

SYNTHESIS AND CHARACTERIZATION OF EGG OIL

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Abstract:

Egg oil is synthesised by heat treatment process. The egg oil is manufactured systematically and various studies are carried out to identify the components present in it. The egg oil consist of triglycerides of fattyacids and minute concentration of vitamins and minerals. The major constituents present in the egg oil is linoleic acid, oleic acid and palmitic acid. The characterisation studies used for the structural elucidation of egg oil are FTIR, NMR,UV,GC-MS and LC-MS analysis. FTIR spectral studies provide information about the functional groups such as acid, esters and alcohol etc. The NMR studies proves the presence of unsaturation and type of protons and carbon present in it. The physico-chemical parameters like viscosity, iodine value, hydroxyl value, acid value, saponification values are determined by standard methods. The GC-MS analysis gives out brief information regarding the fragmentation pattern. LC-MS stadies showed the molecular weight of the egg oil .

Keywords: Triglycerides, LC-MS, heat treatment, GC-MS, physico- chemical parameters.

INTRODUCTION

Egg oil is also known as egg yolk oil (or) ovum oil , is derived from the yolk of chicken eggs consist of mainly triglycerides withtraces of lecithin, cholesterol , xanthophylls such as lutein and zeaxanthin and immunoglobulins. The Word ‘lecithin’ is derived from the greek word lekithos meaning egg yolk. As the name implies lecithin is extracted from whole egg yolks and contains nutrients as Vitamin A, B₁, B₂ , E and D minerals including calcium, iron and phosphorous.¹

The first reported work on essential oils are those of Houton Labillardiere in 1818 who did an elementary analysis of turpentine oil and found a ratio of five carbon to eight hydrogen atoms. More than ten years to pass before any important contribution appears the literature again in 1833². A progress in essential oil research is made when chemists began to allow oils to react with hydrochloric acid . Some of them crystallised and could be further analysed by different techniques. The disadvantage of this method is that very often mixtures of three to four products are considered as pure substances.³

An efficient simplified method used for extracting the maximum amount of oil from egg yolks are done by thermal treatment. The primary product thus obtained is unadulterated, stabilized and medically pure egg oil⁴. Egg oil is manufactured by solvent extraction from egg yolk solid with a fat solvent. The mixture is heated with constant agitation to a temperature not in excess of 160⁰ C. The egg fat is recovered from solvent by distillation process. The extracted egg oil is a by-product and it is suitable for many applications in feeds and industrial uses⁵. Egg oil is produced from other process, in which cholesterol is removed from egg yolk. Wet egg yolks are processed rather than dried egg yolks. A polyunsaturated oil such as safflower oil is employed in conjunction with high energy mixer to extract the cholesterol from the yolk into the processing oil⁶.

Various portions of the avian egg have found use in pharmaceutical and cosmetic preparations. Ointment is synthesised by using egg oil from a dehydrating azeotropic distillation process. The egg oil used to treat wound and heals and it is to be compounded with pharmaceutically acceptable solvents, fillers, antibacterial agents, thickeners, coloring agents (or) other conventional ingredients. Preferably the egg oil preparation is applied at least once a day, more preferably about five times per day⁷.

Egg oil is used as a traditional Chinese medicine for various ailments. Ecovate's conditioning and as a carrier oil for essential oils. Since egg oil is known to be a good oil and absorbs easily into the skin. It can also be used with a variety of essential oils to boost its health and cosmetic benefits⁸. It improves brain and nerve tissue to be a factor in retarding premature aging and senility, body rejuvenation. Lecithin smooths and tones the skin and improves internal functions. It may also be useful in treating burns on the skin. Improves blood circulation contains such essential substances as linoleic, oleic and linolenic acids which are reportedly factors in improvement (or) prevention of such conditions as arteriosclerosis and hypertension.⁹

Result and Discussion :

¹H NMR Structural Analysis:

The ¹H NMR Spectra was recorded on an ADVANCE 400 NMR Spectrometer (Bruker.Co.Germany) operated at 400 MHz using tetramethylsilane as an internal standard and CDCl₃ as the solvent. In this present study, the egg oil was subjected to investigate the structural assignment and purity. The following (figure.1) shows the proton NMR spectra of egg oil in deuterated chloroform at room temperature. The spectrum shows a singlet at 4.80 ppm associated with N-H proton. The δ value for alkyl group (C-H) proton was found to be 1 to 0.96 ppm. The protons feel smaller field strength (i.e) due to shielding effect.¹⁰

The peak at 2.69 ppm corresponds to methylene protons. The peak at 1.73 ppm corresponds to -CH₂ - protons. The peak corresponds to 2.69 ppm corresponds to aromatic C-H proton. The δ value corresponds to 6.83 ppm corresponds to proton in aromatic alcoholic group. The peak at 8.39 ppm corresponds to N-H proton in amide group. The δ value corresponds to 7.25 ppm corresponds to proton in aromatic ring. The δ value corresponds to 10.74 ppm showed the presence of acid group.

In the NMR Spectrum of egg oil, the peak at 1 ppm corresponds to the terminal methyl protons. The peak at 1.73 ppm corresponds to C-H protons then the peak at 2.7-2.8 ppm for protons in CH₂-O group.¹¹

The conclusion made for the structural elucidation of egg oil based on H¹ NMR spectrum is shown in the table.1

Type of protons and groups present in the Egg oil is

Sl.No	Chemical shift (ppm)	Type of proton
1.	$\delta = 4.8$ ppm	-N-H- (amine group)
2.	$\delta = 1$ ppm	-C-H- (Methyl group)
3.	$\delta = 1.73$ ppm	-CH ₂ -R (Secondary methyl)
4.	$\delta = 2.69$ ppm	Ar-H (Aromatic group)
5.	$\delta = 6.83$ ppm	R-O-H (Alcoholic group)
6.	$\delta = 8.39$ ppm	-N-H- (amide group)
7.	$\delta = 7.25$ ppm	Ar-C-H-
8.	$\delta = 0.96$ ppm	-C-H (linolenyl chain)

Table : 1 . H¹ NMR Spectral data

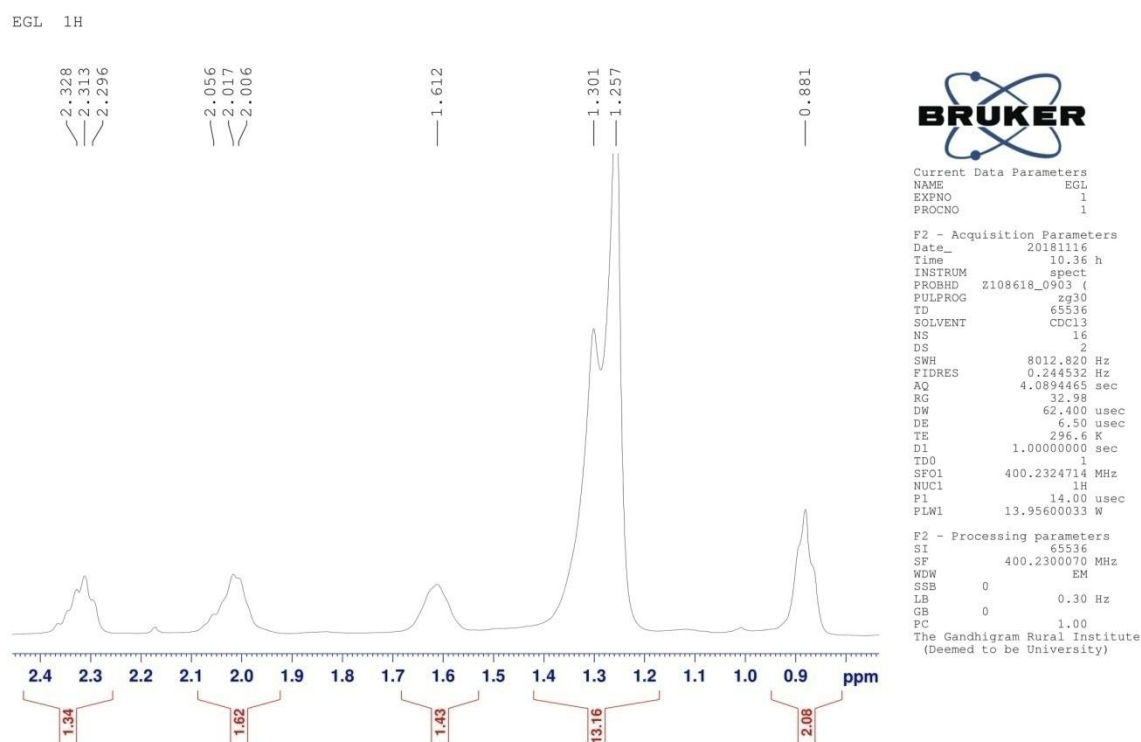


Figure. 1. H¹ NMR Spectrum of Egg Oil

FTIR Spectral Studies:

IR Spectroscopy allows us to corroborate the chemical nature of the products and also to demonstrate that the reaction between the substrates. The carbonyl group appears in the region of 1750- 1735 cm⁻¹ . The

two signals at 1093 cm^{-1} and 1161 cm^{-1} indicate the presence of secondary and tertiary alcohols. The quite intense band in 1171 cm^{-1} represent secondary alcohols.

The IR spectra of egg oil is recorded between 500 cm^{-1} to 4500 cm^{-1} . A strong absorbance band in 2922.15 cm^{-1} is due to the presence of carbonyl group of the glycerol ester moiety. A strong band in 2852.91 cm^{-1} is due to the symmetrical (C-H) bond in $-\text{CH}_2-$ group of the side chain. The peak at 1743 cm^{-1} is due to the presence of carbonyl stretching in esters ¹².

The IR Spectroscopy is not possible to differentiate between the type of alcohols. The peak at 1416 cm^{-1} corresponds to N-H band ¹³.

Serial number	Stretching Frequency	Functional group
1.	3008.03 cm^{-1}	Aromatic C-H stretching vibration.
2.	2922.15 cm^{-1}	C-H stretching in methyl group
3.	2852.51 cm^{-1}	C-H stretching in aldehyde
4.	1463.65 cm^{-1}	C-H vibration in benzene
5.	1377.48 cm^{-1}	CH_2 in olefins
6.	1228 cm^{-1}	C=O stretching in aldehydes.
7.	$1161.23- 1117\text{ cm}^{-1}$	C=O stretching in esters.
8.	1093 cm^{-1}	C=O stretching in anhydride
9.	965.32 cm^{-1}	C-H stretching in trans olefin.

Table. 2. FTIR Spectral Data

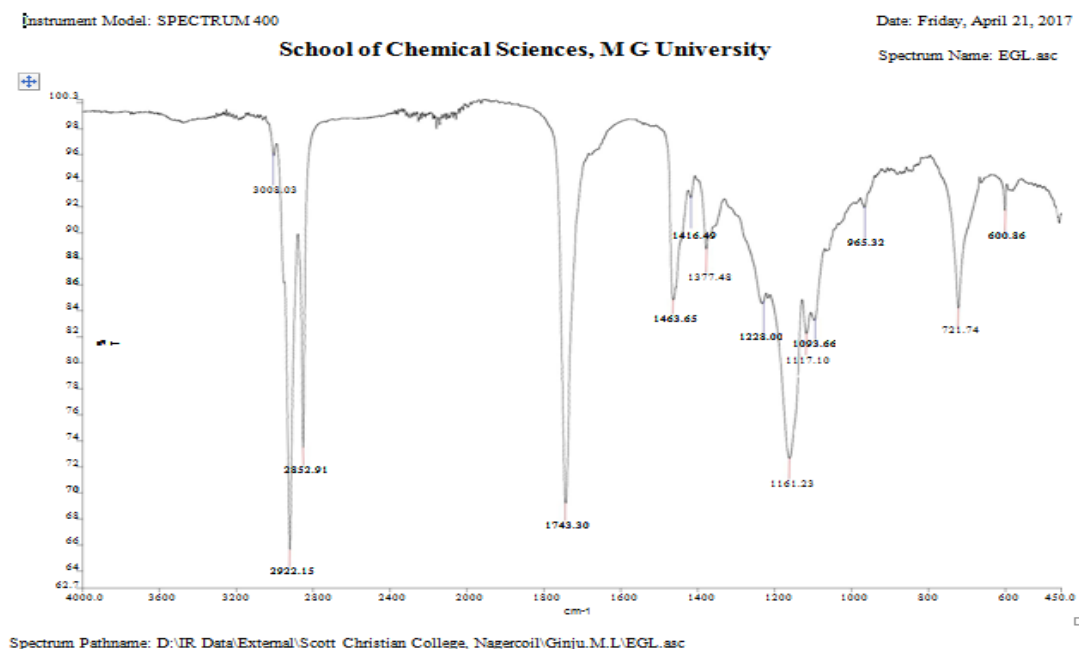


Figure. 2. FTIR Spectrum of Egg oil.

Physico-chemical properties:

The physico-chemical properties of the synthesised egg oil is shown in the following table. (Table.3)

Serial Number	Properties	Egg oil
1.	Colour	Dark brown
2.	Specific gravity	0.976
3.	Intrinsic viscosity	42
4.	Iodine value	145
5.	Molecular weight	290
6.	Acid value	34.02
7.	Saponification value	182

Characterisation of Egg Oil by Mass Spectroscopy:

Gas Chromatograph mass spectrometry (GC-MS) has been used in the separation, identification and quantification of complex mixtures such as essential oils. As a general rule , the identification of these compounds is not precise because the mass spectra of these compounds are very similar and determination with standard MS library is very difficult. For this reason the retention index –IR is used as a parameter for the GC qualitative analysis of the complex mixtures of isomers. ¹⁴

The GC-MS composed of two building blocks such as the gas chromatograph and the mass spectrometer. The gas chromatograph utilises a capillary column which depends on the Column's dimensions (length, diameter, film thickness) as well as the phase properties. ¹⁵

The Gas chromatography rapidly proved to be an efficient method for the analysis of essential oils. ¹⁶

UV Visible Spectroscopy:

Ultraviolet absorption spectroscopy deals with the measurement of energy absorbed when electrons promoted to higher energy levels. The molar absorptivity at 226 nm showed the presence of $n \rightarrow \sigma^*$ transition (trimethyl amine). The λ_{max} at 202 nm indicate the presence of aromatic compound. The conjugation of benzene ring shift the primary band at 203.5nm. The wavelength at 235 nm showed the presence of $\pi \rightarrow \pi^*$ transition. The wavelength at 218nm showed the presence of acyclic dienes. The wavelength at 229 nm indicated the presence of cyclopentadiene. The λ_{max} ranges from 348 – 350 nm indicate the ultraviolet absorption spectra of nitrogen containing aromatic heterocyclic compounds like pyridine and benzene. ¹⁷

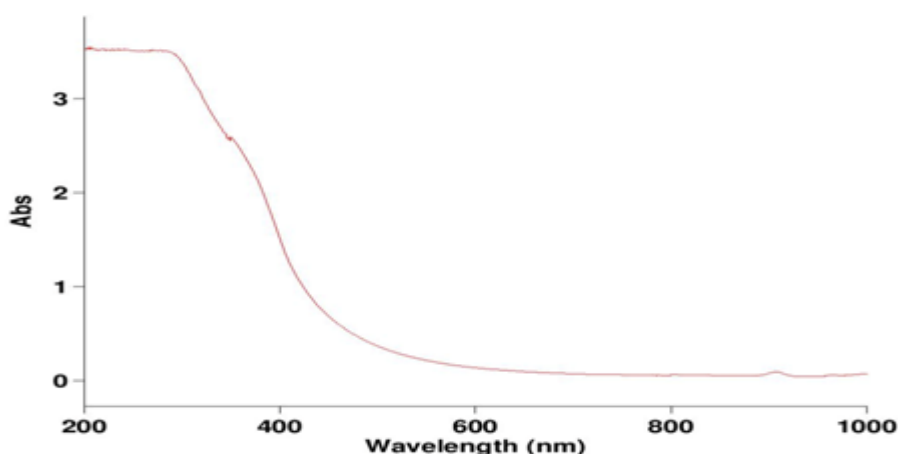


Figure – 3. UV Visible spectrum of Egg oil.

Serial Number	λ_{max}	Type of Electronic Transitions	Functional group
1	202 nm	$n \rightarrow \sigma^*$	Aromatic compound
2	205 nm, 229nm	$n \rightarrow \sigma^*$	Alcoholic group
3	207nm	$n \rightarrow \sigma^*$	Alkyl group
4	218nm	$\pi \rightarrow \pi^*$	Ether
5	226nm	$n \rightarrow \pi^*$	Cycloalkane
6	235nm, 269nm	$\pi \rightarrow \pi^*$	Benzene ring

7	263nm	$\pi \rightarrow \pi^*$	Chlorine based compound
8	908 nm	$\pi \rightarrow \pi^*$	Unsaturated compound

Table -4. UV Visible Spectral Data

C¹³ NMR Spectral studies:

C¹³ NMR Spectroscopy was complement with the H¹ NMR spectroscopy. From the C¹³ NMR spectrum of egg oil the chemical shift at 173 ppm corresponds to triglyceride esters, amides and carboxylic acid. So the chemical shift occurs due to the presence of these groups in egg oil.

The chemical shift value from 127-130 ppm correspond to unsaturated nitriles. The chemical shift at 77 ppm correspond to unsaturated alkynes. The chemical shift at 65 to 68 ppm correspond to alkyl halide or amine. The chemical shift at 34 ppm was due to the saturated alkyl group. The chemical shift at 22 ppm correspond to secondary alkyl group.

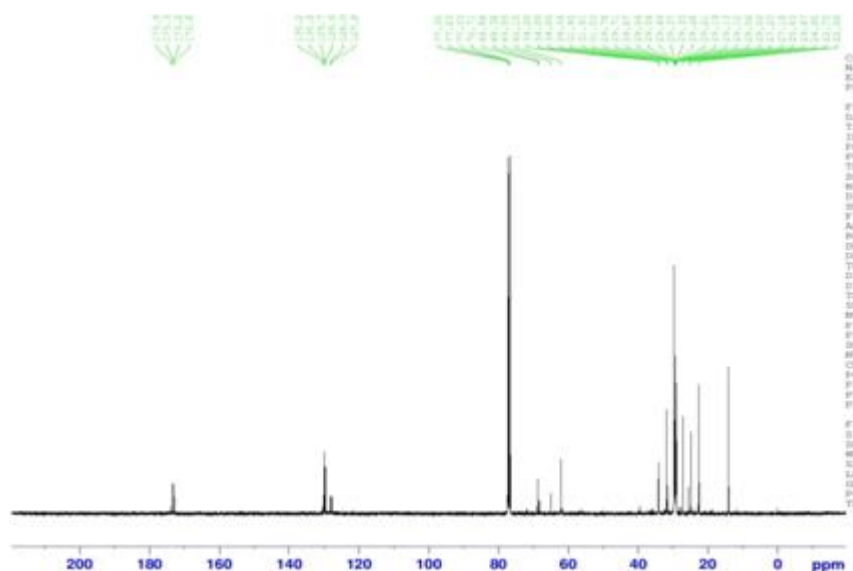


Figure. 4. C¹³ NMR Spectrum of Egg oil.

Serial number	Frequency region	Functional group
1.	173 ppm	Esters, Amide, carboxylic acid.
2.	127- 130 ppm	Nitriles.
3.	77 ppm	Alkyne
4.	65- 68 ppm	Halide, Amine.
5.	22ppm, 34 ppm	Alkyl group.

Table. 5. C¹³ NMR Spectral datas.

Characterisation of Egg Oil by Mass Spectroscopy:

Gas Chromatograph mass spectrometry (GC-MS) has been used in the separation, identification and quantification of complex mixtures such as essential oils. As a general rule , the identification of these compounds is not precise because the mass spectra of these compounds are very similar and determination with standard MS library is very difficult. For this reason the retention index –IR was used as a parameter for the GC qualitative analysis of the complex mixtures of isomers. [14]

The GC-MS composed of two building blocks such as the gas chromatograph and the mass spectrometer. The gas chromatograph utilises a capillary column which depends on the Column's dimensions (length, diameter, film thickness) as well as the phase properties. [15].The Gas chromatography rapidly proved to be an efficient method for the analysis of essential oils.[16] [17]

Mass Spectrometry in Structure Elucidation:

The electron impact (EI) mass spectra of many components and derivatives of known structures are available in mass spectral libraries. The first step in examining a mass spectrum is usually the determination of molecular ion peak. It helps to identify the major fragment ions to elucidate the main fragmentation pathways.

The peak observed at 264 shows a similarity towards the molecular weight of the egg oil. Based on this study , the presence of triglycerides confirmed. The m/z value at 55 showed the presence of oleic acid. (C₁₈H₃₄O₂)

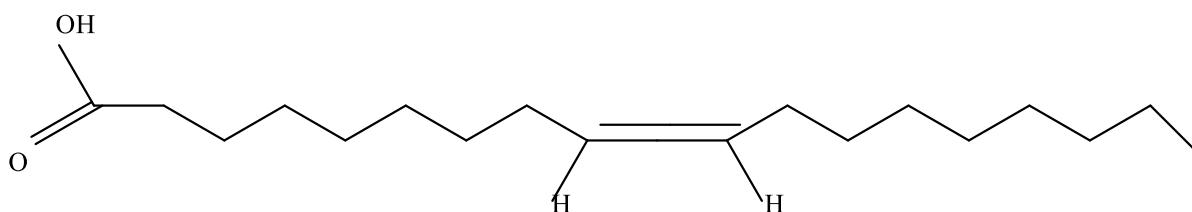


Figure.5. Structure of Oleic acid

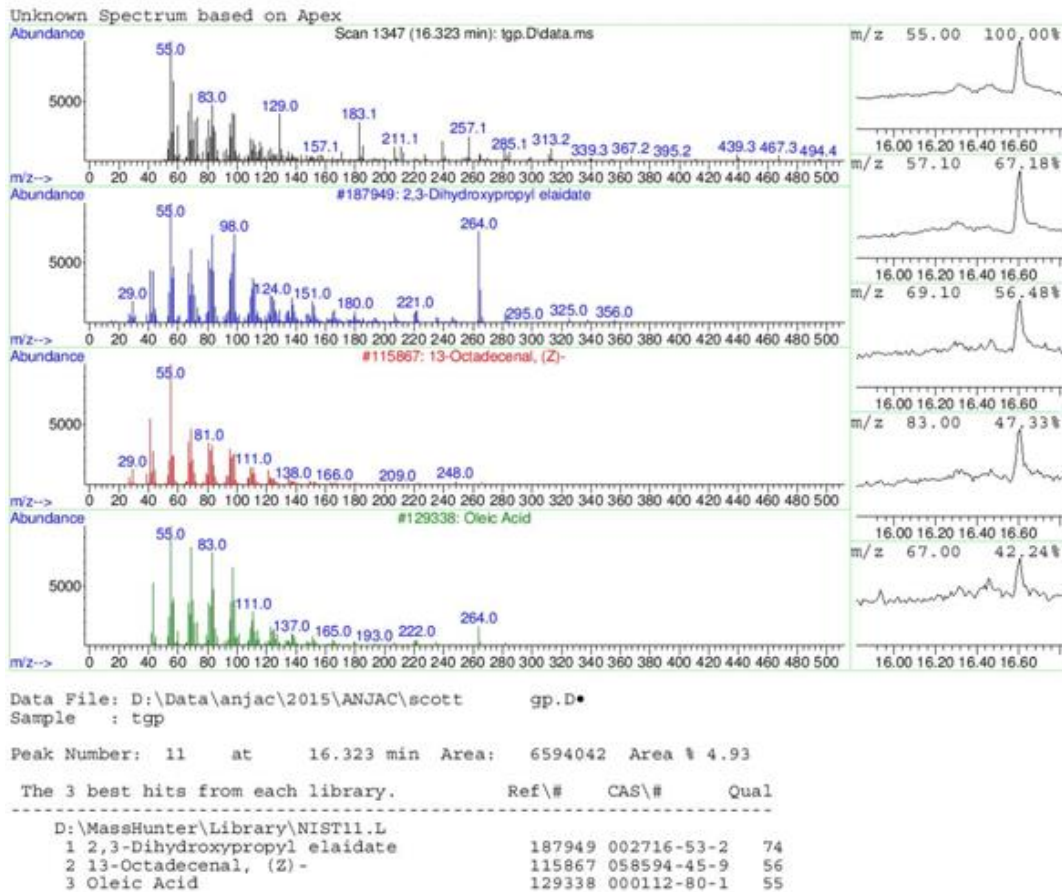


Figure.6. GC-MS Spectrum of Egg Oil.

LC-MS Analysis of Egg oil:

The direct combination of high performance liquid Chromatography with mass spectrometry provides information about the fragmentation pattern. The mass spectrometer act as a major detector for high performance liquid chromatography. LC-MS method to identify the components present in the egg yolk oil. LC-MS technique helped to characterise the multicomponents in the oil. Animal fats and vegetable oils contain triglycerides but are degraded by enzymes into mono and diglycerides and free fatty acids.

Aminoacids are analysed using LC-MS coupled with laser desorption and thermospray. Ultrapure additives and solvents such as acetic acid, acetonitrile, ammoniumacetate and ammonium bicarbonate are used in LC-MS systems. Separation of polycyclic aromatic hydrocarbons and organometallic compound is possible using the technique.

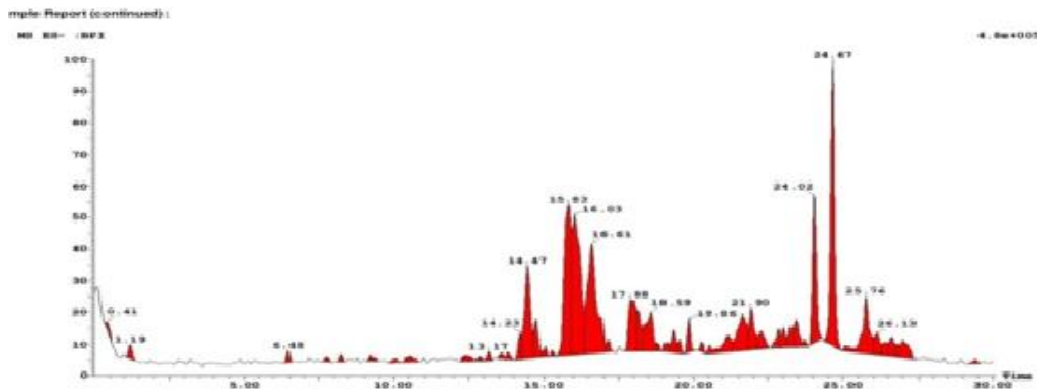


Figure.7. LC-MS Analysis of Egg Oil

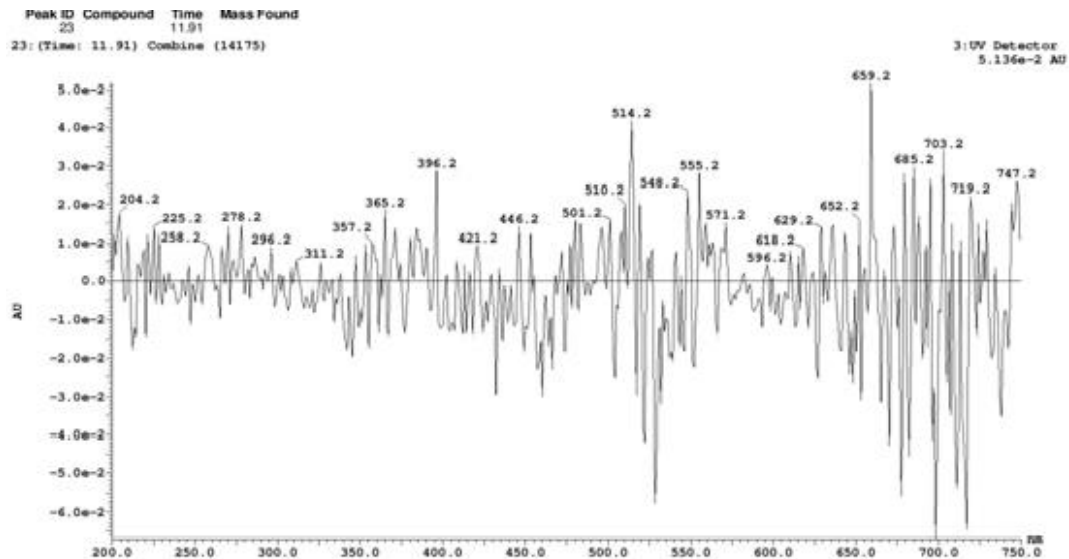


Figure.8. LC-MS Fragmentation Pattern

Edible Vegetable oils constitute an important class of food products. The determination of the triglyceride (TGA) Composition in edible oils provide information of fatty acid composition. Triglycerides helped to analyse components by using HPLC with UV detector. The fatty acid composition of triglycerides are analysed with the help of LC-MS Method. In this present study, LC coupled with MS detection is used for the analysis. This analysis used to realize the identification of triglyceride is vegetable oil ¹⁸ .

The ion fragments m/z 575.5 and m/z 599.5 correspond to linoleic acid and palmitic acid. Mostly LC-MS methods used atmospheric pressure chemical ionization for triglycerides. Triglycerides can be chromatographed by HPLC but their lack of chromophore makes the detection difficult ¹⁹ .

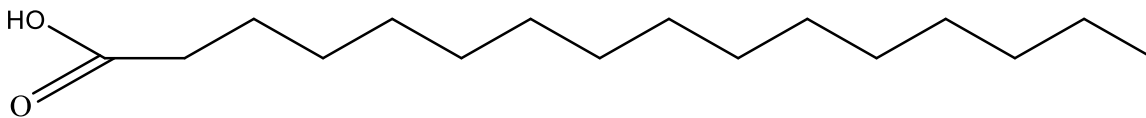


Figure.9. Structure of palmitic acid.

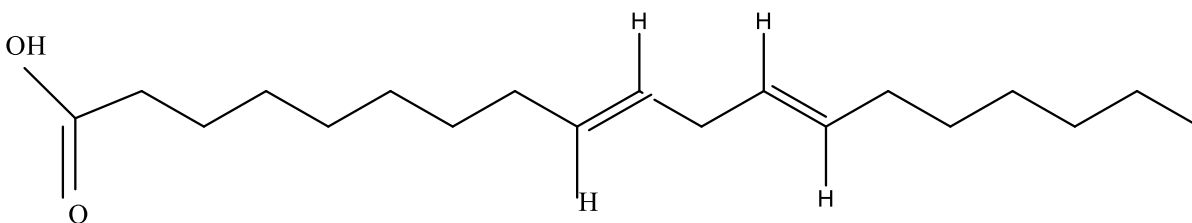


Figure.10. Structure of Linoleic acid.

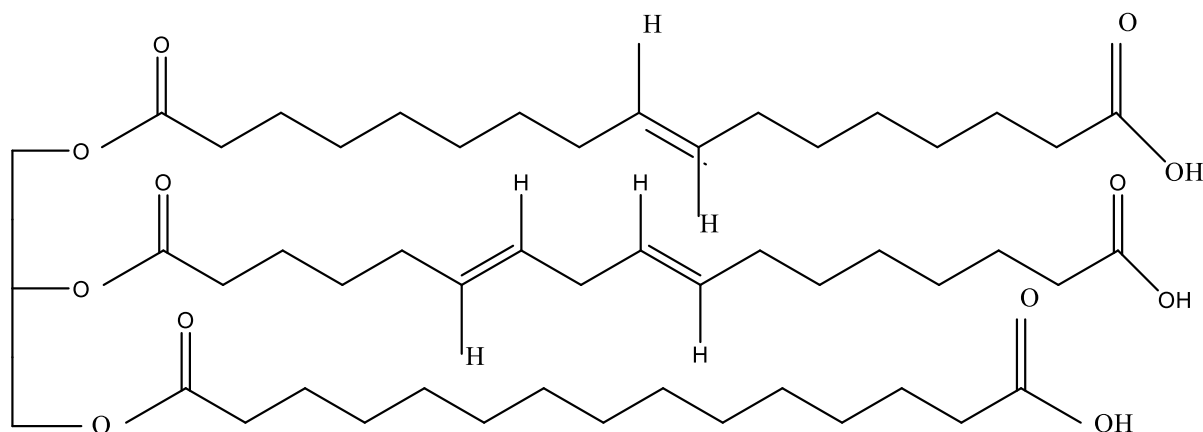


Figure.11 . Structure of Egg oil.

First longest chain corresponds to Oleic acid, then the second chain corresponds to Linoleic acid and the third chain corresponds to palmitic acid.

Conclusion:

The Egg oil is characterised with the spectral studies such as FTIR, ^1H NMR and C^{13} NMR. FTIR studies provide information regarding the functional groups. The presence of acid and ester group is confirmed with the help of FTIR spectral studies. The unsaturated protons are identified with the help of NMR studies. The GC-MS analysis showed the fragmentation pattern present in the egg oil. The GC-MS analysis confirm the presence of triglycerides of fatty acids such as Oleic acid, palmitic acid and linoleic acid. The Molecular weight of Egg oil is analysed by GC-MS analysis. The Liquid Chromatography Mass Spectroscopy showed the presence of multi components of Egg oil. Based on the above studies the structure of Egg oil is elucidated.

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