably <0.05% (w/w).

Chemical interesterification has many industrial applications for the production of margarines, shortenings, and confectionaries (Petrauskaite *et al*., 1998). Newer applications of chemical interesterification include the production of reduced calorie fat substitutes such as Salatrim and Olestra. Salatrim consists of chemically interesterified mixtures of short chain and long chain fatty acid triacylglycerols (Smith *et al*., 1994). Chemically catalyzed interesterification is not selective. Therefore it is sometimes called as randomization process.

Drawbacks of chemical interesterification:

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.

Enzymatic interesterification

Lipases are enzymes; naturally function to catalyze the hydrolysis of TAG. If the water level is reduced it continues to catalyze reactions and at a certain level interesterification begins to dominate over hydrolysis (Quinlan and Moore, 1993).

Interest in enzyme mediated interesterification is increasing from a nutritional and functional stand point since it can be used to produce margarines with no trans fatty acids, synthesize cocoa butter substitutes and improve the nutritional quality of some oils and fats. Enzymatic reactions are more specific, require milder reaction conditions and produce less waste. Also, when immobilized, enzymes can be reused, thereby making them economically beneficial.

Lipase catalyzed interesterification offers possibilities for the transformation of fats and oils beyond those possible using chemical interesterification. Lipase catalyzed interesterification can be random or specific depending on the source of lipase. Examples of random lipases include those from *Geotichum candidum*, *Staphylococcus aureus*, *Candida rugosa*. Specific lipases include pancreatic lipase, *Rhizomucor miehei* lipase (1>3>>2; S>M,L), *Aspergillus niger* lipase (1,3>>2; S,M,L), *Pseudomonas fluorescence* lipase (1,3>2; M,L>S). Some lipases show fatty

acid specificity. e.g. *G. candidum* lipase display specificity towards cis-9 unsaturated fatty acids (Lee and Akoh, 1998; Xu, 2000).

Mechanism of lipase mediated esterification reaction

Lipases operate at oil-water interfaces. In the 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).

Finally, the ester is released and the enzyme reverts back its original state (Figure 9).

Advantages of enzymatic interesterification

Enzymatic interesterification has many advantages such as milder processing conditions and the regio and stereo specificity. One can get products with defined, predictable structures with desired chemical composition (Gunstone, 1989). Enzyme mediated reactions produce products that are more easily purified, generates less waste, and thus make it eco-friendly. Enzymes have higher turnover numbers and are well suited for the production of chiral compounds.

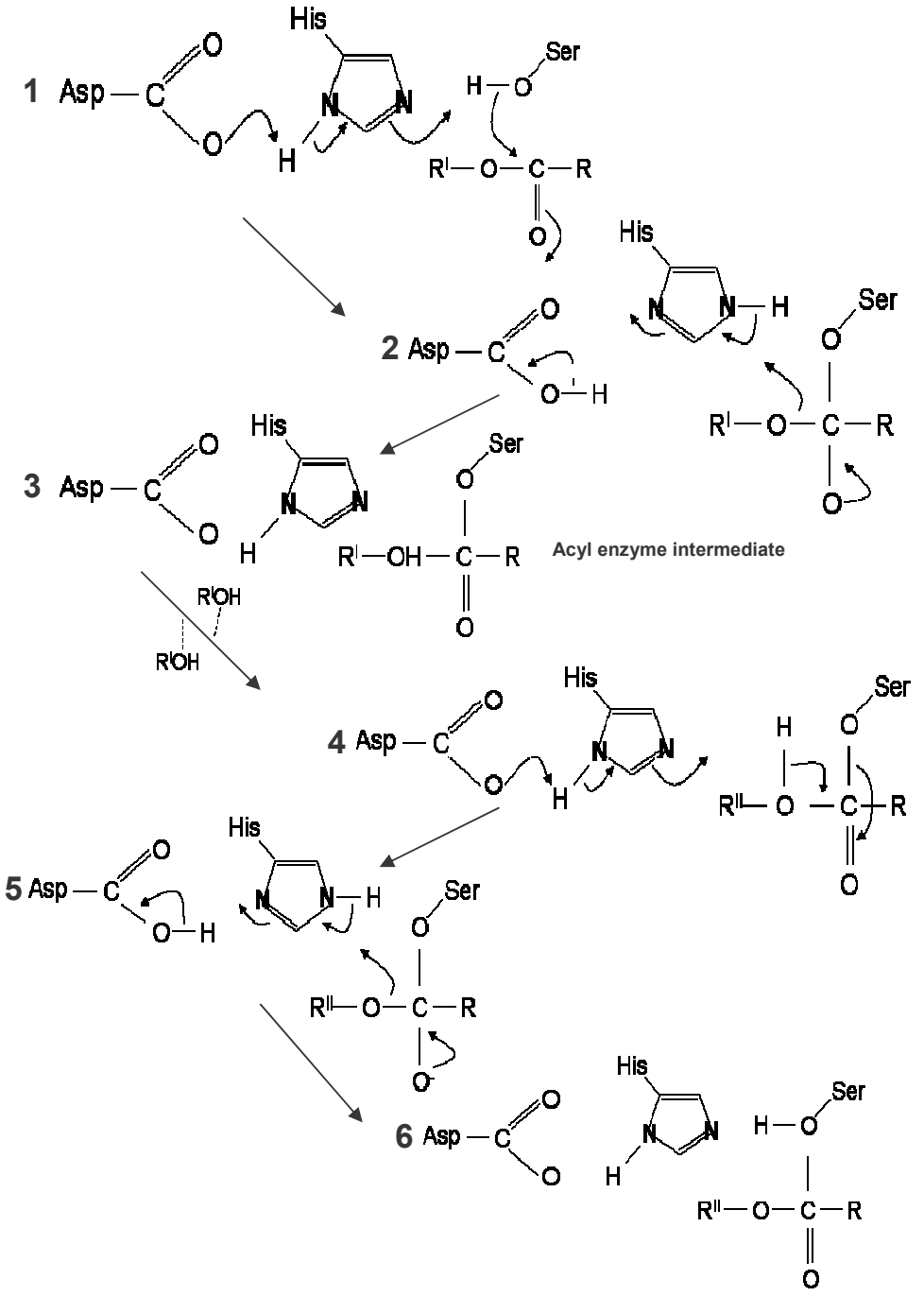


Figure 9. Mode of action of lipases (catalytic mechanism for lipase-mediated enzymatic interesterification). (Source: Marangoni and Rousseau, 1995)

Structured lipids

The industrial applicability of any fat is limited by the nonrandom distribution of fatty acids, which imparts a given set of physical, chemical and functional properties. Natural fats lack the variability desired in the food industry. Technological developments in oil modification methods and increased interest in products beneficial to the health have lead to the production of structured lipids. Structured lipids (SL) can be defined as triacylglycerols restructured or modified to change the fatty acid composition and/or their positional distribution in glycerol molecules by chemical or enzymatic processes (Lee and Akoh, 1998). SL may provide the most effective means of delivering desired fatty acids for nutritive or therapeutic purposes, targeting specific diseases and metabolic conditions (Akoh, 1995). SL can also be synthesized to improve or change the physical and/or chemical characteristics of TAG such as melting point, solid fat contents, iodine value, saponification number. The concept of SL as a new type of lipids for nutritional and medical purposes was introduced by Babayan (1987). An increased understanding of the nutritional properties of lipids, in particular the metabolic effects associated with consumption of TAG with specific fatty acid composition and positional distributions has lead to the development of novel lipid modification technologies for the improvement of the nutritional properties of fats and oils and for medical and food applications. A simple physical mixture of oils results in retention of the original absorption rate of the individual TAG, but SL may lead to different hydrolysis and absorption rates (Tso *et al*., 2001).

Structured lipids have been developed for both medical and food applications. Numerous nutritional products are available for medical applications for patients with HIV/AIDS and other chronic health conditions such as impaired gastrointestinal function, liver disease, congestive heart failure, those recovering from surgery, and for infants with food allergies or digestion problem (Haumann, 1997). Impact, a randomized structured lipid, product of Novartis Nutrition (Minneapolis, Minnesota) is used for patients who have suffered major trauma or surgery, sepsis, or cancer. It is an interesterified product of high lauric acid oil and high linoleic acid oil. Stepan Co. offers structured lipids containing n-3 fatty acids for manufacturers to incorporate in nutritional and medical beverages and in snack bars. Neobee SL 220, a nutritional

product, incorporates both n-6 and n-3 fatty acids as well as small amount of medium chain fatty acids. Abitec Corp. (Columbus, Ohio) has produced structured lipids containing docosahexaenoic and eicosapentaenoic acids, to provide optimum n-6 to n-3 ratio (Haumann, 1997).

A few chemically structured triacylglycerols with low caloric value are commercially available. Salatrim is a family of reduced-calorie fat substitute composed of long and short chain fatty acids produced by the interesterification of hydrogenated vegetable oil with triacylglycerol composed of short chain fatty acids (triacetin and/ or tripropionin and/ or tributyrin). The reduced calories are accounted for by the lower caloric value of the short chain fatty acids and limited absorption of long chain fatty acid (Finely *et al*., 1994b). The caloric content of various salatrim preparations is about 5 kcal/g (Vs. 9 kcal/g for a regular TAG) (Finely *et al*., 1994a). Benefat, a stearic acid (C2:0-C4:0 and C18:0) salatrim is launched in the U.S. market in 1995, is used in a wide range of chocolate confectionary products, including coatings and centers as well as in the baked goods. 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 (Haumann, 1997).

Betapol, a product of enzymatic interesterification is a targeted structured lipid (Unilever, London), closely mimic the fatty acid distribution in human milk fat. Palmitic acid is the most abundant saturated fatty acid in human milk in which about 70% of it is positioned at the sn-2 position of the glycerol back bone. Using 1,3-specific lipase and reacting tripalmitin with unsaturated fatty acids give a triacylglycerol with up to 60% of palmitic acid at the sn-2 position. The resulting structured lipid, betapol, provides improved fat and calcium absorption (Haumann, 1997). Therefore modified oils which are often referred to as designer oils may fulfill needs of food technologists, food industries, nutritional purpose and for therapeutic uses.

Aim and scope of the present investigation

In India, dietary habits especially the fat consumption vary according to geographical region and availability of fat. Each oil used in our culinary purposes is unique in its fatty acid composition and in its minor components. Coconut oil (CNO) is the predominant dietary fat in Kerala and in coastal regions of Karnataka in south India. CNO is rich in lauric acid. Sesame oil is used in southern India. The newer source of edible oil like RBO is also being used in India. These oils contain more unsaturated fatty acids.

To get optimal benefits from oils, Indian Council of Medical Research recommended that the saturated fatty acid (SFA):monounsaturated fatty acid (MUFA):polyunsaturated fatty acid (PUFA) in the oils be in equal proportion and the fat in that diet may provide 20-25% of energy. These guidelines are in tune with the ones promoted by the American Heart Association. However there is no single oil in nature which has a balanced proportion of fatty acids as recommended in these guidelines. Therefore blending of saturated fats with unsaturated oils was taken up as an alternative approach to get an oil with balanced fatty acids.

Blended oils represent a physical mixture of two or more oils. Even though one can balance the fatty acid composition by blending of oils it may not always result in oils with desired physicochemical or nutritional properties. This is because the structure and physical characteristics of TAG molecular species of individual oils will be retained in blended oil. This may be altered by rearranging the fatty acids in the triacylglycerol molecules by enzymatic or chemical interesterification mediated process. Hence, interesterification of oils and fats has attracted the attention of technologists in recent times. Lipase catalyzed interesterification has become a useful tool for the production of designer lipids to improve the physicochemical and functional properties. Rearrangement of fatty acid on the glycerol moiety catalyzed by lipases may change the physical and nutritional properties of the oil. The new triacylglycerol molecules formed in the interesterified oil may be absorbed in a manner which is different than that observed in the native oil or in the blended oils. The lipase mediated reaction also provides specificity with respect to positioning of fatty acids in TAG molecules.

The present investigation is envisaged to prepare oils with balanced fatty acid composition. Such oils are also enriched with nutraceuticals provided by individual oils used for blending. Coconut oil, a saturated fat is blended with either RBO or SESO to get an oil with a fatty acid composition of approximately SFA:MUFA:PUFA in the ratio of 1:1:1. The blended oil is further treated with lipase from *Rhizomucor miehei* to rearrange the fatty acids in the TAG. In this study, the main focus would be towards the physicochemical characterization of blended and interesterified oils, nutritional evaluation with reference to lipids and the mechanism involved in the nutritional effect of blended and interesterified oils.

The main findings in the present investigation are presented as:

- Protocols for the preparation of blended and interesterified oils. Evaluation of physicochemical properties.
- Effect of feeding blended and interesterified oils on serum and tissue lipids in rats.
- Influence of blended and interesterified oils on the transcriptional profiling of genes involved in cholesterol homeostasis.
- Effect of blended and interesterified oils on platelet aggregation and eicosanoid production in rats.
- Effect of blended and interesterified oils on antioxidant enzymes in liver and erythrocyte membrane bound enzymes.

This investigation ultimately gave us an insight on the mechanisms involved in the cholesterol lowering property exhibited by interesterified and blended oil both of which contained similar fatty acid composition. This will enable us to promote oils with balanced fatty acid compositions to reduce risk factors for CVD in Indian population.

MATERIALS

Coconut oil and sesame oil were purchased from a local super market. Physically refined rice bran oil was provided by Foods, Fats & Fertilizers Ltd. Tadepalligudem, A.P., India. Standards α, β, γ, δ tocopherols and tocotrienols were given as gift by Dr. Kalyanasundram, MPOB, Malaysia. Lipozyme IM RM was gift from NOVO Nordisk Bioindustrial Inc., (Danbury, CT, USA). Trilinolein, triolein, tripalmitin, boron trifluoride in methanol, dipalmitoyl phosphatidylcholine, trizma base, zeolite, 5,5'-dithio bis (2-nitrobenzoic acid), collagen, adenosine diphosphate (disodium salt ADP), bovine serum albumin, heparin, thiobarbituric acid, glutathione, glutathione reductase, xanthine, xanthine oxidase, cytochrome C, NADPH, thiobarbituric acid, hydrogen peroxide, cholesterol, fatty acid standards, TRI reagent and deoxy nucleoside triphosphate (dNTP) mixtures were purchased from Sigma Chemical Co., St. Louis, Mo USA. 6-Keto-prostaglandin $F_{1\alpha}$ ($PGF_{1\alpha}$) and thromboxane (TX) B_2 were purchased from Cayman Chemical Co., Ann Arbor, MI, USA. Agarose, choline chloride, DL-methionine, magnesium oxide, manganese carbonate, ferric citrate, calcium phosphate, potassium citrate monohydrate and vitamins were purchased from Himedia Laboratories Mumbai, India. Manganese chloride, ascorbic acid, trichloroacetic acid, EDTA and sodium metaperiodate were obtained from Sisco Research Laboratary Mumbai, India. Ferric chloride, ammonium thiocyanate, bipyridyl pyridine and pyrrogallol were purchased from Qualigens, Mumbai, India. Sodium selinite, and potassium sulphate were from Loba Chemie, Mumbai, India. Casein was purchased from Nimesh Corporation Mumbai, India. All solvents used were of analytical grade and distilled before use.

METHODS

Preparation of blended oils

Blended oils were prepared by mixing coconut oil (CNO) with approximate amount of rice bran oil (RBO) or sesame oil (SESO) to get SFA:MUFA:PUFA in approximately equal proportion (1:1:1). The blending was carried out by mixing predetermined amounts of oils with stirring for 1h at 40 °C on a magnetic stirrer after flushing with nitrogen. The mixing efficiency was monitored by estimating fatty acid composition (by GC) of the blended oils periodically and comparing it with theoretical value.

Preparation of interesterified oils

Interesterified oils were prepared from blended oils using lipase (lipozyme IM RM) *Rhizomucor miehei* at 1% (w/w) (specific activity of lipase 6.2±0.2 μmoles/mg protein). Incubations were carried out in a water bath (BS-06/11/21/31, Jeio Tech Co. Ltd. Seoul-city, South Korea) with a shaker at a speed of 160 rpm for 72 h at 37 °C. After interesterification reaction the oil sample was decanted and the enzyme washed with hexane and dried for reuse. The quality of oils was checked after interesterification. There was no significant change in the peroxide value of interesterified oils compared to the blends. Free fatty acid concentration was increased marginally in interesterified oils. There was no significant change in the nutraceuticals contents and fatty acid composition of oils compared to its blended oil.

Peroxide value of oils

Peroxide value of the sample was determined in terms of milliequivalents (meq) of peroxide per Kg of sample that oxidize potassium iodide under the conditions of test (AOCS official methods, 1997a). Test sample was dissolved in acetic acid:chloroform mixture (3:2, v/v) followed by the addition of saturated potassium iodide and kept closed for a minute and the reaction was terminated by the addition of distilled water. Blue colour appeared after the addition of starch indicator and was titrated against sodium thiosulphate.

Free fatty acid content of oils

Free fatty acid content of oils was determined by following the method described in official methods and recommended practices (AOCS official methods, 1997b). Neutralized alcohol was added to the oil sample to bring out the free fatty acids, heated to boiling and titrated against sodium hydroxide after adding phenolphthalein indicator.

Fatty acid composition of oils

Fatty acid composition of native, blended and interesterified oils were analyzed by gas chromatography (Fison GC fitted with FID) as methyl esters (Morrison and Smith, 1964) prepared by treating oils with 0.5 M potassium hydroxide and methylating with 40% boron trifluoride in methanol. The fatty acid methyl esters

were separated using fused silica capillary column 25 m×0.25 mm (Parma bond FFAP-DF-0.25: Machery-Negal Gm BH co. Duren, Germany). The operating conditions were: initial column temperature 120 °C, raised by 15 °C per min to 220 °C, injection temperature 230 °C and detector temperature 240 °C. Nitrogen was used as the carrier gas. Individual fatty acids were identified by comparing with the retention times of standards (Nuchek Prep, Elysin, Mn USA) and were quantitated by an online Chromatopac CR-6A integrator.

Oryzanol and lignan contents in the oil

Oryzanol content in RBO and RBO containing blended and interesterified oils was estimated spectrophotometrically by dissolving the sample in hexane and measured the absorbance at 340 nm using a uv-visible spectrophotometer (Shimadzu UV-1601). Oryzanol was quantitated using a molar extinction coefficient of 358.9 (Seetharamaiah and Prabhakar, 1986). Sesame lignans sesamin, sesamolin and sesamol in SESO and its blends were analyzed by HPLC (Shimadzu Corp., Tokyo, Japan). Reverse phase C18 column (Phenomenex, Torrance, California, USA., 250 × 4.60 mm, 5 μ) was used for the separation of lignans which were eluted using 70% methanol at a flow rate of 1 mL/min and monitored at 295 nm using a SPD-20A uv-visible detector (Amarowicz *et al*., 2001).

Tocopherol and tocotrienol contents in the oil

Tocopherol and tocotrienol content in the oil were analyzed by HPLC (Shimadzu Corp., Tokyo, Japan) using a RP-C18 column (Phenomenex, Torrance, California, USA., 250 × 4.60 mm, 5μ). The mobile phase used to separate tocopherol and tocotrienol 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 was programmed as described by Chen and Bergman (2005). The isomers of tocopherols and tocotrienols were monitored at 298 nm using SPD-20A uv-visible detector.

Calibration curves were generated using pure standards of α, β, γ and δ-tocopherols and tocotrienols. The tocopherols could be detected at a level of 25 ng and tocotrienols at a minimum concentration of 36 ng.

Triacylglycerol analysis by HPLC

TAG molecular species of native, blended and interesterified oils were monitored according to the method of Dong and Dicesare (1983). Individual TAG molecules were separated on a RP-C18 column (Phenomenex, Torrance, California, USA., 250 mm × 4.5 mm) of 5 μm particle connected to a high performance liquid chromatography (Shimadzu Corp., Tokyo, Japan), equipped with a refractive index detector (RID-10A). Acetone/acetonitrile (65:35, v/v) was used for mobile phase at a flow rate of 1.0 mL/min. The samples were solubilized in acetone.

The elution sequence of TAG was predicted based on their theoretical carbon number (TCN). Fatty acid composition of RBO and SESO showed that more than 90% of the oil is composed of the three fatty acids- palmitic (P), oleic (O) and linoleic (L). Therefore, the TCN of all combinations of P, O and L were calculated according to the equation TCN= ECN- (0.7) L – (0.6) O, where ECN=CN-2n. ECN is the equivalent carbon number, CN is the number of carbons in the three fatty acids of the triacylglycerol, n is the number of double bonds, L is the number of linoleic acids and O is the number of oleic acids (El-Hamdy and Perkins, 1981).

TAG molecules were identified by correlating the TCN and retention time of TAG standards. Three standards LLL, OOO and PPP were used as references for retention time to correlate the TCN values with the TAG retention times (Bland *et al*., 1991).

To positively characterize the composition of TAG molecule separated by HPLC, the individual fractions were collected manually from several runs to get approximately 200 μg of each peak. The fatty acids of TAG molecule in the eluted fraction was esterified to fatty acid methyl esters (FAME) and injected to GC. The fatty acid methyl esters were identified based on the retention time of FAME standards.

Analysis of thermal profile

A Metler (Zurich, Switzerland) differential scanning calorimeter (DSC-30) was used for analyzing the thermal characteristics of the samples. The heat flow of the instrument was calibrated using indium. The PT-100 sensor was calibrated using

indium, zinc and lead. To ensure homogeneity and to destroy all crystal nuclei, the samples were heated to 80 °C. A molten sample of 10 mg was accurately weighed in a standard aluminum crucible and the cover crimped in place. An empty aluminum crucible with pierced lid was used as a reference. For melting characteristics, the samples were stabilized according to the IUPAC method (IUPAC Standard Methods, 1987). The samples were kept at 0 °C for 90 min, at 20 °C for 40 h followed by 0 °C for 90 min prior to introduction into the DSC cell. Thermograms were recorded by heating the sample at a rate of 2 °C /min from –50 °C to 50 °C. The peak temperatures, heat of fusion values (ΔH) and percentage of liquid at various temperatures were recorded directly using a TC-10A data processor and STARe program. The solid fat content (SFC) was calculated from the percentage of liquid, and the melting profiles were drawn by plotting SFC against temperature.

For crystallization studies, the samples are accurately weighed in to standard aluminum crucibles as described above. The samples are kept at 60 °C for 10 min for destroying all crystal nuclei, cooled to -20 °C at the rate of 5 °C/min. the cooling profiles (crystallization) are recorded. The onset, peak temperatures and enthalpy of each peak were calculated using STARe software.

Animal studies

Experimental animals

Male Wistar rats [OUBT-Wistar, IND-cft (2c)] (*Rattus norvegicus*) weighing 45±2 g were grouped (4 rats in each group) by random distribution and housed in individual cages, under a 12 h light/dark cycle, in an approved animal house facility at the Central Food Research Institute Mysore, India. Animals were given fresh diet daily, and left over diets were weighed and discarded. The gain in body weight of animals was monitored at regular intervals. The animals had free access to food and water throughout the study. Each group of rats were fed for a total of 60days, AIN-76 diet (American Institute of Nutrition report, 1977) containing 10% fat from CNO, RBO, SESO, CNO+RBO blended (B), CNO+SESO(B) or CNO+RBO interesterified (I), CNO+SESO(I), sucrose 60%, casein 20%, cellulose 5%, mineral mix 3.5%, vitamin mix 1.0%, choline chloride 0.2%, and methionine 0.3% (Table 5). After 60

days of feeding, rats were fasted overnight and sacrificed under diethyl ether anesthesia. Blood was drawn by cardiac puncture and serum was separated by centrifuging at 700 × g for 20 min at 4 °C. The liver was removed, rinsed with ice-cold saline, blotted, weighed and stored at –20 °C until analyzed. All the experimental protocol was approved by institutional animal ethical committee.

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

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

Analysis of lipid parameters

Serum lipid extraction

Serum lipids were extracted according to Bligh and Dyer, (1959). Methanol and chloroform was added to the serum separately in appropriate amounts (2:2:0.8, v/v/v), mixed well and the extract was filtered using Whatman no. 1 filter paper. The

filtrate was allowed to settle and the lower chloroform layer was separated and used for further analysis.

Liver lipid extraction

Liver lipid was extracted by the method of Folch *et al*. (1957). One gram of liver was homogenized with 1.0 mL of 0.74% potassium chloride, 20 mL of chloroform and methanol (2:1, v/v) was added and homogenized for 1 min. The mixture was left over night and filtered through a Whatman no. 1 filter paper. To the filtrate 3 mL of 0.74% potassium chloride was added and mixed well. The solution was allowed to stand at room temperature. The upper aqueous layer was removed carefully and then lower phase was washed with 3 mL of chloroform: methanol: water (3:48:47, v/v/v) mixture. The chloroform layer was used for lipids analysis.

Total cholesterol estimation

The total cholesterol in the serum and liver were quantified 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 1.5 mL of ferric chloride-acetic acid reagent (504 mg/L). After mixing thoroughly, it was left at room temperature for 15 min. Concentrated sulphuric acid (1 mL) was added, mixed and left at room temperature in the dark for 45 min and the color intensity was measured in the spectrophotometer (Shimadzu 1601 model) at 540 nm. The cholesterol concentration in the sample was quantitated from a standard curve generated with AnalaR cholesterol (30-150 μg).

HDL cholesterol estimation

HDL- cholesterol was estimated after precipitating LDL- cholesterol from serum with a heparin (5000 units/mL)–manganese chloride reagent (2 M) (Warnick and Albers, 1978). The solution was mixed well and kept at 4 °C over night. This was then centrifuged at 3500 × g for 20 min. HDL cholesterol was measured as described earlier after extracting the supernatant with 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 saline and

cholesterol was extracted with acetone alcohol (1:1, v/v) and was estimated as described earlier.

Triacylglycerol estimation

Triacylglycerols were estimated by the method of Fletcher, (1968) using tripalmitin as the reference standard (30-300 μg). An aliquot from chloroform was evaporated and redissolved in isopropanol. Triacylglycerol purifier was added, mixed and centrifuged and the supernatant was saponified with 0.6 mL of 5% potassium hydroxide in isopropanol:water (2:3, v/v) at 65 °C for 15 min. One mL of sodium metaperiodate prepared from the stock solution of 0.025 M in 1 N acetic acid (sodium meta periodate 12 mL and 20 mL of isopropanol and made up to 100 mL with 1 N acetic acid) was added, mixed and 0.5 mL of acetyl acetone was added, and incubated at 50 °C for 30 min. the colour intensity was measured at 405 nm in Shimadzu 1601 spectrophotometer.

Phospholipid estimation

Phospholipids were estimated by ferrous ammonium thiocyanate method (Stewart, 1980) using dipalmitoylphosphatidyl choline (15-100 μg) as the reference standard. The lipid extract in chloroform was evaporated and redissolved in 2 mL of chloroform. Two mL of ferrous ammonium thiocyanate was added and vortexed for 1 min. Following the phase separation, absorbance of chloroform phase was measured at 488 nm in Shimadzu 1601 spectrophotometer.

Fatty acid composition of dietary lipids, serum, liver and tissues were analyzed as methyl esters by GC (Morrison and Smith, 1964) as described earlier.

Assay of HMG-CoA reductase activity

Preparation of rat liver microsomes

Liver microsomes were isolated as described by Shapiro and Rodwell (1971). One gram of liver was homogenized with 4 mL of 0.1 M triethanolamine HCl containing 0.02 M EDTA and 2.0 mM dithiothreitol, pH 7.4. The homogenate was centrifuged (Eppendorf Centrifuge 5415 R, Hamburg, Germany) at 12000 × g for 10 min to remove cell debris and nuclei. The supernatant was carefully removed and

recentrifuged at 12000 × g to ensure the removal of mitochondria. The supernatant was further centrifuged at 60000 × g in Beckman L7-65 R ultracentrifuge for 60 min at 4 ºC. The microsomal pellet was suspended in 0.1 M triethanolamine buffer, pH 7.4, containing 0.02 M EDTA and 10 mM dithiothreitol and allowed to stand for 60 min at 4 ºC and then centrifuged at 60000 × g for 45 min at 4 ºC. The washed microsomes were suspended in 0.1 M triethanolamine buffer containing 0.02 M EDTA and 2 mM dithiothreitol, pH 7.4. Protein concentration was determined using Folin Ciocalteu reagent with bovine serum albumin as reference standard (Lowry *et al*., 1951).

Assay procedure for HMG-CoA reductase

HMG-CoA reductase activity in liver microsome was quantitated by measuring the monothiols with 5,5'-dithiobis (2-nitrobenzoic acid) (Hulcher and Oleson, 1973). Microsomal protein (0.5-1 mg), 0.2 µmoles DTT, 150 nmoles of HMG-CoA and 2 µmoles of NADPH were added to 0.8 mL of 0.1 M triethanolamine buffer containing 0.02 M EDTA, pH 7.4. The reaction mixture was incubated at 37 ºC for 30 min. Sodium arsenite solution (20 µL of 10 mM) was added to facilitate removal of soluble protein and after 1 min. the reaction was terminated by the addition of 0.1 mL of 2.0 M citrate buffer pH 3.5 containing 3% sodium tungstate to precipitate microsomal protein. The mixture was once again incubated at 37 ºC for 10 min and was centrifuged at 10000 × g for 15 min to remove protein. Just before assay 1 mL of supernatant was mixed with 0.2 mL of Tris HCl pH 10.6 and 0.1 mL of Tris buffer pH 8.0. Addition of 50 µL of 0.4 M sodium arsenite facilitated the dithiol-arsenite complex formation. The concentration of monothiol was determined by reacting 5, 5'-dithiobis (2-nitrobenzoic acid) (20 µL of 3 mM in 0.1 M triethanolamine -0.2 M EDTA buffer pH 7.4) with the reaction mixture and measuring the absorbance at 412 nm for 4 min. The absorbance due to monothiols was determined by extrapolating the linear portion of the curve after the addition of DTNB. The difference in absorbance between the complete reaction and that of all the components except NADPH represented the activity due to HMG-CoA reductase.

Gene expression studies

Glassware: All cleaned glassware were individually rinsed with diethyl pyrocarbonate (DEPC) water. Residual DEPC was removed by autoclaving for 15 min at 121 °C, 15 lb pressure.

*Nondisposable Plastic war*e: All plasticware were rinsed with DEPC-water.

Preparation of reagents: All laboratory chemical reagents used were of molecular biology or AR grade or higher grade wherever possible. All stock solutions were sterilized by autoclaving at 121 °C for 15 min at 15 lb pressure. Reagents and buffers which are labile for autoclaving were sterilized by filter sterilization using Millipore disposable sterile filters.

RNase free water: MilliQ water was treated with 0.1 % DEPC and stirred vigorously overnight to bring DEPC into solution. This solution was autoclaved for 15 min at 121 °C, 15 lb pressure to remove traces of DEPC. All solutions were prepared in this RNase free water and then autoclaved.

The following reagents were prepared according to Sambrook and Russel (2001)

a. *50× TAE:* Tris-base (242 g), 57.1 mL glacial acetic acid, 100 mL 0.5 M EDTA dissolved in 1 L distilled water.

b. *MOPS buffer (10×):* MOPS (3-(N-morpholino) propane sulphonic acid) 200 mM, 50 mM sodium acetate, 10 mM EDTA were dissolved in RNase free water, pH adjusted to 7.0 with 2 N NaOH and filter sterilized and stored in dark brown bottle at room temperature.

RNA extraction

Frozen liver (100 mg) was pulverized in liquid nitrogen and homogenized using TRI reagent (Sigma-Aldrich Chemical Pvt. Ltd., Bangalore). (sample volume should not exceed more than 10% of the volume of TRI reagent). After 5 min. incubation at 15-30 ºC, 0.2 mL bromochloropropane was added per mL of TRI reagent, mixed well, incubated at 15-30 ºC for 2-3 min., centrifuged at 12,000 × g for 15 min at 4 ºC. The upper aqueous phase was transferred to a fresh tube to which 0.5 mL isopropyl alcohol was added per mL of TRI reagent, incubated at 15-30 ºC for 10

min. The precipitate was collected by centrifuging at 12,000 × g for 10 min. at 4 ºC. The pellet was washed with 70 % ethanol (1 mL/mL TRI reagent × 2 times) air dried, and then resuspended in RNase free water. RNA was reprecipitated by adding 1/10 volume of 3 M sodium acetate pH 5.2 and 2.5 volumes of 95 % ethanol and stored at –80 °C for long-term storage.

Quantification of DNA/RNA

The yield and purity of RNA was determined using a uv-vis spectrophotometer (Shimadzu 1601), where 1 AU (A_{260}) equals 40 µg of single stranded RNA/mL. The purity was estimated from the relative absorbance at 260 and 280 nm. The acceptable ratio of A_{260}/A_{280} was 1.8-2.1. The integrity of the RNA was assessed by using 1.5% agarose gel electrophoresis and visualized under UV.

Denaturing agarose gel electrophoresis of RNA

Denaturing agarose gel electrophoresis was used to evaluate the isolated RNA.

Preparation of gel

The required amount of agarose was heated in 85 mL water until completely dissolved and then cooled to 60 °C. Then 10 mL of 10 × MOPS running buffer and 5.4 mL of 37% formaldehyde (12.3 M) was added. The gel was assembled; 1 × MOPS running buffer was added to cover the gel. The purified RNA was mixed with 1.0 µL ethidium bromide (10 mg/mL), 4.0 µL formaldehyde and 10 µL formamide and incubated at 85 °C for 10 min and chilled on ice. To this 2 µL of 10 × sample buffer was added (sample buffer 50% glycerol diluted in RNase free water containing 10 mM EDTA pH 8.0, 0.25% (w/v), bromophenol blue and 0.25% (w/v), xylene cyanol FF) and loaded. Electrophoresis was carried out in 1 × MOPS buffer at 100V.

DNA agarose gel electrophoresis

The amplification product was evaluated by agarose gel electrophoresis. Depending on the percentage of the gel, agarose was weighed and added to required volume of 1× (Tris acetate ethylene diamine tetra acetic acid) TAE, heated until completely dissolved and cooled to 55 °C. Ethidium bromide was added to a final concentration of 0.5 µg/mL, mixed well and poured into the gel trough and allowed to solidify.

Samples were mixed with sample buffer and loaded in the well. The gel was electrophoresed for 45-60 min in 1 × TAE buffer at 100V. DNA was visualized under a uv-transilluminator.

cDNA synthesis

Total RNA (10 µg) was used as a template for RT-PCR to generate cDNA using a (High Capacity cDNA Archive Kit) following the manufacturer's protocol (Applied Biosystems, Foster City, CA).

Primer design

Gene specific primers were designed using Primer Express software package version 1.5a (Applied Biosystems). The gene sequences used were retrieved from Gene Bank (http://www.ncbi.nlm.nih.gov/pubmed/), (http://blast.ncbi.nlm.nih.gov/Blast.cgi). These primers synthesized by Sigma-Genosys (Sigma-Aldrich Chemical Pvt. Ltd., Bangalore) were diluted to a final concentration of 1 nmole/µL with nuclease free water and stored at -20 ºC.

The parameters considered during primer design were; The primer length was 18-30 nucleotides and a G/C content of 30-50 %. T_m was calculated using the formula $T_m = 2\ ºC \times (A+T) + 4\ ºC \times (G+C)$. Optimal annealing temperature was calculated as 5 ºC below the estimated melting temperature.

A semiquantitative RNA quantification

A semiquantitative RT-PCR method adapted from that of Powell and Kroon (1992) was used to approximate the HMG-CoA reductase, cholesterol 7-α-hydroxylase (CYP7A1), LDL receptor, sterol regulatory element binding protein (SREBP) -2 and beta actin mRNA abundance. PCR was performed in a final volume of 25 µL containing 1 µL of the reverse transcribed first strand cDNA, 10 pmoles each forward (F) and reverse (R) primer, 200 µM dNTPs, 1× thermopol buffer and 2.0 units of *Taq* polymerase (New England Biolabs). The thermal cycling programme used was: initial denaturation at 94 ºC for 5 min, 30 s of annealing at temperatures and 60 s extension at 72 ºC for 1 min for 35 cycles and completed by a terminal extension at 72 ºC for 7 min. The PCR products were analyzed by electrophoresis on 1.5% agarose gels and viewed under UV light. The gels were scanned in a BioRad Gel

documentation system and intensities of the bands quantified using the software Quantity One, version 4.6.3 (BioRad, Munich, Germany).

Quantitative real time polymerase chain reaction

Four microliters containing 50 ng of cDNA was transferred to a 96 well PCR plates, to which 5 µL of a cocktail of SYBR Green PCR Core reagents (PE Biosystems, Warrington, UK), forward and reverse primers (1 µL of 10 pmoles) were applied and PCR carried out in an ABI PRISM 7700 sequence detection system (Applied Biosystems, Foster City, CA) with 1 cycle of 95 ºC for 30 s and 60 ºC for 30 s each. The reaction mixture with no cDNA was used as the negative control to confirm the absence of primer dimerisation. The cycle threshold was determined by sequence detection system software version 1.7a. Beta actin was used as an internal control. The change in the gene expression levels of HMG-CoA reductase, LDL receptor, CYP7A1 and SREBP-2 were determined by normalizing the mRNA levels of the gene of interest to the mRNA levels of the house keeping gene beta actin. Nucleotide sequence for the various target genes were retrieved from Gene Bank database. The Real Time PCR primers were designed by Primer Express (Applied Biosystems, Foster City, CA) and synthesized by Sigma-Genosys Bangalore India. Qualitative PCR was performed to confirm formation of a single product in each reaction.

Studies on platelet aggregation

Preparation of platelet rich plasma and platelet poor plasma

Blood was collected in heparin as anticoagulant (50 IU/mL, blood) by cardiac puncture from anesthetized rats. It was centrifuged at 150 *g* for 20 min at room temperature to separate platelets from erythrocytes and leucocytes. The platelet rich plasma (PRP) thus obtained was then centrifuged at 150 *g* for 20 min at room temperature to remove any residual erythrocytes and leukocytes. PRP was then utilized as source of platelets to study aggregation. Platelet poor plasma (PPP) is prepared by centrifugation of anticoagulated blood or PRP at 5000 × *g* for 10 min at 4 °C (Gerrard, 1982). The platelet count was adjusted to 400, 000/µL.

Platelet aggregation

The aggregation experiments were performed within two hours of blood collection. Platelet poor plasma used as the blank. After setting the baseline with 450 µL of PRP and PPP in their respective cuvettes the aggregation of platelets were followed by adding 10 µL of 25 µM ADP or 15 µL of collagen (5mg/10 mL in 0.1 N acetic acid) in a Chronolog Dual Channel platelet aggregometer (Denmark) at 37 °C with constant stirring at 1000 rpm. Aggregation was followed for at least 5 min. The light transmission was recorded on a chart paper. The platelet aggregation was quantitated as the maximum change in light transmittance through PRP expressed as a percentage of the light transmittance through the blank (Niranjan and Krishnakantha, 2000).

Fatty acid composition of platelets

Platelets were isolated and washed as described by Brunauer and Huestis (1993) with slight modification. Platelet rich plasma was obtained as described above. The platelets were separated from plasma by centrifuging at 700 × *g* for 10 min. The cells were washed twice in tyrodes buffer pH 7.4 (NaCl 137 mM, KCl 2.7 mM, $NaHCO_3$ 12 mM, EDTA 1 mM, NaH_2PO_4 0.4 mM, $MgCl_2$ 1 mM, glucose 5.6 mM). The final platelet pellet was suspended in the tyrodes buffer and lipid was extracted as described earlier by Bligh and Dyer method (1959). Fatty acid composition was analyzed as methyl esters as described earlier.

Estimation of malondialdehyde (MDA) in agonist challenged platelets

After aggregation, platelet suspension containing PRP (450 µL), which had been challenged by agonist, were transferred to an eppendorf tube containing 20 µL, 1% butylated hydroxyl toluene in ethanol. To this 0.1 mL of 100% TCA in 3 N HCl was added, mixed well and centrifuged at 12000 × *g* for 20 min. Supernatant was treated with 0.1 mL TBA reagent (0.12 M TBA in 0.26 M Tris-HCl), mixed well and incubated in boiling water bath for 30 min. The colour developed was measured at 532 nm (Maguire and Csona-Khalifah, 1987). The amount of malondialdehyde (MDA) formed was calculated using molar extinction coefficient of 1.56×10^{-5} $M^{-1}cm^{-1}$.

Analysis of serum prostaglandins

6-keto-PGF_1 α and TXB_2 were extracted from serum after acidification with 100 µL of 3.0% formic acid to pH 3.0. The extract was purified on Sep Pak C-18 column and eluted in to ethyl acetate (Waters, Millipore Corp., Milford, MA, USA). The combined ethyl acetate extracts were evaporated under the stream of nitrogen and loaded on a RP-C18 column fitted to (Phenomenex, Torrance, California, USA 250 mm × 4.5 mm) of pore size 5 µm fitted to a SPD-20A HPLC (Shimadzu Corp., Tokyo, Japan). The prostaglandins were eluted with potassium dihydrogen phosphate (0.0174 M pH 3.5): acetonitrile (60:40, v/v) at a flow rate of 1 mL/min and monitored at 196 nm. The prostaglandins were identified and quantified using authentic standards (Rajakrishnan *et al*., 2000)

Estimation of lipid peroxides in liver homogenates by TBARS method

One gram of liver was homogenized in 10 mL of 0.15 M potassium chloride, in a teflon homogenizer. The homogenate was filtered through cheesecloth and used for assay. Liver homogenates (4 mg protein) in 0.15 M potassium chloride, 0.025 M Tris-hydrochloride buffer pH 7.5, 2 mM adenosine diphosphate and 10 µM ferrous sulphate were incubated at 37 °C for 5 min. The reaction was initiated by adding 0.1 mM ascorbic acid and incubated at 37 °C for 30 min. The final volume of the reaction mixture was 1 mL. The reaction was terminated using 2 mL of thiobarbituric acid (0.375% thiobarbituric acid, 15% trichloroacetic acid in 0.2 N hydrochloric acid) containing 10 µM butylated hydroxyl anisole. Samples were heated for 15 min in a boiling water bath. Malondialdehyde (MDA) formed was measured at 535 nm and quantitated using an extinction coefficient of 1.56×10^{-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).

Antioxidant enzyme activities

Superoxide dismutase (SOD) activities in the hepatic tissue homogenates were measured by the inhibition of cytochrome C reduction mediated via superoxide anions generated by the xanthine/xanthine oxidase system and monitored at 550 nm. Activities are expressed as units/mg protein, where one unit of SOD, was defined as the amount required to cause half-maximal inhibition of cytochrome C reduction

(Flohe and Oting, 1984). Catalase activity was determined according to Aebi (1984) by following the decomposition of hydrogen peroxide at 240 nm. 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 (Jensson *et al*., 1985). The enzyme activity is expressed as μmoles of CDNB-GSH conjugates formed per minute 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 μmole/min/mg protein.

Isolation of erythrocytes and erythrocyte ghosts

Erythrocyte ghosts were prepared according to the method of Fairbanks *et al*. (1971). After removing plasma from freshly drawn heparinised blood, blood cells were diluted with saline (1:1, v/v), mixed gently and centrifuged at 700 × *g* for 10 min at 4 ºC. Sedimented cells were washed repeatedly till the supernatant became colorless. The cells (0.5 mL) were lyzed by forcing them through 19.5 mL of phosphate buffer (5 mM, pH 8.0) in a centrifuge cup. The contents were centrifuged at 12000 × *g* for 20 min at 4 ºC to sediment erythrocyte ghosts. Ghosts membranes were washed with phosphate buffer (5 mM, pH 8.0) three to four times till they were almost white or pale pink. Finally, the pellet was washed with Tris buffer (10 mM, pH 7.0) and suspended in the same. The samples were used for measuring Na^+/K^+ - and Ca^{2+}/ Mg^{2+} -ATPase activity and for fatty acid analysis.

Determination of Na/K^+-ATPase activity

The activity of Na/K^+-ATPase in RBC membrane was assayed spectrophotometrically as described by Savitha and Panneerselvam (2006). In brief, 1mL reaction mixture containing final concentrations of 5 mM ATP, 100 mM NaCl, 20 mM KCl, 5 mM $MgCl_2$, 0.2 M Tris buffer (pH 7.4) and 30-50 μg of enzyme protein was incubated at 37 °C for 20 min. The reaction was terminated by the addition of 0.5 ml of 5% TCA. Thirty minutes after addition of 1 mL reagent containing 1% (w/v) ammonium heptamolybdate, 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 total ATPase activity was

measured with Na/K^+ and Mg^{2+} present in the reaction mixtures. The Na/K^+ -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 enzyme activity is expressed as μmoles of phosphate liberated/h/mg protein.

Determination of 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, 0.5 mM $CaCl_2$, 5 mM $MgCl_2$, 4 mM ATP and 25-40 μg of protein as an enzyme source. The reaction was carried out at 37 °C for 20 min and then terminated by addition of 5% trichloroacetic acid. Thirty minutes after addition of 1 mL reagent containing 1% (w/v) ammonium heptamolybdate, 40 mg/mL $FeSO_4$ and 1.15 N H_2SO_4 to each tube, the released phosphate was measured using a spectrophotometer at 690 nm. 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 0.5 mM EGTA. Ca^{2+} activity was obtained by subtracting Mg^{2+} activity from total ATPase activity (Savitha and Panneerselvam, 2006).

Statistical analysis

The results were analyzed statistically using ANOVA (Fisher, 1970). A P value < 0.05 was considered statistically significant.

INTRODUCTION

Physicochemical property of an oil or fat is dependent on the triacylglycerol (TAG) molecule it contains. The characteristics of a TAG in turn are dependent on the type of fatty acid molecules; its chain length, degree of unsaturation, geometry of double bond and also on its position in the TAG molecule. The oils containing TAG of higher melting fatty acids tend to show cloudiness or remain as solid at room temperature while that containing unsaturated fatty acids will be in liquid form. The position of fatty acid in the TAG molecule determines the melting point of particular TAG and also its digestion, absorption, metabolism and possibly its role in atherogenesis (Small, 1991). During recent years a shift in consumer preference is seen towards healthier fat. It is well established that fat containing saturated fatty acid (SFA) increase blood total and LDL cholesterol level (Cantwell, 2000) and polyunsaturated fatty acids (PUFA) has beneficial effect in decreasing the cholesterol level (Hegsted *et al*., 1993). Hence the ratio of PUFA/SFA was considered as an important criteria for determining the nutritional benefits from an oil. However, excessive consumption of PUFA leads to oxidative stress if not properly balanced with antioxidants (Scislowski *et al*., 2005), and also being precursors of prostaglandins its excessive intake may cause imbalance in different prostaglandins (Trivedi and Singh, 2005). Therefore there should be a balance between SFA and PUFA in the dietary fat. The consideration of the ratio of PUFA/SFA as a criterion for the nutritional assessment of a fat become insufficient since the monounsaturated fatty acids (MUFA) is present in significant quantities in almost all oils. MUFA have the cushioning effect on the negative qualities of SFA and PUFA. These findings on the effect of individual fatty acids prompted nutritionists and dieticians to search for an ideal oil with SFA:MUFA:PUFA in the ratio of 1:1:1 which is also recommended by Indian Council of Medical Research and American Heart Association (Ghafoorunissa, 1998; Fogli-Cawley *et al*., 2006) to get optimum health effect.

In India, the type of fat consumption varies from region to region. Coconut oil, which contain 90% saturated fatty acid, is the predominant dietary fat in Kerala and coastal regions of Karnataka. Mustard oil is the major cooking oil in north India and the eastern part of the country. Sesame oil (SESO) is used in Tamil Nadu and in other regions of South India. Sunflower oil and ground nut oil are used in most parts of the

country. These oils are rich in unsaturated fatty acids. Rice bran oil (RBO) is recently introduced in Indian market. It is rich in unsaturated fatty acids. Palm oil is also available in Indian market. It contains a higher level of saturated fatty acids. Careful analysis of the fatty acid composition of all these oils indicates that nature has not provided us an oil with the ideal combination of SFA:MUFA PUFA in the ratio of 1:1:1. This underscores the need for developing strategies to balance the fatty acid composition in oil.

The process for modification of fats and oils includes fractionation, hydrogenation, blending and interesterification. Blending of two or more oils has been used as an approach to improve the oxidative stability (Anwar *et al*., 2007) and nutritional properties of the oils (Sugano and Tsuji, 1997). Blending of oils has been used in the preparation of industrial shortenings (Braipson-Danthine and Deroanne, 2004). Blending of saturated fat with unsaturated oil can give oil with balanced fatty acid composition. Even though one can balance the fatty acid composition to desired proportions by blending of suitable oils, it may not always result in oils with desired physicochemical (Kurashige *et al*., 1993) or nutritional properties *(*Marangoni and Rousseau, 1998), because the physical characteristics of individual oils will be retained in blended oil. More over the TAG species from the parent oil remain unaltered in blended oil and these fatty acids will be absorbed in the same manner as that from the TAG of parent oil. This pattern may be changed by rearranging the fatty acids in the triacylglycerol molecules. Rearrangement of fatty acids in TAG may be brought about by enzymatic or chemical interesterification process. The enzyme mediated reaction typically utilizes lipase. Lipase catalyzed interesterification is specific and has become a useful tool for the production of designer lipids to improve the nutritional quality and to enhance the oxidative stability of table spread (Aguedo *et al*., 2008; Shin *et al*., 2009). Farmani *et al*. (2006) prepared zero trans fat Iranian vanaspati through interesterification of palm olein, low erucic rapeseed oil and sunflower oil. Incorporation of γ-linolenic acid and linoleic acid in specialty fats has been achieved through acidolysis and interesterification reaction (Lumor and Akoh, 2005). Enzymatic interesterification of palm stearin and canola oil in varying proportions produced fat products with slip melting point and solid fat content similar to that of margarine, vanaspati and also fat for shortening application (Siew *et al*.,

2007). Rearrangement of fatty acid in the glycerol moiety catalyzed by lipases may change the physical (Marangoni and Rousseau, 1998) and nutritional properties of the oil (Akoh, 1995). The new triacylglycerol molecules formed in the interesterified oil may be absorbed differently than the original TAG from the native oil or in the blend (Kennedy, 1991).

The randomization of fatty acids with different degrees of unsaturation may also affect the physicochemical characteristics of the TAGs in the oil. Coconut oil used in Indian cooking contains relatively higher amounts of saturated fatty acids compared to other oils which are in the main stream of Indian cooking. Rice bran oil and sesame oil which are being mostly used in southern part of India contains higher amount of unsaturated fatty acids. Coconut oil was blended with appropriate amounts of rice bran oil or sesame oil to balance the fatty acid composition and further subjected to transesterification reactions to rearrange the fatty acids in the TAG molecules. The present investigation was undertaken to determine the changes in the physicochemical properties of these blended and interesterified oils thus obtained using Differential Scanning Calorimetry (DSC) and finger printing of TAG molecular species by HPLC.

RESULTS

Quality parameters of the native, blended and interesterified oils

The peroxide value and free fatty acid content of native, blended and interesterified oils were monitored. (Table 1.1). Peroxide value and free fatty acid content of these oils were in the acceptable range of the PFA specifications.

Fatty acid composition of oils

Blended and interesterified oils were prepared with SFA:MUFA:PUFA composition in the ratio of approximately 1:1:1 with PUFA/SFA in the ratio of 0.8-1.0 as per the recommendations of nutritionists for an ideal oil with balanced fatty acid composition. Coconut oil was blended with either rice bran oil or sesame oil. Fatty acid analysis of CNO showed 91% of total fatty acid is saturated. RBO and SESO contain 78% and 84% unsaturated fatty acids respectively (Table 1.2).

Table 1.1. Quality of oils

Oil	PV (meqO_2/Kg oil)	FFA (%)
CNO	0.24±0.09	0.13±0.01
RBO	2.40±0.70	0.31±0.09
CNO+RBO(B)	2.90±0.10	0.32±0.07
CNO+RBO(I)	2.94±0.13	2.02±0.01
SESO	1.40±0.65	2.34±0.13
CNO+SESO(B)	1.76±0.72	2.72±0.03
CNO+SESO(I)	2.08±0.13	4.10±0.62

Values are mean ± SD of three samples. PV-peroxide value, FFA-free fatty acid, meq-milliequivalents, CNO-coconut oil, RBO- rice bran oil, SESO- sesame oil, (B)- blended, (I)-interesterified.

Table 1.2. Fatty acid composition (wt%) of native, blended and interesterified oils

Fatty acids	CNO	RBO	CNO+RBO (B)	CNO+RBO (I)	SESO	CNO+SESO (B)	CNO+SESO (I)
8:0	2.7±0.3	nd	nd	nd	nd	nd	nd
10:0	4.5±0.2	nd	0.5±0.1	0.4±0.1	nd	1.0±0.2	0.9±0.1
12:0	50.3±1.3	nd	9.8±0.4	8.6±0.3	nd	11.6±0.4	11.8±0.5
14:0	21.9±0.4	0.4±0.1	4.7±0.3	4.6±0.2	nd	4.8±0.3	4.6±0.2
16:0	9.1±0.5	19.8±0.4	17.8±0.2	18.9±1.0	9.6±0.1	9.8±0.3	9.9±0.5
18:0	2.7±0.3	1.8±0.1	2.5±0.2	2.6±0.2	5.9±0.1	5.2±0.2	4.9±0.4
18:1	7.0±0.6	41.9±0.3	35.2±0.5	35.9±0.9	41.7±0.2	34.0±0.7	34.6±1.0
18:2	1.8±0.1	35.4±0.2	29.4±0.6	28.9±0.8	42.5±0.3	33.6±0.8	33.2±0.9
18:3	nd	0.6±0.1	nd	nd	0.3±0.1	nd	nd

Values are mean ± SD of three samples. nd- not detected (limit of detection; 0.3%). CNO-coconut oil, RBO- rice bran oil, SESO- sesame oil, (B)- blended, (I)- interesterified.

The SFA: MUFA: PUFA ratio of CNO is 1.00:0.08:0.02, of RBO is 1.0:1.9:1.63 and that of SESO is 1.0:2.69:2.76 (Table 1.3). This shows that none of the native oils used in the Indian culinary are balanced in terms of fatty acid composition recommended for nutritional benefits. Hence the oils are blended to obtain balanced fatty acid composition in which SFA:MUFA:PUFA of CNO+RBO(B) was 1:0.99:0.83 and that of CNO+SESO(B) was 1:1.05:1.04. Interesterification did not change the fatty acid composition of the blended oil (Table 1.2 & 1.3).

Table 1. 3. Distribution of saturated, monounsaturated and polyunsaturated fatty acids in native, blended and interesterified oils

	SFA	MUFA	PUFA	P/S ratio	S:M:P ratio
CNO	91.2	7.0	1.8	0.02	1:0.08:0.02
RBO	22.1	41.9	36.0	1.63	1:1.89:1.63
CNO+RBO(B)	35.3	35.2	29.4	0.83	1:0.99:0.83
CNO+RBO(I)	35.1	35.9	28.9	0.82	1:1.02:0.82
SESO	15.5	41.7	42.8	2.76	1:2.69:2.76
CNO+SESO(B)	32.4	34.0	33.6	1.04	1:1.05:1.04
CNO+SESO(I)	32.1	34.6	33.2	1.03	1:1.07:1.03

S:M:P-saturated: monounsaturated: polyunsaturated fatty acid, SFA- saturated fatty acids, MUFA- monounsaturated fatty acids, PUFA- polyunsaturated fatty acids. P/S- polyunsaturated to saturated fatty acid, CNO- coconut oil, RBO- rice bran oil, SESO- sesame oil, (B)- blended, (I)- interesterified.

TAG molecular species monitored by HPLC

Finger printing of TAG molecular species of native oils is shown in Figure 1.1A, B & C. TAG molecular species present in native, blended and interesterified oils are given in the Table 1.4 & 1.5. TAG profile of coconut oil showed 17 molecular species (Figure 1.1A); eight fatty acids were contributed to their formation. Major TAG in coconut oil accounts for 88.9% saturated TAG molecular species. Twelve TAG species were identified in RBO (Figure 1.1B) and these TAG were comparable with those reported in literature (Marini *et al*., 2003). The major TAG in RBO were triunsaturated (UUU-43.7%) and monosaturated TAG (SUU-43.3%). The major TAG molecular species of SESO were triunsaturated (UUU-56.96%) and monosaturated (SUU-37.15%) TAG (Figure 1.1C).

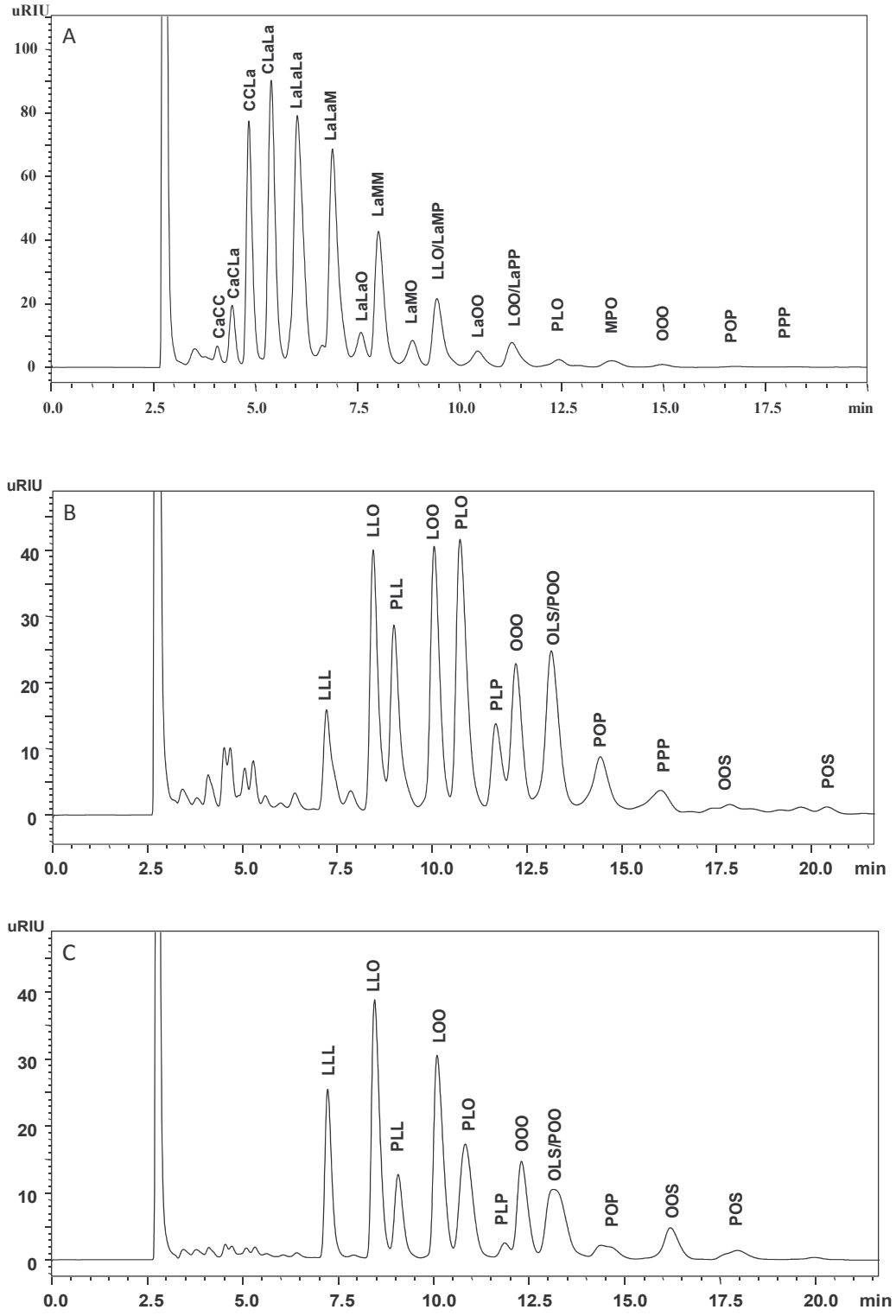


Figure 1. 1. HPLC profile of TAG molecular species of native oils. A: coconut oil; B: rice bran oil; C: sesame oil. Ca- caprylic, C- capric, La- lauric, M-myristic, P- palmitic, S- stearic, O- oleic, L- linoleic.

Table 1.4. TAG molecular species of native, blended and interesterified oils

	Area (%)						
TAG species	**CNO**	**RBO**	**CNO+RBO (B)**	**CNO+RBO (I)**	**SESO**	**CNO+SESO (B)**	**CNO+SESO (I)**
CaCC	1.1±0.1	nd	nd	nd	nd	nd	nd
CaCLa	3.4±0.2	nd	nd	nd	nd	nd	nd
CCLa	12.8±0.4	nd	3.4±0.6	3.4±0.2	nd	3.1±0.2	2.2±0.3
CLaLa	17.8±0.8	nd	3.6±0.5	4.0±0.3	nd	4.3±0.4	1.5±0.1
LaLaLa	20.7±1.2	nd	4.6±0.3	2.6±0.2	nd	4.9±0.3	2.4±0.2
LaLaM	16.6±0.5	nd	3.3±0.2	3.1±0.2	nd	3.8±0.2	4.2±0.2
LaLaO	1.8±0.2	nd	nd	nd	nd	nd	nd
LLL	nd	5.5±0.3	3.9±0.1	5.9±0.1	10.5±0.4	8.9±0.2	8.3±0.3
LaMM	10.1±0.3	nd	3.5±0.1	7.3±0.1	nd	2.8±0.1	7.9±0.2
LaMO	2.1±0.1	nd	nd	nd	nd	nd	nd
LLO/LaMP	6.2±0.2	12.8±0.4	8.8±0.5	10.7±0.9	19.2±0.3	15.0±0.6	15.3±0.8
LaOO	1.6±0.1	nd	nd	nd	nd	nd	nd
PLL	nd	11.2±0.6	9.7±0.4	11.0±0.5	6.7±0.2	6.8±0.5	9.7±0.4
MOL	nd	nd	nd	3.8±0.2	nd	nd	3.1±0.2
MMO/LaPO	nd	nd	nd	3.6±0.4	nd	nd	2.7±0.2
LOO/LaPP	2.9±0.2	15.2±0.7	11.6±1.1	9.4±0.5	17.7±1.1	13.5±0.4	12.2±0.7
PLO	0.9±0.1	18.1±0.4	15.1±0.7	12.1±0.4	13.5±0.6	10.9±0.6	10.5±0.4
PLP	nd	5.9±0.2	5.2±0.1	4.4±0.1	1.3±0.2	1.8±0.1	3.2±0.2
MPO	0.8±0.1	nd	nd	nd	nd	nd	nd
OOO	0.2±0.05	10.2±0.3	8.8±0.3	7.9±0.4	9.6±0.5	7.1±0.3	4.4±0.3
OLS/POO	nd	12.9±0.2	11.6±0.4	8.2±0.2	12.6±0.6	9.4±0.5	7.7±0.2
POP	0.8±0.1	4.9±0.2	4.8±0.1	1.1±0.2	2.6±0.1	2.5±0.2	1.2±0.1
PPP	0.2±0.05	0.9±0.1	0.32±0.03	0.9±0.2	nd	nd	nd
OOS	nd	1.1±0.1	1.6±0.1	0.3±0.05	4.4±0.2	3.5±0.2	2.4±.02
POS	nd	0.5±0.04	nd	0.1±0.05	1.8±0.1	1.5±0.1	1.1±0.1

Values are mean ± SD of three samples. nd- not detected (limit of detection; 0.2%). CNO- coconut oil, RBO- rice bran oil, SESO- sesame oil, (B)- blended, (I)- interesterified, Ca- caprylic, C- capric, La- lauric, M- myristic, P- palmitic, S- stearic, O- oleic, L-linoleic.

Table 1.5. Distribution of trisaturated, monounsaturated, diunsaturated and triunsaturated TAG in native, blended and interesterified oils

TAG species	CNO	RBO	CNO+RBO (B)	CNO+RBO (I)	SESO	CNO+SESO (B)	CNO+SESO (I)
UUU	0.2	43.7	33.1	33.9	56.9	44.5	40.2
SUU	4.5	43.3	38.0	35.2	37.2	30.6	33.4
SSU	5.5	11.3	10.0	9.2	5.7	5.8	8.2
SSS	88.9	0.9	18.7	21.3	-	18.9	18.2

TAG- triacylglycerol, CNO- coconut oil, RBO- rice bran oil, SESO- sesame oil, (B)- blended, (I)- interesterified, U- unsaturated, S- saturated.

Blending of coconut oil with rice bran oil decreased the proportion of the trisaturated TAG by 78.9% compared to native CNO and emergence of 33.1% triunsaturated TAG. The triunsaturated TAG in native CNO were only 0.2% (Table 1.5). Interesterification of CNO+RBO(B) resulted in the decrease in LaLaLa, LOO/LaPP, PLO, and OLS/POO by 43.4, 18.9, 24.7 and 29.3%, respectively compared to blended oils. LaMM TAG species is increased almost double the amount in interesterified oil of CNO+RBO. (Table 1.4 & Figure 1.2).

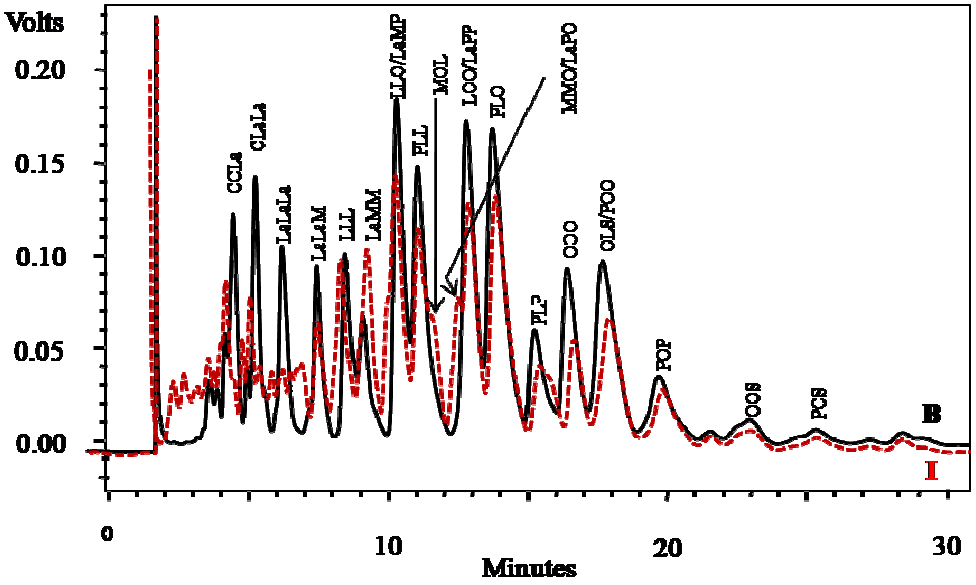


Figure 1.2. TAG molecular species of blended (B —) and interesterified oils (I·····) of CNO with RBO. C- capric, La- lauric, M-myristic, P- palmitic, S- stearic, O- oleic, L- linoleic.

Interesterification also resulted in the increase in the concentration of LLL, LLO/LaMP and PLL by 51.2, 21.5 and 13%, respectively in CNO+RBO blend. The new TAG species emerged in CNO+RBO interesterified oil are MOL (3.8%) and MMO/LaPO (3.6%) (Table 1.4). The blended oil of CNO+SESO showed 78.7% decrease in the trisaturated TAG compared to native CNO. Blending of CNO with SESO resulted in the increase in the monosaturated TAG by 6.8 fold and the triunsaturated TAG increased to 44.5% (Table 1.5), which was only 0.2% in the native coconut oil. Finger printing of TAG molecular species of blended and interesterified oil CNO+SESO by HPLC is shown in Figure 1.3. Interesterification of blended oil resulted in decrease in triunsaturated TAG (9.6%) and increase in monosaturated and disaturated TAG by 9.1 and 41.4%, respectively. The increase in monosaturated TAG is mainly due to increase in the PLL by 42.6% and also due to the occurrence of new TAG MOL by 3.1%. The increase in the disaturated TAG is mainly due to the emergence of comparatively low melting new TAG MMO/LaPO by 2.7% (Table 1.4 & Figure 1.3).

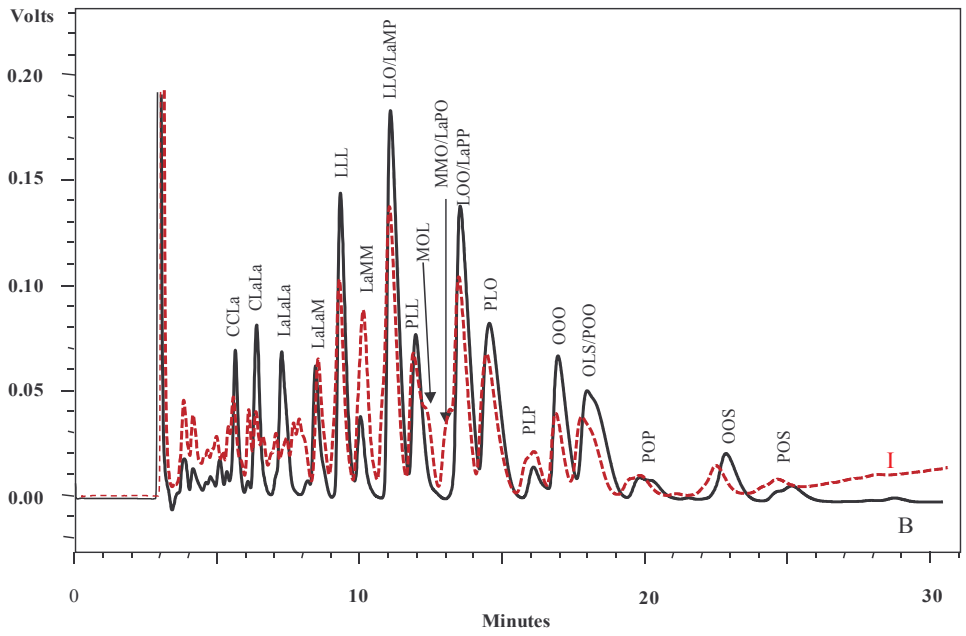


Figure 1. 3. TAG molecular species of blended (B —) and interesterified oils (I ·····) of CNO with SESO. C- capric, La- lauric, M-myristic, P- palmitic, S- stearic, O- oleic, L-linoleic.

Thus blending of CNO with oils containing higher amounts of unsaturated fatty acids resulted in the reduction of trisaturated TAG. Interesterification resulted in

the emergence two new TAG molecules in CNO blends, which were not present in native oils, or in blended oil.

Melting profiles of native, blended and interesterified oils

The consequences of altering the TAG molecular species in blended and interesterified oils on thermal properties were monitored by DSC. Melting endotherms of native oils is shown in Figure 1.4. Saturated fat CNO showed a endothermic melting peak at higher temperature range (16.9 to 24.6 °C) when compared to the unsaturated oils RBO and SESO which showed endothermic melting peak at lower temperature range -25.1 to -15.8 °C and -27.6 to -22.9 °C (Table 1.6).

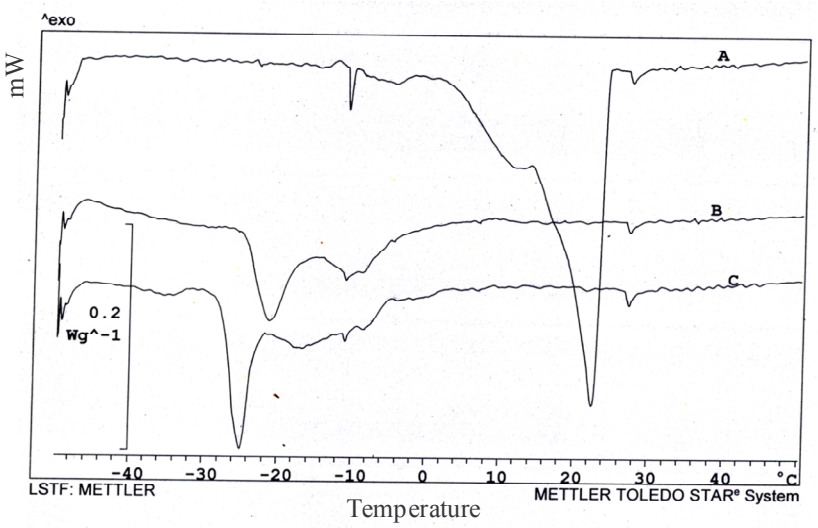


Figure 1.4. Melting endotherm of native oils. A- coconut oil, B- rice bran oil, C- sesame oil.

The solid fat content (SFC) of CNO was 33.1% at 20 °C but at 25 °C the solid content was found to be only 1% (Figure 1.5) indicating that the majority of TAG of CNO is melting between 20-25 °C. The melting peak observed at very low temperatures for RBO and SESO are due to higher levels of unsaturated TAG present in these oils. Solid fat content of RBO (Figure 1.5) showed less than 7.2% solids above 0 °C. In the case of SESO more than 97% solids were melted below 0 °C and no solids were observed above 10 °C, indicating that there is no high melting TAG present in this oil (Figure 1.6).

Table 1.6. Peak temperature and enthalpy of native blended and interesterified oils during melting

Samples	Peak 1				Peak 2			
	Onset (°C)	Peak (°C)	End set (°C)	ΔH [J/g]	Onset (°C)	Peak (°C)	End set (°C)	ΔH [J/g]
CNO	nd	nd	nd	nd	16.9	22.9	24.6	75.5
RBO	-25.1	-21.3	-15.8	51.0	nd	nd	nd	nd
CNO+RBO(B)	-8.3	-4.8	15.7	41.8	nd	nd	nd	nd
CNO+RBO(I)	-15.8	-11.2	-8.4	37.0	nd	nd	nd	nd
SESO	-27.6	-25.0	-22.9	44.6	nd	nd	nd	nd
CNO+SESO(B)	-11.7	-11.2	-10.3	48.0	26.6	27.2	28.2	0.99
CNO+SESO(I)	-17.8	-11.1	-6.9	44.7	26.5	27.2	28.2	0.96

CNO- coconut oil, RBO- rice bran oil, SESO- sesame oil, (B)- blended, (I)- interesterified, nd- not detected.

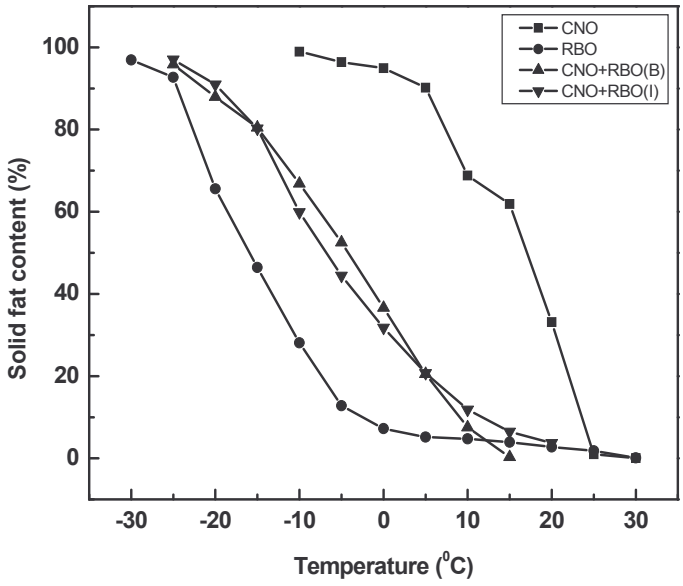


Figure 1.5. Melting profile of blended and interesterified oils of CNO with RBO.
CNO-coconut oil, RBO- rice bran oil, (B)- blended, (I)- interesterified.

The blended oil containing CNO+RBO showed a single endothermic peak (Figure 1.7) in the range of –8 to +15 °C, shifting to lower temperature compared to that observed with CNO alone. The SFC for saturated oil, CNO at 20 °C was 33.12% and that for RBO it was 2.78%. When CNO was blended with RBO the solid fat content was zero at 20 °C (Figure 1.5).

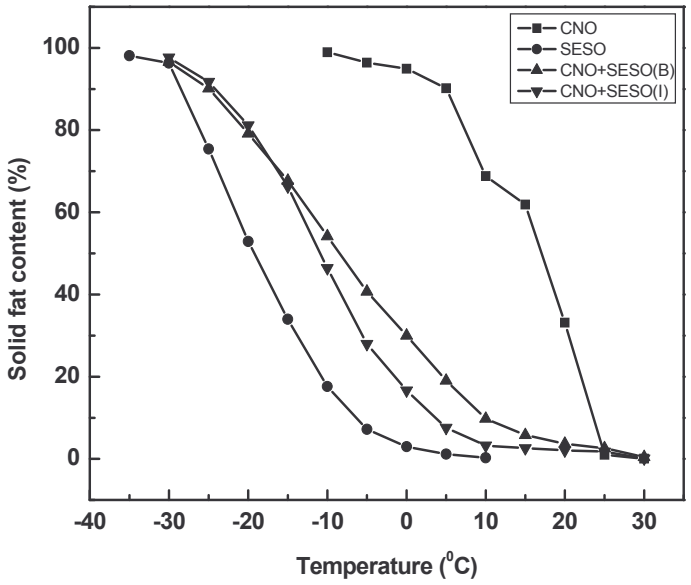


Figure 1.6. Melting profile of blended and interesterified oils of CNO with SESO.
CNO-coconut oil, SESO- sesame oil, (B)- blended, (I)- interesterified.

Blending of CNO with RBO resulted in decrease in the trisaturated TAG (Table 1.5), which lowered the melting temperature of blended oil when compared to native CNO. Interesterification of this blended oil resulted in further lowering of the melting temperature with the endothermic peak being observed in the range of –15.84 to –8.42 °C (Figure 1.7) with a solid fat content of 3.71% at 20 °C (Figure 1.5). The presence of solid fat in interesterified oil at 20 °C may be due to the increase in the disaturated TAG compared to the blended oil. Interesterification of CNO+RBO blend resulted in the decrease in disaturated TAG (SSU- 8%). The decrease in the disaturated TAG are mainly due to the decrease in the individual TAG with higher melting point, POP to 77.0% in interesterified oils compared to the blended oil (Table 1.4).

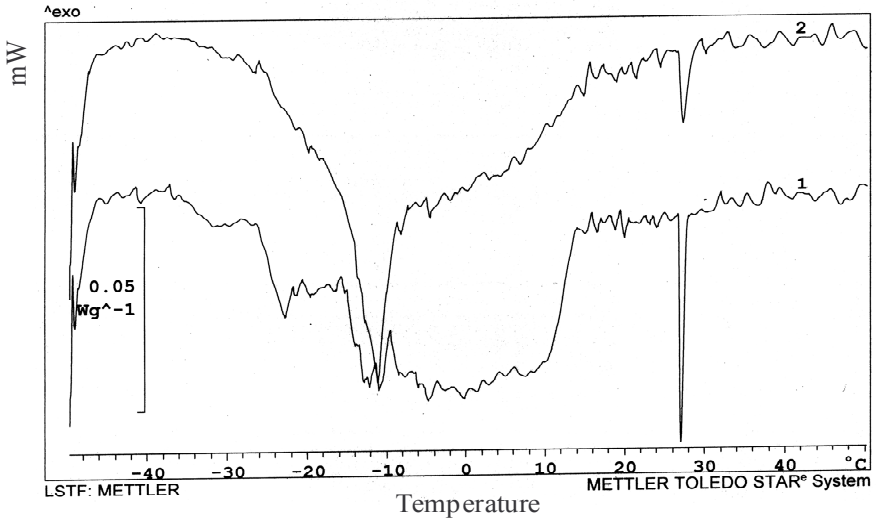


Figure 1.7. Melting endotherm of blended and interesterified oils of CNO with RBO. CNO- coconut oil, RBO- sesame oil, 1- blended, 2- interesterified.

When CNO was blended with SESO, there were two endothermic peaks at different temperatures. The low melting peak, which accounts for 98.9% in terms of energy consumption, is in the range of –11.69 to –10.28 °C (Figure 1.8) Interesterification of CNO+SESO resulted in the shifting of the onset of low melting peak to still lower temperature of –17.78 to –6.97 °C (Figure 1.8). The high melting peak for both blended and interesterified oils was in the similar range of 26.58 to 28.2 °C (Table 1.6).

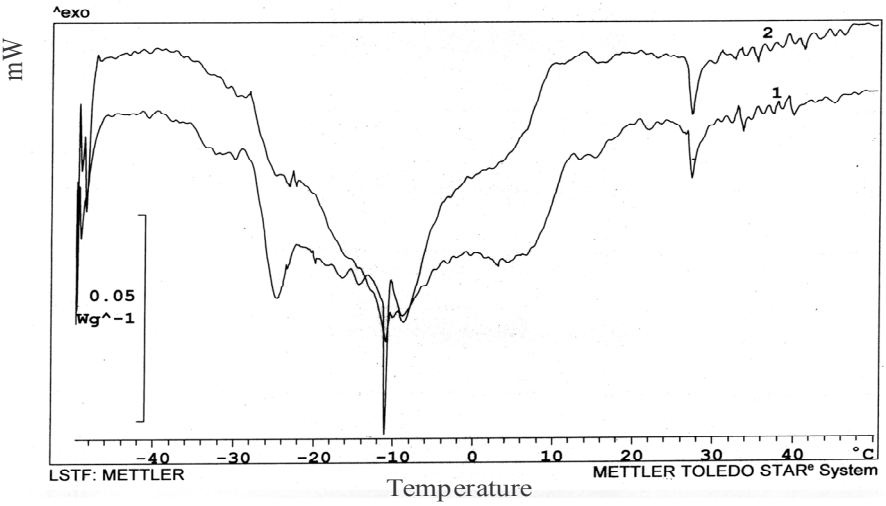


Figure 1.8. Melting endotherm of blended and interesterified oils of CNO with SESO. CNO- coconut oil, SESO- sesame oil, 1- Blended, 2- Interesterified.

Blending of CNO with SESO resulted in the decrease in the solid fat content to 3.67% at 20 °C (Figure 1.6) compared to the 33.1% of CNO. This may be due to the decrease in the trisaturated TAG (78.7%) in the blended oil compared to the native CNO. Interesterification further decreased the solid fat content to 2.09% at 20 °C compared to its blend CNO+SESO (Figure 1.6).

Thus blending of CNO with unsaturated TAG of liquid oils resulted in the decrease in the solid fat content in CNO. Interesterification further reduced the solid fat content in their respective blends.

Crystallization behaviour of native, blended and interesterified oils

Cooling curves of oils show the temperature at which the TAG species start crystallizing. The crystallization of CNO TAG showed a single exothermic peak at 0.97 °C (Figure 1.9 & Table 1.7) with a shoulder peak. No exothermic peak was detected for RBO and SESO, indicating that these two oils did not crystallize under experimental conditions.

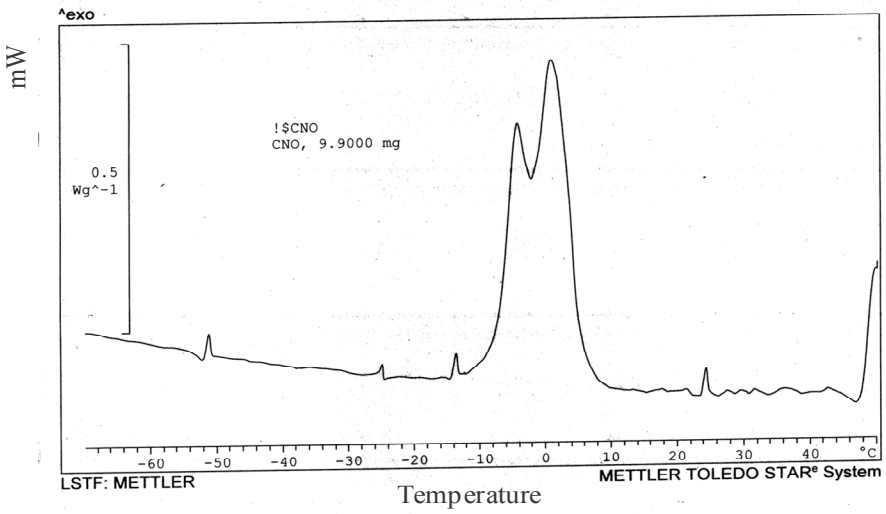


Figure 1.9. Crystallization exotherm of coconut oil

Blending of CNO with RBO resulted in shifting of crystallization temperature to -24.3 and -51.1°C, respectively for peak 1 and peak 2 (Figure 1.10). Interestingly no exothermic peak was observed after interesterification this blended oil with lipase enzyme.

Table 1.7. Crystallization 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]
CNO	5.5	0.97	-3.9	60.7	nd	nd	nd	nd	nd	nd	nd	nd
RBO	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
CNO+RBO(B)	-24.3	-24.9	-23.4	5.9	-50.5	-51.1	-51.9	0.25	nd	nd	nd	nd
CNO+RBO(I)	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
SESO	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
CNO+SESO(B)	-12.1	-13.5	-14.4	2.4	-16.9	-22.9	-29.8	4.3	-42.5	-47.3	-52.6	8.1
CNO+SESO(I)	-12.7	-13.5	14.4	1.4	-28.5	-40.4	-48.2	12.9	nd	nd	nd	nd

CNO- coconut oil, RBO- rice bran oil, SESO- sesame oil, (B)- blended, (I)- interesterified, nd- not detected.

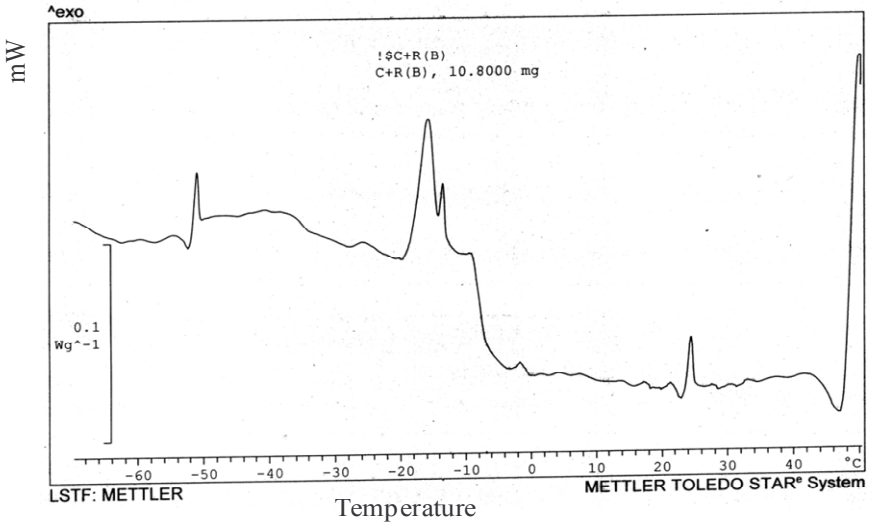


Figure 1.10. Crystallization exotherm of blended oil of CNO with RBO. CNO- coconut oil, RBO- rice bran oil.

The blended oil of CNO+SESO showed three exothermic peaks (Figure 1.11) at -13.46, -22.95 and -47.25 °C, respectively, which were significantly lower than the crystallization temperature of native CNO. Appearance of two peaks in the interesterified oil of CNO+SESO (Figure 1.11) showed that it is more homogeneous

than the corresponding blended oil (Table 1.7). The TAG crystallizing at higher temperature of both blended and interesterified oils of CNO+SESO appeared at -13.5 °C. The TAG crystallizing at low temperature -40.43 °C of CNO+SESO interesterified oil was observed in between the temperature range of the peaks crystallizing at medium and low temperature of CNO+SESO blended oils (Figure 1.11).

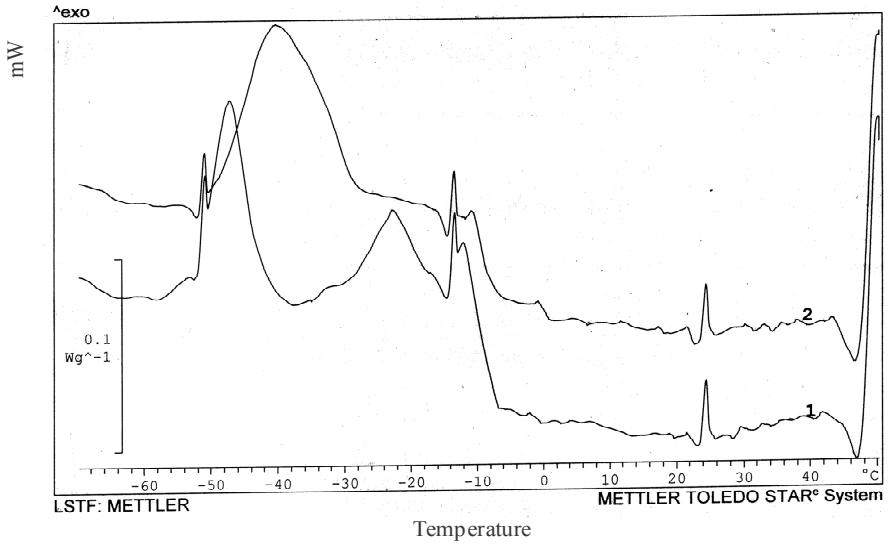


Figure 1.11. Crystallization exotherm of blended and interesterified oils of CNO with SESO. CNO- coconut oil, SESO- sesame oil, 1- Blended, 2- Interesterified.

DISCUSSION

Oils and fats have been modified for various reasons, since the time man came to know about the nature of their constituent molecules. The physicochemical and nutritional properties of an oil or fat are dependent on its constituent molecule, fatty acids. Fatty acids vary in their chain length, unsaturation and configuration of double bonds and also on its stereospecific position on the glycerol molecule. More of saturated fat in the diet is hypercholesterolemic in its effect (Cantwell, 2000) and its replacement by unsaturated fatty acids counteract the effect. However more of PUFA in the diet leads to oxidative stress if not properly balanced with antioxidants (Hegsted *et al*., 1993). Therefore there should be a balance in the fatty acid composition, SFA:MUFA:PUFA, in the oil to get optimum health effect as recommended by ICMR and AHA (Ghafoorunissa, 1998; Fogli-Cawley *et al*., 2006).

In the present study we prepared oils with balanced fatty acid composition of SFA:MUFA:PUFA in the ratio of approximately 1:1:1 by blending and interesterification reaction using CNO with RBO or SESO. The physicochemical properties such as melting and crystallization profiles, solid fat content and TAG molecular species were analyzed for native, blended and interesterified oils.

For many food preparations the food industry needs plastic or solid fats with good stability. Bakery, pastry, margarine and spreads require solid fat, while for deep fat frying liquid oil which is resistant to thermo-oxidation is needed. In recent times the consumer preference has been shifted towards product containing healthier fat. This change in attitude of consumers has challenged the industry to develop new products with favourable physicochemical, organoleptic and nutritional properties (Ronne *et al*., 2005).

CNO is rich in saturated fatty acids, but at the same time it is one of the richest sources of medium chain fatty acids. RBO and SESO contain more of unsaturated fatty acids and also contain minor components such as oryzanol, tocotrienol (RBO), sesamin, sesamolin and sesamol (SESO) which possess nutraceutical properties. Blending CNO with RBO or SESO brought the qualities of both the oils in single oil. Modification of oils and fats have been carried out by manufacturers for various purposes such as to enhance oxidative stability, for desirable physicochemical properties, to improve nutritional properties. Oil blends were prepared to improve the oxidative stability of soybean oil (Chu and Kung, 1998). Butter fat was blended with vegetable oils that are liquid at refrigerator temperature to get spreads with better nutritional property, desirable organoleptic attributes and lowered costs of production (Rousseau *et al*., 1996). CNO solidifies at temperate conditions due to its saturated fatty acids. Blending with unsaturated oils resulted in the decrease in the melting temperature compared to CNO. Blending of high melting palm stearin with sunflower oil, rapeseed oil or soy bean have been used to substitute the trans fatty acids containing vanaspati. Feeding these blended oils to rats showed a significant decrease in the TAG level compared to rats fed with vanaspati (Ray and Bhattacharyya, 1996).

The stereospecific position of fatty acids on TAGs plays a major role in the physicochemical properties and the functionality of fats in food products (Hunter,

2001). For example in the case of lard, the presence of palmitic acid in the sn-2 position contributes desirable flakiness of pie crusts when lard is used as a baking shortening (Small, 1991). The unique positioning of palmitic, oleic and stearic acids in predominant TAGs (POS, SOS, POP) gives cocoa butter a sharp melting point just below body temperature. The melting of cocoa butter at below body temperature is one of the reasons for the pleasant eating quality of chocolate (Dimick and Manning, 1987). Randomization of fats is widely being adopted by the food industry as an alternative to partial hydrogenation for the generation of fats with higher melting points.

The stereospecific position of fatty acids is also crucial because it determines how TAGs are digested. During digestion in the gastrointestinal tract, pancreatic lipase, an enzyme highly specific for the sn-1 and sn-3 esters, hydrolyses TAGs resulting in the formation of 2-monoacylglycerols and free fatty acids that are absorbed in the small intestine (Mattson and Volpenhein, 1964). The 2-monoacylglycerols are reacylated into new TAGs that enter the lymph chylomicrons. Fatty acids released from the sn-1 and sn-3 position positions often have different metabolic fates than fatty acids retained in the sn-2 position. These metabolic fates depend on the fatty acid chain length and stereospecific location on the TAG. Short chain fatty acids and medium chain fatty acids can be solubilized in the aqueous phase of the intestinal contents, where they are absorbed, bound to albumin, and transported to the liver by the portal vein. Longer chain fatty acids, such as palmitic and stearic have low coefficients of absorption because of melting points above body temperature and because of their ability to form calcium soaps. Thus, fats with long chain fatty acids in the sn-1 and sn-3 positions of TAGs may exhibit different absorption patterns as compared to fats with these fatty acids in the sn-2 position.

The food industry uses interesterification to modify the melting and crystallization behavior of fats, which in turn affect the digestion and absorption of fat. In our study interesterification of blended oil resulted in the decrease in the solid fat content in the oil. This was evident from melting and crystallization profiles of native, blended and interesterified oils (Table 1.6. and 1.7). The melting peak in CNO+RBO which was interesterified shifted more towards low temperature range

compared to its blend. Changes in the physical properties of interesterified oil were also observed from crystallization profile. There were no crystals observed in the cooling profile of interesterified oils in CNO+RBO compared to the two melting peak observed in CNO+RBO(B) (Table 1.7.). Interesterification resulted in more homogeneous mixture of oil in SESO combination, as seen in the crystallization profile of blended and interesterified oils of CNO+SESO(I). Crystallization profile of CNO+SESO showed two exothermic peak compared to the three exothermic peak observed in the blend. Reducing the amount of solid fat in the fat phase by blending with vegetable oil or by lowering the total amount of fat have been found to improve both spreadability and nutritional properties of butterfat (Rousseau and Marangoni, 1999).

The change in the physical characteristics of oil is mediated by the changes in the nature of fatty acid in TAGs. Interesterification causes exchange of fatty acid molecule between and within the TAG moiety. This result in the increase or decrease in the proportion of existing TAG molecule or in the emergence of new TAG molecule (Otero *et al*., 2006). Interesterification resulted in the decrease in the trisaturated TAG in the interesterified oil of CNO+RBO by 14% compared to its physical blend. In the case of CNO+SESO there was 10% decrease in triunsaturated TAG and 9% increase in the diunsaturated TAG after interesterification. Thus interesterification provides an opportunity to alter physicochemical properties of oils.

The potential health problems raised due to the consumption of semi-solid fats from partially hydrogenated oils lead to the search of a method for the production of a product with zero trans fatty acids. Interesterification catalyzed by lipase has drawn attention due to its specificity, selectivity, mild reaction condition and ease of product recovery (Yang *et al*., 2003). The hardening of a liquid oil by interesterifying it with a solid fat offers an alternative to the use of partial hydrogenation in the manufacture of margarines and spreads.

The transesterification process does not change the degree of unsaturation or the isomeric state of the fatty acids, as they transfer fatty acids between TAG molecules. Interesterification thus allows one to compare nutritional effects of fats

that are identical in fatty acid composition but differ in the position of fatty acids in triacylglycerol molecules.

In conclusion blended oils were prepared using CNO with RBO or SESO to get an oil with saturated:monounsaturated:polyunsaturated fatty acids in the ratios of approximately 1:1:1. Such a combination is considered to be ideal for providing better nutrition from the oils. These oils were further subjected to interesterification catalyzed by lipase to randomize the fatty acids in the blended oils. The changes in the TAG molecular species brought about by blending and interesterification reaction were monitored by HPLC. Blending of oils retained the TAG molecular species of parent oils without any modifications. Interesterification of blended oils however resulted in the redistribution of fatty acids among TAG molecules resulting in the emergence of altered TAGs. This had an impact on melting and crystallization behaviour and also on solid fat content of oils compared to the native oils or blended oils. These alterations in TAG molecular species of oils may have an impact on nutritional properties. This is being evaluated in subsequent chapters.

INTRODUCTION

Diet and lifestyle are the two modifiable factors which influence the risk factors of cardiovascular diseases. Dietary fat is one of the nutrients which has got maximum blame due to its role in the development and progression of chronic disorders. Hence dietary fat modification has always been advocated for the regulation of the risk factors of various diseases. Several lines of evidence have indicated that types of fat have a more important role in determining risk of coronary heart disease than total amount of fat in the diet. Dietary fatty acids of varying chain length and degree of unsaturation differentially alter plasma lipoprotein profiles and the subsequent risk of developing CVD (Lichtenstein, 2006). Risk factors identified for CVD include elevated blood cholesterol, increased level of low density lipoprotein (LDL) cholesterol in the circulation, decreased high density lipoprotein cholesterol (HDL) and increases in triacylglycerol concentration in the plasma (Varady and Jones, 2005).

The dietary fatty acids influence the plasma cholesterol concentration by exerting its effect on LDL fraction. Long chain SFA with exception of stearate tend to increase LDL concentrations and PUFA tend to decrease them. LDL is produced by the action of lipolytic enzyme on VLDL in the circulation. Therefore the factors which influence the synthesis and secretion of VLDL may play an important role in regulating LDL production. VLDL comprises TAG, phospholipids, cholesterol, cholesterol ester and various apolipoproteins. The availability of these components may influence VLDL synthesis. Considerable evidence suggests that the availability of cholesterol is one of the important factors (Khan *et al*., 1990). VLDL is converted in to intermediate density lipoprotein (IDL). IDL is directly removed from the circulation via interaction with LDL receptor in the liver or further metabolized to LDL and removed from the circulation by LDL receptor mediated uptake. Lipoproteins containing PUFA are better recognized by LDL receptor than those with saturated fatty acid (Small, 1991).

The influence of different fatty acids on cholesterol levels varies according to its chain length, unsaturation, geometry of the double bond, stereospecific position on

the TAG molecule. Metabolic studies have shown that classes of saturated fatty acids exert different effect on plasma lipid and lipoprotein levels. Specifically saturated fatty acids with 12-16 carbon atoms tend to increase plasma total and LDL cholesterol levels, where as stearic acid does not have cholesterol raising effect in comparison with oleic acid (Kris-Etherton and Yu, 1997). Numerous studies have shown strong cholesterol lowering effects of polyunsaturated fatty acids (Zhao *et al*., 2004). Even though polyunsaturated fatty acids (PUFAs) in dietary lipids are essential which help in decreasing serum cholesterol concentration, its consumption in excessive amount results in exerting oxidative stress (Scislowski *et al*., 2005) if not properly balanced with antioxidants. MUFA is neutral in effect and nullify the deleterious effect of both SFA and PUFA (Hegsted *et al*., 1993). Therefore there should be a balance in the amount of saturated:monounsaturated:polyunsaturated fatty acids in the diet to get optimum health benefits. In the present study blended oils were prepared using CNO, the predominant dietary fat in Kerala and coastal regions of Karnataka, with RBO or SESO. RBO and SESO are rich in unsaturated fatty acids and also contain minor components, which possess nutraceutical properties. The minor components present in the oils are shown to have independent health benefits.

The minor constituents present are unique to each oil. For example, tocopherol (vitamin-E), present in almost all edible oils containing unsaturated fatty acids is a potent antioxidant. It inhibits LDL-oxidation in smokers (Liu *et al*., 2004), and prevent ischemic heart disease (Gey *et al*., 1991). Tocotrienol, which is present mainly in palm oil and RBO reduce cholesterol oxidation (Xu *et al*., 2001) and are shown to have antiatherogenic effect (Qureshi *et al*., 1997). γ–Oryzanol, esters of ferulic acid is uniquely present in RBO has hypocholesterolemic activity (Ha *et al*., 2005) by suppressing the HMG-CoA reductase. Sesamin, the lignan molecule in sesame oil is a potent and specific inhibitor of Δ-5 desaturase (Shimizu *et al*., 1991) and it has hypocholesterolemic properties (Chen *et al*., 2005). 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). Thus, the reports in the literature suggest that both saponifiable and unsaponifiable fraction of oils can exhibit beneficial effects.

To get optimal health benefits, Indian Council of Medical Research and also that by American Heart Association have recommended that oil should contain balanced amount of SFA:MUFA:PUFA preferably in equal proportions. Since there is no single oil in nature which has such balanced amount of fatty acids, different oils may be blended to get an oil with balanced fatty acids. Blending also complements the minor components which is lacking in some oils.

Lipase catalyzed interesterification has emerged as a new biotechnological tool for the modification of oils and fats by rearranging fatty acids on the glycerol moiety which result in altered physical (Rodriguez and Gioielli, 2003) and nutritional properties of the oil (Kennedy, 1991). The new triacylglycerol molecules formed in the interesterified oil may be absorbed differently than the original TG from the native oil or in the blend (Liu *et al*., 2004) and may influence its metabolism.

The aim of the present study is to examine whether the blended and interesterified oils with balanced amounts of fatty acids, along with minor components, provide benefits which was monitored by their effect on serum and tissue lipid levels.

RESULTS

Fatty acid composition of dietary lipids

Fatty acid composition of dietary lipids showed that CNO containing diets had 92% saturated fatty acids; RBO and SESO diets contain 75% and 85% unsaturated fatty acids respectively (Table 2.1).

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

Dietary fat	Fatty acid							
	8:0	10:0	12:0	14:0	16:0	18:0	18:1	18:2
CNO	2.5±0.6	4.7±0.4	50.6±0.6	21.6±0.4	9.1±0.5	2.7±0.3	7.0±0.5	1.8±0.1
RBO	nd	nd	nd	1.4±0.3	20.9±2.7	2.5±0.5	41.0±3.5	34.2±1.0
CNO+RBO(B)	nd	nd	8.4±0.1	5.1±0.1	19.0±.01	2.3±0.1	35.6±0.1	29.0±0.7
CNO+RBO(I)	nd	nd	8.8±0.3	4.7±0.7	18.2±1.4	2.4±0.3	36.0±0.1	28.9±0.6
SESO	nd	nd	nd	nd	9.6±0.6	5.7±0.6	41.0±1.2	44.0±2.8
CNO+SESO(B)	nd	nd	14.0±0.1	5.7±0.2	10.0±0.4	5.4±0.5	31.0±0.5	34.0±1.0
CNO+SESO(I)	nd	nd	13.6±0.9	5.6±0.2	9.6±0.1	5.1±1.5	31.3±0.8	34.3±0.3

Values are mean ± SD of three samples. nd- not detected (limit of detection; 0.3%). CNO- coconut oil, RBO- rice bran oil, SESO- sesame oil, (B)- blended, (I)- interesterified.

The SFA: MUFA: PUFA ratio of CNO is 1:0.07:0.02, that of RBO is 1:1.8:1.4 and that of SESO is 1:2.7:2.8 (Table 2.2) showing that none of the oils used in the study contain fatty acids in the proportions desired by nutritionists. When CNO was blended with RBO or SESO in appropriate amounts the SFA:MUFA:PUFA ratios of the resulting blends were in the proportion of 1:1:0.8 and 1:0.9:0.98 for CNO+RBO and CNO+SESO, respectively. The fatty acid composition of interesterified oils was not different from their respective blends.

Table 2.2. Saturated, monounsaturated and polyunsaturated fatty acids in native, blended and interesterified oils

Dietary fat	SFA	MUFA	PUFA	S:M:P ratio	P/S ratio
CNO	91.5	7.0	1.8	1:0.07:0.02	0.02
RBO	24.8	41.0	34.2	1:1.83:1.4	1.5
CNO+RBO (B)	34.8	35.6	29.0	1:1.01:0.83	0.82
CNO+RBO (I)	34.7	36.0	28.9	1:1.03:0.83	0.83
SESO	15.3	41.0	43.6	1:2.67:2.8	2.8
CNO+SESO(B)	35.1	30.9	34.0	1:0.9:0.98	0.96
CNO+SESO(I)	34.3	31.4	34.3	1:0.9:1.0	1.0

SFA- saturated fatty acids, MUFA- monounsaturated fatty acids, PUFA- polyunsaturated fatty acids. S:M:P-saturated:monounsaturated:polyunsaturated fatty acid, P/S ratio-polyunsaturated to saturated fatty acid ratio, CNO- coconut oil, RBO- rice bran oil, SESO-sesame oil, (B)- blended, (I)- interesterified.

Nutraceutical contents

Unique minor components were present in each oil. Blending of two oils resulted in complementing minor components which was lacking in individual oils. Separation of tocopherols and tocotrienols is shown in Figure 2.1. Both β and γ isomers were eluted together (Figure 2.1). Total tocol (tocopherols+tocotrienols) content of CNO was only 3.1 mg/100 g oil (Table 2.3a). However the tocol content in RBO was 100.1 mg/100 g oil and in SESO it was 70.5 mg/100 g oil. When CNO was blended with RBO to balance the fatty acid composition, the resulting blended oil contained 79.9 mg of tocols/100 g oil. When CNO was blended with SESO the tocol concentration was found to be 54 mg/100 g oil. The oryzanol content in RBO was 1.25% (Table 2.3b). Oryzanol was not found in any other oil. When CNO was blended with RBO the blended oil contained 1.02% oryzanol. The major lignans present in SESO is sesamin, sesamolin and sesamol at 98.2, 166.4 and 0.75 mg/100 g oil, respectively (Table 2.3b).

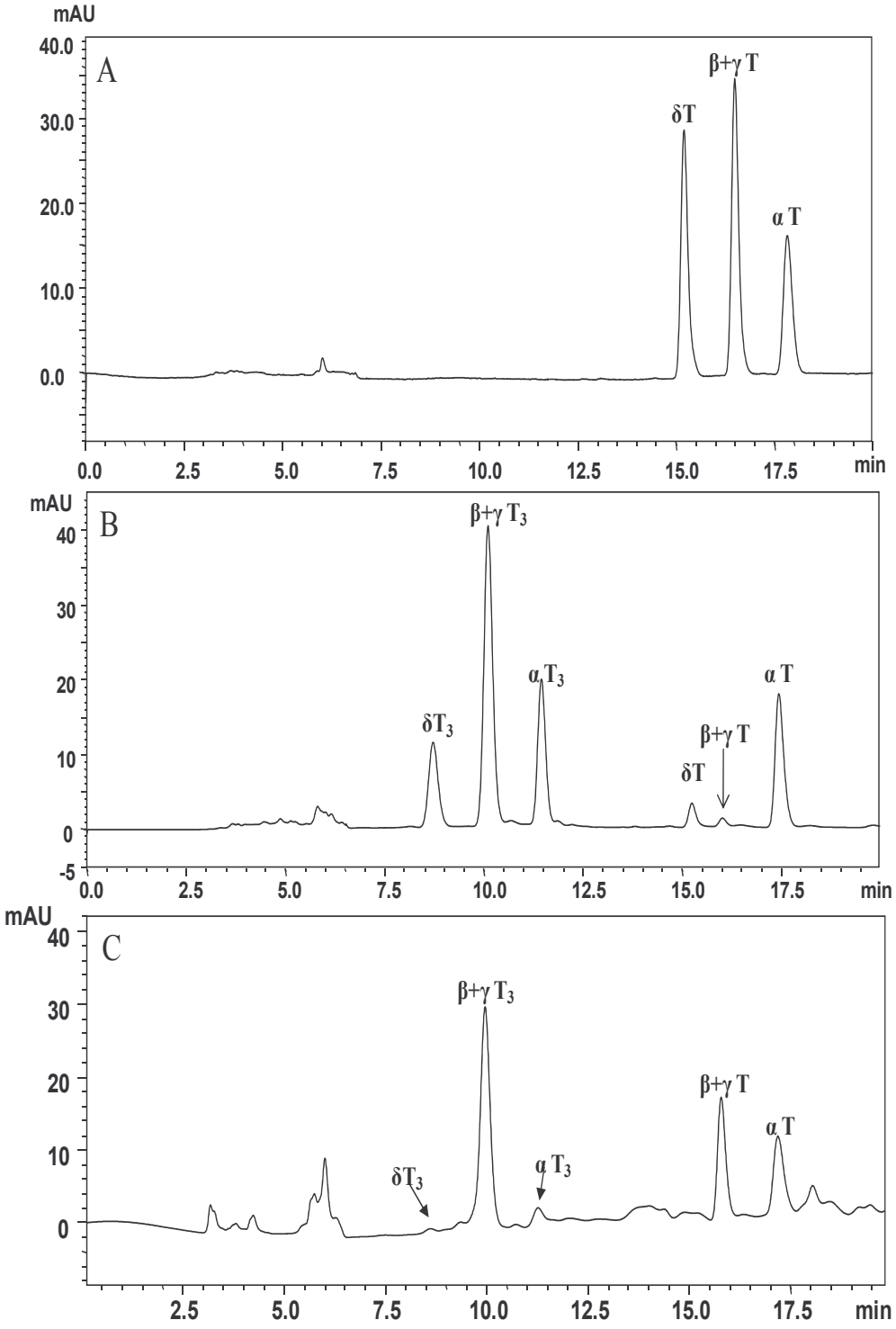


Figure 2.1. HPLC profile of tocopherols and tocotrienols. A: standard tocopherols; B: standard tocotrienols; C: Rice bran oil.

Table 2.3. Minor constituents of oils

a). Tocopherols and tocotrienols (mg/100g oil)

	Tocopherols (T)			Tocotrienols (T_3)			Total ($T+T_3$)
	α	β&γ	δ	α	β&γ	δ	
CNO	nd	nd	nd	2.3±0.3	0.5±0.1	0.3±0.03	3.1
RBO	12±0.6	20.2±1.2	1.8±0.07	14±0.8	51.3±2.4	0.86±0.2	100.1
CNO+RBO(B)	9.6±0.3	16.1±1.1	1.4±0.04	11.1±0.6	41.1±2.3	0.6±0.02	79.9
CNO+RBO(I)	9.1±0.4	15.9±1.1	1.4±0.08	10.6±0.7	41.5±2.1	0.6±0.03	79.1
SESO	nd	67.2±2.3	3.3±0.4	nd	nd	nd	70.5
CNO+SESO(B)	nd	51.4±1.6	2.1±0.2	0.33±0.2	0.11±0.04	0.06±0.01	54.0
CNO+SESO(I)	nd	5 2.2±1.8	2.5±0.1	0.5±0.01	0.1±0.05	0.06±0.01	55.36

Values are mean ± S.D of triplicate samples. nd- not detected. CNO- coconut oil, RBO- rice bran oil, SESO- sesame oil, B- blended, I- interesterified.

b). Oryzanol and sesame lignans

	Oryzanol	Sesame lignans (mg/100g oil)		
	content (%)	Sesamin	Sesamolin	sesamol
CNO	nd	nd	nd	nd
RBO	1.25±0.08	nd	nd	nd
CNO+RBO(B)	1.02±0.07	nd	nd	nd
CNO+RBO(I)	1.02±0.08	nd	nd	nd
SESO	nd	98.2±2.7	166.4±2.3	0.75±0.02
CNO+SESO(B)	nd	72.3±1.3	122.9±2.1	0.49±0.04
CNO+SESO(I)	nd	72.3±1.7	123.0±1.2	0.48±0.02

Values are mean ± S.D of triplicate samples. nd- not detected. CNO- coconut oil, RBO- rice bran oil, SESO- sesame oil, B- blended, I- interesterified.

These lignans were not found in other oils. Blending CNO with SESO resulted in enriching the blended oil with sesamin, sesamolin and sesamol content at 72.3, 122.9 and 0.49 mg/100 g oil, respectively which was not present in coconut oil. The content of these minor constituent in interesterified oils was similar to that found in blended oils.

Effect of dietary lipids on growth parameters

The amount of diet consumed by rats in each group was similar. The average food intake was 12.1±0.23 g/rat daily (combined mean±SD of all groups) (Table 2.4). There was no significant change in the food efficiency ratio measured by gain in body weight to the amount of food consumed. Weight of the liver and heart were similar in rats given different dietary fats. Though marginally higher weight for brain was observed in rats given RBO, it was not statistically significant. Histological examination of organs showed no abnormalities. This study indicated that feeding blended and interesterified oils had no adverse effect on growth and general health of animals.

Table 2.4. Growth and organ weights of rats fed native, blended and interesterified oils

	Food intake g/day	Total gain in body wt. (g)	FER	Liver wt. (g/100g body wt)	Heart wt. (g/100g body wt)	Brain wt. (g/100g body wt)
CNO	12.5±0.95	235±2.0	0.27±0.008	3.3±0.28	0.29±0.02	0.56±0.06
RBO	11.7±1.3	216±49	0.27±0.03	3.4±0.09	0.28±0.05	0.63±0.06
CNO+RBO (B)	12.1±0.69	222±24	0.27±0.01	3.7±0.4	0.28±0.02	0.57±0.06
CNO+RBO (I)	11.9±0.99	219±35	0.27±0.02	3.5±0.2	0.27±0.04	0.61±0.07
SESO	12.2±0.094	219±24	0.27±0.04	3.5±0.02	0.28±0.03	0.56±0.06
CNO+SESO(B)	12.2±1.3	238±27	0.28±0.01	3.5±0.2	0.28±0.01	0.56±0.07
CNO+SESO(I)	12.1±1.0	260±18	0.27±0.02	3.6±0.4	0.27±0.01	0.57±0.06

FER: food efficiency ratio. Values shown are the mean ± S.D of 4 rats. CNO- coconut oil, RBO- rice bran oil, SESO- sesame oil, B- blended, I- interesterified.

Serum lipid profile of rats fed blended and interesterified oils of CNO with RBO

Type of fat consumed altered the cholesterol concentration in serum. Rats fed CNO had serum total cholesterol concentration of 66.2 mg/dL, (Table 2.5a) while those fed RBO had serum cholesterol concentration of 42.7 mg/dL. Thus a 35.5% decrease in serum cholesterol concentration was observed in rats given RBO compared to those fed CNO. The LDL cholesterol and TAG were decreased in rats fed RBO by 49.6 and 25.4%, respectively. Rats fed blended oil consisting of CNO+RBO had a serum cholesterol concentration of 50.4 mg/dL, which was 23.8% lower than in rats given CNO (Table 2.5a). LDL cholesterol and TAG concentration in serum also decreased in rats fed blended oils as compared to rats given CNO. Rats fed blended oils of CNO+RBO showed 32.4 and 13.9% decrease, respectively in LDL cholesterol and TAG in serum compared those fed CNO. The rats fed interesterified oil consisted of CNO+RBO had significantly lower concentration of serum cholesterol by 14.6 and 35% compared to rats fed blended oil of CNO+RBO and CNO alone, respectively (Table 2.5a). The cholesterol concentrations in rats fed interesterified oil of CNO and RBO was comparable to those given RBO alone (Table 2.5a). LDL cholesterol and TAG concentrations in serum of rats fed interesterified oils of CNO+RBO showed 49.2 and 23% reduction compared to those fed CNO and by 24.7 and 10.7% less compared to those fed its blend CNO+RBO.

Table 2.5a. Serum lipid profile (mg/dL) of rats fed blended and interesterified oils of CNO with RBO

	CNO	RBO	CNO+RBO (B)	CNO+RBO (I)
Total cholesterol	66.2±3.6^a	42.7±1.8^b	50.4±3.0^c	43.0±2.0^b
HDL cholesterol	17.1±2.1^a	18.0±1.4^a	17.2±1.0^a	18.1±1.0^a
LDL cholesterol	49.1±2.1^a	24.8±1.8^b	33.2±2.3^c	25.0±1.0^b
TAG	147±5.9^a	110±2.5^b	127±2.6^c	114±1.6^b
Phospholipids	103±8.0^a	104±5.9^a	102±8.0^a	93±2.4^a

Values show the mean ± S.D of 4 rats. Values in the same row with common superscript letters are not significantly different whereas values with different superscript are significantly different at P<0.01. CNO- coconut oil, RBO- rice bran oil, B- blended, I- interesterified.

Serum lipid profile of rats fed blended and interesterified oils of CNO with SESO

Rats fed SESO had a serum cholesterol concentration of 50.7 mg/dL, which was 23.4% lower than that observed in rats, fed CNO. The LDL cholesterol and TAG was decreased in rats fed SESO was reduced by 38.6 and 22.6%, respectively as compared to the rats given CNO. When CNO was blended with SESO to get a balanced fatty acid composition and fed to rats, the serum cholesterol was found to be 52.6 mg/dL, which was at 20.5% lower concentration as compared to rats fed CNO. LDL cholesterol and TAG concentrations in rats fed blended oil consisting of CNO+SESO showed 34 and 12.9% decrease, respectively compared to the rats given CNO (Table 2.5b). Rats given interesterified oils of CNO+SESO showed decrease in the serum cholesterol concentrations by 16% compared to those given blended oil of CNO+SESO. The rats given interesterified oils of CNO+SESO showed a reduction of 47.0 and 19.8% in LDL cholesterol and TAG concentration, respectively compared to the rats given CNO and by 19.6 and 7.8% reduction compared to rats fed blended oil of CNO+SESO. The HDL and phospholipids concentrations however remained unaltered in all the groups (Table 2.5b).

Table 2.5b. Serum lipid profile (mg/dL) of rats fed blended and interesterified oils of CNO with SESO

	CNO	SESO	CNO+SESO (B)	CNO+SESO (I)
Total cholesterol	66.2±3.6^{a}	50.7±2.1^{c}	52.6±3.4^{c}	44.1±2.8^{b}
HDL cholesterol	17.1±2.1^{a}	20.5±1.0^{b}	20.3±1.0^{b}	18.7±1.0^{a}
LDL cholesterol	49.1±2.1^{a}	30.2±1.6^{c}	32.4±2.4^{c}	26.0±2.0^{b}
TAG	147±5.9^{a}	128±2.9^{c}	130±3.7^{c}	118±1.5^{b}
Phospholipids	103±8.0^{a}	101±7.8^{a}	90±5.8^{a}	94±5.9^{a}

Values show the mean ± S.D of 4 rats. Values in the same row with common superscript letters are not significantly different whereas values with different superscript are significantly different at $P<0.01$. CNO- coconut oil, SESO- sesame oil, B- blended, I- interesterified.

Liver lipid profiles of rats fed blended and interesterified oils of CNO with RBO

Liver is an important site for lipid metabolism. Total cholesterol in liver of rats fed CNO was 6.2 mg/g tissue, while those fed RBO had liver cholesterol concentration of 4.6 mg/g (Table 2.6a). Thus a 25.8% decrease in liver cholesterol was observed in rats given RBO compared to those fed CNO. The liver triacylglycerol concentration was also altered by the type of fat given to the rats. Rats fed with RBO showed a decrease in triacylglycerol level by 27% in rats fed RBO as compared to rats fed with CNO (Table 2.6a). Rats fed blended oil consisting of CNO+RBO showed significant decrease in total cholesterol and triacylglycerol by 16% and 13.4%, respectively compared to the rats given CNO (Table 2.6a). The rats fed interesterified oils of CNO+RBO combinations showed decrease in total cholesterol and triacylglycerols by 27.4 and 32.9% compared to the rats given CNO alone and by 13.5 and 17.8% compared to rats fed blended oils of CNO+RBO (Table 2.6a). There was no difference in hepatic phospholipids of rats given different dietary lipids.

Table 2.6a. Liver lipid profiles (mg/g tissue) of rats fed blended and interesterified oils of CNO with RBO

	CNO	RBO	CNO+RBO(B)	CNO+RBO(I)
Total cholesterol	6.2 ± 0.2^{a}	4.6 ± 0.4^{b}	5.2 ± 0.16^{c}	4.5 ± 0.1^{b}
TAG	18.5 ± 1.1^{a}	13.5 ± 0.8^{b}	15.1 ± 0.5^{c}	12.4 ± 0.8^{b}
Phospholipids	18.1 ± 1.5^{a}	16.5 ± 1.7^{a}	17.1 ± 1.2^{a}	18.1 ± 1.4^{a}

Values show the mean ± S.D of 4 rats. Values in the same row with common superscript letters are not significantly different whereas values with different superscript are significantly different at $P<0.01$. CNO- coconut oil, RBO- rice bran oil, SESO- sesame oil, B- blended, I- interesterified.

Liver lipid profiles of rats fed blended and interesterified oils of CNO with SESO

Liver cholesterol concentration of rats fed SESO was 5.5 mg/g tissue, which was 11.2% lower than that observed in rats fed CNO (Table 2.6b). Rats fed with SESO showed a decrease in triacylglycerol level by 16.7% when compared to rats fed CNO. Feeding rats with blended oil of CNO+SESO showed a decrease in cholesterol

and triacylglycerol concentration by 8.1 and 10.8%, respectively compared to rats given CNO alone (Table 2.6b). Rats given interesterified oils of CNO+SESO showed a decrease in cholesterol and triacylglycerols by 17.7 and 25.9%, respectively as compared to rats given CNO alone and by 10.5 and 16.7% compared to rats fed blended oils of CNO+SESO (Table 2.6b). The phospholipids composition in liver remained unaltered irrespective of the type of fat fed to rats.

Table 2.6b. Liver lipid profiles (mg/g tissue) of rats fed blended and interesterified oils of CNO with SESO

	CNO	SESO	CNO+SESO(B)	CNO+SESO(I)
Total cholesterol	6.2 ± 0.2^{a}	5.5 ± 0.2^{c}	5.7 ± 0.1^{c}	5.1 ± 0.1^{b}
TAG	18.5 ± 1.1^{a}	15.4 ± 0.5^{c}	16.5 ± 0.6^{c}	13.7 ± 0.7^{b}
Phospholipids	18.1 ± 1.5^{a}	17.6 ± 0.9^{a}	17.9 ± 0.2^{a}	17.5 ± 0.9^{a}

Values show the mean ± S.D of 4 rats. Values in the same row with common superscript letters are not significantly different whereas values with different superscript are significantly different at $P<0.01$. CNO- coconut oil, RBO- rice bran oil, SESO- sesame oil, B- blended, I- interesterified.

Fatty acid composition of serum and liver lipids.

Fatty acid analysis of serum lipids reflected on the type of fat fed to rats. Linoleic acid (18:2) and arachidonic acid (20:4) content of rats fed CNO was significantly lower than that in rats fed RBO or SESO (Table 2.7). When rats fed blended and interesterified oils of CNO with RBO or SESO there was a significant increase in linoleic and arachidonic content in serum fatty acids compared to rats fed CNO alone (Table 2.7). The linoleic acid concentration in serum lipids was enhanced by 1.94 fold and 2.28 fold in rats given CNO+RBO blended and CNO+RBO interesterified oils, respectively as compared to those given CNO alone. Similarly, the linoleic acid concentration was enhanced by 2.52 fold and 2.91 fold in rats given CNO+SESO blended and interesterified oils, respectively compared to those given CNO alone (Table 2.7). The arachidonic acid concentrations were also enhanced by 1.7 to 2.2 folds in rats given blended or interesterified oils as compared to those given CNO

alone. This indicated the improved EFA status in rats given blended or interesterified oils compared to rats fed CNO.

Table 2.7. Fatty acid composition (wt %) of serum in rats fed native, blended and interesterified oils

	CNO	RBO	CNO+RBO (B)	CNO+RBO (I)	SESO	CNO+SESO (B)	CNO+SESO (I)
12:0	0.9±0.2 [a]	nd	nd	nd	nd	nd	nd
14:0	6.2±0.6[a]	1.7±0.4[b]	0.9±0.03 [b]	0.81±0.02 [b]	2.4±0.7 [b]	2.4±0.2 [b]	1.3±0.01[b]
16:0	28.8±1.4[a]	24.8±1.8[a]	27.0±2.0[a]	27.0±1.8[a]	25.2±1.1[a]	27.9±1.8[a]	27.1±2.1[a]
16:1	5.6±0.2[a]	1.9±0.3[a]	2.8±0.01[a]	2.4±0.1[a]	1.3±0.08[a]	3.2±0.04[a]	2.5±1.1[a]
18:0	10.4±0.7[a]	12.3±1.6[a]	12.8±0.2[a]	10.3±0.3[a]	11.3±0.3[a]	8.9±0.6[a]	9.9±0.4[a]
18:1	34.9±3.0[a]	32.0±2.6[a]	28.4±2.1[a]	32.7±1.2[a]	29.4±1.8[a]	27.6±1.7[a]	27.6±2.0[a]
18:2	6.8±0.1 [a]	16.1±0.5[b]	13.2±0.3[b]	15.5±0.3 [b]	19.0±1.2[b]	17.5±1.6[b]	20.2±0.9[b]
20:4	6.4±0.2 [a]	11.1±0.6[b]	14.3±0.9[b]	11.2±0.5 [b]	11.3±0.6[b]	13.1±0.8[b]	10.9±0.7[b]
P/S ratio	0.29	0.71	0.68	0.70	0.78	0.73	0.81

Values show the mean ± S.D of 4 rats. Values in the same row with common superscript letters are not significantly different whereas values with different superscript are significantly different at $P<0.01$. nd- not detected. CNO- coconut oil, RBO- rice bran oil, SESO- sesame oil, B- blended, I- interesterified.

The fatty acid composition of liver lipids also followed a similar pattern as that of the serum. Rats fed CNO had significantly lower linoleic acid and arachidonic acid content in liver lipids compared to rats fed RBO and SESO (Table 2.8). Feeding rats with blended and interesterified oils of CNO+RBO and CNO+SESO significantly enhanced linoleic and arachidonic acid compared rats given CNO alone. Rats fed blended oils of CNO+RBO or CNO+SESO showed 2.36 and 2.85 fold increase in linoleic acid concentration compared to rats given CNO alone. The rats given interesterified oils of CNO+RBO or CNO+SESO had 2 and 2.9 fold more linoleic acid content compared to those given CNO. Rats fed blended or interesterified oils of CNO+RBO and CNO+SESO had 1.57-2 fold increase in arachidonic acid content compared to rats fed CNO alone (Table 2.8).

Table 2.8. Fatty acid composition (wt%) of liver in rats fed native, blended and interesterified oils

	CNO	RBO	CNO+RBO (B)	CNO+RBO (I)	SESO	CNO+SESO (B)	CNO+SESO (I)
12:0	0.4±0.01 [a]	nd	nd	nd	nd	nd	nd
14:0	1.7±0.25[a]	nd	0.9±0.15[a]	1.2±0.02[a]	0.4±0.01[a]	0.3±0.02[a]	0.7± 0.2[a]
16:0	29.7±1.7[a]	24.3±1.9[a]	26.7±1.5[a]	26.5±1.4[a]	22.1±1.3[a]	25.3±1.0[a]	24.9±2.3[a]
16:1	4.7±0.1[a]	2.7±0.8[a]	2.4±0.04[a]	2.2±0.7[a]	1.3±0.02[a]	1.5±0.03[a]	1.6±0.04[a]
18:0	17.6±1.2[a]	12.0±1.4[a]	15.3±1.5[a]	10.5±0.8[a]	14.3±1.1[a]	12.8±0.5[a]	11.7±0.6[a]
18:1	33.2±1.2[a]	37.0±1.7[a]	29.0±2.1[a]	37.2±3.3[a]	33.2±2.1[a]	30.5±3.0[a]	33.6±2.9[a]
18:2	5.5±0.3[a]	13.6±1.2[b]	13.0±0.5[b]	11.5±0.1[b]	14.9±0.8[b]	15.7±0.9 [b]	16.0±0.3 [b]
20:4	6.8±0.4[a]	10.3±0.1[b]	12.5±0.2[b]	10.7±0.7[b]	13.8±1.0[b]	13.7±1.8 [b]	11.1±1.8 [b]
P/S ratio	0.25	0.64	0.59	0.58	0.78	0.76	0.72

Values show the mean ± S.D of 4 rats. Values in the same row with common superscript letters are not significantly different whereas values with different superscript are significantly different at P<0.01. nd- not detected. CNO- coconut oil, RBO- rice bran oil, SESO- sesame oil, B- blended, I- interesterified.

DISCUSSION

Dietary fat exhibit different physicochemical and nutritional properties depending on the type of fatty acid it contains. CNO is a rich source of medium chain saturated fatty acids. Medium chain fatty acids (MCFA) per se has many desirable characteristics such as high oxidative stability, low viscosity, low melting point and high solubility in water which makes CNO useful for Indian culinary. In India 80% of oil is used for frying dishes where they are subjected high temperatures of 180 °C and above. Hence stability of oil under frying conditions is a desirable quality in Indian cooking. Oils in addition should also provide good nutritional qualities. Even though CNO contains MCFAs, concerns have been expressed as it also contains high amount of myristic acid and palmitic acid, which elevate serum cholesterol (Cantwell, 2000*)*. Coconut oil is limiting in essential fatty acids.

Lesser known oils like RBO and SESO which are also used in India are rich in unsaturated fatty acids and they have hypocholesterolemic effect (Shimizu *et al*., 1991; Penalvo *et al*., 2005). To keep the stability of a saturated fat for culinary purposes and to provide good nutritional qualities one need to balance SFA:MUFA:PUFA, in the oils. Blends of CNO with RBO or SESO were prepared to achieve a balance in different fatty acids to get SFA:MUFA:PUFA in the ratio of 1:1:1 and PUFA/SFA ratio 0.8-1.0. No single oil used in India provides such a balanced amount of fatty acids. The blended oils were also enriched with nutraceuticals, which was not found in CNO.

Blending and interesterification has been used as a useful tool for the modification of the physicochemical properties of oils and fats. Earlier workers have also noticed that blending of oils improve the physicochemical (Rodriguez and Gioielli, 2003; Qi *et al*., 2006) and nutritional properties of the oil. Koba *et al*. (2000) have shown that feeding rats with a diet containing blended oils comprising rice bran oil and safflower oil in the ratio of 70:30 increased the HDL concentrations and increased the HDL/TC ratio significantly (Kang *et al*., 2005). Sugano and Tsuji (1996) explained the specific cholesterol lowering effect of this blended oil based on the combined effect of the linoleic acid from sunflower oil and the unsaponifiable matter present in the RBO. Our present studies have similarly shown a significant cholesterol lowering effect of blended oils consisting of CNO+RBO and CNO+SESO in comparison with rats given CNO alone.

The polyunsaturated/saturated (P/S) fatty acid ratio is critical in determining the atherogenic potency of an oil. Even though increasing dietary PUFA:SFA ratio has been recommended by earlier investigators for reducing risk factors for CVD, a high PUFA:SFA ratio of diet may enhance oxidative stress because PUFAs are highly susceptible to oxidation. Feeding rats with fats having different concentrations of PUFA:SFA, Kang *et al*. (2005) showed that a PUFA:SFA ratio of 1.0-1.5 is a desirable range to reduce risk factors for CVD. It is also interesting to note that when CNO with a P/S ratio of 0.02 was fed to rats the P/S ratio of serum fatty acids were 0.25. However, when this P/S ratio was enhanced to 0.8-1.0 by blending CNO with RBO or with SESO, the P/S ratio in serum fatty acids was increased to 0.68-0.81.

RBO with a P/S ratio 1.5 and SESO with P/S ratio of 2.8 could increase the P/S ratio of serum lipids to 0.71 and 0.78, respectively indicating higher concentrations of P/S ratio of oils and this has limitations to what extent the serum fatty acid composition can be altered. Hence a P/S ratio of 0.8-1.0 in the oil may be suffice to maintain EFA status in the animals.

It is well established that both the quality and quantity of dietary triacylglycerols in the oils can influence plasma cholesterol concentrations in humans and in a range of animal species (Grundy and Denke, 1990). In a blended oil, the individual triacylglycerols from the parent oils retain their native structure. However interesterification reaction rearranges the fatty acids in the triacylglycerols molecules and produces fats with altered properties. Interesterification using lipases randomizes the fatty acid distribution in the triacylglycerols leading to modifications in the chemical composition hence may also change nutritional properties of the oil(s) (Ronne *et al*., 2005; Marangoni and Rousseau, 1998).

These changes in the TAG molecular species making the interesterified oil different from blended oil, result in the changes in the physical properties which in turn influence the digestion and absorption of fat (Small, 1991) and thereby nutritional properties. Enzymatic interesterification lowered the solid fat content of butter fat above 20 °C when it is interesterified with 40 and 30 % canola oil (Rousseau and Marangoni, 1998). Earlier studies by Kritchevski *et al*. (1982) have shown that randomization of peanut oil reduces its atherogenecity by 37%. Similarly atherogenecity of lard was reduced when subjected to interesterification and fed to rabbits on cholesterol enriched diets (Kritchevski and Tepper, 1977). Randomized butter has been shown to reduce serum cholesterol in man by 211% (Christophe *et al*., 1978). All these studies show that structure of fat and composition can influence the cholesterolemia and atherogenesis. Straarup and Hoy, (2000) have shown that structured TAG containing MCFAs may have some beneficial effects, improve fat absorption in normal and malabsorbing rats. Structured lipids containing medium and long chain fatty acids prevent body fat accumulation in healthy subjects (Kasai *et al*., 2003). Oba and Witholt (1994) showed the incorporation of about 50% oleic acid in milk fat which was interesterified with oleic acid using immobilized *Rhizopus oryzae*

lipase. Randomized structured triacylglycerols improved the digestion, absorption and lymphatic transport of lipids and fat-soluble vitamins (Tso *et al*., 2001). Structured triacylglycerol that contain one or two medium chain fatty acids may provide a vehicle for rapid hydrolysis and absorption due to their smaller molecular size and greater water solubility in comparison to long chain triacylglycerol (Tso *et al*., 1995). Feeding rats with structured lipid significantly reduced the liver cholesterol concentration by 27% compared to the blend of coconut and safflower oil (1:0.7 ratio) with similar fatty acid composition (Rao and Lokesh, 2003).

In the present investigation we studied the effect of feeding the interesterified oils on serum and liver lipid parameters. Rats fed interesterified oils of CNO+RBO showed decreased cholesterol concentration compared to rats fed blended oil of CNO+RBO. Similarly rats fed interesterified fats containing CNO+SESO showed a significant decrease in serum and hepatic cholesterol concentrations compared to rats given blended oil of CNO+SESO of similar composition. Therefore interesterified fats showed higher hypolipidemic effect compared to blended oils. This is in agreement with earlier observations on the efficacy of interesterified oil compared to blended oil in beneficially modifying many metabolic effects (Rao and Lokesh, 2003).

The ability of a fat to alter serum lipids depends not only on the type of fatty acid but also on the minor constituent present in the oil. Studies on humans (Most *et al*., 2005) have shown that the cholesterol reducing property of RBO is due to the unsaponifiable compounds present in the oil. Oryzanol lowers the cholesterol concentration by suppressing cholesterol absorption and enhancing the fecal sterol excretion (Wilson *et al*., 2007; Ausman *et al*., 2005). RBO is also rich in β- and γ-tocotrienols (Sugano and Tsuji, 1997). Studies on humans have shown that tocotrienols lower cholesterol through the inhibition of HMG-CoA reductase, the rate limiting enzyme in cholesterol biosynthesis (Kerckhoffs *et al*., 2002). The tocotrienol rich fraction of RBO is also reported to lower serum total cholesterol and LDL cholesterol concentrations in hypercholesterolmic individuals (Qureshi *et al*., 1997; Wilson *et al*., 2007). Feeding oryzanol at 0.5% concentration showed cholesterol lowering effect in hamsters (Qureshi *et al*., 2002) and 0.2% concentration of oryzanol

was effective in reducing the cholesterol concentration in rats (Seetharamaiah and Chandrasekhara, 1988). In the present study rats consumed a diet containing 0.125% oryzanol in the groups given RBO alone and 0.102% by rats given blended and interesterified oils of CNO+RBO. This may also have been contributed to cholesterol lowering effect of CNO+RBO combinations.

Sesamin from SESO has been proven to be hypocholesterolemic. Feeding rats with 0.5% sesamin showed significant decrease in cholesterol absorption by 30% compared with control rats (Hirose *et al*., 1991). Studies by Chen *et al.* (2005) have shown that consumption of 0.6% of sesamin and sesamolin exhibit hypolipidemic effect in humans (Chen *et al*., 2005). In the present study rats consumed a diet containing 0.2% and 0.33% sesamin and sesamolin daily when fed with SESO alone, 0.16 and 0.26% sesamin and sesamolin when rats were given blended and interesterified oils of CNO+SESO. Hence sufficient lignans were provided to rats given blended and interesterified oils containing SESO. Sesamin is the precursor of enterolactone (Penalvo *et al*., 2005), which reduces the risk of acute coronary events (Varharanta *et al*., 1999). Hence sesamin may have also contributed to cholesterol lowering effect of CNO+RBO combinations. However the individual contribution of oryzanol in CNO+RBO and sesamin in CNO+SESO are yet to be quantified.

In conclusion the present study was under taken to balance the fatty acid composition of saturated:monounsaturated:polyunsaturated fatty acids in 1:1:1 ratio by blending CNO with RBO or SESO. This also resulted in an oil with P/S ratio of 0.8-1.0. Feeding rats with these blended oils resulted in lowering the hypercholesterolemic effect of CNO. When the blended oils were subjected to interesterification and fed to rats it further reduced serum and liver cholesterol. A higher hypolipidemic effect of interesterified oils was observed even though both blended and interesterified oils had similar fatty acid compositions. The molecular mechanism involved in the cholesterol lowering property of blended and interesterified oils is discussed in next chapter.

INTRODUCTION

Dietary lipids influence many aspects of lipid metabolism and the extent of its effect is dependent on the chain length, number and position of double bonds and geometry of double bonds of fatty acid they contain. The fatty acids act as substrates in several metabolic pathways and their conversion to biologically active molecules such as eicosanoids, represents mechanisms whereby they exert their effect (Salter and Tarling, 2007). However, studies during last decade suggest that fatty acids and/ or their derivatives regulate the various metabolic pathways at the genomic level (Jump and Clarke, 1999; Pegorier *et al*., 2004; Davidson, 2006). Several studies have shown that polyunsaturated fatty acids (PUFAs) affect the expression of proteins involved in lipid metabolism (Sampath and Ntambi, 2005). PUFAs down regulate the expression of genes for enzymes involved in fatty acid synthesis such as acetyl-CoA carboxylase, fatty acid synthase, and stearoyl-CoA desaturase (Jump and Clarke, 1999; Sampath and Ntambi, 2005). Studies by Sanderson *et al*. (2008) have shown that the expression of peroxisome proliferator activated receptor (PPAR) -α gene is regulated by unsaturated fatty acids in mouse liver. In this study it was found that eicosapentaenoic acid is the most potent activator of PPAR-α.

Dietary fat plays an important role in modulating risk factors for cardiovascular diseases (CVD) (Lairon *et al*., 2009). Long chain saturated fatty acids (SFA) increase serum total cholesterol and low density lipoprotein cholesterol, important risk factors for CVD (Cantwell, 2000), whereas unsaturated fatty acids decrease serum lipids (Hegsted *et al*., 1993). Therefore the levels of cholesterol in the serum need to be controlled.

The liver plays a central role in cholesterol homeostasis, which is modulated by coordinated changes in the levels of mRNAs encoding multiple enzymes involved in the cholesterol biosynthesis, uptake and efflux pathway (Matsuyama *et al*., 2005). HMG-CoA reductase is the rate limiting enzyme in cholesterol biosynthesis. Cholesterol 7-α-hydroxylase (CYP7A1), a liver specific enzyme catalyzes the first and rate limiting step in bile acid biosynthesis, a major cholesterol efflux pathway (Cheema *et al*., 1997). LDL receptors play a vital role in the hepatic uptake and clearance of plasma cholesterol (Rudling, 1992). Sterol regulatory element binding

protein (SREBP) -2, a transcription factor, is involved in cholesterol homeostasis by virtue of its ability to bind and activate the promoters of the genes encoding for LDL receptor and HMG-CoA reductase (Miserez *et al*., 2002). Both the LDL receptor and HMG-CoA reductase genes have a sterol regulatory element in their promoter regions and are therefore commonly regulated by SREBP-2 (Sato *et al*., 1999).

We have earlier prepared blended oils to contain equal proportions of SFA, MUFA and PUFA. These oils were subjected to interesterification reactions using lipase. We noticed that interesterified oils showed better hypocholesterolemic effect as compared to that found in rats given blended oils with similar fatty acid composition reported in chapter II. Most of the research work reported in literature has been focused on the effect of native oils on total and LDL cholesterol level and on the expression of genes involved in cholesterol metabolism. However the reason for higher cholesterol lowering property of interesterified oil as compared to that observed by feeding blended oil with similar fatty acid composition reported in our study is not known. The present investigation was therefore undertaken to study the molecular mechanism involved in the hypocholesterolemic effects observed with blended and interesterified oil by monitoring the mRNA abundance of hepatic HMG-CoA reductase, CYP7A1, the LDL receptor, SREBP-2, proteins involved in cholesterol metabolism.

RESULTS

Fatty acid composition of dietary lipids

The fatty acid composition of the dietary fat used in this study is similar to that used in previous studies (Chapter II) (Table 3.1)

Serum lipid profile

Changes in the serum cholesterol content in rats which were given different dietary lipids are given in Table 3.2**.** The values obtained for serum cholesterol concentration in rats fed with CNO, RBO, SESO, blended and interesterified oils were comparable with the results of previous study (Chapter II). Rats fed diet containing CNO showed higher level of total cholesterol and VLDL+LDL cholesterol as compared to the rats fed with RBO or SESO.

Table 3.1. Fatty acid composition (wt%) of dietary lipids

	CNO	RBO	CNO+RBO (B)	CNO+RBO (I)	SESO	CNO+SESO (B)	CNO+SESO (I)
8:0	2.9±0.4	nd	0.6±0.1	0.5±0.1	nd	0.8±0.1	1.0±0.2
10:0	5.1±0.3	nd	1.2±0.1	1.2±0.2	nd	1.5±0.1	1.4±0.1
12:0	50.1±1.4	nd	10.2±0.3	10.5±0.3	nd	13.3±0.3	11.7±0.4
14:0	21.2±0.9	nd	4.3±0.2	4.7±0.3	nd	4.9±0.2	4.3±0.2
16:0	9.0±0.3	20.2±1.2	17.7±0.4	17.3±0.5	10.3±0.3	9.5±0.4	9.3±0.2
18:0	2.7±0.2	1.6±0.2	1.7±0.1	1.7±0.2	5.6±0.3	4.6±0.2	4.7±0.3
18:1	7.2±0.4	42.6±2.3	35.1±1.1	34.3±1.3	42.6±1.5	33.5±0.6	34.5±1.2
18:2	1.8±0.1	35.1±1.8	28.8±0.9	29.4±0.7	41.1±1.0	31.6±0.9	32.8±0.8
18:3	nd	0.5±0.1	0.4±0.1	0.4±0.1	0.4±0.1	0.3±0.1	0.3±0.1
S:M:P ratio	1:0.08:0.02	1:1.95:1.6	1:0.98:0.81	1:0.96:0.83	1:2.7:2.6	1:0.96:0.91	1:1.06:1.02

Values are mean ± SD of three samples. nd- not detected (Limit of detection; 0.3%). CNO- coconut oil, RBO- rice bran oil, SESO- sesame oil, (B)- blended, (I)- interesterified, S:M:P- saturated:monounsaturated:polyunsaturated fatty acids.

The cholesterol levels were also reduced significantly in rats fed with the CNO+RBO and CNO+SESO blends compared to those fed with CNO alone. Rats fed interesterified oils CNO+RBO (I) or CNO+SESO(I) showed a further decrease in serum cholesterol when compared to those fed blended oils of CNO with RBO or SESO (Table 3.2a & 3.2b). It is interesting to note here that the rats fed interesterified oil had significantly lower levels of cholesterol when compared to those fed blended oils with similar fatty acid composition. The rats fed with blended oils showed a decrease in total cholesterol by 24-26%, while rats fed interesterified oils showed a decrease in total cholesterol by 36-39% when compared to rats given CNO. These decreases were observed in LDL cholesterol while HDL cholesterol remained unaffected.

Table 3.2a. Serum lipid profile (mg/dL) of rats fed blended and interesterified oils of CNO with RBO

	CNO	RBO	CNO+RBO(B)	CNO+RBO(I)
Total Cholesterol	76.5±3.1 ^{a}	46.2±1.6 ^{b}	56.3±2.1 ^{c}	48.6±2.4 ^{b}
HDL cholesterol	22.1±1.9 ^{a}	20.2±1.3 ^{a}	20.5±1.7^{a}	19.8±0.64^{a}
LDL+VLDL cholesterol	54.4±2.2 ^{a}	28.3±3.3 ^{b}	35.7±1.1^{c}	26.4±1.9 ^{b}

Values show the mean ± S.D of 4 rats. Values in the same row with common superscript letters are not significantly different whereas values with different superscript are significantly different at P<0.01. CNO-coconut oil, RBO- rice bran oil, B- blended, I-interesterified.

Table 3.2b. Serum lipid profile (mg/dL) of rats fed blended and interesterified oils of CNO with SESO

	CNO	SESO	CNO+SESO(B)	CNO+SESO(I)
Total Cholesterol	76.5±3.1 ^{a}	50.1±1.9^{b}	57.2±2.6 ^{c}	46.3±2.4 ^{b}
HDL cholesterol	22.1±1.9 ^{a}	20.5±1.1 ^{a}	21.1±1.0^{a}	19.8±1.1^{a}
LDL+VLDL cholesterol	54.4±2.2 ^{a}	29.6±1.3 ^{b}	36.1±2.1^{c}	26.5±1.4 ^{b}

Values show the mean ± S.D of 4 rats. Values in the same row with common superscript letters are not significantly different whereas values with different superscript are significantly different at P<0.01. CNO-coconut oil, SESO- sesame oil, B- blended, I-interesterified.

HMG-CoA reductase activity

HMG-CoA reductase is the rate limiting enzyme in cholesterol biosynthesis. Feeding of rats with a diet containing CNO lowered the activity of HMG-CoA reductase as compared to that found in rats given RBO (by 23%) or SESO (by 18%) (Table 3.3). This may be due to feed back inhibition of the enzyme by high amount of cholesterol found in serum of rats given CNO (Table 3.2). Rats fed with blended oils showed marginally increased HMG-CoA reductase activity by 5-10% compared to those fed with CNO (Table 3.3). Rats fed interesterified oils showed increased activity of HMG-CoA reductase by 17-18% as compared to those fed CNO. However the difference observed in HMG-CoA reductase activity in rats given blended oil and interesterified oils were not statistically significant.

Table 3.3. Influence of native, blended and interesterified oils on HMG-CoA reductase activity

Dietary fat	HMG-CoA reductase activity (pmoles of CoA formed/min/mg protein)
CNO	220 ± 12^{a}
RBO	285 ± 20^{b}
CNO+RBO(B)	231 ± 14^{c}
CNO+RBO(I)	258 ± 18^{ed}
SESO	267 ± 21^{e}
CNO+SESO(B)	244 ± 18^{d}
CNO+SESO(I)	260 ± 17^{d}

Values show the mean ± S.D of 4 rats. Values in the same column with common superscript letters are not significantly different whereas values with different superscript are significantly different at $P<0.01$. CNO-coconut oil, RBO- rice bran oil, SESO- sesame oil, B-blended, I- interesterified.

RNA isolation

In order to evaluate effect of dietary lipids on mRNA abundance of genes involved in cholesterol metabolism, total RNA was extracted from liver stored at -70 °C, following the TRI reagent method (Figure 3.1). The presence of two bands of 18S and 5.8S RNA indicated that the preparation was suitable for further studies.

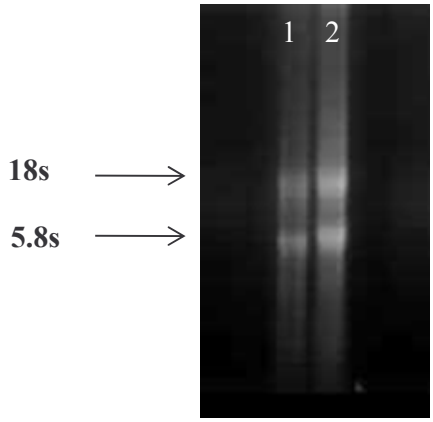


Figure 3.1. Agarose gel electrophoresis of total RNA isolated from liver tissue. Lane 1 CNO fed and Lane 2 CNO+SESO(B) fed.

A semiquantitative hepatic mRNA quantification

The hepatic mRNA abundance of the major genes involved in cholesterol homeostasis was studied as affected by feeding different dietary lipids. HMG-CoA reductase, LDL receptor and CYP7A1 are the major enzymes/proteins involved in the biosynthesis and clearance of whole body cholesterol. SREBP-2 is a key transcription factor which regulates the HMG-CoA reductase and LDL receptor. Beta actin was used as the house keeping gene for normalization. The primer sequences used for the amplification of genes with the expected amplicon length are listed in Table 3.4. Significant differences in the hepatic mRNA abundance were observed when PCR product intensity was measured using Quantity One software. (Figure 3.2). The mRNA levels of HMG-CoA reductase were increased in rats fed on diets containing RBO or SESO when compared to the rats given CNO. These differences in the mRNA abundance were further confirmed by a semiquantitative multiplex–PCR (Figure 3.3) for simultaneous amplification of HMG-CoA reductase and LDL receptor in the same reaction. Rats fed with diet containing blended and interesterified oils showed increased mRNA abundance for LDL receptor. Rats fed with

interesterified oils showed higher elevation of mRNA abundance for LDL receptor and for CYP7A1 compared to those fed with blended oil. The intensity of the beta actin band remained unaltered in all the dietary groups. These results indicated that no artifacts were generated in the experiment.

Table 3.4. List of primers used for PCR

Gene	Accession number	PCR primers	Oligonucleotide length (base pair)
Beta Actin	(EF156276)	F-5'GCC AAC CGT GAA AAG ATG A3' R-5'ATG CCA CAG GAT TCC ATA CCC3'	480
HMG-CoA R	(NM_013134)	F-5'TTT GAA GAG GAC GTG CTG AGC3' R-5'CCT GAC ATG GTG CCA ACT CC3'	401
LDL-R	(NM_175762)	F-5'CAT CTC CCG GCA GTT TGT GT3' R-5'GGC GGC TAC CGT GAA TAC AG3'	401
C-7-alpha H	(U01962)	F-5'TTG CCG TGT TGG TGA GCT GT3' R-5'CCA AAT GCC TTC GCA GAA GTA3'	301
SREBP-2	(NM_001033694)	F-5'CCC AGG TAC ACC AGG CTT TC3' R-5'TCT CAG TCA CTT CCA GGG CC3'	351
Multiplex PCR			
Beta actin-239	(EF156276)	F-5'CAT TGA ACA CGG CAT TGT CAC3' R-5'CAG TGG TAC GAC CAG AGG CAT CA3'	239
HMG-CoA R-286	(NM_013134)	F-5'GTG GTT GGA ATT ATG AGT GCC CA3' R-5'GCA CTC GCT CTA GAA AGG TCA ATC A3'	286
LDL-R485	(NM_175762)	F-5'CTG TGA AAA TGG CTC GGA TGA ACT A3' R-5'ACT GGC GGC TAC CGT GAA TAC A3'	485
Real-time PCR			
Beta actin-116	(EF156276)	F-5'CCT AAG GCC AAC CGT GAA AA3' R-5'CCA GTG GTA CGA CCA GAG GC3'	116
HMG-CoA R-136	(NM_013134)	F-5'AGG TAA TTG TGG GAA CGG TG3' R-5'ATG ATG ATG TCG CTG CTC AG3'	136
LDL-R121	(NM_175762)	F-5'TGA AAA TGG CTC GGA TGA ACT3' R-5'CAG GCA ATC CCA GTC TTG GT3'	121
C-7 alpha H 111	(U01962)	F-5'TAC TTC TGC GAA GGC ATT TGG3' R-5'GAA CAC AGA GCA TCT CCC TGG3'	111
SREBP-2 126	(NM_001033694)	F-5'TGG GCT TCT TGG CTA GCT ACT T3' R-5'TTC GCT CCA TGA AAA ACT TCT G3'	126

F-forward primer, R- reverse primer, HMG-CoA R- HMG-CoA reductase, LDL-R- LDL receptor, C-7 alpha H- cholesterol 7-α-hydroxylase, SREBP-2- sterol regulatory element binding protein-2.

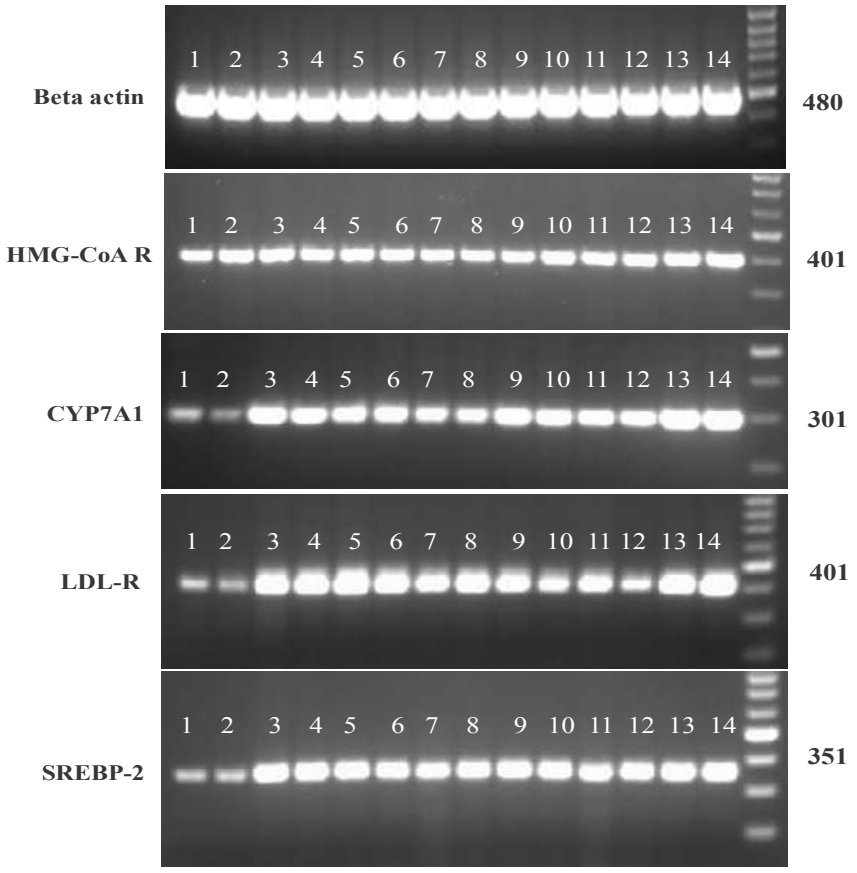


Figure 3.2. Expression of hepatic genes involved in cholesterol homeostasis. HMG-CoA R- HMG-CoA reductase, LDL-R- LDL receptor, CYP7A1- cholesterol 7-α-hydroxylase, SREBP-2- sterol regulatory element binding protein-2, 1&2: coconut oil (CNO); 3&4: rice bran oil (RBO); 5&6: sesame oil (SESO); 7&8: CNO+RBO blended (B); 9&10: CNO+SESO(B); 11&12: CNO+RBO interesterified (I); 13&14: CNO+SESO(I).

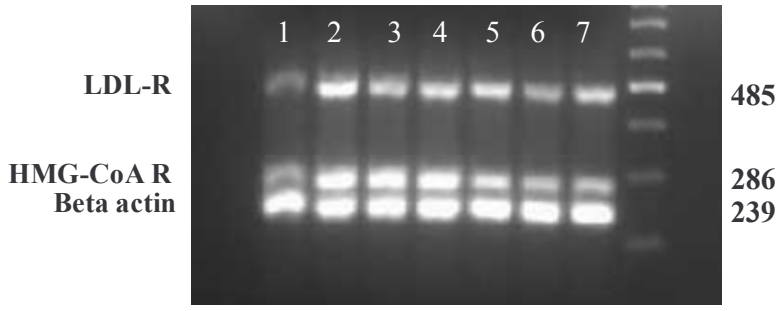


Figure 3.3. Expression of hepatic genes involved in cholesterol homeostasis by multiplex PCR. HMG-CoA R- HMG-CoA reductase, LDL-R- LDL receptor, 1: coconut oil (CNO); 2: rice bran oil (RBO); 3: sesame oil (SESO); 4: CNO+RBO blended (B); 5: CNO+SESO(B); 6: CNO+RBO interesterified (I); 7: CNO+SESO(I).

Influence of dietary fat on hepatic mRNA abundance

Based on semi quantitative analysis reported in the previous section, gene expression studies were carried out for enzymes/proteins involved in cholesterol metabolism using Real Time PCR to quantify the mRNA abundance.

Hepatic HMG-CoA reductase mRNA abundance

The relative mRNA expression of HMG-CoA reductase, the rate limiting enzyme in the cholesterol biosynthetic pathway, as affected by dietary lipids is shown in Figure 3.4a. The mRNA abundance for HMG-CoA reductase was lower in rats fed with CNO compared to that found in other groups. The suppression of HMG-CoA reductase mRNA in rats fed CNO reflected on the lower HMG-CoA reductase activity observed in these groups (Table 3.3). Rats fed with diet containing interesterified oil showed marginally higher mRNA abundance than those fed with blended oil, though this difference is not statistically significant (Figure 3.4a).

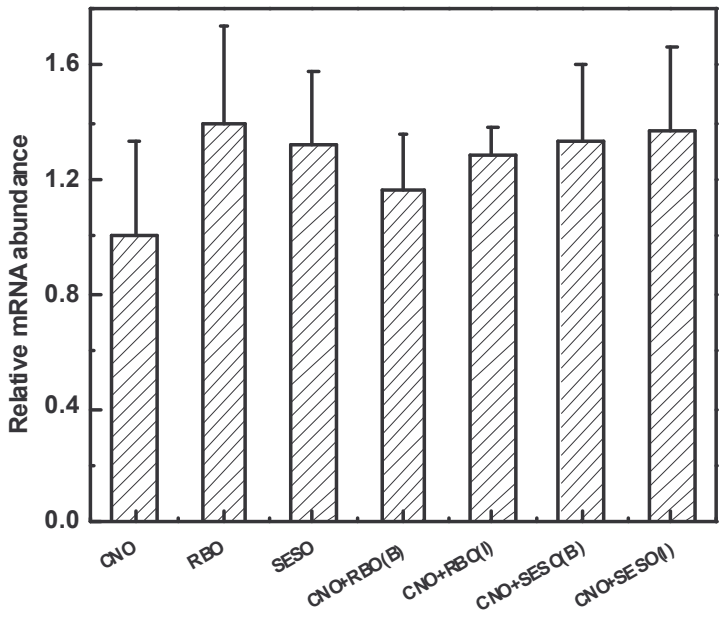


Figure 3.4a. HMG-CoA reductase mRNA expression determined by RT-PCR. CNO- coconut oil, RBO- rice bran oil, SESO- sesame oil, (B)- blended, (I)- interesterified. The data represents the mean of four replicates ±SD from each dietary group. Values are not significantly different at $P<0.05$. The mRNA abundance was calculated in relation to the house keeping gene β-acting. The relative abundance of the CNO fed group was set at 1.

The reduction in mRNA abundance for HMG-CoA reductase for rats on CNO was found to be by 1.39 and 1.33 fold as compared to that found in rats given RBO and SESO respectively. Similarly rats given blended oils of CNO+RBO and CNO+SESO showed reduction in mRNA abundance for HMG-CoA reductase by 1.16 and 1.34 fold respectively when compared to rats fed with CNO. The reduction in mRNA abundance for HMG-CoA reductase for rats given interesterified oils CNO+RBO and CNO+SESO was found to be by 1.29 and 1.37 fold, respectively as compared to that found in rats on CNO.

Cholesterol 7-α-hydroxylase mRNA abundance

CYP7A1 is involved in the catabolism of cholesterol through bile acid synthesis. The mRNA abundance of CYP7A1 increased by 3.7 and 3.4 fold in the rats given RBO and SESO respectively, when compared to the rats given CNO (Figure 3.4b).

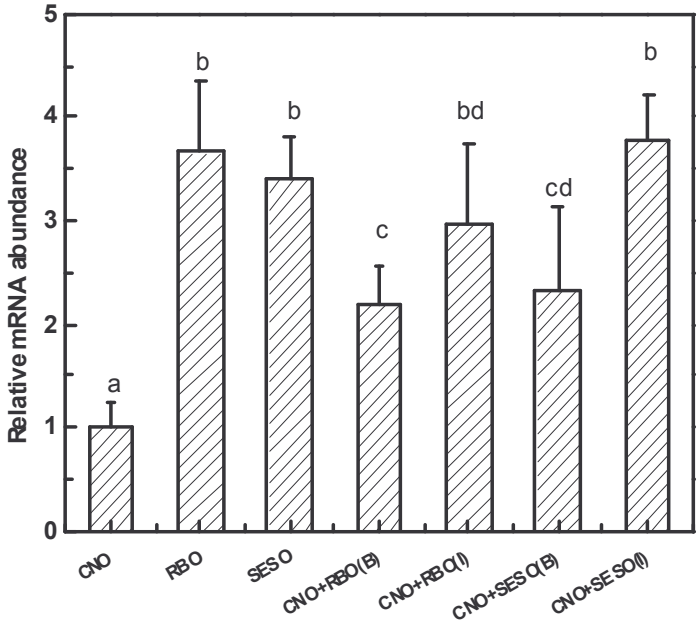


Figure 3.4b. Cholesterol 7-α-hydroxylase mRNA expression determined by RT-PCR. CNO-coconut oil, RBO- rice bran oil, SESO- sesame oil, (B)- blended, (I)- interesterified. The data represents the mean of four replicates ±SD from each dietary group. Values not sharing the common superscript are significantly different whereas values with different superscript are significantly different at $P<0.05$. The mRNA abundance was calculated in relation to the house keeping gene β-acting. The relative abundance of the CNO fed group was set at 1.

Feeding rats with blended oils consisting of CNO with RBO or SESO increased the mRNA levels by 2.2 and 2.3 fold compared to that found in the rats given CNO. Rats fed with interesterified oils showed markedly upregulated expression of the CYP7A1 compared to those fed with blended oils. CYP7A1 mRNA was increased by 3.0 fold in rats given interesterified oil of CNO+RBO and 3.8 fold in rats given CNO+SESO as compared to those given respective blends. These results indicate that the CYP7A1 is upregulated in rats given blended and interesterified oils, but the upregulation was significantly higher in rats given interesterified oils compared to those given blended oils.

Hepatic LDL receptor mRNA abundance

LDL receptor plays a crucial role in the cholesterol uptake from plasma. Hepatic LDL receptor expression in the rats fed CNO was significantly lower than that observed in the rats fed RBO, SESO or their blends with CNO (Figure 3.4c).

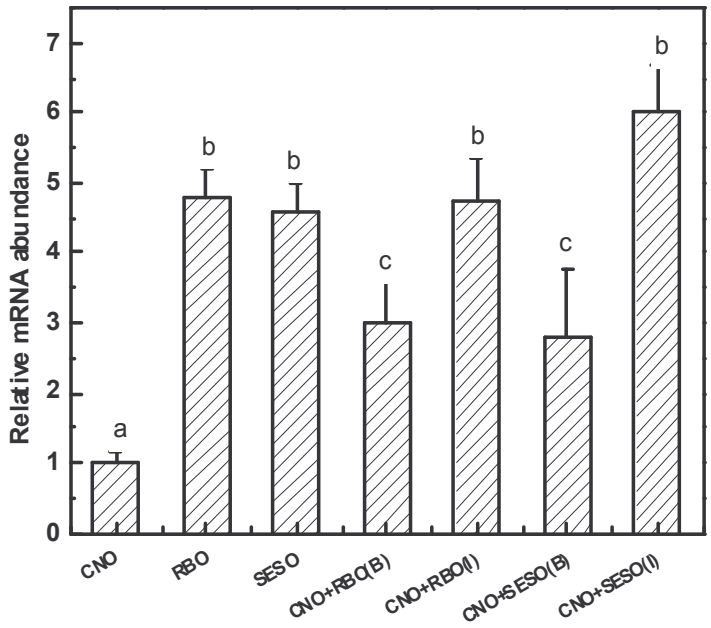


Figure 3.4c. LDL receptor mRNA expression determined by RT-PCR. CNO-coconut oil, RBO- rice bran oil, SESO- sesame oil, (B)- blended, (I)- interesterified. The data represents the mean of four replicates ±SD from each dietary group. Values not sharing the common superscript are significantly different whereas values with different superscript are significantly different at $P<0.05$.The mRNA abundance was calculated in relation to the house keeping gene β-acting. The relative abundance of the CNO fed group was set at 1.

Rats fed blended oil consisting either CNO+RBO(B) or CNO+SESO(B) showed a threefold increase in mRNA abundance for LDL receptor as compared to those given CNO. However, when interesterified oils were fed to rats there was a further increase in mRNA abundance for LDL receptor by 4.7 fold in CNO+RBO fed rats and by 6.0 fold in CNO+SESO fed rats as compared to those given CNO. Rats given CNO+SESO(I) showed a 6 fold increase in the expression of LDL mRNA, which was the maximum change observed in these studies which also coincided with maximum decrease in serum total cholesterol (Table 3.2). This indicated that dietary fat influences the clearance of cholesterol by increasing its uptake by LDL receptors

Sterol regulatory element binding protein (SREBP)-2 mRNA abundance

SREBP-2, a transcription factor is involved in the regulation of the genes involved in cellular uptake and biosynthesis of cholesterol. The response of SREBP-2 gene expression to different dietary lipids was similar to that observed with LDL receptor expression.

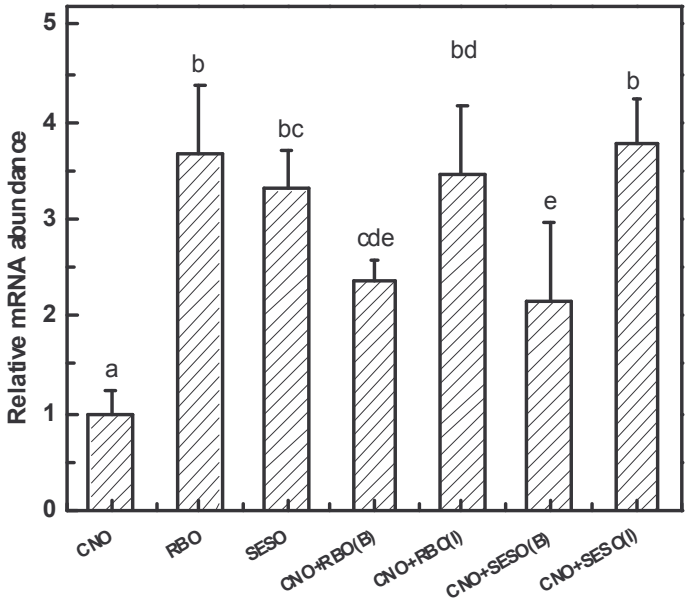


Figure 3.4d. **Sterol regulatory element binding protein (SREBP)-2 mRNA expression determined by RT-PCR.** CNO-coconut oil, RBO- rice bran oil, SESO- sesame oil, (B)-blended, (I)- interesterified. The data represents the mean of four replicates ±SD from each dietary group. Values not sharing the common superscript are significantly different whereas values with different superscript are significantly different at $P<0.05$.The mRNA abundance was calculated in relation to the house keeping gene β-acting. The relative abundance of the CNO fed group was set at 1.

The SREBP-2 mRNA levels in rats fed with RBO or SESO were increased by 3.7 and 3.3 fold, respectively in comparison to that found in rats given CNO. Rats fed with blended oil increased the expression levels of SREBP-2 mRNA abundance by 2.4 fold in rats given CNO+RBO and by 2.1 fold in CNO+SESO respectively as compared to those given CNO (Figure 3.4d). The expression of these genes were increased markedly by 3.5 and 3.7 fold when rats were fed a diet containing CNO+RBO(I) and CNO+SESO(I) as compared to those given CNO. These increases in mRNA abundance for SREBP-2 were significantly higher than that observed in rats given blended oils.

The mRNA concentration for beta actin was similar in rats fed with different types of dietary fats (Figure 3.4e). These results also show that the expression of beta-actin as the value of cycle threshold (C_T) was not influenced by the dietary lipids. The expression signal of the house keeping gene beta-actin served as internal control for normalization.

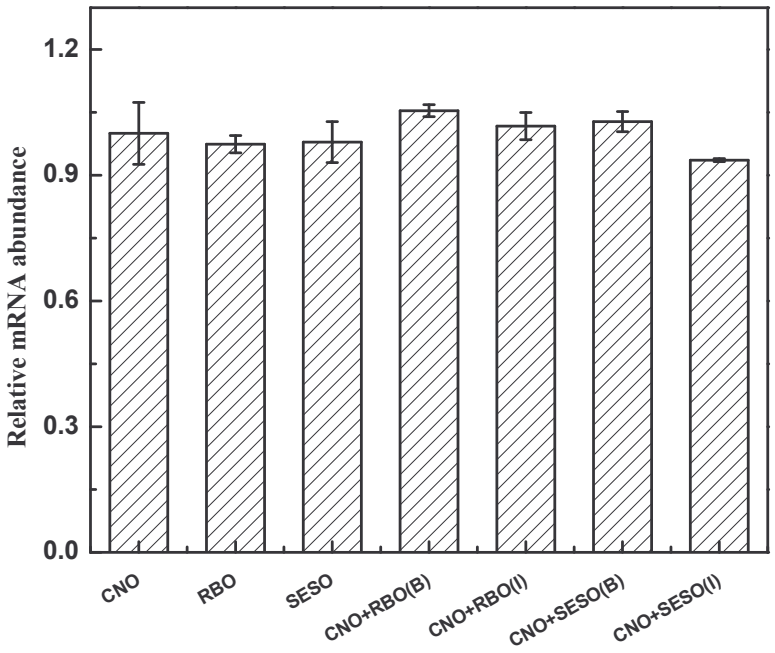


Figure 3.4e. B**eta actin mRNA expression determined by RT-PCR.** CNO-coconut oil, RBO- rice bran oil, SESO- sesame oil, (B)- blended, (I)- interesterified. The data represents the mean of four replicates ±SD from each dietary group. Values are not significantly different at $P<0.05$.The relative abundance of the CNO fed group was set at 1.

Correlation analysis

The changes in the mRNA abundance of major enzymes/proteins involved in cholesterol homeostasis were weighed against serum total and LDL cholesterol levels by Pearson correlation analysis (Table 3.5). Correlation analysis showed that the decrease in serum total cholesterol negatively correlates to the decrease in both the HMG-CoA reductase activity (r=0.6629), and mRNA abundance (r=0.7699), and also with the LDL receptor mRNA abundance (r=0.8095). The mRNA abundance of CYP7A1 also exhibit negative correlation to the total cholesterol (r=0.8539). The correlation analysis of LDL cholesterol to HMG-CoA reductase activity and mRNA abundance of HMG-CoA reductase, LDL receptor and CYP7A1 followed the changes in total cholesterol. LDL cholesterol level showed a negative correlation with HMG-CoA reductase activity (r=0.5758), HMG-CoA reductase mRNA abundance (r=0.7143), LDL receptor mRNA abundance (r=0.8203) and also with CYP7A1 (r=0.8618). A positive correlation is observed between HMG-CoA reductase activity and HMG-CoA reductase mRNA abundance (r=0.8737), indicating that the lowered activity observed was due to decrease protein synthesis.

Table 3.5. Correlation coefficients of mRNA abundance of major enzymes/ proteins involved in cholesterol metabolism with cholesterolemic parameters

		HMG-CoA reductase activity	HMG-CoA reductase mRNA abundance	Cholesterol 7-α-hydroxylase	LDL receptor abundance
Total cholesterol	r value	-0.6629	-0.7699	-0.8539	-0.8095
	p value	0.1045	0.0428	0.0144	0.0273
LDL cholesterol	r value	-0.5758	-0.7144	-0.8618	-0.8203
	p value	0.1761	0.0713	0.0126	0.0238
HMG-CoA reductase activity	r value	-	0.8737	0.8203	0.8182
	p value	-	0.0101	0.0238	0.0244

DISCUSSION

The objective of the study is to understand molecular mechanism for cholesterol homeostasis influenced by dietary lipids with different degrees of unsaturation as well as those containing modified TAG molecular species as has been observed in interesterified oils but with similar fatty acid composition as that of blended oils. Circulating plasma cholesterol concentrations depend on the balance between cholesterol synthesized by the liver, the rate of its conversion in to bile acid, and also its clearance from plasma by LDL receptor mediated uptake (Wong *et al*., 2006). In the present investigation we studied the expression level of HMG-CoA reductase, CYP7A1, LDL receptor and SREBP-2, the major proteins involved in cholesterol homeostasis. HMG-CoA reductase is the rate limiting enzyme in cholesterol biosynthesis. CYP7A1 is the first and regulatory enzyme in the conversion of cholesterol in to bile acid (Russell and Setchell, 1992). In normal animals and humans, receptor-dependent mechanisms account for 70-80% of total LDL turn over (Spady *et al*., 1985). The transcription factor SREBP-2 regulates the expression of several genes involved in cholesterol synthesis and uptake (Wong *et al*., 2006).

HMG-CoA reductase activity was altered by dietary fats. Rats fed with CNO showed a lower HMG-CoA reductase activity which coincided with the lower levels of mRNA. This may be due to the feedback inhibition of HMG-CoA reductase caused by high level of cholesterol found in CNO fed animals. The mRNA abundance for HMG-CoA reductase increased in rats fed with RBO, SESO or blends of CNO+RBO or CNO+SESO by 8-26% and showed a negative correlation with the cholesterol concentration observed in serum. While cholesterol concentrations were reduced by 23-46% in rats given RBO or SESO or blends with CNO+RBO, CNO+SESO or the corresponding interesterified oils, the mRNA levels for HMG-CoA reductase changed by only 8-26% as compared to that found in rats given CNO. This indicated that though the dietary lipids did affect HMG-CoA reductase activity, the mRNA levels in rats given lipids of different degrees of unsaturation was marginally affected. Hamsters fed SFA also showed lowered mRNA abundance for the HMG-CoA reductase gene compared to those fed a diet containing high levels of MUFA and PUFA (Dorfman and Lichtenstein, 2006).

The dietary fatty acids also influenced the mRNA abundance for CYP7A1. Cheema *et al*. (1997) have compared the effect of SFA, MUFA and PUFA rich diet on CYP7A1 in mice. Feeding mice with diet rich in PUFA resulted in significantly increased CYP7A1 activity compared to those fed with diet containing SFA or MUFA containing diet. Mice fed with PUFA containing diets had significantly higher mRNA abundance compared to those fed with SFA or MUFA rich diet. In the present study we noticed that rats fed with RBO and SESO showed significantly higher levels of mRNA abundance for CYP7A1 as compared to those given CNO. Blending CNO with RBO or SESO and feeding to rats resulted in significantly increased expression of CYP7A1 as compared to those given CNO. Feeding rats with a diet containing interesterified oils also showed a significant increased in mRNA abundance for CYP7A1 compared to those given CNO. Rats fed interesterified oil showed a higher increase in mRNA abundance for CYP7A1 compared to those fed with blended oils of similar fatty acid composition. These studies indicated that PUFA regulate the cholesterol level by enhancing the activity and mRNA abundance for CYP7A1. These observations are similar to that made in the studies of Cheema *et al*. (1997).

The circulating LDL concentration in plasma is also controlled by its uptake by LDL receptors. This clearance is influenced by the amount and type of TAG in the dietary lipids. TAG containing predominantly unsaturated fatty acids accelerate the rate of receptor dependent LDL uptake by the liver (Horton *et al*., 1993). Long chain SFA with exception of stearic acid increase plasma LDL concentrations and PUFA tend to decrease them. This was shown to be mediated by modulating LDL receptor activity (Woollett *et al*., 1992). Dietary fats containing unsaturated fatty acids upregulate LDL receptors. Bennett *et al*. (1995) have shown that diet containing triglyceride enriched in specific fatty acids differentially influence the plasma lipoprotein concentration and also modulate the expression of genes involved in hepatic lipoprotein metabolism. Feeding hamsters with tripalmitin and trimyristin caused significant increase in LDL cholesterol by 1.37 fold 2.11 fold, respectively when compared to hamsters fed triolein. The increase in cholesterol level was accompanied by increased expression of hepatic apolipoprotein B gene by 14.9 and 66.8%, respectively when compared to those fed triolein. A significant decrease in the

LDL receptor mRNA abundance is observed to an extent of 46.9 and 54.3 in hamsters given trimyristin and tripalmitin as compared to those given triolein. Horton *et al*. (1993) have shown that hamsters fed with CNO had decreased mRNA abundance for LDL receptors compared to those fed sun flower oil. Our results are in agreement with these reports indicating that dietary lipids containing unsaturated fatty acids upregulate LDL receptor mRNA abundance and helps in lowering plasma cholesterol. However we also noticed that interesterified fats have similar fatty acid composition as that of blended oils, but still had greater influence in enhancing mRNA abundance for LDL receptor which coincided with its ability to lower serum cholesterol further. The reason for these differential effects on LDL receptor mRNA by interesterified and blended oils are yet to be evaluated.

Expression of key proteins involved in cholesterol metabolism is regulated by SREBPs (Brown and Goldstein, 1997). SREBP-2 is a transcription factor which influence cholesterol synthesis and uptake (Tamehiro *et al*., 2007). Activation of SREBP-2 is dependent on the cholesterol status of the cell (Goldstein *et al*., 2006). Studies by Dorfman and Litchenstein (2006) have shown that dietary PUFA increases the mRNA abundance for the gene SREBP-2. The expression pattern for SREBP-2 followed the same pattern as that of LDL receptor. In the present study also it was observed that similar to the pattern observed with LDL receptor mRNA abundance, the SREBP-2 mRNA concentration was also increased in rats fed with oils that contain higher levels of PUFA compared to rats fed with CNO. The increased mRNA abundance for SREBP-2 correlated with increased mRNA abundance for LDL receptors.

The minor components present in oils are also reported to have effect on cholesterol metabolism at the genomic level. Studies by Chen and Cheng (2006) have shown that RBO diet containing 0.4%, (weight percent of diet) γ-oryzanol and 1.5 mg γ-tocotrienols per day significantly increased the hepatic LDL receptor, CYP7A1 and HMG-CoA reductase mRNA expression in diabetic rats. In the present study rats ingested 0.13% (weight percent of diet) γ-oryzanol and 0.77 mg/d γ-tocotrienol in RBO fed group. Rats fed blended and interesterified oil of CNO+RBO ingested 0.104% (weight percent of diet) γ-oryzanol and 0.66 mg γ-tocotrienol per day.

Lim *et al*. (2007) have shown that sesamin and sesamolin present in SESO are capable of modulating the expression of genes involved in the fatty acid oxidation. Increased fatty acid oxidation reflects on the decreased lipid level in serum. In this study rats were given a diet containing 2 g/kg sesamin or 0.6 g/kg sesamolin which significantly increased the mRNA abundance for genes involved in fatty acid oxidation. Sesamin is shown to have hypocholesterolemic effect. Feeding rats with 0.5% sesamin significantly decreased the cholesterol absorption by 30% in rats (Hirose *et al*., 1991). In the present study rats were given a diet contain 98 mg sesamin and 0.166 mg sesamolin /kg diet. The minor components present in RBO or SESO may also have exerted their effect on the expression of genes which resulted in the reduction of cholesterol level in rats fed blended and interesterified oils. This aspect however was not addressed in this investigation.

In conclusion the present study gives us an insight into the molecular mechanism involved in the cholesterol lowering properties of blended and interesterified oils. The hypocholesterolemic effect observed in rats fed blended oils can be mainly attributed to increased uptake of LDL cholesterol by liver through the upregulation of LDL receptor and by enhancing the catabolic pathway via activation of CYP7A1 gene expression. Thus interesterified and blended oils lowered serum cholesterol in rats by upregulating cholesterol clearance pathways. However the effectiveness of interesterified oils to lower serum cholesterol is significantly higher than that observed with blended oil. The reasons for these differential effects are now being pursued.

INTRODUCTION

Atherosclerosis and thrombosis are two important events responsible for the progression and development of cardiovascular disease (CVD). The circulating platelets and their interaction with vascular endothelium play a significant role in the formation of coronary thrombosis (Svaneborg *et al*., 2002). Essential polyunsaturated fatty acids influence thrombotic events and are found to be protective against atherogenesis (McLaughlin *et al*., 2005). Platelet hyperactivity is considered to be a risk factor for thrombosis (Misikangas *et al*., 2001). It was demonstrated that the thrombotic tendency of the blood platelets is influenced by fatty acid composition of the dietary lipids (Thijssen *et al*., 2005). Dietary fatty acids modify the aggregation of platelets.

Dietary fat changes the fatty acid composition of cellular membranes. Such modifications in the fatty acid composition of membranes can lead to a variety of functional changes, including membrane fluidity, ion transport, cellular responses and biosynthesis of eicosanoides (Piche and Mahadevappa, 1990). Dietary lipids influence the hemostatic systems and platelet function (Miller, 1997). Saturated fatty acids are atherogenic and favour platelet aggregation by decreasing prostacyclin production and increasing thromboxane production. They can thus be considered prothrombotic substances. Polyunsaturated fatty acids reduce platelet and thrombogenic activity of the arterial wall (Bertomeu *et al*., 1990; De La Cruz *et al*., 1997). The type and level of eicosanoids synthesized in the system influence the platelet aggregation and there by modulate thrombotic tendency (Zhou and Nilsson, 2001). It has been established that dietary saturated fat and serum cholesterol correlated well with cardiovascular disease and polyunsaturated fatty acids have been associated with alterations in atherogenic indexes such as plasma lipoproteins, thrombosis, and blood pressure (Stallones, 1983). However, excessive consumption of PUFA leads to oxidative stress if not properly balanced with antioxidants (Scislowski *et al*, 2005). PUFA being precursors of prostaglandins, its intake in excess may cause changes in prostaglandin levels (Trivedi and Singh, 2005).

The effects of individual fatty acids on thrombotic tendency have been evaluated in animal models and in human subjects. Studies in human have shown that

ex vivo platelet aggregation time as measured by filtragometry was favorably prolonged during consumption of the linoleic acid diet compared with the stearic or oleic acid diet (Thijssen *et al*., 2005). In rats, arterial thrombosis tendency as measured with the aortic loop technique, was decreased by (n-6) and (n-3) PUFA, whereas SFA with 12–16 carbon atoms promoted arterial thrombus formation. The effects of oleic acid were neutral or even antithrombotic compared with SFA (Hornstra and Kester, 1997).

Role of dietary polyunsaturated fatty acids in platelet aggregation and vascular function has been greatly advanced by the identification and characterization of prostaglandins generated by platelet and endothelial cells. Thromboxane A_2 is a vasoconstrictor and a powerful inducer of platelet aggregation. This is produced by platelets. Prostacyclin is a platelet antagonist and synthesized by endothelial cells. Both these compounds are synthesized from the cyclooxygenase pathway from arachidonic acid and the ratio of these eicosanoides is considered to reflect on thrombosis (Bunting, *et al*, 1983). Arachidonic acid may be transported in the circulation either in the free fatty acid form bound to albumin or esterified in lipoprotein phospholipids and cholesterol esters (Willis, 1981). Direct uptake of free arachidonic acid by platelets or the vessel wall leads to its incorporation into membrane phospholipids (Bills *et al*., 1977). When acted up on by phospholipases, arachidonic acid released from phospholipids becomes available to the enzyme cyclooxygenase and is rapidly converted to the labile cyclic endopexides, PGG_2 and PGH_2. Thromboxane synthetase and prostacyclin synthetase convert the endoperoxides to biologically active thromboxane A_2 and PGI_2. Although linoleic acid is rapidly converted to arachidonic acid in the liver and in cells such as fibroblast, similar elongation and desaturation does not occur in platelet membranes. Experimental data shows that feeding diet high in linoleic acid leads to increased concentrations of linoleic acid in the platelet membrane and decreased arachidonic acid (Zhou and Nilsson, 2001).

CNO is rich in saturated fatty acid and contain less than 2% linoleic acid. Blending of CNO with RBO or SESO resulted in increasing the linoleic acid content of blended oils to about 29-33%. These oils were also subjected to interesterification

reaction mediated through lipase. Because of increased levels of linoleic acid these modified oils may influence platelet function and thereby show their effect on thrombotic parameters. The present study was undertaken to assess the effect of feeding blended and interesterified oils on thrombotic factors such as platelet aggregation and eicosanoid production.

RESULTS

Fatty acid composition of diet

Fatty acid composition of the oils used in the present study was similar in their composition as that of earlier studies (Chapter III). The linoleic acid content of the blended oils were 29 and 33% respectively for CNO+RBO(B) and CNO+SESO(B). The fatty acid composition of interesterified oils was not different from their respective blends (Table 4.1).

Table 4.1. Fatty acid composition (wt %) of dietary fat

Fatty acid	CNO	RBO	CNO+RBO (B)	CNO+RBO (I)	SESO	CNO+SESO (B)	CNO+SESO (I)
8:0	2.6±0.3	nd	0.6±0.1	0.5±0.1	nd	0.9±0.2	1.0±0.3
10:0	5.3±0.2	nd	1.1±0.1	1.3±0.2	nd	1.3±0.1	1.3±0.1
12:0	50.4±1.7	nd	10.4±0.2	10.5±0.4	nd	13.3±0.5	12.6±0.3
14:0	20.5±0.9	0.4±0.1	4.5±0.3	4.4±0.3	nd	4.7±0.3	4.4±0.2
16:0	9.2±0.4	19.9±1.1	17.2±0.3	17.1±0.6	10.1±0.4	9.6±0.3	9.6±0.3
18:0	2.6±0.2	1.8±0.2	1.7±0.2	1.7±0.3	5.4±0.3	4.4±0.2	4.5±0.3
18:1	7.5±0.3	42.6±2.4	35.0±1.3	34.7±1.2	42.4±2.1	33.1±1.4	33.4±1.3
18:2	1.9±0.1	34.8±1.6	29.1±1.1	29.4±0.9	41.7±1.1	32.4±1.3	32.8±1.2
18:3	nd	0.5±0.2	0.4±0.1	0.4±0.1	0.4±0.2	0.3±0.1	0.4±0.1

Values are mean ± SD of three samples. nd- not detected, CNO- coconut oil, RBO- rice bran oil, SESO- sesame oil, (B)- blended, (I)- interesterified.

Fatty acid profile of platelets of rats fed native, blended and interesterified oils

Fatty acid composition of platelets was influenced by the fatty acid composition of dietary lipids (Table 4.2). Rats fed CNO showed a significant decrease in the amount of linoleic and arachidonic acid level compared to rats fed RBO, SESO or blended and interesterified oils of CNO+RBO or CNO+SESO. Rats on CNO diet showed 50% lower amount of linoleic acid and arachidonic acid as compared to those fed RBO and SESO. Rats fed blended oils containing CNO+RBO(B) showed an increase in the linoleic acid content by 1.89 fold and by 2.15 fold in the case of rats given CNO+SESO(B) as compared to those given CNO. The arachidonic acid content in rats fed blended oil was increased by 1.69 fold and by 1.75 fold, in rats given CNO+RBO(B) and CNO+SESO(B) respectively as compared to rats given CNO. Rats fed interesterified oil also showed significantly higher amount of linoleic acid by 1.9 fold when given CNO+RBO(I) and by 2.2 fold in rats given CNO+SESO(I).

Table 4.2. Fatty acid composition (%) of platelets of rats fed native, blended and interesterified oils

	Fatty acid						
Dietary fat	**14:0**	**16:0**	**16:1**	**18:0**	**18:1**	**18:2**	**20:4**
CNO	2.3±0.6^{a}	27.7±1.9^{a}	3.7±0.4^{a}	21.7±1.2^{a}	29.8±1.2^{a}	6.9±0.7^{a}	7.9±0.5^{a}
RBO	nd	23.9±2.1^{ac}	1.9±0.7^{b}	21.3±2.3^{a}	24.6±2.4^{bcd}	13.8±1.1^{b}	14.5±1.3^{b}
CNO+RBO(B)	1.3±0.3^{b}	22.5±1.6^{bc}	1.4±0.3^{b}	23.2±1.9^{a}	26.0±1.5^{abd}	13.1±1.3^{b}	13.4±1.1^{b}
CNO+RBO(I)	1.1±0.4^{b}	21.4±1.8^{bc}	1.8±0.6^{b}	23.6±1.4^{a}	26.7±1.9^{ab}	13.2±1.0^{b}	12.2±0.8^{b}
SESO	nd	24.1±2.4^{ac}	1.1±0.4^{b}	20.4±2.4^{a}	23.4±2.1^{cd}	16.7±1.2^{c}	14.3±0.9^{b}
CNO+SESO(B)	0.9±0.3^{b}	24.7±1.3^{ac}	1.9±0.3^{b}	22.9±2.7^{a}	20.8±1.6^{c}	14.9±1.3^{bc}	13.9±1.1^{b}
CNO+SESO(I)	0.6±0.5^{b}	24.4±2.2^{ac}	2.0±0.5^{b}	23.1±1.6^{a}	22.6±1.1^{c}	15.2±0.9^{bc}	12.1±0.6^{b}

Values are mean ± SD of four rats. nd- not detected. Values in the same column with common superscript letters are not significantly different whereas values with different superscript are significantly different at $P<0.01$. CNO- coconut oil, RBO- rice bran oil, SESO- sesame oil, (B)- blended, (I)- interesterified.

The arachidonic acid level in platelets increased by 1.54 and 1.53 fold, respectively in rats fed with CNO+RBO(I) and CNO+SESO(I) when compared to rats given CNO. However, the arachidonic acid content in platelets of rats fed interesterified oil was marginally lower than that in rats fed blended oils but was not statistically significant.

Platelet aggregation in rats fed native, blended and interesterified oils

ADP and Collagen induced platelet aggregation in rats fed native, blended and interesterified oils is shown in Figure 4.1 & 4.2.

The rate of ADP induced platelet aggregation was decreased by 47 and 40% respectively in rats fed RBO and SESO as compared to those given CNO. The collagen induced platelet aggregation was decreased by 43 and 35% respectively, in rats fed RBO and SESO compared to those fed with CNO (Table 4.3a & 4.3b). Rats fed blended oil also showed significant decrease in ADP induced platelet aggregation by 34 and 30% respectively in CNO+RBO(B) and CNO+SESO(B) fed animals as compared to rats given CNO. Similarly collagen induced platelet aggregation rate was reduced by 25 and 22% respectively in rats given CNO+RBO(B) and CNO+SESO(B), respectively as compared to rats given CNO.

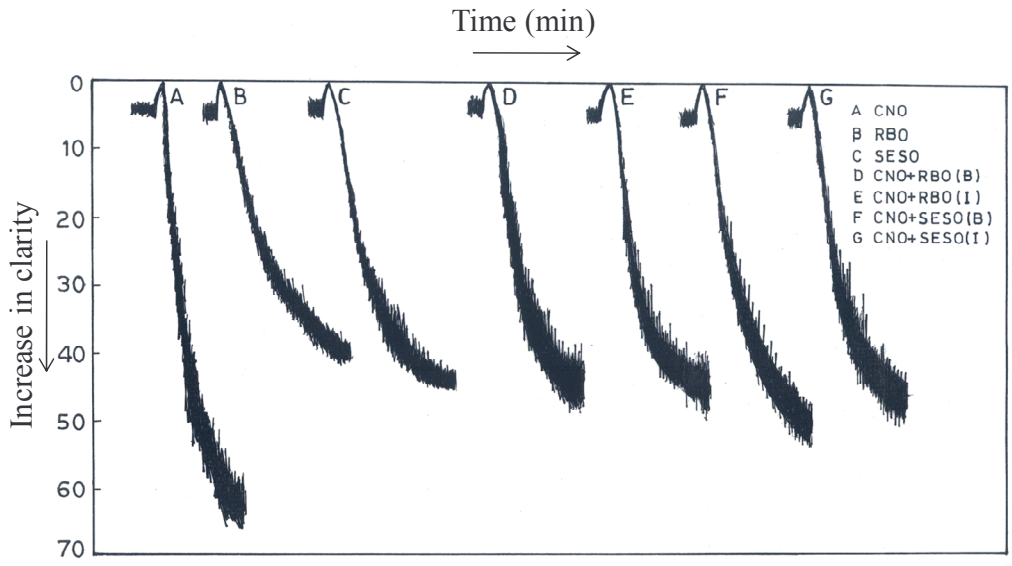


Figure 4.1. ADP induced platelet aggregation in native, blended and interesterified oils. CNO- coconut oil, RBO- rice bran oil, SESO- sesame oil, (B)- blended, (I)- interesterified.

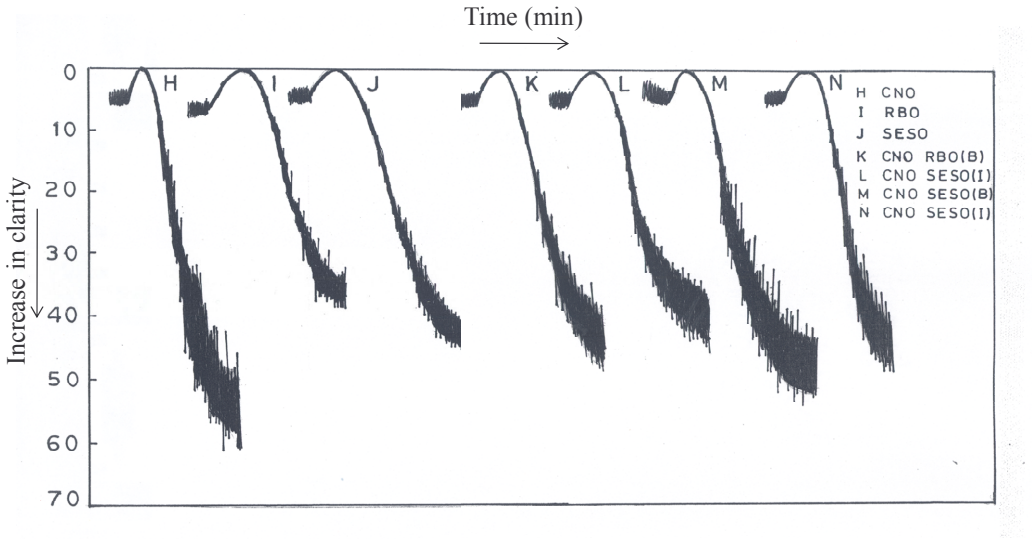


Figure 4.2. Collagen induced platelet aggregation in native, blended and interesterified oils. CNO- coconut oil, RBO- rice bran oil, SESO- sesame oil, (B)- blended, (I)- interesterified.

Table 4.3a. Platelet aggregation in rats fed CNO+RBO

	CNO	RBO	CNO+RBO(B)	CNO+RBO(I)
Rate of aggregation				
ADP induced	11.6±0.2[a]	6.1±0.40[b]	7.6±0.32[c]	7.3±0.29[c]
Collagen induced	7.9±0.62[a]	4.5±0.58[b]	5.9±0.30[c]	5.6±0.28[c]
Percent aggregation				
ADP induced	66.2±3.8[a]	41.0±2.1[b]	50.6±2.4[c]	49.6±1.6[c]
Collagen induced	61.1±2.5[a]	39.1±1.4[b]	47.8±1.8[c]	45.1±1.6[c]

Values show the mean ± S.D of 4 rats. Values in the same row with common superscript letters are not significantly different whereas values with different superscript are significantly different at $P<0.01$. CNO- coconut oil, RBO- rice bran oil, B- blended, I- interesterified.

Table 4.3b. Platelet aggregation in rats fed CNO+SESO

	CNO	SESO	CNO+SESO(B)	CNO+SESO(I)
Rate of aggregation				
ADP induced	11.6±0.2[a]	7.0±0.34[b]	8.1±0.56[c]	7.6±0.51[c]
Collagen induced	7.9±0.62[a]	5.1±0.31[b]	6.2±0.62[c]	5.8±0.38[c]
Percent aggregation				
ADP induced	66.2±3.8[a]	45.6±1.8[b]	53.2±3.1[c]	50.3±1.2[c]
Collagen induced	61.1±2.5[a]	45.1±1.3[b]	51.6±2.6[c]	49.7±2.3[c]

Values show the mean ± S.D of 4 rats. Values in the same row with common superscript letters are not significantly different whereas values with different superscript are significantly different at $P<0.01$. CNO- coconut oil, SESO- sesame oil, B- blended, I- interesterified.

The rate of ADP induced platelet aggregation was decreased by 37 and 34% respectively in rats fed CNO+RBO(I) and CNO+SESO(I), where as the collagen induced aggregation was decreased by 29 and 26% respectively, in rats fed CNO+RBO(I) and CNO+SESO(I) compared to those fed with CNO (Table 4.3a & 4.3b). However the rate of platelet aggregation observed in rats given blended and interesterified oils were comparable.

The percent of platelet aggregation was significantly reduced in rats fed with RBO or SESO or blended oils. The percent of ADP induced platelet aggregation was decreased by 38 and 31% in rats fed with RBO or SESO compared to rats given CNO. Rats fed with blended oils showed a decrease in the percent of ADP induced platelet aggregation by 24 and 20%, respectively in CNO+RBO(B) and CNO+SESO(B) groups compared to rats fed with CNO. Feeding interesterified oils to rats resulted in similar decrease in the percent aggregation. The percent of ADP induced aggregation was decreased by 25 and 24%, respectively in rats fed with CNO+RBO(I) and CNO+SESO(I) compared to rats given CNO (Table 4.3a & 4.3b).

Similarly the percent of collagen induced aggregation was decreased by 36 and 26% in rats fed with RBO and SESO compared to those given CNO (Table 4.3a & 4.3b). The percent of collagen induced aggregation was decreased by 24 and 15%, respectively in rats fed with CNO+RBO(B) and CNO+SESO(B) compared to rats given CNO. Rats fed with interesterified oils showed a decrease in the percent of collagen induced platelet aggregation by 26 and 19%, respectively in CNO+RBO(I) and CNO+SESO(I) groups compared to rats fed with CNO (Table 4.3a & 4.3b).

Lipid peroxides level in platelets of rats fed native, blended and interesterified oils

Dietary lipids influence the fatty acid composition of platelets which in turn affect the peroxide level in the system. Lipid peroxides level in platelets which was aggregated using ADP were increased by 2.1 and 2.2 fold respectively, in rats fed RBO and SESO compared to rats fed CNO (Table 4.4a & 4.4b).

Table 4.4a. Lipid peroxide level in platelets of rats fed CNO+RBO

Lipid peroxide level in platelets	CNO	RBO	CNO+RBO (B)	CNO+RBO (I)
nmoles of MDA formed/1.5 × 10^9 platelets (ADP induced)	1.8±0.3[a]	3.9±0.4[b]	3.4±0.3 [c]	3.1±0.2 [c]
nmoles of MDA formed/1.5 × 10^9 platelets (Collagen induced)	1.6±0.2 [a]	2.9±0.3 [b]	2.6±0.2 [c]	2.4±0.3 [c]

Values show the mean ± S.D of 4 rats. Values in the same row with common superscript letters are not significantly different whereas values with different superscript are significantly different at P<0.01. CNO- coconut oil, RBO- rice bran oil, B- blended, I- interesterified.

Table 4.4b. Lipid peroxide level in platelets of rats fed CNO+SESO

Lipid peroxide level in platelets	CNO	SESO	CNO+SESO (B)	CNO+SESO (I)
nmoles of MDA formed/1.5 × 10^9 platelets (ADP induced)	1.8±0.3[a]	4.1±0.5 [b]	3.6±0.4 [c]	3.3±0.2 [c]
nmoles of MDA formed/1.5 × 10^9 platelets (Collagen induced)	1.6±0.2 [a]	3.2±0.4 [b]	2.9±0.2 [c]	2.5±0.3 [c]

Values show the mean ± S.D of 4 rats. Values in the same row with common superscript letters are not significantly different whereas values with different superscript are significantly different at P<0.01. CNO- coconut oil, SESO- sesame oil, B- blended, I- interesterified.

Rats fed blended oil showed a increased lipid peroxide level by 1.8 and 2 fold respectively in CNO+RBO(B) and CNO+SESO(B) groups compared to rats given CNO. The lipid peroxide level in rats fed interesterified oils also increased by 1.7 and 1.8 fold in CNO+RBO(I) and CNO+SESO(I), respectively as compared to those fed CNO (Table 4.4a & 4.4b). Similar changes in malondialdehyde levels were observed in platelets which were aggregated using collagen (Table 4.4a & 4.4b). The LPO was increased by 1.8 and 2.0 folds in rats fed RBO and SESO respectively. In rats given blended oil of CNO+RBO or CNO+SESO had higher amount of LPO by 1.6 and 1.8 fold respectively as compared to those given CNO. Feeding rats with interesterified oils comprising CNO+RBO(I) and CNO+SESO(I) showed an increase in LPO level by 1.5 fold and 1.6 fold respectively as compared to rats given CNO (Table 4.4a & 4.4b).

Serum prostaglandin level in rats fed native, blended and interesterified oils

Cyclooxygenase catalyzed arachidonic acid metabolites such as thromboxane and prostacyclin regulate the functions of platelets, arterial and endothelial cells, which are all involved in atherosclerosis and thrombosis (Kinsella *et al*., 1990; Lawson *et al*., 1985). Their levels in serum are influenced by dietary fat. Rats fed with CNO showed higher amount of TXA_2 in serum compared to those given RBO, SESO or blended oils (Figure 4.3a & 4.3b).

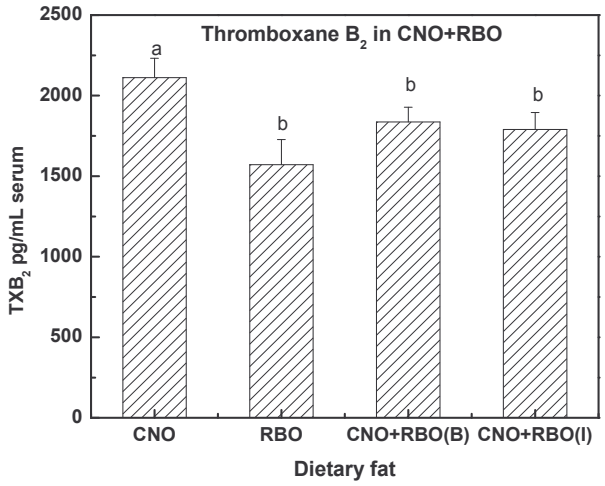


Figure 4.3a. Thromboxane B_2 level in rats fed blended and interesterified oils of CNO+RBO. Values not sharing the common superscript are significantly different whereas values with different superscript are significantly different at P<0.05. CNO- coconut oil, RBO- rice bran oil, (B)- blended, (I)- interesterified.

Thus thromboxane levels decreased by 25 and 20%, respectively in rats fed with RBO or SESO compared to rats given CNO. Rats given blended oil showed a decrease in thromboxane level by 13 and 12% in CNO+RBO(B) and CNO+SESO(B) groups respectively. Feeding interesterified oils also resulted in decrease in thromboxane level by 15 and 14%, respectively in rats given CNO+RBO(I) and CNO+SESO(I) when compared to rats fed with CNO diet.

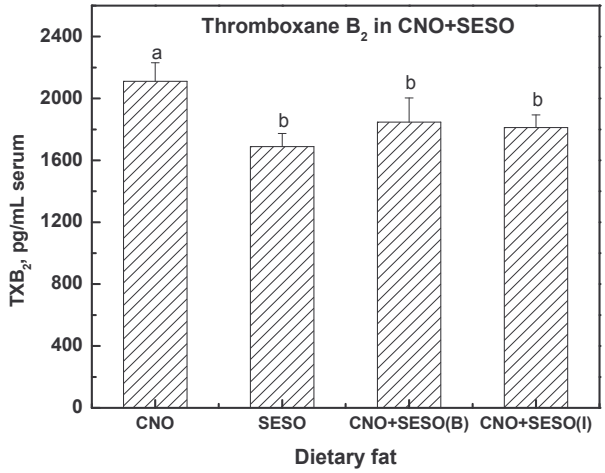


Figure 4.3b. Thromboxane B_2 level in rats fed blended and interesterified oils of CNO+SESO. Values not sharing the common superscript are significantly different whereas values with different superscript are significantly different at $P<0.05$. CNO- coconut oil, oil, SESO- sesame oil, (B)- blended, (I)- interesterified.

Prostacyclin is an antagonist for platelet aggregation; its level can counteract the effects of thromboxane in thrombosis. Rats fed with RBO, SESO, blended or interesterified oils showed significantly increased prostacyclin level compared to rats fed with CNO (Figure 4.4a & 4.4b). The prostacyclin concentration was increased by 1.9 and 1.8 fold in rats given RBO and SESO compared to those given CNO. Rats fed with blended oils consisting of CNO+RBO(B) and CNO+SESO(B) showed an increase in the prostacyclin level by 1.7 and 1.6 fold, respectively compared to rats given CNO. The prostacyclin level was increased by 1.6 and 1.6 fold respectively in rats fed with interesterified oils of CNO+RBO(I) and CNO+SESO(I) compared to rats given CNO.

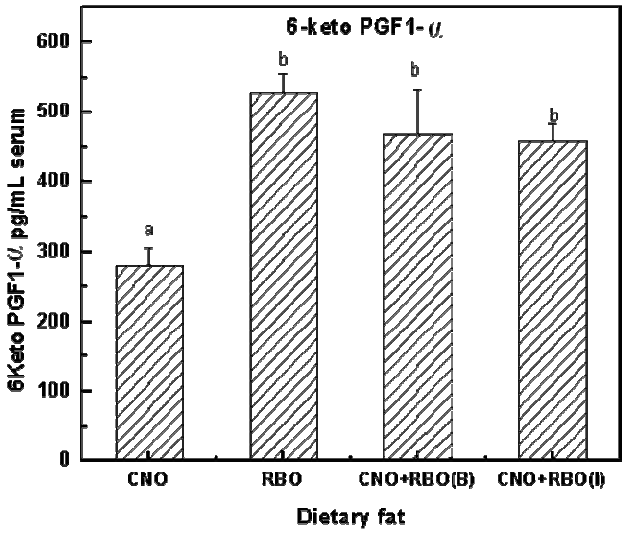


Figure 4.4a. 6-Keto PGF1-α level in rats fed blended and interesterified oils of CNO+RBO. Values not sharing the common superscript are significantly different whereas values with different superscript are significantly different at P<0.05. CNO- coconut oil, RBO- rice bran oil, (B)- blended, (I)- interesterified.

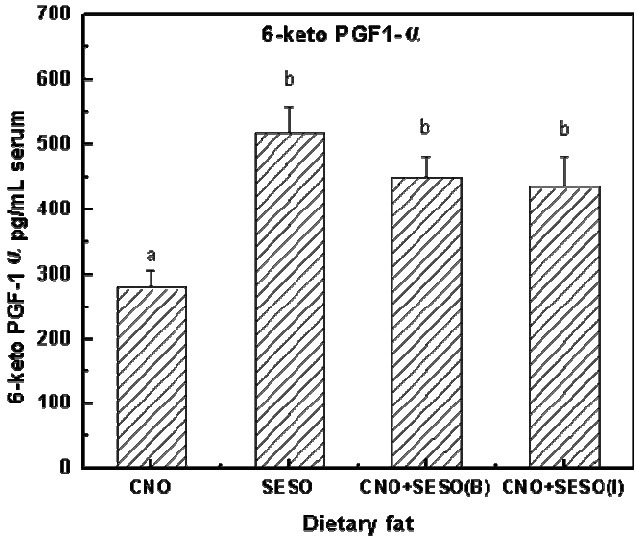


Figure 4.4b. 6-Keto PGF1-α level in rats fed blended and interesterified oils of CNO+SESO. Values not sharing the common superscript are significantly different whereas values with different superscript are significantly different at P<0.05. CNO- coconut oil, SESO- sesame oil, (B)- blended, (I)- interesterified.

The ratio of prostacyclin to thromboxane is considered as an indicator for thrombosis (Bunting, *et al*, 1983). The ratio for PGI_2/TXA_2 in rats given CNO was 0.13, while this ratio for rats given RBO and SESO was 0.34 and and 0.31, respectively. PGI_2/TXA_2 ratio in rats given CNO+RBO(B) and CNO+SESO(B) was 0.25 and 0.24 respectively. The rats given interesterified oils of CNO+RBO(I) and CNO+SESO(I) showed ratio of 0.26 and 0.24, respectively (Figure 4.5a & 4.5b).

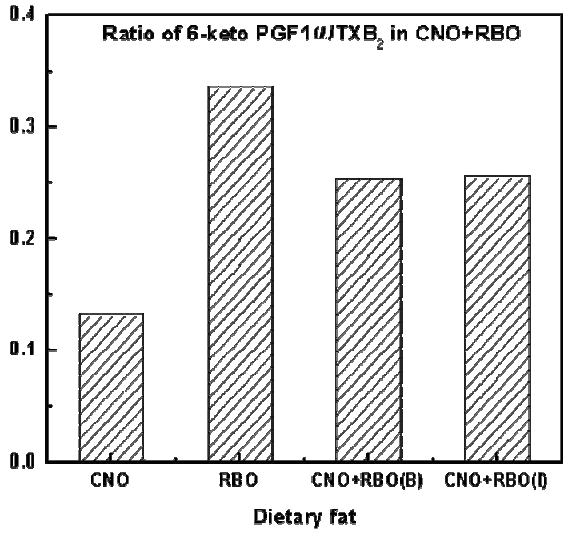


Figure 4.5a. Ratio of 6-keto PGF1-α/ thromboxane B_2 in rats fed blended and interesterified oils of CNO+RBO. CNO- coconut oil, RBO- rice bran oil, (B)- blended, (I)- interesterified.

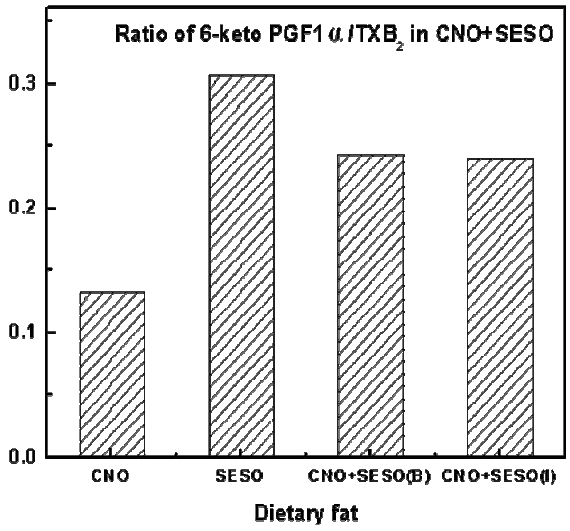


Figure 4.5b. Ratio of 6-keto PGF1-α/ thromboxane B_2 in rats fed blended and interesterified oils of CNO+SESO. CNO- coconut oil, SESO- sesame oil, (B)- blended, (I)- interesterified.

DISCUSSION

Both atherosclerosis and thrombosis produce occlusive vascular lesions in humans, which may lead to adverse clinical outcomes, such as stroke, myocardial infarction, or peripheral vascular disease. It is well accepted that among other factors, dietary fatty acids influence the development and progression of atherosclerosis (Moreno and Mitjavila, 2003). The effect of dietary fat upon atherosclerosis may be mediated through two key processes: first, the influx of lipids and lipoproteins, such as low density lipoproteins, from the plasma into the arterial wall and, second the formation of platelet thrombi in advanced atherosclerosis. Blood platelet function is thought to be critical in the mechanisms involved in atherosclerosis and arterial thrombosis.

One of the complications of CVD results from the formation of arterial thrombus (Badimon, 2001), which is initiated by disturbances in the hemostatic balance. Key regulators of this delicate balance are the endothelial wall, blood platelets, coagulation and fibrinolytic factors (Hornstra *et al*., 1998). It was demonstrated that the thrombotic tendency of the blood is influenced by total fat intake as well as the fatty acid composition of the diet. Although the biochemical basis of the effects of dietary fatty acids on thrombotic tendency have not been fully elucidated, dietary fatty acids can modulate the fatty acid compositions of platelets and other cell membranes, thereby changing the availability of arachidonic acid. This fatty acid is a precursor for eicosanoid synthesis, which is involved in platelet aggregation (Zhou and Nilsson, 2001).

The present investigation was undertaken to assess the influence of dietary fatty acids on platelet aggregation and eicosanoid levels in blood plasma. Effect of native, blended and interesterified fats were assessed on thrombotic factors such as rate and extent of platelet aggregation and eicosanoid levels in blood plasma in rats. It was found that rats fed with RBO or SESO or blended and interesterified oil, showed a reduction in platelet aggregation compared to that observed in rats fed with CNO. Earlier studies have reported that saturated fatty acids are atherogenic in nature and favour platelet aggregation, while polyunsaturated fatty acids reduce platelet and thrombogenic activity of the arterial wall (Bertomeu *et al*., 1990; De La Cruz *et al*.,

1997 & 2000). Experiments conducted by Hornstra and Wierts (1993) by loop insertion method demonstrated that rats fed hydrogenated coconut oil increase the platelet aggregation by 29%, where as rats fed sunflower showed a decrease in platelet aggregation. Platelets from rats fed a diet high in saturated fat showed increased sensitivity to thrombin induced platelet aggregation and were able to release platelet factor III more readily than controls (McGregor *et al*., 1980). Burri *et al*. (1991) have shown that consumption of the diet high in linoleic acid decrease ADP- and collagen-induced platelet aggregation relative to diet containing oleic acid. Studies by Kwon *et al*. (1991) have found that consumption of oleic acid as well as linoleic acid decreased collagen-induced platelet aggregation compared to that found in subjects consuming diet high in SFA. The reason why diet containing saturated fatty acids increases platelet aggregation may be attributed to the hypercholesterolemic effect caused by the saturated fatty acids. studies have shown that hypercholesterolemia increases platelet activity above normal levels and enhances the role of platelets in CVD. High LDL level increases the sensitivity of platelets to activating agents via receptor mediated signaling cascade and lipid exchange between LDL particles and plasma membrane (Lacoste *et al*., 1995).

Blending of CNO with RBO or SESO reduced the thrombotic potential of CNO. The blended oils contained lesser amount of saturated fatty acids compared to CNO. Rats given blended oil of either CNO+RBO or CNO+SESO in the present study showed a significant decrease in the rate and extent of platelet aggregation as compared to rats fed with CNO. Interesterification of blended oil did not alter the fatty acid composition or minor components. Feeding interesterified fats to rats also resulted in significant decrease in the rate and extent of platelet aggregation when compared to rats fed with CNO. The rate and extent of platelet aggregation observed in rats fed with interesterified oils was comparable with that observed in rats given blended oils.

Eicosanoides, the oxygenated metabolites of arachidonic acid have been implicated in several physiological processes which influence cardiovascular system. This includes vascular thrombosis, myocardial ischemia, and tissue injury as well as sudden death. 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 athero-thrombogenesis (Spector, 1992). Fatty acids in the diet influence the endothelial cell damage and consequent atherosclerotic disease (Badimon, 2001; Hornstra *et al*., 1998). Linoleic acid was reported to inhibit platelet adhesion and aggregation by reducing the formation of the vasoconstrictor eicosanoid TXB_2 (Zhou and Nilsson, 2001). Studies by Bayindir *et al.* (2002) have shown that feeding a saturated fat such as butter to rabbit resulted in significant increase in the TXB_2 concentration by 18.3% over and above the basal value.

Linoleic acid is precursor for arachidonic acid which in turn is the substrate for the generation of eicosanoids formed by the action of cyclooxygenase. Arachidonic acid is mainly occurring at the sn-2 position of phospholipids. The concentration of eicosanoid precursors in membrane depends on the dietary supply of these fatty acids, and on the desaturation-elongation of ingested precursor PUFA such as linoleic acid. The distribution of arachidonic acid in membrane varies among tissues and even within different phospholipid classes. Phosphatidylinositol has the highest content of arachidonic acid of all phospholipids (Chilton and Murphy, 1986). The selectivity in tissue distribution of arachidonic acid is achieved by a number of collaborating mechanisms based on relative specificity of acylation reaction and lipolytic enzymes in combination with the regulation of desaturation elongation reactions. The metabolism of arachidonic acid in lipoproteins by enzymes and lipoprotein receptors is different from the predominant C_{16}-C_{18} fatty acids. The lipoprotein independent transport as plasma FFA and 2-acyl lysophosphatidylcholine represents important pathways for the transport of eicosanoid precursors to tissues. Considerable amount of eicosanoid precursor formation is taking place in liver and several extrahepatic tissues (Zhou and Nilsson, 2001). Blended and interesterified oils used in this study contained higher levels of linoleic acid as compared to CNO. Feeding these blended and interesterified oils significantly enhanced linoleic as well as arachidonic acid levels in platelets compared to that found in rats given CNO.

However the aggregatory compound TXB_2 observed in the serum of rats given CNO were significantly higher than that observed in rats given oils containing linoleic acid. Significantly the antiaggregatory compound, prostacyclin was found to be in higher amounts in rats given oils containing linoleic acid such as RBO, SESO or blended or interesterified oils containing CNO+RBO or CNO+SESO. This higher

production of prostacyclin tilted the balance for prostacyclin, thromboxane ratio favourably for reducing aggregation of platelets in rats given unsaturated lipids. This was reflected on lower aggregation of platelets observed in rats given blended or interesterified oils as compared to that found in rats given CNO. It is also interesting to note that the decrease in platelet aggregation observed in blended or interesterified oils are comparable since the changes introduced by feeding these oils in terms of arachidonic acid levels in platelets and prostacyclin to thromboxane ratio is also comparable.

In addition to the fatty acids component, minor constituents in the oil may also influence the thrombotic parameters. Studies have shown that the presence of minor compounds in oils could change platelet activation (Oubina *et al*., 2001).Vitamin E in the oils provide health benefits against CVD through its inhibitory effect on platelet aggregation (Clarke *et al*., 2008). Studies have shown that α-tocopherol modulate the expression of protein kinase C, a factor, which activate platelet aggregation at the transcriptional level (Zingg and Azzi, 2004). Supplementation of 50 µg/d of tocotrienol rich fraction from rice bran significantly decreased the serum TXB_2 and PF4 levels in swine (Qureshi *et al*. 1991). γ-Oryzanol in rice bran oil is reported to have inhibitory effect on platelet aggregation (Cicero and Gaddi, 2001). Dietary lignans is reported to have antithrombotic properties. Studies by Noguchi *et al*. (2004) have shown that 1000 mg/kg diet is effective in suppressing the thrombotic tendency in hypertensive rats. The dietary fat RBO and its blend and interesterified oil were a source of tocotrienols, and oryzanol and SESO and its blends and interesterified oils were containing sesamin (Chapter II). The minor constituents present in RBO, SESO and their blends and interesterified oils might have also contributed to the antithrombotic properties shown by these dietary groups.

In conclusion the present investigation shows that rats given RBO, SESO or blended and interesterified oils lowered the rate of platelet aggregation compared to that found in rats given CNO. Rats fed with CNO showed an increase in thromboxane but decreased prostacyclin levels in serum. The ratio of prostacyclin/ thromboxane A_2 ratio was however enhanced in rats given blended and interesterified oils of CNO with RBO or SESO. This resulted in a favourable decrease in platelet aggregation observed in rats given blended or interesterified oils as compared to those given CNO.

INTRODUCTION

The generation of reactive oxygen species (ROS) such as super oxide anion ($O^{\cdot-}_2$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\cdot}$) during metabolism is an essential and normal process (Shih *et al*., 2008). Low level of ROS is indispensible in many biochemical processes, including intracellular signaling in the cell differentiation and cell progression or the arrest of growth, apoptosis (Ghosh and Myers, 1998) and defense against microorganisms (Bae *et al*., 1997). In contrast, high doses and/or inadequate removal of ROS result in oxidative stress, cause severe metabolic malfunctions and damage biological macromolecules (Mates *et al*., 1999). Oxidative stress has been shown to be involved in the pathophysiology of a number of chronic diseases including cardiovascular diseases (Willcox *et al*., 2004) that account for the leading causes of death in many developing countries (Joshi *et al*., 2008). It also accelerates the process of aging (MacDonald-Wicks and Garg, 2002). The biological effects of free radicals are controlled by various cellular defense mechanisms consisting of enzymatic and nonenzymatic scavenger components (Mates *et al*., 1999). The antioxidant enzymes in animal cell that prevent deleterious effect of ROS include superoxide dismutase, catalase glutathione peroxidase, glutathione transferase and glutathione reductase (Ibrahim *et al*., 2000).

Superoxide dismutase (SOD) catalyzes the dismutation of the highly reactive superoxide anion, $O^{\cdot-}_2$ to less reactive species H_2O_2 (Mahfouz and Kummerow, 2000). Peroxide can be removed by catalase or glutathione peroxidase (GSH-Px) reactions (Fridovich, 1995).

$$2O^{\cdot-}_2 + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

Catalase converts H_2O_2 to water and molecular oxygen,

$$2\ H_2O_2 \xrightarrow{Catalase} 2\ H_2O + O_2$$

$$ROOH + AH_2 \xrightarrow{Catalase} H_2O + ROH + A$$

In animals, hydrogen peroxide is detoxified by catalase and GSH-Px. Catalase protects cells from hydrogen peroxide generated from within. GSH-Px catalyzes the reduction of hydroperoxides using GSH, thereby protecting mammalian cells against oxidative damage.

$$ROOH + 2GSHO \xrightarrow{\text{GSH-Px}} ROH + GSSG + H_2O$$

The glutathione transferases catalyze the conjugation of glutathione with a variety of hydrophobic compound bearing an electrophilic center. It can utilize any ligand with carbon, sulfur, nitrogen or oxygen as the electrophile (Jakoby, 1985).

$$GSH + RSSR' \xrightarrow{\text{GST}} GSSR + R'SH$$

One of the suggested mechanisms for atherosclerosis is the increased generation of oxidized LDL (Steinberg, 1997) which leads to the development of foam cells from macrophages in blood vessels (Lusis, 2000). Low-fat diets are generally recommended to decrease circulating total and LDL cholesterol levels. Decreasing the consumption of dietary cholesterol and saturated fatty acid (SFA)s was found to be helpful in realizing the health benefits from low-fat diet (Kang *et al*., 2005). Consumption of monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFA)s has also been demonstrated to have beneficial effects on health by decreasing plasma lipids, exhibiting anti-inflammatory effects on the endothelium resulting in improvements in vascular function (De Caterina *et al*., 2000; Christon, 2003). However studies have also shown that PUFA containing lipids are prone to lipid peroxidation. The susceptibility of the fatty acid to lipid peroxidation increases in proportion to its degree of unsaturation (Richard *et al*., 2008). The ratio of PUFA to SFA in the diet determines the susceptibility of LDL to peroxidation (Esterbauer *et al*., 1992). Therefore there should be a balance in the P/S ratio in the diet.

The composition and organization of lipids in biological membranes are important factors that determine the fluidity. This influences the activity of membrane bound proteins such as enzymes, receptors, carriers or ion channels. Because

membrane phospholipids are composed of unsaturated fatty acids and are located in an oxygen containing environment, membranes are susceptible to lipid peroxidation. This process is a free radical chain reaction initiated by reactive oxygen species such as superoxide, hydroxyl radical or singlet oxygen. ROS mediated reactions can significantly damage the polyunsaturated fatty acids and proteins in the membranes (Sevanian and Hochestein, 1985). Thus peroxidation of lipids is known to alter membrane fluidity and to affect the function of integral proteins associated with the membrane (Rice-Evans and Hochestein, 1981). The control of lipid peroxidation is an essential process in aerobic organisms, as lipid peroxidation products can damage DNA. Lipid peroxidation can also directly inhibit activities of enzymes such as Na^+/K^+-ATPases (Da Silva *et al*., 1998; Esterbauer etal., 1992).

Oxidative stress is one of the causative factors that link hypercholesterolemia with atherogenesis (Halliwell, 1996). Erythrocytes are constantly exposed to both extracellular and intracellular sources of reactive oxygen species (ROS). Hypercholesterolemia leads to increased cholesterol accumulation in the erythrocytes and endothelial cells and thereby activating them to produce oxygen free radicals (Kay, 1991). Thus, erythrocytes are extremely vulnerable to these oxidative challenges and hypercholesterolemia (Vijayakumar and Nalini, 2006).

The blended and interesterified oils prepared by us as described in chapter 1 contained higher amount of unsaturated fatty acids compared to CNO. This may influence the unsaturated fatty acid composition of membranes when fed to rats. The influence of such a change on parameters regulating lipid peroxidation was monitored. The effects of feeding rats with modified lipids were also monitored on the activities of erythrocyte membrane bound enzymes.

RESULTS

Fatty acid composition of diet

Fatty acid compositions of dietary lipids (native, blended and interesterified oils) used in this study is shown in Table 5.1.

Table 5.1. Fatty acid composition (wt%) of dietary fat

Fatty acid	CNO	RBO	CNO+RBO (B)	CNO+RBO (I)	SESO	CNO+SESO (B)	CNO+SESO (I)
8:0	2.8±0.4	nd	0.5±0.1	0.5±0.1	nd	0.9±0.2	1.0±0.2
10:0	5.7±0.3	nd	1.2±0.1	1.1±0.2	nd	1.2±0.1	1.1±0.3
12:0	51.2±2.1	nd	10.1±0.4	10.3±0.5	nd	12.7±0.6	12.9±0.4
14:0	19.1±1.1	nd	4.6±0.4	4.7±0.2	nd	4.5±0.2	4.6±0.2
16:0	8.9±0.3	20.1±1.3	18.1±0.6	17.3±0.5	9.6±0.6	9.7±0.5	9.4±0.4
18:0	2.4±0.2	2.0±0.3	1.5±0.2	1.7±0.3	5.4±0.4	4.6±0.3	4.4±0.2
18:1	7.3±0.4	42.1±2.1	34.5±1.8	34.2±1.2	43.2±1.9	33.0±1.4	33.4±1.3
18:2	1.9±0.1	35.3±1.2	29.1±1.1	29.8±1.2	41.8±2.1	33.1±1.3	32.9±1.2
18:3	nd	0.5±0.1	0.4±0.2	0.4±0.1	0.4±0.2	0.3±0.1	0.3±0.1
P/S ratio	0.02	1.62	0.83	0.84	2.73	0.99	0.99

Values are mean ± SD of three samples. nd- not detected. CNO- coconut oil, RBO- rice bran oil, SESO- sesame oil, (B)- blended, (I)- interesterified, P/S - polyunsaturated to saturated fatty acid.

Fatty acid profile of liver lipids in rats fed native, blended and interesterified oils

As indicated in earlier chapter (chapter II) feeding blended and interesterified oils to rats altered fatty acid composition in liver lipids (Table 5.2). The unsaturated fatty acids, linoleic acid and arachidonic acid levels were significantly enhanced in rats fed RBO, SESO or blended or interesterified oil as compared to rats fed with CNO diet. The P/S ratio of liver lipids was increased by 3.1 and 4.3 fold, respectively in rats fed with RBO and SESO. Feeding blended oils of CNO with RBO or SESO improved the P/S ratio which was increased by 2.6 and 3.3 fold, when given CNO+RBO(B) and CNO+SESO(B), respectively. P/S ratio of rats fed interesterified oil was also increased by 2.7 and 3.4 fold when given CNO+RBO(I) and CNO+SESO(I) respectively, compared to rats fed with CNO. No significant

differences in P/S ratio was observed in liver lipids of rats fed blended and corresponding interesterified oil.

Table 5.2. Fatty acid composition (wt%) of liver lipids in rats fed native, blended and interesterified oils

Fatty acid	CNO	RBO	CNO+RBO (B)	CNO+RBO (I)	SESO	CNO+SESO (B)	CNO+SESO (I)
12:0	0.8±0.2 [a]	nd	nd	nd	nd	nd	nd
14:0	1.4±0.4[a]	nd	0.9±0.2[a]	1.1±0.2[a]	nd	0.4±0.3[a]	0.6± 0.2[a]
16:0	30.2±1.9[a]	26.1±1.4[a]	25.9±1.1[a]	24.9±1.2[a]	22.7±1.4[a]	24.1±1.5[a]	23.0±1.9
16:1	4.2±0.6[a]	1.9±0.4[a]	2.3±0.3[a]	2.2±0.4[a]	1.5±0.2[a]	1.7±0.3[a]	1.6±0.04[a]
18:0	19.8±1.5[a]	13.4±1.6[a]	15.3±1.2[a]	13.1±0.9[a]	12.1±1.2[a]	14.3±0.7[a]	11.7±0.8[a]
18:1	31.4±2.1[a]	30.0±1.9[a]	30.1±2.3[a]	33.6±2.3[a]	32.9±2.3[a]	30.1±2.4[a]	33.6±2.4[a]
18:2	5.9±0.4[a]	14.0±0.6[b]	12.6±0.9[b]	12.9±0.3[b]	14.6±0.6[b]	15.1±0.9 [b]	14.7±0.7 [b]
20:4	6.3±0.5[a]	14.6±0.7[b]	12.9±0.7[b]	11.7±0.8[b]	16.2±1.0[b]	14.3±1.4 [b]	12.4±1.1 [b]
P/S ratio	0.23	0.72	0.61	0.63	0.99	0.76	0.77

Values show the mean ± S.D of 4 rats. Values in the same row with common superscript letters are not significantly different whereas values with different superscript are significantly different at $P<0.01$. nd- not detected. CNO- coconut oil, RBO- rice bran oil, SESO- sesame oil, B- blended, I- interesterified, P/S - polyunsaturated to saturated fatty acid.

The fatty acid compositions of the erythrocyte membrane

The fatty acid compositions of the erythrocyte membranes in rats fed with native, blended and interesterified oils are shown in Table 5.3. The unsaturation index of the membrane fatty acids was significantly increased in rats fed with RBO, SESO, blended and interesterified oils. The linoleic acid content increased by 2.0 and 2.1 fold, respectively in rats fed with RBO and SESO compared to rats given CNO. The rats given blended oils showed an increase in linoleic acid content by 1.7 and 1.9 fold, respectively when given CNO+RBO(B) and CNO+SESO(B) compared to those fed

with CNO. Similarly rats fed with interesterified oils also showed an increase in the linoleic acid content by 1.8 and 1.9 fold respectively in CNO+RBO(I) and CNO+SESO(I) compared to rats given CNO. Dietary fatty acid composition influenced the arachidonic acid content of erythrocyte membrane in rats.

Table 5.3. Fatty acid composition (wt%) of erythrocyte membrane in rats fed native, blended and interesterified oils

Fatty acid	CNO	RBO	CNO+RBO (B)	CNO+RBO (I)	SESO	CNO+SESO (B)	CNO+SESO (I)
14:0	2.2±0.6^a	nd	0.5±0.3^a	0.6±0.3^a	nd	0.5±0.2^a	0.4± 0.2^a
16:0	26.4±2.1^a	23.3±1.7^a	24.6±2.3^a	23.7±1.4^a	21.9±1.6^a	22.6±1.4^a	23.4±1.8
16:1	3.6±0.5^a	1.5±0.6^a	1.7±0.4^a	1.6±0.7^a	1.4±0.4^a	1.8±0.2^a	1.4±0.3^a
18:0	19.2±2.0^a	14.4±0.8^a	16.3±1.4^a	16.8±1.1^a	14.9±1.5^a	15.9±0.5^a	16.1±1.2^a
18:1	33.1±2.5^a	28.9±2.1^a	29.6±1.8^a	28.8±1.3^a	28.1±2.7^a	29.4±3.1^a	29.2±2.1^a
18:2	8.2±0.9^a	16.6±1.3^b	14.2±0.6^b	14.9±0.9^b	17.8±1.2^b	15.4±1.3^b	15.5±0.9^b
20:4	7.3±1.0^a	15.3±1.1^b	13.1±0.4^b	13.6±0.7^b	15.9±0.8^b	14.3±1.1^b	14.0±1.3^b
P/S ratio	0.32	0.85	0.65	0.69	0.92	0.76	0.74

Values show the mean ± S.D of 4 rats. Values in the same row with common superscript letters are not significantly different whereas values with different superscript are significantly different at $P<0.01$. nd- not detected. CNO- coconut oil, RBO- rice bran oil, SESO- sesame oil, B- blended, I- interesterified, P/S - polyunsaturated to saturated fatty acid.

The arachidonic acid content of erythrocyte membrane was increased by 1.8 to 2.2 fold in rats fed RBO, SESO and in blended and interesterified oils fed rats compared to those fed with CNO (Table 5.3). These changes in the fatty acid composition resulted in a significant increase in the P/S ratio of erythrocyte membrane in rats fed RBO, SESO, blended and interesterified oils compared to those fed with CNO (Table 5.3).

Lipid peroxide level in liver and erythrocyte membranes of rats fed native, blended and interesterified oils

Dietary fat influenced the fatty acid composition of the liver lipids which in turn changed the lipid peroxide level. Rats fed with RBO or SESO had significantly higher lipid peroxide level by 1.6 and 2 fold respectively, when compared to those fed with CNO (Table 5.4). Feeding blended oils containing CNO+RBO(B) and CNO+SESO(B) to rats showed increased lipid peroxide level in liver by 1.3 and 1.6 fold, respectively when compared to rats fed with CNO. Rats fed with interesterified oils had increased lipid peroxide level by 1.3 and 1.7 fold respectively as compared to that found with CNO (Table 5.4). However the lipid peroxide levels in rats fed blended and respective interesterified oils were comparable. The rats fed diet containing RBO and SESO showed 1.7 and 2.2 fold higher lipid peroxides in erythrocyte membrane when compared with rats given CNO enriched diets (Table 5.4).

Table 5.4. Lipid peroxide level in liver and erythrocyte membrane of rats fed native, blended and interesterified oils

CNO	RBO	CNO+RBO (B)	CNO+RBO (I)	SESO	CNO+SESO (B)	CNO+SESO (I)
LPO levels in liver (nmoles of MDA formed/mg protein)						
3.2 ± 0.3^{a}	5.2 ± 0.4^{b}	4.2 ± 0.3^{c}	4.3 ± 0.2^{c}	6.4 ± 0.5^{d}	5.1 ± 0.4^{c}	5.3 ± 0.2^{c}
LPO levels in erythrocytes (nmoles of MDA formed/mg protein)						
2.2 ± 0.2^{a}	3.9 ± 0.3^{b}	3.4 ± 0.2^{c}	3.1 ± 0.2^{c}	4.9 ± 0.4^{d}	4.2 ± 0.2^{b}	3.9 ± 0.3^{b}

Values show the mean ± S.D of 4 rats. Values in the same row with common superscript letters are not significantly different whereas values with different superscript are significantly different at $P<0.01$. CNO- coconut oil, RBO- rice bran oil, SESO- sesame oil, B- blended, I- interesterified.

LPO level in rat erythrocytes membrane were increased by 1.4 and 1.9 fold respectively, when CNO+RBO(B) and CNO+SESO(B) diets were fed as compared to rats given CNO diet. Feeding interesterified oil of CNO+RBO(I) and CNO+SESO(I)

also increased the LPO content of erythrocyte membrane by 1.5 and 1.9 fold respectively, as compared to those fed CNO (Table 5.4).

Correlation between dietary fatty acid, liver lipid composition and lipid peroxide level

A positive correlation (r=0.9226, Figure 5.1) between P/S of dietary fat fed to rats and LPO level in liver was observed in this study. Similarly a positive correlation between P/S ratio of liver lipids and LPO level in liver was also observed (Figure 5.2).

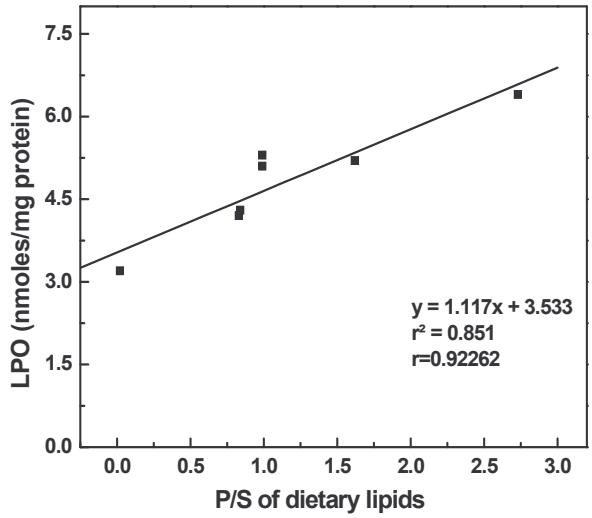


Figure 5.1. Correlation of P/S ratio of dietary lipids with lipid peroxides (LPO) in liver. P/S-polyunsaturate/saturated fatty acids.

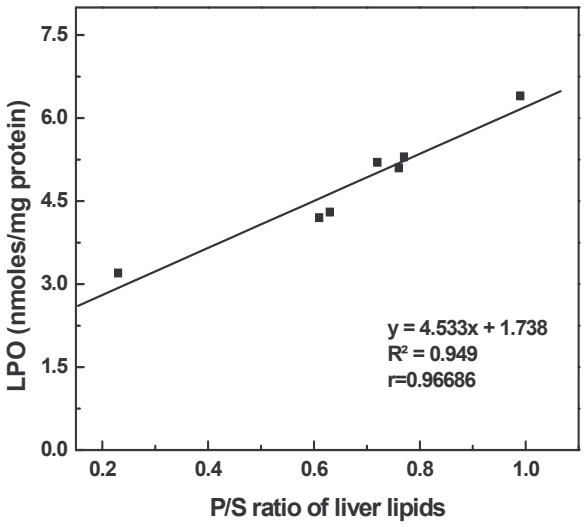


Figure 5.2. Correlation of P/S ratio of liver lipid with lipid peroxides (LPO) in liver. P/S-polyunsaturate/saturated fatty acids.

A positive correlation between P/S ratio of erythrocyte membrane lipids and LPO (Figure 5.3) were also observed. This indicated that the P/S ratio of dietary fat influences the P/S ratio of liver and membrane lipids and this in turn correlates with lipid peroxide levels observed in hepatic tissue and in erythrocyte membrane.

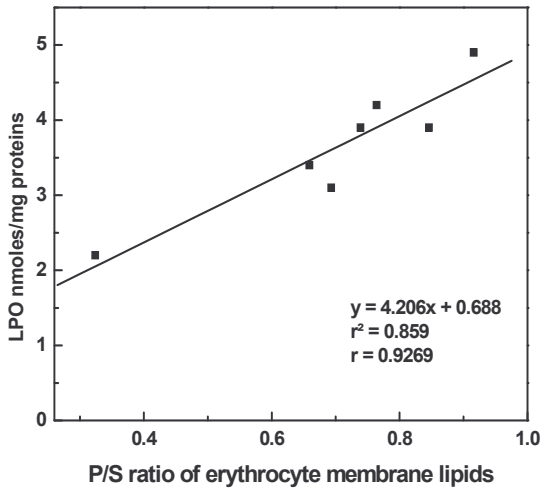


Figure 5.3. Correlation of P/S ratio of erythrocyte membrane lipid with lipid peroxides (LPO) in erythrocytes. P/S-polyunsaturate/saturated fatty acids.

Hepatic antioxidant enzyme activity of rats fed native, blended and interesterified oils

Feeding diet containing unsaturated fatty acids increased the levels of unsaturated fatty acids in tissues and cells which rendered them susceptible to lipid peroxidation. To compensate for changes in the lipid peroxide levels the endogenous antioxidant systems were found to be affected. Rats fed RBO, blended or interesterified oils showed increased activity of antioxidant enzymes such as SOD, catalase, GSH-Px, GST as compared to that found in rats fed with CNO. The SOD activity was increased by 45% in rats fed with RBO compared to rats given CNO. Feeding blended oil and interesterified oils resulted in increase in the activity by 24 and 26%, respectively in rats consuming CNO+RBO(B) and CNO+RBO(I) compared to rats given CNO (Table 5. 5a). The catalase activity was increased by 25% in rats fed with RBO compared to rats given CNO diet. Rats given blended and interesterified oils also showed increased activity for catalase by 12 and 13% when fed diet containing CNO+RBO(B) and CNO+RBO(I), respectively compared to rats given CNO (Table 5.5a).

Table 5. 5a. Effect of dietary fat on hepatic antioxidant enzymes in rats fed CNO+RBO

Parameters	CNO	RBO	CNO+RBO(B)	CNO+RBO(I)
SOD (Units/min/mg)	34.2±1.3[a]	49.6±1.9[b]	42.4±2.5[b]	43.2±2.9[b]
Catalase (μmoles/min/mg protein)	47.6±3.2[a]	59.8±2.6[b]	53.1±3.1[c]	53.9±2.4[c]
GSH-Px (μmoles/min/mg protein)	63.4±3.4[a]	76.7±2.9[b]	71.0±2.6[c]	71.9±3.7[c]
GST (μmoles/min/mg protein)	48.6±2.9[a]	61.6±2.1[b]	56.7±1.7[bc]	57.4±2.3[c]

Values are the mean ± S.D of 4 rats. Values in the same row with common superscript letters are not significantly different whereas values with different superscript are significantly different at $P<0.05$. SOD-superoxide dismutase, GSH-Px-glutathione peroxidase, GST-glutathione transferase, CNO- coconut oil, RBO- rice bran oil, B- blended oil, I-interesterified oil.

Feeding rats with RBO increased the GSH-Px activity by 21% compared to those given CNO. Rats fed with blended oil and interesterified oils increased the GSH-Px activity by 11 and 13%, respectively when fed a diet containing CNO+RBO(B) and CNO+RBO(I) compared to rats given CNO (Table 5. 5a). The GST activity increased in rats fed with RBO by 27% compared to those given CNO. Rats fed blended interesterification also showed increase in GST activity by 17 and 18%, respectively when given CNO+RBO(B) and CNO+RBO(I) compared to rats given CNO (Table 5.5a).

Rats fed with SESO, blended and interesterified oils containing CNO+SESO also showed increased activity for the endogenous antioxidant enzymes. Feeding SESO resulted in the increase in SOD activity by 58% compared to rats given CNO. Rats given blended and interesterified oils of CNO+SESO showed increased activity for SOD by 41 and 43%, respectively when given a diet containing CNO+SESO(B) and CNO+SESO(I) compared to rats given CNO (Table 5.5b). The catalase activity was increased by 33% in rats given SESO. Feeding blended and interesterified oils also showed increased activity for catalase by 20 and 22% in rats fed CNO+SESO(B) and CNO+SESO(I), respectively compared to rats given CNO (Table 5.5b). The GSH-Px activity was increased by 30% in rats given SESO compared to rats fed with

diet containing CNO. The rats fed with CNO+SESO(B) and CNO+SESO(I) also showed increased activity for GSH-Px by 21 and 24%, respectively compared to rats given CNO (Table 5.5b). The GST activity in rats given SESO was increased by 34% compared to rats given CNO. Feeding blended and interesterified oils also increased the GST activity by 22 and 24%, respectively in rats given CNO+SESO(B) and CNO+SESO(I) compared to rats fed with CNO (Table 5.5b). There was no significant difference in the activity of antioxidant enzymes in rats fed blended and their respective interesterified oils. Thus the antioxidant enzyme activities in a concerted manner were enhanced in rats given RBO, SESO, blended or interesterified oils as compared to rats given diet containing CNO.

Table 5.5b. Effect of dietary fat on hepatic antioxidant enzymes in rats fed CNO+SESO

Parameters	CNO	SESO	CNO+SESO(B)	CNO+SESO(I)
SOD (Units/min/mg)	34.2±1.3^{a}	54.2±2.4^{b}	48.1±3.5^{b}	49.2±1.7^{b}
Catalase (μmoles/min/mg protein)	47.6±3.2^{a}	63.2±2.6^{b}	56.6.0±2.8^{c}	57.9±3.9^{c}
GSH-Px (μmoles/min/mg protein)	63.4±3.4^{a}	82.3±3.1^{b}	76.8±3.7^{c}	78.6±2.4^{c}
GST (μmoles/min/mg protein)	48.6±2.9^{a}	65.3±3.7^{b}	59.2±1.9^{c}	60.4±2.7^{c}

Values show the mean ± S.D of 4 rats. Values in the same row with common superscript letters are not significantly different whereas values with different superscript are significantly different at $P<0.05$. SOD-superoxide dismutase, GSH-Px-glutathione peroxidase, GST-glutathione transferase, CNO- coconut oil, SESO- sesame oil B- blended oil; I- interesterified.

Influence of native, blended and interesterified oils on Na^{+}/K^{+}-ATPase and Ca^{2+}/Mg^{2+}-ATPase activities in erythrocytes

The effect of feeding blended and interesterified oils to rats on the Na+/K+-ATPase activity of erythrocytes is given in Table 5. 6. The activity of Na^{+}/K^{+}-ATPase was significantly increased by 22 and 31% respectively in rats fed RBO and SESO compared to those fed CNO (Table 5. 6). The rats fed blended oils containing CNO+RBO(B) and CNO+SESO(B) showed 11 and 19% increase in the activity of

this enzyme as compared to that found in CNO fed rats. Feeding interesterified oils also similarly increased the activity of Na^+/K^+-ATPase by 13 and 23% in rats fed CNO+RBO(I) and CNO+SESO(I), respectively as compared to those given CNO (Table 5. 6). The activity of Ca^{2+}/Mg^{2+}-ATPase was increased in rats fed RBO and SESO by 19 and 22% respectively, compared to rats fed CNO.

Table 5.6. Na^+/K^+-ATPase activities (nmole Pi/mg protein/h) in erythrocyte membrane of rats fed native, blended and interesterified oils

CNO	RBO	CNO+RBO (B)	CNO+RBO (I)	SESO	CNO+SESO (B)	CNO+SESO (I)
264 ± 1.5^a	321 ± 5.9^b	293 ± 9.8^c	298 ± 4.6^c	347 ± 3.9^d	315 ± 8.3^b	324 ± 7.5^b

Values show the mean ± S.D of 4 rats. Values in the same row with common superscript letters are not significantly different whereas values with different superscript are significantly different at $P<0.05$. Pi-phosphorus, CNO- coconut oil, RBO- rice bran oil, SESO- sesame oil, B- blended, I- interesterified.

Table 5.7. Ca^{2+}/Mg^{2+}-ATPase activities (nmole Pi/mg protein/h) in erythrocyte membranes of rats fed native blended and interesterified oils

CNO	RBO	CNO+RBO (B)	CNO+RBO (I)	SESO	CNO+SESO (B)	CNO+SESO (I)
424 ± 6.2^a	506 ± 5.7^b	478 ± 4.9^c	482 ± 9.8^c	519 ± 6.8^b	484 ± 7.2^c	492 ± 4.6^c

Values show the mean ± S.D of 4 rats. Values in the same row with common superscript letters are not significantly different whereas values with different superscript are significantly different at $P<0.05$. Pi-phosphorus, CNO- coconut oil, RBO- rice bran oil, SESO- sesame oil, B- blended, I- interesterified.

Feeding blended oils CNO+RBO(B) and CNO+SESO(B) increased the activity of this enzyme by 13 and 14% as compared to those fed CNO. Rats fed interesterified oils also showed increased activity of Ca^{2+}/Mg^{2+}-ATPase by 14 and 16% in rats fed CNO+RBO(I) and CNO+SESO(I) compared to rats given CNO (Table 5. 7). This

study again indicates that feeding rats with blended and interesterified oil has similar effect on the activities of membrane bound enzymes.

DISCUSSION

Dietary fat affects the fatty acid composition, lipid peroxidation and antioxidant defense systems of the body (Pulla Reddy and Lokesh, 1994). Presence of unsaturated fatty acids in cell membrane and in plasma lipoprotein renders them highly susceptible to peroxidation mediated by reactive oxygen species. The generation of reactive oxygen species is recognized as being involved in vascular disorders (Halliwell and Chirico, 1993; Witztum and Steinberg, 1991). The oxidative modification of LDL has been found to play a key role in the development of atherosclerosis (Berliner and Heinecke, 1996). Inherent antioxidant defense system such as SOD, catalase, GSH-Px and GST remove superoxides and peroxides before they react with metal catalysts to form more reactive species and thus protect cells against oxidative stress. A decrease in the activity of these enzymes may predispose cells to free radical damage (Huang and Fwu, 1993).

The present investigation was under taken to study the effect of dietary fatty acids on oxidative stress measured in terms of lipid peroxide levels and its impact on the endogenous antioxidant systems. We also looked at the effect of modified oils on membrane bound enzymes such as Na^{+}/K^{+}-ATPase and Ca^{2+}/Mg^{2+}-ATPase.

It is well known that PUFA containing lipids are prone to lipid peroxidation. The susceptibility of the fatty acid to lipid peroxidation increases in proportion to its degree of unsaturation (Richard *et al*., 2008). Our present study indicated that the P/S ratio of dietary fat influenced the P/S ratio of tissue lipids. As the P/S ratio of dietary lipids was increased there was a corresponding increase in P/S ratio of liver and in erythrocyte membrane lipids. This in a linear fashion increased the formation of LPO in liver and erythrocyte membrane.

Oxidative stress is an important contributor to the development of cardiovascular pathologies, such as atherosclerosis (Byon *et al*., 2008). ROS-initiated oxidative stress can however be regulated by cell defense mechanisms, which include

superoxide dismutase (SOD), catalase, and glutathione peroxidase and glutathione transferase (Mates *et al*., 1999). Alteration in the dietary lipids influenced the activities of the antioxidant enzymes involved in scavenging the oxygen free radicals which initiates lipid peroxidation. The activity of the antioxidant enzyme was elevated in rats fed with oils containing unsaturated lipids compared to rats fed with CNO. Feeding rats with blended oils and interesterified oils containing CNO with RBO or SESO showed an increased activity of SOD, catalase, GSH-Px and GST compared to the rats given CNO. The increased activity of these antioxidant enzymes observed in rats given unsaturated lipids may partly nullify the effects higher lipid peroxide level generated in these groups. There was no significant difference in LPO or antioxidant enzyme activities in rats given blended oils or interesterified oils. However feeding interesterified oils to rats showed a higher hypocholesterolemic activity when compared to the rats given blended oils of similar composition (Chapter II).

The minor components present in oil may also exhibit antioxidant properties. RBO contains tocopherols, tocotrienols and γ-oryzanol in its unsaponifiable matter (Minhajuddin *et al*., 2005; Juliano *et al*., 2005). Tocopherols and lignans such as sesamin, sesamolin and sesamol are the minor components present in sesame oil (Ahmad *et al*., 2006). The health benefits of tocopherol and tocotrienols are well documented. Tocopherols and tocotrienols are known for their antioxidant activity (Khanna *et al*., 2006). α-Tocopherol is a lipid-soluble antioxidant and it functions as a chain-breaking antioxidant for lipid peroxidation in cell membranes and also as a scavenger of reactive oxygen species (ROS) such as singlet oxygen (Liebler, 1993). Studies have shown that tocotrienols possess hypocholesterolemic, antioxidant, anticancer and neuroprotective properties that are often not exhibited by tocopherols (Osakada *et al.*, 2004). Several studies have shown that tocotrienol possesses more antioxidant activity than their counterparts, tocopherol (Kamal-Eldin and Appelqvist, 1996). *Invitro* studies by Serbinova *et al*. (1991) have shown that α-tocotrienol possesses 40-60 times higher antioxidant activity than α-tocopherol against ferrous/ascorbate and ferrous NADPH induced lipid peroxidation in rat liver microsomal membrane. Peroxyl radical scavenging activity followed by

chemiluminescence method using azobisdimethyl valero nitrile showed that α-tocotrienol is 3.3 times more potent in scavenging peroxyl radical than that of α-tocopherol in membranes (Suzuki *et al*., 1993). γ-Oryzanol is known to be a powerful inhibitor of iron-driven hydroxyl radical formation, and it was also reported to possess antioxidant activity in stabilizing lipids (Duve and White, 1991). Sesamin, sesamolin the unique minor components of sesame oil are known for their antioxidant activity (Kamal–Eldin *et al*., 1994; Shyu and Hwang, 2002). The blended and interesterified oils used in our studies contained significant amounts of these antioxidant molecules which also might have contributed in modifying lipid peroxidation observed in this study. This needs to be elaborated in further studies.

The fatty acid composition of membranes lipids plays an important role in maintaining fluidity (Lemaitre *et al*., 2008). Membrane fluidity can be altered by dietary lipids. PUFAs modulate membrane physicochemical properties particularly membrane fluidity which in turn affect a number of membrane related factors, including membrane bound enzymes (Hashimoto *et al*., 2001). Erythrocyte membrane fluidity was found to be decreased in patients with coronary artery disease (Luneva *et al*., 2007). Increasing the amount of PUFA levels in the diet increases the ratio of polyunsaturated to saturated fatty acids in the membranes. This may affect membrane fluidity (Leger *et al*., 1990). This in turn may have influenced the membrane associated enzyme activities such as Na^+/K^+-ATPase and Ca^{2+}/Mg^{2+}-ATPase as observed in this study. Rats fed with RBO and SESO had a significantly higher activity of Na^+/K^+-ATPase and Ca^{2+}/Mg^{2+}-ATPase compared to rats fed CNO diet. Rats fed with blended and interesterified oils also showed higher activity of Na^+/K^+-ATPase and Ca^{2+}/Mg^{2+}-ATPase compared to those given CNO. This indicated that these enzymes show higher activity in an unsaturated lipid environment.

In conclusion the present study shows that the P/S ratio of dietary fat influences the P/S ratio of tissue lipids. This in turn influences the lipid peroxide level. The increase in LPO in rats given unsaturated lipids resulted in higher levels of antioxidant enzyme activities in liver. This may partly blunt the effects of increased LPO observed in rats given unsaturated lipids.

The study of lipids and their major structural elements, the fatty acids, remains one of the most enigmatic research fields in biology and nutrition. As a specific component in the diet, fat provides essential fatty acids. Fats also dissolve and assist the absorption of fat-soluble vitamins and essential nutrients. Fatty acids are also required for membrane synthesis, construction of various structural elements in cells and tissues, production of signaling compounds and fuel.

Consumer interest in dietary fat beneficial to the health is increased in recent time. This is mainly because of the link between fat and risk factors of various chronic diseases. The nutritional quality of a fat is dependent on the physicochemical properties of the TAG molecules it contains. The physicochemical property of a TAG molecule is dependent not only on the fatty acid chain length, unsaturation and geometry of double bond but also on the stereospecific position in the TAG molecule. Oils containing TAG of higher melting fatty acids tend to show cloudiness or remain as solid at room temperature, while that containing unsaturated fatty acids will be in liquid form. It is well recognized that intake of saturated fatty acids increase the cholesterol level and its replacement with polyunsaturated fatty acids decrease the cholesterol level. There is no fat or oil in nature with balanced fatty acids (SFA:MUFA:PUFA in the ratio of 1:1:1) to get optimum health benefits as recommended by Indian Council of Medical Research and/or American Heart Association (Ghafoorunissa, 1998, Fogli-Cawley *et al*., 2006).

Several methods are available for the modification of oils and fats to improve the quality. Blending of fats have also been used to modify physical and chemical properties of natural fats. Blended oils prepared using corn oil and butter fat resulted in a base for spreads, with no trans fatty acids but, keeping the inherent qualities of butter, with higher EFA content (Rodrigues and Gioielli, 2003). Binary mixture of milk fat and phytosterol esters was used to improve the nutritional properties of milk fat with decreased saturation, and a softer consistency than pure milk fat (Rodrigues *et al*., 2007). Blending of *Moringa oleifera* oil with sunflower oil and soybean oils have been carried to enhance the oxidative and thermal stability of PUFA rich oils (Anwar *et al*., 2007). However, blending of oils may not always result in required physicochemical properties. The TAG from parent oils retains its physicochemical

properties there by its absorption rate. (Kennedy, 1991). In the present study the blended oils prepared using CNO with RBO or SESO showed altered physicochemical properties such as decreased solid fat content in CNO+RBO(B) and CNO+SESO as compared to CNO (Reena *et al*., 2009).

Rearrangement of fatty acid on the TAG molecule caused by interesterification has been used as an approach for the modification of oils fats. Interesterification results in altered TAG composition of the oils by altering the amount of existing TAG species and/or result in the generation of new TAG species (Chen *et al*., 2007). Enzymatic production of structured lipids is attaining wider attention due to the specificity of enzymes in carrying out reactions. Sahin *et al*., (2005) have prepared fat substitute by enzymatic acidolysis of tripalmitin with hazelnut oil fatty acid and stearic acid. Enzymatic interesterification has been used for the production of trans free fat and also for the production of fat with improved functional properties (Shin *et al*., 2009). Randomized lipids prepared using fish oil and medium chain structured TAG had improved digestion, absorption and lymphatic transport (Tso *et al*., 2001).

Dietary lipids influence various metabolic pathways depending on total fat content, fatty acid composition, and individual variation. The individual fatty acids vary markedly in their physical characteristics, absorbability, metabolic fate, and regulatory effect. The effects of dietary fat on plasma lipids and lipoproteins have been the subject of numerous studies. It is generally accepted that saturated fatty acids raise and polyunsaturated fatty acids reduces blood cholesterol level in humans (Zhao *et al*., 2004) and animals (Trautwein *et al*., 1999). However, not all saturated fatty acids share hypercholesterolemic property. Saturated fatty acids with < 12 carbon atoms do not raise serum cholesterol concentrations. This is because medium chain fatty acids are readily absorbed from the small intestine directly into the portal system and transported to the liver for metabolism (Matulka *et al*., 2006). The position of lauric acid (C12:0) as a medium chain or long chain fatty acid is also a matter of debate due to the way it is metabolized. Lauric acids enter the circulation partly as a component of chylomicron TAG and partly as free fatty acids (Grundy, 1994). Therefore the cholesterolemic effect of lauric acid is still controversial. Keys *et al*.

(1965) reported that lauric acid is as hypercholesterolemic as that of palmitic and myristic acids. Hegsted *et al*. (1965) in contrast showed that lauric acid increases cholesterol concentrations much less than that mediated by other long chain fatty acids. Later on Denke and Grundy (1992) studied the effect of lauric acid, in which the lauric acid was incorporated in to a synthetic fat. This lauric acid enriched fat was compared with palm oil, which differed in composition only by having palmitic acid in the place of lauric acid. The effects of these two saturated fatty acids were compared with oleic acid rich safflower oil. It was observed that lauric acid increases LDL cholesterol concentrations only by about two-thirds as observed when palmitic acid rich fats are fed. Among the cholesterol raising saturated fatty acids myristic acid is considered as the most potent than lauric or palmitic acid (Hu *et al*., 1999). Myristic acid is however present in much smaller amounts in most of the fats. Studies have shown that palmitic acid raises the serum total cholesterol concentration. Palm oil, which contains about 40% palmitic acid resulted in the increased total and LDL cholesterol compared to subjects who consumed diet rich in MUFA or PUFA oils such as canola and soybean. It also promotes the secretion of lipoproteins containing apolipoprotein B-l00 (apo B) (Vega-lo Pez *et al*., 2006). The mechanism by which these long chain saturated fatty acids increase cholesterol level is by increasing the LDL fraction. They also decrease LDL receptor protein, LDL receptor activity, and LDL receptor mRNA (Dorfman *et al*., 2006, Mustad *et al*., 1996). After reaching the liver the fatty acids, recirculates in the form of lipoprotein and modifies plasma and LDL cholesterol concentration (Nicolosi *et al*., 1998). Studies have also shown that stearic acid, an 18 carbon saturated fatty acid does not raise plasma cholesterol concentrations and considered as a neutral fatty acid. The reason for neutral effects of stearic acid has been studied by many investigators. Earlier investigations have suggested that stearic acid is not well absorbed (Bonanome and Grundy, 1988), but later investigations indicated that > 90% of the stearic acid available in the diet is absorbed (Baer *et al*., 2003). Another mechanism for neutral effect of stearic acid is attributed to its desaturation to oleic acid, which does not raise cholesterol concentration (Rhee *et al*., 1997). The cholesterol raising properties of dietary SFAs is therefore attributed to lauric acid (12:0), myristic acid (14:0), and palmitic acid (16:0).

The biological effects of MUFA depend on whether it is in *cis* or *trans* configuration. *Cis*-MUFA are relatively neutral or can lower LDL without affecting HDL cholesterol, but *trans*-MUFA have been shown to increase LDL and decrease HDL (Mensink, 2005). *Trans*-MUFA also increases plasma TAG level (Nicolosi *et al*., 1998) and also increase levels of lipoprotein(a) (Tholstrup and Sammman, 2004). *cis* MUFA like oleic acid may not raise serum LDL-cholesterol concentrations as it is a preferred substrate for acyl CoA: cholesterol acyltransferase (ACAT) enzyme in the liver which esterifies cholesterol (Rumsey *et al*., 1995). In the presence of excess of oleic acid, free cholesterol is readily esterified (Lee and Carr, 2004). Oleic acid allows normal expression of LDL receptors. Dietary oleic acid however does not affect serum VLDL concentrations relative to saturated fatty acids, and hence the synthesis of VLDL by the liver is not curtailed with diets high in oleic acid (Spady *et al*., 1993).

Dietary PUFAs are known for their lipid lowering effect. Several metabolic studies have shown that linoleic acid is hypocholesterolemic in its effect. It reduces total and LDL cholesterol (Harris, 2008). It also beneficially influence the platelet function (Micha, 2008). However experiments have shown that high intake of linoleic acid was associated with an increased risk of ulcerative colitis (Hart, 2009). Studies in hamsters also have shown that high levels of PUFA in the diet lower HDL concentration compared to that found in animals given a diet containing more of MUFA or SFA (Terpstra *et al*., 2000). High concentrations of total and LDL cholesterol and low concentrations of HDL are risk factors for coronary disease. Concern has also been raised that excessive PUFA consumption could be detrimental if it decreases HDL cholesterol (Grundy and Denke, 1990). Excessive intake of PUFA has undesirable effects such as oxidative stress (Park *et al*. 1999) because of its high susceptibility to lipid peroxidation (Saito and Kubo, 2003). The presence of linoleic acid in LDL lipids makes them more prone to oxidation, which could promote the development of atherosclerosis (Parthasanathy *et al*., 1990). All these studies indicate that there should be a balance in the fatty acid composition of diet with respect to saturated, monounsaturated and polyunsaturated fatty acids in the lipids.

In the present investigation we prepared modified oils by blending coconut oil with rice bran oil or sesame oil in a proportion which gave saturated:monounsaturated:polyunsaturated fatty acids in the ratio of approximately

1:1:1. This ratio was in tune with recommendation of ICMR (Ghafoorunissa, 1998). The blended oils were further treated with lipase from *Rhizomucor miehei* in non aqueous medium to rearrange the fatty acids on the glycerol molecule (Reena *et al*., 2009). The effects of these oils on serum and tissue lipids were monitored by feeding them to rats and comparing the effect with rats given CNO (Reena and Lokesh, 2007).

Blending of selected oils are reported to provide additional benefits when consumed. Studies have shown that oil prepared by blending of vegetable oils (MCT + coconut oil) and plant sterols (tall oil) was effective in obesity prevention. It was found that subjects consuming these modified oils showed lower cholesterol levels compared to subjects who consumed diet containing olive oil (St-Onge *et al*., (2003).

The physicochemical properties of a TAG molecule are dependent on the chain length, unsaturation, geometry of the double bond and stereospecific position of the fatty acid in the TAG. In addition to overall fatty acid composition, the stereospecific distributions of fatty acids in a particular fat also need to be considered for their effect in biological systems. The metabolic fate of a fatty acid is dependent on its position in the TAG. (Small, 1991). Studies have shown that the fatty acid in the sn-2 position of a TAG is well absorbed (Hunter, 2001). Lein and coworkers (1993) studied the effect of feeding combinations of coconut oil and palm olein either in their native or interesterified form on cholesterol level in rats. In the physical mixture of 25:75 coconut oil/palm olein, 93% of the palmitic acid was in the sn-1 and sn-3 positions and in interesterified form 65% palmitic acid was in the sn-1 and sn-3 positions. Feeding interesterified fat to rats significantly decreased the fecal excretion of saturated fatty acids, suggesting that increasing the saturated fatty acid in the sn-2 position could increase its absorption. Support for this hypothesis has been provided by studies showing a better absorption of total dietary fat or16:0 by infants which were fed formula containing 16:0 esterified in the sn-2 position of the TAG (Carnielli *et al*., 1995). Structured lipids prepared using rapeseed oil and MCT improved the fat absorption in normal rats and rats having malabsorption problems (Straaup and Hoy, 2000). Recently Cho *et al*. (2009) have reported that structured lipid containing monoacylglycerol enriched with monounsaturated fatty acids not only lower blood lipid level and also exert antioxidant effect. In the present investigation we noticed

that feeding interesterified oil resulted in a significant decrease in the serum and hepatic cholesterol levels when compared to the rats given a physical blend of same oil (Reena and Lokesh, 2007).

Dietary fatty acids have a considerable effect on plasma LDL cholesterol concentrations and therefore influence the risk factors for coronary heart disease (Fernandez and West, 2005). Dietary PUFA have a major impact on the activity of enzymes associated with lipid biosynthesis and oxidation (Sampath and Ntambi, 2005). Dietary fat influence the mRNA levels involved in cholesterol metabolism. The capacity of fats to regulate the cholesterol metabolism is dependent on chain length, position and geometry of double bonds with in the fatty acids they contain (Salter and Tarling, 2007). In mammals the expression of several genes has been shown to be modulated by fatty acids in a positive or negative manner (Duplus *et al*., 2000). Dietary polyunsaturated fatty acid down regulate the expression of the protein NPC1L1 involved in intestinal cholesterol absorption (Alvaro *et al*., 2009). Studies by Hsu *et al*. (2006) have shown that feeding safflower oil to rats resulted in the upregulation of genes coding for enzymes of fatty acid oxidation in the liver. Dietary fatty acids affect the activity and abundance of specific transcription factors involved in expression of genes for many key enzymes, receptors and transport proteins (Sampath and Ntambi, 2005; Deckelbaum *et al*., 2006).

High cholesterol concentration in the serum is identified as one of the risk factor of CVD. Cholesterol homeostasis is controlled in mammalian cells by cholesterol synthesis, uptake and efflux pathways. Liver plays a central role in these processes and is the major site for controlling cholesterol levels. The rate limiting step in the cholesterol biosynthesis is catalyzed by the enzyme HMG-CoA reductase. Cholesterol can be either excreted in to the bile directly or after conversion in to bile acids. Conversion of cholesterol to bile acid is an irreversible process and cholesterol 7-α hydroxylase (CYP7A1) is the rate limiting enzyme involved in the biosynthesis of bile acids and occurs exclusively in the liver. Cheema *et al*. (1997) compared the effect of SFA, MUFA and PUFA rich diet on CYP7A1 in mice. Cholesterol supplementation increased the activity and the mRNA abundance of CYP7A1. Feeding mice with diet rich in PUFA resulted in significantly increased CYP7A1

activity compared to those fed with diet containing higher amounts of saturated or monounsaturated fatty acid containing diet. The activity of CYP7A1 in mice given monounsaturated fats however was found to be significantly higher compared to mice fed with diet containing saturated fatty acids. Mice fed with PUFA fed group had significantly high mRNA abundance for CYP7A1 compared to those fed with SFA or MUFA diet. This indicates that monounsaturated and polyunsaturated fatty acids upregulate the expression of CYP7A1 and thereby lower the cholesterol level by converting it to bile acids. In the present study we observed that rats fed with RBO and SESO showed a significantly high mRNA abundance for CYP7A1 as compared to those given CNO. Rats fed blended oils containing CNO with RBO or SESO also showed an increased expression of CYP7A1 as compared to those fed CNO. Feeding rats with interesterified oils showed a further increase in mRNA abundance for CYPA7A1 compared to those given blended oil with similar fatty acid composition. This indicates that PUFA lowers the cholesterol level by influencing the CYPA7A1.

Clearance of LDL from the plasma is mediated by the LDL receptors (Tocher, 2003). Activity and expression of LDL receptor are modulated by fatty acids. In mammals, saturated fatty acids tend to decrease LDL receptor activity, protein and mRNA abundance for LDL while oleic acid and PUFA increase them (Dietschy, 1998; Fernandez and West, 2005). Dorfman and Lichtenstein (2006) have studied the effect of different dietary fats on the regulation of the expression of genes involved in cholesterol homeostasis. They found that saturated fatty acids decreases the mRNA abundance for LDL receptor compared to monounsaturated or polyunsaturated fatty acids. Hamsters fed saturated fatty acids showed lower mRNA abundance for HMG-CoA reductase gene compared to those fed diet containing MUFA and PUFA. The mRNA abundance for LDL receptor increased in rats fed with RBO and SESO compared to those fed CNO diets. Feeding rats with blended oil containing CNO+RBO or CNO+SESO also showed a significant increase in the mRNA abundance for LDL receptor compared to rats fed CNO. Feeding interesterified oils to rats also showed increase in the mRNA abundance for LDL receptor compared to CNO. The mRNA abundance for LDL receptors in rats fed interesterified oil was found to be significantly higher when compared to rats fed blended oil with similar fatty acid composition. A striking point observed in the present study is that rats fed

interesterified oils containing of CNO+SESO showed high mRNA level for LDL receptor coinciding with maximum decrease observed in cholesterol level. The mRNA abundance for HMG-CoA reductase did not show a remarkable difference as that observed with LDL receptor in spite of the fact that HMG-CoA reductase activity was significantly influenced by the dietary fatty acids.

Dietary fat regulates gene expression by controlling the activity or abundance of key transcription factors (Jump *et al*., 2005). The sterol regulatory element binding proteins play a major role in regulating the expression of genes associated with lipid and lipoprotein metabolism (Brown and Goldstein, 1999; Horton *et al*., 2002). SREBP-2 is predominantly involved in the regulation of cholesterol metabolism (Horton *et al*., 1998). At high concentrations, intracellular sterols inhibit SREBP-2 expression (Sato *et al*., 1999) and inhibit maturation of its inactive precursor molecule (Brown *et al*., 2002). Dietary fatty acids also influence the mRNA abundance of SREBP-2. Studies by Dorfman and Lichtenstein (2006) have shown that dietary PUFA increase the expression of SREBP-2. In the present study the mRNA abundance for SREBP-2 followed the same pattern as that of LDL receptor. Rats fed with RBO, SESO, blended and interesterified oils showed elevated mRNA abundance for SREBP-2 compared to rats fed with CNO. This shows that dietary lipids influence the lipid metabolism at the genomic level. The mRNA abundance for SREBP-2 followed the pattern similar to that of LDL receptor. We found an increase in the mRNA abundance for LDL receptor and SREBP-2 after feeding rats with a diet containing unsaturated fatty acids.

Thus the type of fat in the diet has an impact on many aspects of lipid metabolism including that on lipoprotein pathways, lipid synthesis and oxidation and cholesterol metabolism (Salter, 2007). Dietary long chain saturated fatty acids increase total and LDL cholesterol in the blood suppressing the LDR receptor protein synthesis, LDL receptor activity and also mRNA abundance for LDL receptor, whereas dietary PUFA showed opposite effect.

High concentration of plasma TAG is considered as a risk factor for CVD (Furtado *et al*., 2008). Clinical trials have shown that apolipoprotein B containing lipoprotein contribute to the development and progression of CVD. Elevated

apolipoprotein B is associated with the risk factors of CVD obesity, dyslipidemia, thrombosis and LDL cholesterol (Williams *et al*., 2003). Unsaturated fatty acids in the diet are found to reduce TAG level in the serum (Kotronen *et al*., 2009). Feeding unsaturated fatty acid rich diet resulted in the decrease in the TAG concentration in serum compared to that found in rats given CNO. Blending of CNO with RBO or SESO and feeding to rats showed decreased TAG level in CNO+RBO(B) and CNO+SESO(B). Rats fed with interesterified oils showed a further decrease in TAG level compared to CNO and also compared to that observed after feeding the blended oil. Therefore blended and interesterified oil can be used as an approach to reduce the risk factors of CVD in Indian population who consume saturated fats.

Thrombosis is another risk factor for CVD (Badimon, 2001). Studies have shown that factors which enhance thrombotic tendency such as increased blood platelet aggregation (Thaulow *et al*., 1991; Elwood *et al*., 1991) and eicosanoid production are positively associated with cardiovascular risk. The thrombotic tendency of the blood is influenced by total fat intake as well as the fatty acid composition of the diet (Thijssen *et al*., 2005). Dietary fatty acids can modulate the fatty acid composition of platelets, thereby changing the availability of arachidonic acid. Arachidonic acid is a precursor for eicosanoid synthesis, which is involved in platelet aggregation (Zhou and Nilsson, 2001). In rats, arterial thrombosis as measured with the aortic loop technique, was decreased by (n-6) and (n-3) PUFA, whereas SFA with 12–16 carbon atoms promoted arterial thrombus formation (Hornstra and Kester, 1997). Stearic acid is reported have beneficial effect on platelet aggregation (Kelly *et al*., 2001). The effects of oleic acid as a major monounsaturated fatty acid were neutral or even antithrombotic compared with SFA (Hornstra and Kester, 1997). Studies have shown that dietary palmitic acid increases the palmitic acid content of platelet. This in turn increases the platelet volume, coagulation factor FVII activity (Kelly *et al*., 2001). Rats fed with CNO showed increased rate of platelet aggregation. However rats fed blended and interesterified oils showed a significant decrease in the thrombotic effect as compared to rats given CNO.

Arterial thrombosis contributes to the genesis and complications of cardiovascular disease (Davies, 1997). Studies have shown that dietary fatty acids

influence the production of eicosanoides by platelets and endothelial cells. The balance between the production of thromboxane A_2, a vasoconstrictor and platelet aggregating agent, and prostacyclin, a vasodilator and an inhibitor of platelet aggregation, are shown to be critical in the development of thrombotic disorders (McDonald *et al*., 1989). Studies by MacDonald *et al*. (1989) have been shown that fatty acid composition of the diet influence the bleeding time which in turn is influenced by the prostacyclin and thromboxane levels. Subjects given mixed fat containing high level of saturated fatty acid (37%) showed increased thromboxane level and decreased prostacyclin concentration compared to subjects who were given diet containing canola oil (14% SFA) or sunflower oil (19% SFA). Prostacyclin/thromboxane ratio was also high in subjects given diet containing canola or sunflower oil diet (1.8 fold and 2.0 fold respectively) compared to those who were on mixed diet containing higher levels of saturated fatty acids. In the present study we observed that rats fed diet containing, RBO and SESO showed a high prostacyclin/thromboxane ratio compared to rats fed CNO. Feeding rats with blended and interesterified oils containing CNO with RBO or SESO improved the prostacyclin/thromboxane ratio as compared to those given CNO. This indicated that the dietary lipids with balanced proportions of fatty acids favourably influence eicosanoid level.

Dietary fat also influences the risk of coronary heart disease by several other mechanisms. One such effect is seen on the susceptibility of LDL to oxidation. Despite the favorable effects on lipid profiles contributed by diets which are high in unsaturated fat, concerns are expressed that such diets could increase the susceptibility of LDL to peroxidation, thereby putting strain on antioxidant defense systems. Oxidative modification of low density lipoprotein (LDL) is thought to play an important role in the development of atherosclerosis (Roche, 1999). Key initial events during early atherogenesis are the recruitment and differentiation of circulating monocytes, which take up oxidized LDL to form lipid loaded- foam cells, results in atheroma plaque formation (Moreno and Mitjavila, 2003). The degree of unsaturation of dietary fatty acids affects lipoprotein composition and there by its susceptibility to undergo oxidation (Scislowski *et al*., 2005). Oxidation of lipids begin at the PUFA rich phospholipids present on the surface of LDL particles, and propagate to the

lipophilic core containing cholesteryl ester and TAG. The resulting aldehydes, especially 4-hydroxynonenal, bind to apolipoprotein B moiety of LDL. This modified LDL particles are recognized by the scavenger receptors of macrophages and is rapidly taken up by the macrophages present in the subendothelial space. Thus intracellular cholesterol accumulates and converts the macrophages into lipid loaded foam cells (Witztum and Steinberg, 1991; Westhuyzen, 1997). Studies in our laboratory (Nagaraju and Belur, 2008) have shown that feeding groundnut oil rich in unsaturated fatty acids to rats increased the susceptibility of LDL to oxidation. Feeding blended and interesterified oils containing groundnut oil with coconut oil resulted in decrease in the LDL oxidation by 7.4 and 13%, respectively compared to rats fed with groundnut oil. In the present investigation we noticed that the lipid peroxides in rat liver homogenate showed a decrease when given blended and interesterified oils of CNO+RBO and CNO+SESO compared to rats fed with RBO or SESO. This indicates that blended and interesterified oils can be used for controlling lipid peroxides, which in turn can modulate the risk factors of CVD.

The biological effects of free radicals are controlled by various cellular defense mechanisms consisting of enzymatic and non enzymatic scavenger molecules. Antioxidant enzymes remove or transform ROS into less toxic metabolites. The antioxidant enzymes include SOD, catalase, GSH-Px and GST (Mates *et al*., 1999). The decreases in the activity of antioxidant enzymes reflect on the sensitivity of these enzymes to radical induced inactivation (Ceriello *et al*., 1991). Lipids containing unsaturated fatty acids are prone to oxidation (Kang *et al*., 2005). The antioxidant molecules present in the system inhibit the oxidation of these lipid molecules. Rats given tocotrienol rich fraction showed significantly higher activity for SOD, catalase and GSH-Px (Lee *et al*., 2009). Hsu *et al*. (2004) have shown that sesame oil administration increased the antioxidant enzymes such as SOD and catalase in rats. In the present study also we observed that rats given RBO and SESO showed increased activity for SOD, catalase, GSH-Px and GST compared to rats fed CNO. The increased activity of these antioxidant enzymes seen in rats given RBO and SESO may be partly the result of presence of tocopherols and tocotrienols and oryzanol in the RBO and lignans and γ-tocopherol present in the SESO. Feeding blended and interesterified oils of CNO+RBO and CNO+SESO to rats showed increased

antioxidant activity for SOD, catalase, GSH-Px and GST compared to rats fed CNO. This indicates that endogenous antioxidant system act as compensatory mechanism to protect the cell from oxidative stress caused by lipid peroxidation.

Dietary fat affect physicochemical properties of membranes including fluidity and can modulate membrane associated physiologic processes (Berlin *et al*., 1998). Membrane fluidity is a major determinant of many membrane associated functions, such as binding of proteins to receptors, neurotransmitter release and reuptake, ion transport, protein phosphorylation and membrane-bound enzyme activity (Ghosh *et al*., 1993). Polyunsaturated fatty acids are important membrane components that influence membrane integrity and fluidity (da Rocha *et al*., 2009). Erythrocyte membrane fluidity is altered by unsaturated fatty acids in the membrane and it was shown that dietary linoleic acid increase the membrane fluidity (Berlin *et al*., 1998). Studies by Broncel *et al*. (2007) have shown that increased membrane fluidity increase the activity of Na^+/K^+-ATPase of erythrocyte membrane. They have also reported that a significant decrease in the Na^+/K^+-ATPase in hyperlipidemic patients. Rats fed with CNO showed a significant decrease in the activity for Na^+/K^+-ATPase compared to rats fed with diet containing unsaturated fatty acids. Rats fed with blended and interesterified oils containing CNO with RBO or SESO increased the activity of Na^+/K^+-ATPase compared to rats fed CNO.

Apart from fatty acids, oils also contain specific minor constituents in the unsaponifiable fraction. Each oil has unique minor components. Some of these minor components are reported to have nutraceutical properties. For example rice bran oil contains oryzanol, tocopherols and tocotrienols. Oryzanol the ferulic acid esters of triterpene alcohol exhibit cholesterol lowering property (Seetharamaiah and Chandrasekhara, 1988; Sugano and Tsuji, 1997). Tocotrienols is also reported to have hypocholesterolemic effect. It is postulated that tocotrienols especially γ-tocotrienols lower cholesterol through the inhibition of HMG-CoA reductase, the rate limiting enzyme in endogenous cholesterol synthesis (Kerckhoffs *et al*., 2002) The tocotrienol rich fraction of RBO is also reported to lower serum total cholesterol and LDL cholesterol concentrations in hypercholesterolmic individuals (Qureshi *et al*, 1997; Wilson *et al*., 2007). Tocopherols and tocotrienols, the vitamin E compounds are recognized for their effective inhibition of lipid peroxidation (Xu *et al*., 2001). The

antioxidant activity of the tocopherols and tocotrienols is attributed to their ability to donate their phenolic hydrogen to lipid free radicals (Burton and Ingold, 1981).

Sesamin, sesamolin and sesamol are the major lignans present in sesame oil. Sesame lignans are reported to have antioxidant (Kang *et al*., 1999) and hypocholesterolemic (Hirata *et al*., 1996) effect. Hemalatha *et al*, (2004) have shown that dietary sesame oil inhibits iron-induced oxidative stress in rats. Sesamin present in sesame oil is shown to inhibit Δ5-desaturation of (n-6) fatty acids (Shimizu *et al*., 1991) that interrupt the formation of arachidonic acid which is the substrate for proinflammatory 2-series prostaglandins (Utsunomiya *et al*., 2000). The sesamin also exhibit hypocholesterolemic activity via inhibition of cholesterol synthesis and absorption (Hirata *et al*., 1996). It shows antihypertensive effect (Nakano *et al*., 2002). It improves the bioavailability of α-tocopherol (Kamal-Eldin *et al*., 1995). The degradation product of sesamin in liver (Nakai *et al*., 2003) and the metabolite of sesamolin (sesamol and sesamolinol) have been shown to exhibit antioxidant effects in vivo (Kang *et al*., 1998). The cholesterol lowering effect of sesamin is mediated mainly by inhibiting the absorption of dietary cholesterol in the intestinal tract, by increasing the excretion of cholesterol in bile and by decreasing the activity of HMG-CoA reductase (Hirose *et al*., 1991). Dietary sesamin reduces total and LDL cholesterol concentrations in hypercholesterolemic patients (Hirata *et al*., 1996). The blended and interesterified oils used in our study contained these specific minor components. However the effects of these minor constituents individually were not evaluated in our studies.

In India the dietary habit in particular fat consumption vary widely. Coconut oil is the predominant dietary fat in Kerala and coastal regions of Karnataka. Coconut oil is considered as an atherogenic fat due to its high (about 90%) saturated fatty acid content. Sesame oil is used in few states of south India. Rice bran oil, which is recently introduced in Indian market, is also a source of cooking fat for Indian population. Sesame oil and rice bran oil are rich in unsaturated fatty acids and also contain minor components possessing nutraceutical properties. Sesame lignans are reported to have hypolipidemic and antioxidant properties and γ-tocopherol a compound with potent antioxidant properties is present in sesame oil. Oryzanol, a

compound with hypolipidemic property is uniquely present rice bran oil. It also contains tocotrienols, possessing hypocholesterolemic and antioxidant properties. However it is also known that consumption of excess amounts of polyunsaturated fatty acid leads to oxidative stress, if not balanced with antioxidants. In this investigation we prepared blended oils consisting of coconut oil with RBO or SESO, to give saturated:monounsaturated:polyunsaturated fatty acids in the proportion of approximately 1:1:1. Blending of CNO with RBO or SESO increased the linoleic acid level and also enriched the coconut oil with specific nutraceuticals derived from oils used for blending. The blended oils were further treated with lipase to rearrange the fatty acid molecules on the TAG. Interesterification of blended oils did not change the fatty acid composition and the nutraceutical content of the oils. However interesterification rearranged the fatty acids in the TAG molecular species. This altered the physical properties of blended oils. Melting profile of oils indicated that interesterification improved the homogeneity of blended oil. Analysis of TAG molecular species by HPLC showed the generation of new TAG molecular species in the interesterified oil which was not present in the native oil and/ or in blended oils. These modified oils were fed to rats to study their effect on cholesterol and found that interesterified oils show higher cholesterol lowering effects than blended oils. Rats fed with RBO or SESO had significantly lower serum and liver cholesterol level compared to that in CNO fed rats. Rats given blended or interesterified oils containing CNO with RBO or SESO showed a significant decrease in the cholesterol levels compared to the rats given CNO. Interesterified oils however showed better hypocholesterolemic effect than blended oil even though both blended and interesterified oil had similar fatty acid composition. Interesterified oils showed modified TAG molecular species due to interchange of fatty acids between TAG species of oils. Whether it has any impact on cholesterol lowering property of interesterified oils is yet to be ascertained. The mechanism of hypocholesterolemic action of modified oils were studied by monitoring, the molecular profiling of hepatic genes involved in cholesterol metabolism. It was observed that rats fed modified oils had an increased expression for the genes involved in cholesterol clearance pathway such as LDL receptor and cholesterol 7-α-hydroxylase compared to those fed with CNO. Interesterified oil changed the expression of genes responsible for cholesterol

homeostasis which coincided with their greater efficiency in lowering cholesterol compared to rats given blended oils. Therefore it is interesting to note that the nutritional quality of an oil or fat is not only dependent on the fatty acid composition per se but also on distribution of fatty acids in TAG molecular species. The mechanism of cholesterol lowering property of modified oils indicated that there is a significant increase in the mRNA abundance for LDL receptor and cholesterol 7-α hydroxylase and thus influencing cholesterol clearance pathways. The blended and interesterified oil feeding also modulated the thrombotic factors measured in terms of rate and extent of platelet aggregation and eicosanoid production in rats. It was found that rats fed with blended and interesterified oils produce less thrombogenic effect compared to those fed with coconut oil. The antithrombotic effect observed after feeding rats with blended and interesterified oils may be mediated by the favourable ratio of prostacyclin/thromboxane as compared to rats given CNO. The analysis of lipid peroxidation level showed that rats fed with blended and interesterified oil had increased lipid peroxide level when compared to rats given CNO. However the activities of endogenous antioxidant enzymes were also increased in rats fed with blended and interesterified oil as a compensatory mechanism to partly control the higher levels of peroxides produced. Feeding modified oils changed membrane fluidity, which affected the membrane bound enzyme activities.

Further studies are needed to understand the reason for greater efficiency of interesterified oils as compared to blended oils with similar fatty acid composition to lower serum lipids. The structure of TAG molecular species may be an important factor in understanding their influence on lipid metabolism which need to be further pursued.

Summary of the major findings of the present study

- Blended oils containing approximately equal proportions of saturated:monounsaturated:polyunsaturated fatty acids were prepared using coconut oil, a fat rich in saturated fatty acid, with rice bran oil or sesame oil, which are rich in unsaturated fatty acids. Rice bran and sesame oil individually contained unique minor components having nutraceutical properties, which was complemented when blended oils were prepared.

- Blending of oils resulted in the enrichment of minor components such as tocopherols, tocotrienols and oryzanol in CNO+RBO and tocopherols and sesame lignans like sesamin, sesamolin and sesamol in CNO+SESO combinations.

- The blended oils were subjected to interesterification reaction using immobilized lipase, which resulted in the rearrangement of the fatty acids on the TAG.

- Quality of oils monitored by peroxide value and free fatty acid content were comparable in the blended and interesterified oils. Lipase catalyzed interesterification did not alter the fatty acid composition and minor components present in the oil.

- Physicochemical analysis of modified oils indicated that interesterification resulted in the change in some of the TAG molecular species with the emergence of newer TAG molecules, which were not present in the parent or in blended oil.

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

- Changes observed in the TAG molecules affected the physical characteristics such as melting, crystallization properties and solid fat content in the interesterified oil.

- Feeding rats with modified oils at 10% level for a period of 60 days resulted in the significant decrease in the total and LDL cholesterol in serum as compared to rats fed CNO.

- Rats fed interesterified oils showed significantly lower cholesterol level compared to those given blended oil with similar fatty acid composition.

- The molecular mechanism involved in the hypocholesterolemic effects of oils were studied by transcriptional profiling of genes involved in cholesterol homeostasis (HMG-CoA reductase, LDL receptor, cholesterol 7-α-hydroxylase and SREBP-2).

- Transcriptional profiling indicated a marginal increase in the mRNA abundance of HMG-CoA reductase, the rate limiting enzyme in cholesterol biosynthesis in animals fed with blended and interesterified oils as compared to those given CNO.

- The mRNA abundance measured by RT-PCR indicate an increase in the LDL receptor, responsible for hepatic clearance leading to lower serum cholesterol in rats fed with blended and interesterified oils.

- The increase in the LDL receptor expression was more profound in rats given interesterified oil compared to those given blended oils.

- Cholesterol 7-α-hydroxylase, the first and rate limiting enzyme involved in bile acid biosynthesis, also showed an increase in the mRNA abundance in rats given blended and interesterified oils.

- SREBP-2 a transcription factor involved in cholesterol homeostasis was upregulated in rats given blended or interesterified oils when compared to the rats given CNO.

- Thrombotic parameters as influenced by feeding blended and interesterified oils showed a significant decrease in the rate and percentage aggregation of platelets as compared to those given CNO.

- The ratio of prostacyclin/thromboxane in rats fed blended and interesterified oil was significantly higher than those found in CNO fed rats.

- The lipid peroxide level in rats fed blended and interesterified oil increased compared to those fed CNO.

- The activity of endogenous antioxidant enzymes such as SOD, catalase, GSH-Px, GST in the liver were elevated in rats fed blended and interesterified oils compared to rats fed CNO.

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

- 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 results were discussed in the light of findings emerging from this investigation and the reports available in scientific literature.

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