The Thesis contains following Chapters:-

CHAPTER-I : INTRODUCTION

Functional role of fat is manifold. Fat is most concentrated source of energy. The energy supplied by fat is almost double that of carbohydrates and proteins. Fat works as a vehicle to carry the fat soluble vitamins, nutrients and antioxidants in the body. Fat helps in raising High Density Lipoprotein (HDL) level (good cholesterol). Further, fat imparts textural qualities and taste to food. Fats and oils are lubricants of foods – through use as release agents, as part of cooking process and as lubricant during chewing (mastication). Fats and oils modify flavour release and improve palatability.

Fatty acids\textsuperscript{1,2} are long chain aliphatic carboxylic acids which are part of all lipid species. The fatty acids of plant, animal and microbial origin generally contain even number of C atoms in straight chains, with a carboxyl group at one extremity and with double bond in specific position in relation to this. In animal tissues, the common fatty acids vary in chain length from 14 to 22, but can span from 2 to 36 or even more. Individual groups of microorganisms can contain fatty acids with 80 to more carbon atoms, but higher plants usually exhibit a more limited chain length distribution. Fatty acids from animal tissues may have one to six double bonds, those from algae may have upto five, while those of higher plants may have more than three. Microbial fatty acids occasionally have more than one. Plants and microbial fatty
acids can contain a wide variety of functional groups. It is the non-polarity of acyl-chain, which makes liquid miscible in organic solvents and immiscible in aqueous systems. The non-polarity of the acyl-chain is attributed to the many C-H bonds which are non-polar due to minimal electronegativity differences and also to the many C-C bonds. In general the acids contain even number of monocarboxylic acids of chain length C₄-C₂₄. There are rare variations – longer or shorter chains, branched or cyclic chains or odd numbered chains. The substitutent group are incorporated into the aliphatic chains by enzyme systems. The delta (Δ) notation indicate position of double bond in chain counting from the carboxyl (-COOH) end whereas ω (omega) or η notations indicate position of the same from methyl end. Methyl esters of fatty acids which are volatile are used in gas-liquid chromatography for profile of the constituents fatty acids.

Triacylglycerols (TAG) are neutral type of lipids. The fat is stored in the form of TAG for energy and insulation. Different fatty acids esterify with glycerol giving a number of possible species.

The phosphoglycerides⁴⁻⁶ includes all derivatives of glycerophosphoric acid containing one or more acyl or related group. Phosphatidyl choline (lecithin), Phosphatidyl ethanolamine (cephalin), Phosphatidyl serine and Phosphatidyl inositol groups are important in plants and animals. The phospholipids constitute the cell membrane and are carriers of biologically active agents. Brain contains a protein phospholipids complex receptor⁷⁻⁹
Plants are able to synthesize the needed unsaturated fatty acids which are all incorporated in the phospholipids, which in turn are used for membrane building. The specific physical properties of the membrane is due to the chain length and degree of unsaturation of the fatty acids. In the animals, linoleic acid as precursor of arachidonic acid, can not be synthesized de novo by animals and must be taken up from vegetable sources. Such acids (ω3 or ω6) are termed as essential fatty acids (EFA) and serve as presursors of prostanoids. They have also an effect on hyperlipidemia. Aquatic animals also posses essential fatty acids. Several workers have reviewed the chemistry, biochemistry and biotechnology of lipids.\textsuperscript{11-17}

Oleochemicals are nonedible products – developed from the modification of fatty acids, glycerol and alkyl esters of fatty acids – by reactions like hydrolysis, esterification and oxidation. Oleochemicals have utility in various products – leather, cosmetics, pharmaceuticals, detergents, polymers and paints.\textsuperscript{18}

Oleochemicals are less toxic and easily biodegradable than Petrochemicals. Oleochemicals have promising future as value added products of oils and fats and for production of materials with environmental benefits.\textsuperscript{19, 20}

Shortage of traditional oil has created considerable interests in developing new sources of oils and fats evaluating their suitability for edible purposes.
Chemical investigations of many non-traditional seed oils of Madhya Pradesh e.g. *Feronia limonia,^{21,22} Brassica oleracea,^{23} Aegle marmelos,^{24} Conium maculatum,^{25} Balanites aegytiaca,^{26} Pithecellodium dulce,^{27} Buchanania lanzan,^{28,29} Annona squamosa,^{30} Shorea robusta,^{30} Tectona grandis,^{30} Basella rubra,^{31} Garcinia indica,^{32} Syzygium cuminii,^{33} Wrightia tinctoria^{34} and *Boswellia serrata^{35} have been carried out in our laboratory. Substantial work leading to Doctoral Thesis on "Fats" have been done in the laboratory during the last decade.^{36-41}

India has emerged as a major importer of edible oil. The Government hopes to increase the area under major oil seed crops to more than 30 million hectares in the near future.^{42}

Recently triglyceride types of seed oils have been chosen from some plants of Solanaceae^{43} and oil of *Psoralea* (Leguminosae) investigated.^{44} Alkyl resin have been studied from *Pithecellodium^{45*} and lipid peroxidation products of Soyabean oil determined.^{46} Preparation of oleochemicals for special application and making of structured lipids have been discussed in a recent seminar.^{19} Latest is report of triterpenoids from *Orchid-Eria,^{47*} from *Ixora finitarysoniana^{48*} and from *Echinops echinatus^{49*}. We have attempted aqueous exaction of Corn oil which is economic and ecofriendly.^{50} Microorganism mediated formation of hydroxy fatty acids from Corn oil^{51} and analysis of monoglycerides using different analytical techniques^{52} have recently been attempted in this laboratory.
SUMMARY

The latest trend of work includes lipid polyester content composition,\(^5^3\) role of bioactive molecules in liposome preparation,\(^5^4\) link between cognitive impairment and cardiovascular disorder,\(^5^5\) characterization of fatty acyl composition of complex lipids,\(^5^6\) role of biologically active oxidized lipid (phytoprostanes) in parental lipid nutrition,\(^5^7\) expression of acyl Co-A binding protein\(^5^8\) and alkaline sphingomyelinase and sphingolipid metabolites in human meconium.\(^5^9\)

CHAPTER II: METHODS

Extraction:

Various solvents or solvent combinations have been suggested for extracting lipids.\(^6^0\) Common methods include extraction with chloroform-methanol\(^6^1\) or hexane : isopropanol\(^6^2\) followed by addition of salt solution to result in partition between an aqueous and an organic phase. These may be regarded as general methods for the extraction of both neutral and polar lipids, but there are a variety of more specialized extraction methods. Steam distillation is used to produce the volatile essential oil, in which mono and sesquiterpenes dominate while continuous extraction with hot solvents is accomplished by Soxhlet extraction. A very simple extraction method is the short-duration dip in organic solvents for epicuticular lipids. In some cases separation of lipid classes can be accomplished by partition during an extraction procedure e.g separation of neutral and polar lipids from very polar lipids.\(^6^3\) Sometimes extraction can
involve a chemical treatment e.g. the dipolymerization of cutin and suberin (complex polyesters of hydroxy fatty acids) is required before the monomers are soluble in organic solvents. There are several points worth considering regarding extractions. These include problems of incomplete extraction, the release of lypoalytic enzymes, and oxidative compounds. An extraction method should be standardized for exhaustive extraction of the lipids and, where possible, with regarding to tissues. Losses in partition methods may include highly polar lipids which partition into the aqueous phase e.g. sphingolipids and lyso phospholipids require direct extraction with water saturated n-butanol. Homogenization of plant tissues releases lipolytic enzymes.

Phospholipase is active even in organic solvents. A short heat treatment is usually done to inactive the lipolytic enzymes prior to homogenization. Moreau has demonstrated the variability of phospholipase activity in a range of plant tissues. Another problem in extraction is that of chemical stability. Oxidation is a problem, with polysaturated fatty acids. Precautions to minimize oxidation include the use of peroxide free solvents, the addition of antioxidants such as ethoxyquin or butyalated hydroxy toluene, shielding from strong light in order to prevent photooxidation and photoisomerization and the use of nitrogen to evaporate solvents.

Exhaustive procedures for extraction of different plant tissues have been described by Zhukov et al.
SUMMARY

Chromatographic Methods:

A description of chromatographic methods has been given by Willard et al.\textsuperscript{73} and Poole and Schuette\textsuperscript{74}, Braithwaite and Smith\textsuperscript{75} and Weston and Brown.\textsuperscript{76} Application in the field of lipids has been comprehensively described by Kates,\textsuperscript{77} Christie\textsuperscript{78} and Mangold.\textsuperscript{79}

There are various solvent systems for the separation of lipid classes\textsuperscript{80} by TLC. Some solvent systems for plant lipids using silica gel TLC have been given by Wilson and Rinne.\textsuperscript{81} One dimensional TLC can also utilize multiple developments with several solvent systems.\textsuperscript{82} Two dimensional TLC increases the potential resolution of a complex lipid mixture.\textsuperscript{83} Determination of phospholipids on two dimensional thin layer chromatographic plates by imaging densitometry has been tried.\textsuperscript{84} A three way system has recently been proposed.\textsuperscript{85}

Weerheim\textsuperscript{86} has suggested a method for HPTLC. For analysis of isomeric phosphoinositides, 1.8% boric acid impregnation has been suggested.\textsuperscript{87} A review of phospholipid separation by TLC has been recently given.\textsuperscript{88, 89}

HPLC is used not only in the analysis of thermally labile, non-volatile ionic compounds but for all types of molecules from the smallest ions to macromolecules. Most important, HPLC has opened horizon in separations and has helped to make possible the development of biotechnology, where there is a great need for ultra pure products. Johnson and Stevenson\textsuperscript{90} described the basic principles.
Reviews of HPLC separations \(^{91-93}\) and advances in the field have recently been appeared.\(^{94}\) Double piston pumps are the latest in HPLC system.\(^{95}\) Recently, separation of liposomes, \(^{54}\) free sterols,\(^{96}\) assessment of lipid classes\(^{97}\) and measurement of labelled fatty acids \(^{98}\) have been carried.

Development in GLC has been reviewed by Ackman\(^{99}\) and Jamieson\(^{100}\) along with packed and capillary column\(^{101}\) and gas chromatography\(^{102}\).

Automated analysis of FAME\(^{103}\) and an effective method for the same shift in ECL on capillary column\(^{104}\) have been suggested. Application of different temperature and pressure programs, on a single capillary column has been proposed.\(^{105}\) Losses of PUFA (DHA) from high temperature of column during GLC of methyl esters of long-chain Omega-3 fatty acids has been reported.\(^{106}\) Analysis of isomeric diacylglycerol classes to evaluate the quality of olive oil in relation to storage, has been carried out.\(^{107}\)

**Spectroscopic Methods:**

A comprehensive text on the technique has been given by Waller\(^{108}\) and Waller and Derner\(^{109}\). The technique in essence involves the analysis of the mass/charge ratios of ions produced from the sample molecules. A detailed review on the recent development have been given by Burlingame et al.\(^{110}\)

Recent work in the field includes - mass spectroscopy of fatty acid derivatives,\(^{111}\) analysis of conjugated linoleic acid
derivatives,\textsuperscript{112} oxazoline derivative of highly unsaturated fatty acids with determination of double bond positions,\textsuperscript{113} elucidation of chlorine location in dichloro fatty acids with DMOX derivatization,\textsuperscript{114} characterization of fatty acids distribution,\textsuperscript{115} software tools for analysis of Mass Spectroscopy data,\textsuperscript{116} rapid characterization of fatty acyl composition of complex lipids\textsuperscript{117} and application of Capillary Electrophoresis (CE) – Mass Spectroscopy (MS) to characterization of bacterial lipopolysaccharides.\textsuperscript{118}

For lipids, the most commonly studied nuclei are $^1\text{H}\textsuperscript{119}$ and $^{13}\text{C}$-NMR\textsuperscript{120} is more useful in biological studies. The use of high resolution NMR in the study of the chemistry and biochemistry of lipids has been reviewed by Pollard.\textsuperscript{121} Studies on biological molecules particularly membrane have been carried.\textsuperscript{121-124} The texts of Silverstein\textsuperscript{125} Dyke\textsuperscript{126} and Gordon\textsuperscript{127} et al., contain general information, while Gunstone,\textsuperscript{128} has data more pertinent to acyl lipids.

**Degradative Methods:**

These constitute analysis of constituent moieties of complex lipids, degradation of acyl chain\textsuperscript{129} and stereospecific analysis.\textsuperscript{130-132}

Recently TLC on zinc ferrocyanide–silica gel G plates have been attempted.\textsuperscript{133} New technique in fatty oil refining, lipid processing and preparation of oleochemicals were discussed in a recent seminar.\textsuperscript{134} Applications of Chromarod-Iatroscan TLC-FID in lipid analysis have recently been presented.\textsuperscript{135} Latest is the parametric study of polysulphone membranes for their separation
SUMMARY

performance. Separation of alkenyl glycerol ether lipid content have been attempted and a method for analysis of phospholipid and its products have recently been proposed. Recently from this laboratory, Lariya has completed studies on lipids from some nonconventional sources, Soni has presented worked on linoleic acid isomers, Thakur on microorganism mediated formation of hydroxy fatty acid and Vaidya on analytical technique for analysis of monoglycerides. Latest work is on quantification of diacylglycerols and acyl lipid composition.

CHAPTER-III: STUDIES ON LOCAL SOYABEAN OIL

Soyabean (Glycine max) - also known as Soybean (U.S.) belongs to Leguminosae. The cotyledons are the parts of the seed that emerge from soil as the seedling develops. The valued portion of the plant is seed which is rich in protein and fatty oil.

The oil is associated with reduced cardiac risk. The oil also contains antioxidants. Although lot of work has been done on the plant, some of the unexplored aspects have been taken in the present study as follows:-

(A) Determination of Phospholipid Content from Oil:

Phospholipids present in oil precipitate during storage. Their removal enhances oxidative stability. The residual phosphorous determination in processed oil has been carried by FTIR. Phospholipid determination by TLC/FID has earlier been done in our laboratory. The present method uses less solvents and is faster.
(B) Adsorption of Heated Oil Components on Magnesium Silicate:

In earlier studies from this laboratory\textsuperscript{148} it has been demonstrated that on high temperature heating, the chemical and nutritional utility of oils get deteriorated. In the present study, the frying oil has been passed through the adsorbent – magnesium silicate, so that many of the constituents – harmful to health are retained by it. FTIR has been used to observe the retention behaviour.

(C) Determination of Lipid Peroxidation Products from Heated Oil:

On high temperature heating, peroxidation\textsuperscript{149} products are formed. Attempt has been made to identify the non-polar and polar peroxidation\textsuperscript{150} products using a single (HPLC) method. Hexenal, Hexanal, Heptaenal, Octanal, Nonenal and Decanal in nonpolar fraction and Hydroxyhexenal, Hydroxyoctenal and Hydroxynonenal have been identified in polar fraction.

CHAPTER-IV: STUDIES ON ACACIA ARABICA SEED OIL

\textit{Acacia arabica} belongs to family – Leguminosae.\textsuperscript{151} Natural oils contain complex mixtures of triacylglycerols and a complete determination of triglyceride profile would require combination of procedures. However, using Liquid Chromatography, improved resolution in shorter time has been achieved, in the present attempt. Different columns with mobile phase selectivities have been studied by workers.\textsuperscript{152,153}

CHAPTER-V: STUDIES ON LOCAL CORN OIL

Corn (\textit{Zea mays}) belongs to family – Gramineae.\textsuperscript{154} Corn is a well known plant and its oil is in use. Locally grown corn is used
for edible purposes in various forms – baked, roasted and as oil. The work on local kernels (seeds) have been divided into :

(A) **Aqueous Extraction of Oil**:

Enzyme assisted aqueous extraction with good yield of oil has been attempted as an economic and ecofriendly alternative to solvent extraction.

(B) **Microorganism Mediated Formation of Hydroxy Fatty Acids from Oil**:

Corn oil yielded three hydroxy fatty acids (11 hydroxy 9 ene, 10 hydroxy 8 ene and 9 hydroxy 10 ene) different from known source – ricinoleic acid, in presence of microorganism *Pseudomonas*, possibly by trasformation of oleic acid.

(C) **Oleochemical-Diester Formation from Isolated Hydroxy Fatty Acid and its Identification**:

Estolides have high viscosity indices and find use in Pharmaceutical and Polymeric preparations. Estolide formation with hydroxy fatty acid and unsaturated (oleic) acid present in the oil, has been carried out in presence of clay and the product characterised by HPLC.

**CHAPTER VI: STUDIES ON OLEOCHEMICAL-SUCROCHEMICAL**

(A) **Preparation of Sucrose Fatty Acid Ester (Sucrochemical) using Sucrose and Annona squamosa Seed Oil and its Characterisation**:

The synthesis of sucrose fatty acid esters which can be used as food emulsifier, results in complex mixtures. Difficulty arises in choosing a solvent – which should ideally dissolve the sugar as
well as the methyl ester of fatty acid. A solvent (methanol) has been chosen which is less toxic than the solvents in use. A detection method – other than GC – suitable for nonvolatile compounds has been tried. As natural source of desired fatty acids – *Annona squamosa* seed oil has been taken.\textsuperscript{156,157} The product has been identified by TLC and HPLC.

**(B) Crystal Pattern Studies:**

Fatty acid methyl ester crystal patterns as such and in presence of sucrose fatty acid ester has been studied using DSC and XRD.