EVALUATION OF N-3 UNSATURATED FATTY ACIDS IN OILS AND CHOLESTEROL OXIDES IN GHEE

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CERTIFICATE

This dissertation entitled "Evaluation of n-3 **unsaturated fatty acids in Oils and Cholesterol oxides in Ghee"**, embodies the work carried out at the School of Environmental Science, Jawaharlal Nehru University, New Delhi. This work has not been submitted in part or full for any degree or diploma of any university.

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CONTENTS

AIM AND SCOPE:

Today scientific understanding of Human Nutrition is one of the most important aspects. It has saved countless human lives or improved their quality. In the most effluent countries people are malnourished, not through deficiency of food but through over consumption and other imbalances like in the composition and amount of intake.

As the body can not synthesize all the necessary precursor molecules required for building of large molecules some has to be supplied through food like essential fatty acids precursors for building up of triglecerides, eicosanoids etc.Previously the essential fatty acids constituted linoleic acid(n-6), linolenic acid(n-3) previously. But recently the other members of n-3 family Eicosa pentaenoic acid, Decosa hexaenioc acid ,are reported to be essential for the development of human.

Most of essential fatty acids required for the body comes from plant sources especially vegetable oils contribute large percentage of human daily fat intake. and would be one of the causes for CHD incidence either due to excess intake or composition of the oil which is consumed. As the n-3 fatty acids are reported to reduce the cholesterol content in blood thus decreasing the incidence

of CHD.In India mainly north India oil consumption is higher. Nearly 10 varieties of oils are being used. Hence evaluation of n-3 fatty acids in oils is vital to explore beneficial properties to heart.

So the present study aimed at evaluation of the amounts of n-3 fatty acids in oils. The study would help in correlating the type of oil consumed in a particular area and incidence of CHD and assessing the risk of CHD due to consumption of variety of oils. It would also help in recommending dietary practice for normals to endure good health.

From this study we could learn the amount of n-3 unsaturated fatty acids and other mono unsaturated fatty acids. As the study is done commercial oils available we could learn the effects of different processing methods on oil composition by comparison between two types of commercial oils.Since the fatty acid composition is known which is useful to future studies.

REVIEW OF LITERATURE:

The essentiality of Fat in diet was demonstrated by Burr&Burr(1929) nearly sixty years ago. The rats which are reared on fat free diet are failed to grow, developed renal disease, dermatitis and necrosis of the tails and eventually died.

Later studies identified the critical components termed as essential fatty acids and are shown to be polyunsaturated fatty acids with two or more double bonds. (Holman 1968).

Formerly linoleic acid (n-6) precursor of other n-6 fatty acids, commonly found in vegetable oils and in many other food stuffs, is shown to be primarily essential. However there is a second series of highly polyunsaturated fatty acids the n-3 fatty acids that know also appear to be essential. The fatty acids of this family are derived from linlenic acid which is present in many foods or can be obtained directly from the diet.

CHEMISTRY OF ESSENTIAL FATTY ACIDS:

The fatty acids which are found commonly in plants and animals contain even number of carbon atoms(4-24) arranged in straight chains with a terminal carboxyl group. These may be fully saturated or contain one,two or more (up to six) double bonds which generally but not always have a cis-configuration.

In the unsaturated fatty acids of given chain length, the double bonds may be present in a number of different positions and a full description of any acid must specify the position and configuration of the double bond.In the

short hand nomenclature, the acid for example cis 9 octa decenoic acid (oleic acid) structure

$$CH_3(CH_2)_7 - CH = CH - (CH_2)_7 - COOH$$

is designated as 18:1 or indicating the position of the double bond $18:1^{\Delta 9}$. In addition, the position of double bond can be denoted in the form of (n-x), where n is the chain length of the acid and x the number of carbon atoms from the last double bond to the terminal methyl group i.e. 18:1(n-9) The (n-x) nomenclature is only used with fatty acids containing cis double bonds.

In the fatty acid containing more than one double bond the double bonds are positioned either in conjugated fashion or in nonconjugated fashion. The fatty acids originated from plants and animals usually contain the double bonds positioned in non conjugated fashion. Poly unsaturated fatty acids (PUFA) all have low melting points. The more double bonds they possesses the greater their susceptibility to oxidative deterioration (auto oxidation). If the acids or their derivatives are subjected to high temperature or to alkaline hydrolysis under conditions which are too vigorous, migration of stereo mutation of double bonds can occur.

The non conjugated fatty acids (often abbreviated to PUFA) of animal and plant origin can be subdivided into several simple families according to their biosynthetic

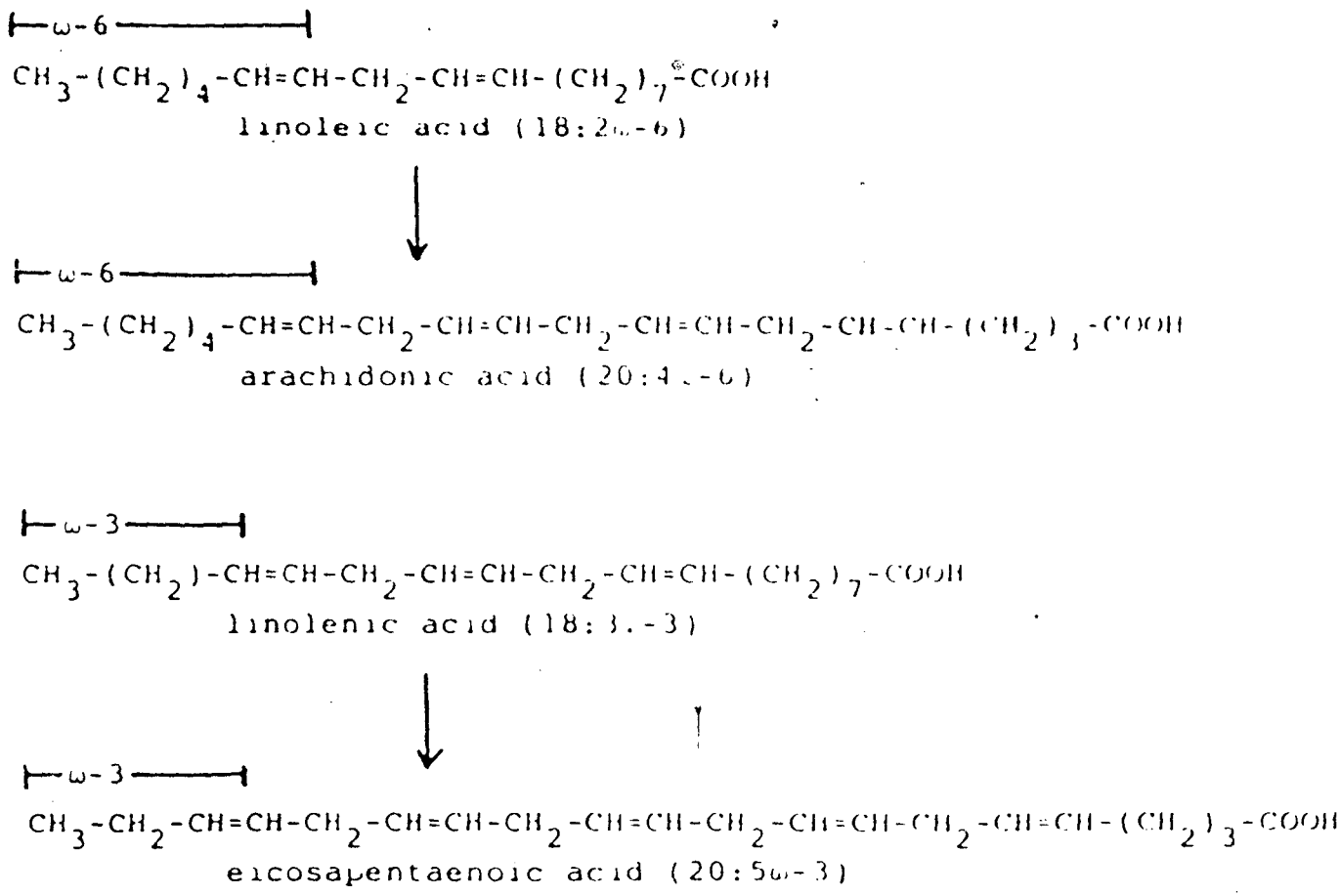


Figure 1. Structures of ω-6 and ω-3 fatty acids.

derivation from single specific fatty acid precursor. The acids in each family contain two or more cis-double bonds, generally separated by single methylene group (CH 2) and have the same terminal structure (Christie, 1982).

LINOLEIC ACID FAMILY :

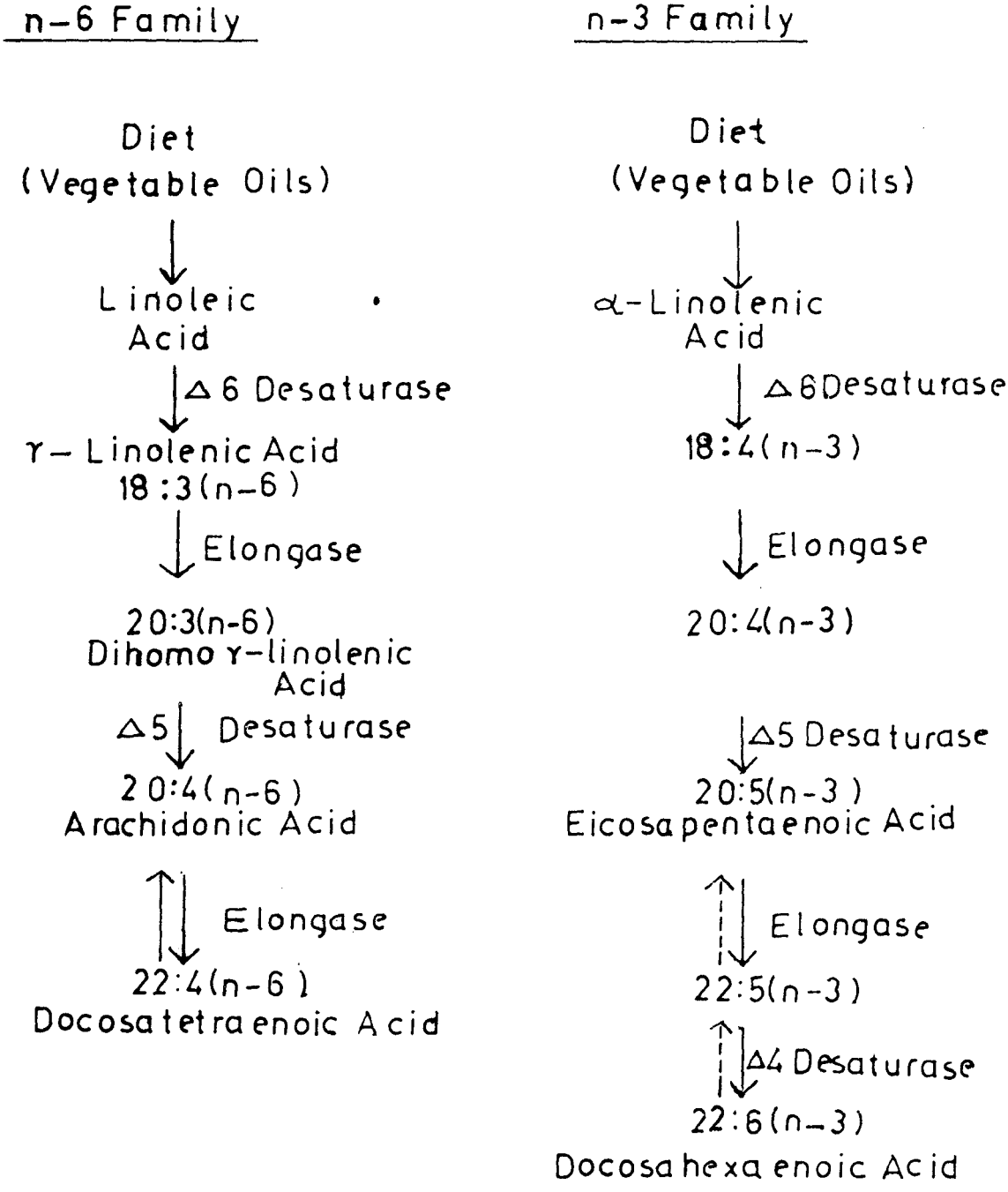
The structure of linoleic acid is shown in figure.1. This is the commonest and simplest fatty acid of this family and is found in most plant and animal tissues. It is an essential fatty acid in animal diets, as it can not be synthesized by the animal required for growth, reproduction and healthy development. In animal tissues, it is the precursor of a family of other fatty acids which are produced from it by desaturation and chain elongation (figure.2) All having the terminal n-6 structure can also function us essential fatty acids. Linolenic acid is found in appreciable amounts in seed oils. Isomers of linoleic acid, in which one or more double bonds have a trans-configuration, have been isolated from certain seed oils.

LINOLENIC ACID FAMILY :

The structure of linolenic acid is shown in figure.1. It is primary precursor of another important family of

FIG. 2

PATHWAY OF ELONGATION AND DESATURATION



polyunsaturated fatty acids. Linolenic acid and/or polyunsaturated fatty acids of the (n-3) family are essential fatty acids in fish.(Sinnhuber et al.,1972). But it is still a matter of debate whether they have an essential role in mammals. Cis 5, 8, 11, 14, 17, Eicosa pentaenoic acid (EPA) and cis 4, 7, 10, 13, 16, 19 Docosahexaenoic acid (DHA) in particular are found prominent in the brain and retina. Apart from essential amino acids n-3 fatty acids are the largest components of the cerebral cortex and retina that can only be derived from the diet as the body can not synthesize. Dietary sources of n-3 fatty acids are many plants of the land, phytoplankton and algae of the sea and fish oils.

ESSENTIALITY OF N-3 PUFA :

The essentiality of n-3 fatty acids has been fully demonstrated in fish (sinnhuber et al., 1972). There is some evidence for their importance in retinal and brain function in rats (wheeler et al., 1975, lamptey and walker 1976). But no data is available previously for primates. Recently the essentiality of n-3 fatty acids is shown in primates rhesus monkeys because that primate species provides the best available model for the human situation. Conner and Neuringer fed the monkeys with control diet

having 7/1 ratio of n-6/n-3, 250/1 ratio deficient diet before 2 months of conception and throughout pregnancy and resulting infants until 22 months. They found no difference in birth weight and in postnatal growth between the control and deficient. However the levels of n-3 fatty acids found to be depleted from plasma and tissues, including RBC, liver, skin,fat,cerebral cortex and retina. In particular the very long chain n-3 fatty acid decosoahexaenoic acid, (DHA) was selectively found depleted from neural and retinal phospholipids and was replaced by n-6 fatty acids 22:4,22:5. These are associated with significant impairment in the development of visual acuity and in the recovery of the electro retinogram. The change in membrane phospholipid composition produced by n-3 fatty acid deficiency might alter the transmission of information through the brain visual pathways as well as affect the photoreceptive process in the retina.

However the applicability of these findings to human nutrition is not yet fully understood. The data which is available now seems sufficient to regard them as essential nutrients with their own functions distinct from those of linoleic acid and other fatty acids of the n-6 series. Thus n-3 fatty acids appear to be essential nutrients for retinal and brain functions, especially during foetal and postnatal development.

IMPORTANCE OF N-3 FATTY ACIDS :

1. In Cardio Vascular Disease :

Cardio vascular disease reflects deleterious interactions among plasma lipids, arterial endothelium, plate lets and monocytes, which gradually result in atherosclerosis thrombosis and myocardial ischemia and infarction. The preventive measures emphasize the modifiable risk factors which include modification of dietary Fat. All the above changes are exacerbated by risk factors especially high intake of food fat with saturated fatty acids. Thus the rate and severeness of atherogenesis, thrombosis and Infarction is greatly influenced by dietary fats (Stamler,1983).

Dietary lipids are transported to liver and tissues as chylomicroms and are reassembled in the liver to lipoproteins that is the trigleceride rich VLDL, cholesterol rich LDL and HDL. The metabolism of these lipoproteins is genetically determined to some extent butis also markedly influenced by dietary fat intake. Excess of synthesis over clearance results in hyperlipodimia (Grundy,1986;Shaefer,et al.,1985). Consumption of food high in fats, especially rich in saturated fatty acids and cholesterol, considerably increases lipid levels especially LDL levels. Extensive

studies and epidomological data have shown that the elevated levels of plasma cholesterol due to high intake of saturated fatty acids, genetic defects are closely correlated with coronary artery diseases. (CAD). These elevated LDL cholesterol and VLDL triglecerides both are major risk factors for CAD(Shaefer1985).

A substantial amount of research has been conducted to develop preventive methods for CAD. Since 1950 modification of dietary fat intake as a lipid lowering measure has been studied. Reduction in the saturated fatty acid intake decreases lipid levels of blood When the essential fatty acids were discovered, people thought atherogenisis and CAD are due to deficiency of these essential fatty acids linoleic, lenolenic acid.

However early studies showed that fish oils low in essential fatty acids lowered plasma lipid levels eventhough they contained high concentrations of cholesterol (Shaefer 1985). But fish oils are not encouraged for consumption because they contain high amount of cholesterol. Later n-6 PUFA which depress Very Low Density Lipoproteins (VLDL), Low Density Lipoproteins (LDL) levels (Ahrens, et et.,1959: Grundy,1986.) have dominated dietary recommondation in past 25 years.

Recently from 2 years the potentiality of n-3 PUFA of marine origin in reducing the atherogenisis, thrombosis and CAD is gradually being appreciated. Fish lipids were found to exert ameliorative effects in patients with advanced atherosclerosis. Storm and Jenson 1951 reported a sharp decrease in the mortality from circulatory diseases in Norway associated with a marked increase in fish consumption that correlated with a simultaneous decrease in the rate of mayocardial infarctions. Bang and Dyer Berg (1972) reported that the incidence of atherosclerosis, infarction and CAD in Eskimos in Greenland was, 10 fold less than in their counterparts living in Denmark.

This diet was associated with a marked reduction in arochidonic acid (20:4 n-6) concentration in lipids of plasma, platelets and tissues with a concurrent increase in n-3 PUFAs, Eicosa pentaenoic acid and decosa hexaenoic acid in lipids. These observations suggested that displacement of archidonic acid from tissue lipids by dietary n-3 PUFAs somehow reduced the risk of CAD and associated problems.

The possible mechanism by which n-3 PUFAs may reduce Heart disease(Kinsella,1987.).

1. Modifications of serum lipid levels
2. Reduction of arachidonic acid synthesis

3. Alteration of tissue fatty acids, reduction of arachidonic n-3 PUFA ratios.
4. Alteration of thromboxane : prostacyclin ratio and decrease in platelet aggregation tendency.
5. Reduction in thrombotic tendency.
6. Modification of events in atherogenesis reduction in cell/cell interactions.
7. Modification of vasopressin, blood pressure and blood viscosity.
8. Alteration of membrane fluidity and of receptor and membrane bound enzyme activity.

The n-3 fatty acids reduce the serum lipid levels and arachidonic acid synthesis by reducing fatty acid synthesis and trigleceride synthesis and thereby limit the assembly and secretion of Very Low Density Lipoprotiens (VLDL) significantly, two n-3 PUFAs eicosapentaenoic acid and to a greater extent, decosahexaenoic acid- suppress fatty acid and trigleceride synthesis in rat liver and enhance the clearance of VLDL in the peripheral vasculature by improving vaso dilatation, By suppressing the production of VLDL n-3 PUFA indirectly affect the production of Low Density Lipoproteins (LDL) and by reduced competition, may facilitate the clearance of LDL. They may also enhance turnover of LDL receptors and thereby improve receptor-mediated removal of LDL.

As these n-3 fatty acids affect the metabolism of eicosanoids which mediate the physiologic phenomena effecting the coronary vasculature, thus improving the vasodialation.

The Poly Unsaturated fatty acids (PUFA) are precursors of prostaglandins (PG). There are three different series of prostaglandins 1 st and 2nd series of PG are derived from lenoleic acid and 3rd series are derived from Eicosa pentaenoic acid which is derivative of lenolenic acid (PUFA). The prostaglandins (eicosanoids) Thromboxane A2 and prostacyclin PGI2 which are pertains to maintain the vascular tone, hemodynamics, integrity of blood vessels. These two prostaglandins are derived from arachidonic acid(AA) which intern derived from lenoleic acid (Generally small amounts of arachidonic acid is released in response to specific agonist stimulation or some may be released in response to injury, inflammation or stress). In platelets of blood, the arachidonic acid released in response to stimulation by thrombin or collagen or by the impact of platelets with the vessels of wall is rapidly converted to thromboxane A2. This is the most potent platelet - aggregatory substance. In addition to the formation of transient thrombi, it promotes platelet interaction (adhesion) to the walls of the blood vessel, causing

vascular constriction. Thus it may play critical role in the development of atherosclerosis and CAD.

The effects of this thromboxane A2 is counteracted by production of prostacyclin by the endothelial cells of arterial tissue or released from platelet upon interaction with endotheliem. This is a potent antiaggregatory, antiadhesive agent and also vasodialatory (Moncada et al., 1920: chierchia et al., 1986).

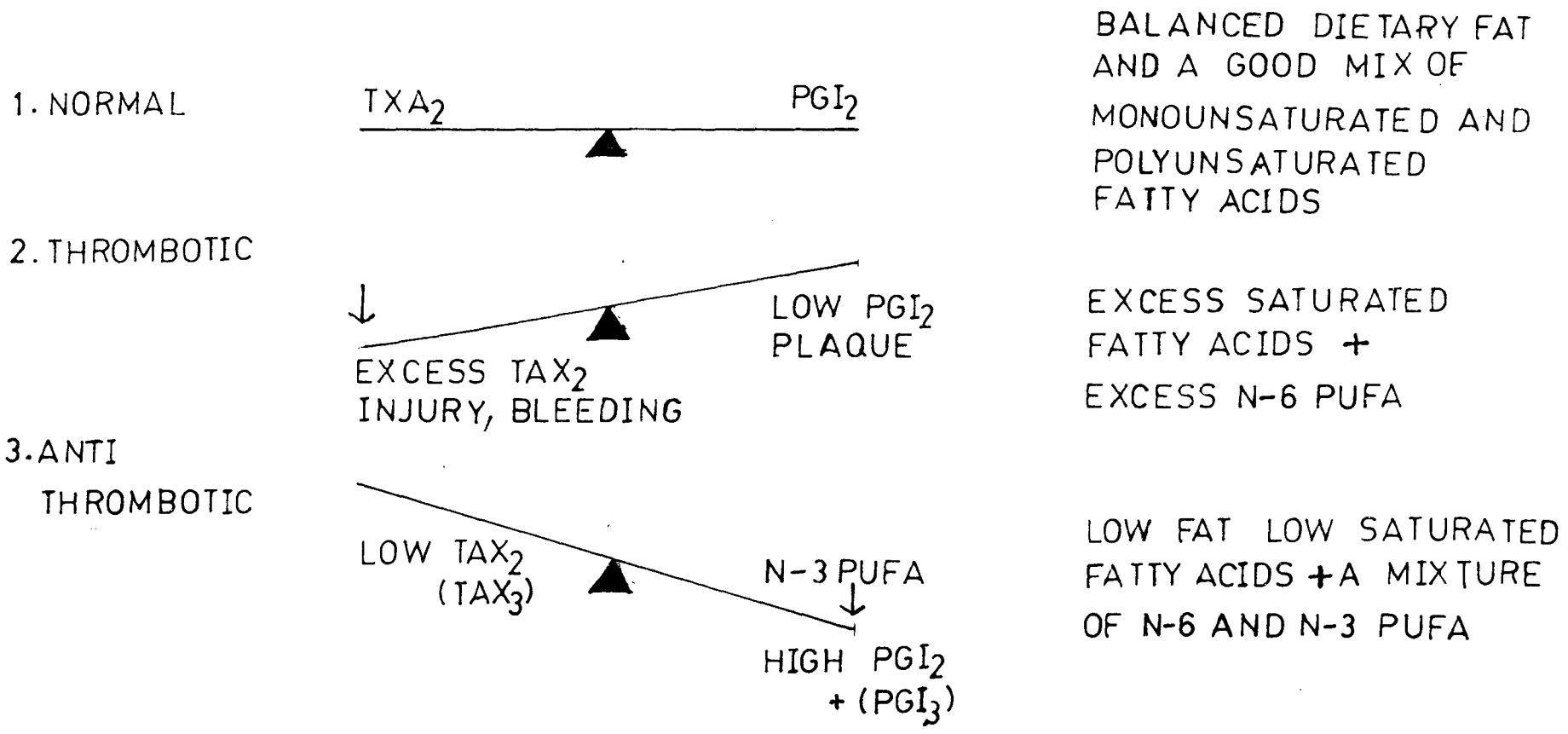
The balance between these two aggregatory and antiaggregatory prostaglandins play important role in maintaing vascular tone.(Kinsella,1987) as shown in the figure 3.

The relative amounts of Thromboxane A2 and prostacyclin synthesized are greatly influenced by the availability of the precursor arachidonic acid which in turn is largely determined by the amount of dietary linoleic acid, the n-6 fatty acid occurring abundantly in vegetable oils.

In persons who consume relatively large amounts of linoleic acid, excess thromboxane A2 is produced. Patients who have angina and atherosclerosis have increased rates of synthesis and excretion of thromboxane A2, reflecting extensive platelet/vessel wall interactions (Fitzgerald at al., 1987: fitzgerald et al., 1983) prostacyclin excretion

FIG.3
EICOSANOID HOMEOSTASIS AND PLATELET BEHAVIOUR :-

ADHESIVE	ANTIADHESIVE
PROAGGREGATORY	ANTIAGGREGATORY
VASOCONSTRICTION	VASODILATION



is also increased in these instances. There are some questions to whether this perturbation results in an imbalance favouring aggregation of platelets. Thus suppression of thromboxane A2 and enhancement of prostacyclin synthesis seems desirable to minimize these pathologic events. A pragmatic approach for this, a study of dietary means. The n-3 PUFA of fish oils and sea food lipids and vegetable oils may provide such a means.

When fish oils which contain large amounts of EPA and DHA administered there is a progressive reduction of platelet arachidonic acid and a concomitant increase in the content of EPA and DHA in the phospholipid pools and thereby dilute the thromboxane A2 precursor pool (Dyerberg,1978:Deyerberg ,1986:Yamori,1985: Sanders,1984). The reduced thromboxane A2 synthesis, reflecting decreased substrate availability and competitive inhibition from the free n-3 PUFAs, occurs especially if high levels of plasma n-3 PUFAs are achieved plasma concentrations of 5 to 20μM may reduce blood pressure and facilitate VLDL clearance in peripheral vascular tissue.

DIETARY EFFECTS OF N-3 PUFA FROM FISH OIL:

1. Replace saturated fatty acids and decrease endogenous fatty acid synthesis.

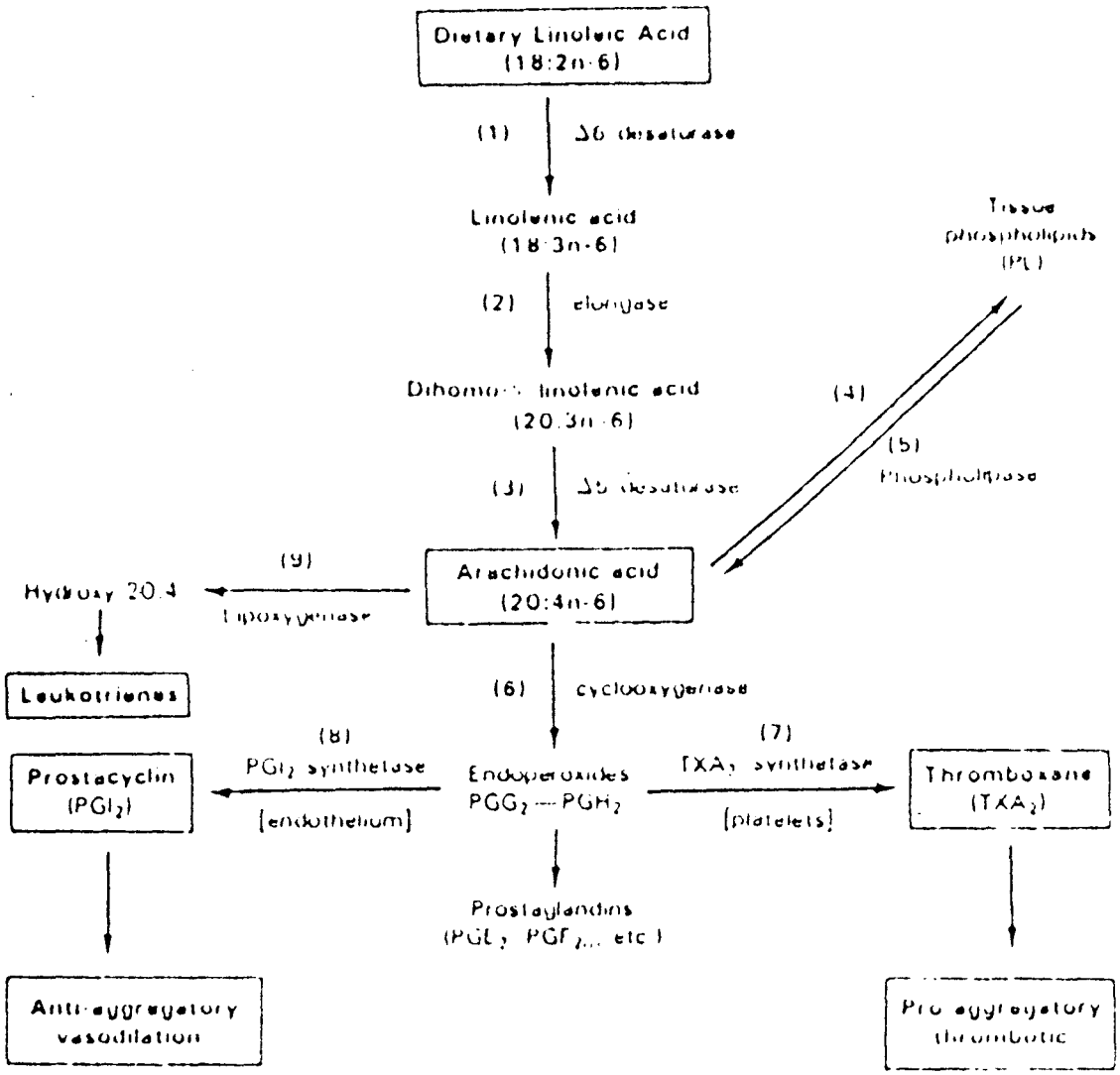


Fig. 4—**Key Steps** *(boldface numbers) in conversion of dietary linoleic acid (18:2) to arachidonic acid (20:4) and thence to prostanoids and leukotrienes. The key enzymatic steps which may be directly affected by dietary n-3 polyunsaturated fatty acids are 1 and 6*

2. Suppress hepatic trigleceride synthesis and production of VLDL.
3. Compete with 6 desaturase and reduce arachidonic acid
4. Displace arachidonic acid from ecosanoid pools in tissue.
5. Inhibit eicosanoid synthesis as free fatty acids compete for cyclo-oxygenase and lipo-oxygenase.
6. Serve as precursor of eicosanoid analogs of varying potencies (PGI3, Thromboxane A3, Lukotriene B 3). (Kinsella,1987)

There is negligible effects of fish oils on the anti aggregatory, vasodialatory prostacyclin PGI 2. In addition there is concurrent production of PGI3 from EPA in human plasma which has antiaggregatory effects (Needlman at al.,1979). Similarly there is a another concurrent production of Thromboxane A3, generated from EPA by platelets is a very week agonist of aggregations than A2. This A3 by binding to thromboxane A2 receptors on platelets not only does not evoke response but also prevent the binding of thromboxane A2. Thus the overall anti adhesion, antiaggregation status is greatly improved by dietary n-3 PUFAs. This important discovery was confirmed by Von Schacky et al.,1985.

Effect on leukotriens :

In addition to effect on the thromboxane-prostacyclin balance, these n-3 fatty acids also effect on lenkotriene B4 production. The prostanoids and leukotrienes modulate cell to cell communications, interactions and signaling. The Thromboxane A2 and lenkotriene B4 both promote adhesion of platelets and monocytes to the blood vessel wall, effects that are counteracted by prostacyclin. (Robertson etal., 1977: Ross, 1986: Willis et al., 1986). The leukotrienes B4 is a chemokinetic and chemotactic agent and when produced in the blood vessels, rapidly attracts additional monocytes and macrophages. A balanced production of these bioactive agents modulates the physiologic functioning of the vascular cell system. When there is a perturbation, results in excessive platelet-monocyte / vessel wall interaction (Lands 1986).

The arachidonic acid which is formed from lenoleic acid is converted into a series of leukotriens, peptido leukotriens by monocytes and macrophages. (Lewis., 1986)

These leukotrienes at concentrations of 10 to 30 nM elicit chemotaxis and the adherence of leukocytes to surface is enhanced and at higher concentrations, causes lysosomal degranulation and superoxide generation (Ross, 1986). These

may injure endothelial tissue. The EPA and DHA reduce the synthesis of leukotriene B4 but also result in the synthesis of leukotriene B5 from EPA. Leukotriene B5 is very week in terms of chemotaxis adherence and aggregation which compete with B4 thus diluting the effect of B4 DHA is a poor substrate for lipoxygenase, so it can inhibit the conversion of arachidonic acid to lenkotriene B4 by competing with AA. (Lewis and Austen, 1986) The diet containing 3 gm. EPA 2 gr DHA has been shown to reduce the levels of arachidonic acid in leukocytes and monocytes and inhibit synthesis of leu. B4 significantly while increasing leu.B5 synthesis (Lewis, 1986).

On the basis of epidomological data consumption of 2 to 5 gr of n-3 PUFA daily in conjunction with reduced levels of saturated and n-6 PUFAs may be effective in maintaining reduced levels of plasma lipids and retarding the development of athero sclerosis.

ROLE IN KILLING OF CANCER CELLS:

Recently the role of n-3 and n-6 PUFA in killing of human breast tumor cells is reported (presented in symposium). ϒ-linolenate, 18:3n-4 (GLA), arachidonate 20:4 n-6 (AA) Eicosa pentaenoic acid 20;5n-3(EPA), linoleate 18:2n-6 (LA), Decosahexanoate 22:6n-3 (DHA) are the fatty acid

which are reported to kill the tumor cells. In these GLA, AA, EPA were the most effective killing agent among the essential fatty acids tested, DHA was least effective. The effects of GLA and AA are faster than those induced by EPA. As these fatty acids are unsaturated which are easily prone to peroxidation. When these amounts is more in tumor cells, stimulate the superoxide anion production. Thus the effectiveness of a given PUFA in killing cancer cells correlate with the extent of lipid peroxidation of the PUFA substrate in the cells. (Begin, 1987).

OTHER ROLES :

These n-3 fatty acids reduce the whole blood viscosity and increase the erythrocyte deformability,which reported in volunteers and in patients of peripheral vascular disease (Terano et al.1983).

Due to the alteration of red cell membrane composition thus influencing fluidity which may play an important role in various cell functions, cell permeability, enzyme activity, hormone receptors.(Gensberg et al.,1982)

Possible Side Effects :

1. Yellow fat disease, a disorder affecting the fatty depots, has been reported in certain wild animals that consume high amounts of polyenoic fatty acids (Dormandy,

1978).

2. The consumption of large amounts of unsaturated fatty acids increases the requirement for antioxidants, as peroxidized fatty acids may have harmful side effects (Hey et al., 1982).

3. At high concentration of unsaturated fatty acids prolonged cutaneous bleeding time is observed in human dietary experiments has been very moderate(Reardon et al.,1985)

4. The unsaturated fatty acids n-6, n-3 stimulate endogenous production of hydroperoxids and regarded as a risk factor for lung cancer. The peroxidative damage to pulmonary tissue is prevented by selenium containing enzyme glutathione-peroxides abolishing the anticarcinogenic effects of selenium (Selenium was reported to be anticarcinogenic). (Reardon et al.,1985).

Correlation of the mortalities of lung cancer with the blood selenium data and per capita consumption of fat and oils shown regression equations which account for more than 80% of the mortality variations.

The people even though they have sufficient concentrations of selenium which influence the host resistance against cancer, to prevent the hydroperoxide

effect on lungs formed from PUFAs, is diverted to Glutathione peroxides, thus making them susceptible to cancer and abolishing anticarcinogenic effects of selenium.

As a rule the diets having more oils and fats are deficient of selenium showing antagonistic relationship between selenium and fats(Schrauzer,1987).

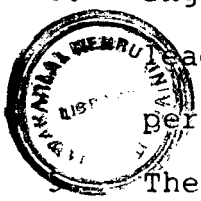
SOURCES OF N-3 FATTY ACIDS:

From the past few years fish oils are known to be the primary sources for n-3 fatty acids particularly EPA and DHA. But they are not recommended in dietary guide lines to lower the cholesterol and prevention of atherosclerosis upto 1972 because of the following reasons.

1. The populations concerned with preventing atherosclerotic disease might not be ready to accept eating seafood.
2. The n-3 PUFA have low melting points and are easily oxidized.This properties create problem in manufacture of margarine.

Latter they are included after reports of their effects on coronary atherosclerosis . Although they are effective in lowering atherosclerosis incidence there are some possible side effects

1. High intake of fish oils may lead to inhibition of platelet aggregation and prolonged cutaneous bleeding time.
2. They may contain contaminats such as pesticides and other pollutants.
3. The products such as cod liver oil prepared from cod liver is high in vitamin A and D which are toxic in excess.High concentrations of vitamin D may lead to nephrocalcinosis in subjects with renal disorders.
4. Ingestion of fish oil over long time period can lead to stress on the vit. E status and increased peroxide formation which is toxic in vivo.
5. The fish oils are not palatable.



Due to the disadvantages investigations are going on for alternatives which are equivalently effective. In daily life most of the fats which are comsumed in large amounts by human being are vegetable origin like vegetable oils. The health benefits of these oils depends on their composition i.e the ratios of different fatty acids including saturated and unsaturated. Those with low percentage of saturated fatty acids and having cosiderable amounts of n-3 fatty acids (PUFA) are especially beneficiary for CHD patients. A knowledge of the fatty acid composition i.e percentages of

saturated, unsaturated (MUFA, PUFA) is prerequisite in revealing their health benefits.The composition of some vegetable oils reported by some authors are shown in figures 1 to 5.

The experiments with feeding of (David Krichovsk,1982) corn oil,Palmkernal oil, coca butter and coconut oil which are low in cholesterol constitute 14% of diet with other necerry constituents to raabbits show that cocoa butter is cholesterolemic and atherogenic than Palm oil or coconut oil. This may due to the fact that about half of the fatty acids of palm oil are c16 or shorter where as 76% of fatty acids of cocoa butter are c18 or longer.Corn oil is less atherogenic than other three fats and increase in cholesterol is not significant. The usage of fish oil lowers the levels of both triglecerides and serum cholesterol. But unsaturated vegetable oils achieve only the latter i.e., reduce the serum cholesterol and the ischemic damage that follows interruption of blood flow to a tissue perhaps by increasing the fluidity of blood.

Increased consumption of salad oils such as soyabean oil and corn oil have become widely accepted as part of cholesterol lowering diets, but they may also be influential in improving cardiovascular health in man through the contribution of linolenic acid to EPA suggest that vegetable

oil containing linolenic acid may be biologically effective similar to those of fish oils.

There are no published studies that shows whether linolenic acid intake from vegetable oil diets is sufficient to benefit health. According to the fat supplies in India,an average fat available is 15 - 16g daily made up of roughly (10g) of vegetable oil and 3g of hydrogenated fat and ghee (butter fat) 2.5g. The average fat intake is reported to be 3-19g daily and for rural population it is reported to be 10.5gm by National Nutrition Monitering bureau, Hyderabad. Report for the year 1979, and it contribute an average of 14.7% of totalenergy (Atchaya, 1987).

The common edible oils which are available in India vary considerably in their percentage content of essential fatty acids lin and len

Oil	lin%	len%
Sunflower oil	60	-
Soyabean oil	55	5
Sesame oil	42	-
Ground nut oil	27	-
Rape mustard	16	.15
Palm oil	10	-

Vanaspathi	2-5	2
Ghee\Butter	2	2

(Atchaya,1987)

In the total rural dietaty calories in 10 Indian states dietary lin contribute to an avarage of 4.8%. This is the well above the 3%en % level of lin recommended by the Joint FAO/WHO consultation of 1977. For len no figure was suggested since data were lacking. The present range of len contribution to energy in 10 Indian States is 0.23 - 0.44 en% average 0.28 en%.

TABLE -1 : FATTY ACID COMPOSITION OF SOME VEGETABLE OILS

ACIDS,	CORN OIL	RAPESEED OIL	SOYABEAN OIL	PEANUT OIL	PALM OIL	SUNFLOWER OIL	SESAME OIL
14:0	-	-	Tr	Tr	Tr	-	-
16:0	13	4	11	6	14	11	10
18:0	4	2	4	5	2	6	5
20:0	Tr	-	Tr	2	Tr	-	-
22:0	Tr	-	Tr	3	-	-	-
24:0	-	-	-	1	-	-	-
14:1	-	-	-	-	-	-	-
16:1	-	-	-	Tr	2	-	-
18:1	29	19	25	61	64	27	40
20:1	-	13	-	-	-	-	-
22:1	-	40	-	-	-	-	-
18:2	54	14	51	22	16	52	45
18:3	-	8	9	-	-	-	-

Tr:Trace
Source (Fedeli et al.,1971)

TABLE-2
SELECTED FATTY ACID COMPOSITION OF SOME EDIBLE FATS AND OILS(g/100g)

ACID	MENHADEN,	SOYA, OIL	PEANUT, OIL	BUTTER,	CORN, OIL	SUNFLOWER, OIL	RAPESEED, OIL
4;0	-	-	-	3	-	-	-
6:0	-	-	-	2	-	-	-
8:0	-	-	-	1	-	-	-
10:0	-	-	-	3	-	-	-
12:0	-	-	-	3	-	-	-
14;0	9	-	-	10	-	-	-
16:0	19	11	10	26	11	6	5
18:0	4	4	2	12	2	4	2
20:0	-	-	1	2	-	-	1
16:1	13	-	-	2	-	-	1
18:1	16	23	46	25	25	22	53
20:1	2	-	1	-	-	-	1
22:1	1	-	-	-	-	-	-
18:2	2	51	31	2	57	66	22
18:3	1	7	-	1	1	-	11
18:4	2	-	-	-	-	-	-
20:4	1	-	-	-	-	-	-
20:5	13	-	-	-	-	-	-
22:5	2	-	-	-	-	-	-
22:6	8	-	-	-	-	-	-

Sourse: (Bimbo, 1987).

TABLE 3: POLY UNSATURAED FATTY ACIDS IN SOME EDIBLE OILS AND FAT'S

No.	FAT OR OIL	PUFA CONTENT(gm/100gm)
1.	COTTONSEED OIL	50
2.	GHEE(BUTTER FAT)	4
3.	GROUNDNUT OIL	28
4.	MAIZE OIL(CORN OIL)	45
5.	MUSTARD OIL	25
6.	SAFFLOWER OIL(KUSUMA OIL)	75
7.	SESAME(GINGELY) OIL	42
8.	SOYA BEAN OIL	55

Sourse: personal comunication.

TABLE-4: THE FATTY ACID COMOPOSITION OF SOYABEAN OIL

COLUMS	PALMITIC	STERIC	OLEIC	LNOLEIC	LINOLENIC
A*	13.4	2.5	24.9	53.1	6.1
B	11.1	2.9	21.8	55.4	8.8
C*	13.7	2.3	23.9	54.2	5.9

A-50% NaOH, 25%DEGS, B-50% KOH , 25%DEGS, C-25% DEGS

* mean values of two results
Source: (Kato et al.,1970).

TABLE -5 : FATTY ACID COMPOSITION OF VEGETABLE OILS

FATTY ACID	GROUNDNUT OIL	MAIZE OIL	SUNFLOWER OIL	SESAME OIL	SOYABEAN OIL	PALM OIL
6:0	-	-	-	-	-	-
8:0	-	-	-	-	-	-
10:0	-	-	-	-	-	-
12:0	-	-	-	-	-	<0.2
<14	<0.4	<0.1	<0.4	<0.1	<0.1	
14:0	<0.6	<0.1	<0.5	<0.5	<0.5	0.5-5.9
14iso	-	-	-	-	-	-
14:1	-	-	-	-	-	-
15:0	-	-	-	-	-	-
15:iso	-	-	-	-	-	-
15:antiso	-	-	-	-	-	-
16:0	6-16	8-19	3-10	7-12	7-14	32-59
16:1	<1:0	<0.5	0.1	0.5	0.5	0.06
16:2	-	-	-	-	-	-
16:iso	-	-	-	-	-	-
17:0	<0.1	-	-	-	-	-
17:1	<0.1	-	-	-	-	-
17:iso	-	-	-	-	-	-
17:antiso	-	-	-	-	-	-
18:0	1.3-6.5	0.5-4	1-10	3.5-6	1.4-5.5	1.5-8
18:1	35-72	19-50	14-65	35-50	19-30	27-52
18:2	13-45	34-62	20-75	35-50	44-62	5-14
18:3	<1:0	<2:0	<.7	<1.0	4-11	<1.5
20:0	1-3	<1;0	<1.5	<1:0	<1:0	<1:0
20:1	.5-2.1	<0.5	<0.5	<0.5	<1:0	-
20:2	-	-	-	-	-	-
20:4	-	-	-	-	-	-
22:0	1-5	<0.5	<1.0	<0.5	<0.5	-
22:1	<2.0	-	<0.5	-	-	-
22:2	-	-	-	-	-	-
24:0	0.5-3	<0.5	<0.5	-	-	-
24:1	-	-	<0.5	-	-	-

Source: (Egan, 1986)

QUALITATIVE EVALUATION OF CHOLESTEROL OXIDES IN COMMERCIAL FATS

Introduction :

Cholesterol oxides are the oxidized products of cholesterol. This oxidation readily occurs in solution, in aqueous disperision and in foods. When cholesterol is exposed to air, elevated temparatures, free redical initiators, light or a combination of these. Recently the interest on these cholesterol oxides has been intensified due to the reports implicating cholesterol oxidation products in adverse human health effects (Addis et al., 1983). These effects include cytotoxicity (peng et al., 1978: Barankowski et al., 1982 : Hill et al., 1984: peng et al., 1985), angiotoxicity, mutagenicity (smith et al., 1979; Ansari etal., 1982: sevanian, 1984), carcinogenicity (Bischoff, 1977), and others (peng et al., 1979: parsons and Goss., 1978: peng et al., 1982: peng et al., 1985). There is also a speculation that a link may exist between ingested cholesterol oxidation products and Coronary Heart disease(CHD). Basing on these implications, the present study evaluation of cholesterol oxides in commercial fats (ghee) was undertaken. This study would help in kowing the status of cholesterol oxides in different commercially prepared ghees subjected to heat and /or oxidizing agents during processing or storage. The work is mainly

concentrated on animal origin products as they are rich in cholesterol.

REVIEW :

The autoxidation of cholesterol has been recognized and studied from last 90 years, but systematic studies begun from 1960 due to the development of Thin layer chromatography (Smith, 1981). The adverse effects which are implicated by the chlesterol oxides in human body has evoked the estimation of these in different foods such as ghee.

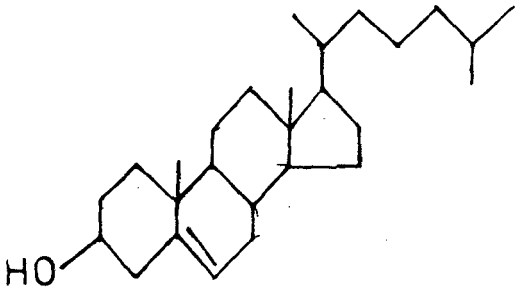
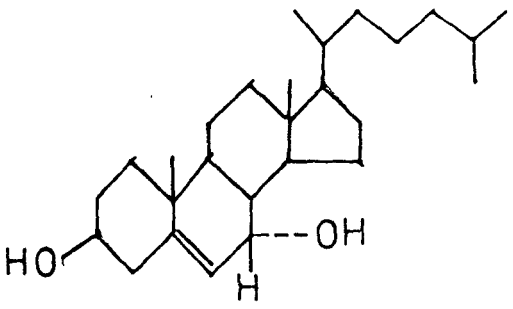
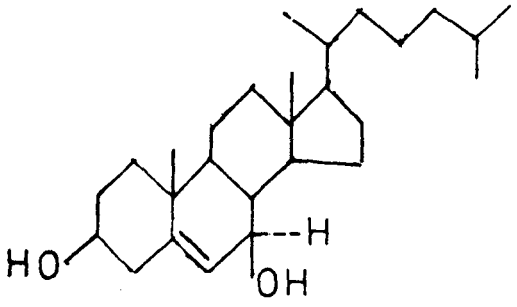
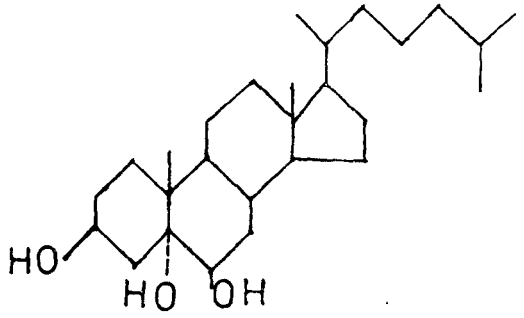
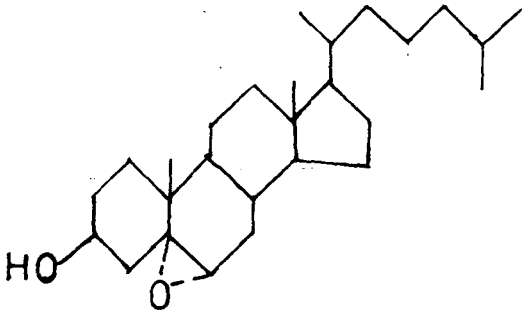
Chemistry of cholesterol oxides :

The chemical structure of cholesterol is shown in figure.5. The cholesterol molecule consists of a polycyclic nucleus with 4 fused rings, a branched aliphatic side chain attached to D-ring at C-17, a hydroxyle group that is bonded to C-3 of the A ring and is in B configuration, a 5 carbon doublebond in the B-ring.

Mechanism of cholesterol oxides formation :

The cholesterol autoxidation is initiated by abstraction of Hydrogen atom linked to carbon atom followed by attack of molecular oxygen initially forming

FIG.5

STRUCTURE	NAME OF THE COMPOUND
	CHOLESTEROL
	7α HYDROXY CHOLESTEROL (3β 7α DIHYDROXY CHOLESTA-5ENE)
	7β HYDROXY CHOLESTEROL (3β 7β DIHYDROXY CHOLESTA-5ENE)
	CHOLESTANE TRIOL (3β 5α 6β TRIHYDROXY CHOLESTANE)
	5,6 EPOXY CHOLESTEROL (5,6α EPOXY 5α CHOLESTA-3β-OL)

hydroperoxides, later they are converted to Hydroxides and/or ketones (Maerker, 1987).

In general, autoxidation involves free radical species generated through one-electron transfer process in chain reaction sequence which ultimately yields peroxide (or) hydroperoxide products. Subsequent reactions of these initial products may then promote more extensive autoxidations and moderate the process through product inhibition and chain reaction termination.

The free radical reactrion of an organic compound RH proceeds through three stages :

1. Initiation
2. Propagation
3. Termination.

Similarly the autoxidation of cholesterol also proceeds in three stages (Smith, 1981)

1. Initiation.
2. Reaction with moleculer 02 of air,
3. Subsequent transformations of initially formed products.

In the formal autoxidation reactions the initiation involves the homolysis of a susceptiable carbon hydrogenbond thus forming free radicals. But this process does not occur at measurable rates in the autoxidations of many

stable organic compounds. There are otherways of initiation i.e, formation of free radicals.

In the presence of a catalyst which can remove an electron from even electron containing substrate, thus transforming it to an odd-electron containing radical wahich is a free radical. Such agents i.e, catalysts may be stable free radicals themselves (Nitroxides, azocompounds, peroxides, hydroperoxides readily subject to bond homolysis, trasitional metal ions or excited oxygen species) capable of intiating free radical process. Other conditions which promote free radical formation enzyme transformations, rediolysis and photolyisis reactions.

There is no evidence in literature regarding the chlesterol autoxidation. Infact there are several agencies by which chlesterol autoxidation may beinitiated only general speculations can be provided for the case of cholesterol.

The initiation reaction in the case of cholesterol autoxidation may not be simple one, may involve a multistep sequence, such as initial oxidation reaction not involving free radicals to form a peroxide (or) hydroperoxide which via subsequent homolysis provides free radicals which inturn initiate a general free radical chain reaction involving cholesterol.

RH +02--> R. +HOO. ------- 1

R. +.OOH--> ROOH ------- 2

RH + 02 ---> ROOH Hydroperoxide.

ROOH -------> RO. + .OH ------ 3

ROOH -------> ROO. + .H ------ 4

The homolysis of Hyroperoxides occurs in several means through natural instability, thermal sensitivety, transition metal ion catalysis etc.

The uncatalysed hydroperoxide homolysis can occur in two ways (3) & (4). The formation of alkoxyl and hydroxyl radicals is more likely than formation of ROO. and .H radicals because the energy required for the formation of RO. + .OH is less than the formation of ROO. + .OH. In the presence of transition metal ion the homolysis occurs as follows :

$$Mn^{+} + ROOH \longrightarrow M^{(n+1)+} + RO. + OH^{-} \quad \text{--------- 5}$$

$$Mn^{+} + ROOH \longrightarrow M^{(n-1)+} + ROO. + H^{+} \quad \text{--------- 6}$$

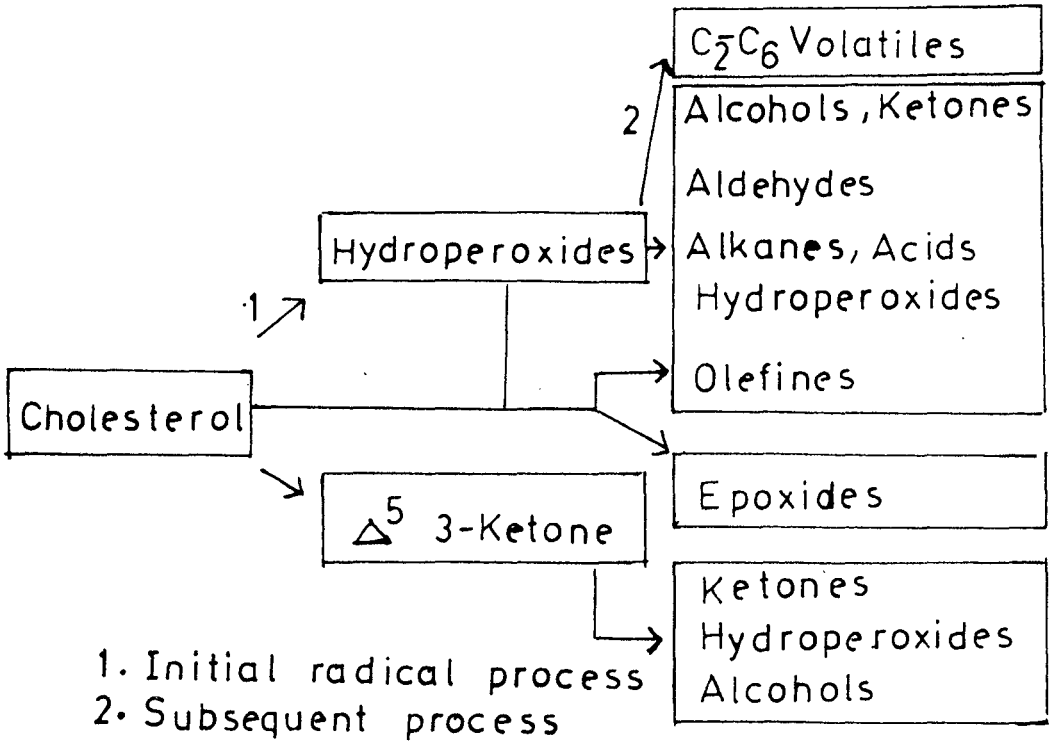
Given initiating peroxide bond homolysis to alkyl and hydroxyle radicals, propagation reactions takes place.

RO. + RH ------> ROH + R. -------7

By subsequent termination reactions form Alcohols, Ketones, epoxides, etc. Overall summary of cholesterol autoxidation reaction:

FIG.6

Summary of Cholesterol Auto oxidation Reaction:-



Studies have been conducted inorder to provide better model of cholesterol autoxidation for the state of cholesterol in foods and in aqueous environment of animal tissue.

PRINCIPAL CHOLESTEROL OXIDES :

There are about 60 products which are resulting from autoxidation, photooxidation, enzymatic action. There are some reports providing evidence only to certain of these cholesterol oxides as toxic substances.

The following are the principle cholesterol oxides which are formed.

The carbon atom or atoms oxidized in solid phase of cholesterol are different from carbons which are oxidation occurs at the tertiary c-25 position and forms 25-Hydroperoxide and by degradation of the latter 25-hydroxide is formed. Similarly the autoxidation was seen at c-20 carbon also. By contrast the side chain oxidation is not observded in autoxidation was seen at c-20 carbon also. By contrast the side chain oxidationis not observed in autoxidations cxarried out in solution or, in aqueous dispersions. (Maerker, 1987).

In aqueous colloidal dispersions with sodium staearate and cholesterol stirred in the presence of molecular oxygen at 85 C and PH 8, epimers of 7-hydroperoxides are found after 3 hours in the ratio α:β: 1:2. As these hydroperoxides are thermally unstable, they are converted to stable products which are found to be 7 hydroxy cholesterol and epimeric 7-hydroxy cholesterol. The β-isomer is thermodynamically more stable than form and are readily interconvertible. But the equlibrium is in favour of 7-β epimer. similarly some others found small amounts of (6-7%) 5-6 epoxides and traces of other derivatives when cholesterol is dispersed with sodium stearate at PH-8.

On reaction of cholesterol with singlet oxygen in pyridine and in the presence of hematoporphyrin as photosensitizer (kulig and Smith, 1973) forms 5 hydroperoxide 74-75% in which the double bond has moved to the position and 1 to 2% both isomers of 6 hydroperoxy 4-enes. These isomers are stable not inter convertable. The 5 hydroperoxide 6-enes in the presence of acid or on heating, isomerise to 7α hydroperoxy 5- ene which then epimerizes to the 7β-hydroperoxide as shown in the flow chart.

There are other types of cholesterol oxides, 5-6, epoxides which are formed as autoxidation products both in

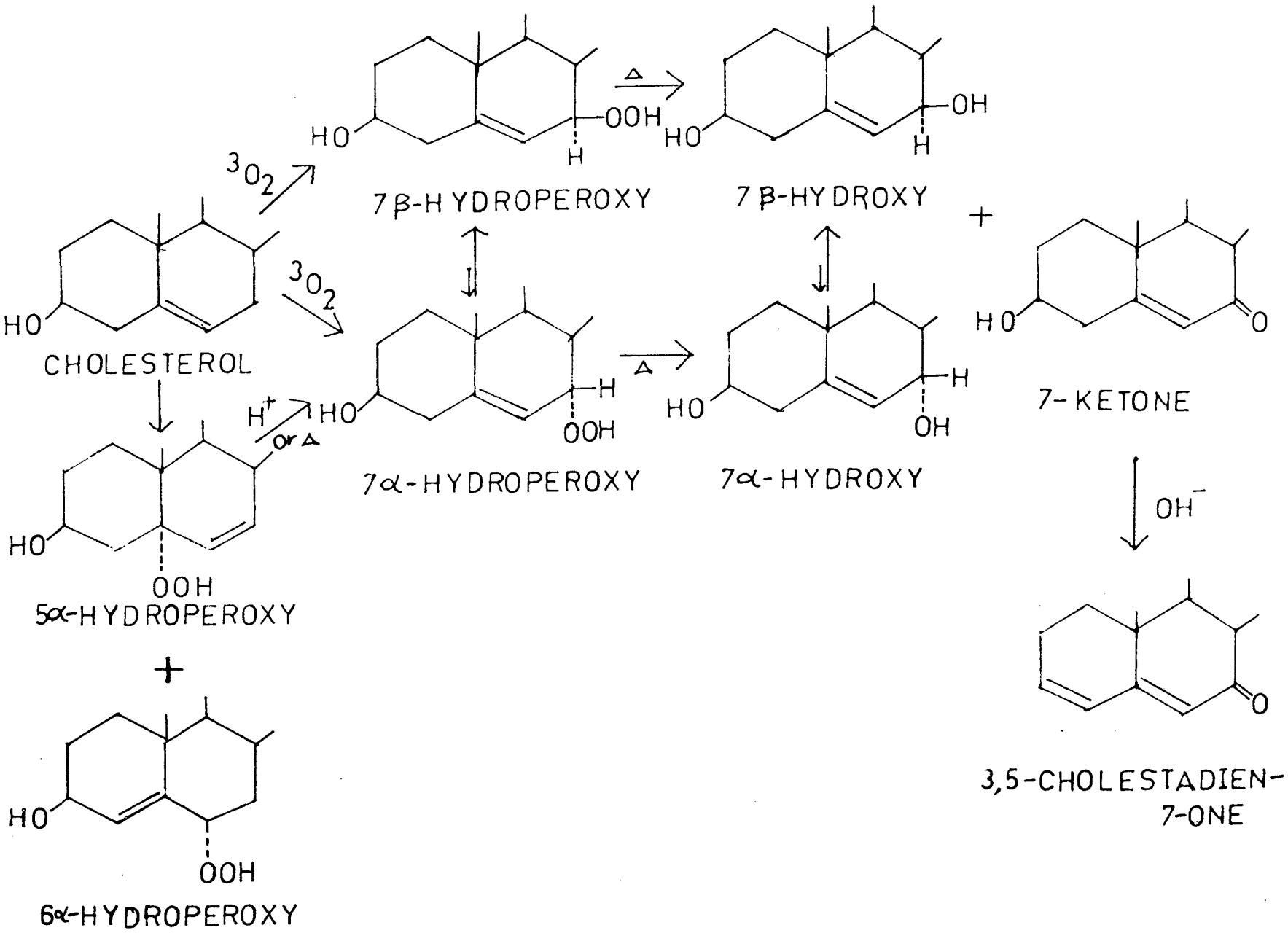


FIG-7. CONVERSION OF CHOLESTEROL TO OXIDES

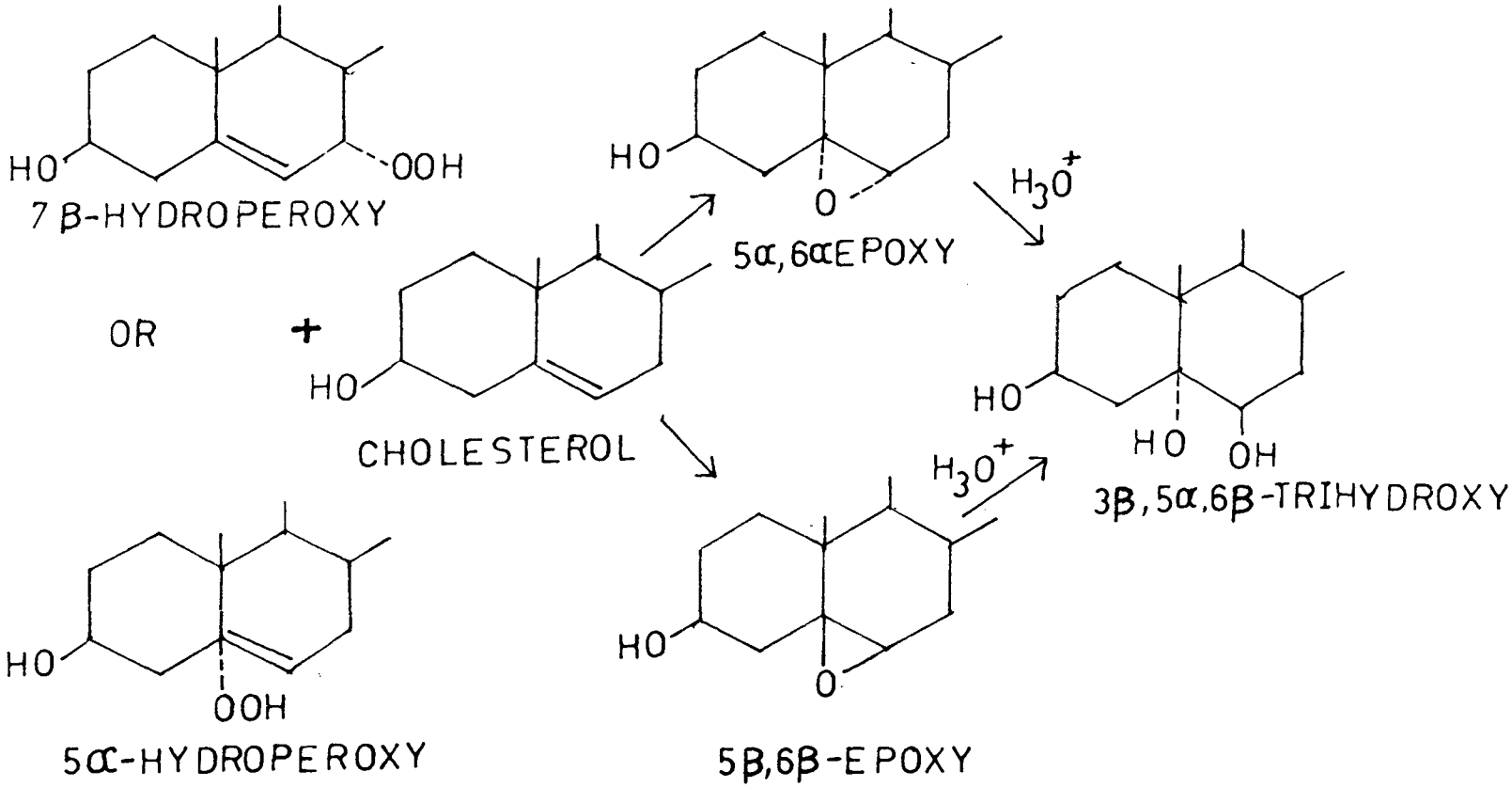


FIG.8 CONVERSION OF CHOLESTEROL TO OXIDES

crystalline state and in solution or dispersion. These epimers of 5-6, epoxides are formed in small amounts when cholesterol is treated with either epimer of 7 hydroxycholesterol or with the 5 hydroxy 6-ene in chloroform. The epimers of 5,6 depoxides are concluded to be secondary oxidation products.

The hydration of these 5,6 epoxides form another type of cholesterol oxide called 3β ,. 5α , 6βtrihydroxy cholesterol which is the most toxic of the cholesterol oxides tested today (Smith 1979).

There are also reports (Maerker, 1987) of cholesterol oxides which are formed due to oxidation of A ring, in aged cholesterol naturally in air irradiated in air 60° c, gamma radiation (or) under other circumstances (Ansari and Smith, 1978). But these are not reported in solutions (or) dispersions.

TOXIC EFFECTS OF CHOLESTEROL OXIDES :

Even before appreciation of the effects of individual cholesterol oxides there were reports of such compounds in foods. Chicoya (1968) has shown that exposure of spray dried egg yolk tallow to ultraviolet irradiation gave rise to a number of cholesterol exides including the B-epoxides and the triol. Tsai and Hudson (1985) found a range of 3-74

p-p-m of cholesterol 5,6 epoxide in a number of commercial dry whole egg samples, 3-166 ppm of some oxides in dry yolk powders. There are also reports of presence of cholesterol oxides in several foods such a s various meats, sausages, dairy products, curd, french fries that are wholly of partially derived. On all these products cholesterol oxides were detected only after they had been subjected to heat, light air, or oxydizing agents.

ANGIOTIXICITY :

The Angiotoxicity of cholesterol oxides was reported in 1976 by Hideshige Imai.

Pure cholesterol was shown to be relatively ineffective in inducing cell death and lesions. However the concentrate of impurities obtained from recrystallization of impure cholesterol seperated by the thin layer chromatography are shown to cause angiotoxicity. Similarly cholesterol oxides of synthetically prepared 3β , 5 ∝, 6β cholesterol triol, 25 Hydroxy cholesterol, cholesterol 5∝ , 6∝ epoxide and others also shown to be angiotoxic., In the experiment with rabbits of two different strains initially, necrotic lesions, later smooth muscle death was observed after 24 hours of 3 doses of injections, after two weeks hypocellularity and cellular debris in the aorta was

increased, and after 10 weeks, fibromuscular thickening, pulmonary artery thickening has been observed., thus iniating atherosclerotic lesions. (Hideshige 1980).

Studies on white carnean pegions (Jocobson M.S. et al, 1985) also sugest that dietary exposure to low levels of cholestane-triol, is atherogenic to a greater degree than exposure to pure cholesterol alone.

The ultra structuaral changes which have been brought about by these small amounts of cholesterol oxides in aorta of chicken was reportdø by Tokuyasu K (1980) thus leading to atherosclerosis. The endogenous cholesterolemia induced by feeding cholesterol containing diets (Tokuyasu, et al, 1980).

After 24 hours of intravøenous injection of 25 hydroxy cholesterol, in rabbit shown with scanning electron microscopy the luminal surface of the aorta having numerous ballon like protrusions, and injection of triol øalso shown similar effects but more frequently compared to 25-OH cholesterol. Intracytoplasamic vacuoles and deffuse subendothelial edema is observed. under transmission electron microscope. (Shikangpeng, 1985).

CARCINOGENICITY :

The issue of carcinogenicity of cholesterol and its derivatives as been controversial over fifty years, an issue yet to be accorded definitive treatment. A bit more satisfactory state of affairs has developed for the evaluations of cholesterol autoxidation products than is the case for cholesterol or heated chkolesterol.

The feeding experiments of food subjected to irradication in air, demonstration of carcinogencity in marsh-buffalo mice of a subcutaneously injected preparation of crude progestron derived from cholesterol by autoxidation focused attention to cholesterol oxides as potential carcinogens.

The cholesterol oxide 5α , 6α cholestane 3βol is shown to be locally carcinogenic at the subcutaneous site in both mice and rat, aloso in intra testicular site in mice. (Bischoff et al 1977). This was supported by the finding, that the chol . oxides were developed in vitro when human skin is exposed to UV as does the skin of hairless mice when exposed in Vivo. Chronic suberythemic levels of UV light on the skin of hairless albino mice produced increase of cholesterol α oxides content and reached maximum at 10 weeks, later squamus cell carcinomas has been observed (Bischoff, and Byron, 1977)

CYTOTOXICITY :

Cholesterol oxides are also known to cause cytotoxicity. The experiments with tissue culture have shown (Baranowski et al., 1982) oxysterols promote a greater inflammatory reaction than purified cholesterol. These cholesterol oxides are cytotoxic for fibroblasts (Kandutsch et al, 1978) and inhibition of growth (chem et , al ., 1974) and Vascular smooth cells (peng et al., 1978). This may be due to lack of membrane renewal or formation resulting from inhibition of cholesterol synthesis (HMG CoA reductase) brought about by certain of these sterols. This results in defective membrane formation and defective growth. The studies with tissue cultures of mouse fibroblasts and macropheges, pig vascular smooth muscle cells (Baranowski, et al., 1982), have shown the necrotic lessions and the neutroplhil polymorphonuclear infiltration which is an acute inflammatory response to the necrosis caused by oxysterols. The experiments with indivdidual cholesterol oxides, 25 hydroxy cholesterol choloesterol 5α , 6β -epoxide and cholestane triol shown that triol was most active in its cytopathic effect on cells in culture, in size of granuloma formation and in producing necrosis (Baranowski, et al., 1982).

The cell death and necrosis brought about by oxysterols is possibly relevant to advdanced human atherosclerotic lesions. Such ulceration may play a role in many of the clinical complications of advanced atherosclerosis. Thus an ulcer would form a nidus for thrombus formation might provide a portal for intimal haemoirrhage, and its contents could cause atheromatus and cholesterolemboli. It remains to be determined whether the fissures and cracks in the intima that under lie human coronary thrombosis (costantinides, 1966) constitute an earlier manifestation of necrotic damage than gross overt ulceration.

The significance and importance of oxysterols in the diet and in the human atheromatus plaque has yet to be firmely established.

Although oxysterols shown to be highly toxic, there is a contradictory view that large subcutaneous implants of cholesterol mixed with oxysterol are rapidly resorbed in comparision with an implanted equal weight of cholesterol alone (Krut, 1980).

This may be due to detergent action of oxysterols and also cholesterol oxides increase the solubility of cholesterol in aqueous suspensions.

So some of these cytopathic effects might be due to such detergent induced damage to cell membrane (Baranowski, et al., 1982).

A number of autoxidation products are recognisedrecently which are capable of indeucing rabbits aortic smoothcell death in vitro (Peng, et al., 1979). Different grades of toxicity was shown by these chyolesterol oxides as follows :

Compounds	Grade of cytotoxicity			
Concentration in culture Medium (Mg/ml)	10	20	50	100
25-OH Cholesterol	1	2	4	4
20 α OH Cholesterol	0	1	2	4
4 Cholestenk-3 one	0	0	1	3
7α OH Cholesterol	0	0	1	2
7β OH Cholesterol	0	0	1	3
7 Keto cholesterol	0	0	1	3
3,5, Choestadien -7 one	0	0	0	0
5,6 epoxy Cholesterol	0	0	0	0
Cholesterol 3β, 5α, 6β triol	1	2	4	4

A degreed of cytotoxicity was gradede as percentage of dying and dead cells,

0 : less than 5%

1 : 5 - 25%

2 : 25 - 50%

3 : 50 - 75%

4 : 75% - 100%

According to these invitro experiments the most toxic cholesterol oxides are 25 hydroxy cholesterol and cholestane triol.

MUTAGENICITY :

The experiments with V 79 chinese hamster cell cultures, epoxide (12, 4μM concentration). It induced 8 -azaguanine resistant mutants at frequencies 4,6 to 11.8 fold higher than the spontaneous mutation rate. (Savanian et al, 1984). They also shown that this 5,6 epoxide is converted to cholosterol triol which is cytotxic, less mutagenic in vivo in order to prevent mutation by epoxide.

In addition cholesterol oxides are also shown to be active as frame shift mutagens towards S. typhimarium. (Savanian, et al., 1984).

Inhibitory to the enzymes of sterol biosynthesis :

Cholesterol oxidation products have been demonstrated to be inhibitory towards several enzymatic step implicated

in the biosynthesis of cholesterol from lanosterol.

In cholesterol biosynthesis these cholesterol oxides are shown to depress the activity of key enkzyme of HMG Co.A reductase in mouse liver cells (kandutsch et al, 1974: 1973), fibroblasts (Brown et al. 1974), hepatomeacells (Bell et al., 1976) and smooth muscle cells (peng et al., 1979).

The potency of inhibition of HMG CoA reductase vary with type of cholesterol oxide 25 hydroxy cholesterol is one of the most toxic sterols tested as well as the most potent inhibitor of cholesterol biosynthesis. Howevder cholestane triol also shown to have remarkble cytotoxic effects on the aortic smooth muscle cells, but it only moderately inhibits cholesterol biosynthesis. (Peng et at., 1979).

In addition the cholestane 3β 5α 6β triol shown to be inhibitory to sterol Δ^{24} reductase in cultures of rat hepatocytes. (Smith, 1981) . The oxygenated sterols are also shown to act as moderator of the esterification of cholesterol by fatty acids catalyzed by both extra cellular and intra cellular enzymes. (Smith, 1981).

MATERIALS AND METHODS

Scope of the study:

The present study is a preliminary study for the future study of commercial oils and fat consumption and coronary heart disease. The commercial oils manufactured by standard companies are selected to determine their fatty acid composition and fats to determine cholesterol oxides qualitatively. These commercially available oils and fats are consumed in large percentage by the people. So these could be the best samples for analysis. There are varieties of oils extracted from different sources are consumed by the people distributed in different states of the country. So the typical 7 varieties of oils and fats(ghee) are taken for analysis. The following are the 7 varieties of oils

S.No	Oil	Brand-1	Brand-2
1.	Sunflower oil	Saffola	Flora
2.	Corn oil	Cornola	-
3.	Sasame oil	Inder Dhanush	-
4.	Groundnut oil	Postman	Dalda
5.	Mustard oil	Kanodia	Jaumbo
6.	Soyabean oil	Vital	-
7.	Palm oil	Palmolein	Imported through State Trading Corporation, N.Delhi.

In order to compare the composition of single variety of oil manufactured by different companies, each variety of oil manufactured by two companies having different brand names are taken except palm oil, corn oil (single company is available).

Fats:

S.NO	Fat	Brand -1	Brand -2
1.	Ghee	DMS	VITA

Standard compounds and other chemicals:

The standard compounds of fatty acids and cholesterol oxides which are 99% pure are obtained from sigma company and solvents for analysis are obtained from authentic companies and are redistilled in all glassware apparatus.

Apparatus used :

Thin layer chromatography :

Principle:

This technique is basing on the principle that different components will have different adsorption capacities on a solid phase.

The adsorbent is held on glass plates in a thin layer, and the mobile solvent phase moves up the plate by capillary action taking the various components of the sample applied with it at differing rates, according to the extent to which they are adsorbed by the adsorbent. The component which is having less adsorption capacity will move large distance from the origin, and vice versa. The separation depends on the nature of the compounds to be seperated, solid support and polarity of mobile phase.

Description of the Apparatus :

The basic equipment contains the following items

1. TLC - spreader with adjustment of layer thickness from 0 to 2mm.
2. Aligning tray for coating five 20x20cm plates
3. Ten glass plates 20x20cm pf equal thickness.
4. Two end plates 20x5cm.
5. A normal chamber with ground edges.
6. Spotting divice for marking, sample application.
7. A drying rack
8. A microletre pipette.

Gas liquid chromatography:

Principle:

The compounds to be seperated are volatalized and passed in a stream of inert gas (the mobile phase) through a column in which a high boiling point liquid is coated onto a solid supporting material. The substances are separated according to their volatilities and on their relative solubilities in liquid phase. They emerge from the column as peaks of concentration, ideally exibiting a poision distribution. These peaks are detected by some means which converts the concentration of the component in the gas phase into an electrical signal which is amplified and passed to a continuous recorder so that a tracing is obtained with an individual peaks bear a direct relationship to the mass of the component present.

Description of the apparatus:

Gas chromatograph is from shimadzu corporation, JAPAN. Gaschromatograph GC-9A is highly efficient controlled by microcomputer. The instrument can be used to temparature programmed analysis and automatic analysis. Every laboratory GC consists of essentially of six parts:

1. Carrier gas system
2. Sampling device

3. Column
4. Thermostat
5. Detector
6. Recording and evaluating system.

The presently used GC has the following major constituent units.

1. Oven section - Column oven, sample injection port, detector, power controller.
2. Flow control section-carrier gas flow control, FID flow control.
3. Electric controll portion-power unit,Key and display unit, Detector controlunit

Column Oven:

This is for heating the columns. Thes can accommodate 3 types of columns

1. Stainless steel column 10mx2
   (Outer diameter 4mm,Inner diameter 3mm)
2. Glass column 5mx2
   (Outer diameter 5mm,Inner diameter 2.6mm)
3. Capillar column
   (stainless steel or glass,quartz, 100mx1)

The temparature controlling range is $-100^{O}+399^{O}C$.
Sample injection port: Where sample is injected with a

syringe and the range of temparature- room temparature +399°C

The injection port is made up of silicon rubber.

Detector:

Where the sample after separating through the column individual components are detected. There are four types of detectors which can be installed.

Flame Ionisation Detector:

This is based on the measurement of the electrical conductivity of a hydrogen flame. The electrical conductivity of hydrogen burniung in air is low, when organic vapours are mixed with the hydrogen, the conductivity increases.The commonly used carrier gas with this is nitrogen and air. The increased conductivity is measured and amplified recorded as peak on the recorder.This is very sensitive detector most commonly used. The minimum detectable level is 3×10^{-12} g/sec

The maximum temparature which can be obtaind is 400°C

Thermal conductivity detector:

This is based on the difference in thermal conductivity between a carrier gas and a gas and solute mixture. A heated

sensing element is placed in the gas stream and maintained at a temparature above that of the detector cell wall. A reference element and reference stream is necessary. In this chromatograph the element is made up of Tangstenrhenium. The maximum temparature which can be obtained is 400^{o}c. The sensitivity of the device is with in limits, proportional to the difference in temparature between the sensing elementand the cellwall.

Helium is preferred as a carrier gas for detectors of this kind.

Electron capture detector :

This detector used for detection of compounds with high electron affinity.Under the influence of radiation free electrons and positive ions are produced in the carrier gas ussually nitrogen,the charged particles migrate to the appropriate electrodes. The recombination of these two is about ten thousasnd times greater than the velocity of the positive ions. and there will be a constant ion current.When electronegative compound reachs the detector this will capture the electrons with the production of negetive ions. These negetive ions combine with the positive ions which has been formed in carrier gas and ion current will be deminished. Thus the detector system responds to the

decrease in the costant ion current.The ECD detector is highly sensitive to only certain molecules.

Flame photometric detectors:

In this detector which measure the intensity of the light which accompanies the cumbustion of the organic compound by a selenium photo cell.This is useful for detection of organic compounds.

Flow control section:

This contain carrier gasflow control, FID flow control with the carrier gas folw controll, we can put required flow of carrier gas in the column. So that the component to be separated are seperated well. The FID flow control is used to control flow of hydrogen and air so that the sensitivity is more.

Analysis :

The general procedure for the isolation of fatty acids from the complex lipids include the following steps

1. Saponification
2. Transesterification
3. Thin layer choromatographic separation
4. Gas liquid choromatographic separation

(christie,w.w.1982)

In 1986 Peers and Coxon modified by adding one step i.e silvernitrate partition for separating unsaturated (highly) fatty acids from saturated fatty acids so that the recovery of highly unsaturated fatty acids such as EPA, DHA are more, in addition to the silver ion chromatography. This is only for confirmation.

Saponification:

100mg of lipid sample was taken in a flat bottomed 150ml flask. 2ml of 1m solution of KOH in 95% ethonal is added. Then reflux condnser is attached and refluxed for 1 hour.The solution is cooled, 5ml water is added and is extracted throughly with diethyl ether 3x5ml. Some times it may require centrifugation break the emulsion into layers.The upper diethyl ether layer is separated and washed with water. This water is added to the aqueous layer.This diethyl ether layer removes nonsaponifiable matter.

The aqueous layer which is left is acdified with 6M hydrochloric acid and extracted with diethyl ether or hexane (3x5ml). The free fatty acids are recovered after washing the extract with water,drying it over anhydrous sodium sulphate and removed the solvent in a rotary evaporator.

Transesterification:

The sample was then dissolved in 1ml of tetrahydro furan in a test tube and 5ml of acetyle chloride methonal in 5:100 ratio. is added, then refluxed for 2hr, After refluxing 5ml of water containing sodium chloride 5% is added. The the esters are extracted with hexane added .Then the esters are exracted with hexane 2x5ml using pasture pipettes to separate thelayers.Latter the hexane layer is washed withwater (4ml) containing potassium bicarbonate 2% and dried over anhydrus sodium sullhate. The solution is evaporated on rotary evaporator.

In adddition to this transesterification canalso be done with boron trifloride, conc. H2So4 etc.

Silvernitrate partition:

The fatty acid methyl esters upto 1g dissolved in 10ml of 2,2,4,trimethyl pentane was shaken with an equal volume of silvernitrate solution (25%w/v in ethanol, water1:1) and allowed to stand for a few minutes to allow phase separation.The upper organic layerwas removed and lower aqueous layer is diluted with water(10ml). Then this is extracted with 3x10ml of hexane. Both organic layers were dried and evaporated. This step can be eliminated when there is no highly unsaturated fatty acids in the sample.

Thin layer choromatography:

The sample obtained after silvernitrate partition can be directly analysed by GLC. Before that the presence of fatty acids are conformed by doing silver ion thin layer choromatography. and saturated by conventional chromatography.

Preparation of TLC plates:

23.75gm of silicagel G is mixed with a solution of 1.25gm silvernitrate(AgN03) in 50ml of water and made it as slurry. This slurry is spread on the glass plates 20x20cm with help of applicator. The applicator must be made up of either anodized aluminium or stainless steel so that it is resistant to the silvernitrate corrossive action. After spreading the slurry the plates are left onthe tray until the transperency of the layer has disappeared. then the plates are kept in a drying rack and these plates are stored in moistureless chamber or air tight glass chamber.

Activation of plates: Just before the spotting the sample the plate is activated at 110°C in oven for about 30 minutes after cooling sample is applied. Sample application: About 20µl sample was applied either as a spot or a band at 1.5cm from one margin with the help of microsyringe. After spotting the plate was allowed to dry then they are subjected to developing.

Developing of plates :

The plates are developed in developing chamber. 200ml of solvent containing 40:60v/v hexane :diethyle ether was poured and allowed to saturate for 1 hour. Then plates were kept gently inside and allowed until the solvent front reaches upto below 2cm from the upper margin.Then removed and allowed to dry.

Visualization of spots:

The spots are visualized by spraying 2,7,dichloro florescene (.2% solution in 95% ethanol) with sprayer under UV light. Yellow spots are seen on a purple colour background.

Identification: The fatty acids are identified by their Rf values compared with that of standards which are run parallely.

$$Rf = \frac{\text{Distance of spot centre from origin}}{\text{Distance of solvent front from origin}}$$

The above procedure is lengthy and cumbersome as it has several extraction and purification steps. Recently in 1984 Lepage,G., reported a one step reaction that is carried out in the same tube and bypasses all the extraction and purification steps before analysing with GC. The

recoveries by this method is better than 96% and there is no need to add an antioxident to protect unsaturated lipids.In the present study this procedure is followed.

Procedure:

100mg of lipid sample was taken in a screw capped test tube. 1ml of internal standard Heptadecanoic acid 17:0, containing 1mg/ml prepared in 3:2 methanol: benzene is added. then 2ml of freshly prepared acetyle chloride: methonal 5:100 (v/v) is added to each tube and are capped subjected to mehanolysis at 100°C for 1hr in boiling water bath. The caps are put in such a way so that there would not be any leakage.After heating the tubes were allowed to cool for 10minuters and 2ml of water is added shaken well. Then 2ml of hexane is added to extract the esters of fatty acids. The layer of hexane canbe separated or it can be left in the same tube and stored at 4 till they are injected in GC.

Preparation of standards:

A standard mixture of 9 fatty acid esters is prepared by mixing the solutions in hexane as follows

16:0	0.2ml	1mg/ml
16:1	0.2ml	1mg/ml
17:0	0.2ml	1mg/ml

18:0	0.2ml	1mg/ml
18:1	0.2ml	1mg/ml
18:2	0.2ml	1mg/ml
18:3	0.1ml	10mg/ml
20:5	0.5ml	0.1mg/ml
22:6	0.2ml	1mg/ml

GC separation:

The analysis is performed on a stainless steel packed column packed with 10% diethylene glycol succinate (100-200 mesh) of length 2m and internal diameter 3mm
Prior to injection of the sample the GC is adjusted to the following conditions which are standardized previously where the separation is maximum. The components are best separated in temparature program.The temparature program is shown

Initial column temparature	:150°C for 15 minuts
Rate of increase	:.2°C/min
Column final temparature	:165°C for 30 min.
Injection port temparature	:230°C
detector temparature	:230°C
Carrier gas flow (Nitrogen)	: 40ml/min

First the instrument is calibrated with standard mixture by injecting 1 . Then sample 1 is injected with the

help of syringe. The peaks are recorded on a automatic recorder attached to the chromatograph.

Identification of peaks:

The peaks obtaind after sample run are identified by comparing the retention times with that standards.

Quantitative caliculations:

The peaks which are obtaind are essentially a Gausian distribution curves. The caliculation of the area is programmed already in the GC. so areas willbe given directlty.The formula is

Area A = $\sqrt{2\pi}\sigma h = 2.507\sigma h$

A: Peak area
H:Peak height
σ:Standerd deviation

By knowing the areas of different components in the sample and area of internalstandard the amount of the component is calculated by using the formula.

$$\text{Content(mg)} = \frac{\text{ACP X RF X WIS X 100}}{\text{AISP X WS}}$$

ACP = Area of the component peak

RF = Response factor

WIS = Weight of internal satandard

AISP = Area of the internal standard peak

WS = Weight of sample.

Qualitative analysis of cholesterol oxides:

In the present study the presence of cholesterol oxides in commercially prepared ghees (two varieties) and home made ghee (which is conventionally prepared in the home)are studied qualitatively by using thin layer chromatography.

Procedure:

The cholesterol oxides were isolated from the ghee by previously described procedure after little modification.(Jocobson ,M S,1987).

The procedure include the following steps

1. Saponification
2. Extraction of nonsaopnifiable components
3. Separation by Thin layer choromatography

Saponification:

2gm of ghee was taken in a flat bottomed 150 ml flask and 25 ml of 15% KOH in ethanol was added. A reflux condenser was attached and heated at 40 c under vacume (or) nitrogen for 2 hrs. After cooling the non saponifiable components are extracted.

Extraction of nonsaponifiable components :

25 ml of distilled water is added and poured into a separatory funnel. Then 2x25 ml of Diethyl ether was added shaken, and allowed for layer separation. The lower aqueous layer is discarded and upper ether layer is washed with distilled water for two to three times. The organic layer is passed through Sodium Sulphate to remove traces of water and then evaporated at low temparature, dissolved in 1 ml of hexane and subjected to Thin layer Chromatography.

Preparation of standard compounds:

5 mg of each standard compond is dissolved in 1 ml of haxane or ethanol. A mixture of the standard compounds is prepared by mixing 0.25 ml of each standard compond. The individual compounds and mixture 10μl each is spotted.

Thin layer chromatography :

It is the most versatile techneque for the study of cholesterol oxides.

Preparation of Thin layer on glassplates:

30 gm of silica Gel G is mixed with 75 ml of water and made it as slurry. This slurry is spread on glass plates 20x20 cm with applicator evenly. The plates are left on the

tray until the transperency of the layer has disappeared. These plates are stored in a moisture free chamber. At the time of requirement before application of sample are heated or activated at 110°C for one hour and sample is applied.

Sample application:

About 10 µl of standard mixture and 20µl of sample is applied as a spot with a µl syringe. After drying they are subjected to developing.

Development of plates:

The plates are kept in developing chamber for development. The solvent used is diethyl ether (chicoye.E. et al, 1968) insted of hexane: diethyl ether 1:1 (v/v) (Jacobson.M.S, 1987) as the resolution is more in the former case. Initially the solvent is poured into the chamber and allowed to saturate for 1 hour and the plates are kept inside. The plates were removed after the solvent front reaches 1.5 cm from the upper margin and allowed to dry.

Visualization of spots:

The spots are visualized by sparying 10% CuSO4 in 8% H2PO4 and charred in oven at 180°c for 30 to 60 minutes. Brownish black spots are seen.

Identification:

The cholesterol oxides are identified comparing the Rf values of sample with that of standard which are run parallel.

$$\text{Rf value} = \frac{\text{Distance of spot centre from origin}}{\text{Distance of solvent front from origin}}$$

RESULTS

Evaluation of n-3 fatty acids in oils and cholesterol oxides in ghee:

The amount of n-3 unsaturated fatty acids are quantified by the following procedure of lepage and Roy C.C (1984), instead of other procedures (Christe 1982: Freedman, et al. 1984: Iverson, et al. 1964; Peers, et al. 1986) as this procedure eliminates the cumbersome and lengthy steps. More over it is one step reaction that is carried out in the same tube bypassing all the extraction and purification steps. The recovery of fatty acids were shown to be around 96%.

It is not necessary to degum or purify the oils as these are refined, and are directly used for transesterification and also quantified by GC internal standard method.

The instrument was initially calibrated with standard fatty acids so that linear response of the detector was achieved to avoid overloading of column.

1 μl of mixture solution of standard fatty acids (concentration 1 mg/ml) is injected on to the column. The figure 9. shows the GC signal patterns for fatty acids.

The correlation of the log retention times of saturated fatty acids versus carbon number has been shown in figure 10. representing the linear structural relationship between retention time and carbon numbers saturated fatty acids.

After calibration with standard mixture and calculation of response factors 1 µl of sample substance was injected in sensitivity range, 10^2

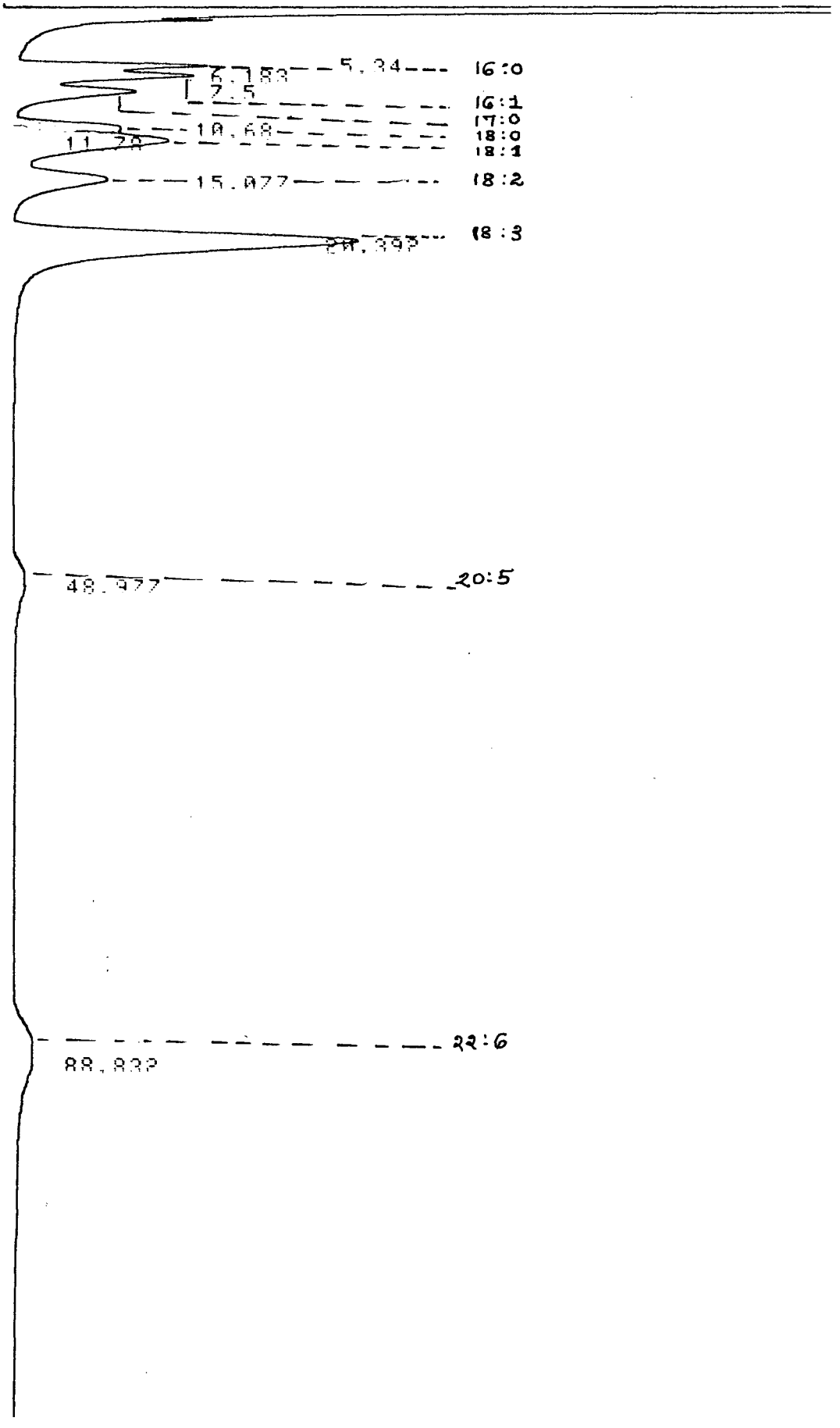
Each variety of oil was prepared in triplicates, and injected twice in GC. The area of the peaks of the samples were calculated by computer using the formula mentioned previously in Materials and Methods. After knowing the area of sample and standard fatty acids, the amount was calculated. The results were shown in Table 6 to Table 12.

The fatty acid composition of sunflower oil manufactured by two companies having brand names saffola and Flora were shown in table 7. In the Flora oil the palmitic acid constitutes 5.3 mg/100 mg which was in consistent with previously reported value of 6 mg/100 mg (Bimbo, P.A., 1986), where as saffola it was 5.5 mg/100 mg. Stearic acid and oleic acid together constitute 35.7 mg/100 mg in flora oil, but the reported value was only 26 mg/100 mg,where as in saffola it was 16.2 mg/100 mg which was not in consistent

FIG.9

START 1

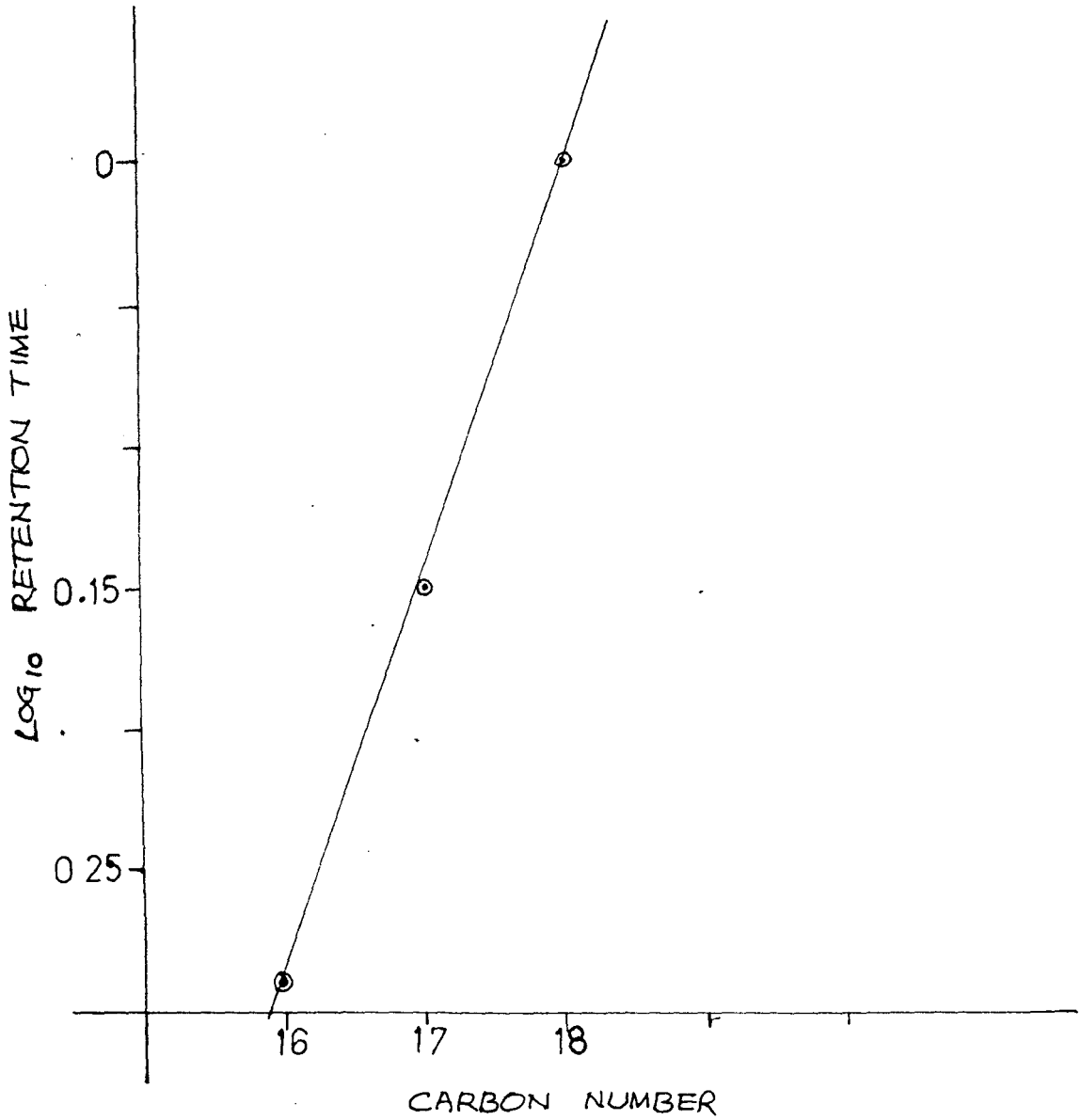
17/05/89 18:41:01



C-R2A

FIG.9

FIG. 10 THE CORRELATION OF THE LOG RETENTION TIME VERSUS CARBON NUMBER



with the previously reported value. Linoleic acid constitutes in flora, saffola, 55.1 mg/100 mg and 56.5 mg/ 100 mg respectively while the reported value was 66 mg/100 mg. No peaks were observed corresponding to the n-3 fatty acids suggesting the complete absence of n-3 fatty acids which was in aggrement with the previously reported (table 2). The above results were graphically represented in fig.12

The fatty acid composition of Groundnut oil manufactured by two companies having brand names Postman and Dalda were shown in the table 6.

The amount of palmitic acid which was found to be 8.7 mg/100 mg, 10.3 mg/100 mg in postman and dalda respectively which was in consistent with the reported value 6-16 mg/160 mg (table.5). Stearic acid and oleic acid together constitute 50.3 mg/100 mg, 56.27 mg/100 mg respectively in Postman of the reported value of Groundnut oil from different source (table 5). The content of linoleic acid in postman and Dalda were 25 mg/100 mg, 28 mg/100 mg respectively, while the reported value of Groundnut oil was 13-45 mg/100 mg (table 5). No peaks were observed corresponding to the n-3 fatty acids suggesting the absence of the same which is in agreement with the previous report

(table 2). The above results were graphically represented in Fig.11.

The fatty acid composition of Til oil (Sesame oil) manufactured by single company having brand name Indradhanush were also observed and quantified (table 12). The content of palmitic acid which is found 7.9 mg/100 mg the previously reported value is 7-12 mg/100 mg. (Table 5).

The stearic and oleicacid together constitutes 35.99 mg/100 mg where as the reported value is 38-50 mg/100 mg (table 5). The content of linoleic acid was found to be 42 mg/100 mg where as the reported value is 35-50 mg/100 mg. No peaks are observed corresponding to the n-3 unsaturated fatty acids. The results were shown graphically in fig.13.

The fatty acid composition of soybean oil manufactured by two companies having brand names Vittal and Surya was shown in the table 9.

The amount of palmitic acid which was found in Vittal, Surya was 7.9 mg/100 mg, 6.76 mg/100 mg respectively, the reported value is 7-14 mg/100 mg (table 5). Stearic acid and oleic acid together constitute 21.5 mg/100 mg,

20.7 mg/100 mg, in Vittal, Surya respectively, the reported value is20-35mg/100 mg. The amount of linoleic acid in both brands Vittal and Surya is 47.5 mg/100 mg, 43 mg/100 mg respectively, where as reported value is44-62mg/100 mg. (Table 5) In this oil peak was observed corresponding to the lenolenic acid and was found to be 5.2 mg/100 mg in Vittal and 4.6 mg/100 mg in Surya, where as reported value is4-11mg/100 mg. The above results were shown graphically in fig. 14.

The fatty acid composition of Mustard oil manufactured by two companies having brand names kanodia, Jaumbo.

The palmitic acid constitutes 1.9 mg/100 mg in Kanodia and 27 mg/100 mg in Jumbo. The stearic and oleic acid together constitutes 11.9 mg/100 mg in Kanodia, 12.42 mg/100 mg in Jumbo. The content of linoleic acid was found to be 14.7 mg/100 mg in Kanodia and 16.6 mg/100 mg in Jumbo. Lenolenic acid was found to be 10.4 mg in Kanodia, 9.3 mg in Jumbo. These results were shown graphically in fig. 15.

The fatty acid composition of corn oil manufactured by single company having brand name Carnola was also studied (Table 11).

The palmitictic acid content of this oil was found to be 13.4 mg/100 mg, where as reported value is 11 mg/100 mg. The amount stearic acid and oleic acid together were found to be 27.4 mg/100 mg, reported value is 27 mg/100 mg (Table 2). The content of lenoleic acid and linolenic acid were found to be 55.4 mg/100 mg and 1.02 m/100 mg respectively which is consistent with the reported value 57 mg/100 mg and 1 mg/100 mg. These results were shown graphically in fig. 16.

The fatty acid composition of palm oil, brand name Palmolein was shown in Table 12. The palmitic acid content of this oil was found to be 36.9 mg/100 mg where as reported value is 32.59 mg/100 mg. The stearic + oleic acid together constitute 46 mg/100 mg, the reported value is 28.5-60 mg/100 mg (Table 5). The linoleic acid amount is found to be 9 mg/100 mg, the reported value is 5-14 mg/100 mg. The n-3 fatty acid content i.e., linolenicacid is found to be 0.4 mg/100 mg, the reported value is <1.5 mg/100 mg. These results were shown graphically in Fig. 17. The amounts of linolenic acid in 4 oils were shown in fig.18. similarly the amounts of other fatty acids were shown in fig. 19,20,21 in 7 oils.

TABLE-6:FATTY ACID COMPOSITION OF GROUNDNUT OIL (mg/100mg)

Fatty Acid	No.of carbons &Double bonds	Brand-1 POSTMAN	Brand-2 DALDA
Palmitic Acid	16:0	8.78 ±.12	10.32 ±.15
Stearic Acid + Oleic Acid	18:0 + 18:1 (n-9)	50.32 ±.86	56.27 ±.71
Lenoleic Acid	18:2 (n-6)	25.11 ±.56	28.41 ±.42

TABLE-7:FATTY ACID COMPOSITION OF SUNFLOWER OIL(mg/100mg)

Fatty Acid	No.of Carbons &Double bonds	Brand-1 FLORA	Brand-2 SAFFOLA
Palmitic Acid	16:0	5.35 ±.07	5.51 ±.1
Stearic Acid + Oleic Acid	18:0 + 18:1 (n-9)	35.73 ±.32	16.20 ±1.4
Lenoleic Acid	18:2 (n-6)	55.11 ±.48	56.53 ±.83

TABLE-8:FATTY ACID COMPOSITION OF TIL OIL (mg/100mg)

Fatty Acid	No.of Carbons &Double Bonds	Brand-1 INDERDHANUSH
Palmitic Acid	16:0	7.99 ±.18
Stearic Acid + Oleic Acid	18:0 + 18:1 (n-9)	35.99 ±1.1
Lenoleic Acid	18:2 (n-6)	42.72 ±.84

TABLE-9:FATTY ACID COMPOSITION OF SOYABEAN OIL (mg/100mg)

Fatty Acid	No.of Carbons &Double Bonds	Brand-1 VITAL	Brand-2 SURYA
Palmitic Acid	16:0	7.92±.04	6.76±.32
Stearic Acid + Oleic Acid	18:0 + 18:1 (n-9)	21.55 ±.37	20.77 ±1.8
Linoleic Acid	18:2 (n-6)	47.57 ±.27	43.30 ±1.8
Linolenic Acid	18:3 (n-3)	5.23 ±.05	4.61 ±.34

TABLE-10:FATTY ACID COMPOSITION OF MUSTARD OIL (mg/100mg)

Fatty Acid	No.of Carbons &Double Bonds	Brand-1 KANODIA	Brand-2 JUMBO
Palmitic Acid	16:0	1.90 ±.08	2.27 ±.08
Stearic Acid + Oleic Acid	18:0 + 18:1 (n-9)	11.99 ±.46	12.42 ±.19
Linoleic Acid	18:2 (n-6)	14.78 ±.62	16.65 ±.2
Linolenic Acid	18:3 (n-3)	10.41 ±.44	9.38 ±.24

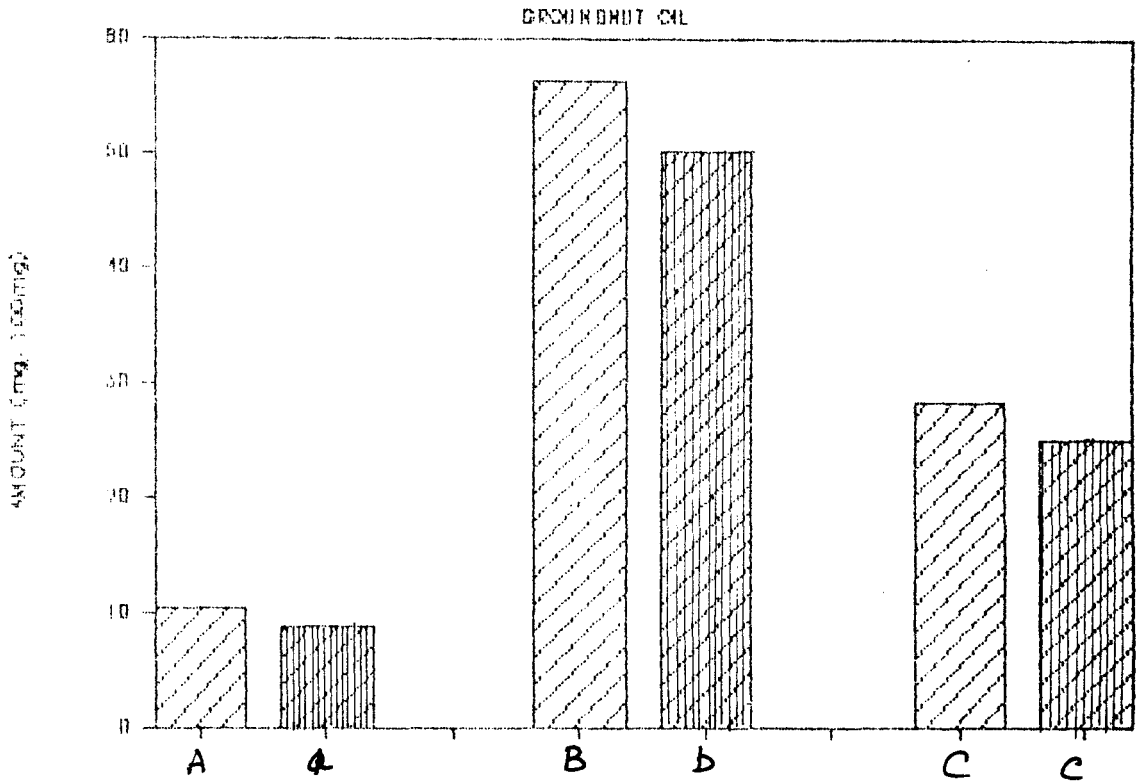
TABLE-11:FATTY ACID COMPOSITION OF CORN OIL (mg/100mg)

Fatty Acid	No.of Carbons &Double Bonds	Brand-1 CORNOLA
Palmitic Acid	16:0	13.45 ±.11
Stearic Acid + Oleic Acid	18:0 + 18:1 (n-9)	27.41 ±.25
Linoleic Acid	18:2 (n-6)	55.49 ±.33
Linolenic Acid	18:3 (n-3)	1.02 ±.08

TABLE-12:FATTY ACID COMPOSITION OF PALM OIL (mg/100mg)

Fatty Acid	No.of Carbons &Double Bonds	Brand-1
Palmitic Acid	16:0	36.90 ±.52
Stearic Acid + Oleic Acid	18:0 + 18:1 (n-9)	46.10 ± 1
Lenoleic Acid	18:2 (n-6)	9.09 ±.55
Linolenic Acid	18:3 (n-3)	0.04 ±.1

FIG. 11. FATTY ACID COMPOSITION OF



A, a : PALMITIC ACID

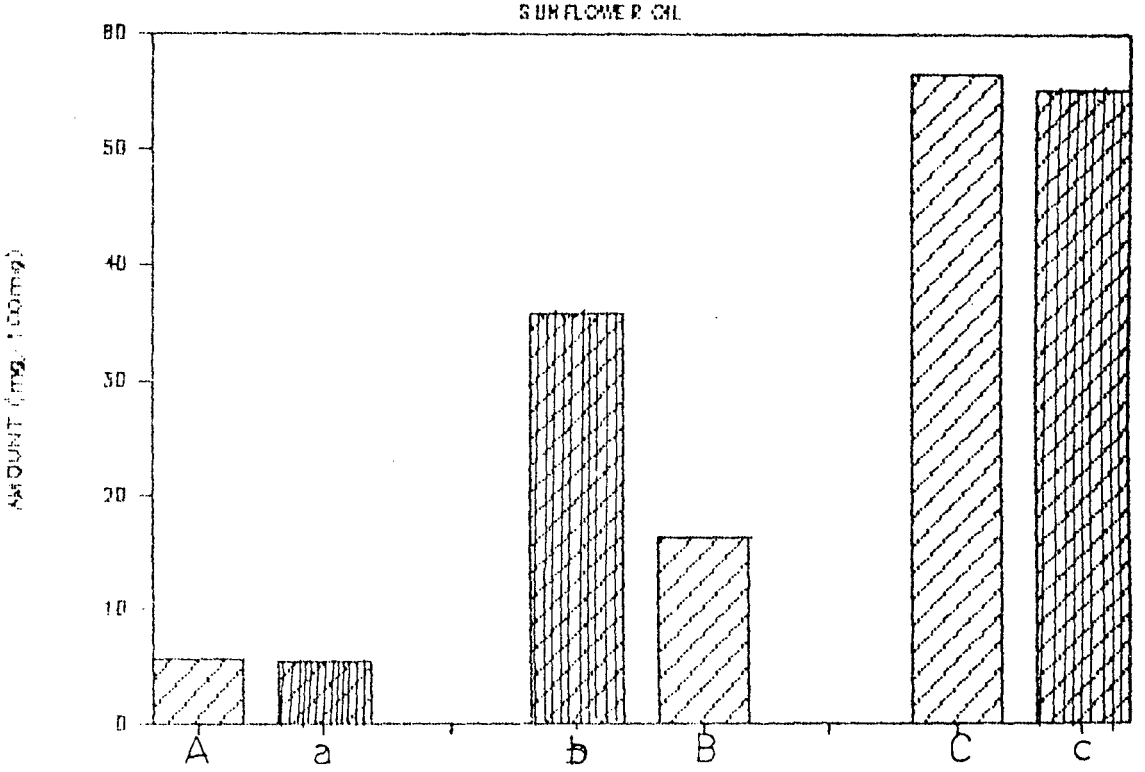
B, b : STEARIC ACID
+OLEIC ACID

C, c : LINOLEIC ACID

— DALDA

— POSTMAN

FIG.12.FATTY ACID COMPOSITION OF SUNFLOWER OIL



A, a : PALMITIC ACID

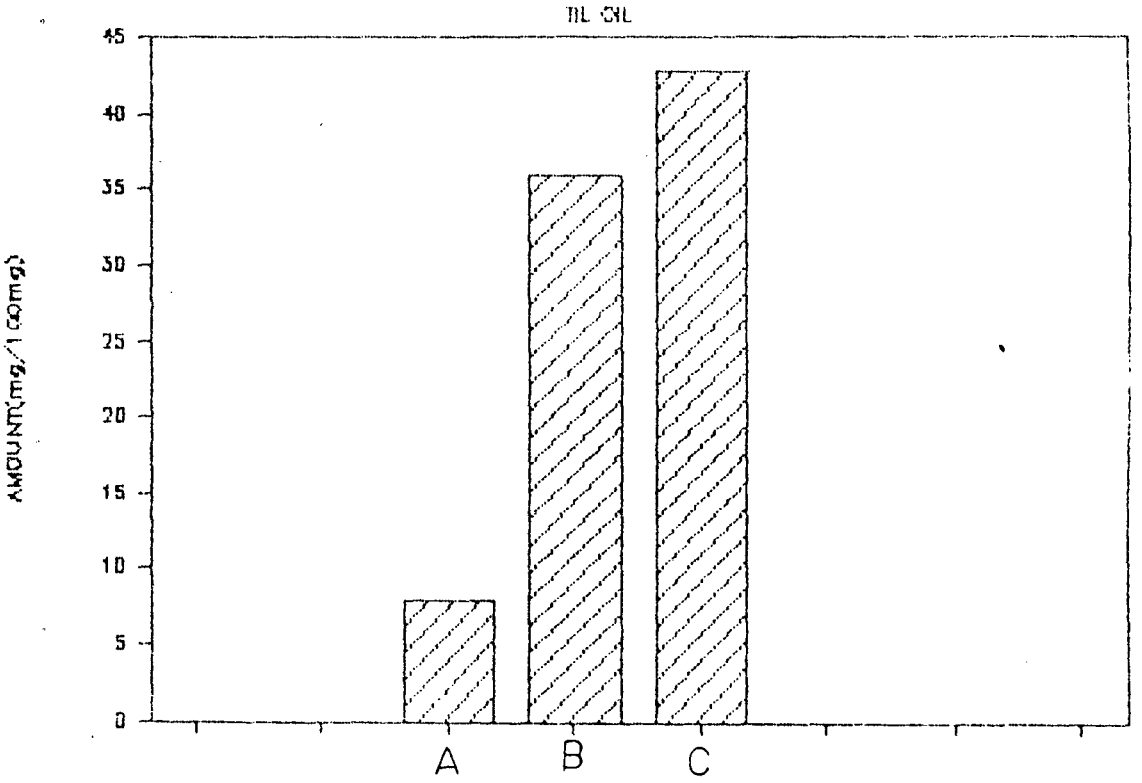
B, b : STEARIC ACID
+OLEIC ACID

C, c : LINOLEIC ACID

– SAFFOLA

– FLORA

FIG.13.FATTY ACID COMPOSITION OF



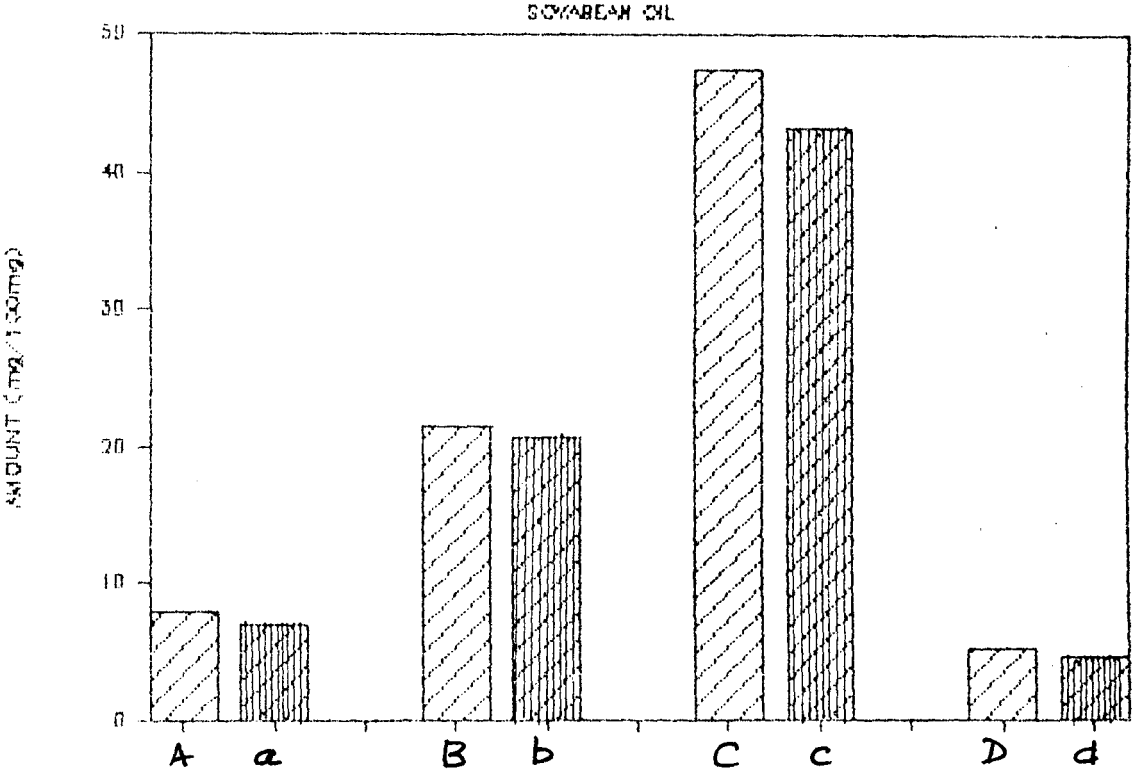
A : PALMITIC ACID

B : STEARIC ACID
+OLEIC ACID

C : LINOLEIC ACID

- INDERDHANUSH

FIG.14.FATTY ACID COMPOSITION OF SOYABEAN OIL



A, a : PALMITIC ACID

B, b : STEARIC ACID +OLEIC ACID

C, c : LINOLEIC ACID

D, d : LINOLENIC ACID

– VITAL

– SURYA

FIG.15.FATTY ACID COMPOSITION OF

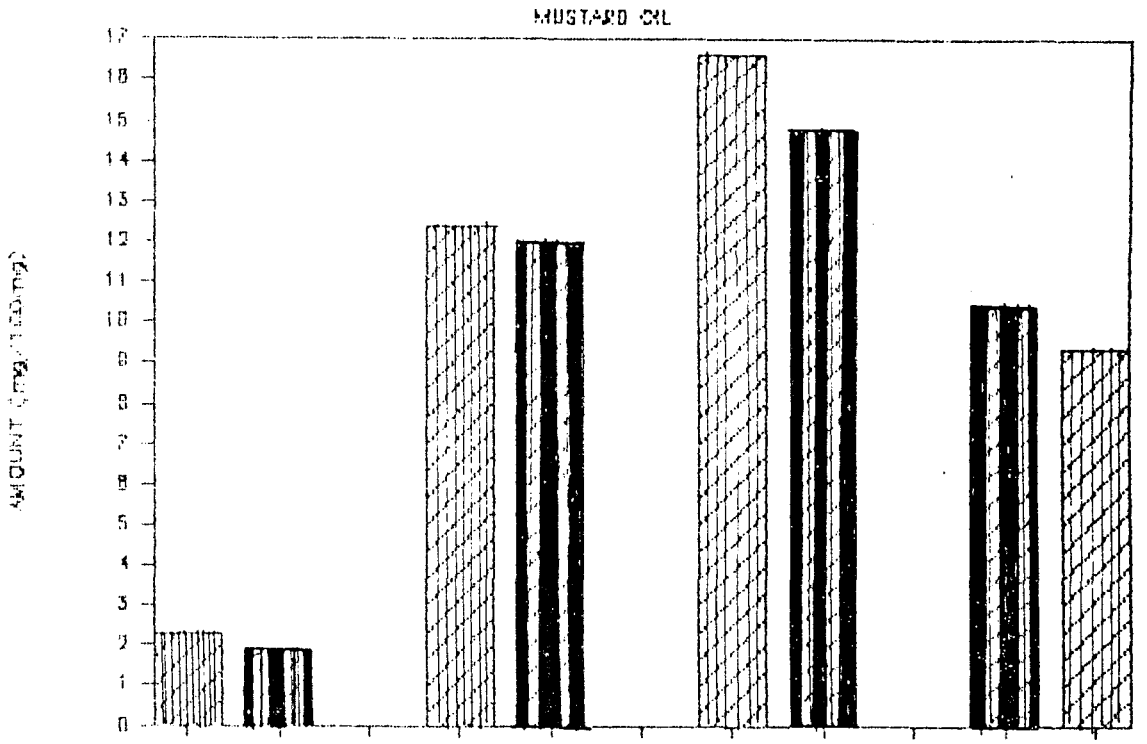
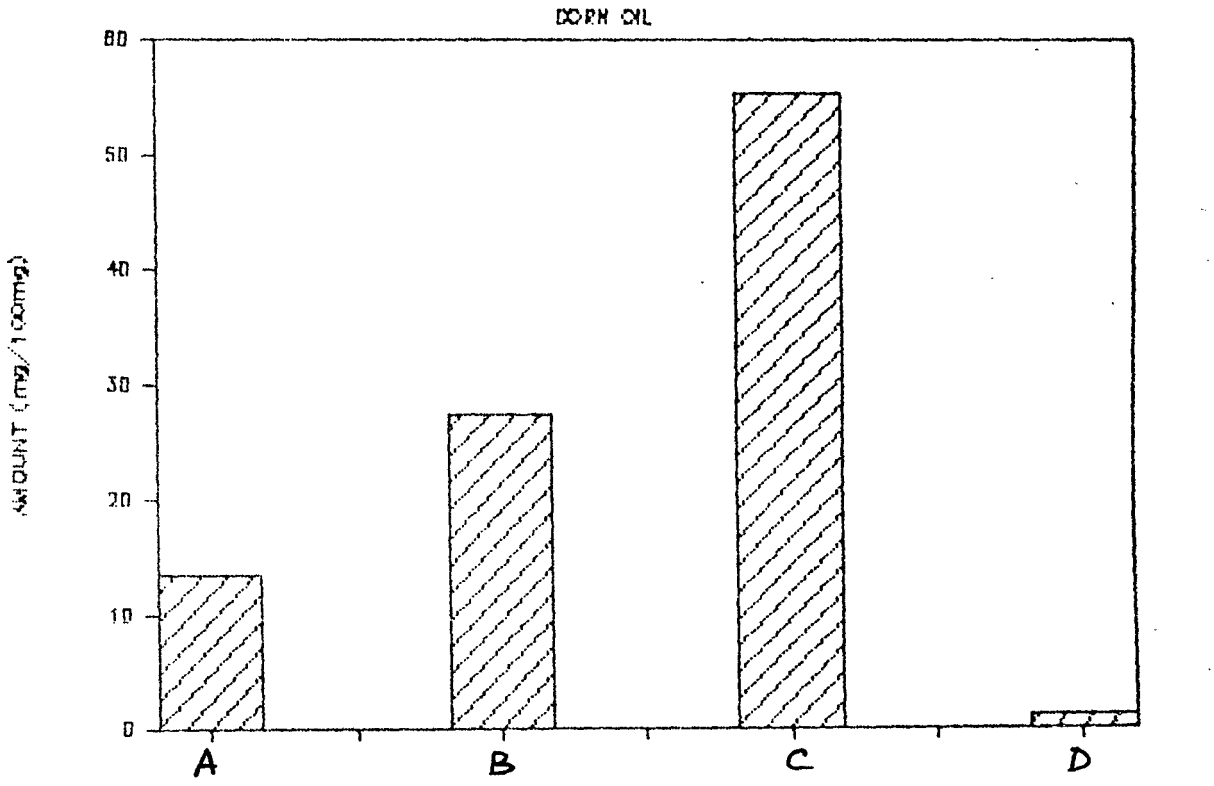


FIG.16.FATTY ACID COMPOSITION OF



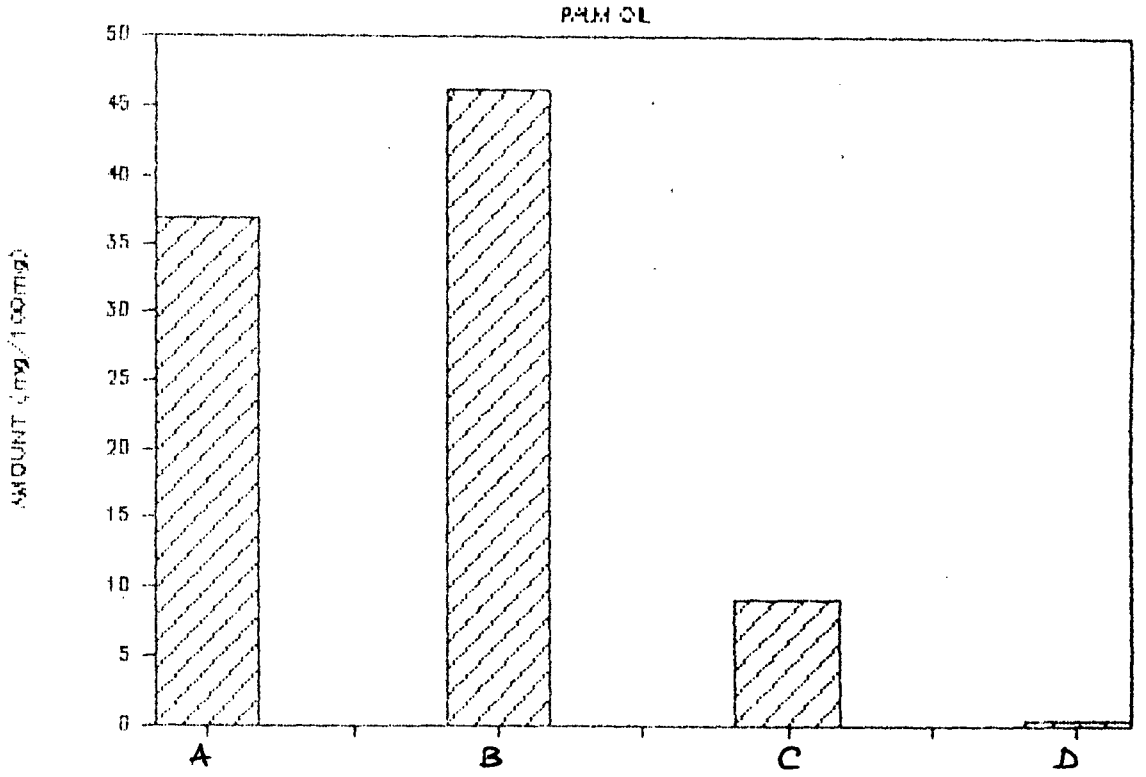
A : PALMITIC ACID

B : STEARIC ACID
+ OLEIC ACID

C : LINOLEIC ACID

D : LINOLENIC ACID

FIG.17.FATTY ACID COMPOSITION OF



A : PALMITIC ACID

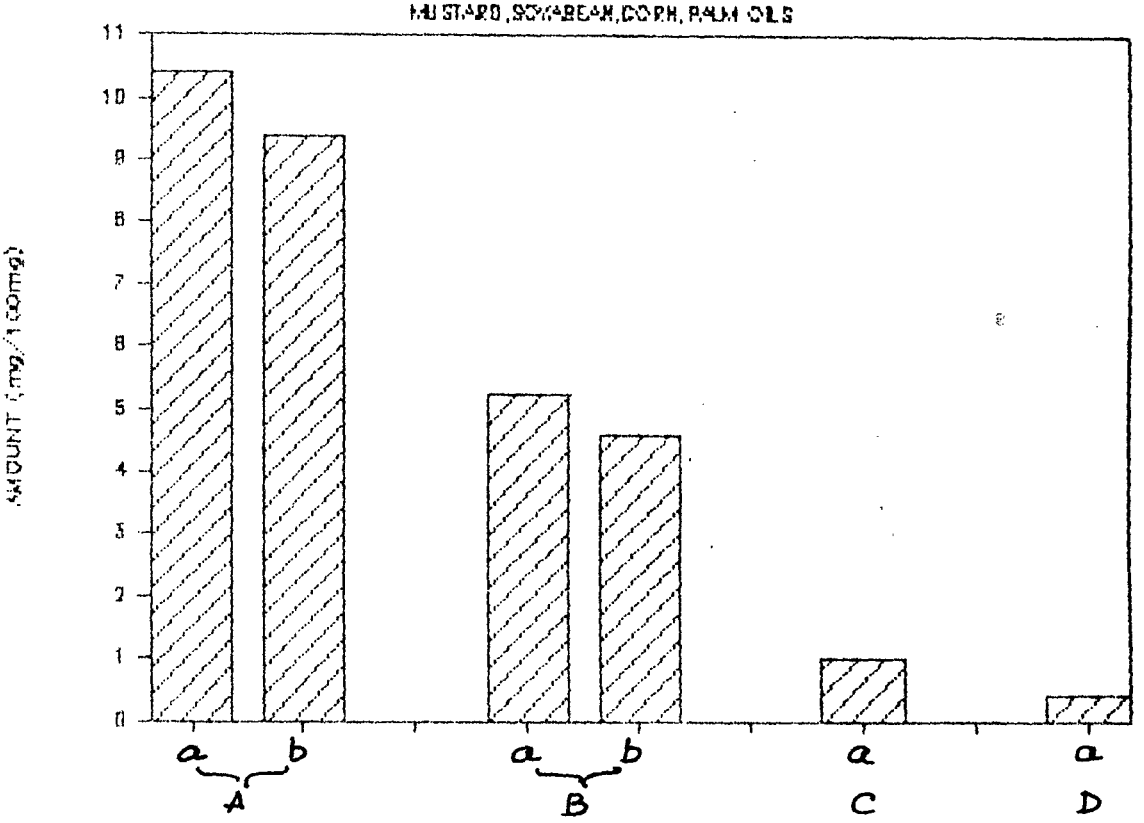
B : STEARIC ACID
+OLEIC ACID

C : LINOLEIC ACID

D : LINOLENIC ACID

PALMOLEIN

FIG.1B. LINOLENIC ACID COMPOSITION OF MUSTARD, SOYABEAN, CORN, PALM OILS



A : MUSTARD OIL a. KANODIA
b. JUMBO

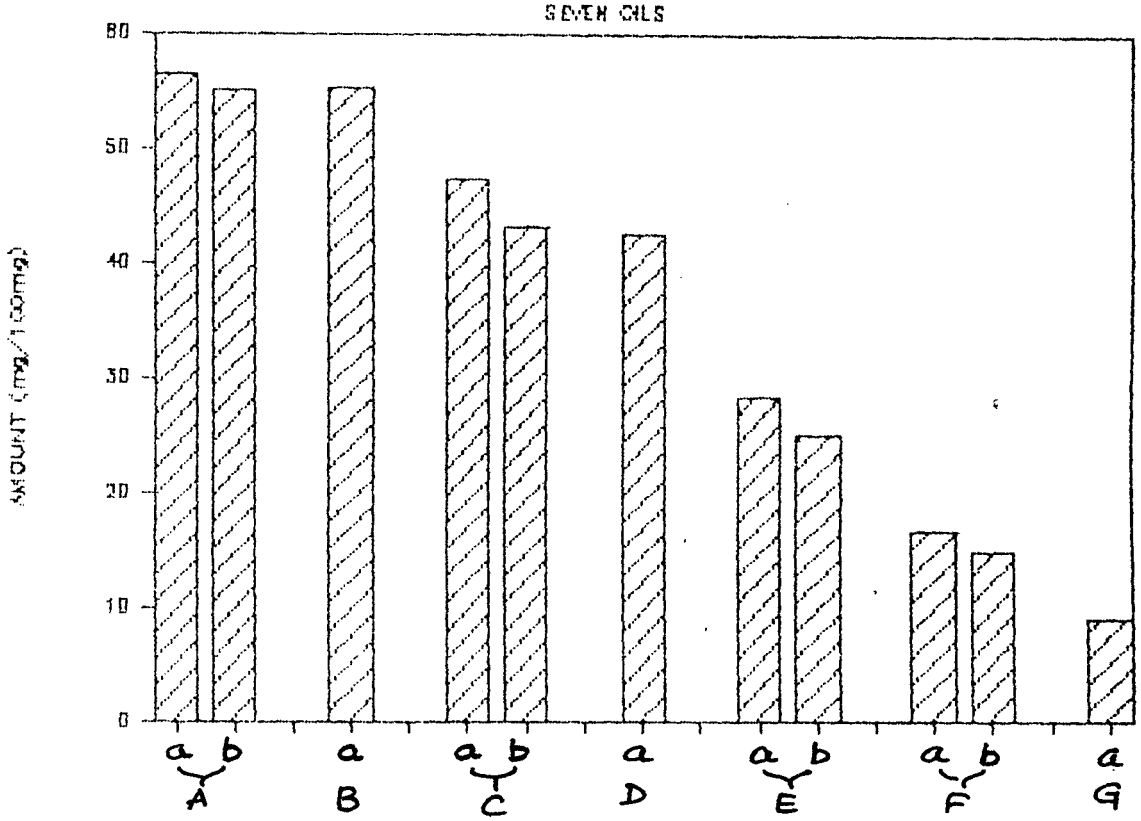
B : SOYABEAN OIL a. VITAL
b. SURYA

C : CORN OIL a. CORNOLA

D : PALM OIL a. PALMOLEIN

▨ – LINOLENIC ACID

FIG.19.LINOLEIC ACID COMPOSITION OF SEVEN OILS



A : SUNFLOWER OIL — a. SAFFOLA; b. FLORA

B : CORN OIL — a. CORNOLA

C : SOYABEAN OIL — a. VITAL; b. SURYA

D : TIL OIL — a. INDERDHANUSH

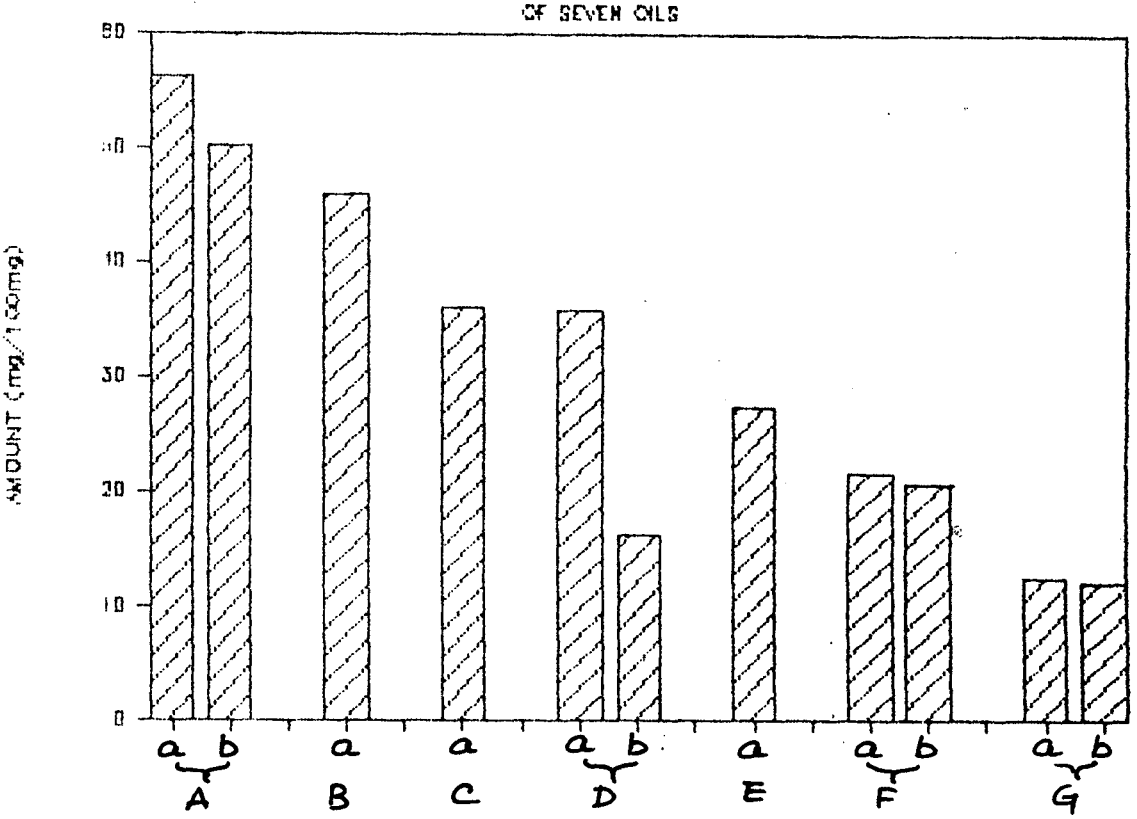
E : GROUNDNUT OIL — a. DALDA; b. POSTMAN

F : MUSTARD OIL — a. JUMBO; b. KANODIA

G : PALM OIL — a. PALMOLEIN

▨ - LINOLEIC ACID

FIG. 20. STEARIC+OLEIC ACID COMPOSITION OF SEVEN OILS



STEARIC +OLEIC ACIDS

A : GROUNDNUT OIL
- a. DALDA
- b. POSTMAN

B : PALM OIL
- a. PALMOLEIN

C : TIL OIL
- a. INDERDHANUSH

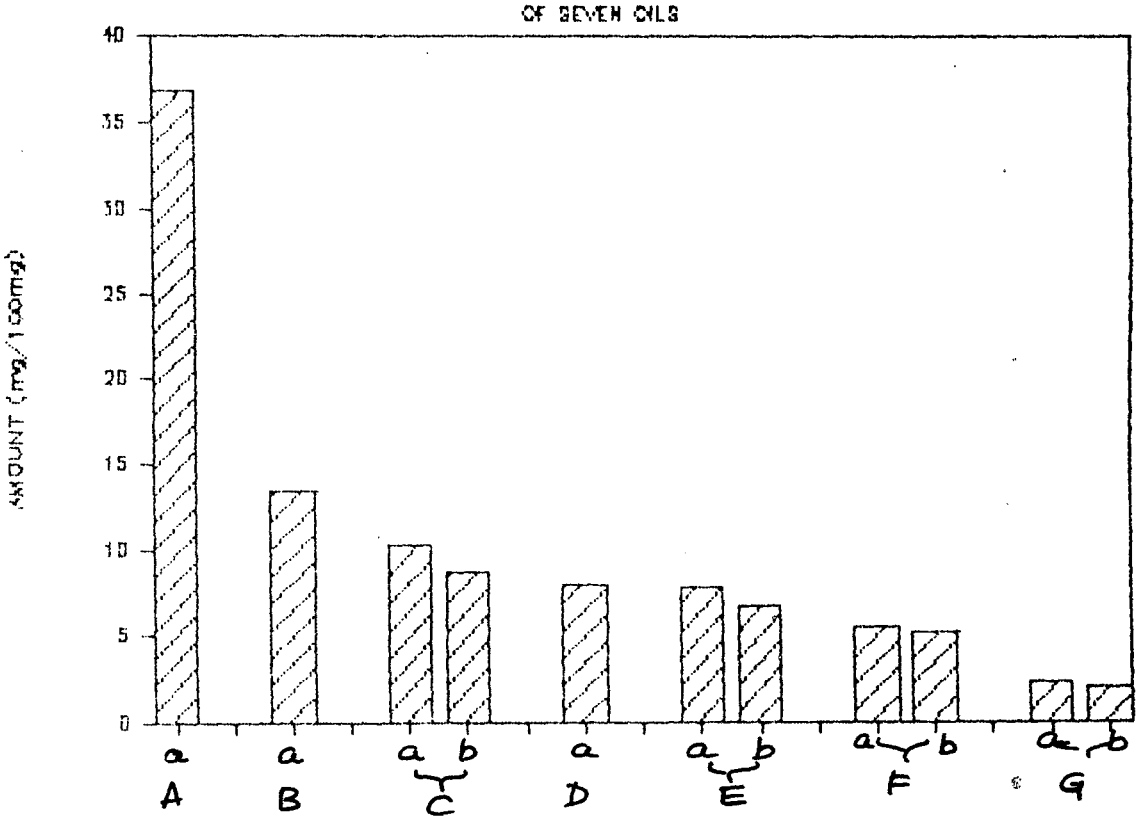
D : SUNFLOWER OIL
- a. FLORA
- b. SAFFOLA

E. CORN OIL
- a. CORNOLA

F. SOYABEAN OIL
- a. VITAL
- b. SURYA

G. MUSTARD OIL
- a. JUMBO
- b. KANODIA

FIG.21 .PALMITIC ACID COMPOSITION OF SEVEN OILS



A : PALM OIL a . PALMOLEIN

B : CORN OIL a . CORNOLA

C : GROUNDNUTOIL a . DALDA
b . POSTMAN

D : TIL OIL a . INDERDHANUSH

E : SOYABEAN OIL a . VITAL
b . SURYA

F : SUNFLOWER OIL a . SAFFOLA
b . FLORA

G : MUSTARD OIL a . JUMBO
b . KANODIA

– PALMTIC ACID

Qualitative study of cholesterol oxides in Ghee :

The qualitative study of cholesterol oxides was carried out by the following procedure of Jacobson, 1987 with some modifications.

In this study 4 varieties of ghee were taken for analysis.

1. Home made (conventionally prepared)
2. DMS ghee (Delhi Milk Scheme)
3. Vita ghee
4. Fresh butter obtained from Buffalo milk

The butter is extracted from milk and extracted by methanol chloroform water (Folch, 1957) then subjected to saponification. The remaining varities of ghee were directly subjected to saponification under nitrogen.

After extraction with diethyle ether subjected to Thin layer chromatography

Standard compounds :

This study was restricted to only two types of cholesterol oxides 1. 5α 6κ epoxide 2. cholestane triol Both standard and samples are spotted on TLC plate and

developed. The results were shown in photographs. T[redacted] value of standard compounds were given below.

	Rf value
5∝6∝ epoxy cholesterol	0.78
cholestane triol	0.10

Fig. 23 shows separation of cholesterol oxides.

A . Standard compounds

B . DMS Ghee

C . DMS Ghee

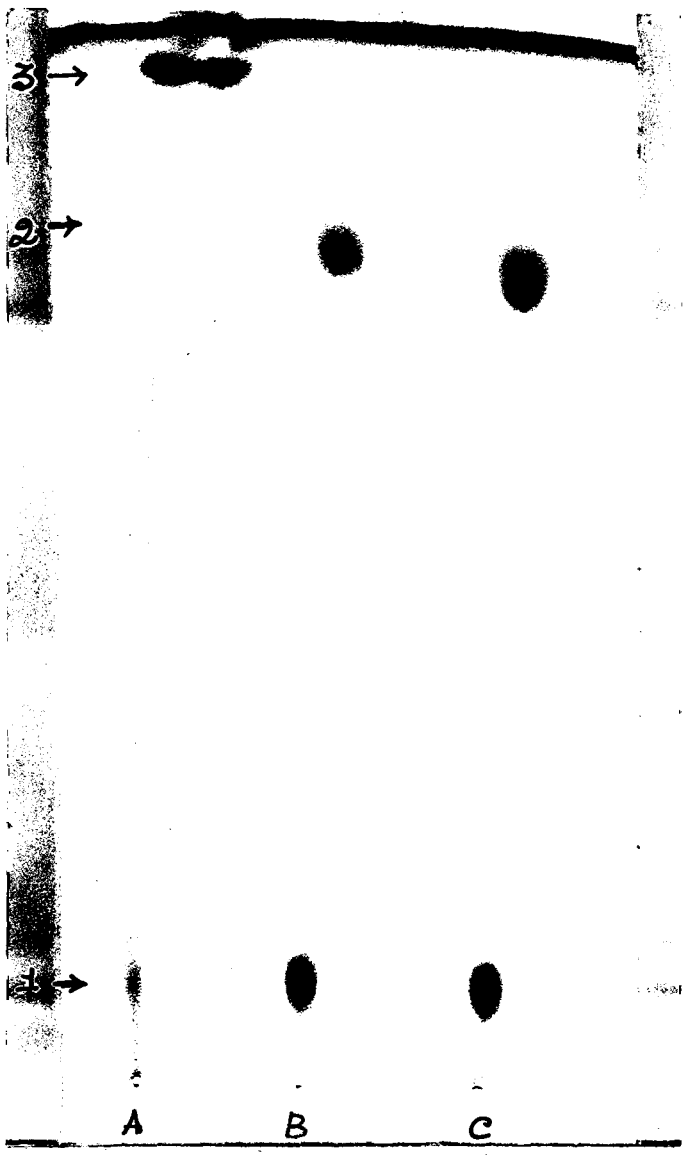
D . Vita Ghee

E . Fresh butter

In all three varieties DMS ghee, Vita ghee and fresh butter the absence of the two cholesterol oxides epoxide, cholestane triol is observed whereas these two oxides were detected in home made ghee and shown in fig. 22.

FIG. 22.

SEPARATION PATTERNS OF CHOLESTEROL OXIDES



A : SAMPLE (HOME MADE GHEE)

C & B : STANDARD CHOLESTEROL OXIDES

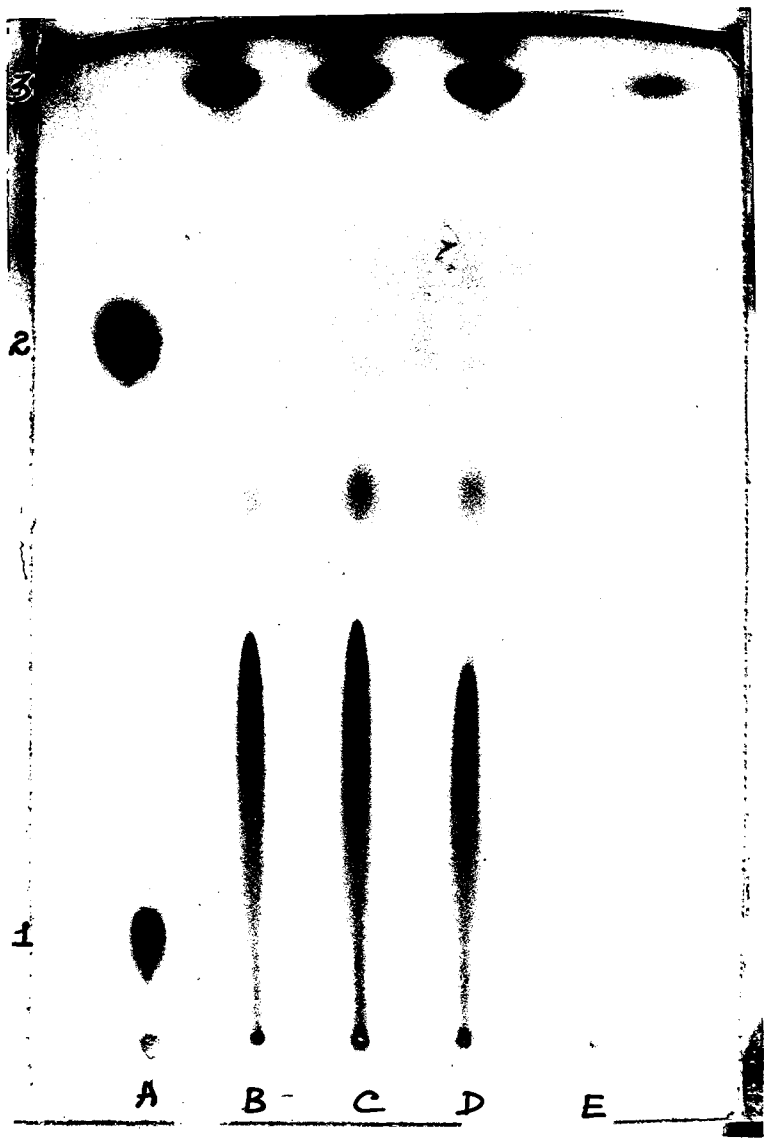
1. CHOLESTANE TRIOL

2. 5,6. EPOXY CHOLESTEROL

3. CHOLESTEROL

FIG. 23.

SEPARATION PATTERNS OF CHOLESTEROL OXIDES



A : STANDARD CHOLESTEROL OXIDES

B&C : SAMPLE (DELHI MILK SCHEME GHEE)

D : VITA GHEE (SAMPLE)

E : FRESH BUTTER

1. CHOLESTANE TRIOL
2. 5,6 EPOXY CHOLESTEROL
3. CHOLESTEROL

DISCUSSION

We have adapted the direct transesterification procedure described by Guy lepage 1984 who used this procedure for the quantitative evaluation of fatty acids from adipose tissue and human milk, for the evaluation fatty acids from oils. The results suggest that this procedure is suitable for the evaluation of fatty acids from the refined oils which bypasses all lengthy extraction and purification process which may leads the loss of fatty acids. As this procedure was carried out in the same test, tube the chances of loss is very less. Another advantage of this procedure is consumption of less time compared to the other procedures. Hence several samples can be easily monitored.

The general procedures which are usually followed for the analysis of fatty acids Ce 1-62 and Ce 2-66 methods of American oil chemical socity. The principle of the technique involves alkali catalysed methonolysis of the trigleceride and further mathylation with methanolic BF3. The validity of this procedure for the routine analysis of EPA and DHA in marine oils were carried out by Enig R.G and Ackman R.G. (1987). The unsaturated fatty acid can also be quantified by using procedures Urea fractionation (Iverson, 1964) and derivative preparations like methoxy mercuration

of unsaturated fatty acids (Harold B, et al 1966) and there are other procedure for making derivatives of fatty acids (Pears, et al, 1986; Christe 1982)

The presently used procedure also does not require the usage of antioxidants like BHT to prevent oxidation of highly unsaturated fatty acids like EPA and DHA, and linolenic acid and eliminates problems which are faced due to BHT peaks in GC. As this procedure is single step procedure, i.e., once methylated, the unsaturated fatty acid no longer subject to autoxidation (Wren, J.J.1964). This was confirmed by Guy lepage in 1984.

After extraction of fatty acids from sample and preparation of derivatives by the above said procedures, subjected to Gas Chromatography.

Usually for the separation of unsaturated fatty acid polar polyester liquid phases are much more suitable as they allow clear separations of ester of the same chain length, but with zero to six double bonds unsaturated components eluting after the related saturated ones. In the present study Diethylene glycol succinate (DEGS) column is used for the separation of fatty acids. There are other phases which can also be used for the seperation of fatty acids (Christie

1982). The column is packed with 10% Diethylene Glycol succinate (DEGS) supported on 100-200 mesh Diatomic C-AW, operated under temperature program (shown previously) as the resolution was better than in isothermal conditions The figure shows the peaks (which are traced by auto matic recorder), of individual standard fatty acids.

The figure suggests that stearic and oleic acid resolution is less than expected. The resolution of other fatty acids were inconsistent with the expected.

Similarly in the sample also the resolution of stearic acid and oleic acid were not upto the expectations. This may be due to the high amount of oleic acid which overlaps with former peak of stearic acid, or it may be due to the defect in operation or it may also be due to the age of the column.

The quantification of the fatty acids was done by internal standard method by taking Heptadecanoic acid (17:0) as internal standard as this fatty acid is not experienced in vegetable oils (Haken 1974).

As this study is restricted to some specific fatty acids area normalization method is not used for

quantification. The area normalization procedure requires study of all the individual fatty acids. So for this study internal standard method is chosen (Deans 1968)

Each oil is analyzed six times on GC. Peaks of fatty acids were identified by comparing the retention times with standard compounds. Then quantified by automatic integrator. The results obtained are reported in Results

From the results we can classify the oils on the basis of presence of n-3 fatty acids. In class -1 where the n-3 fatty acid are absent. Sunflower oil, Ground nut oil and Til oil comes under this class where as class II has n-3 fatty acids i.e., linolenhic acid is present, the rest of the n-3 fatty acid are EPA and DHA are absent. Soybean oil, Mustared oil , Corn oil, Palm oil comes under this class. In these 4 oils the amount of n-3 fatty acid i.e., linolenic acid was found to be more in mustared oil than other 3 oils, next comes soybean oil then cornoil, then palmoil as represented in figure 18.

In mustard oil the amount of n-3 fatty acids i.e., linolenic acid was found to be 10 mg/100 mg.in one and other brand it is 9.38 mg/100 mg.

In addition to the n-3 fatty acids, the n-6 fatty acid i.e.,linoleic acid, was also quantified. Results suggest that this fatty acid is present in all the oils which were studied. The amount of n-6 fatty acid was found to be maximum in sunflower oil and cornoil, 55.8mg/100 mg, 55.4mg/100 mg respectively. The decreasing order of the amount of n-6 fatty acid i.e., lenoleic acid is as follows. Sunflower oil >cornoil >soyabeanoil > Til oil > Ground nut oil> mustard oil > palmoil. With respect to the palmitic acid the results suggest that the palmoil has maximum amount compared to other types of oils. The decreasing order of the amount of palmitic acid is as follows :

Palmoil > Corn oil > Groundnut oil > Til oil > Soyabean oil> Sunflower oil > Mustard oil.

Similarly, with respect to stearic + oleic acid, the results suggest the maximum amount is observed in Groundnut oil. The decreasing order of the amount of stearic + oleic acid is as follows:

Groundnut oil > Palm oil > Til oil > Corn oil > Soyabean oil > Sun flower oil > Mustard oil.

The discrepancy between the fatty acid composition of different brands of same oil is not significant except in

case of sunfloweroil where the amount of stearic+oleic acid was found to be 35.7mg in brand flora, 16.2 mg in suffola. The discrepancy may be due to the different processing methods or it may be due to the artifacts.

The n-6/n-3 ratio of 4 oils is as follows :

Mustard oil	1.588
Soybean oil	9.23
Palm oil	22.32
Corn oil	54.40

These results shows that mustard oil has less ratio 1.58 where as cornoil has 54.4 maximum.

The amounts of individual fatty acids in these oils are in agreement with the reported values except few cases. The differences may either due to the species from which the oils are extracted or may be experimental artifacts.

Codex committee of Fats and oils, England conducted a meeting in which there are some agreements regarding the composition of vegetable oils that is mandatory, rather than advisory.

The following are the fatty acid ranges for some major oils (Mounts, T. 1987)

Soyabean oil:

Fatty acid	approved value	obtained value
18:0	3.0-5.5	21.55
18:1	18 -26	
18.2	50-57	47-57
18:3	5.5-10	5.23

Peanut oil (or) Ground nut oil :

18:3	< .3	0

Sunflower oil :

18:1	14-35	35
18:2	55-75	55
18:3	< .3	0

Corn oil :

16:0	9-14	13.45
18:1	24-42	27.41

If the sample composition is with in the mandatory fatty acid ranges then it is compliance with the standard the result obtained in the present study of the oils shown above are in agreement with the standard ranges.

From the past one year people are concentrated on n-3 fatty acids due to their health benefits. Fish oils were

shown to be primary source of the fatty acids. The emphasis made on EPA and DHA there was seldom mention of vegetable oil sources of the parent of the family. Due to some disadvantages fish oils, people are looking for alternative.

The ability of humans to convert alpha linolenic acid to EPA suggest that vegetable oils containing alfa linolenic acid may have biological effects similar to those of fish oils. While there is no published studies showing that the alfa linolenic acid intake from vegetable oil diets is sufficient to benefit health.

Cholesterol, which is present in the foods derived from animal such as ghee, butter etc. is converted to cholesterol oxides when they're subjected to high temperatures, free radical initiators, Light etc. These are of the current interest because of their toxic properties. There is also a speculation that a link may exist between ingested cholesterol oxidation products and coronary heart decease.

The cholesterol oxides are qualitatively studied by adapting the procedure of Jocobson M.S. (1987). As cholesterol is sensitive to oxygen the seponification is carried out under nitrogen. After extraction with diethyl ether, TLC was performed. The separations of cholesterol

oxide was better in case when ether solvent is used as mobile phase.

From the figure, the results suggest that the ghee obtained from commercial preparations, (no spots were identified corresponding to the standard compounds 5∝ 6∝ epoxy cholestane, Cholestane triol) does not have these epoxy cholestane, cholestane triol. This infers that the ghee which was prepared by commercial companies are not subjected to high temperature and antioxidants are added in order to prevent autoxidation. Although there is a spot of cholesterol oxide which was expected to be 7-ketone, by comparing with literature, which is intermediate stable products of cholesterol auto oxidation process. 5 ,6 epoxy cholesterol and triol are final stable products also called secondary products. The experimental study of 5∝, 6∝, epoxide and cholestane triol shows that triol was more active in its cytopathic effect on cells in cultures (Baranowski, 1982) then other cholesterol oxides. The epoxide is also shown to cause mutations in V 79 Chinese hamster cell culture.

In the figure.23, the fresh butter separation suggests that there are no corresponding spots of any cholesterol oxides infering that there is no auto oxidation.

When home made ghee which is prepared by heating at high temperature in copper vessel is spotted the separation figure suggests that both the Epoxide and triol are present as represented in figure 22. As these two oxides are secondary products formed when oxidation rate is more.

Summary and Conclusions:

The present study evaluation of n-3 fatty acids in oils and cholesterol oxides in ghee reveals that the oils which are subjected to GLC can be classified into two groups 1. Sunflower oil, Groundnut oil, Til oil, which does not have n-3 fatty acids. 2. Mustard oil, Soyabean oil, Corn oil, Palm oil which has n-3 fatty acids. The n-3 fatty acids are shown to be beneficial to the heart by reducing the platelet interaction with the arterial cell walls thus reducing the constriction of artery which leads to coronary heart disease (CHD) and also shown to be essential for the development of retina and brain (where these n-3 fatty acids especially EPA and DHA are more) in reshus monkeys. In addition there are several other roles which are very important in medical services. In the present study the **Mustard oil** show more n-3 fatty acids concentrations compared to other oils suggests that this is more beneficial to the heart.

Similarly, the cholesterol oxides which are formed due to heating of ghee at high temperature or exposure to air, light, oxygen, are shown to be likely one of the risk factors of coronary heart disease. In the present study of these oxides qualitatively by using thin layer chromatography suggests that home made ghee which was conventionally prepared by heating at high temperature, has cholestane triol, 5 6 epoxy cholesterol. This cholestane triol, 5 6 epoxy cholestrol are shown to be angiotoxic and cholestane triol is the most active substance in cytotoxicity compared to other oxides. Whereas in other brands of ghee which are commercially available, 5 6 epoxy cholesterol and cholestance triol compounds are not identified, suggesting that these ghees may not be subjected to that much high temperature during processing. Although these ghees has expected to have other types of oxides such as ketones. From this it can be suggested that consumption of Home made ghee in large amounts may be one of the risk factors lead to coronary heart disease through atherosclerotic mechanisms.

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